

Regulation of Hepatic Drug Metabolism by the Interaction of Host- and Gut Microbiome-Derived Bile Acids and Hepatic LncRNAs

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Abstract

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Liver is a critical organ for nutrient homeostasis and xenobiotic biotransformation. The gut microbiome regulates important host metabolic pathways in liver including xenobiotic metabolism and intermediary metabolism, such as the conversion of primary bile acids (BAs) into secondary BAs and is an important factor of healthy development. The nuclear receptors pregnane X receptor (PXR/NR1I2) and constitutive androstane receptor (CAR/Nr1I3) are well-known regulators for xenobiotic metabolism and disposition in liver. However, little is known regarding the age specificity of secondary BA metabolism or other microbial metabolites and the potential effects of PXR and CAR on the composition and function of the gut microbiome. Additionally, altered expression of long noncoding RNAs (lncRNAs) by environmental chemicals modulates the expression of xenobiotic metabolizing genes and transporters; yet little is known regarding how lncRNAs are regulated by PXR, affecting xenobiotic metabolism. Therefore, the goal of my dissertation was to use a multi-omics approach, including transcriptomics (RNA-seq), targeted metabolomics (UPLC-MS/MS for BAs and GC-MS/MS for short-chain fatty acids), targeted

proteomics (LC-MS), and 16S rRNA gene sequencing as well as bioinformatics, to strategically investigate the modulation of hepatic xenobiotic metabolizing genes through gut microbiome-mediated, age-dependent, and transcriptional mechanisms. To achieve this goal, multi-omics analysis of various tissues were performed in male and female conventional (CV) and germ-free (GF) mice at various ages: postnatal days 1, 5, 10, 15, 25, 60, and 120. With increasing age, the gut microbiome in mice become more diverse and had increased functional capacity to produce secondary BAs, including the family *S24-7*. Twenty-seven bacteria were positively associated with secondary unconjugated, deoxy, and keto BAs with nine of these BAs only produced in CV mice. Short-chain fatty acids (SCFAs) were found in livers of CV mice only with six SCFAs increasing with age. Global analysis of hepatic RNA expression showed an age-specific enrichment of KEGG pathways, including xenobiotic metabolism for adult CV and GF mice. Differentially expressed drug-metabolizing enzymes and transporters exhibited age and sex specific expression patterns with more genes differentially expressed with increasing age. Overall, the age-specific gut microbiome impacts host xenobiotic metabolism likely through circulation of metabolites (e.g. BAs and SCFAs). Inversely, to test how the liver (specifically PXR and CAR) regulates gut microbiota and secondary BA synthesis, nine-week-old male CV and GF mice were orally gavaged with corn oil, PXR agonist pregnenolone-16 α -carbonitrile (PCN; 75 mg/kg), or CAR agonist 1,4-bis-[2-(3,5-dichloropyridyloxy)]benzene, 3,3',5,5'-tetrachloro-1,4-bis(pyridyloxy)benzene (TCPOBOP; 3 mg/kg) once daily for four days. PCN and TCPOBOP decreased two taxa in the *Bifidobacterium* genus, which corresponded with decreased gene abundance of the BA-deconjugating enzyme bile salt hydrolase (*bsh*). In liver and small intestinal content of GF mice, there was a TCPOBOP-mediated increase in total, primary, and conjugated BAs corresponding with increased Cyp7a1 mRNA. Bacteria from the genera *Bifidobacterium*, *Dorea*, *Peptococcaceae*, *Anaeroplasma*, and *Ruminococcus* positively correlated with T-UDCA in LIC, but negatively correlated with T-CDCA in serum. Therefore, PXR and CAR activation down-

regulates BA-metabolizing bacteria in the intestine and modulates BA homeostasis in a gut microbiota-dependent manner.

To understand how lncRNAs may impact hepatic xenobiotic metabolism, RNA-Seq was performed from livers of adult male C57BL/6 mice treated with corn oil, the PXR agonist PCN, or the CAR agonist TCPOBOP. PXR activation differentially regulated 193 lncRNAs with 40% also regulated by CAR. Among differentially expressed lncRNAs, the lncRNA-PCG pairs displayed a high co-regulatory pattern by PXR and CAR activation. Combining the RNA-Seq data with a published PXR ChIP-Seq dataset (Cui et al., 2010b), 774 expressed lncRNAs with direct PXR-DNA binding sites, and 26.8% of differentially expressed lncRNAs had changes in PXR-DNA binding following PCN exposure. Therefore, some lncRNAs are regulated by PXR and CAR activation and that they may be important regulators of xenobiotic metabolism. Taken together, there is an age-dependent interaction in the gut-liver axis that exhibits a circulatory pattern such that BA homeostasis is both governed by both the gut microbiome and the host liver, with modifications to either biocompartment impacting the other biocompartment. Targeting the gut-liver axis and lncRNAs may lead to the design of novel therapies alleviated toxicities through control of hepatic xenobiotic metabolism.

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List of Abbreviations

5MeC	DNA methylation
Abcc3/Mrp3	ATP binding cassette subfamily C member 3
AhR	aryl hydrocarbon receptor
ApoA4	Apolipoprotein A4
AS-1	antisense 1
ASVs	amplicon sequence variants
Atp8b1	ATPase, class I, type 8B, member 1
bai	BA-inducible
BAs	bile acids
BH-FDR	Benjamini–Hochberg false discovery rate
BSA	bovine serum albumin
Bsep/Abcb11	bile salt efflux pump
bsh	bile salt hydrolase
C/EBP	CCAAT-enhancer-binding proteins
CA	cholic acid
CAR/Nr1i3	constitutive androstane receptor
CDCA	chenodeoxycholic acid
CITCO	6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime
CREB	cAMP responsive element binding protein
CV	conventional
Cyp3a11	cytochrome P450, family 3, subfamily a, polypeptide 11
Cyps	cytochrome P450s
DCA	deoxycholic acid
DE	Differentially expressed
DPGs	drug processing genes
ESI	electrospray ionization
EWAS	epigenome wide association studies
FGF19	fibroblast growth factor 19
FishTaco	Functional Shifts' Taxonomic Contributors
FPKM	fragments per kilobase of transcript per million mapped reads
FXR	farnesoid x receptor
G6Pase	glucose-6-phosphatase catalytic subunit
GAL	galactose
Gapdh	glyceraldehyde 3-phosphate dehydrogenase
GF	germ-free
GLP-1	glucagon-like peptide 1
GPR	G-protein coupled receptors
Gsts	glutathione-s-transferases
Gstz1	glutathione S-transferase zeta 1
H3K4me2	histone H3 lysine 4 di-methylation
HCA	hyocholic acid
HDCA	hyodeoxycholic

hESC-Hep	human embryonic stem cell-derived hepatocytes
HNF1 α	hepatocyte nuclear factor 1 α
HSD3B7	3 β -hydroxy- Δ 5-C27-steroid dehydrogenase
IACUC	Institutional Animal Care and Use Committee
IPA	Ingenuity Pathway Analysis
IS	internal standards
KEGG	Kyoto Encyclopedia of Genes and Genomes
LCA	lithocholic acid
LIC	large intestinal content
lncRNAs	long noncoding RNAs
LXRs	liver X receptors
MRM	multiple-reaction-monitoring mode
NASH	non-alcoholic steatohepatitis
Nfe2l2/Nrf2	nuclear factor, erythroid derived 2, like 2
Nqo1	NAD(P)H dehydrogenase, quinone 1
Nr0b2/SHP	nuclear receptor subfamily 0, group B, member 2
Ntcp/Slc10a1	sodium taurocholic transporting polypeptide
Oatp	organic anion transporting polypeptide
OTUs	operational taxonomic units
PAVIS	peak annotation and visualization
PBDEs	polybrominated diphenyl ethers
PCA	principle component analysis
PCGs	protein-coding genes
PCN	pregnenolone-16 α -carbonitrile
PEPCK1	phosphoenolpyruvate carboxykinase 1
PICRUSt	Phylogenetic Investigation of Communities by Reconstruction of Observed States
Ppara α	peroxisome proliferator activated receptor alpha
PXR/NR1I2	pregnane X receptor
qPCR	quantitative polymerase chain reaction
RIN	RNA integrity
RNApol II	RNA polymerase II
SAA1	serum amyloid A1
SCFAs	Short-chain fatty acids
SHP	small heterodimer partner
shRNA	short hairpin RNA
SIC	small intestinal content
siRNA	small interfering RNA
Slc27a5/Bal	solute carrier family 27 member 5
SMRT	silencing mediator of retinoid and thyroid hormone receptors
SNPs	single nucleotide polymorphisms
TCPOBOP	1,4-bis-[2-(3,5-dichloropyridyloxy)]benzene, 3,3',5,5'-tetrachloro-1,4-bis(pyridyloxy)benzene
TFs	transcription factors
TGR5	Takeda G protein receptor 5

TSS	transcription start site
TTS	transcriptional termination site
UDCA	ursodeoxycholic acid
Ugt	UDP glucuronosyltransferase
UTR	5'-untranslated region
VDR	vitamin D receptor
α MCA	alpha-muricholic acid

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Chapter 1: Introduction

1.1 Gut Microbiome

The gut microbiome is increasingly recognized as an important regulator of host nutrient homeostasis and xenobiotic metabolism and transport. Dysbiosis of the gut microbiome is associated with poor detoxification of persistent environmental chemicals and onset of chronic diseases, such as irritable bowel disease, metabolic syndrome, cancer, hepatic steatosis, and cirrhosis. Recent literature suggests that early life exposures to toxic environmental chemicals and antibiotics, as well as malnutrition, modulate the gut microbiome leading to delayed onset of diseases (Arrieta et al., 2014). Despite the growing recognition that gut microbiome is a new research paradigm for the developmental origins of health and disease, there lacks basic biological research establishing the developmental role of the gut microbiome and hepatic health. The expression of important host xenobiotic metabolism and transport genes in liver is age- and gut microbiome-dependent (Toda et al., 2009b; Toda et al., 2009c; Selwyn et al., 2015a; Selwyn et al., 2015c; Selwyn et al., 2016).

The gut microbiome is a key modifier of host xenobiotic metabolism and transport through direct and indirect mechanisms (Spanogiannopoulos et al., 2016; Koppel et al., 2017). For direct mechanisms, intestinal bacteria use their own enzymes that are distinct from the host to metabolize ingested compounds, such as dietary components, pharmaceuticals, and environmental chemicals (Spanogiannopoulos et al., 2016; Koppel et al., 2017). These microbial modifications, many of which remain poorly characterized, can either increase or decrease the toxicity or efficacy of drugs. The PharmacoMicrobiomics database has identified over 90 drugs in the literature that are modified by the gut microbiome, but only 28 have identifiable interactions with a specific bacterial species (<http://pharmacomicrobiomics.com/>). For example, the first drug-bug interaction was reported by Drs. Lindenbaum and Saha for the inactivation of digoxin by *Eggerthella lenta* (Lindenbaum et al., 1981; Saha et al., 1983). Characterization of these microbial enzymes has led to better understanding of the pharmacokinetics as well as improved

efficacy and safety of digoxin (Haiser et al., 2013; Haiser et al., 2014; Koppel et al., 2018). Furthermore, microbial β -glucuronidases deconjugate host Phase-II glucuronidation metabolites and modulate the potential toxicity of circulating endogenous and xenobiotic compounds (Wallace et al., 2015; Pollet et al., 2017; Little et al., 2018).

For indirect mechanisms, the gut microbiome can modify host metabolites that are subsequently absorbed into systemic circulation and enter various host organs to interact with host receptors involved in xenobiotic metabolism and transport (Spanogiannopoulos et al., 2016; Fu and Cui, 2017). The presence of the gut microbiome is necessary in regulating the constitutive expression of many drug processing genes (DPGs), evidenced from altered expression of DPGs in mice lacking a gut microbiome (i.e. germ-free [GF] mice) (Bjorkholm et al., 2009; Toda et al., 2009c; Selwyn et al., 2015a; Selwyn et al., 2015c). For example, the expression of the major Phase-I oxidation enzyme cytochrome P450, family 3, subfamily a, polypeptide 11 (Cyp3a11)—a PXR-target gene and the mouse ortholog to human CYP3A4—was decreased in GF mice compared to CV mice. This effect was also observed in CV mice treated with antibiotics and was correlated with decreased bacteria in the feces that produce the PXR-activating bile acid (BA) lithocholic acid (LCA) (Toda et al., 2009b; Toda et al., 2009a). Therefore, modulation of the gut microbiome and the bacterial metabolites can alter xenobiotic metabolism and transport of the host.

1.2 Nuclear Receptors and Drug Metabolism

Biotransformation of xenobiotics, such as drugs, dietary factors, and environmental chemicals, often mitigates potential toxicities to the body, preventing the occurrence of xenobiotic-induced injury. Liver is a critical organ for nutrient homeostasis and xenobiotic metabolism and transport. Regarding metabolism of xenobiotics, there are two major categories of metabolism: Phase I and Phase II. Phase I xenobiotic-metabolizing enzymes catalyze oxidation, reduction,

and hydrolysis reactions, which generally produce a small functional group on the substrate for further reactivity. Phase II xenobiotic-metabolizing enzymes catalyze various conjugation reactions, including glucuronidation, sulfonation, glutathione conjugation, acetylation, methylation, amino acid conjugation, and fatty acid conjugation. In general, phase I and II metabolism make the initial substrate more water soluble for excretion. The nuclear receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR) are well-known xenobiotic-sensing transcription factors with overlapping target genes involved in the Phase I and Phase II xenobiotic metabolism as well as transporters (collectively called drug-processing genes [DPGs]) (Kliewer et al., 1998; Wei et al., 2000; Moore et al., 2003; Petrick and Klaassen, 2007; Hernandez et al., 2009; Cui and Klaassen, 2016). The vast majority of DPG research has focused on adult humans and animal models; however, it is known that age and gut microbiome are significant modifiers of the expression of DPGs.

PXR was first identified as a steroid-activating receptor before its function in xenobiotic metabolism was discovered (Kliewer et al., 1998; Kliewer et al., 2002). PXR is a promiscuous transcription factor that is activated by various xenobiotics, including therapeutic drugs such as dexamethasone, paclitaxel, and rifampicin (Moore et al., 2003), environmental toxicants such as the flame retardant polybrominated diphenyl ethers (PBDEs) (Pacyniak et al., 2007), some pesticides (Lemaire et al., 2006), and herbal supplements such as St. John's Wort (Moore et al., 2000). PXR is also activated by metabolites produced by the gut microbiome, such as the secondary BA LCA and the tryptophan metabolite indole-3-propionic acid (Staudinger et al., 2001; Venkatesh et al., 2014). Pregnenolone-16 α -carbonitrile (PCN) is recognized as a specific PXR ligand in rodents, whereas rifampicin is specific to human PXR activation (Moore et al., 2003).

CAR was identified as a mediator of drug metabolism specifically for altering the sensitivity to barbiturates (Tzameli et al., 2000; Wei et al., 2000). Repressive endogenous ligands for constitutive activity of CAR include androstenol and androstanol (Forman et al., 1998), whereas endogenous activators of CAR are not well characterized. Phenobarbital and LCA can indirectly

facilitate the nuclear localization of CAR and up-regulation of CAR-target genes (Swales and Negishi, 2004; Beilke et al., 2009a; Beilke et al., 2009b). A potent and selective CAR activator in mice is 1,4-bis-[2-(3,5-dichloropyridyloxy)]benzene, 3,3',5,5'-tetrachloro-1,4-bis(pyridyloxy)benzene (TCPOBOP) (Tzameli et al., 2000), whereas 6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime (CITCO) is a selective CAR activator in humans (Swales and Negishi, 2004).

Activation of PXR and CAR either endogenously or pharmacologically can protect the liver from toxicity. For example, microbial secondary BAs, which are toxic to the liver at elevated concentrations, can activate PXR or CAR as a compensatory mechanism against cholestasis (Staudinger et al., 2001; Beilke et al., 2009a). Neonatal activation of PXR and CAR interact with histone epigenetic marks to modulate target gene transcription and permanently change drug metabolism in mouse liver (Cui et al., 2009; Li et al., 2009; Wada et al., 2009; Cui et al., 2010a; Cui et al., 2010b; Chen et al., 2012; Giebel et al., 2016). Neonatal activation of CAR has shown to permanently induce the expression and activity of Cyp2b, Cyp2c, and Cyp3a enzymes in mouse liver (Chen et al., 2012; Tien et al., 2015; Tien et al., 2017), while neonatal activation of PXR induces the expression of PXR target genes, but decreases the expression of PPAR α target genes (Giebel et al., 2016). Furthermore, there is an age-dependent regulation of histone methylation at the *Cyp3a* locus in mice, and permissive epigenetic marks around the *Cyp3a* locus increase following PXR activation in human liver cells (Yan et al., 2017).

1.3 Biosynthesis and disposition of bile acids

Host liver, which is the major site for xenobiotic metabolism and transport and nutrient homeostasis, mediates circulating metabolites with the gut microbiome through enterohepatic circulation. Within the gut-liver axis, the circulating metabolites produced by the host, gut microbiome, or both, create a dynamic signaling network that is sensitive to changes in external environment such as diet and xenobiotics. One of the important classes of circulating metabolites

jointly produced by host liver and intestinal bacteria is BAs. Primary BAs are produced in the liver from cholesterol and are modified by the gut microbiome to secondary BAs before being reabsorbed into the blood and “repaired” by the liver (Hofmann, 2009a).

BAs are amphipathic molecules synthesized in liver *de novo* from cholesterol to primary BAs through two distinct pathways: 1) the classic pathway with the overall rate-limiting BA synthesis enzyme Cyp7a1 and 2) the alternative pathway initiated by Cyp27a1. Primary BAs are conjugated with taurine, glycine, or other co-substrates and undergo enterohepatic circulation by secretion into the bile and re-absorption from the intestine, facilitating the uptake of dietary fats, steroids, drugs, and lipophilic vitamins (Hofmann, 2009b; Li and Chiang, 2014). In the intestine, bacteria further metabolize primary BAs to secondary BAs through deconjugation, oxidation, and epimerization reactions; this, in general, increases the hydrophobicity of the BA pool (Ridlon et al., 2006; Hofmann, 2009b; Li and Chiang, 2014). Primary and secondary BAs also include their conjugate forms. In humans, the majority of BAs are conjugated with taurine, glycine, or 3'-phosphoadenosine-5'-phosphosulfate, whereas in mice, the majority of BAs are conjugated with taurine (Thakare et al., 2018). Primary BAs all have a 7-hydroxy substituent (from Cyp7a1), and in humans, the majority of primary BAs are chenodeoxycholic acid (CDCA) and cholic acid (CA). CA is the only primary BA from the classic pathway in liver and is metabolized by intestinal bacteria to the secondary BA deoxycholic acid (DCA). CDCA is produced from the alternative pathway of BA synthesis, and in mice, CDCA is metabolized to α -muricholic acid (α MCA), β MCA, and ursodeoxycholic acid (UDCA) (Hofmann, 2009b). UDCA has been found increased in both genders of GF compared to CV mice, suggesting that a host mechanism exists to produce UDCA (Zhang and Klaassen, 2010; Selwyn et al., 2015b; Wahlstrom et al., 2017). There is no known host enzyme for the synthesis of UDCA in mice and humans. Secondary BAs produced from the alternative pathway of BA synthesis include ω MCA, LCA, hyocholic acid (HCA), and hyodeoxycholic (HDCA). Because BAs in high concentrations can be toxic the BA pool is tightly controlled. BA homeostasis is regulated by the major BA sensor farnesoid x receptor (FXR),

which is responsible for a network of genes in hepatic BA synthesis, biliary BA secretion, intestinal BA absorption, and hepatic BA uptake (Kuipers et al., 2004; Rizzo et al., 2005; Matsubara et al., 2013). In general, FXR is activated by hydrophobic BAs with CDCA a much more potent activator of FXR than CA, and global binding patterns of FXR are similar in primary human hepatocytes and mouse liver (Li et al., 2013; Ding et al., 2015).

Transporters are important to the distribution of BAs throughout the enterohepatic organs. Majority of conjugated BAs absorbed from the portal blood into hepatocytes, occurs from the basolateral uptake transporter sodium taurocholic transporting polypeptide (Ntcp/Slc10a1), whereas unconjugated BAs are transported into hepatocytes by the organic anion transporting polypeptide (Oatp) 1b2 (Klaassen and Aleksunes, 2010; Csanaky et al., 2011). Biliary excretion of BAs from hepatocytes to storage in the gall bladder is through the rate-limiting transporter bile salt efflux pump (Bsep/Abcb11). Transport of BAs into blood as a compensatory mechanism during cholestasis occurs on the on the basolateral membrane: the solute carriers Slc51a/Ost α and Slc51b/Ost β and the efflux transporter multidrug resistance-associated protein ATP binding cassette subfamily C member 3 (Abcc3/Mrp3) and Abcc4/Mrp4 (Halilbasic et al., 2013). Interestingly, the regulation of BAs and transporters in mice is age dependent such that there is a surge in the primary conjugated BA T-CA at birth which increases the expression of the BA transporters Ntcp, Bsep, Ost β , and Mdr2 in an FXR dependent manner, modifying enterohepatic circulation (Cui et al., 2012). However, how gut microbiome modifies BA homeostasis early in life is unknown.

Several studies have shown the importance of the xenobiotic-sensing nuclear receptors PXR and CAR in BA homeostasis. Pharmacological activation of PXR by pregnenolone-16 α -carbonitrile (PCN) represses hepatic Cyp7a1 mRNA and activity in mice (Li and Chiang, 2013; Li and Chiang, 2014), and endogenous activation of PXR by the secondary BA LCA protects the liver against toxicity (Staudinger et al., 2001). PXR activation in intestine/enterocytes induced the intestinally secreted hormone fibroblast growth factor 19 (FGF19; Fgf15 in mice), which represses

the transcription of Cyp7a1 in liver (Wistuba et al., 2007; Li and Chiang, 2013). Activation of CAR has also been shown to repress the expression of *CYP7A1* in human hepatocytes (Miao et al., 2006). CAR activation in male and female CV mice by TCPOBOP decreased the BA pool, primarily through decreased T-CA, while the expression of Cyp7a1 increased, attempting to compensate for the decreased BAs (Lickteig et al., 2016). The BA ligase solute carrier family 27 member 5 (Slc27a5/Bal), which regulates BA conjugation, was decreased following TCPOBOP exposure (Cui and Klaassen, 2016), possibly contributing to the improved metabolic health following CAR activation (Dong et al., 2009; Gao et al., 2009). BAs are not known to serve as ligands of CAR (Li and Chiang, 2013).

However, little is known regarding the regulation of gut microbiota and the microbial metabolism of BAs by age and xenobiotic exposure. Furthermore, production of BAs is age-dependent, however the ontogeny of primary BAs and the associated bacteria responsible for secondary BA synthesis is poorly understood (Cui et al., 2012), yet little is known regarding to what extent secondary BAs modulate the ontogeny of hepatic xenobiotic metabolism and transport and intermediary metabolism related genes.

1.4 Long noncoding RNAs

Excerpts taken from Dempsey JL and Cui JY (2017). Long Non-Coding RNAs: A Novel Paradigm for Toxicology, *Toxicological Sciences*, 155:1 (3–21).

Previously thought to be transcriptional noise, lncRNAs have been recently found to be integral to human health and disease. The NONCODE database has identified over 90,000 human lncRNA genes and over 140,000 human lncRNA transcripts (Zhao et al., 2016). lncRNAs have diverse regulatory functions in cell physiology, including chromatin remodeling, gene transcription and translation, protein trafficking, and cellular signaling (Geisler and Coller, 2013; Karlsson and Baccarelli, 2016). Dysregulation of lncRNAs due to environmental exposures,

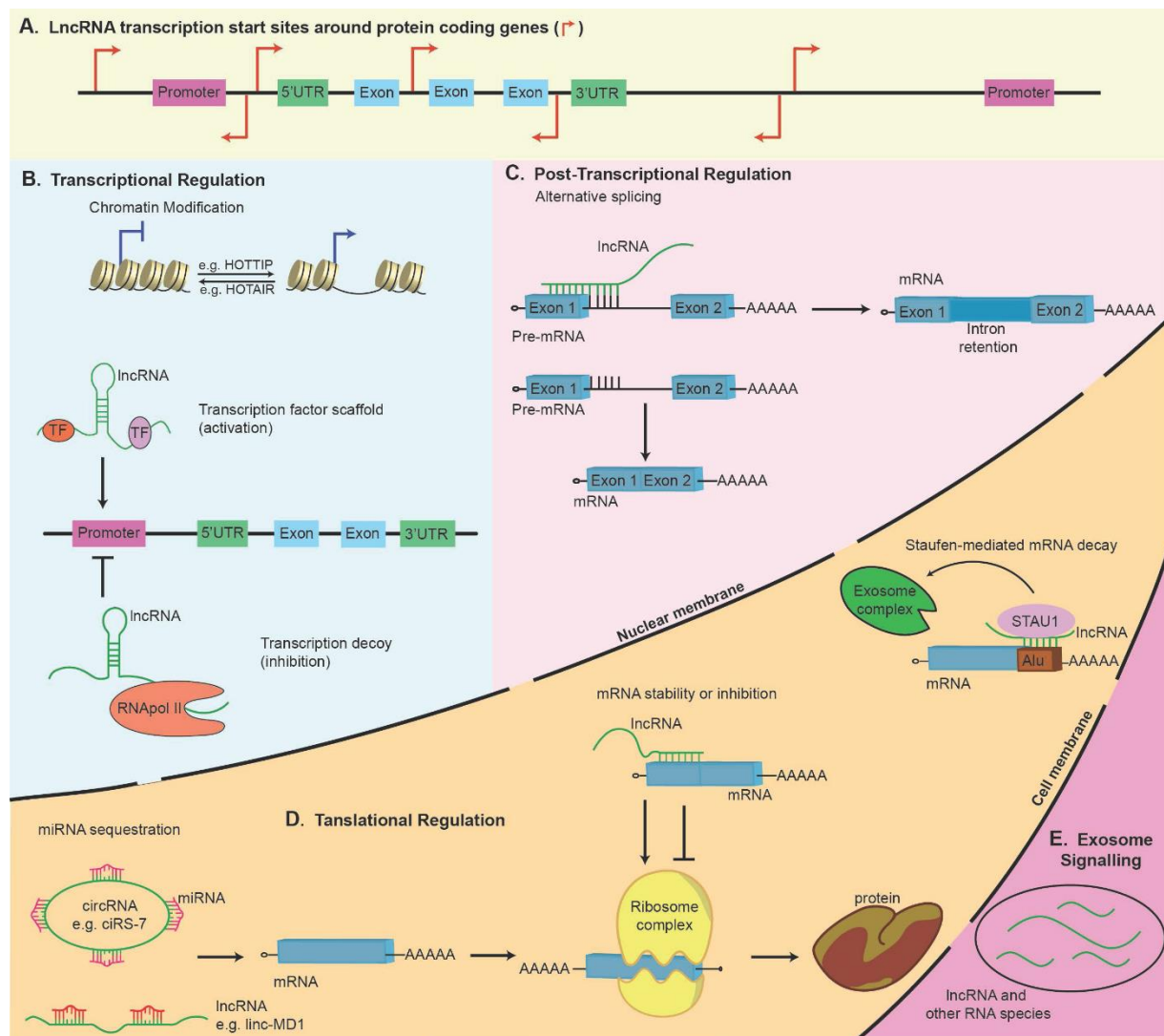
genetic mutations, or other causes are associated with various human diseases, such as cancer, neurological disorders, and metabolic syndrome (Geisler and Collier, 2013).

LncRNAs can be transcribed from either the Watson or the Crick DNA strand and can be intergenic, exonic, in enhancer regions, or in regions distal to protein-coding genes (Figure 1.1A) (Wang and Chang, 2011; Geisler and Collier, 2013). Similar to mRNAs, lncRNAs are transcribed by RNA polymerase II (RNAPol II), can undergo alternative splicing, may carry single nucleotide polymorphisms (SNPs), have 5' caps, and are usually polyadenylated (Derrien et al., 2012; Karlsson and Baccarelli, 2016). It has been estimated that the majority of lncRNAs have more than 2 exons, and can have secondary and tertiary structures (Derrien et al., 2012; Novikova et al., 2012).

LncRNAs have their own promoters, unique DNA-binding motifs, and preferred transcription factors (TFs) (Derrien et al., 2012; Popadin et al., 2013; Alam et al., 2014). Computational analysis of RNA-Seq data has suggested that lncRNA transcription may be independent from and influence the transcription of neighboring protein-coding genes, and may have more *cis* expression quantitative trait loci than protein-coding genes (Derrien et al., 2012; Popadin et al., 2013). LncRNAs are also regulated by epigenetic mechanisms including DNA methylation (Popadin et al., 2013). Only 11-29% of lncRNAs are ubiquitously expressed in all tissues, as compared to 65% of protein-coding genes, and lncRNAs are generally expressed at much lower levels than protein-coding mRNAs (Derrien et al., 2012; Djebali et al., 2012).

LncRNAs can act as transcriptional inhibitors by acting as a lncRNA decoy that bind to RNAPol II, such as the short interspersed response element Alu transcripts during heat shock (Figure 1.1B), or a lncRNA-DNA triplex obstructing the assembly of the pre-initiation complex (Martianov et al., 2007; Mariner et al., 2008). However, in the *Saccharomyces cerevisiae* galactose (GAL) gene cluster, GAL lncRNAs form lncRNA-DNA hybrids (R-loops) on the GAL gene promoter, activating transcription (Cloutier et al., 2016). LncRNAs also bind and localize transcription factors for gene activation or repression (Martens et al., 2004; Willingham et al.,

2005). After the formation of the nascent transcripts from the protein-coding genes, lncRNAs can regulate mRNA processing through alternative splicing (Figure 1.1C) and other RNA editing



processes (Faghihi and Wahlestedt, 2009).

Figure 1.1. LncRNA transcriptional start sites and examples of lncRNA functions. **A.** LncRNAs are transcribed from the Watson or Crick strand and the arrows represent lncRNA transcriptional start sites that are intergenic, exonic, in enhancer regions, or in regions distal to protein-coding genes. **B.** LncRNAs, such as HOTAIR and HOTTIP, modify nuclear architecture through organization of histone modification proteins (top), whereas other lncRNAs (bottom) influence transcription by localizing transcription factors or inhibiting RNA polymerase II. **C.** LncRNAs regulate alternative splicing of nascent protein-coding transcripts by intron retention. **D.** LncRNAs modulate translation through miRNA sequestration (left), interacting with mRNA to increase transcript stability or inhibit translation (middle), and mRNA turnover (right). **E.** LncRNAs are also emerging as possible extracellular signals in exosomal shuttle RNA to regulate the biological

functions of neighboring or distal cells. From Dempsey JL and Cui JY (2017). Long Non-Coding RNAs: A Novel Paradigm for Toxicology, *Toxicological Sciences*, 155:1 (3–21).

LncRNA regulation is impacted by exposures to various chemicals such as polycyclic aromatic hydrocarbons (Gao et al., 2013; Gao et al., 2016), benzene (Bai et al., 2014), cadmium (Zhou et al., 2015), chlorpyrifos-methyl (Shin et al., 2014), bisphenol A (Doshi et al., 2013; Kumamoto and Oshio, 2013), phthalates (LaRocca et al., 2014), and BAs (Li et al., 2015; Zhang et al., 2016). It has been suggested that some lncRNAs act in cis to regulate the expression of neighboring protein-coding genes (PCGs) in a mechanism that fine-tunes gene expression. However, little is known regarding the transcriptional regulation of lncRNAs related to the metabolism of xenobiotics – in particular, how the critical xenobiotic-sensing nuclear receptors PXR or CAR may regulate the expression of lncRNAs.

Many lncRNAs are highly expressed in liver, which is a major organ for xenobiotic metabolism and transport and nutrient homeostasis. A stringent computational pipeline identified 15,558 lncRNAs that are expressed in mouse liver, based on an analysis of 186 mouse liver RNA-Seq datasets ranging over 30 biological conditions (Yu et al., 2017). Interestingly, Yu et al. also demonstrated greater inter-species conservations within DNA sequences and higher frequency of proximal binding by hepatic transcription factors in the liver-expressed lncRNA gene promoters as compared to PCG promoters (Yu et al., 2017). The liver-enriched lncRNAs were predicted to be metabolically sensitive regulators with diverse functions in physiological homeostasis, especially energy metabolism (Yang et al., 2016). Regulation of genes involved in energy metabolism and nutrient homeostasis by lncRNAs found in the literature include gluconeogenesis (Goyal et al., 2017), cholesterol and BA homeostasis (Ananthanarayanan, 2016; Lan et al., 2016; Yu et al., 2017; Zhang et al., 2017), lipid metabolism (Li et al., 2015; Chen, 2016; Yang et al., 2016; Li et al., 2017; Zhao et al., 2017), and non-alcoholic fatty liver disease (Chen et al., 2017).

In addition to modulating intermediary metabolism in liver, lncRNAs are suggested to modulate hepatic xenobiotic metabolism. For example, two lncRNAs that are transcribed from

the anti-sense strands of the DNA encoding the hepatocyte nuclear factor 1 α (HNF1 α) and HNF4 α are important in regulating the major drug-metabolizing cytochrome P450s (Cyps) in human liver cancer-derived HepaRG cells (Chen et al., 2018). Specifically, short hairpin RNA (shRNA) knockdown of the lncRNA gene *HNF1 α antisense 1 (AS-1)* decreased the mRNA expression of PXR and CAR, as well as the expression of seven major P450s (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1, and CYP3A4) in HepaRG cells. In addition, small interfering RNA (siRNA) knockdown of the lncRNA gene *HNF4 α -AS1* increased the mRNA expression of PXR as well as the expression of six P450s (CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2E1, and CYP3A4). This indicated that there is a complex network between transcription factors and lncRNAs that regulate the expression of P450s (Chen et al., 2018).

1.5 Dissertation Outline

My long-term goal is to identify key regulatory mechanisms of the gut-microbiome-liver axis that regulate host xenobiotic metabolism and disposition and nutrient homeostasis, so as to identify novel probiotic therapies that protect and/or treat diseases in vulnerable populations such as newborns and malnourished people. The objective of this research is to use a multi-omics approach to investigate the gut microbiome-mediated and age-dependent mechanisms that modulate hepatic xenobiotic metabolism and transport related genes. My central hypothesis is that the age-dependent interaction of the gut-liver axis is governed by BA homeostasis in a circulatory pattern that ultimately modulates the expression of hepatic drug processing genes.

Chapter 1: Introduction.

Chapter 2: Ontogenic mechanisms for the regulation of hepatic drug metabolism through host- and gut microbiome-derived BAs. Liver is a critical organ for nutrient homeostasis and xenobiotic metabolism and transport. The gut microbiome regulates important host metabolic pathways in

liver including xenobiotic metabolism and intermediary metabolism, such as the conversion of primary BAs into secondary BAs and is an important factor of healthy development. However, little is known regarding the age specificity of secondary BA metabolism or other microbial metabolites. In this chapter, I investigate how the age-specific gut microbiome impacts host xenobiotic metabolism through circulation of metabolites in male and female CV and GF mice, 16S rRNA gene sequencing, LC-MS/MS target metabolomics for BAs, and GC-MS/MS target metabolomics for SCFAs, and RNA-Seq.

Chapter 3: Pharmacological activation of PXR and CAR down-regulates distinct BA-metabolizing intestinal bacteria and alters BA homeostasis. The gut microbiome regulates important host metabolic pathways including xenobiotic metabolism and intermediary metabolism, such as the conversion of primary BAs into secondary BAs. The nuclear PXR and CAR are well-known regulators for xenobiotic metabolism and transport in liver. However, little is known regarding the potential effects of PXR and CAR on the composition and function of the gut microbiome. In this chapter, I used male CV and GF mice, 16S rRNA gene sequencing, LC-MS/MS target metabolomics for BAs, and RT-qPCR for gene expression to investigate how PXR and CAR activation regulates BA metabolizing bacteria and modulates BA homeostasis.

Chapter 4: Regulation of Hepatic Long Non-coding RNAs by PXR and CAR Agonists in Mouse Liver. Altered expression of lncRNAs by environmental chemicals modulates the expression of xenobiotic metabolism and transport related genes and may serve as therapeutic targets and novel biomarkers of exposure. PXR is a critical xenobiotic-sensing nuclear receptor that regulates the expression of many drug-processing genes, and it has similar target gene profiles and DNA binding motifs with another xenobiotic-sensing nuclear receptor, namely CAR. However, little is known how lncRNAs are regulated by PXR in concert with PCGs to compare the PXR-targeted lncRNAs with CAR-targeted lncRNAs. In this chapter, I characterize lncRNAs that are regulated

by PXR and CAR and that may be important regulators of protein-coding genes involved in xenobiotic metabolism using RNA-seq performed on livers of mice exposed to corn oil, PXR, or CAR ligands.

Chapter 5: Conclusions and future directions.

1.6 Specific Aims and Hypotheses

To address my research goals, I have developed three related specific aims and hypotheses.

Chapter 2

Specific Aim: Determine the developmental regulation of individual BA species as well as their effect on host xenobiotic metabolism and transport and nutrient homeostasis by targeting the gut-liver axis.

Hypothesis: Age-specific bacteria produce unique patterns of secondary BAs during development that may regulate xenobiotic metabolism.

Chapter 3

Specific Aim: Determine to what extent the xenobiotic-sensing nuclear receptors PXR and CAR modulate BA homeostasis.

Hypothesis: Activation of PXR and CAR regulates gut microbiota and secondary BA synthesis.

Chapter 4

Specific Aim: Characterize the effect of PXR and CAR on the regulation of hepatic lncRNAs.

Hypothesis: lncRNAs are regulated by PXR in concert with protein-coding genes and PXR- and CAR- regulate differing sets of lncRNAs.

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Chapter 2: Ontogenic Mechanisms for the Regulation of Hepatic Drug Metabolism through Host- and Gut Microbiome-derived Bile Acids

2.1 Introduction

Biotransformation of xenobiotics, such as drugs, dietary factors, and environmental chemicals, often mitigates potential toxicities to the body, preventing the occurrence of xenobiotic-induced injury. Liver is a critical organ for nutrient homeostasis and xenobiotic metabolism and transport. Regarding metabolism of xenobiotics, there are two major categories of metabolism: Phase I and Phase II. Phase I xenobiotic-metabolizing enzymes catalyze oxidation, reduction, and hydrolysis reactions, which generally produce a small functional group on the substrate for further reactivity. Phase II xenobiotic-metabolizing enzymes catalyze various conjugation reactions, including glucuronidation, sulfonation, glutathione conjugation, acetylation, methylation, amino acid conjugation, and fatty acid conjugation. In general, phase I and II metabolism make the initial substrate more water soluble for excretion. The nuclear receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR) are well-known xenobiotic-sensing transcription factors with overlapping target genes involved in the Phase I and Phase II xenobiotic metabolism as well as transport (collectively called drug-processing genes [DPGs]) (Kliewer et al., 1998; Wei et al., 2000; Moore et al., 2003; Petrick and Klaassen, 2007; Hernandez et al., 2009; Cui and Klaassen, 2016). The vast majority of DPG research has focused on adult humans and animal models; however, it is known that age and gut microbiome are significant modifiers of the expression of DPGs.

The gut microbiome is a critical component of first-pass metabolism and is a mediator for “indirect xenobiotic metabolism” through modulation of hepatic signaling via remote-sensing mechanisms (Spanogiannopoulos et al., 2016; Fu and Cui, 2017). A chemical, nutritional, antimicrobial gradient shapes the microbiome composition and function along the gastrointestinal tract (Hayashi et al., 2005; Wang et al., 2005; Donaldson et al., 2016; Hillman et al., 2017; Crespo-

Piazuelo et al., 2018; Seekatz et al., 2019). Although the ratio of bacterial to human cells is approximately 1:1, there are nearly 10 million genes in the human microbiome (Lloyd-Price et al., 2017) with 3.3 million genes in the human gut microbiome (Qin et al., 2010).

Within the vast pool of microbial genes, a subset is capable of directly metabolizing drugs, dietary factors, environmental chemicals, and other xenobiotics independent from the host, often through reduction or hydrolysis reactions. This metabolism can occur before the first pass metabolism of the liver, redefining pharmacokinetics outside of host genetic polymorphisms, xenobiotic partitioning, distribution, and other factors of inter-individual variability. Indirectly, the gut microbiome produces metabolites that can interact with host receptors, acting as signaling molecules to regulate the expression and activity of xenobiotic metabolizing enzymes. For example, primary bile acids (BAs) are produced in the liver from cholesterol and are excreted into the intestines through the bile duct wherein the microbiota convert them to secondary BAs that can be reabsorbed to the liver (Chiang and Ferrell, 2019). The secondary BA lithocholic acid (LCA; made from the primary BA chenodeoxycholic acid [CDCA]) can activate the major xenobiotic sensing nuclear receptor pregnane X receptor (*PXR/NR1I2*), which is responsible for regulating the expression of many xenobiotic metabolizing enzymes (Staudinger et al., 2001).

Administration of xenobiotics, primarily oral antibiotics that are poorly absorbed, initially established the indirect relationship between gut bacteria and indirect signaling control of drug metabolism in the liver. In rats, the administration of the antibiotic ciprofloxacin decreased the hepatic expression of *CYP2C11* and *CYP3A1* (Xie et al., 2003). In mice treated with ciprofloxacin, the expression and activity of *Cyp3a11* between conventional (CV; i.e. mice with a microbiome) and germ-free (GF; i.e. mice without a microbiome) mice was microbiome-dependent (Toda et al., 2009b). The expression of many *Cyp1*, *2*, and *3* enzymes as well as the major xenobiotic sensing transcription factors *PXR*, constitutive androstane receptor (*CAR/NR1I3*), and *AHR* were decreased in the livers of GF compared to CV mice (Toda et al., 2009c). A microarray study showed that the expression of 112 xenobiotic metabolizing genes were differentially expressed

between CV and GF mice (Bjorkholm et al., 2009). In addition, conventionalization or restoration of the microbiome of GF to create exGF mice restored some of the differentially regulated drug metabolizing enzymes. GF female mice habituated in cages housing CV mice normalized the hepatic RNA expression of many hepatic P450s to CV levels, including *Cyp3a11*, but it is unclear how the microbiome of the ex-GF mice differed from CV mice (Claus et al., 2011). GF mice exposed to CV mouse feces conventionalized the RNA expression of the PXR-target genes *Cyp3a* cluster and decreased the PPAR α -target genes *Cyp4a* cluster, whereas GF mice exposed to the probiotic VSL3 did not have a major impact on hepatic P450 gene expression (Selwyn et al., 2016). RNA-Seq in livers of male and female CV and GF mice from the ages of day 1 to day 90 showed that the xenobiotic metabolism was the most downregulated pathway in the absence of bacteria, and the differential regulation of DPGs could be age-specific and gut microbiome dependent (Selwyn et al., 2015a). This demonstrates that specific commensal bacteria in the gut – and by extension the metabolites produced by bacteria – have unique signaling capabilities relevant to host drug metabolism and that the gut microbiome at a specific age may impact the development of DPGs.

Among the metabolites unique to bacteria and circulating in hosts are BAs. BAs are amphipathic molecules synthesized in the liver from cholesterol to primary BAs through two distinct pathways: 1) the classic pathway (neutral pathway) (Chiang and Ferrell, 2018) with the overall rate-limiting BA synthesis enzyme Cyp7a1 and 2) the alternative pathway (acidic pathway) (Chiang and Ferrell, 2018) initiated by Cyp27a1. Primary BAs are conjugated with taurine, glycine, or other co-substrates and are secreted into the bile and Intestines. BAs are reabsorbed from the intestine, undergoing enterohepatic circulation, and facilitate the uptake of dietary fats, steroids, drugs, and lipophilic vitamins (Hofmann, 2009; Li and Chiang, 2014). In the intestine, bacteria metabolize primary BAs to produce secondary BAs through deconjugation, oxidation, and epimerization reactions (Ridlon et al., 2006; Hofmann, 2009; Li and Chiang, 2014). Secondary BAs tend to increase the hydrophobicity of the BA pool. Regarding the conjugate

forms of BAs, in humans, the majority of BAs are conjugated with taurine, glycine, or 3'-phosphoadenosine-5'-phosphosulfate; in mice, the majority of BAs are conjugated with taurine (Thakare et al., 2018). All primary BAs have a 7-hydroxy substituent (from Cyp7a1), and in humans, the majority of primary BAs are chenodeoxycholic acid (CDCA) and cholic acid (CA). CA – from the classic pathway – is produced by the liver and is metabolized by intestinal bacteria to the secondary BA deoxycholic acid (DCA). In mice, CDCA – from the alternative pathway – is metabolized to alpha-muricholic acid (α MCA), β MCA, and ursodeoxycholic acid (UDCA) (Hofmann, 2009). Previously classified as a secondary BA, UDCA has been found increased in GF compared to CV mice of both sexes, suggesting that a host mechanism exists to produce UDCA (Zhang and Klaassen, 2010; Selwyn et al., 2015b; Wahlstrom et al., 2017; Dempsey et al., 2019). There is no known host enzyme for the synthesis of UDCA in mice and humans. Because of the revelation that UDCA is not a *bona fide* secondary BA, it is possible that other secondary BAs can be produced by the host. Secondary BAs produced from the alternative pathway of BA synthesis include ω MCA, LCA, hyocholic acid (HCA), and hyodeoxycholic (HDCA). Because BAs in high concentrations can be toxic the BA pool is tightly controlled. BA homeostasis is regulated by the major BA sensor farnesoid x receptor (FXR), which is responsible for a network of genes in hepatic BA synthesis, biliary BA secretion, intestinal BA absorption, and hepatic BA uptake (Kuipers et al., 2004; Rizzo et al., 2005; Matsubara et al., 2013). In general, FXR is activated by hydrophobic BAs with CDCA a much more potent activator of FXR than CA (Li et al., 2013b; Ding et al., 2015).

Transporters are important to the distribution of BAs throughout the enterohepatic organs. Majority of conjugated BAs absorbed from the portal blood into hepatocytes, occurs from the basolateral uptake transporter sodium taurocholic transporting polypeptide (Ntcp/Slc10a1), whereas unconjugated BAs are transported into hepatocytes by the organic anion transporting polypeptide (Oatp) 1b2 (Klaassen and Aleksunes, 2010; Csanaky et al., 2011). Biliary excretion of BAs from hepatocytes to storage in the gall bladder is through the rate-limiting transporter bile

salt efflux pump (Bsep/Abcb11). Transport of BAs into blood as a compensatory mechanism during cholestasis occurs on the basolateral membrane: the solute carriers Slc51a/Ost α and Slc51b/Ost β and the efflux transporter multidrug resistance-associated protein ATP binding cassette subfamily C member 3 (Abcc3/Mrp3) and Abcc4/Mrp4 (Halilbasic et al., 2013). Interestingly, the regulation of BAs and transporters in mice is age dependent such that there is a surge in the primary conjugated BA T-CA at birth which increases the expression of the BA transporters Ntcp, Bsep, Ost β , and Mdr2 in an FXR dependent manner, modifying enterohepatic circulation (Cui et al., 2012). However, how gut microbiome modifies BA homeostasis early in life is unknown.

Several studies have shown the importance of the xenobiotic-sensing nuclear receptors PXR and CAR in BA homeostasis. Pharmacological activation of PXR by pregnenolone-16 α -carbonitrile (PCN) represses hepatic Cyp7a1 mRNA and activity in mice (Li and Chiang, 2013; Li and Chiang, 2014), and endogenous activation of PXR by the secondary BA LCA protects the liver against toxicity (Staudinger et al., 2001). PXR activation in intestine/enterocytes induced the intestinally secreted hormone fibroblast growth factor 19 (FGF19; Fgf15 in mice), which represses the transcription of Cyp7a1 in liver (Wistuba et al., 2007; Li and Chiang, 2013). Activation of CAR has also been shown to repress the expression of *CYP7A1* in human hepatocytes (Miao et al., 2006). CAR activation in male and female CV mice by 1, 4-bis[2-(3, 5-dichloropyridyloxy)]benzene (TCPOBOP) decreased the BA pool, primarily through decreased T-CA, while the expression of Cyp7a1 increased, attempting compensate for the decreased BAs (Lickteig et al., 2016). The BA ligase solute carrier family 27 member 5 (Slc27a5/Bal), which regulates BA conjugation, was decreased following TCPOBOP exposure (Cui and Klaassen, 2016), possibly contributes to the improved metabolic health following CAR activation (Dong et al., 2009; Gao et al., 2009). BAs are not known to serve as ligands of CAR (Li and Chiang, 2013). CV and GF male mice exposed to PCN or TCPOBOP revealed decreased *Bifidobacterium* genus in the large intestinal content and decreased abundance of the bile salt hydrolase gene (Dempsey

et al., 2019). In addition, the genres *Bifidobacterium*, *Dorea*, *Peptococcaceae*, *Anaeroplasma*, and *Ruminococcus* positively correlated with T-UDCA in large intestinal content (Dempsey et al., 2019).

BAs in the host are age-dependent, and therefore, characterization of the microbiome at specific ages is important. Human infants fed breast milk have up to 90% colonization of the genus *Bifidobacterium*, which uses milk oligosaccharides as an energy source, compared to up to 14% in the infant gut who do not receive breast milk (Yatsunenkov et al., 2012; Kirmiz et al., 2018; Taft et al., 2018; Gopalakrishna and Hand, 2020; Lawson et al., 2020). *Bifidobacterium* produce milk glycans which in turn are fermented to short-chain fatty acids (SCFAs) by other bacteria (Gopalakrishna and Hand, 2020). Supplementation of *Bifidobacterium* strains in infants have yet to show long-term health effects (Bazanella et al., 2017), yet infants at three months old with a high composition of Bifidobacteriaceae had fewer reported morbidities and medications up to 12 months of age (Berger et al., 2020). In adults, SCFAs are produced primarily by anaerobic bacteria that help produce a healthy and diverse microbiome that is instrumental to immune health and other metabolic processes (Clemente et al., 2012; Belkaid and Hand, 2014; Tan et al., 2014). Interestingly, SCFAs may indirectly affect xenobiotic metabolism. For example, SCFAs act as ligands for two G-protein coupled receptors (GPR41 and GPR43) and modify histone acetylation that impacts signaling of the transcription factor aryl hydrocarbon receptor (AHR), which is activated by planar xenobiotics such as polycyclic aromatic hydrocarbons (Boffa et al., 1978; Sealy and Chalkley, 1978; Siavoshian et al., 2000). Interestingly, using metagenomic sequencing it was shown that centenarians (aged 99 to 104 years) and semisupercentenarians (aged 105 to 109 years) have a gut microbiome shifted towards degrading xenobiotics, which coincides with decreased capacity of the liver to metabolize xenobiotics (Rampelli et al., 2020). This suggests that the microbiome adapts to changes in the host to protect both the host and the microbiome, indicating that a probiotic or stimulant may alter the gut microbiome at younger ages to protect the host at younger similar to centenarians.

As a mature host, BA homeostasis and xenobiotic metabolism are partially regulated by microbial metabolites, including secondary BAs. Furthermore, secondary BAs and other microbial metabolites regulate DPGs by activation and repression of xenobiotic sensing nuclear receptors and transcription factors. However, the relationship of how gut microbiome develops in concordance with BA homeostasis and the maturation of DPGs is unknown. Therefore, the goal of this study was to determine the developmental relationship of the gut microbiome and microbial metabolites (e.g. BAs) in relation to the developmental regulation of DPGs in mice.

2.2. Methods

Animals

CV C57BL/6J male and female mice were purchased from Jackson Laboratory (Bar Harbor, ME) at 8 weeks of age and were bred one week after arrival. These initial mice were used to create a CV mouse colony for this study. GF mice in the C57BL6 background from a colony maintained by the University of Washington Gnotobiotic Core facility (the initial GF breeding colony was established with mice purchased from the National Gnotobiotic Rodent Resource Center at the University of North Carolina at Chapel Hill. Mice were housed with a 12-hour light/dark cycle in a temperature and humidity-controlled environment in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited facility at the University of Washington. Studies were approved by the Institutional Animal Care and Use Committee at the University of Washington. The CV and GF mice were exposed to the same diet (laboratory autoclaved rodent diet #5010 by LabDiet, St. Louis, MO), water (non-acidified autoclaved water), and bedding (autoclaved Enrich-N'Pure by Andersons Lab, Maumee, OH). At 1, 5, 10, 15, 25, 60, and 120 days of age, mice were euthanized, and tissues were collected. Serum was collected by cardiac puncture followed by centrifugation at 4°C at 2000 *g* for 10 minutes in MiniCollect blood collection tubes (Greiner Bio-One, Monroe, NC). Livers were flash frozen in liquid nitrogen. Duodenum, jejunum, ileum, and large intestine were collected and immediately frozen in liquid nitrogen with intestinal content remaining inside. For consistency, the intestinal content was not flushed due to the small size and fragile nature of the intestines in the younger mice. All samples were stored at -80°C until further analysis.

RNA isolation and sequencing

Total RNA was isolated from liver using RNA-Bee reagent according to the manufacturer's instructions (Tel-Test, Inc., Friendswood, TX). RNA concentration was determined using a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Waltham, MA) at 260 nm, and RNA

integrity was determined by gel electrophoresis visualization of the 28S and 18S rRNA bands under ultraviolet light, as well as Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Samples with an RNA integrity number greater than 7 were used for sequencing. The cDNA libraries were prepared by depleting sample of rRNA and using the SMARTer® (Switching Mechanism at 5' End of RNA Template) Stranded RNA-Seq kit (formerly Clontech, now Takara Bio USA, Inc., Nojihigashi, Shiga, Japan). Sequencing was performed on an Illumina HiSeq2000 sequencer using a 50-base pair, paired-end multiplexing strategy at Dr. Nickerson's Sequencing Facility at the Northwest Genomics Center (University of Washington). FASTQ files were demultiplexed, concatenated by sample, and quality control of files was performed using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). RNA-Seq reads were mapped to the reference genome GRCm38/mm10 M11 (National Center for Biotechnology Information; NCBI) using STAR (Spliced Transcripts Alignment to a Reference; v2.5.2b) (Dobin et al., 2013). Sequencing alignment/map files were converted to binary format using SAMtools v1.5 (Li et al., 2009). The files were subsequently analyzed by Cufflinks v2.2.1 to estimate transcript abundance expressed as fragments per kilobase of transcript per million mapped reads (FPKM) (Trapnell et al., 2010). Differential gene expression was determined between age- and gender-matched CV and GF mice using Cuffdiff (Trapnell et al., 2010). Genes were considered expressed if the average FPKM across all groups was greater than 1. Differentially expressed genes were considered significant with a Benjamini–Hochberg false discovery rate (BH-FDR) less than 0.05. Expressed genes were used for hierarchical clustering using R package ComplexHeatmap (Gu et al., 2016) and for gene ontology enrichment using the R package topGO (<https://bioconductor.org/packages/release/bioc/vignettes/topGO/inst/doc/topGO.pdf>). Principle component analysis plots were made using ggplot2 (v 3.0.0).

16S rDNA Sequencing and data analysis

Total DNA from the large intestine with content was isolated from conventional mice using the Omega E.Z.N.A.® Stool DNA Kit (Omega Bio-tek, Inc., Norcross, GA) according to the manufacturer's instruction. DNA concentration was determined using a Qubit 2.0 Fluorometer (Life Technologies/Thermo Fisher Scientific, Grand Island, NY), and the integrity and quality of DNA samples were confirmed using an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA). The V4 region of the 16sRRNA gene was amplified and sequenced using a HiSeq 2500 platform (250-bp paired-end) (BGI Americas, Cambridge, MA) (n = 3 per group). Analysis of 16S FASTQ data was performed with QIIME 2 2017.11 (Bolyen et al., 2019). The paired-end sequence reads were de-multiplexed, merged, and quality checked by FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) followed by denoising by DADA2 (Callahan et al., 2016). Amplicon sequence variants (ASVs) were aligned (Katoh et al. 2002) (via q2-alignment) and used to construct a phylogeny (Price et al. 2010). Alpha-diversity (Faith's Phylogenetic Diversity) (Faith, 1992) and beta diversity (weighted UniFrac) (Lozupone et al., 2007) were estimated after samples were rarefied (subsampling without replacement) to 4000 sequences per sample. Taxonomy was assigned to ASVs (Bokulich et al., 2018) by a naïve Bayes taxonomy classifier against the Silva 99 version 128 reference 99% OTUs reference sequences (Quast et al., 2013). Functional pathways were predicted from the 16S data using Phylogenetic Investigation of Communities by Reconstruction of Observed States (PICRUSt) (Langille et al., 2013). The taxonomic drivers of shifts in functions between groups identified in PICRUSt was predicted by Functional Shifts' Taxonomic Contributors (FishTaco) (Manor and Borenstein, 2017). Bar plots and line plots were made using SigmaPlot.

Bile acid extraction and quantification

BAs were extracted from four bio-compartment (liver, ileum, large intestine, and serum) as previously described (Zhang and Klaassen, 2010; Dempsey et al., 2019). Briefly, for liver, ileum, and large intestine, tissues were homogenized in ice-cold and mixed with the internal

standards (CA-d4, DCA-d4, GCA-d4, G-CDCA-d4, and LCA-d4). Samples were precipitated with 5% NH₄OH in acetonitrile and centrifuged to obtain pellets. Pellets were resuspended in ice-cold methanol and centrifuged again. The supernatants were combined and dried under vacuum. Serum samples were mixed with internal standard, precipitated with methanol, and the supernatant was dried under vacuum. After drying, the precipitates were reconstituted in 50% methanol water solution prior to LC-MS. LC-MS quantification was reported previously (Dempsey et al., 2019). Briefly, LC-MS analysis was performed on a Water Acquity I-Class UPLC TQS-micro MS (Waters, Milford, MA) system at the Northwest Metabolomics Research Center. Chromatographic separation was performed with a Waters XSelect T3 column (2.5 µm, 2.1 x 150 mm) using 5 mM ammonium acetate in H₂O with 0.1% acetic acid and acetonitrile with 0.1% acetic acid. The mass spectrometer was equipped with an electrospray ionization source, and targeted data acquisition was performed in multiple-reaction-monitoring mode (MRM), monitoring for 56 BAs. The extracted MRM peaks were integrated using the TargetLynx software (Waters, Milford, MA). Statistical analysis for differences between CV and GF mice stratified by age and sex was performed initially by an ANOVA followed by a generalized linear model using Tukey post-hoc test. Sex was not found to be a covariate in BA analysis. Bar plots were made using SigmaPlot.

Short-chain fatty acid extraction and quantification

Chemicals. Acetic acid was bought from Thermo Fisher Scientific (Fair Lawn, NJ). Propionic acid, isobutyric acid, butyric acid, 2-methylbutyric acid, isovaleric acid, valeric acid, 2-methylpentanoic acid, 3-methylpentanoic acid, isocaproic acid, caproic acid, 2-methylhexanoic acid, 4-methylhexanoic acid, heptanoic acid, hexanoic acid-6,6,6-d₃ internal standard, N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA), and methoxyamine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO).

Sample Preparation. Each fecal sample (~20 mg) was mixed with 20 μ L NaOH solution (0.5 M in water), 20 μ L IS solution (hexanoic acid-6,6,6-d₃ in water; 200 μ M), and 460 μ L MeOH in an Eppendorf tube (1.5 ml). Then stainless beads were added into the tube, and homogenization (2 min) was performed using a Bullet Blender homogenizer (Next Advance, Averill Park, NY). After homogenization, 400 μ L 80% MeOH in water (v:v) were added into the sample. Then the sample was vortexed for 30 s, stored under -20 °C for 30 min, and sonicated in an ice bath for 10 min. After centrifugation (14,000 rpm, 10 min), 700 μ L supernatant was collected into a new Eppendorf tube. The sample was then dried under vacuum at 37 °C for 240 min using a CentriVap Concentrator (Labconco, Fort Scott, KS).

GC-MS. Each sample was first derivatized with 40 μ L of methoxyamine hydrochloride solution in pyridine (MeOX, 20 mg/mL) under 60 °C for 90 min. Next, 60 μ L of MTBSTFA was added, and the mixture was incubated under 60 °C for 30 min. Then the sample was vortexed for 30 s, followed by centrifugation at 14,000 rpm for 10 min. Finally, 70 μ L supernatant was collected into a new glass vial for GC-MS analysis.

GC-MS experiments were performed using an Agilent 7820A gas chromatography system coupled to an Agilent 5977B mass spectrometer (Agilent Technologies, Santa Clara, CA). Chemical derivatives in the samples were separated using an HP-5 ms capillary column coated with 5% phenyl-95% methylpolysiloxane (30 m $\times$ 250 μ m i.d., 0.25 μ m film thickness, Agilent Technologies). 1 μ L of each sample was injected, and the solvent delay time was set to 5 min. The initial oven temperature was held at 60 °C for 1 min, ramped up to 325 °C at a rate of 10 °C/min, and finally held at 325 °C for 10 min. Helium was used as the carrier gas at a constant flow rate of 20 mL/min through the column. The temperatures of the front inlet, transfer line, and electron impact (EI) ion source were set at 250 °C, 290 °C, and 230 °C, respectively. The electron energy was -70 eV, and the mass spectral data were collected in the full scan mode (m/z 30-600).

Agilent MassHunter Workstation Software Quantitative Analysis (B.09.00) was used to process the GC-MS data for compound identification, peak picking, and quantification. The signal-to-noise ratio (S/N) was set to S/N=3. The retention time and quantification mass for each SCFA were determined using its chemical standard. The concentrations of SCFAs in biological samples were calculated using the calibration curves constructed from the corresponding SCFAs standards.

Targeted Proteomics by LC-MS/MS

The procedure is described in previous publications (Li et al., 2017; Li et al., 2018). Briefly, mouse hepatic membrane proteins were isolated using a Mem-PER plus membrane protein extraction kit (Pierce Biotechnology, Rockford, IL). Tissue extract (80 μ l at 2 mg/ml total protein) was mixed with 10 μ l DTT (250 mM), 20 μ l of 0.2 mg/ml bovine serum albumin, 10 μ l of 10 mg/ml human serum albumin, and 40 μ l ammonium bicarbonate buffer (100 mM, pH 7.8). The solution was incubated at 95 °C for 5 min. Subsequently, 20 μ l iodoacetamide (500 mM) was added, and the sample was incubated for 30 min at room temperature in the dark. Ice-cold methanol (0.5 ml), chloroform (0.1 ml) and water (0.4 ml) were added to each sample and then centrifuged (16,000 g) for 5 min at 4°C. The pellet was washed with ice-cold methanol (0.5 ml) and centrifuged at 8,000g for 5 min at 4°C. The pellet was resuspended with 60 μ l 50 mM ammonium bicarbonate buffer. Trypsin (20 μ l) was added at a 1:80 trypsin:protein ratio (w/w), and samples were incubated for 16 h at 37 °C. Trypsin digestion was quenched with 10 μ l of 80% acetonitrile with 0.5% formic acid followed by 20 μ l heavy peptide internal standards. Samples were centrifuged for 5 min at 4,000 g at 4°C, and the supernatant was collected into a LC-MS vial. The stable isotope-labeled heavy peptides were used as internal standards (Thermo Fisher Scientific, Rockford, IL). LC-MS/MS consisted of an Acquity LC (Waters Technologies, Milford, MA) coupled to an AB Sciex Triple Quadrupole 6500 MS system (Framingham, MA). One to three surrogate peptides per protein were designed for the quantification (Supplemental Table 3 of Li et al., 2018).

Peptide separation was achieved on an Acquity UPLC column (HSS T3 1.8 μm , 2.1x100 mm, Waters, Hertfordshire, UK). Mobile phase A (water with 0.1% formic acid; v/v) and mobile phase B (acetonitrile with 0.1% formic acid; v/v) were used with a flow rate of 0.3 ml/min in a gradient manner. Peak integration and quantification were performed using Analyst (Version 1.6, Mass Spectrometry Toolkit v3.3, Framingham, MA, USA). Ion suppression was addressed by using heavy peptide. Bovine serum albumin (BSA) was used as an exogenous internal standard, to correct for protein loss during processing and digestion. In total, three-step data normalization was used; first average peak areas of light peptides were divided by corresponding average heavy peptide peak areas. This ratio was then divided by BSA light/heavy peak area ratio. Finally, the data were normalized to average quality control values (pooled representative sample) for a robust method to ensure reproducibility (Bhatt and Prasad, 2018). Statistical analysis for differences between CV and GF mice stratified by age and sex was performed initially by an ANOVA followed by a generalized linear model using Tukey post-hoc test. Bar plots were made using SigmaPlot.

Activity-based protein profiling

Samples were processed by removing S9 fractions, and then separating the cytosol from microsomes. Microsomes were pooled together and a protein quantification via Pierce™ BCA Protein Assay was used to determine microsomal protein concentrations. All samples were then normalized using 1X PBS and underwent probe-labeling with 2EN-ABP, ATW8-ABP and ATW12-ABP. NADPH+ samples were given the requisite cofactor, NADPH, and the NADPH- control samples were given an equivalent volume of DMSO. All samples were then incubated at 37°C for 60 min, with 1000 rpm agitation. After incubation, samples underwent click chemistry via the addition of biotin-azide (60 mM), sodium ascorbate (10 mM), THPTA (2mM), and CuSO₄ (4mM). Samples were incubated at 37°C for 1.5 h with 500 rpm agitation. After click chemistry incubation, samples underwent a methanol wash to further precipitate proteins. Once precipitated proteins

were isolated, a BCA was conducted to quantify protein concentrations and the sample proteins were again normalized across all sample groups. Normalized proteins underwent streptavidin enrichment, reduction and alkylation and overnight trypsin digestion. Streptavidin beads were then removed from samples and the remaining peptides were vialled and submitted for MS/LCMS analysis.

Preparation of Lung Lysate S9. Methods outlined in the following paper under the *Preparation of Lung Lysate S9* section. <https://pubs.acs.org/doi/10.1021/acs.jproteome.8b00086>

Probe-Labeling, Click Chemistry and Methanol Wash. Liver microsomes from each sample group (F Germ-Free, M Germ-Free, F Conventional, M Conventional) were pooled, split in half (NADPH+, NADPH-) and normalized to equivalent protein concentrations. All samples were given the following probes: 2EN-ABP (20 μ M), ATW8-ABP (20 μ M) and ATW12-ABP (20 μ M). NADPH+ samples were given the requisite cofactor, NADPH (2 μ M), and NADPH- samples were given an equivalent volume of DMSO. All samples were then incubated at 37°C for 60 min, with 1000 rpm agitation. The click-chemistry reaction was carried out with the addition of biotin-azide (60 mM), sodium ascorbate (10 mM), THPTA (2mM), and CuSO₄ (4mM). Samples were then agitated at 500 rpm, 37°C for 1.5 h. Proteins were precipitated with the addition of cold MeOH and stored in a -80 freezer overnight. Samples were then spun down at 4°C, 15,000 *g* for 4 min. The precipitates were isolated and air-dried for 5 min. 1.2% SDS in PBS was added and proteins were resolubilized by probe sonication. Samples were then heated at 95°C for 5 min to further induce protein precipitation and spun down for 5 min at 14,000 *g*, rt. Supernatant containing precipitated proteins was collected, and proteins were re-quantified via BCA. Samples were then normalized to equal protein concentrations.

Streptavidin Enrichment. Methods outlined in the following paper under the ***Streptavidin Enrichment*** section.

<https://pubs.acs.org/doi/10.1021/acs.jproteome.8b00086>

2.3 Results

Functional analysis of bacteria in the large intestine

To understand the developmental patterns of the gut microbiome is related to the age of the mouse host, DNA from the large intestine of male and female CV mice at ages day 1, 5, 10, 15, 25, 60, and 120 was isolated and subjected to 16S rRNA gene sequencing. The subsequent data was analyzed through QIIME2 according to the Materials and Methods section. The 16S rRNA gene sequencing analysis revealed age-specific patterns during the development of CV mice. In general, there were no global differences in the gut microbiome by sex for diversity (alpha and beta diversity), nor for functional analyses.

With sequences rarefied to a depth of 4000, day 1 mice had the greatest alpha diversity with Faith's Phylogenetic Diversity for richness and evenness greater than 7 for both sexes (Figure 2.1A). The alpha diversity decreased below 4 for days 5 and 10, before increasing to less than 7 for days 15, 25, 60, and 120. The weighted UniFrac principle coordinates analysis of beta diversity showed that sexes clustered together by age groups. There were four major clusters that separated by age for day 1, 5, and 10 before clustering as the mature microbiome at days 15, 25, 60, and 120 (Figure 2.1B). The percent composition of the major bacteria in the microbiome are consistent for both males and females (Supplemental Figure 2.S1). In concordance with the alpha diversity, day 1 mice had a relatively even and rich distribution of bacteria before being dominated by the genus *Acinetobacter* (~65%) at day 5 and the genus *Lactobacillus* (~90%) at day 10. The latter bacteria sharply decreased at day 15 with the initial development of the mature microbiome, as indicated by the alpha and beta diversity, and likely corresponded with the transition of the diet from milk to solid food.

To predict the functional differences of the gut microbiome with respect to host age, PICRUST was used to predict the number of genes present in various KEGG pathways. Specifically, bacteria metabolize hepatic primary BAs to secondary BAs, which are then re-absorbed and transported to the host liver. This circular pattern allows for BAs to be a

communication medium between the host and the gut bacteria. For the KEGG pathway secondary BA synthesis relative to day 1 mice, there was a significant increase in the number of genes capable of performing the function with a sharp and significant increase occurring at day 15 and a continued upward trend through day 120 for males and females (Figure 2.1C).

FishTaco compared the composition of the microbiome in day 1 and day 120 CV mice in relation to the gene counts created by PICRUST to identify which KEGG pathways experience a significant functional shift between age groups and the bacteria responsible for that shift (Figure 2.1D). For each pathway, the top right bar indicates the bacteria at day 120 that drive function (positive Wilcoxon Score; taxa predicted to have genes with the function) or attenuate the function (negative Wilcoxon Score; taxa predicted to no have genes with the function). The bottom bar indicates bacteria at day 1 that drive the difference in function (positive Wilcoxon Score; taxa predicted to no have genes with the function) or attenuate the change in function (negative Wilcoxon Score; taxa predicted to have genes with the function). The Wilcoxon test statistic, which is an indicator for the magnitude of the functional shift from day 1 to day 120, is represented by the diamonds. For secondary BA biosynthesis (ko00121), the shift magnitude is ~2.6 with many bacteria in day 120 mice responsible for the function from the family S24-7. Taxa in day 120 mice attenuating the shift in secondary BA synthesis do include the family S24-7, the order Clostridiales, and Other Taxa (a group of 304 other possible taxa). Whereas in day 1 mice most bacteria contributing to the lack of secondary BA biosynthesis are a sum of 304 other taxa, as well as the order Clostridiales and the family *Enterobacteriaceae*. In addition, taxa present in day 1 mice that are attributed to secondary BA synthesis include the *Enterococcus* and the family *Ruminococcaceae*. Other KEGG pathways with significant functional shifts between day 1 and day 120 mice include proximal tubule bicarbonate reclamation, nucleotide excision repair, nitrogen metabolism, zeatin biosynthesis, vitamin B6 metabolism, one carbon pool by folate, glycosphingolipid biosynthesis - ganglio series, glycosphingolipid biosynthesis - globo and isoglobo series, lipopolysaccharide biosynthesis, glycosaminoglycan degradation, polyketide

sugar unit biosynthesis, streptomycin biosynthesis, other glycan degradation, D-Glutamine and D-glutamate metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, alanine, aspartate and glutamate metabolism, and oxidative phosphorylation. Overall, the gut microbiome in mice has altered composition and diversity corresponding to the age of the host and with functions, such as secondary bile acid metabolism, that are drastically different between a newborn and a sexually mature adult.

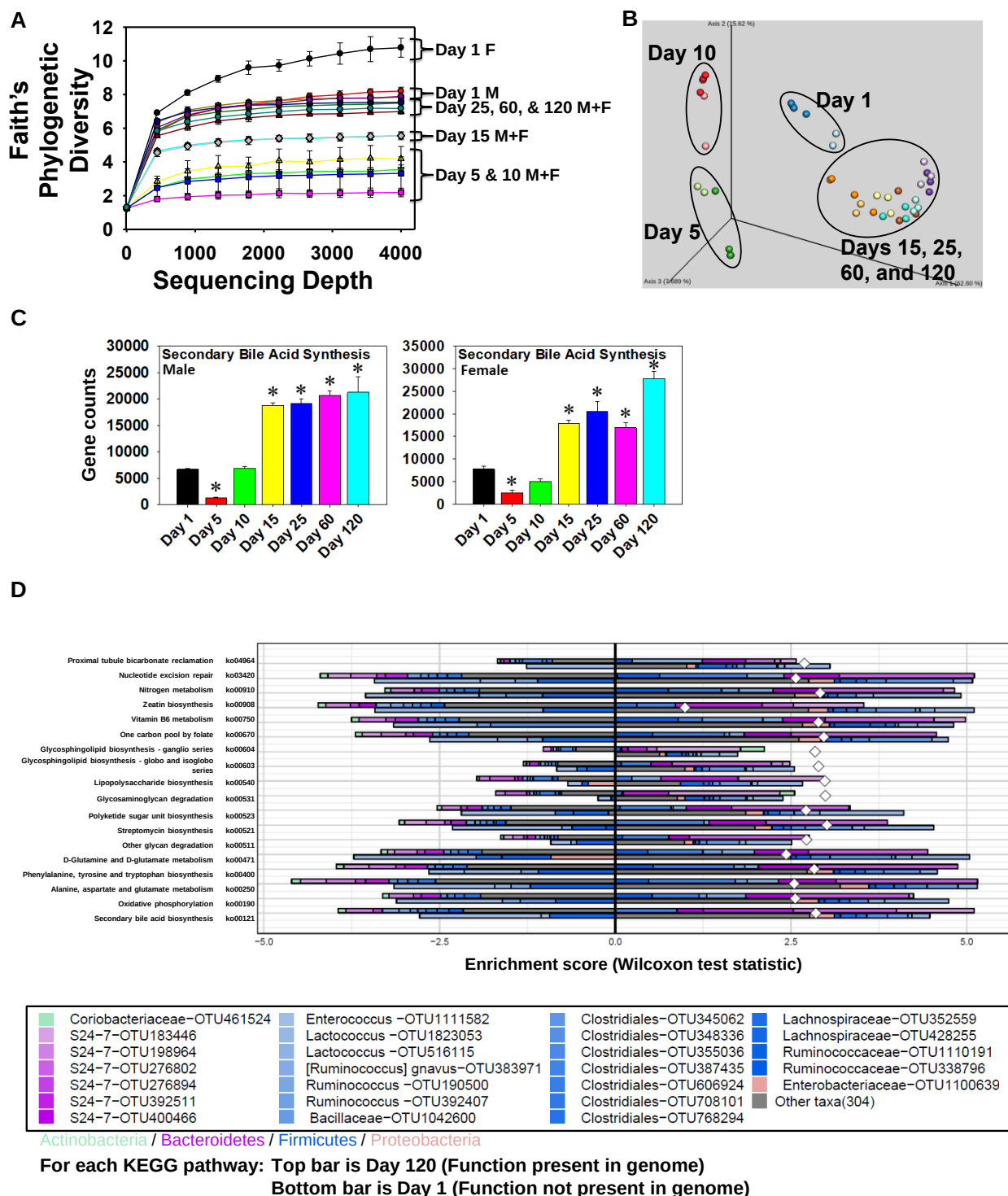


Figure 2.1. A. Alpha diversity of large intestinal microbiota in CV male and female mice at seven ages (days 1, 5, 10, 15, 25, 60, and 120; $n=3$ per group). The 16S rRNA gene sequencing data were analyzed using QIIME2 as described in Materials and Methods. **B.** Beta diversity of large intestinal microbiota in CV male and female mice at seven ages ($n=3$ per group). The 16S rRNA

gene sequencing data were analyzed using QIIME2 as described in Materials and Methods. **C.** The total number of microbial genes in secondary BA synthesis identified from the 16S rRNA gene sequencing data by PICRUSt (n=3 per group). Asterisks (*) represent statistically significant differences as compared to day 1 group within each gender (one-way ANOVA followed by Dunnet's post hoc test; statistical significance was considered at $p < 0.05$). **D.** Comparative analysis by FishTaco to identify the taxonomic drivers of shifts in KEGG functions identified by PICRUSt between day 1 and day 120 CV mice (male and female; n=6). Enrichment scores are a Wilcoxon test statistic (rank sum). For each KEGG function with a significant difference in function, the top bar is day 120 mice and the bottom bar is day 1 mice. The colors making up bar are the score for the bacteria (OTU) attributed to drive the function (day 120) or attenuate the function (day 1). The diamond is the total enrichment score for a given function (rank score driving function minus rank score attenuating function).

Developmental interaction of bacteria and bile acids

The functional development of the gut microbiome in a maturing host indicated that secondary BA synthesis may be an important signaling mechanism between bacteria and the host liver. Many bacteria are known to synthesize secondary BAs from host primary BAs, but little is known about the age specificity of secondary BA metabolism. To test the hypothesis that age-specific bacteria produce unique patterns of secondary BAs during development, metabolites were isolated from the large intestines and 56 BAs were quantified by LC-MS at days 1, 5, 10, 15, and 120. These ages were selected for BA quantification because the gut bacteria differed the greatest at the neonatal and adolescent ages before forming the adult microbiome around day 25. A Spearman correlation was performed between BAs that were significantly different between ages (one-way ANOVA) and bacteria that had an average abundance >0.01% for all ages and sexes. The Spearman correlation identified 3 major clusters of BAs (Figure 2.2A). Cluster 1 (green) consists mostly of conjugated primary BAs (T- α/β MCA, T-CDCA, and T-CA) as well as T-HDCA and T-UDCA. Cluster 2 (purple) consists mostly of secondary unconjugated, deoxy, and keto BAs, including HDCA, HCA, UDCA, 3-ketocholanic acid, DHCA, 3,12-diketocholanic acid, 5 α -Cholanic acid-3, 6-dione, 3,7-diketocholanic acid, 3 α -hydroxy-12 ketolithocholic acid, 3 α -hydroxy-7 ketolithocholic acid, 5 α -cholanic acid-3 α -ol-6-one, 8(14),(5 β)-cholanic acid-3 α , 12 α -diol, LCA, ω MCA, and 3-DHCA, as well as T-DCA. Cluster 3 (yellow) consists of 3-DHCA, CA, G-DCA, and G-DHCA.

Twenty-seven bacteria were positively associated with Cluster 2 BAs (Figure 2.2B), which were primarily detected at the adult age in the large intestines (Supplemental Figure 2.S2-4) and negatively associated with Cluster 3 BAs, which were most abundant at neonatal ages and decrease into adulthood, generally. These bacteria included *Lachnospiraceae*, *Lachnospiraceae*, *Lachnospiraceae* NK4A136 group 1, *Bacteroidales* S24-7 group 3, *Erysipelotrichaceae* group 1, *Ruminococcaceae* *Ruminiclostridium* 9, *Lachnospiraceae* *Lachnospiraceae* NK4A136 group 3, *Lachnospiraceae* [*Eubacterium*] *xylanophilum* group, *Ruminococcaceae* *Ruminococcus* 1,

Lachnospiraceae Roseburia group 2, *Ruminococcaceae UCG-014*, *Lachnospiraceae Lachnospira*, *Ruminococcaceae Ruminiclostridium* 5 group 1, *Mollicutes RF9* group 1, *Coriobacteriaceae Coriobacteriaceae UCG-002*, *Ruminococcaceae Ruminococcaceae UCG-013*, *Ruminococcaceae Ruminococcaceae UCG-014*, *Ruminococcaceae Anaerotruncus* group 2, *Lachnospiraceae Tyzzerella* 3, *Bifidobacteriaceae Bifidobacterium*, *Family XIII Family XIII UCG-001*, *Ruminococcaceae Ruminococcaceae UCG-004*, *Ruminococcaceae Ruminococcaceae UCG-010*, *Lachnospiraceae Dorea*, *Coriobacteriaceae Enterorhabdus*, *Ruminococcaceae Ruminococcaceae UCG-005*, *Mollicutes RF9* group 2, *Defluviitaleaceae Defluviitaleaceae UCG-011*. The first 18 bacteria were present in the large intestine of day 1 mice, but they had low abundance or were not detected for days 5-25. At days 60 and 120, these 18 bacteria had their highest abundance relatively to age, matching the correlated BAs (Figure 2.2B and Supplemental Figure 2.S2-4). Being positively associated with Cluster 2 BAs (secondary unconjugated, deoxy, and keto BAs), these bacteria likely have a competitive advantage for metabolizing primary BAs. The latter nine bacteria are present at late adolescent and adult ages (days 25, 60, and 120). In addition to Cluster 2 BAs, these nine bacteria are positively associated with Cluster 1 BAs (T- α/β MCA, T-CDCA, T-CA, T-HDCA, and T-UDCA), which are most abundant in CV mice at day 1 relative to other ages (Supplemental Figure 2.S2-4). These bacteria may consume or thrive with low concentrations of the Cluster 1 BAs in adult mice compared to the bacteria only positively associated with Cluster 2 BAs. Comparing the Spearman correlation to the FishTaco attributed functions for secondary BA synthesis (Figure 2.2.1D), the family S24-7 was strongly attributed to secondary BA synthesis and is positively associated with Cluster 2 (Figure 2.2.1A), indicating that this family of bacteria is predicted to have genes that may produce secondary unconjugated, deoxy, or keto BAs.

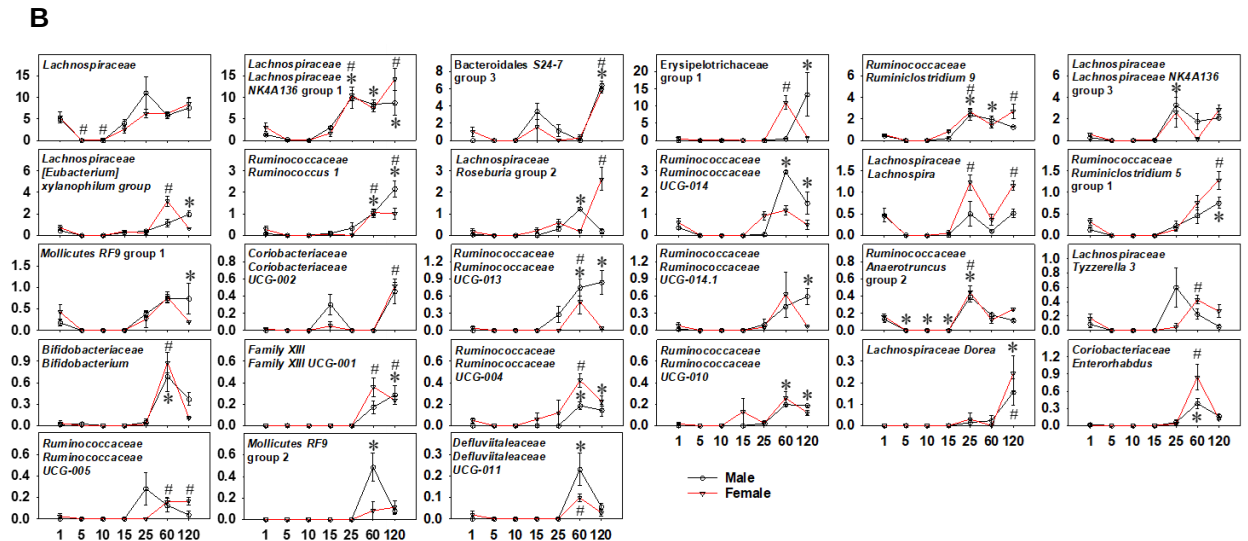
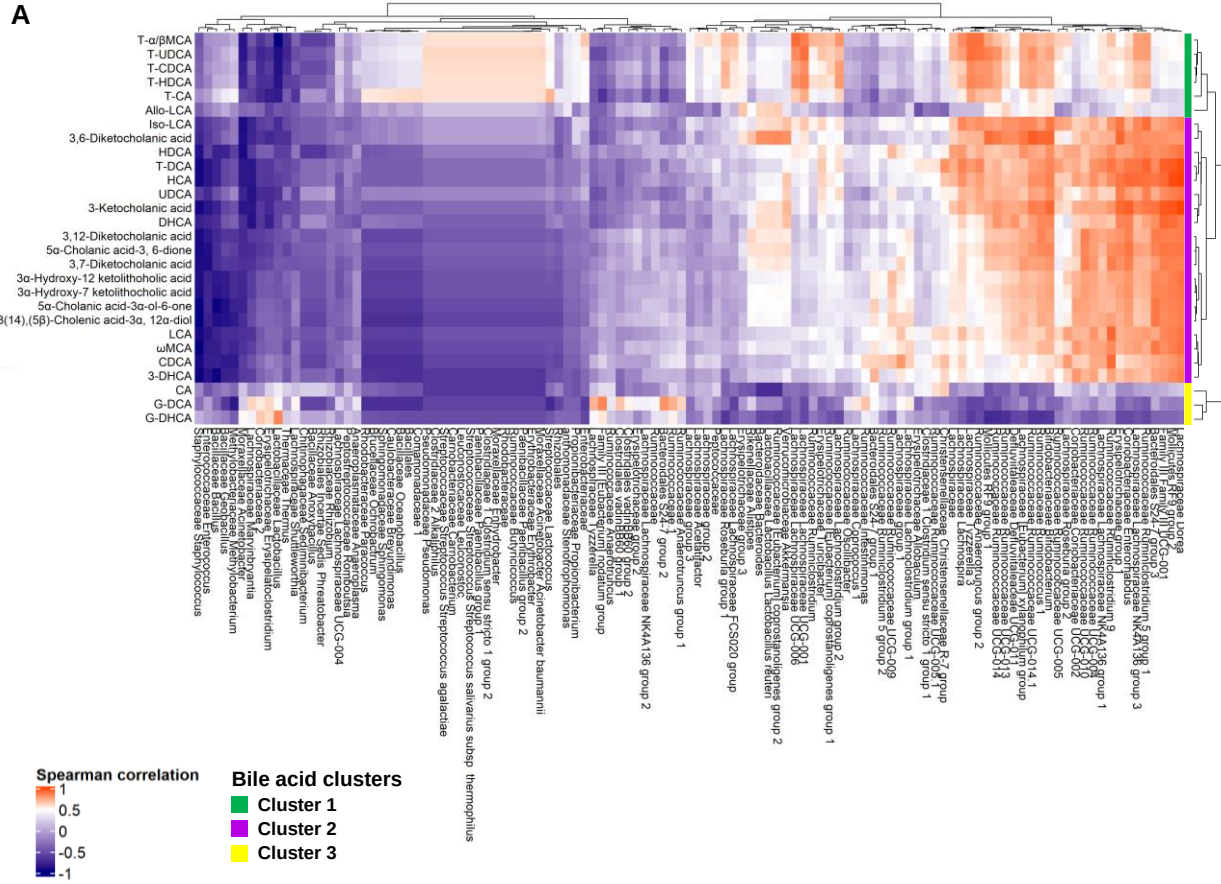


Figure 2.2. A. Spearman correlation of BAs in CV mice significantly different between ages (days 1, 5, 10, 15, and 120) in both sexes (male and female) and bacteria present in the large intestine greater than 0.01%. Positive Spearman correlation is red and negative correlation is blue. BAs formed three main clusters with Cluster 1 as green, Cluster 2 as purple, and Cluster 3 as yellow. **B.** The percent composition of bacteria that are positively correlated with BA Cluster 2. The percent composition of bacteria in large intestines of males is shown in black (n=3) and females

is shown in red (n=3). Significant difference by ANOVA compared to day 1 is indicated by asterisks (*) in males and by the pound symbol (#) in females.

The microbial genuses *Enterococcus* and *Lactococcus* were negatively associated with all significant BAs in the large intestine and were functionally considered to not drive secondary BA synthesis. *Lachnospiraceae* and *Ruminococcaceae* were found to drive secondary BA synthesis but were positively associated with Cluster 2 BAs. These bacteria may thrive in the presence of BAs, colonizing in proximity to BA metabolizing bacteria, but not contributing to the production of secondary BAs. Overall, there appeared to be age-specific bacteria corresponding to the development and presence of secondary BAs in the large intestines of mice.

Secondary BAs in liver, serum, ileum, and large intestine

To determine how the presence of the microbiome affects BA homeostasis in developing male and female mice and to test the hypothesis that specific BAs are microbiome dependent, a targeted metabolomics of 56 BAs in four biocompartments (serum, liver, and ileum, and large intestine) of CV and GF, male and female mice at ages 1, 5, 10, 15, and 120 was performed. Individual BAs were classified into seven major categories: total, primary conjugated, primary unconjugated, secondary conjugated, secondary unconjugated, deoxy- and keto-, 6-OH, and 12-OH BAs. The sums of these categories are shown in Supplemental Figure 2.S5. The total concentration of BAs per gram of tissue in CV mice decreased with increased age in serum and liver, but BAs had an initial surge in concentration at day 1 in the ileum and large intestine, before decreasing during early development and then increasing at Day 120 to reveal a “U-shaped” curve. The surge is likely due to increased primary unconjugated, secondary conjugated, secondary unconjugated, and deoxy- and keto-BAs. In the ileum and large intestine of GF mice, primary unconjugated, secondary conjugated, secondary unconjugated, and deoxy- and keto-BAs are not detected. In GF mice, BA concentrations by category are relatively stable during the younger ages (days 1, 5, 10, and 15) before increasing to their adult concentrations at Day 120. This large increase is due to primary conjugated BAs. The concentration of individual BAs for large

intestines are in Supplemental Figures 2.S2-4 and for serum, liver, and ileum in Supplemental Figures 2.S6-12.

Because bacteria metabolize primary conjugated BAs to primary unconjugated, secondary conjugated, secondary unconjugated, and deoxy- and keto-BAs, we tested the hypothesis that a subset of these BAs are produced only in the presence of bacteria. Of the 56 BAs analyzed, nine BAs were detected only in CV mice in all four biocompartments (Figure 2.3). Specifically, these BAs were the conjugated secondary BA T-DCA, the unconjugated secondary BA ω MCA, and the deoxy- and keto-BAs 3,6-diketocholanic acid, 3-ketocholanic acid, 3 α -hydroxy-7 ketolithocholic acid, 3 α -hydroxy-7, 12-diketolithocholic acid, 3 α -hydroxy-12-ketolithocholic acid, 5 α -cholanic acid-3, 6-dione, and 5 α -cholanic acid-3 α -ol-6-one. All nine BAs are not present or are minimally detected at younger ages (days 1, 5, 10, and 15) before reaching their highest monitored quantity at day 120 in both male and female CV mice, indicating that a mature microbiome is necessary for their synthesis. Furthermore, these BAs differed in detection by biocompartment, although none were detected in serum. The keto- and deoxy-BAs were detected in ileum and/or large intestine: 3 α -hydroxy-7 ketolithocholic acid and 5 α -cholanic acid-3 α -ol-6-one found in both; 3,6-diketocholanic acid, 3-ketocholanic acid, 3 α -hydroxy-12-ketolithocholic acid, and 5 α -cholanic acid-3, 6-dione are found only in large intestine; and 3 α -hydroxy-7, 12-diketolithocholic acid was only found in ileum. T-DCA and ω MCA were quantified in liver, ileum, and large intestine. In addition to requiring microbial enzymes to be produced, the distribution indicates that these keto- and deoxy-BAs may not be reabsorbed into the blood since they were not detected in the serum or liver, whereas T-DCA and ω MCA are reabsorbed to liver. This may indicate that T-DCA and ω MCA are two major BA signaling molecules between bacteria and mouse liver.

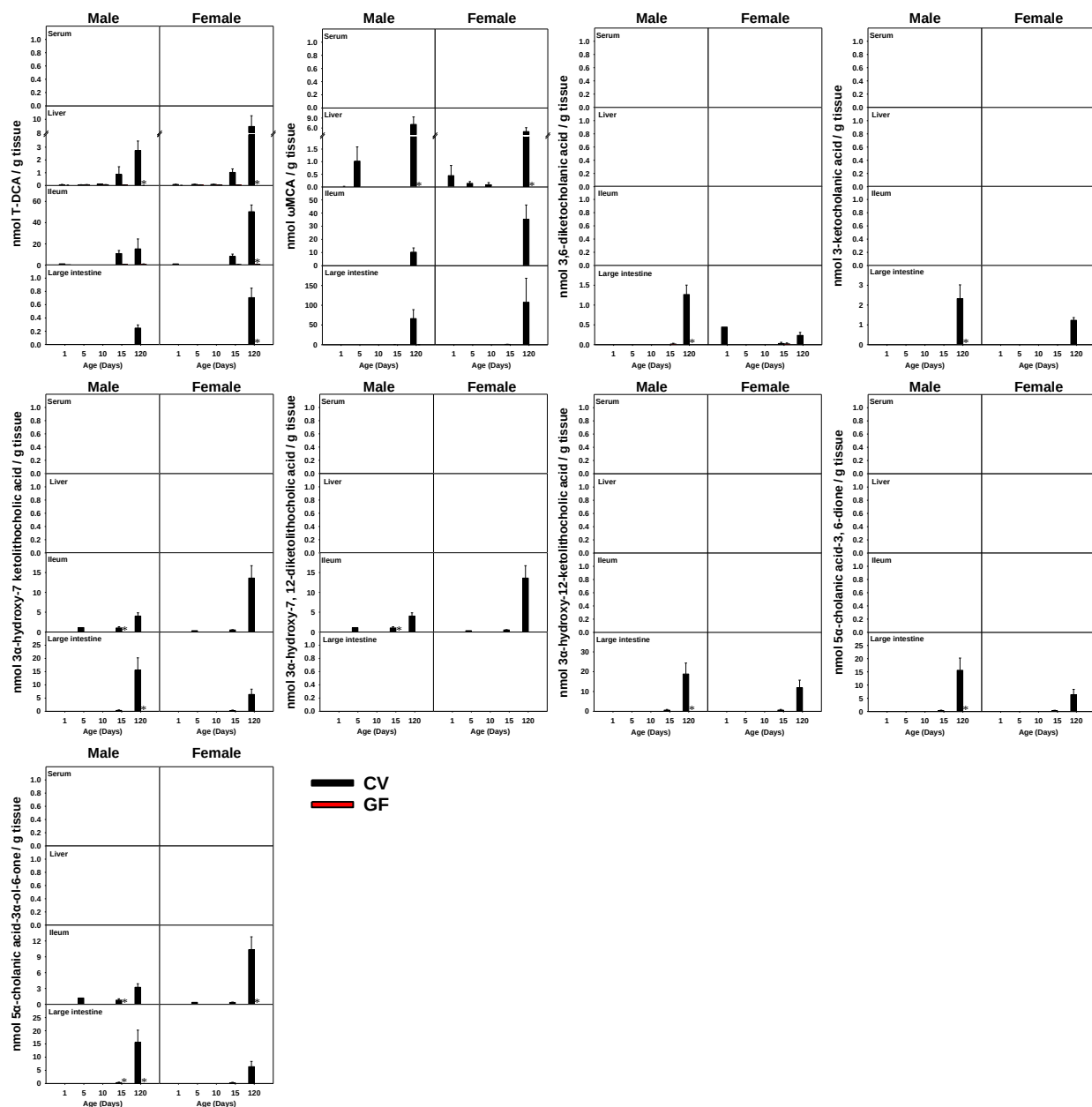


Figure 2.3. LC-MS quantification of CV-specific BAs detected in the four biocompartments: serum, liver, ileum, and large intestine for both male and female mice at days 1, 5, 10, 15, and 120 (n=3 per group) as described in Materials and Methods. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex. A generalized linear model was used to identify BAs with different concentrations followed by Tukey post-hoc test.

Host bile acid levels

Primary BAs are produced in the host liver through two main pathways: 1) the classic pathway (neutral pathway) (Chiang and Ferrell, 2018) with the overall rate-limiting BA synthesis enzyme Cyp7a1 and 2) the alternative pathway (acidic pathway) (Chiang and Ferrell, 2018) initiated by Cyp27a1. The classic pathway generates CA and its derivatives whereas the alternative pathway generates CDCA and its derivatives. Because of the chemical structure of these BAs, the properties and functions can affect health different. Therefore, a ratio comparison of CDCA to CA including the sum of their derivatives (Σ CDCA: Σ CA) may identify potential signaling and health differences between ages and the presence of the microbiome (Figure 2.4A). For CA, this includes G-CA, T-CA, CA, T-HCA, and HCA. For CDCA, this includes G-CDCA, T-CDCA, CDCA, T- α/β MCA, α MCA, β MCA, T-UDCA, and UDCA. For both male and female mice in serum, liver, and ileum, the Σ CDCA: Σ CA ratio is significantly increased or tends to increase in GF compared to CV mice at the adult age (day 120), but there is no difference at the younger ages (day 1, 5, 10, and 15). In these three biocompartments, the Σ CDCA: Σ CA ratio increases from less than 2 to greater than 4 in serum, greater than 6 in liver, and greater than 8 in ileum, indicating that the Σ CDCA increases and/or Σ CA decreases and that the magnitude of this difference is increased in the liver and ileum compared to the serum. There is no difference in the Σ CDCA: Σ CA ratio in large intestine. This is due to an increase in the Σ CDCA: Σ CA ratio in CV mice at the adult age relative to the other biocompartments, indicating that the BA environment may be more similar in the large intestine between CV and GF mice than other organs.

To look at the affect BAs may have on the acidity of each biocompartment, the hydrophobicity index was analyzed (Heuman, 1989). Increased BA hydrophobicity is associated with cytotoxicity (Stenman et al., 2013). In general, the hydrophobicity index was maintained between -0.25 and -0.50 for all ages and sexes in both CV and GF mice with few exceptions (Figure 2.4B). The hydrophobicity index was decreased in all four biocompartments of both male and female day 120 GF mice. In serum, this decrease was from about -0.25 to less than -0.60.

In liver, this decrease was from about -0.40 to less than -0.60. In ileum and large intestine, this decrease was from about -0.40 to almost -0.75. Interestingly, in large intestine of CV mice for both sexes, the hydrophobicity index of pooled samples (3 intestinal samples pooled together per quantification) at day 1 was at about -0.50, before becoming nearly 0 at days 5 and 10, and then increased at days 15 and 120 to almost 0.45. Whereas in GF mice, the hydrophobicity index was at -0.25 at days 1 and 5 and increased to between -0.50 and -0.75 at days 15 and 120, respectively. The difference in hydrophobicity index patterns between CV and GF mice is indicative of a different acidic environment in the large intestines that may be a health difference.

Hepatic bile acid metabolism and transport related genes

To determine the difference in BA production and transport by the CV or GF mouse host, protein expression and RNA expression were quantified (Figure 2.4C). The rate of BA production from cholesterol in the liver is controlled by the overall rate-limiting BA synthesizing enzyme Cyp7a1. The amount of Cyp7a1 protein in the liver is too low to detect using the peptide method (as described in Materials and Methods), therefore RNA expression was used to determine the difference in expression of Cyp7a1 between CV and GF mice at the seven developmental ages. Apart from female day 120 mice, which had a decrease in Cyp7a1 RNA expression from CV (~40 FPKM) to GF (~10 FPKM), there were no differences in Cyp7a1 expression. Protein expression between CV and GF mice of other major hepatic BA metabolizing enzymes varied. The first major protein downstream of Cyp7a1 in the classical pathway, 3 β -hydroxy- Δ 5-C27-steroid dehydrogenase (HSD3B7), had slightly increased protein expression in GF mice in male mice at days 10, 15, 25, 60, and 120 and in female mice at days 10, 15, 60, and 120. The protein expression of Cyp8b1, which produces CA and determines the ratio between CA (classic pathway) and CDCA (alternative pathway), was significantly decreased in male day 120 mice (~210 in CV to ~125 FPKM in GF) only and matches the ratio of increased Σ CDCA: Σ CA. For the alternative pathway of BA synthesis, the rate-limiting enzyme Cyp27a1 was slightly decreased in

females at days 60 and 120 and in general, the expression was greatly increased at ages 25, 60, and 120. Regarding BA conjugation, Slc27a5/Bal was increased in GF male mice at day 10 and in female mice at days 1, 5, 10, 15, and 25, with a tendency to increase with age in both male and female CV and GF mice. Other key hepatic BA metabolizing enzymes quantified that did not have major changes in protein expression include Cyp7b1, Akr1d1, and Akr1c14 (Supplemental Figure 2.S13); similarly, there were no major changes in RNA expression between CV and GF mice of other hepatic BA metabolizing enzymes, including Cyp17a1, Cyp26a1, and Cyp39a1 (Supplemental Figure 2.S14).

Regarding the protein expression of BA transporters, the expression of the cholesterol efflux dimer Abcg5/g8 was increased or tended to be higher in livers of GF mice at ages 1, 10, 15, 60, and 120 for both male and female mice (Figure 2.4C). The basolateral uptake transporter sodium taurocholic transporting polypeptide (Ntcp/Slc10a1), which is responsible for the uptake of conjugated BAs from the portal blood into hepatocytes, had increased protein expression in GF mice at day 5 in males and Day 1 in females. Overall, the difference in magnitude for the protein expression for these transporters is relatively low and is reflected in limited to no differences in their RNA expression (Supplemental Figure 2.S14). Additional BA transporters were evaluated for differences in RNA expression between CV and GF mice. The hepatic basolateral efflux transporters from the multidrug resistance-associated protein ATP-binding cassette, sub-family C (CFTR/MRP) member 3 (Abcc3/Mrp3) and Abcc4/Mrp4 had limited to no difference in RNA expression between CV and GF mice (Supplemental Figure 2.S15). The Slc transporters organic cation export transporter Slc22a1/Oct1, equilibrative nucleoside transporter Slc29a1/Ent1, and canalicular efflux transporter Slc47a1/Mate1 similar had limited no differences in RNA expression. The hepatic basolateral uptake transporters Slco1a1/Oatp1, Slco1a4/Oatp1a4, Slco1b2/Oatp2, and Slco2b1/Oatp2b1 also had no differences limited no differences in RNA expression.

In mice, Cyp2c70 was shown to produce the murine specific primary BAs α MCA and β MCA, but not Cyp2c37 (Takahashi et al., 2016), which are vastly increased in GF mice in the conjugated and unconjugated forms (i.e. in day 120 male mice, T- α/β MCA is less than 100 nmol/g liver in CV mice and increases to 2000 nmol/g liver in GF mice; Supplemental Figure 2.S8). Yet protein expression of Cyp2c70 is not significantly different, indicating that other Cyp2c enzymes may be responsible for the production of murine-specific BAs (Wahlstrom et al., 2017). The sum of the RNA expression of the Cyp2c genes revealed that GF mice had greater Cyp2c expression at days 10, 25, 60, and 120 in male mice and days 25 in 60 in female mice (Figure 2.4D). This matches the pattern of murine BAs having a larger magnitude of increase in male mice compared to female mice (Supplemental Figure 2.S8). Cyp2c genes that are increased at day 120 in male mice include Cyp2c37, 50, 54, 67, 68, 69, and 70; in female mice, Cyp2c genes expression increase at day 120 include Cyp2c29, 37, 38, 54, and 67. This may indicate that some Cyp2c genes other than Cyp2c70 produce muricholic acids in a sex-specific manner.

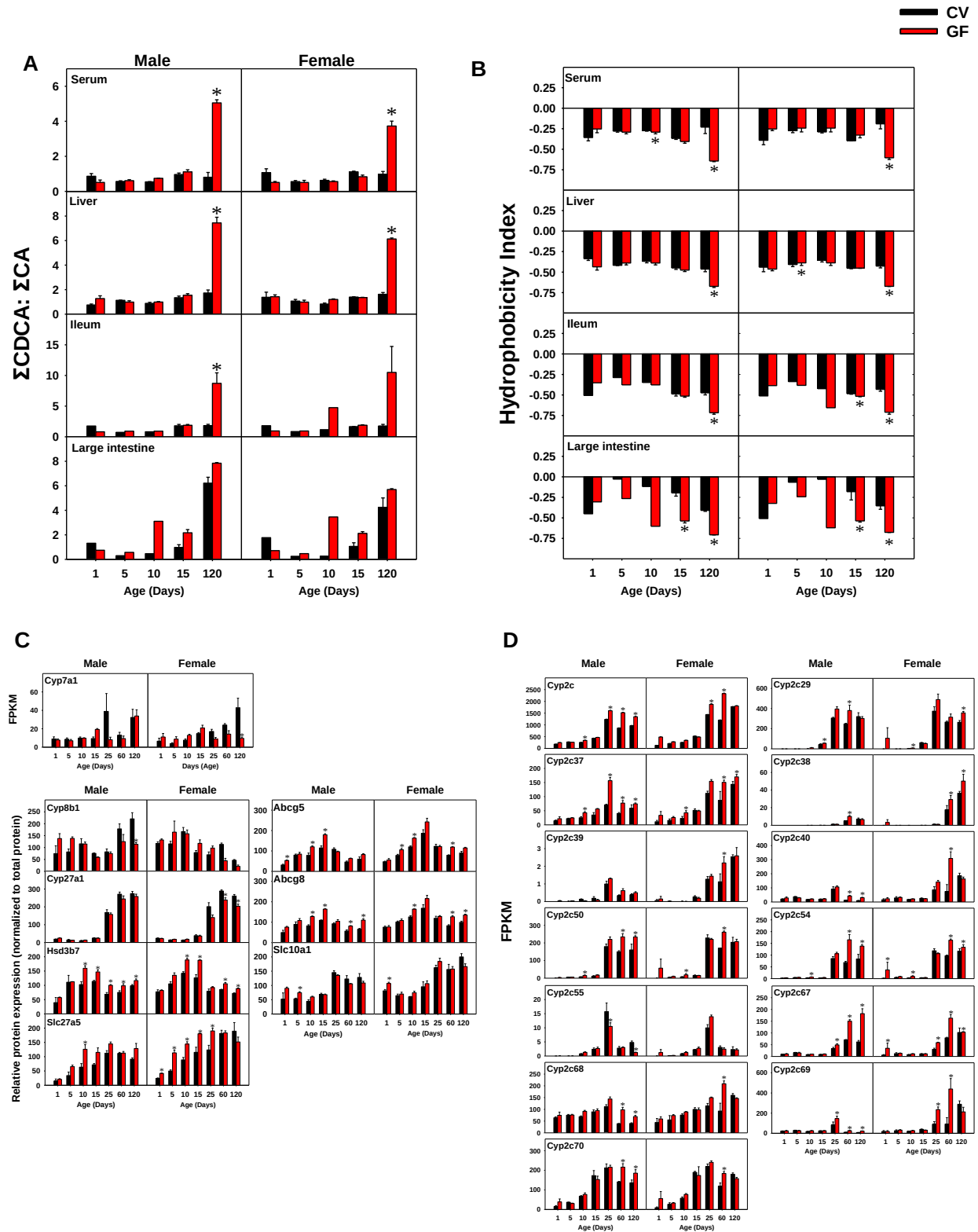
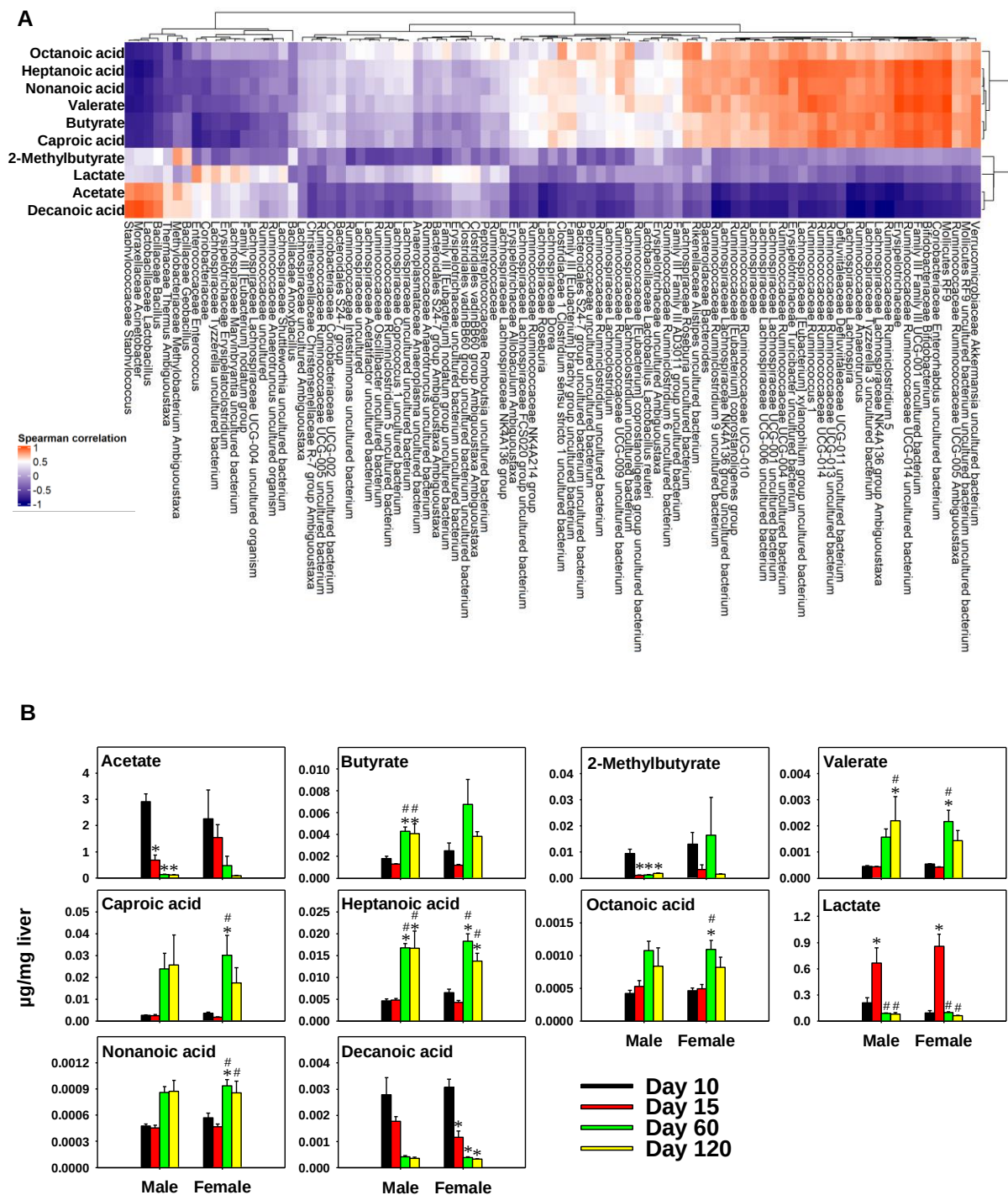


Figure 2.4. A. Ratio comparison of the sum of primary BA CDCA and its derivatives over the sum of the primary BA CA and its derivatives. **B.** Comparison of the hydrophobicity index of BAs in each biocompartment (serum, liver, ileum, and large intestine) and sex. Hydrophobicity values

were calculated (Heuman, 1989). **C.** Targeted proteomics of differentially regulated BA metabolizing genes in the livers of CV and GF, male and female mice at days 1, 5, 10, 15, 25, 60, and 120. The RNA expression of Cyp7a1 from RNA-sequencing is presented due to the protein not being detected. **D.** RNA expression of Cyp2c genes in the liver of CV and GF, male and female mice at days 1, 5, 10, 15, 25, 60, and 120. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex. A generalized linear model was used to identify the biocompartment and sex that have significantly different values followed by Tukey post-hoc test to identify the specific sex and age for differences between CV and GF mice. For RNA expression, asterisks (*) represent statistically significant differences between CV and GF mice at age determined by Cuffdiff (Trapnell et al., 2010).

Hepatic SCFAs and gut microbiome

SCFAs are microbially derived metabolites from fibers not digestible by the host, and many can act as signaling molecules that regulate inflammation, epigenetics (Kasubuchi et al., 2015; Oleskin and Shenderov, 2016; Fu and Cui, 2017; Remely and Haslberger, 2017; Spiljar et al., 2017). SCFAs were quantified in the liver of CV mice and days 10, 15, 60, and 120 by GC-MS, and a Spearman correlation was conducted comparing the relationship between SCFAs that were significantly different by age (one-way ANOVA) and large intestinal bacteria with a composition greater than 0.01%. The analysis revealed three patterns within SCFAs and their relationship with the detected large intestinal bacteria (Figure 2.5A). 1) C 8.0/Octanoic acid, heptanoic acid, C 9.0/nonanoic acid, valerate, butyrate, and caproic acid were positively associated with 31 bacteria, including the genus *Akkermansia* that produces propionate and acetate (Derrien et al., 2004; Louis and Flint, 2017) and *Roseburia*. In general, these SCFA shared a similar concentration pattern during development with low SCFA concentrations at days 10 and 15, increasing at days 60 and 120 (Figure 2.5B). 2) 2-Methylbutyrate and lactate were not positively associated with any cluster of the detected bacteria (Figure 2.5A). The SCFA 2-methylbutyrate in male mice had decreased levels at days 15, 60 and 120 compared to day 10, but there was no observed pattern in female mice (Figure 2.5B). Lactate was increased in day 15 compared to day 10 mice before decreasing at Day 60 and Day 120 to concentrations less than day 10, indicating that lactate may be critical to development at Day 15. 3) Acetate and decanoic acid were positively associated with 4 bacteria, including the genera *Bacillus*, *Lactobacillus*, *Acinetobacter*, and *Staphylococcus*, whereas most bacteria were negatively associated with acetate and decanoic acid (Figure 2.5A). Interestingly, acetate (the most abundant SCFA) and decanoic acid (the least abundance SCFA) had decreasing concentrations from nearly 3 to >0.5 $\mu\text{g}/\text{mg}$ liver and 0.003 to >0.0005 $\mu\text{g}/\text{mg}$ liver, respectively. This may indicate that the third pattern of SCFAs are important at young ages whereas the first pattern of SCFAs is needed for adult ages.



Age is a major determinant of hepatic gene expression during development

It is known that age and microbiome impact the expression of genes in the liver and that these changes in expression may be due to BAs or SCFAs. However, a comprehensive, global analysis integrating age and gene expression has not been evaluated. RNA from liver samples of the seven developmental ages (days 1, 5, 10, 15, 25, 60, and 120) in male and female, CV and GF mice was sequenced and analyzed. Using genes with an average expression value >1 FPKM, principle component analysis (PCA) showed that most of the variation within the samples was due to age (Figure 2.6A). Principle component 1 explained 30.1% of the variation in gene expression, and principle component 2 explained 10.2% of the variation in gene expression. The samples grouped in 4 distinct clusters, which is attributed to their age groups: 1) days 1 and 5; 2) day 10; 3) day 15; 4) days 25, 60, and 120. Neither enterotype nor sex had a global effect on gene expression. Of note, these age clusters based on hepatic gene expression also matched the age clusters for microbiome in CV mice (Figure 2.1B), demonstrating that development (age) is a large determinant for hepatic development and that the microbiome may be more influenced by factors from the host than exogenous materials (diet).

Using k-means clustering and KEGG pathway enrichment analysis, the heatmap revealed 3 distinct age distributions across the 4 k-means clusters (Figure 2.6B). Age was the defining variable for clustering based on gene expression. Cluster 1, which was enriched for genes in the KEGG pathways monocarboxylic acid metabolic process, fatty acid metabolic process, xenobiotic metabolic process, and organic acid catabolic process, was highest in days 25, 60, and 120 for both sexes and enterotypes. Cluster 3, which was enriched for genes in the KEGG pathways tissue morphogenesis, blood vessel development, and cell morphogenesis involved in differentiation, was highest in days 1 and 5 both sexes and enterotypes. Days 25, 60, and 120 for both sexes and enterotypes were most enriched for Cluster 4, which was the KEGG pathways ribonucleoprotein complex biogenesis, chromosome segregation, mRNA processing, and DNA repair. Cluster 2, which was enriched for genes in the KEGG pathways mitochondrial gene

expression mitochondrial translation, and mitochondrial RNA processing, had gene expression evenly distributed across all ages, sexes, and enterotypes. Days 5 and 10 for both sexes and enterotypes were not preferentially enriched for any of the 4 clusters, which may indicate that variables other than age and enterotype have a significant influence on hepatic gene expression.

KEGG enrichment for cluster 1 identified 300 enriched KEGG pathways (Supplemental Table 1) with the top 10 enriched pathways listed in Figure 2.6C. These pathways include monocarboxylic acid metabolic process, cofactor metabolic process, fatty acid metabolic process, sulfur compound metabolic process, organic acid catabolic process, carboxylic acid catabolic process, small molecule catabolic process, xenobiotic metabolic process, cellular amino acid metabolic process, and alpha-amino acid metabolic process. KEGG enrichment for cluster 2 identified 3 enriched KEGG pathways (Supplemental Table 2) with the top 10 enriched pathways listed in Figure 2.6C. These pathways include mitochondrial gene expression mitochondrial translation, and mitochondrial RNA processing. KEGG enrichment for cluster 3 identified 500 enriched KEGG pathways (Supplemental Table 3) with the top 10 enriched pathways listed in Figure 2.6C. These pathways include tissue morphogenesis, cell morphogenesis involved in differentiation, vasculature development, blood vessel development, heart development, neuron projection morphogenesis, cell projection morphogenesis, plasma membrane bounded cell projection morphogenesis, blood vessel morphogenesis, and cell morphogenesis involved in neuron differentiation. KEGG enrichment for cluster 4 identified 463 significantly enriched KEGG pathways (Supplemental Table 4) with the top 10 enriched pathways listed in Figure 2.6C. These pathways include ribonucleoprotein complex biogenesis, chromosome segregation, nuclear division, mRNA processing, DNA replication, DNA repair, nuclear chromosome segregation, regulation of chromosome organization, mitotic sister chromatid segregation, and DNA-dependent DNA replication. Interestingly, Clusters 3 and 4 were enriched for days 10 and 15 when development and cell differentiation is occurring in the liver, and mature, metabolic pathways are most enriched in adult age mice (days 25, 60, and 120) in Cluster 1.

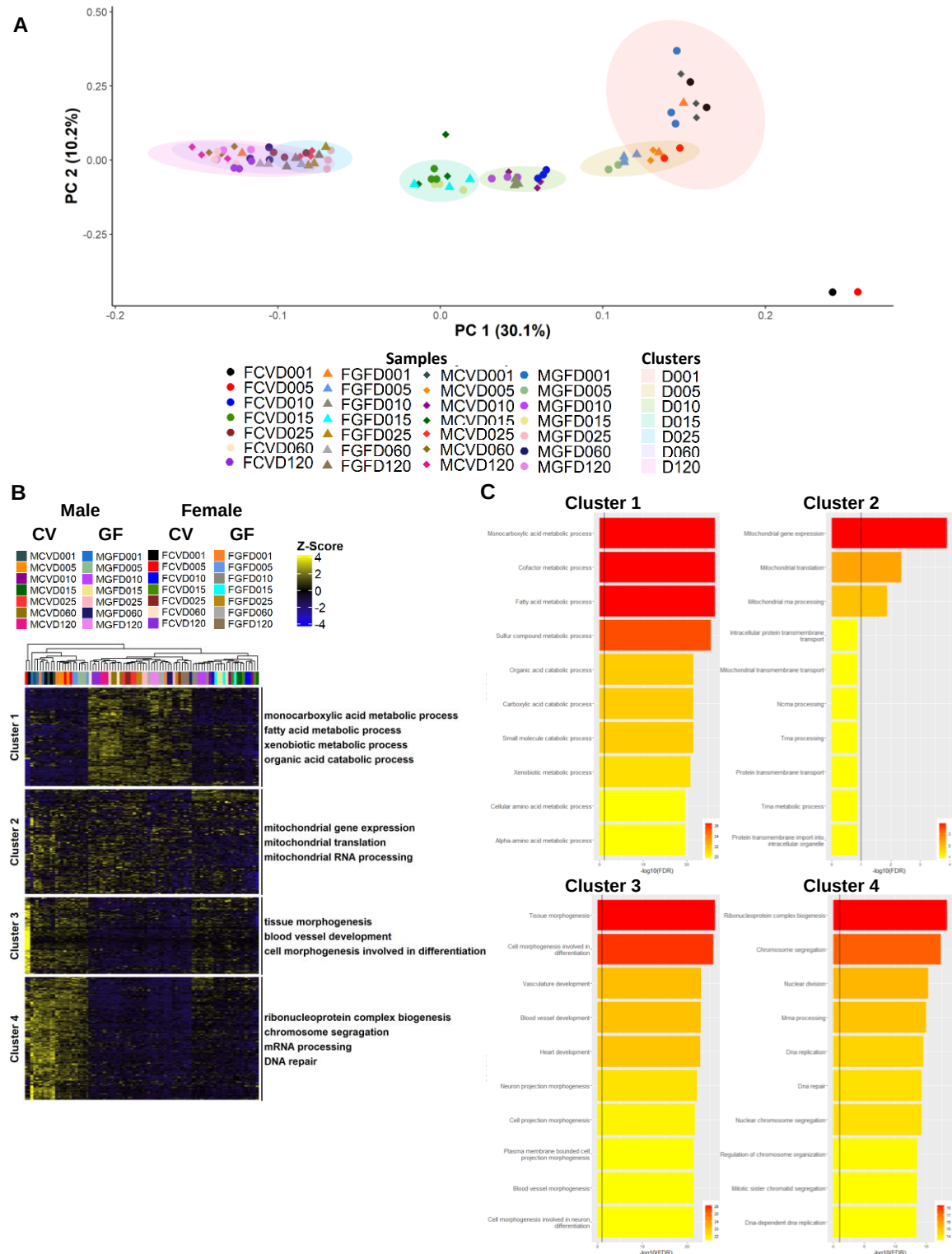


Figure 2.6. A. Principle component analysis of each age (day 1, 5, 10, 15, 25, 60, and 120) and sex (male and female; $n=3$) using hepatic RNA expression. Inclusion of a gene required an average RNA expression among all ages and both sexes greater than or equal to 1 FPKM. **B.** Heatmap of K-means clustering (4) and **C.** KEGG pathway enrichment analysis.

Gut microbiome regulates hepatic xenobiotic metabolism related genes

Included for KEGG pathways in Cluster 1, which was enriched primarily in adult mice at days 25, 60, and 120 (Figures 2.6B and C), is xenobiotic metabolic process. The secondary BAs LCA and DCA are known to activate the xenobiotic sensing nuclear receptor PXR (Staudinger et al., 2001), and inversely, activation of the nuclear receptors PXR, CAR, peroxisome proliferator activated receptor alpha (Ppar α), as well as the transcription factor AhR alter the regulation of BA metabolism (Lickteig et al., 2016; Csanaky et al., 2018; Zhang et al., 2018; Dempsey et al., 2019). AhR and PXR were not altered by the lack of gut microbiome; CAR and Ppar α were increased in GF male mice at day 120 (Supplemental Figure 2.S19). Other nuclear receptors that form a heterodimer with PXR to regulate the expression of drug metabolizing enzymes, such as Rxra and Rxrg were altered increased or decreased in day 120 male GF mice only.

To investigate the hypothesis that lack of gut microbiome alters xenobiotic metabolism in an age specific manner, we identified known xenobiotic metabolizing genes, transporters, genes involved in antioxidants, and nuclear receptors (collectively called drug processing genes; DPGs), as well as BA synthesis genes (Supplemental Table 5), and examined this subset of genes for differentially expressed (DE) RNA between CV and GF mice stratified by age and gender (Supplemental Figures 2.S16-20). Overall, more DPGs were differentially regulated by the lack of microbiome in day 120 mice compared to the other six ages (Figure 2.7A). In males the number of differentially regulated genes increased in an exponential pattern from day 1, 5, 10, and 15 (about 10 to 30 DE genes) to about 100 DE genes at day 120. In females, the increase in the number of DE genes was less pronounced with less than 80 DE DPGs at day 120. Phase I and Phase II genes had the most prominent increase in DE genes. Overall, this matched the global clustering such that the DE genes are most likely to occur at adult ages.

Comparing the DPGs for each age group showed some genes were uniquely DE by gender. At day 1, 31 genes were DE with 5 DPGs DE solely in males, 24 in females, and 2 in both sexes. This is reversed at day 5 with 24 genes DE solely in males, 4 and females, and 6 in

both. Up to 21 genes were DE at days 10 and 15 with a relatively equal distribution of the number of DE DPGs at days 25 and 60. At day 120 65 genes were uniquely differentially expressed in males, 36 in females, and 38 in both sexes. Overall, this demonstrates that DPGs can be differentially expressed by gut microbiome as well as by sex, and that the number of genes influenced by gut microbiome and sex increases with age.

However, changes in hepatic RNA expression does not always indicate that there will be changes in protein expression or function of xenobiotics. Therefore, a subset of key P450s was evaluated for protein expressed as well as activity. The RNA expression of 4 major P450s (Cyp1a2, Cyp2b9, Cyp3a11, and Cyp4a10) was compared to the targeted protein expression and was relatively similar for all four genes (Figure 2.7C). These examples were chosen because they are targets of the xenobiotic sensing transcription factors AHR, CAR, PXR, and PPAR α , respectively. For Cyp1a2, RNA and protein expression in males and females was low at days 1, 5, 10, and 15, before increasing to adult levels; GF mice tended to have increased expression at adult ages only. For Cyp2b9, RNA and protein expression in males increased from days 1 to 25 and was barely detected at days 60 and 120 for both CV and GF mice. In females, Cyp2b9 increased linearly from day 1 through day 120 with no clear differences in CV and GF mice. This shows that Cyp2b9 is age- and gender-specific, but the lack of gut microbiome does not influence its expression. For Cyp3a11, the RNA and protein expression in males and females was low at days 1, 5, 10, and 15 for both CV and GF mice, before increasing in CV mice only; GF mice maintained low level, immature expression of Cyp3a11, indicating that the development of age-specific bacteria between days 15 and 25 and is maintained into adulthood is necessary to regulate the basal expression of Cyp3a11. For Cyp4a10, RNA and protein expression in males and females was high at day 1 initially before decreased at days 5, 10, and 15. The expression increased again at day 25, however the increased expression was only maintained in the RNA of GF mice (both males and females). This indicates that Cyp4a10 is influenced by the presence of

the mature microbiome, however, post translational processes regulate the protein expression such that the bacteria cannot alter its expression.

To validate that the changes in RNA expression reflect functional differences, activity-based protein profiling followed by untargeted proteomics was used for P450s and glutathione-s-transferases (Gsts). The livers of male CV and GF mice at day 120 were pooled (4 equal mass samples per pool), and total proteins were isolated before probes that covalently bind to either P450s or Gsts were applied to the samples. Covalently bound proteins were then isolated and subjected to untargeted proteomics via LC-MS. This technique quantitatively identifies only the active proteins. The relatively LOG2 fold change of the GF/CV protein activity (LFQ intensity) was compared to the RNA expression (FPKM) (Figure 2.7D). RNA expression was considered significant with an FDR-BH less than 0.05 and activity-based protein abundance was significant with an absolute value LOG2 change of 30%. For P450s, the proteins of 19 P450s were quantified and generally matched the RNA expression direction of change. For example, the Cyp1a2 RNA differentially increased in GF mice compared to CV mice and the protein activity also increased. Other P450s that had matching increased or decreased expression and activity included Cyp2c40 (increased), Cyp3a11 (decreased), Cyp4a10 (increased), Cyp4a14 (increased), and Cyp4f14 (increased). There were six P450s that had altered protein activity, but no change in RNA expression: Cyp2c29 (increased), Cyp2c70 (increased), Cyp2d10 (increased), Cyp2d9 (increased), Cyp2f2 (decreased), and Cyp4a12a (increased).

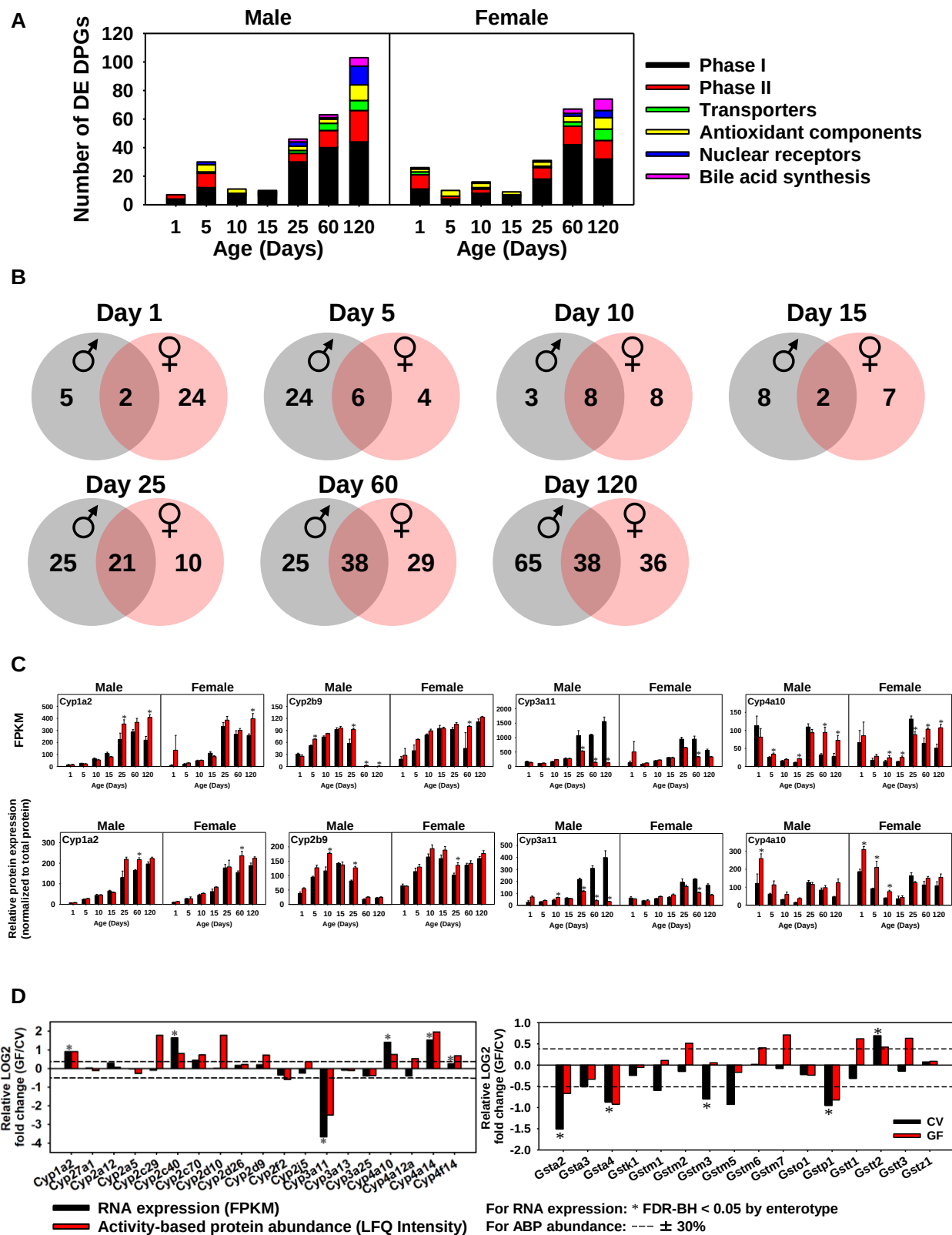


Figure 2.7. A. The number of drug processing genes (DPGs) differentially expressed (DE) between CV and GF at days 1, 5, 10, 15, 60, and 120 for males and females. DPGs were selected

by investigators from the literature and are listed in Supplemental Table 5. **B.** Venn diagrams of the number of DPGs that are DE uniquely in males, females or by both at each age (day 1, 5, 10, 15, 25, 60, and 120). **C.** RNA and protein expressions for select DPGs (Cyp1a2, Cyp2b9, Cyp3a11, and Cyp4a10). These examples were chosen because they are targets of the xenobiotic sensing transcription factors AHR, CAR, PXR, and PPAR α , respectively. For RNA expression, asterisks (*) represent statistically significant differences between CV and GF mice at each age determined by Cuffdiff (Trapnell et al., 2010). **D.** The relative LOG2 fold change of RNA expression and active protein abundance for GF to CV Day 120 male mice for detected P450s and Gsts. For RNA expression, asterisks (*) represent statistical significance between CV and GF mice using FDR-BH < 0.05 (n=3 per group) calculated by Cuffdiff (Trapnell et al., 2010). For active protein abundance, the dotted line represents a 30% increase or decrease in activity (single pooled sample of 4 mouse livers).

For Gsts, 16 proteins were quantified. There were four Gsts that had matching altered RNA expression and protein activity, which included Gsta2 (decreased), Gsta4 (decreased), Gstp1 (decreased), and Gstt2 (increased). Interestingly, five Gsts had increased protein activity, but no change in RNA expression, including Gstm2, Gstm6, Gstm7, Gstt1, and Gstt3. Gstm3 had decreased RNA expression and no change in protein activity; however, the decrease in RNA expression was small in Day 120 male mice (from about 30 FPKM in CV to about 19 in GF mice). Overall, among the detected Gst proteins, six had increased protein activity and three had decreased activity in GF mice, indicating an altered hepatic environment in the liver that may be specific to the increased activity of Gstm enzymes and Gstt enzymes compared to the decreased activity of Gsta enzymes and Gstp1.

2.4 Discussion

In summary, age dependent xenobiotic metabolism may be regulated by an age-specific microbiome (Figure 2.8). With increasing age, the gut microbiome in mice became more diverse and had increased functional capacity to produce secondary BAs, including the microbial family S24-7. Specifically, these age groups consisted of day 1, day 5, day 10, and days 15, 25, 60 and 120. In relation to age, 27 bacteria, primarily present in day 120 mice, were positively associated with secondary unconjugated, deoxy, and keto BAs. Nine of these BAs were produced in CV mice only, among which only T-DCA and ω MCA were detected in the liver, suggesting they may act as a feedback signaling molecule from bacteria. Comparing CV and GF mice, there was an increase in the Σ CDCA: Σ CA ratio in serum, liver, and ileum of day 120 GF mice, but not CV mice, and this corresponded with a decrease in the CA-producing P450 in Day 120, GF male mice. SCFAs were also found in livers of CV mice only with six SCFAs increasing with age. Global analysis of hepatic RNA expression showed an age-specific enrichment of KEGG pathways with 4 clusters grouped by age similar to the gut microbiome. Known to be impacted by the lack of gut microbiome, xenobiotic metabolism was an enriched pathway for adult CV and GF mice (day 25, 60, and 120). The DE DPGs exhibited age and sex specific expression patterns with more DPGs being DE with increasing age. Finally, the functional capacity of individual P450s and Gsts was altered by gut microbiome. Overall, the age-specific gut microbiome impacts host xenobiotic metabolism likely through circulation of metabolites (e.g. BAs and SCFAs).

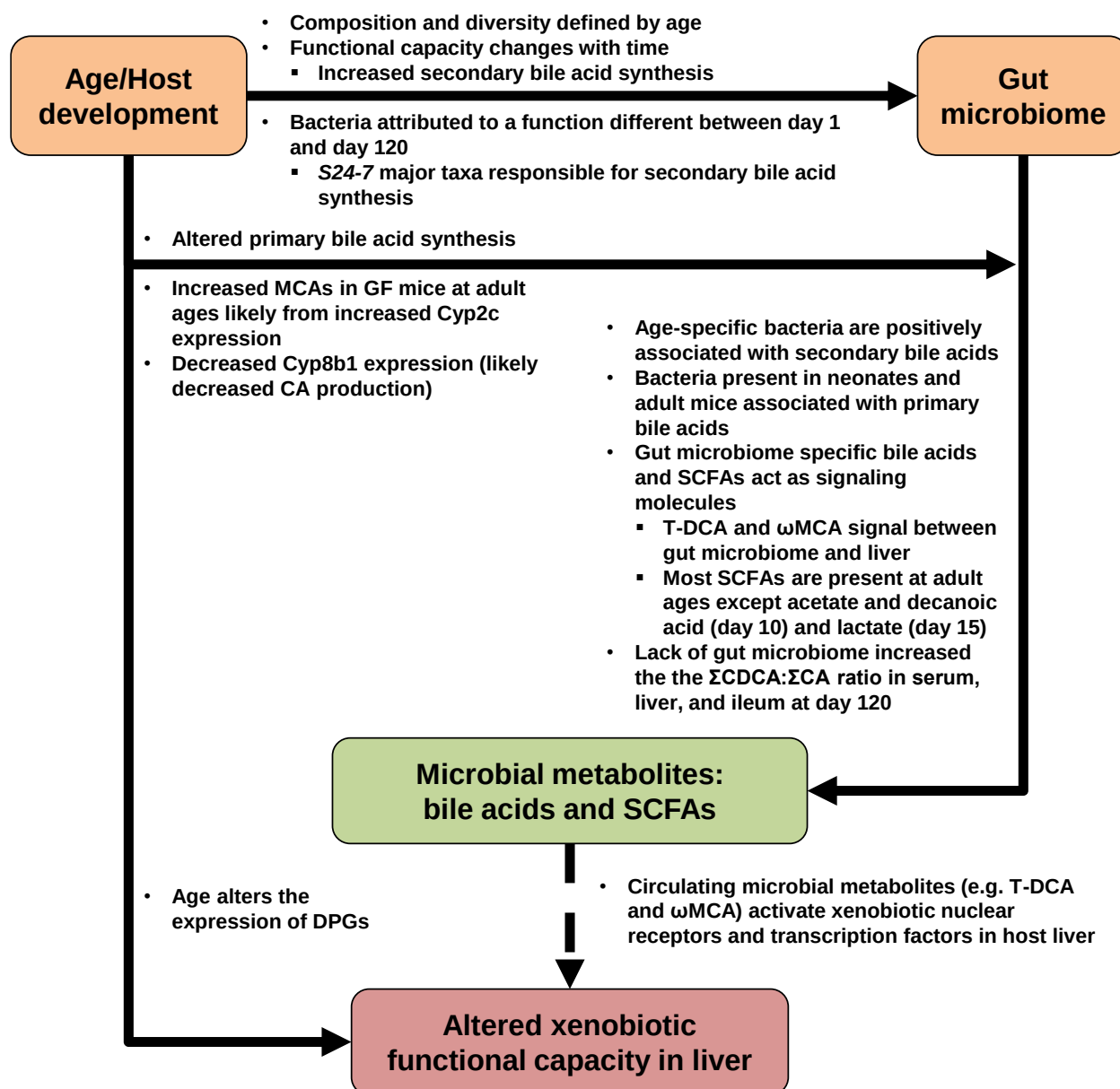


Figure 2.8. Diagram illustrating the major findings of the present study. Briefly, 1) the murine gut microbiome with increased age had greater capacity to produce secondary BAs, primarily by the family S24-7, and bacteria primarily present in day 120 mice were positively associated with secondary unconjugated, deoxy, and keto BAs. 2) Nine of these BAs were only produced in CV mice, among which only T-DCA and ω MCA were detected in the liver. 3) SCFAs were also only found in livers of CV mice with six SCFAs increasing with age. 4) Global analysis of hepatic RNA expression showed an age-specific enrichment of xenobiotic metabolism for adult CV and GF mice, and the DE DPGs exhibited age and sex specific expression patterns with more genes being DE with increasing age.

BAs are prominent metabolites that are important for the regulation of lipid and glucose metabolic homeostasis and energy metabolism in the liver and extra hepatic organs, such as adipose tissues and muscle (Chiang and Ferrell, 2018). Nine BAs were detected only in CV mice: T-DCA, ω MCA, 3,6-diketocholanic acid, 3-ketocholanic acid, 3 α -hydroxy-7 ketolithocholic acid, 3 α -hydroxy-7, 12-diketolithocholic acid, 3 α -hydroxy-12-ketolithocholic acid, 5 α -cholanic acid-3, 6-dione, and 5 α -cholanic acid-3 α -ol-6-one. T-DCA is a well-known secondary BA that activates FXR and Takeda G protein receptor 5 (TGR5) in the intestine. Activation of FXR in intestine induces fibroblast growth factor 19 (FGF19, or Fgf15 in mice) which goes to the liver to inhibit CYP7A1 and CYP8B1 (Chiang and Ferrell, 2018). Activation of TGR5 stimulates the secretion of glucagon-like peptide 1 (GLP-1) which activates β -cell secretion of insulin and improves insulin sensitivity (Chiang and Ferrell, 2018). Regarding the murine secondary BA ω MCA, it is converted in the large intestine from α MCA and β MCA for secretion (Eyssen et al., 1983; Chiang and Ferrell, 2018). Among the keto-BAs, 3-ketocholanic acid is produced from the metabolism of LCA, which is toxic to the liver, by the gut microbiome (Sakai et al., 1980; Akao et al., 1986) and is demonstrated to have less toxicity to bacteria in the cecum (Tian et al., 2020). 3-ketocholanic acid was detected minimally in day 120 CV mice in the large intestine (Supplemental Figure 2.S4), whereas LCA was detected in CV mice at day 15 and day 120, as well as at day 1 male GF mice, although this may be due to carry-over in the LC-MS. 3-ketocholanic acid – also referred to as 3-ketolithocholic acid – and LCA are activators of PXR and FXR, but are potent ligands of vitamin D receptor (VDR) with 3-ketocholanic acid having a binding affinity for VDR 3.5 times more than LCA (Makishima et al., 2002; Masuno et al., 2013). It is unknown how the other keto-BAs with LCA-like structures react with VDR, PXR, and FXR. Activation of VDR by LCA and 3-ketocholanic acid increase the expression of CYP3A enzymes and CYP24A1, primarily in ileum, and this occurs through a VDR-RXR heterodimer (Xie et al., 2001; Makishima et al., 2002; Ishizawa et al., 2018). Along with many xenobiotics, CYP3A4 (Cyp3a11 in mice) can inactivate vitamin D, indicating LCA and its derivatives may regulate vitamin D levels (Sawada et al., 2004; Xu et al.,

2006). VDR, which is not expressed in liver (Zhou et al., 2006), is a crosstalk mechanism between bacteria and the host in the intestines. Conditional deletion of VDR in mice altered BA, carbohydrate, protein, and lipid metabolites (84 of 765 monitored) and increased the expression of FXR in liver and intestine (Chatterjee et al., 2020). VDR interacts with other nuclear receptors for the regulation of BA metabolism and the regulation of DPGs in intestine (Schmidt and Mangelsdorf, 2008; Staudinger et al., 2013). How these nuclear receptors, in particular VDR, interact with the microbiome and their metabolites to maintain health is unclear. More research is needed to understand how secondary BAs – LCA its derivatives – and VDR may regulate xenobiotic metabolism in the intestine. This is particularly important as it is now recognized that xenobiotics may be first metabolized by the gut microbiome and intestines before the liver (Dempsey and Cui, 2019).

Because BAs undergo enterohepatic circulation, are metabolized by bacteria, can be toxic to both bacteria and the host, and are necessary for proper nutrition, understanding the communicative interaction between bacteria and host is paramount for identifying the healthy dynamic relationship between the host and gut microbiome. Furthermore, understanding how bacteria affect BA metabolism during development may aid in the understanding of physiology and the development of therapeutics to treat metabolic diseases (Chiang and Ferrell, 2018). In this study, functional analysis of the detected bacteria from day 1 to day 120 showed an exponential increase in the capacity of the gut microbiome to produce BAs (Figure 2.1C), and the microbial family attributed to driving secondary BA synthesis between day 1 and day 120 was S24-7 (Figure 2.1D). This *Bacteroidales* family was first sequenced and characterized as an anaerobic bacteria that was a major portion of the murine gut and had enzymes involved in complex carbohydrate degradation, primarily glycans (Lagkouvardos et al., 2016; Ormerod et al., 2016; Lagkouvardos et al., 2019). Recently, members of S24-7 were cultured and strains showed specificity to the mouse intestines, covering up to 75% of the functional portion of the mouse metagenome (Lagkouvardos et al., 2016).

In this study, three different families were identified from 16S rRNA gene sequencing data, and a total of 59 species have been identified thus far by metagenomic sequencing (Lagkouravdos et al., 2019). S24-7 was identified as a SCFA-producing bacteria that may impact the development of type 2 diabetes (Xiao et al., 2020). Mice fed a high-fat diet supplemented with a mixture of soluble fibers (polysaccharides) had increased S24-7, but this family was not related to insulin sensitivity or adiposity (Xu et al., 2020). Regarding BAs, a liver regeneration study showed a positive association between S24-7, hepatic metabolism, and the primary conjugated BA T-CDCA and the conjugated secondary BA T-DCA (Liu et al., 2016). Interestingly, male hamsters given a hypercholesterolemic diet supplemented with wheat gluten had a positive association between S24-7 and decreased cholesterol (serum and liver). In the present study, S24-7 group 3 was positively associated with secondary unconjugated, deoxy, and keto BAs (Cluster 2, Figure 2.2A), which excluded T-CDCA and T-DCA, and none of the three S24-7 groups were correlated with SCFAs (Figure 2.5A). Correlation with S24-7 may be difficult as it will be positively associated with any metabolites only present in adult mice and negatively associated with metabolites only present in neonatal mice. Because S24-7 is a dominant bacteria in the murine gut, is found in humans, and is associated with SCFA metabolism and BA metabolism, future research is needed to characterize the species within S24-7 to understand their direct hepatic health effects and potential for developing therapeutics.

Very few bacteria in the gut microbiome relative to the number of bacteria in the gut microbiome have been experimentally confirmed as having the capability of metabolizing BAs; the majority of bacteria confirmed with secondary BA synthesis capacity are from the families *Bacteroidaceae*, *Clostridiaceae*, *Lactobacillaceae*, and *Bifidobacteriaceae* (Ridlon et al., 2006; Jones et al., 2008; Just et al., 2018; Vital et al., 2019) (Tazuke et al., 1998; Grill et al., 2000; Kitahara et al., 2001; Knarreborg et al., 2002; Lepercq et al., 2004; Ridlon et al., 2010; Li and Chiang, 2014; Xu et al., 2016). In general, these microbial genes are activated by BAs and have an operon of BA-inducible (*bai*) genes (Wells and Hylemon, 2000; Vital et al., 2019). Gut bacteria

that metabolize BAs were traditionally identified using *in vitro* and *in vivo* techniques. More recently, *in silico* analyses and gene prediction from metagenomic sequencing and 16S rRNA gene sequencing, respectively, have gained popularity and could be used as screening methods for BA metabolizing bacteria before confirmation by traditional culturing methods. In this study, 27 bacteria had a positive correlation with the Cluster 2 BAs, which were mostly secondary unconjugated, deoxy, and keto BAs and included all nine BAs not detected in GF mice (Figure 2.2A). Of the 27 bacteria, 10 are from the family *Ruminococcaceae* and eight are from the family *Lachnospiraceae*. *In silico* analysis of metagenome-assembled genomes for *bai* genes demonstrated that the most species with *bai* genes were from the family *Ruminococcaceae* with a few species from *Lachnospiraceae* (Vital et al., 2019), indicating that these two family may play more prominent roles in secondary BA synthesis than was traditionally considered. However, *Lachnospiraceae* was decreased in mice fed lard or bile acids whereas the this treatment increased the family *Desulfovibrionaceae* (Just et al., 2018). *Desulfovibrionaceae* was also positively correlated with Cluster 2 BAs, but there is no evidence of bile acid metabolizing genes at this time. The metagenomic study also found significant *bai* horizontal gene transfer, which may indicate that bacteria of other species may adapt to metabolize BAs (Vital et al., 2019). The family S24-7, in this study, was attributed to the increase in secondary BA synthesis in day 120 mice (Figure 2.1D), and S24-7 group 3 was positively associated with Cluster 2 BAs. A *bai* operon or alternative genes have yet to be identified in S24-7 (Lagkouvardos et al., 2019; Vital et al., 2019), and *in vitro* studies are needed to confirm if S24-7 can metabolize secondary BAs. Among the 27 bacteria, the genus *Bifidobacterium* can deconjugate BAs through *bsh*, but it is not known if it contains BA dehydrogenases or other *bai* enzymes (Ridlon et al., 2006). The abundance of *bsh* genes is associated with ten human diseases: gastrointestinal diseases, obesity, type 2 diabetes, liver diseases, cardiovascular diseases, and neurological diseases (Jia et al., 2020). Other families positively correlated with the Cluster 2 BAs, including *Erysipelotrichaceae*, *Clostridiales*

Family XIII, and *Mollicutes*, do not have evidence for the capacity of secondary BA synthesis, although they are correlated to other microbial metabolites (Lin et al., 2016).

Regarding primary BAs, there was an increase in the Σ CDCA: Σ CA ratio in day 120 GF mice relative to CV mice in serum, liver, and ileum, which was attributed to a marked increase in CDCA BA derivatives (Figure 2.4A). Examination of individual BA sums in these compartments showed the increased Σ CDCA BAs was due to the murine BAs, specifically T- α/β MCA. Secondary BA synthesis is initiated by *bsh* and produces secondary BAs that are FXR agonists leading to the regulation of the BA pool. In mice without bacteria, there is no *bsh* activity. This prevented the metabolism of primary BAs, such as T- α/β MCA, that are FXR antagonists, resulting in an increase in the BA pool size and a feed-forward loop that is overproducing CDCA and its derivatives, which was demonstrated in mice given tempol that targets the *bsh*-containing bacteria *Lactobacilli* (Li et al., 2013a; Ridlon et al., 2014). Without an alternative method of metabolism, the Σ CDCA BAs become more abundant. However, in large intestine there is no difference in the Σ CDCA: Σ CA between CV and GF day 120 mice. In the large intestine of GF mice, there is increased T- α/β MCA (Supplemental Figure 2.S2.2), whereas in large intestine of CV mice, there is an increase in the unconjugated forms – α MCA and β MCA. The difference in ratio may be due to fewer primary BAs being absorbed in the ileum of CV mice. Interestingly, the ileal BA transporter Slc10a2 was found to be related to the genus *Turicibacter* in relation to genetic diverse mice (Kemis et al., 2019), but may be indicative of the relationship of BA reabsorption and the presence of specific bacteria in the gut microbiome. More research is needed to elucidate the relationships between specific bacteria and the expression of genes in intestines, specifically BA transporters in the ileum.

The presence of the gut microbiome is necessary to regulate the constitutive expression of many DPGs. This is shown by the altered expression of DPGs in mice lacking a gut microbiome (GF mice) (Bjorkholm et al., 2009; Toda et al., 2009c; Selwyn et al., 2015a; Selwyn et al., 2015c). For example, in mice, the expression of the Phase I oxidation enzyme, Cyp3a11 – a PXR-target

gene and the mouse ortholog to human CYP3A4 – was decreased in GF mice compared to CV mice. This effect was also observed in CV mice treated with antibiotics and was correlated with decreased bacteria in the feces that produce the PXR-activating BA LCA (Toda et al., 2009b; Toda et al., 2009a). GF mice housed in a formerly CV mouse habituated environment to inoculate with the expected adult microbiome restored the expression of DPGs with some alterations; however, GF mice treated with the probiotic VSL3 did not restore the expression of all DPGs, including *Cyp3a11* (Selwyn et al., 2016). This indicates that there are microbial metabolites made in the complete, natural microbiome that do not exist in the VSL3 probiotic. RNA-Seq of day 90 male mouse livers showed that xenobiotic metabolism was the most down-regulated pathway (Selwyn et al., 2015a). The expression of DPGs in livers of CV and GF mice has previously shown four different patterns of expression. First, known PXR-regulated genes had a gradual increase in expression in CV mice, such as the *Cyp3a* family, whereas in GF mice, these genes were down-regulated. The second pattern were genes were upregulated in GF mice, primarily at the adult age and included *Cyp1a2* and *Cyp2c* family. Pattern 3 had expression that was increased at day 15, and pattern 4 were neonatally enriched genes (Selwyn et al., 2015a). Western blots for protein expression and activity assays showed similar observations.

In this study, global analysis of expressed hepatic genes showed that xenobiotic metabolic processes were found to be enriched for adult ages (day 25, 60, and 120) for both sexes and in male and female mice (Figure 2.6B and C), confirming that xenobiotic metabolism capacity is altered by age. Further examination showed that there was an increase in the number of DE DPGs in both males and females (Figure 2.7A) and that many of the differentially expressed genes were sex dependent at each age (Figure 2.7B). Because specific microbial metabolites at the adult ages in mice regulate xenobiotic metabolism, it can be assumed that these metabolites and others similarly regulated xenobiotic metabolism at younger ages, and this occurs in a sex specific manner. In addition, the prototypical P450 target genes of *AhR*, *CAR*, *PXR*, and *PPAR α* – *Cyp1a2*, *Cyp2b9*, *Cyp3a11*, and *Cyp4a10* – had similar hepatic protein expression and mRNA

expression (Figure 2.7C), and the expressions of *Cyp3a11* and *Cyp4a10* match previous findings (Selwyn et al., 2015a). The decreased activity in GF mice of *Cyp3a11* and increased activity of *Cyp4a* was also confirmed (Figure 2.7D).

Other DPGs are known to be gut microbiome and sex specific. The mRNAs *Cyp2c* in liver were shown to be upregulated in adult male GF mice but not female GF mice (Selwyn et al., 2015a). In mice, *Cyp2c70* was shown to be responsible for the production of the murine specific primary BAs α MCA and β MCA, but not *Cyp2c37*, from CDCA and UDCA (Takahashi et al., 2016) but not *Cyp3a11* (Wahlstrom et al., 2017) or *Cyp2c37* (Takahashi et al., 2016). Yet, protein expression of *Cyp2c70* is not significantly different between CV and GF adult mice, which may indicate that other *Cyp2c* enzymes may be responsible for the production of murine-specific BAs (Wahlstrom et al., 2017). In this study, the mRNA expression of all *Cyp2c* genes were increased in male GF mice and days 25, 60, and 120, whereas the expression was increased in female GF mice at days 25 and 60 (Figure 2.5D). Among individual *Cyp2c* enzymes, the mRNA in male GF mice, but not female GF mice, was differentially increased at day 120 for *Cyp2c40*, 50, 68, 69, and 70. Recently, two *Cyp2c70* knockout mouse model studies showed a humanization of the BA pool, showing that *Cyp2c70* is primarily responsible for murine BAs (de Boer et al., 2020; Straniero et al., 2020). Overall, the sex-specific and microbiome influence expression of the xenobiotic and BA metabolizing enzymes, *Cyp2c*, demonstrate the diverse capabilities of DPGs in affecting host health. Understanding genes with multiple roles in drug metabolism and health may complicate the efficacy and safety of therapeutics.

In summary, many BAs are only produced in the presence of bacteria, along with SCFAs. These BAs and SCFAs are age dependent, corresponding to the age-specific microbiome. Expression and function of DPGs is gut microbiome- and age dependent, and it is known that BAs can interact with nuclear receptors regulating DPGs. Age dependent hepatic xenobiotic metabolism may be regulated by the age-specific microbiome through a mixture of microbial metabolites, including BAs and SCFAs. More studies are needed to confirm the specific

metabolites, and likely mixture of metabolites, that help regulate the metabolism of xenobiotic of a healthy host.

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2.8 Supplemental Figures
Supplemental Figure 1

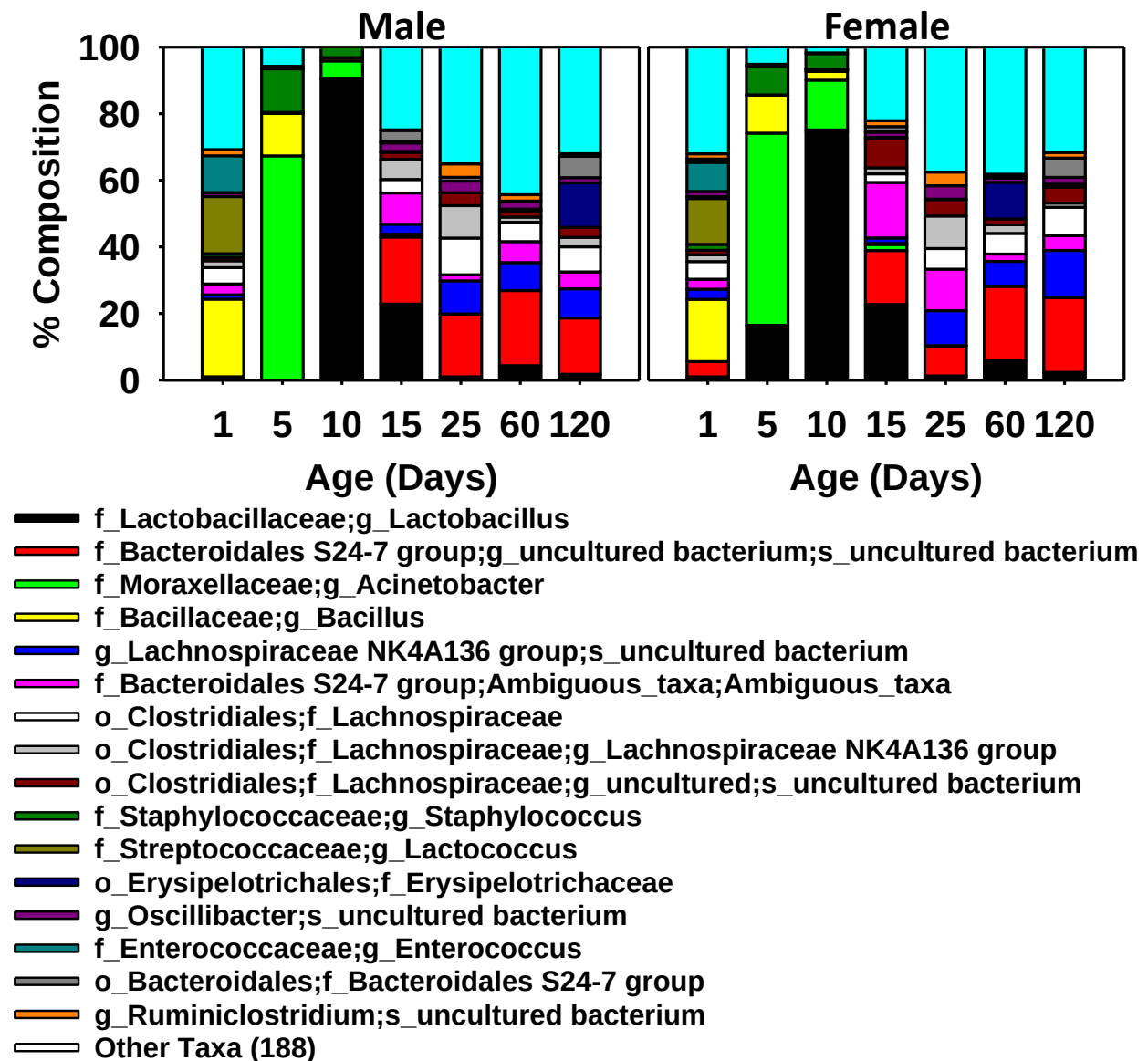
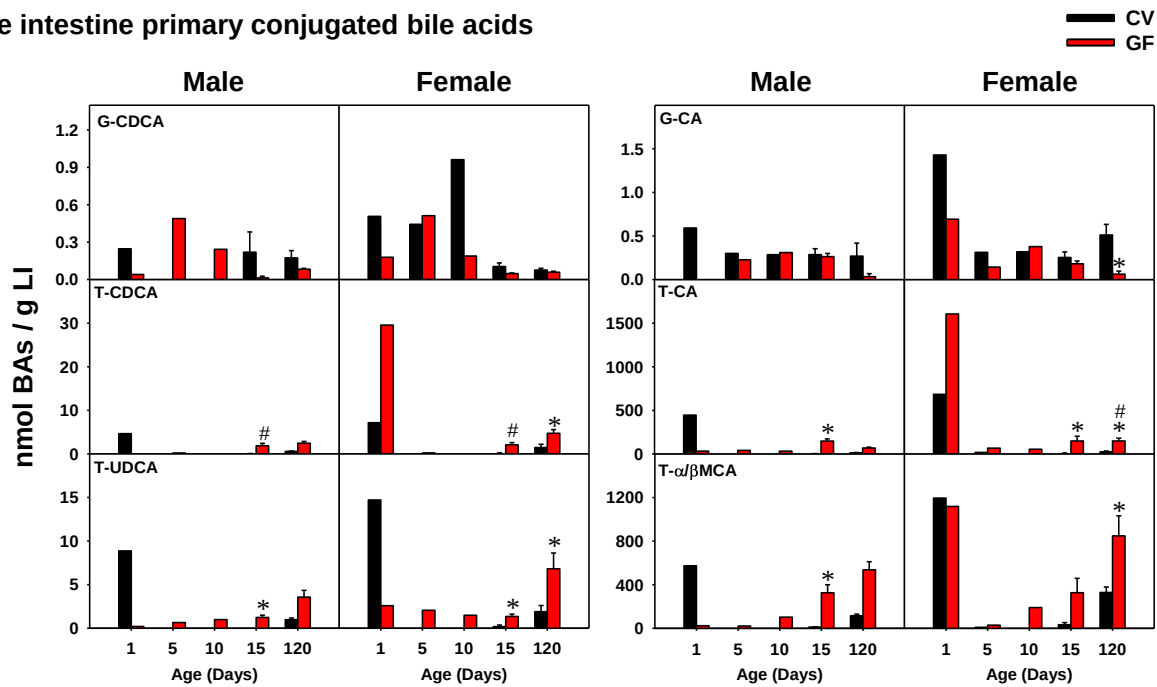


Figure 2.S1. Compositional abundance of large intestinal bacteria in male and female CV mice at postnatal days 1, 5, 10, 15, 25, 60, and 120 (n=3) to the species level (L7). The 16S rDNA sequencing data were analyzed using QIIME as described in Materials and Methods. There were 204 bacteria (OTUs) identified, and the top 16 bacteria (averaged across all groups) are shown with the remaining 188 OTUs summed together in the “Other Taxa” category.

Supplemental Figure 2

Large intestine primary conjugated bile acids



Large intestine primary unconjugated bile acids

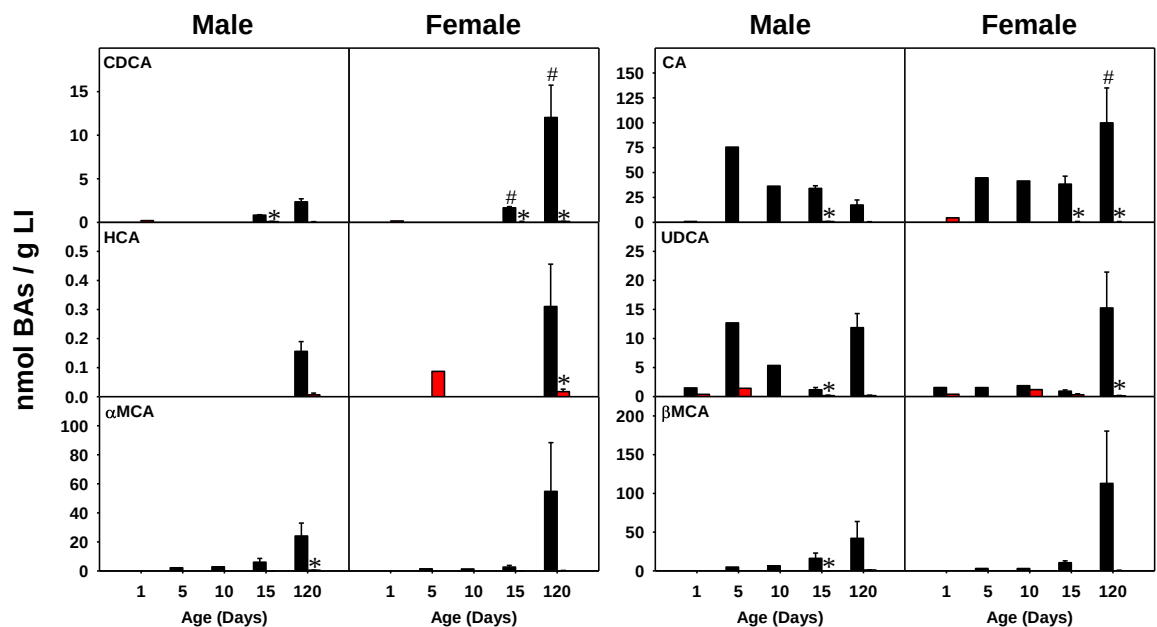
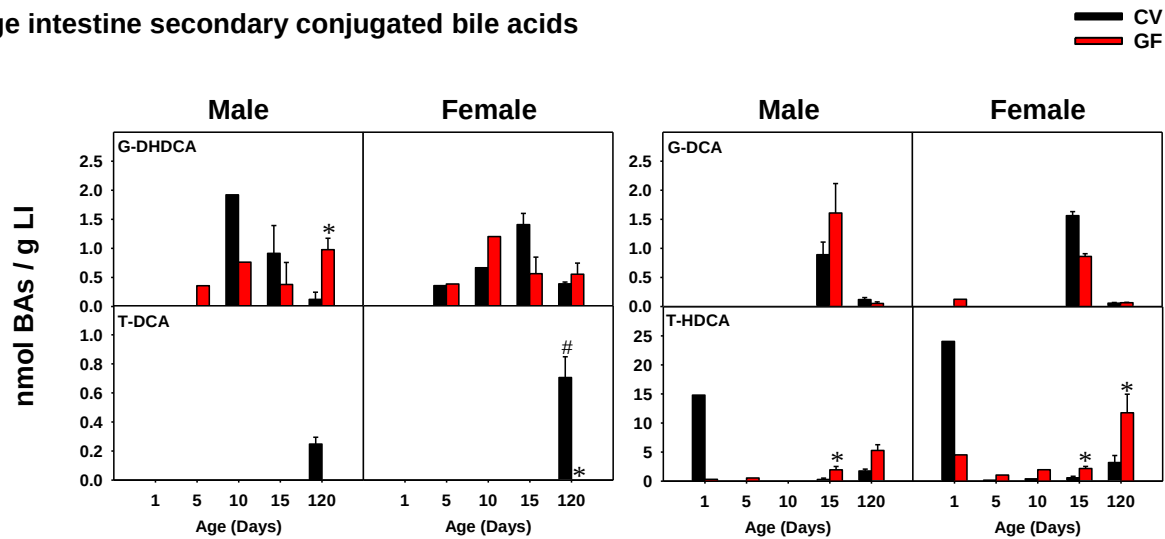


Figure 2.S2. LC-MS quantification of large intestinal primary conjugated and unconjugated BAs in CV and GF, male and female mice at days 1, 5, and 10 (n=1 pooled sample of 3 mice), as well as 15 and 120 (n=3 per group). A generalized linear model was used to identify BAs with different concentrations followed by Tukey post-hoc test. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex.

Supplemental Figure 3

Large intestine secondary conjugated bile acids



Large intestine secondary unconjugated bile acids

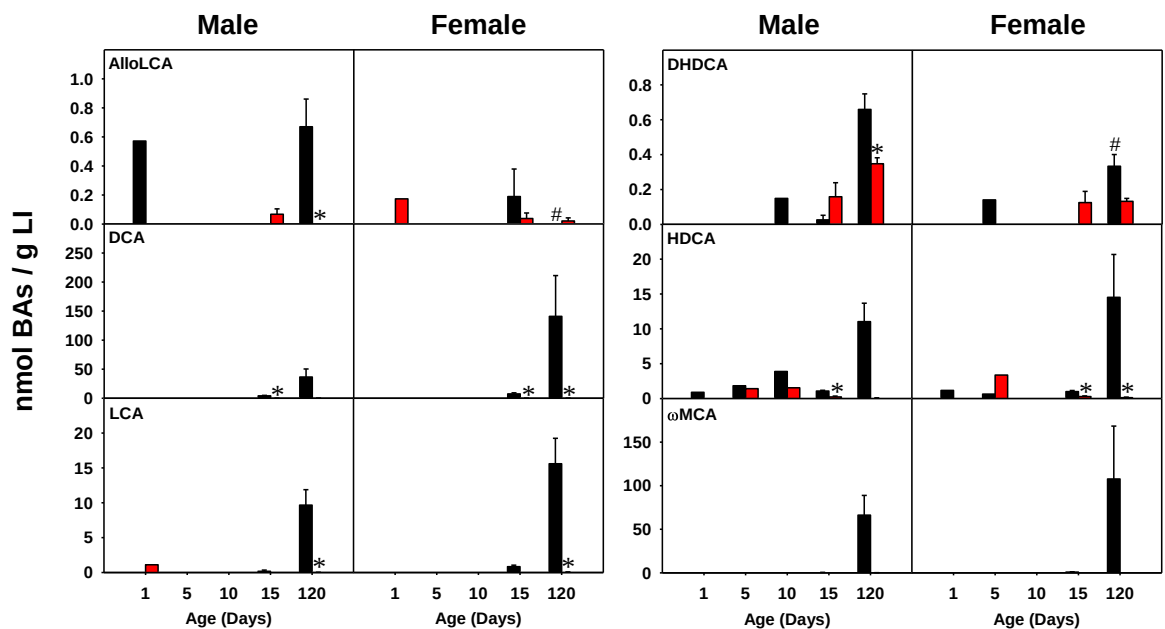


Figure 2.S3. LC-MS quantification of large intestinal secondary conjugated and unconjugated BAs in CV and GF, male and female mice at days 1, 5, and 10 (n=1 pooled sample of 3 mice), as well as 15 and 120 (n=3 per group). A generalized linear model was used to identify BAs with different concentrations followed by Tukey post-hoc test. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex.

Supplemental Figure 4

Large intestine deoxy and keto bile acids

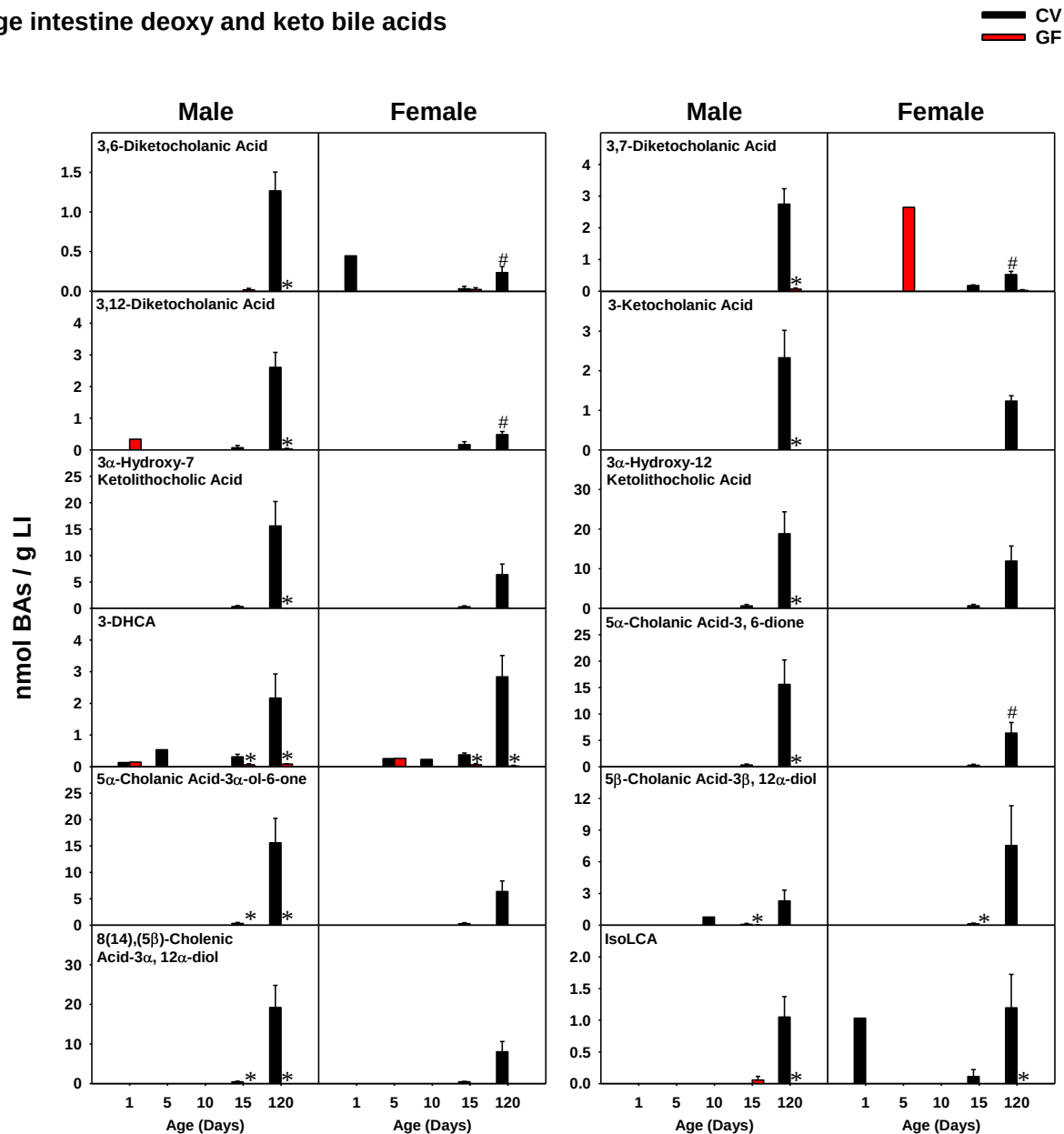


Figure 2.S4. LC-MS quantification of large intestinal deoxy and keto BAs in CV and GF, male and female mice at days 1, 5, and 10 (n=1 pooled sample of 3 mice) as well as 15, and 120 (n=3 per group). A generalized linear model was used to identify BAs with different concentrations followed by Tukey post-hoc test. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex.

Supplemental Figure 5

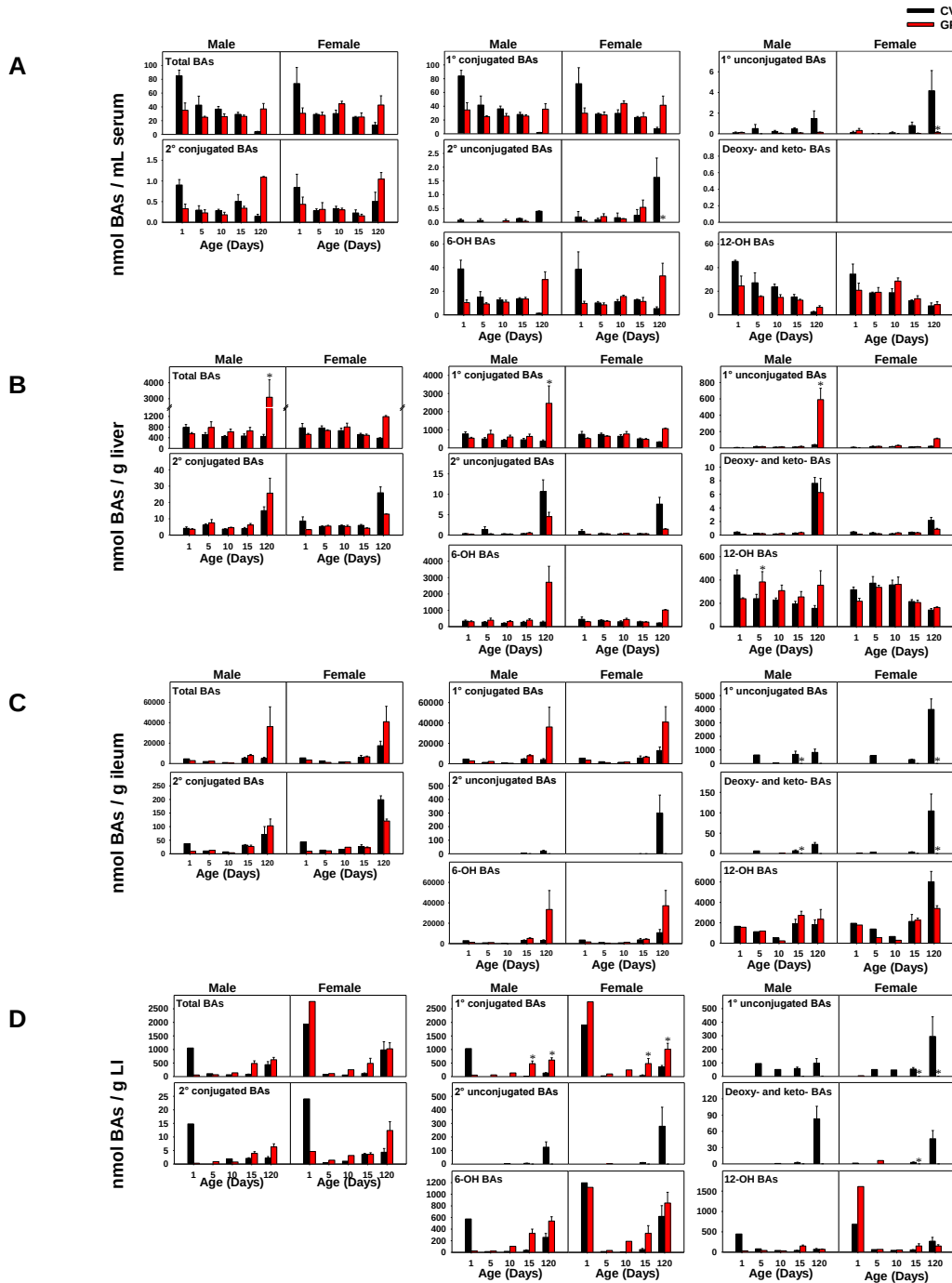
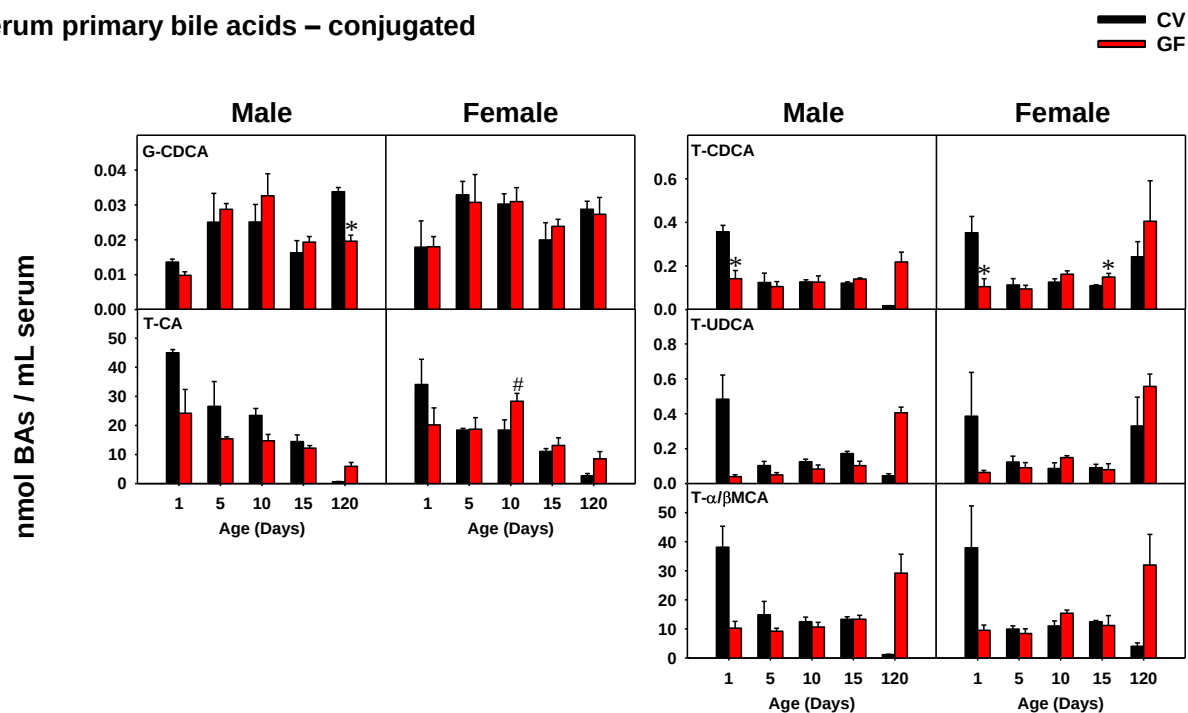


Figure 2.S5. LC-MS quantification and sums of total, primary (1°) conjugated, 1° unconjugated, secondary (2°) conjugated, 2° unconjugated, deoxy and keto, 6-OH, and 12-OH BAs in CV and GF, male and female mice at days 1, 5, 10, 15, and 120 in **A.** serum, **B.** liver, **C.** ileum, and **D.** large intestine. Serum and liver have n=3 for each group. Ileum and large intestine have n=3 per group for days 15 and 120 and a pooled sample of 3 mice for days 1, 5, and 10. A generalized linear model was used to identify BAs with different concentrations followed by Tukey post-hoc test. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex.

Supplemental Figure 6

Serum primary bile acids – conjugated



Serum primary bile acids – unconjugated

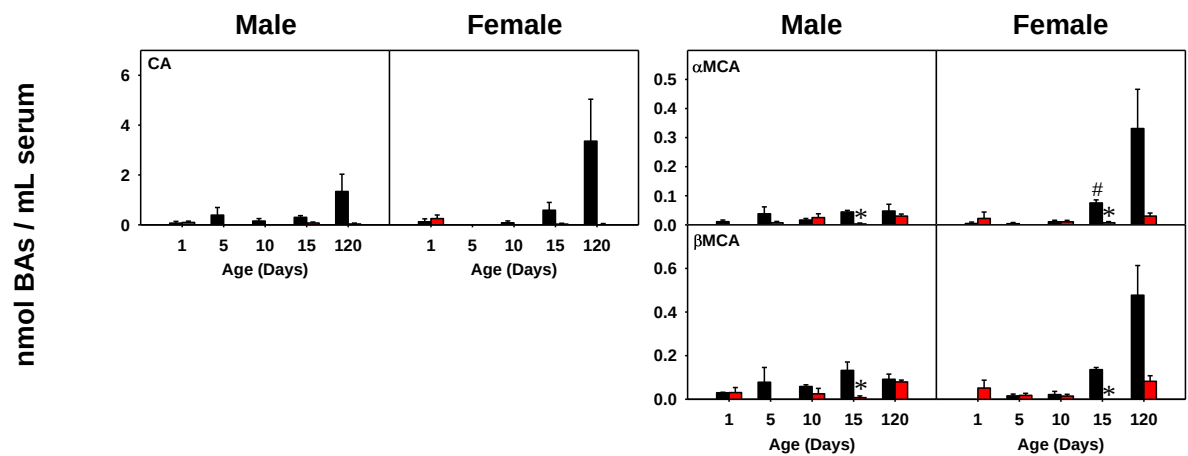
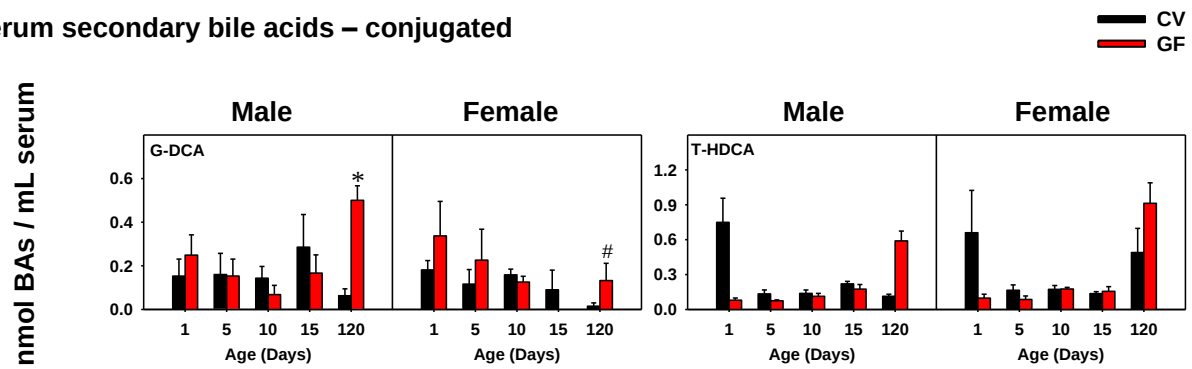


Figure 2.S6. LC-MS quantification of serum primary conjugated and unconjugated BAs in CV and GF, male and female mice at days 1, 5, 10, 15, and 120 (n= 3 per group). A generalized linear model was used to identify BAs with different concentrations followed by Tukey post-hoc test. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex.

Supplemental Figure 7

Serum secondary bile acids – conjugated



Serum secondary bile acids – unconjugated

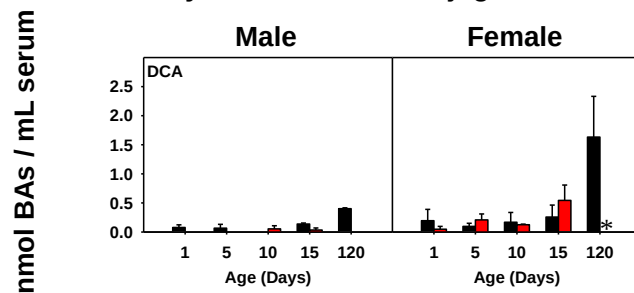
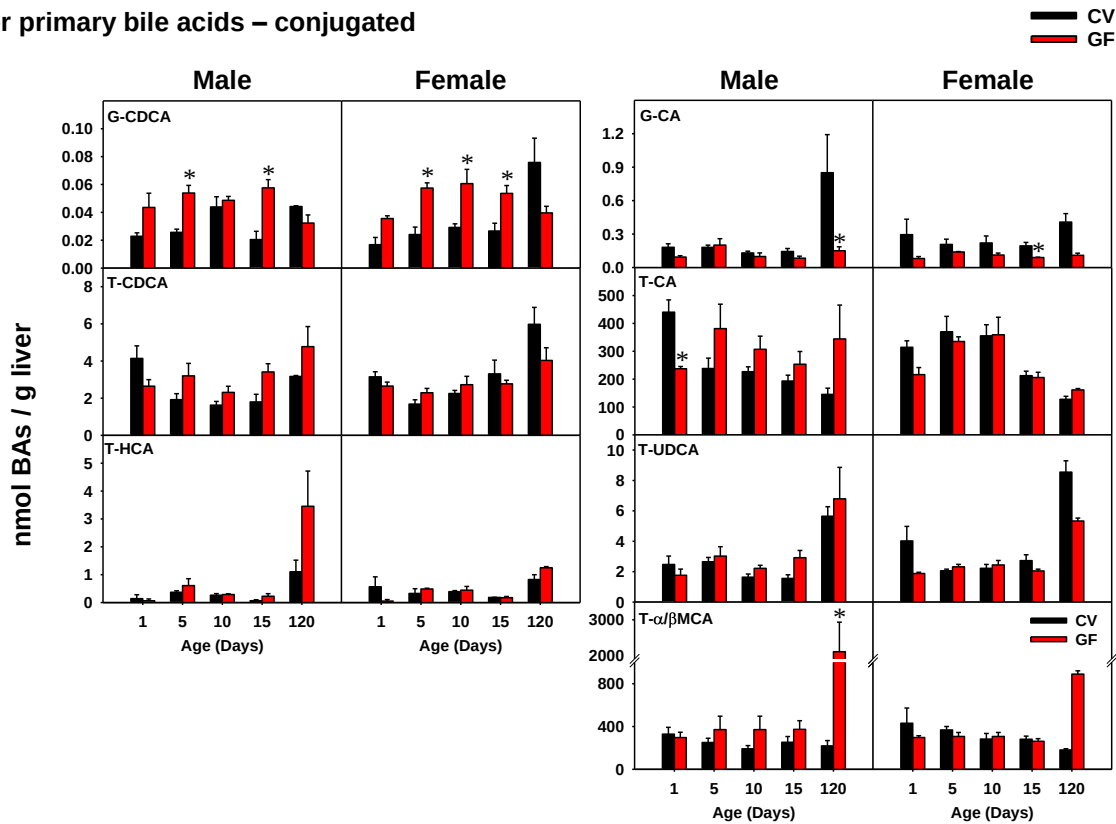


Figure 2.S7. LC-MS quantification of serum secondary conjugated and unconjugated BAs in CV and GF, male and female mice at days 1, 5, 10, 15, and 120 (n= 3 per group). A generalized linear model was used to identify BAs with different concentrations followed by Tukey post-hoc test. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex.

Supplemental Figure 8

Liver primary bile acids – conjugated



Liver primary bile acids – unconjugated

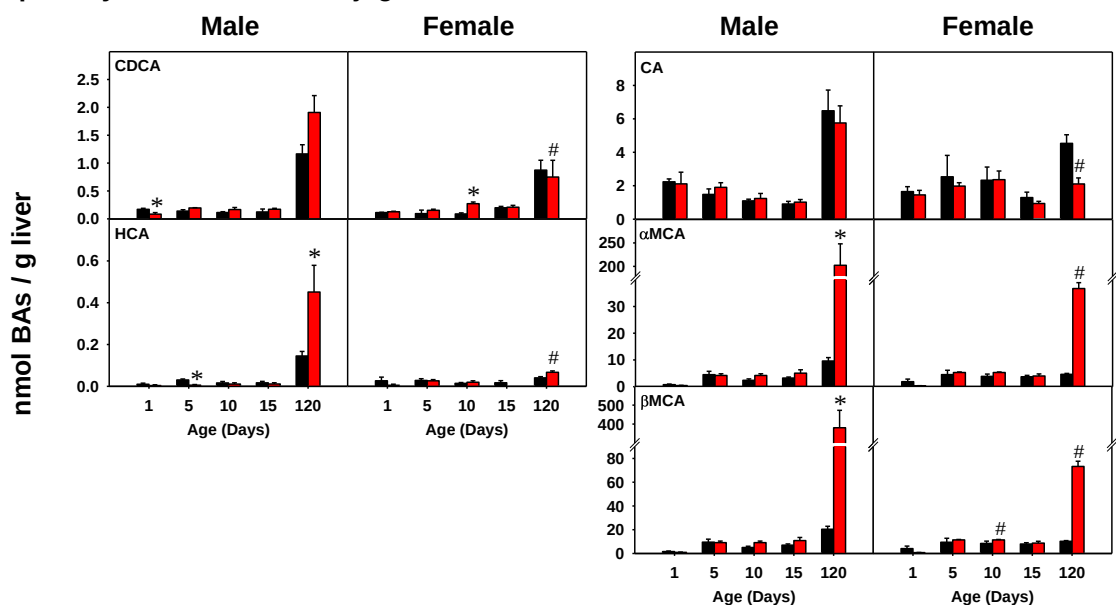


Figure 2.S8. LC-MS quantification of liver primary conjugated and unconjugated BAs in CV and GF, male and female mice at days 1, 5, 10, 15, and 120 (n= 3 per group). A generalized linear model was used to identify BAs with different concentrations followed by Tukey post-hoc test. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex.

Supplemental Figure 9

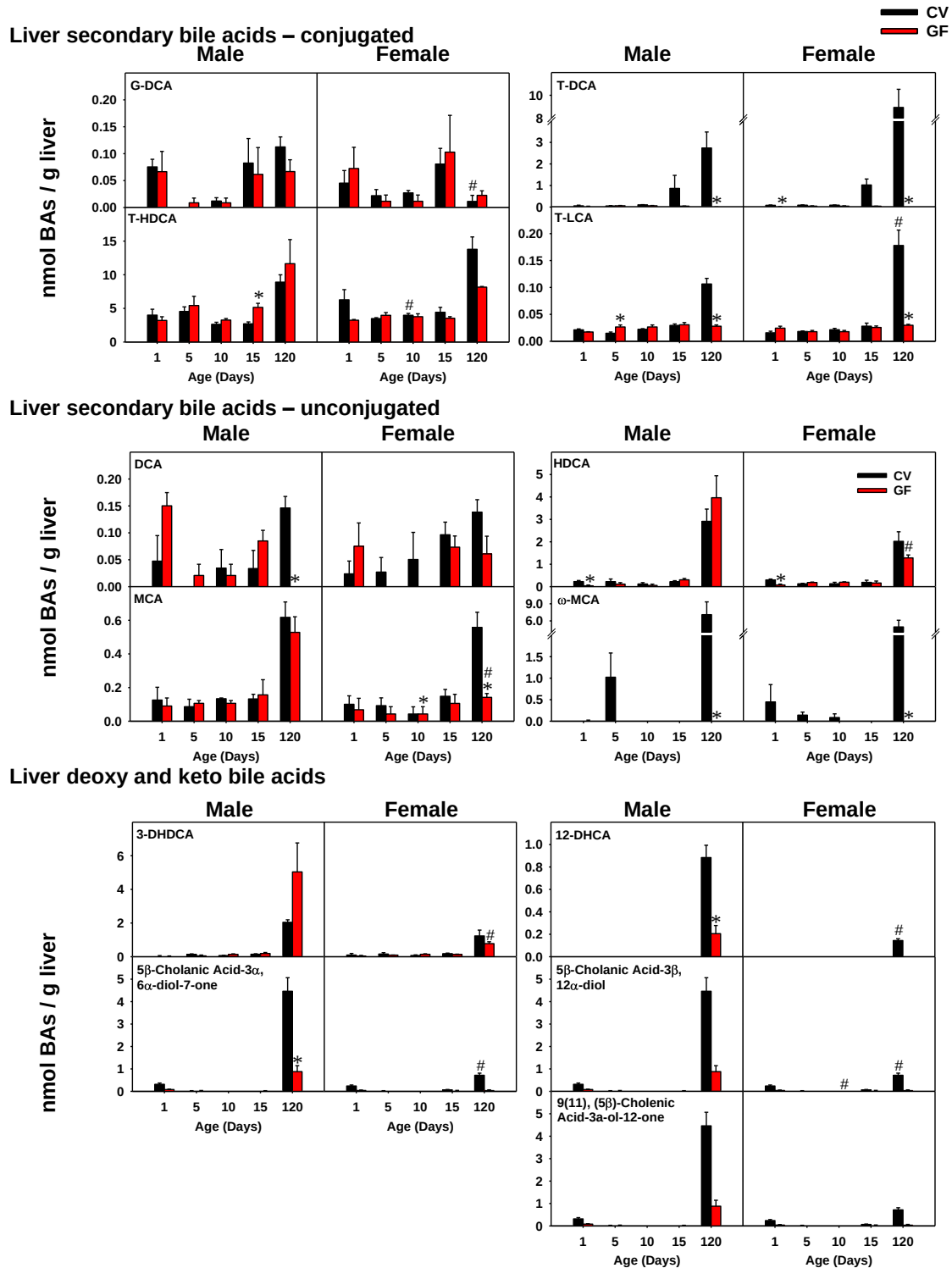


Figure 2.S9. LC-MS quantification of liver secondary conjugated and unconjugated BAs, as well as deoxy and keto BAs, in CV and GF, male and female mice at days 1, 5, 10, 15, and 120 (n= 3 per group). A generalized linear model was used to identify BAs with different concentrations followed by Tukey post-hoc test. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex.

Supplemental Figure 10

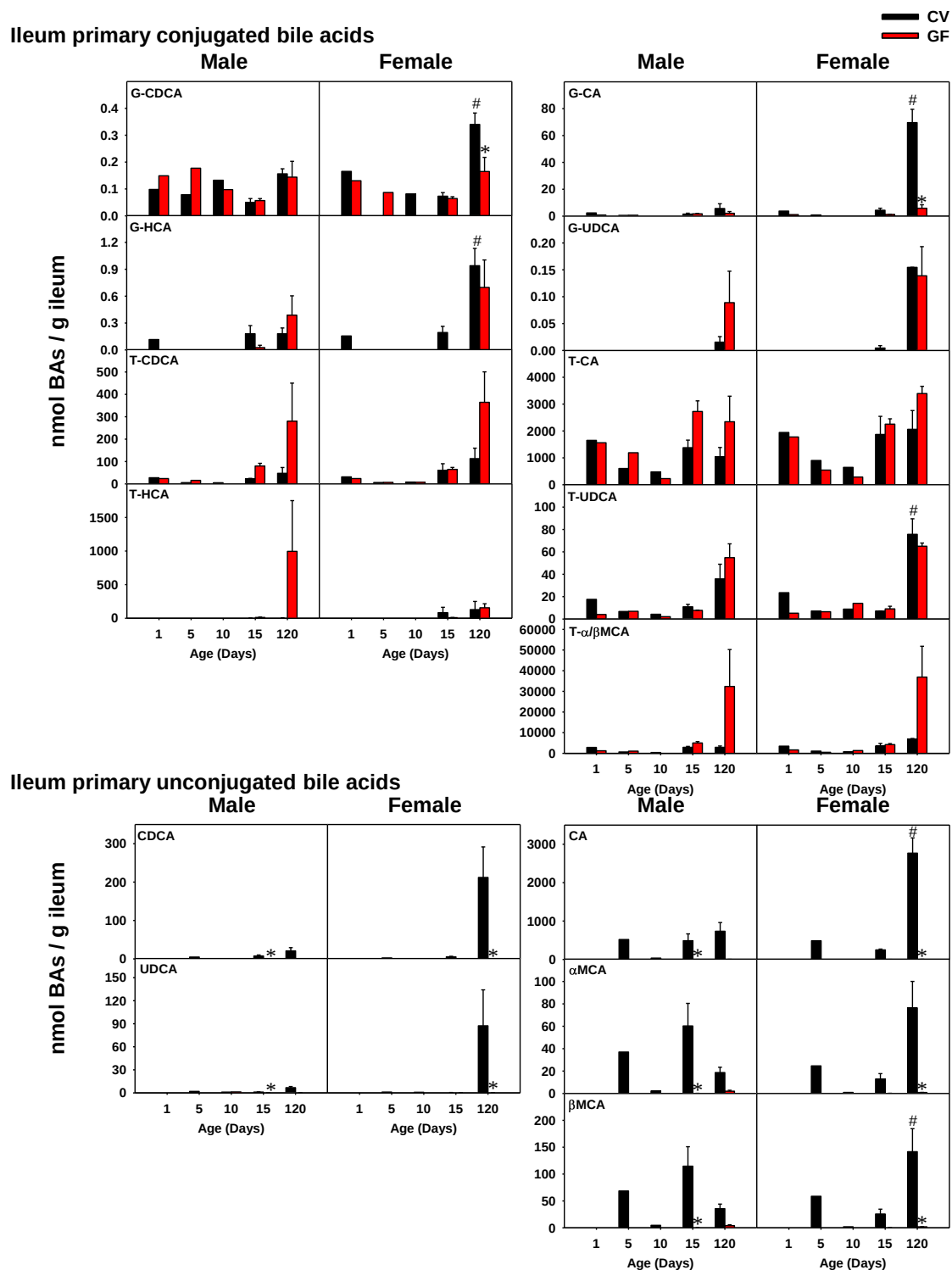
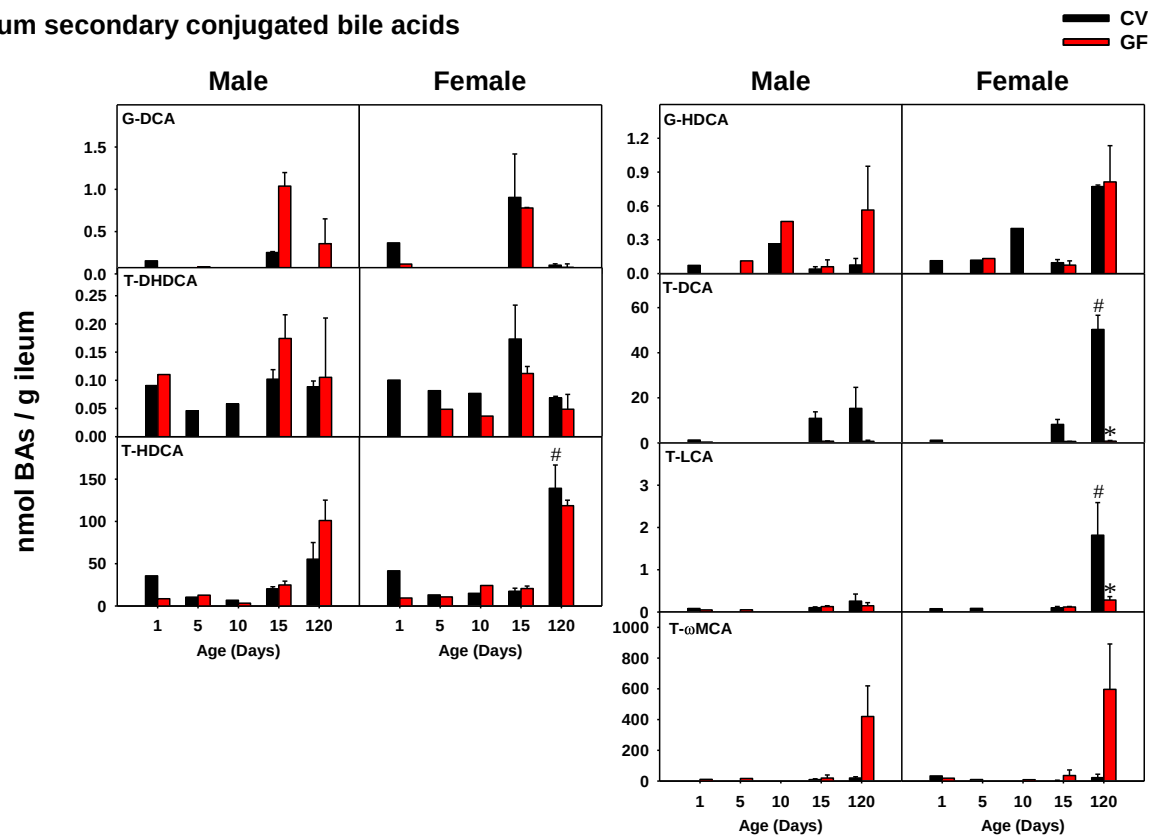


Figure 2.S10. LC-MS quantification of ileal primary conjugated and unconjugated BAs in CV and GF, male and female mice at days 1, 5, and 10 (n=1 pooled sample of 3 mice), as well as 15 and 120 (n=3 per group). A generalized linear model was used to identify BAs with different concentrations followed by Tukey post-hoc test. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex.

Supplemental Figure 11

Ileum secondary conjugated bile acids



Ileum secondary unconjugated bile acids

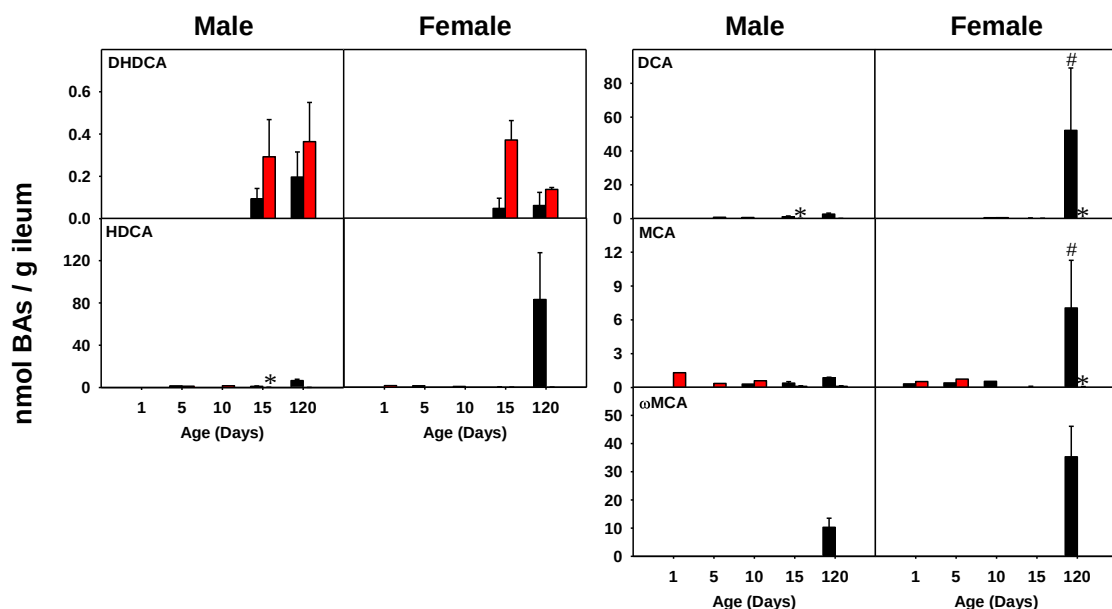


Figure 2.S11. LC-MS quantification of ileal secondary conjugated and unconjugated BAs in CV and GF, male and female mice at days 1, 5, and 10 ($n=1$ pooled sample of 3 mice), as well as 15 and 120 ($n=3$ per group). A generalized linear model was used to identify BAs with different concentrations followed by Tukey post-hoc test. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex.

Supplemental Figure 12

Ileum deoxy and keto bile acids

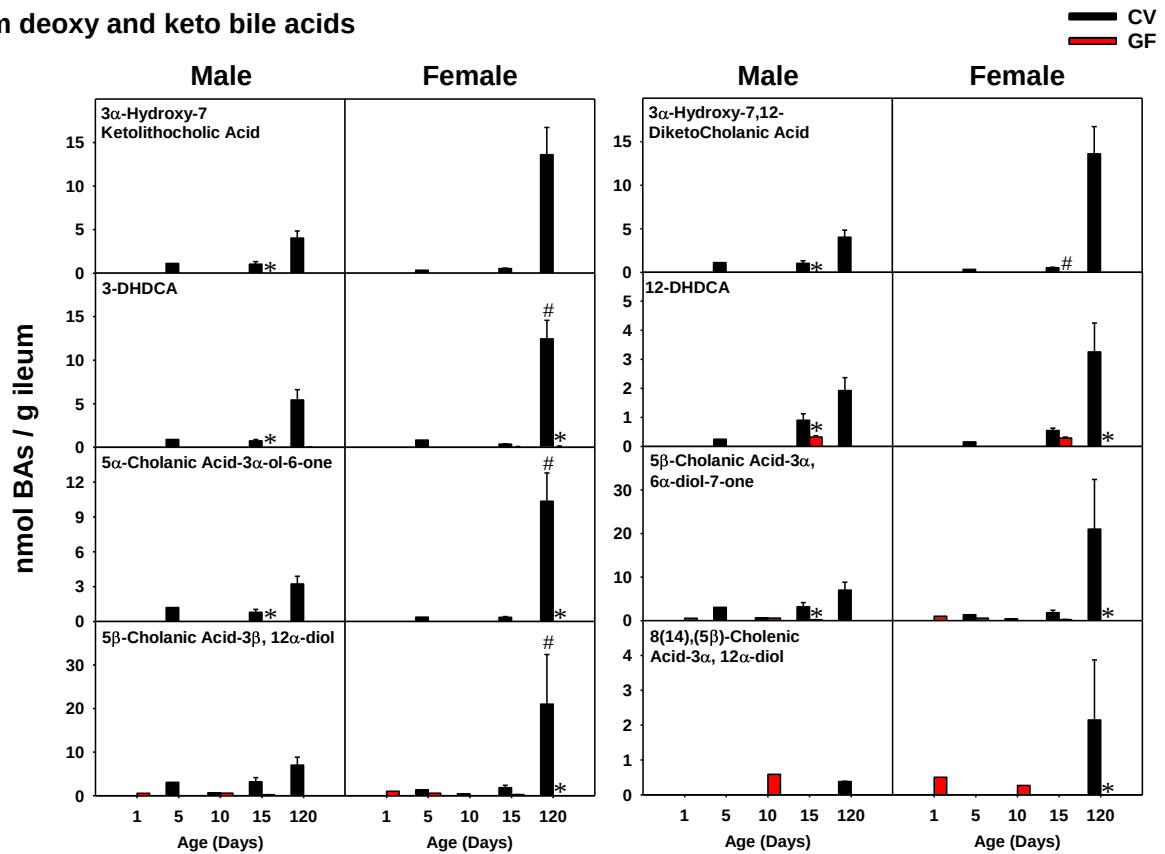
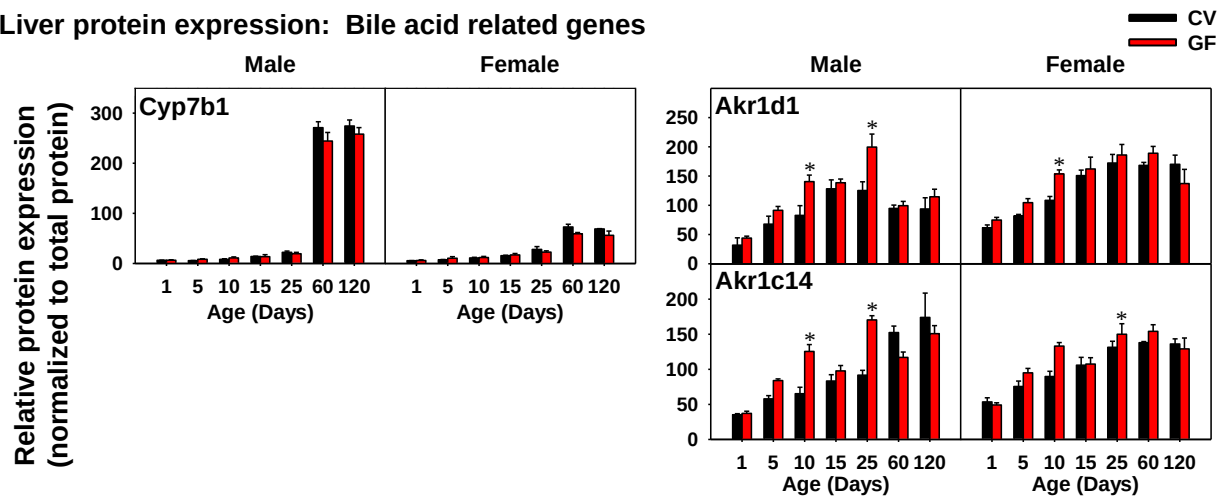


Figure 2.S12. LC-MS quantification of ileal deoxy and keto BAs in CV and GF, male and female mice at days 1, 5, and 10 (n=1 pooled sample of 3 mice), as well as 15 and 120 (n=3 per group). A generalized linear model was used to identify BAs with different concentrations followed by Tukey post-hoc test. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex.

Supplemental Figure 13

Liver protein expression: Bile acid related genes



Liver protein expression: Bile acid related transporters

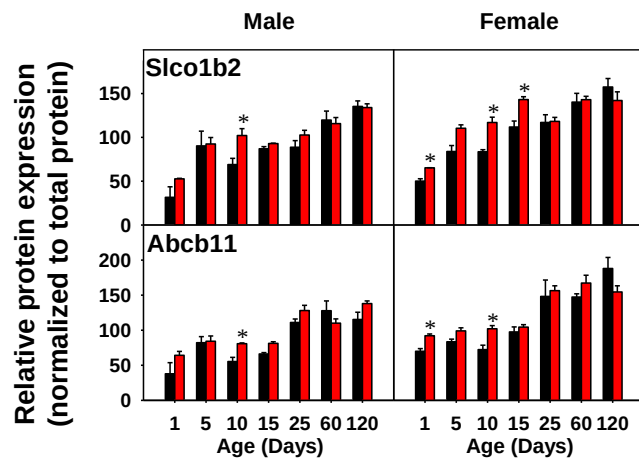
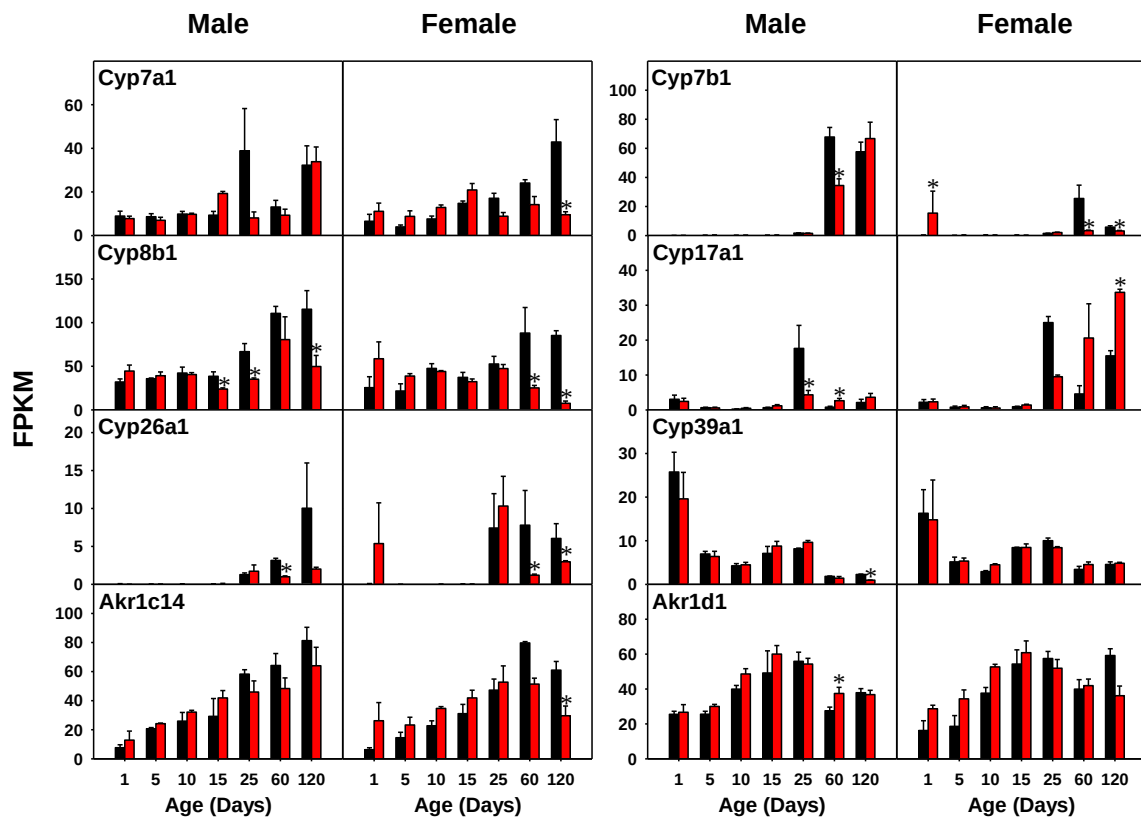


Figure 2.S13. Targeted proteomics in livers of CV and GF, male and female mice at days 1, 5, 10, 15, 25, 60, and 120 for primary BA synthesis and transporter genes products (n=3 per group). A generalized linear model was used to identify BAs with different concentrations followed by Tukey post-hoc test. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex.

Supplemental Figure 14

Liver RNA expression: Bile acid metabolism related genes

CV
GF



Liver RNA expression: Other bile acid related genes

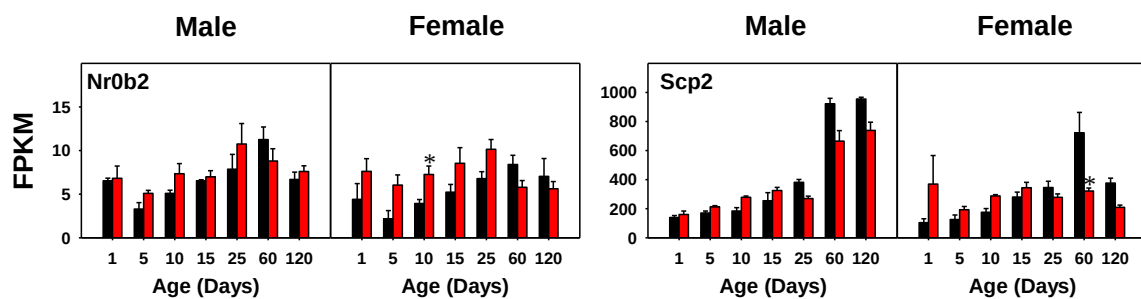


Figure 2.S14. RNA-seq quantification of mRNAs in livers of CV and GF, male and female mice at days 1, 5, 10, 15, 25, 60, and 120 for primary BA synthesis and transporter genes. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex as determined by Cuffdiff (FDR-BH < 0.05).

Supplemental Figure 15

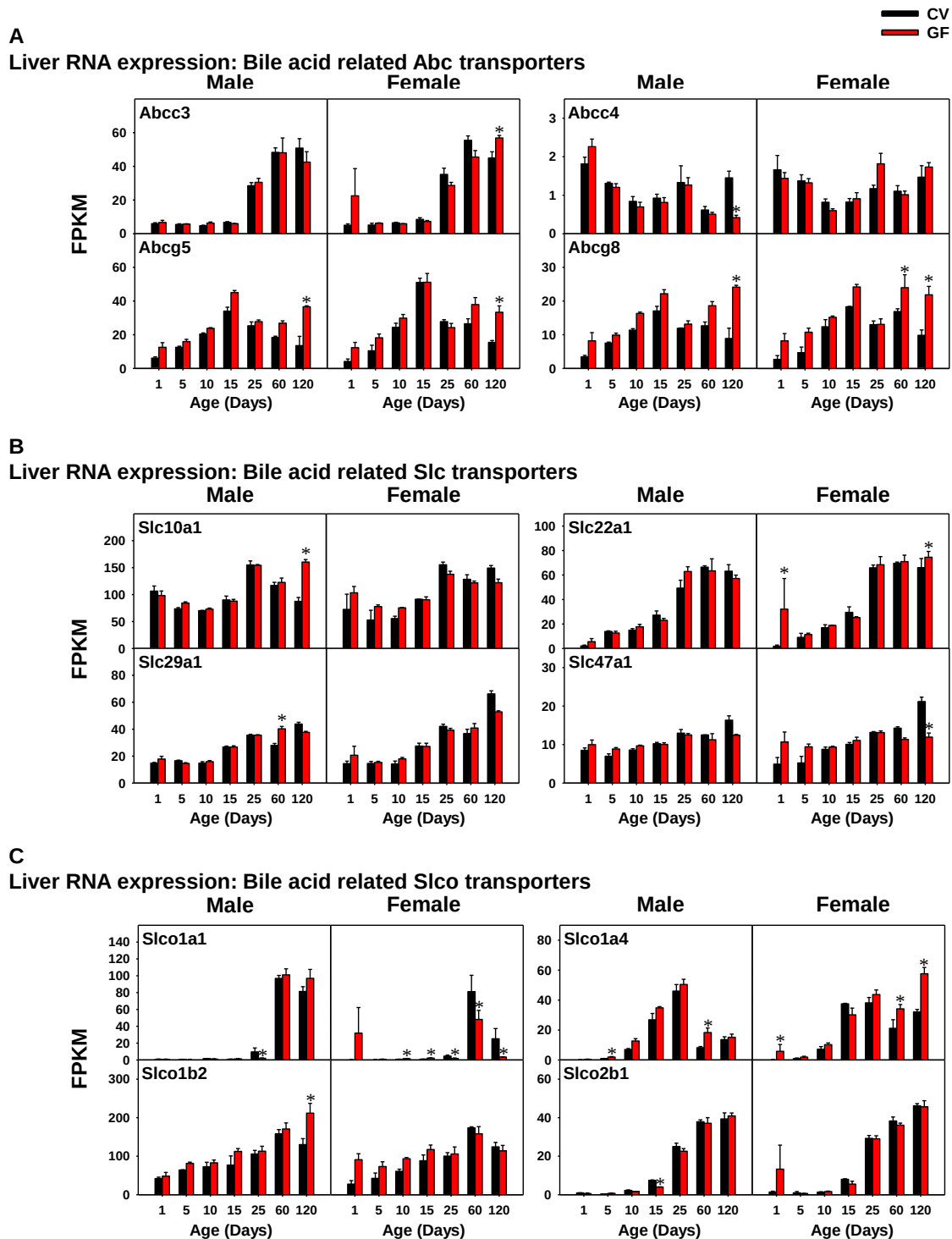


Figure 2.S15. RNA-seq quantification of mRNAs in livers of CV and GF, male and female mice at days 1, 5, 10, 15, 25, 60, and 120 for BA synthesis transporter genes: **A.** Abc transporters, **B.** Slc transporters, and **C.** Slco transporters. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex as determined by Cuffdiff (FDR-BH < 0.05).

Supplemental Figure 16

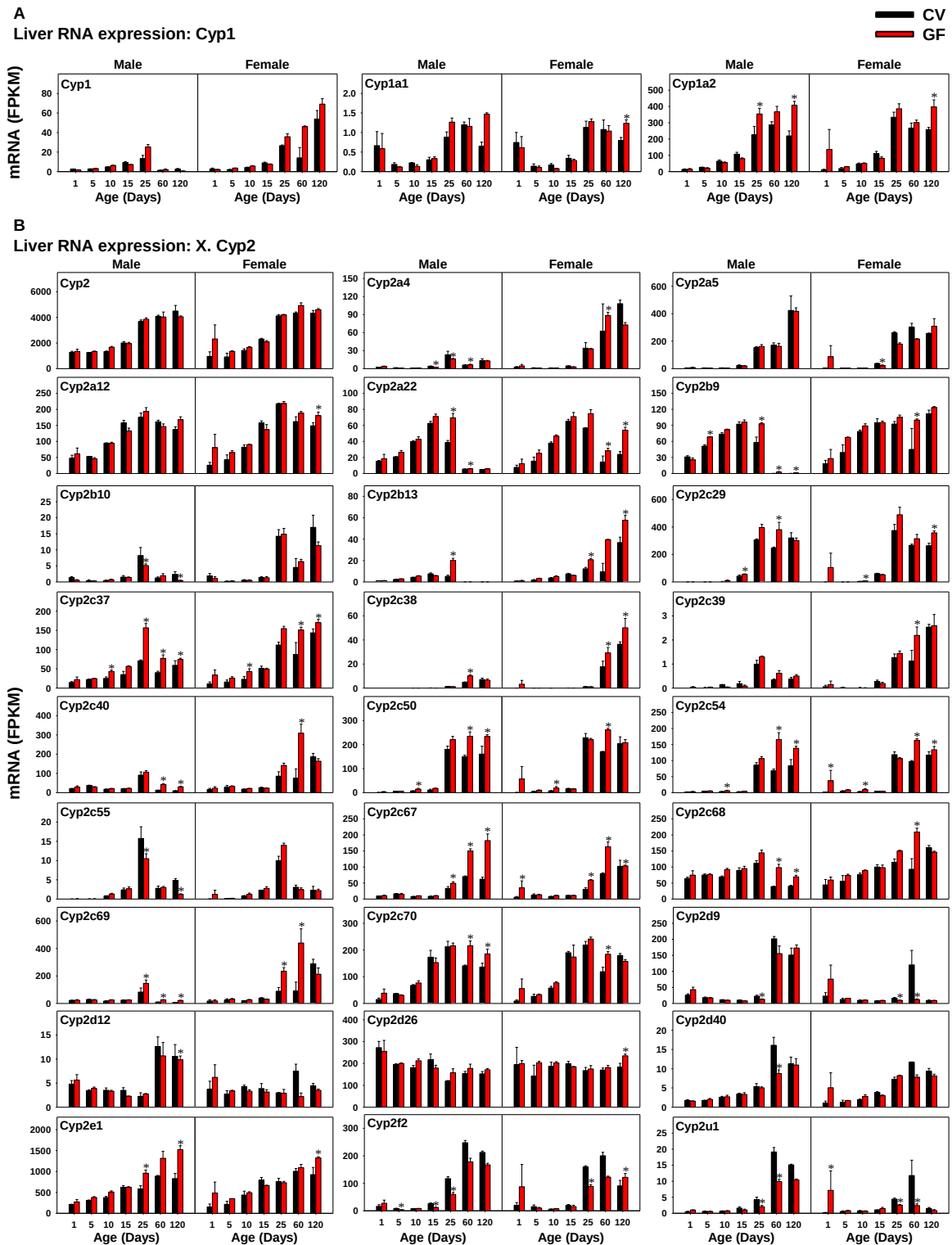


Figure 2.S16. RNA-seq quantification of mRNAs in livers of CV and GF, male and female mice at days 1, 5, 10, 15, 25, 60, and 120 for P450s: **A.** Cyp1 and **B.** Cyp2. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex as determined by Cuffdiff (FDR-BH < 0.05).

Supplemental Figure 17

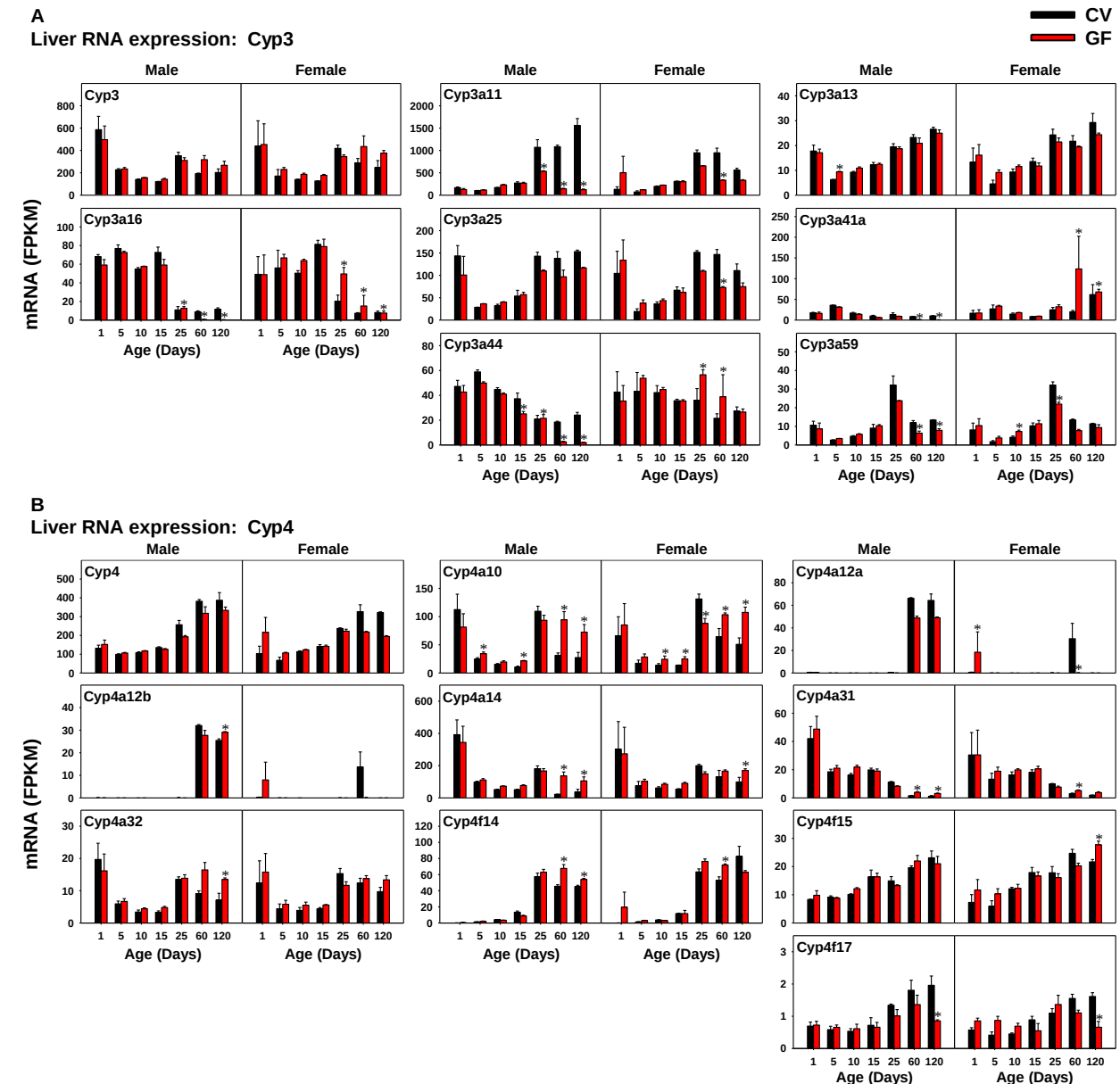


Figure 2.S17. RNA-seq quantification of mRNAs in livers of CV and GF, male and female mice at days 1, 5, 10, 15, 25, 60, and 120 for P450s: **A.** Cyp3 and **B.** Cyp4. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex as determined by Cuffdiff (FDR-BH < 0.05).

Supplemental Figure 18

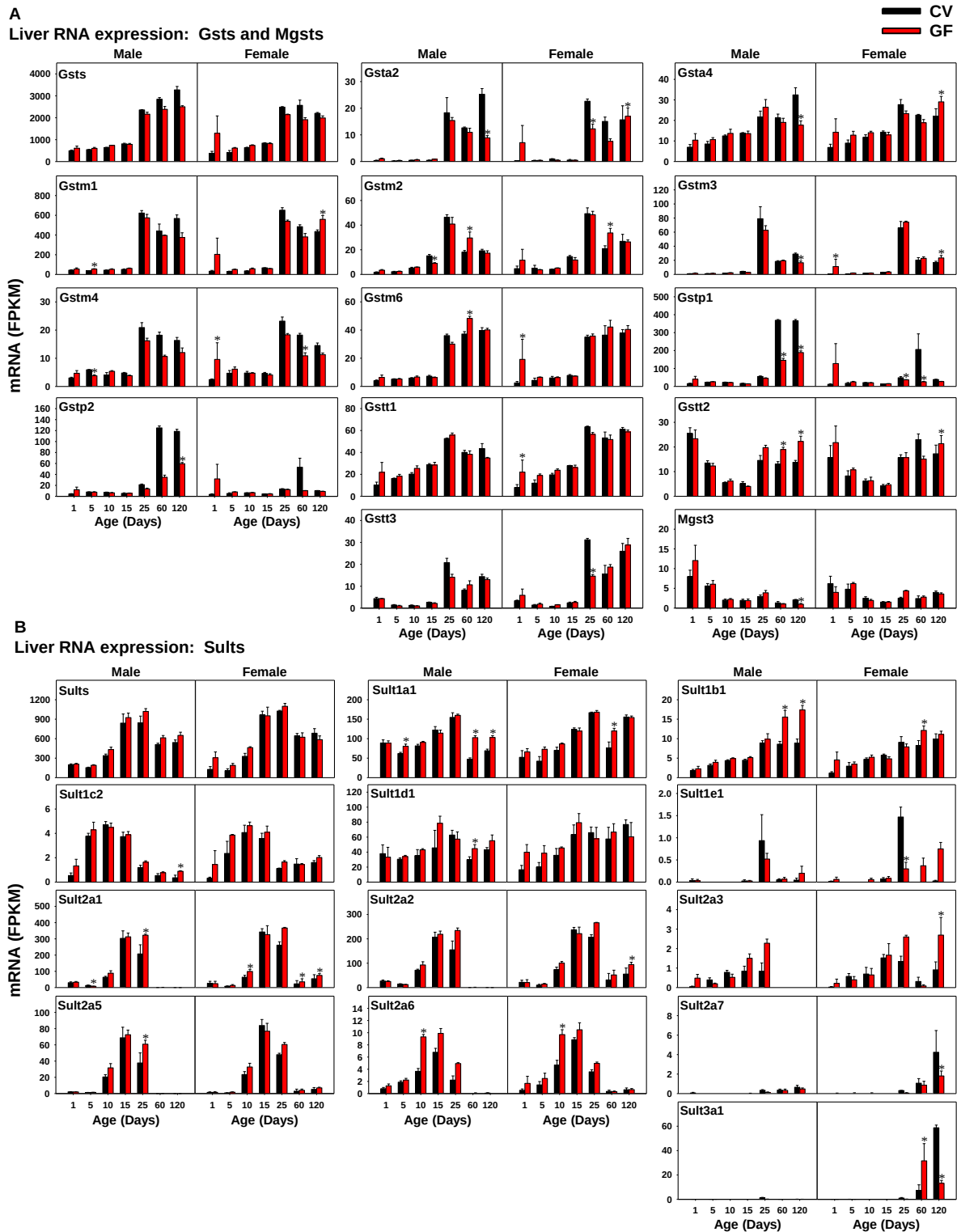


Figure 2.S18. RNA-seq quantification of mRNAs in livers of CV and GF, male and female mice at days 1, 5, 10, 15, 25, 60, and 120 for **A.** Gsts and Mgsts and **B.** Sults. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex as determined by Cuffdiff (FDR-BH < 0.05).

Supplemental Figure 19

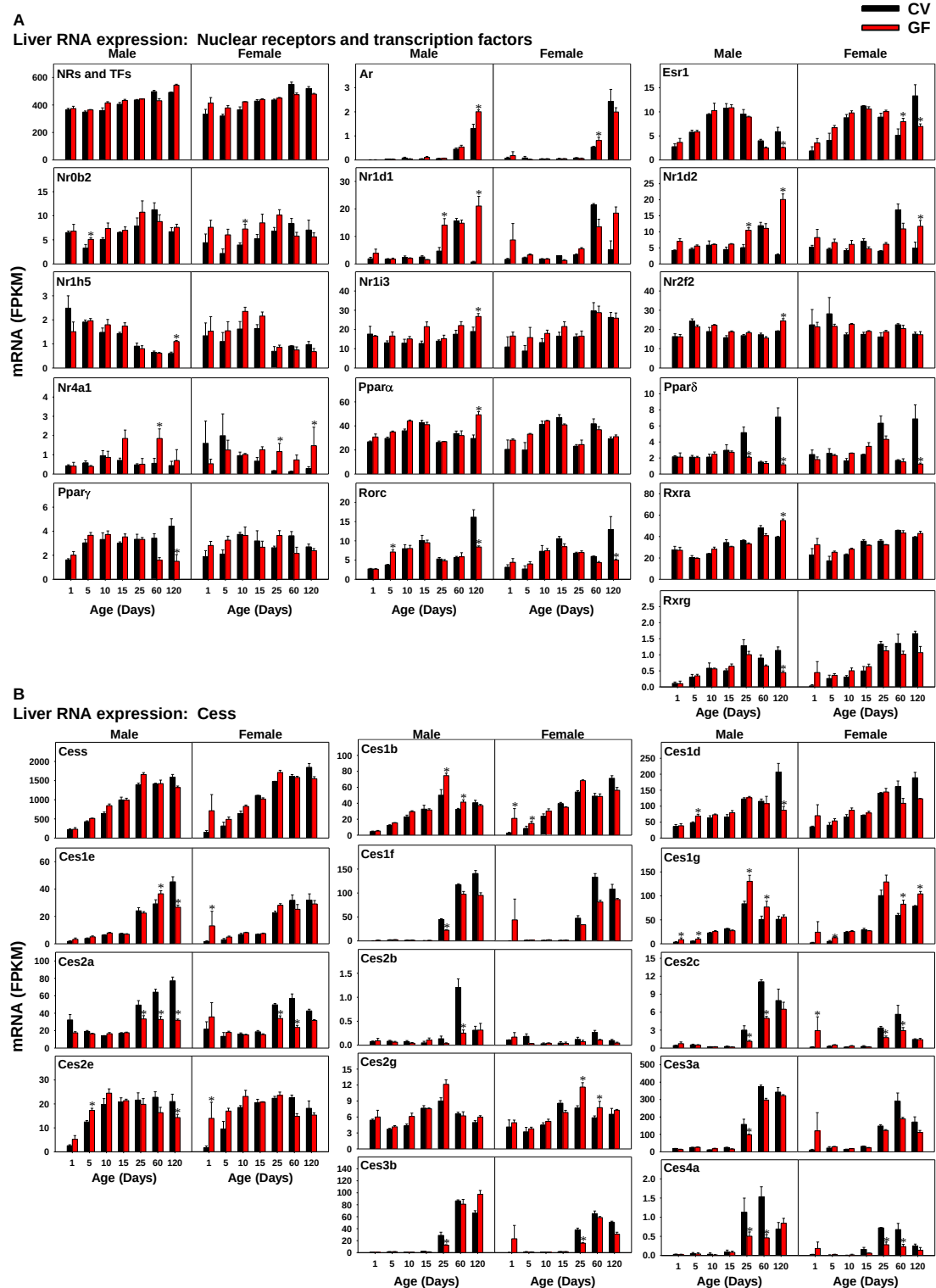


Figure 2.S19. RNA-seq quantification of mRNAs in livers of CV and GF, male and female mice at days 1, 5, 10, 15, 25, 60, and 120 for **A.** nuclear receptors and transcription factors and **B.** Cess. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex as determined by Cuffdiff (FDR-BH < 0.05).

Supplemental Figure 20

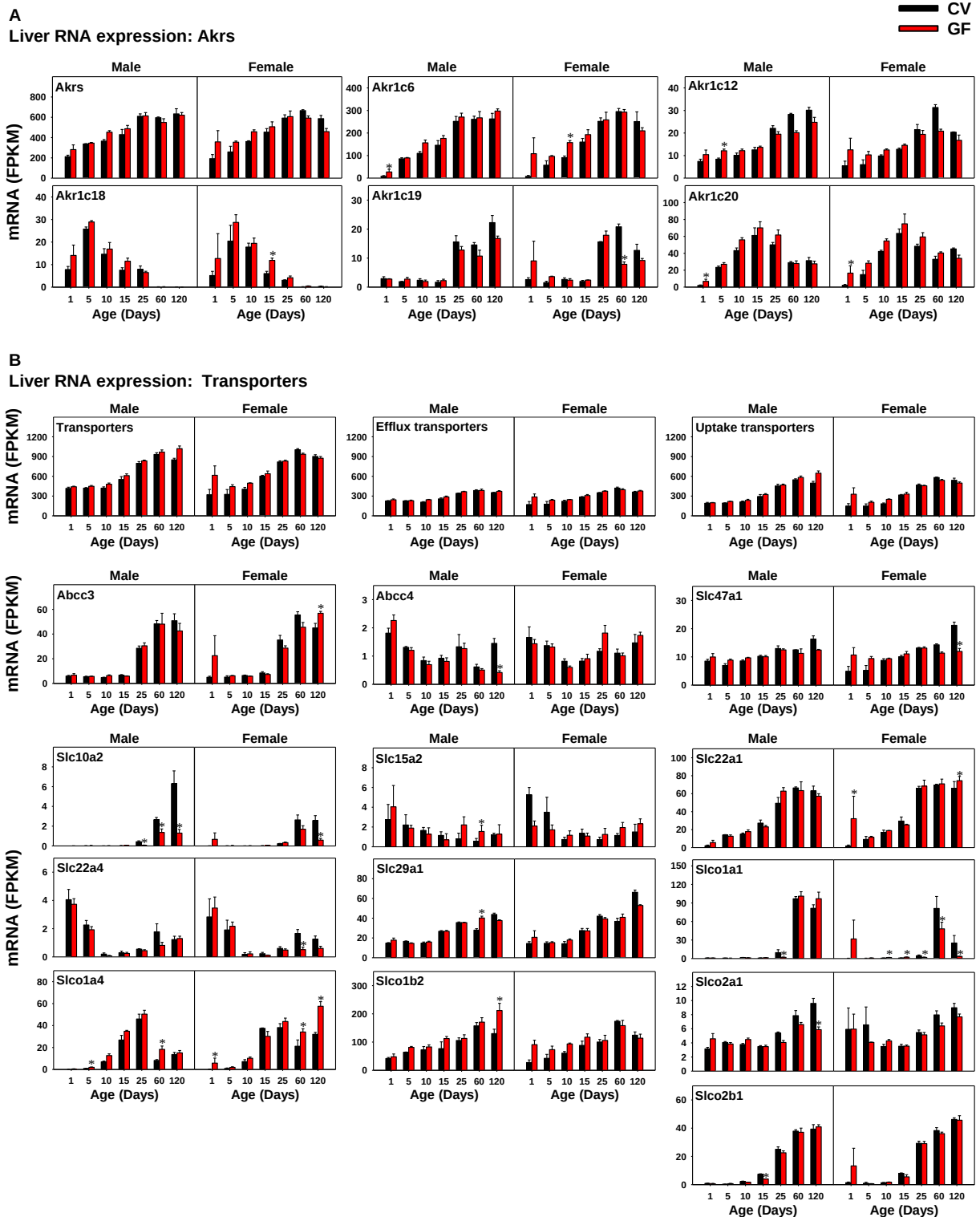


Figure 2.S20. RNA-seq quantification of mRNAs in livers of CV and GF, male and female mice at days 1, 5, 10, 15, 25, 60, and 120 for **A.** Akrs and **B.** transporters. Asterisks (*) represent statistically significant differences between CV (black) and GF (red) mice by each age and sex as determined by Cuffdiff (FDR-BH < 0.05).

2.9 Supplemental Tables

Supplemental Table 1

Table 2.S1. Enriched KEGG pathways from Cluster 1 from the k-means cluster analysis of genes expressed in the liver averaged from all groups (CV and GF, male and female, and days 1, 5, 10, 15, 25, 60, and 120).

GO.ID	Term	Annotated	Significant	Expected	result1	FDR
GO:0032787	monocarboxylic acid metabolic process	437	236	110.93	1.00E-30	3.42E-27
GO:0051186	cofactor metabolic process	404	213	102.56	1.00E-30	3.42E-27
GO:0006631	fatty acid metabolic process	294	166	74.63	1.40E-30	3.42E-27
GO:0006790	sulfur compound metabolic process	210	130	53.31	1.60E-29	2.93E-26
GO:0016054	organic acid catabolic process	177	110	44.93	2.40E-25	2.93E-22
GO:0046395	carboxylic acid catabolic process	177	110	44.93	2.40E-25	2.93E-22
GO:0044282	small molecule catabolic process	256	142	64.99	3.10E-25	3.25E-22
GO:0006805	xenobiotic metabolic process	84	66	21.32	1.60E-24	1.47E-21
GO:0006520	cellular amino acid metabolic process	214	122	54.32	3.20E-23	2.35E-20
GO:1901605	alpha-amino acid metabolic process	156	98	39.6	3.20E-23	2.35E-20
GO:0006732	coenzyme metabolic process	271	140	68.79	5.70E-21	3.80E-18
GO:0008202	steroid metabolic process	223	121	56.61	1.40E-20	8.56E-18
GO:0008610	lipid biosynthetic process	394	183	100.02	2.40E-20	1.35E-17
GO:0055086	nucleobase-containing small molecule metabolic process	483	213	122.61	4.00E-20	2.10E-17
GO:1901615	organic hydroxy compound metabolic process	312	153	79.2	6.00E-20	2.93E-17
GO:0072521	purine-containing compound metabolic process	366	171	92.91	2.10E-19	9.63E-17
GO:0044283	small molecule biosynthetic process	483	205	122.61	3.90E-17	1.68E-14
GO:0006066	alcohol metabolic process	230	116	58.39	1.50E-16	6.11E-14
GO:0006749	glutathione metabolic process	49	40	12.44	2.00E-16	7.68E-14
GO:0009117	nucleotide metabolic process	420	182	106.62	2.20E-16	7.68E-14
GO:0071466	cellular response to xenobiotic stimulus	111	69	28.18	2.20E-16	7.68E-14
GO:0006753	nucleoside phosphate metabolic process	426	183	108.14	5.20E-16	1.73E-13
GO:0009063	cellular amino acid catabolic process	74	52	18.78	5.50E-16	1.75E-13
GO:0033865	nucleoside bisphosphate metabolic process	83	56	21.07	7.40E-16	2.09E-13
GO:0033875	ribonucleoside bisphosphate metabolic process	83	56	21.07	7.40E-16	2.09E-13
GO:0034032	purine nucleoside bisphosphate metabolic process	83	56	21.07	7.40E-16	2.09E-13
GO:0016042	lipid catabolic process	214	108	54.32	1.60E-15	4.35E-13
GO:0042737	drug catabolic process	100	63	25.38	1.80E-15	4.71E-13
GO:0045333	cellular respiration	129	75	32.75	2.10E-15	5.31E-13
GO:0006694	steroid biosynthetic process	110	67	27.92	2.70E-15	6.60E-13
GO:0006575	cellular modified amino acid metabolic process	132	76	33.51	2.90E-15	6.86E-13
GO:0006163	purine nucleotide metabolic process	332	149	84.28	3.80E-15	8.71E-13
GO:1902652	secondary alcohol metabolic process	106	65	26.91	4.40E-15	9.71E-13
GO:0072329	monocarboxylic acid catabolic process	99	62	25.13	4.50E-15	9.71E-13
GO:0006637	acyl-CoA metabolic process	68	48	17.26	5.60E-15	1.14E-12
GO:0035383	thioester metabolic process	68	48	17.26	5.60E-15	1.14E-12
GO:0016125	sterol metabolic process	109	66	27.67	6.50E-15	1.29E-12

GO:0019693	ribose phosphate metabolic process	347	153	88.09	1.00E-14	1.93E-12
GO:0008203	cholesterol metabolic process	103	63	26.15	1.40E-14	2.57E-12
GO:0009410	response to xenobiotic stimulus	145	80	36.81	1.40E-14	2.57E-12
GO:1901606	alpha-amino acid catabolic process	65	46	16.5	1.80E-14	3.22E-12
GO:0046394	carboxylic acid biosynthetic process	271	126	68.79	2.20E-14	3.84E-12
GO:0042738	exogenous drug catabolic process	36	31	9.14	2.80E-14	4.78E-12
GO:0016053	organic acid biosynthetic process	272	126	69.05	3.10E-14	5.05E-12
GO:0015980	energy derivation by oxidation of organic compounds	194	98	49.25	3.10E-14	5.05E-12
GO:0009150	purine ribonucleotide metabolic process	322	143	81.74	3.90E-14	6.22E-12
GO:0044242	cellular lipid catabolic process	155	82	39.35	1.50E-13	2.34E-11
GO:0007005	mitochondrion organization	379	160	96.21	2.40E-13	3.59E-11
GO:0009259	ribonucleotide metabolic process	331	144	84.02	2.40E-13	3.59E-11
GO:0009062	fatty acid catabolic process	82	52	20.82	3.60E-13	5.28E-11
GO:0033559	unsaturated fatty acid metabolic process	71	47	18.02	4.70E-13	6.76E-11
GO:0019369	arachidonic acid metabolic process	37	30	9.39	1.80E-12	2.54E-10
GO:0009072	aromatic amino acid family metabolic process	25	23	6.35	3.20E-12	4.43E-10
GO:0006091	generation of precursor metabolites and energy	281	124	71.33	3.50E-12	4.75E-10
GO:0042445	hormone metabolic process	120	66	30.46	3.60E-12	4.80E-10
GO:0016126	sterol biosynthetic process	44	33	11.17	6.80E-12	8.91E-10
GO:0001676	long-chain fatty acid metabolic process	66	43	16.75	1.00E-11	1.26E-09
GO:0022900	electron transport chain	66	43	16.75	1.00E-11	1.26E-09
GO:0022904	respiratory electron transport chain	64	42	16.25	1.30E-11	1.62E-09
GO:0006695	cholesterol biosynthetic process	41	31	10.41	2.10E-11	2.52E-09
GO:1902653	secondary alcohol biosynthetic process	41	31	10.41	2.10E-11	2.52E-09
GO:0043648	dicarboxylic acid metabolic process	73	45	18.53	5.70E-11	6.74E-09
GO:0033108	mitochondrial respiratory chain complex assembly	71	44	18.02	7.20E-11	8.38E-09
GO:1901568	fatty acid derivative metabolic process	83	49	21.07	7.40E-11	8.48E-09
GO:0006635	fatty acid beta-oxidation	59	38	14.98	2.80E-10	3.16E-08
GO:1901617	organic hydroxy compound biosynthetic process	144	71	36.55	4.50E-10	5.00E-08
GO:0010257	NADH dehydrogenase complex assembly	40	29	10.15	5.20E-10	5.61E-08
GO:0032981	mitochondrial respiratory chain complex I assembly	40	29	10.15	5.20E-10	5.61E-08
GO:0051188	cofactor biosynthetic process	205	92	52.04	7.70E-10	8.18E-08
GO:0046165	alcohol biosynthetic process	100	54	25.38	8.20E-10	8.59E-08
GO:0072330	monocarboxylic acid biosynthetic process	197	89	50.01	9.60E-10	9.92E-08
GO:0009074	aromatic amino acid family catabolic process	18	17	4.57	9.90E-10	1.01E-07
GO:0016999	antibiotic metabolic process	96	52	24.37	1.50E-09	1.51E-07
GO:0006084	acetyl-CoA metabolic process	31	24	7.87	1.80E-09	1.78E-07
GO:0006690	icosanoid metabolic process	69	41	17.52	2.00E-09	1.96E-07
GO:0006633	fatty acid biosynthetic process	102	54	25.89	2.10E-09	2.03E-07
GO:0042180	cellular ketone metabolic process	151	72	38.33	2.20E-09	2.10E-07
GO:0019373	epoxygenase P450 pathway	22	19	5.58	3.10E-09	2.91E-07
GO:0009108	coenzyme biosynthetic process	161	75	40.87	3.80E-09	3.53E-07
GO:0055088	lipid homeostasis	125	62	31.73	4.20E-09	3.85E-07

GO:0042773	ATP synthesis coupled electron transport	47	31	11.93	5.30E-09	4.80E-07
GO:0009060	aerobic respiration	54	34	13.71	5.80E-09	5.19E-07
GO:0019395	fatty acid oxidation	84	46	21.32	8.10E-09	7.16E-07
GO:0034440	lipid oxidation	85	46	21.58	1.30E-08	1.14E-06
GO:0042775	mitochondrial ATP synthesis coupled electron transport	46	30	11.68	1.40E-08	1.21E-06
GO:0007031	peroxisome organization	29	22	7.36	1.70E-08	1.45E-06
GO:0006119	oxidative phosphorylation	76	42	19.29	2.50E-08	2.11E-06
GO:0006120	mitochondrial electron transport, NADH to ubiquinone	18	16	4.57	2.60E-08	2.12E-06
GO:0042178	xenobiotic catabolic process	18	16	4.57	2.60E-08	2.12E-06
GO:0042537	benzene-containing compound metabolic process	18	16	4.57	2.60E-08	2.12E-06
GO:0009069	serine family amino acid metabolic process	36	25	9.14	3.40E-08	2.74E-06
GO:0034976	response to endoplasmic reticulum stress	192	83	48.74	4.20E-08	3.35E-06
GO:0019216	regulation of lipid metabolic process	256	104	64.99	4.60E-08	3.63E-06
GO:0034754	cellular hormone metabolic process	66	37	16.75	1.00E-07	7.80E-06
GO:0046890	regulation of lipid biosynthetic process	132	61	33.51	1.50E-07	1.16E-05
GO:0090181	regulation of cholesterol metabolic process	25	19	6.35	1.60E-07	1.22E-05
GO:0062012	regulation of small molecule metabolic process	268	106	68.03	1.70E-07	1.29E-05
GO:0009066	aspartate family amino acid metabolic process	36	24	9.14	2.20E-07	1.65E-05
GO:1903008	organelle disassembly	78	41	19.8	2.40E-07	1.78E-05
GO:0044272	sulfur compound biosynthetic process	63	35	15.99	3.10E-07	2.27E-05
GO:0006639	acylglycerol metabolic process	98	48	24.88	3.80E-07	2.76E-05
GO:0010876	lipid localization	253	100	64.22	4.00E-07	2.88E-05
GO:0019883	antigen processing and presentation of endogenous antigen	20	16	5.08	4.70E-07	3.35E-05
GO:0006720	isoprenoid metabolic process	59	33	14.98	5.40E-07	3.81E-05
GO:0006638	neutral lipid metabolic process	99	48	25.13	5.60E-07	3.91E-05
GO:0000096	sulfur amino acid metabolic process	31	21	7.87	8.40E-07	5.70E-05
GO:0072350	tricarboxylic acid metabolic process	31	21	7.87	8.40E-07	5.70E-05
GO:0030258	lipid modification	173	73	43.92	8.40E-07	5.70E-05
GO:0019748	secondary metabolic process	45	27	11.42	8.80E-07	5.92E-05
GO:0006641	triglyceride metabolic process	82	41	20.82	1.30E-06	8.67E-05
GO:0015721	bile acid and bile salt transport	12	11	3.05	2.60E-06	0.000171787
GO:0090407	organophosphate biosynthetic process	367	132	93.16	3.00E-06	0.000196446
GO:0006536	glutamate metabolic process	24	17	6.09	3.70E-06	0.00024014
GO:0008206	bile acid metabolic process	22	16	5.58	4.20E-06	0.00026954
GO:0006558	L-phenylalanine metabolic process	9	9	2.28	4.30E-06	0.00026954
GO:1902221	erythrose 4-phosphate/phosphoenolpyruvate family amino acid metabolic process	9	9	2.28	4.30E-06	0.00026954
GO:0046889	positive regulation of lipid biosynthetic process	74	37	18.78	4.30E-06	0.00026954
GO:0002483	antigen processing and presentation of endogenous peptide antigen	18	14	4.57	4.70E-06	0.000289662
GO:0019885	antigen processing and presentation of endogenous peptide antigen via MHC class I	18	14	4.57	4.70E-06	0.000289662
GO:0008652	cellular amino acid biosynthetic process	53	29	13.45	4.80E-06	0.00029336
GO:0015711	organic anion transport	240	92	60.92	5.30E-06	0.000318608
GO:0055092	sterol homeostasis	80	39	20.31	5.30E-06	0.000318608
GO:0009144	purine nucleoside triphosphate metabolic process	215	84	54.58	5.70E-06	0.000339868

GO:0042133	neurotransmitter metabolic process	86	41	21.83	6.30E-06	0.000372615
GO:0006869	lipid transport	222	86	56.35	6.60E-06	0.000384162
GO:0034308	primary alcohol metabolic process	41	24	10.41	6.60E-06	0.000384162
GO:0006101	citrate metabolic process	27	18	6.85	7.40E-06	0.000423997
GO:0046034	ATP metabolic process	188	75	47.72	7.40E-06	0.000423997
GO:0071941	nitrogen cycle metabolic process	11	10	2.79	9.30E-06	0.00052873
GO:1901293	nucleoside phosphate biosynthetic process	208	81	52.8	9.60E-06	0.000541588
GO:0009165	nucleotide biosynthetic process	205	80	52.04	9.90E-06	0.000554249
GO:0042632	cholesterol homeostasis	79	38	20.05	1.00E-05	0.000555606
GO:0009123	nucleoside monophosphate metabolic process	218	84	55.34	1.10E-05	0.000606571
GO:0006081	cellular aldehyde metabolic process	55	29	13.96	1.30E-05	0.000706237
GO:0019218	regulation of steroid metabolic process	74	36	18.78	1.30E-05	0.000706237
GO:0009064	glutamine family amino acid metabolic process	50	27	12.69	1.40E-05	0.000754971
GO:0006699	bile acid biosynthetic process	17	13	4.32	1.50E-05	0.000791439
GO:0009205	purine ribonucleoside triphosphate metabolic process	210	81	53.31	1.50E-05	0.000791439
GO:0010565	regulation of cellular ketone metabolic process	103	46	26.15	1.50E-05	0.000791439
GO:0062013	positive regulation of small molecule metabolic process	109	48	27.67	1.60E-05	0.000838171
GO:0009126	purine nucleoside monophosphate metabolic process	208	80	52.8	1.90E-05	0.000954425
GO:0009167	purine ribonucleoside monophosphate metabolic process	208	80	52.8	1.90E-05	0.000954425
GO:0033866	nucleoside bisphosphate biosynthetic process	33	20	8.38	1.90E-05	0.000954425
GO:0034030	ribonucleoside bisphosphate biosynthetic process	33	20	8.38	1.90E-05	0.000954425
GO:0034033	purine nucleoside bisphosphate biosynthetic process	33	20	8.38	1.90E-05	0.000954425
GO:0010817	regulation of hormone levels	299	108	75.9	1.90E-05	0.000954425
GO:0006099	tricarboxylic acid cycle	26	17	6.6	2.00E-05	0.000991081
GO:0032527	protein exit from endoplasmic reticulum	38	22	9.65	2.00E-05	0.000991081
GO:0000045	autophagosome assembly	81	38	20.56	2.10E-05	0.00102676
GO:0036503	ERAD pathway	81	38	20.56	2.10E-05	0.00102676
GO:1901607	alpha-amino acid biosynthetic process	51	27	12.95	2.30E-05	0.001117099
GO:0005975	carbohydrate metabolic process	374	130	94.94	2.50E-05	0.00120625
GO:0030433	ubiquitin-dependent ERAD pathway	62	31	15.74	2.60E-05	0.001246301
GO:0006820	anion transport	301	108	76.41	2.70E-05	0.001285831
GO:0015850	organic hydroxy compound transport	120	51	30.46	2.90E-05	0.001372168
GO:0009199	ribonucleoside triphosphate metabolic process	214	81	54.32	3.20E-05	0.00150441
GO:0006063	uronic acid metabolic process	10	9	2.54	3.30E-05	0.001522151
GO:0019585	glucuronate metabolic process	10	9	2.54	3.30E-05	0.001522151
GO:0019627	urea metabolic process	10	9	2.54	3.30E-05	0.001522151
GO:0009161	ribonucleoside monophosphate metabolic process	211	80	53.56	3.40E-05	0.001548795
GO:0072522	purine-containing compound biosynthetic process	173	68	43.92	3.40E-05	0.001548795
GO:0009141	nucleoside triphosphate metabolic process	228	85	57.88	4.00E-05	0.001810864
GO:0042558	pteridine-containing compound metabolic process	27	17	6.85	4.10E-05	0.001844748
GO:0006625	protein targeting to peroxisome	16	12	4.06	4.40E-05	0.001909444
GO:0043574	peroxisomal transport	16	12	4.06	4.40E-05	0.001909444
GO:0045540	regulation of cholesterol biosynthetic process	16	12	4.06	4.40E-05	0.001909444

GO:0072662	protein localization to peroxisome	16	12	4.06	4.40E-05	0.001909444
GO:0072663	establishment of protein localization to peroxisome	16	12	4.06	4.40E-05	0.001909444
GO:0106118	regulation of sterol biosynthetic process	16	12	4.06	4.40E-05	0.001909444
GO:0006164	purine nucleotide biosynthetic process	168	66	42.65	4.50E-05	0.001927794
GO:0015849	organic acid transport	168	66	42.65	4.50E-05	0.001927794
GO:0006525	arginine metabolic process	14	11	3.55	4.60E-05	0.001927794
GO:0042430	indole-containing compound metabolic process	14	11	3.55	4.60E-05	0.001927794
GO:0000422	autophagy of mitochondrion	58	29	14.72	4.60E-05	0.001927794
GO:0061726	mitochondrion disassembly	58	29	14.72	4.60E-05	0.001927794
GO:0002455	humoral immune response mediated by circulating immunoglobulin	37	21	9.39	4.70E-05	0.001958511
GO:0006767	water-soluble vitamin metabolic process	25	16	6.35	5.20E-05	0.002154621
GO:0015718	monocarboxylic acid transport	84	38	21.32	5.90E-05	0.002430933
GO:0035384	thioester biosynthetic process	23	15	5.84	6.50E-05	0.002648389
GO:0071616	acyl-CoA biosynthetic process	23	15	5.84	6.50E-05	0.002648389
GO:0006559	L-phenylalanine catabolic process	7	7	1.78	6.80E-05	0.002695741
GO:0009812	flavonoid metabolic process	7	7	1.78	6.80E-05	0.002695741
GO:0043651	linoleic acid metabolic process	7	7	1.78	6.80E-05	0.002695741
GO:0046113	nucleobase catabolic process	7	7	1.78	6.80E-05	0.002695741
GO:1902222	erythrose 4-phosphate/phosphoenolpyruvate family amino acid catabolic process	7	7	1.78	6.80E-05	0.002695741
GO:0046942	carboxylic acid transport	167	65	42.39	7.20E-05	0.002838968
GO:0006721	terpenoid metabolic process	43	23	10.92	7.40E-05	0.002902225
GO:1905037	autophagosome organization	85	38	21.58	8.00E-05	0.003120851
GO:0006739	NADP metabolic process	33	19	8.38	8.30E-05	0.003220751
GO:0006733	oxidoreduction coenzyme metabolic process	140	56	35.54	9.40E-05	0.0036284
GO:0015918	sterol transport	60	29	15.23	1.00E-04	0.003819792
GO:0030301	cholesterol transport	60	29	15.23	1.00E-04	0.003819792
GO:0000050	urea cycle	9	8	2.28	0.00012	0.004490204
GO:0006568	tryptophan metabolic process	9	8	2.28	0.00012	0.004490204
GO:0006586	indolalkylamine metabolic process	9	8	2.28	0.00012	0.004490204
GO:0052695	cellular glucuronidation	9	8	2.28	0.00012	0.004490204
GO:0030970	retrograde protein transport, ER to cytosol	24	15	6.09	0.00013	0.0047671
GO:1903513	endoplasmic reticulum to cytosol transport	24	15	6.09	0.00013	0.0047671
GO:0006534	cysteine metabolic process	15	11	3.81	0.00013	0.0047671
GO:0016236	macroautophagy	170	65	43.15	0.00013	0.0047671
GO:0001505	regulation of neurotransmitter levels	183	69	46.45	0.00014	0.005033137
GO:0002486	antigen processing and presentation of endogenous peptide antigen via MHC class I via ER pathway, TA	11	9	2.79	0.00014	0.005033137
GO:0042135	neurotransmitter catabolic process	11	9	2.79	0.00014	0.005033137
GO:0002428	antigen processing and presentation of peptide antigen via MHC class Ib	13	10	3.3	0.00014	0.005033137
GO:0006958	complement activation, classical pathway	29	17	7.36	0.00015	0.005314493
GO:0046486	glycerolipid metabolic process	252	90	63.97	0.00015	0.005314493
GO:0097164	ammonium ion metabolic process	108	45	27.42	0.00015	0.005314493
GO:0006730	one-carbon metabolic process	22	14	5.58	0.00017	0.005965455
GO:0009308	amine metabolic process	53	26	13.45	0.00017	0.005965455

GO:0045834	positive regulation of lipid metabolic process	122	49	30.97	0.00022	0.007683238
GO:0044106	cellular amine metabolic process	51	25	12.95	0.00023	0.007956698
GO:0046390	ribose phosphate biosynthetic process	176	66	44.68	0.00023	0.007956698
GO:0035966	response to topologically incorrect protein	110	45	27.92	0.00024	0.008263662
GO:0051289	protein homotetramerization	77	34	19.55	0.00025	0.008567757
GO:0006569	tryptophan catabolic process	6	6	1.52	0.00027	0.00896009
GO:0042436	indole-containing compound catabolic process	6	6	1.52	0.00027	0.00896009
GO:0046218	indolalkylamine catabolic process	6	6	1.52	0.00027	0.00896009
GO:0052696	flavonoid glucuronidation	6	6	1.52	0.00027	0.00896009
GO:0052697	xenobiotic glucuronidation	6	6	1.52	0.00027	0.00896009
GO:0070189	kynurenine metabolic process	6	6	1.52	0.00027	0.00896009
GO:0071569	protein ufmylation	6	6	1.52	0.00027	0.00896009
GO:0005996	monosaccharide metabolic process	200	73	50.77	0.00029	0.009494911
GO:0048002	antigen processing and presentation of peptide antigen	38	20	9.65	0.00029	0.009494911
GO:0098754	detoxification	38	20	9.65	0.00029	0.009494911
GO:1905897	regulation of response to endoplasmic reticulum stress	69	31	17.52	0.00032	0.010430578
GO:0002475	antigen processing and presentation via MHC class Ib	16	11	4.06	0.00033	0.010522696
GO:0006555	methionine metabolic process	16	11	4.06	0.00033	0.010522696
GO:0008299	isoprenoid biosynthetic process	23	14	5.84	0.00033	0.010522696
GO:0006914	autophagy	308	105	78.19	0.00033	0.010522696
GO:0061919	process utilizing autophagic mechanism	308	105	78.19	0.00033	0.010522696
GO:0009152	purine ribonucleotide biosynthetic process	162	61	41.12	0.00034	0.010794632
GO:0061912	selective autophagy	44	22	11.17	0.00038	0.012012586
GO:0015919	peroxisomal membrane transport	14	10	3.55	0.00039	0.012223333
GO:0042559	pteridine-containing compound biosynthetic process	14	10	3.55	0.00039	0.012223333
GO:0045833	negative regulation of lipid metabolic process	64	29	16.25	0.00041	0.012795489
GO:0006570	tyrosine metabolic process	8	7	2.03	0.00042	0.012888201
GO:0006995	cellular response to nitrogen starvation	8	7	2.03	0.00042	0.012888201
GO:0043562	cellular response to nitrogen levels	8	7	2.03	0.00042	0.012888201
GO:0046146	tetrahydrobiopterin metabolic process	8	7	2.03	0.00042	0.012888201
GO:0000266	mitochondrial fission	31	17	7.87	0.00044	0.013225246
GO:0002476	antigen processing and presentation of endogenous peptide antigen via MHC class Ib	12	9	3.05	0.00044	0.013225246
GO:0002484	antigen processing and presentation of endogenous peptide antigen via MHC class I via ER pathway	12	9	3.05	0.00044	0.013225246
GO:0042402	cellular biogenic amine catabolic process	12	9	3.05	0.00044	0.013225246
GO:0046459	short-chain fatty acid metabolic process	12	9	3.05	0.00044	0.013225246
GO:0006986	response to unfolded protein	88	37	22.34	0.00045	0.013415854
GO:0050810	regulation of steroid biosynthetic process	50	24	12.69	0.00045	0.013415854
GO:0002474	antigen processing and presentation of peptide antigen via MHC class I	26	15	6.6	0.00046	0.013658462
GO:0018958	phenol-containing compound metabolic process	42	21	10.66	0.00051	0.015082016
GO:0032371	regulation of sterol transport	34	18	8.63	0.00053	0.01554808
GO:0032374	regulation of cholesterol transport	34	18	8.63	0.00053	0.01554808
GO:0034311	diol metabolic process	19	12	4.82	0.00056	0.016297778
GO:0042493	response to drug	493	157	125.15	0.00056	0.016297778

GO:0006766	vitamin metabolic process	48	23	12.18	0.00061	0.017682767
GO:1902930	regulation of alcohol biosynthetic process	32	17	8.12	0.00071	0.020420157
GO:0006085	acetyl-CoA biosynthetic process	17	11	4.32	0.00071	0.020420157
GO:0019217	regulation of fatty acid metabolic process	75	32	19.04	0.00078	0.022345781
GO:0008207	C21-steroid hormone metabolic process	27	15	6.85	0.00079	0.022544202
GO:0009260	ribonucleotide biosynthetic process	170	62	43.15	0.00081	0.023025349
GO:0006956	complement activation	46	22	11.68	0.00082	0.023219614
GO:0007033	vacuole organization	141	53	35.79	0.00085	0.023976538
GO:0019362	pyridine nucleotide metabolic process	125	48	31.73	0.00086	0.02416567
GO:0042573	retinoic acid metabolic process	15	10	3.81	9.00E-04	0.02519313
GO:0016101	diterpenoid metabolic process	38	19	9.65	0.00093	0.025933916
GO:0072524	pyridine-containing compound metabolic process	129	49	32.75	0.00101	0.028058106
GO:0006067	ethanol metabolic process	5	5	1.27	0.00105	0.028207692
GO:0006145	purine nucleobase catabolic process	5	5	1.27	0.00105	0.028207692
GO:0006526	arginine biosynthetic process	5	5	1.27	0.00105	0.028207692
GO:0006572	tyrosine catabolic process	5	5	1.27	0.00105	0.028207692
GO:0009092	homoserine metabolic process	5	5	1.27	0.00105	0.028207692
GO:0015722	canalicular bile acid transport	5	5	1.27	0.00105	0.028207692
GO:0019346	transsulfuration	5	5	1.27	0.00105	0.028207692
GO:1990564	protein polyufmylation	5	5	1.27	0.00105	0.028207692
GO:1990592	protein K69-linked ufmylation	5	5	1.27	0.00105	0.028207692
GO:0009310	amine catabolic process	13	9	3.3	0.00111	0.02971073
GO:0072523	purine-containing compound catabolic process	33	17	8.38	0.00112	0.029869382
GO:0051055	negative regulation of lipid biosynthetic process	36	18	9.14	0.00127	0.033747029
GO:0016558	protein import into peroxisome matrix	11	8	2.79	0.00132	0.034823309
GO:0034982	mitochondrial protein processing	11	8	2.79	0.00132	0.034823309
GO:0046496	nicotinamide nucleotide metabolic process	124	47	31.48	0.00133	0.034961362
GO:0006144	purine nucleobase metabolic process	18	11	4.57	0.00141	0.03667
GO:0009404	toxin metabolic process	18	11	4.57	0.00141	0.03667
GO:0033617	mitochondrial respiratory chain complex IV assembly	18	11	4.57	0.00141	0.03667
GO:0000038	very long-chain fatty acid metabolic process	23	13	5.84	0.00143	0.037058728
GO:0006627	protein processing involved in protein targeting to mitochondrion	7	6	1.78	0.00146	0.03730885
GO:0006729	tetrahydrobiopterin biosynthetic process	7	6	1.78	0.00146	0.03730885
GO:0052803	imidazole-containing compound metabolic process	7	6	1.78	0.00146	0.03730885
GO:0110095	cellular detoxification of aldehyde	7	6	1.78	0.00146	0.03730885
GO:0046415	urate metabolic process	9	7	2.28	0.00147	0.037433958
GO:0009112	nucleobase metabolic process	31	16	7.87	0.00153	0.038296997
GO:0046460	neutral lipid biosynthetic process	31	16	7.87	0.00153	0.038296997
GO:0046461	neutral lipid catabolic process	31	16	7.87	0.00153	0.038296997
GO:0046463	acylglycerol biosynthetic process	31	16	7.87	0.00153	0.038296997
GO:0046464	acylglycerol catabolic process	31	16	7.87	0.00153	0.038296997
GO:0010823	negative regulation of mitochondrion organization	48	22	12.18	0.00166	0.04140966
GO:0045940	positive regulation of steroid metabolic process	26	14	6.6	0.00177	0.043855338

GO:1901661	quinone metabolic process	26	14	6.6	0.00177	0.043855338
GO:0015748	organophosphate ester transport	63	27	15.99	0.00181	0.044695421
GO:0019682	glyceraldehyde-3-phosphate metabolic process	16	10	4.06	0.00185	0.045377592
GO:0034312	diol biosynthetic process	16	10	4.06	0.00185	0.045377592
GO:0009991	response to extracellular stimulus	250	84	63.46	0.00203	0.049626733
GO:0019432	triglyceride biosynthetic process	29	15	7.36	0.00207	0.050436478
GO:0015914	phospholipid transport	49	22	12.44	0.0023	0.055854967
GO:0006851	mitochondrial calcium ion transmembrane transport	14	9	3.55	0.0024	0.0579
GO:0042572	retinol metabolic process	14	9	3.55	0.0024	0.0579
GO:0031667	response to nutrient levels	231	78	58.64	0.00248	0.059633836
GO:0035634	response to stilbenoid	19	11	4.82	0.00259	0.062075359
GO:0042304	regulation of fatty acid biosynthetic process	38	18	9.65	0.00275	0.06569544
GO:0006544	glycine metabolic process	12	8	3.05	0.00307	0.071934121
GO:0009071	serine family amino acid catabolic process	12	8	3.05	0.00307	0.071934121
GO:0018904	ether metabolic process	12	8	3.05	0.00307	0.071934121
GO:0042447	hormone catabolic process	12	8	3.05	0.00307	0.071934121
GO:0044241	lipid digestion	12	8	3.05	0.00307	0.071934121
GO:0050667	homocysteine metabolic process	12	8	3.05	0.00307	0.071934121
GO:0072376	protein activation cascade	56	24	14.22	0.00318	0.074038476
GO:1990748	cellular detoxification	30	15	7.62	0.00318	0.074038476
GO:0001916	positive regulation of T cell mediated cytotoxicity	22	12	5.58	0.00326	0.075185031
GO:0010499	proteasomal ubiquitin-independent protein catabolic process	22	12	5.58	0.00326	0.075185031
GO:0045723	positive regulation of fatty acid biosynthetic process	22	12	5.58	0.00326	0.075185031
GO:0042157	lipoprotein metabolic process	97	37	24.62	0.00363	0.08342425
GO:0016485	protein processing	166	58	42.14	0.00364	0.08342425
GO:0001523	retinoid metabolic process	36	17	9.14	0.00372	0.08499215
GO:0009068	aspartate family amino acid catabolic process	10	7	2.54	0.00383	0.085637866
GO:0009109	coenzyme catabolic process	10	7	2.54	0.00383	0.085637866
GO:0030299	intestinal cholesterol absorption	10	7	2.54	0.00383	0.085637866
GO:0033240	positive regulation of cellular amine metabolic process	10	7	2.54	0.00383	0.085637866
GO:0035999	tetrahydrofolate interconversion	10	7	2.54	0.00383	0.085637866
GO:0036444	calcium import into the mitochondrion	10	7	2.54	0.00383	0.085637866
GO:0051044	positive regulation of membrane protein ectodomain proteolysis	10	7	2.54	0.00383	0.085637866
GO:0006855	drug transmembrane transport	42	19	10.66	0.00404	0.089785939
GO:0046503	glycerolipid catabolic process	42	19	10.66	0.00404	0.089785939
GO:0050994	regulation of lipid catabolic process	45	20	11.42	0.00414	0.091454096
GO:0055081	anion homeostasis	45	20	11.42	0.00414	0.091454096
GO:0051881	regulation of mitochondrial membrane potential	57	24	14.47	0.00419	0.092223952
GO:0001910	regulation of leukocyte mediated cytotoxicity	48	21	12.18	0.0042	0.092223952
GO:0008535	respiratory chain complex IV assembly	20	11	5.08	0.00444	0.097202866
GO:0045542	positive regulation of cholesterol biosynthetic process	8	6	2.03	0.00458	0.098215556
GO:0051409	response to nitrosative stress	8	6	2.03	0.00458	0.098215556
GO:0090153	regulation of sphingolipid biosynthetic process	8	6	2.03	0.00458	0.098215556

GO:0090205	positive regulation of cholesterol metabolic process	8	6	2.03	0.00458	0.098215556
GO:0106120	positive regulation of sterol biosynthetic process	8	6	2.03	0.00458	0.098215556
GO:0110096	cellular response to aldehyde	8	6	2.03	0.00458	0.098215556
GO:1905038	regulation of membrane lipid metabolic process	8	6	2.03	0.00458	0.098215556
GO:0006103	2-oxoglutarate metabolic process	15	9	3.81	0.00466	0.099350116
GO:0055089	fatty acid homeostasis	15	9	3.81	0.00466	0.099350116
GO:0055090	acylglycerol homeostasis	31	15	7.87	0.00474	0.100471561
GO:0070328	triglyceride homeostasis	31	15	7.87	0.00474	0.100471561
GO:0006015	5-phosphoribose 1-diphosphate biosynthetic process	6	5	1.52	0.00497	0.101531978
GO:0006531	aspartate metabolic process	6	5	1.52	0.00497	0.101531978
GO:0006547	histidine metabolic process	6	5	1.52	0.00497	0.101531978
GO:0006642	triglyceride mobilization	6	5	1.52	0.00497	0.101531978
GO:0009698	phenylpropanoid metabolic process	6	5	1.52	0.00497	0.101531978
GO:0009804	coumarin metabolic process	6	5	1.52	0.00497	0.101531978
GO:0019626	short-chain fatty acid catabolic process	6	5	1.52	0.00497	0.101531978
GO:0019852	L-ascorbic acid metabolic process	6	5	1.52	0.00497	0.101531978
GO:0045046	protein import into peroxisome membrane	6	5	1.52	0.00497	0.101531978
GO:0046391	5-phosphoribose 1-diphosphate metabolic process	6	5	1.52	0.00497	0.101531978
GO:0052805	imidazole-containing compound catabolic process	6	5	1.52	0.00497	0.101531978
GO:1901029	negative regulation of mitochondrial outer membrane permeabilization involved in apoptotic signaling...	6	5	1.52	0.00497	0.101531978
GO:1990144	intrinsic apoptotic signaling pathway in response to hypoxia	6	5	1.52	0.00497	0.101531978
GO:1905953	negative regulation of lipid localization	34	16	8.63	0.00505	0.102879722
GO:1901137	carbohydrate derivative biosynthetic process	399	124	101.29	0.00521	0.105845263
GO:0006584	catecholamine metabolic process	23	12	5.84	0.00526	0.10598033
GO:0009712	catechol-containing compound metabolic process	23	12	5.84	0.00526	0.10598033
GO:0070861	regulation of protein exit from endoplasmic reticulum	23	12	5.84	0.00526	0.10598033
GO:0042446	hormone biosynthetic process	37	17	9.39	0.00528	0.106091836
GO:0035967	cellular response to topologically incorrect protein	83	32	21.07	0.00544	0.109008087
GO:0042136	neurotransmitter biosynthetic process	58	24	14.72	0.00546	0.109013533
GO:0010498	proteasomal protein catabolic process	371	116	94.18	0.00547	0.109013533
GO:0090207	regulation of triglyceride metabolic process	43	19	10.92	0.00555	0.11030813
GO:0051262	protein tetramerization	119	43	30.21	0.00579	0.114767189
GO:0006515	protein quality control for misfolded or incompletely synthesized proteins	26	13	6.6	0.00593	0.117225391
GO:0010893	positive regulation of steroid biosynthetic process	18	10	4.57	0.00605	0.1189563
GO:0046653	tetrahydrofolate metabolic process	18	10	4.57	0.00605	0.1189563
GO:0008210	estrogen metabolic process	13	8	3.3	0.00622	0.120681164
GO:0009081	branched-chain amino acid metabolic process	13	8	3.3	0.00622	0.120681164
GO:0009083	branched-chain amino acid catabolic process	13	8	3.3	0.00622	0.120681164
GO:0046185	aldehyde catabolic process	13	8	3.3	0.00622	0.120681164
GO:0070863	positive regulation of protein exit from endoplasmic reticulum	13	8	3.3	0.00622	0.120681164
GO:0015893	drug transport	87	33	22.08	0.00642	0.124232929
GO:0140053	mitochondrial gene expression	97	36	24.62	0.00681	0.131433
GO:0006576	cellular biogenic amine metabolic process	32	15	8.12	0.00686	0.132050499

GO:0043467	regulation of generation of precursor metabolites and energy	94	35	23.86	0.00714	0.13566
GO:0001912	positive regulation of leukocyte mediated cytotoxicity	35	16	8.88	0.00714	0.13566
GO:0017001	antibiotic catabolic process	35	16	8.88	0.00714	0.13566
GO:0045923	positive regulation of fatty acid metabolic process	35	16	8.88	0.00714	0.13566
GO:0051187	cofactor catabolic process	35	16	8.88	0.00714	0.13566
GO:0006760	folic acid-containing compound metabolic process	21	11	5.33	0.00721	0.136636021
GO:1903573	negative regulation of response to endoplasmic reticulum stress	38	17	9.65	0.00733	0.138552113
GO:0006006	glucose metabolic process	151	52	38.33	0.00784	0.147811208
GO:0043457	regulation of cellular respiration	24	12	6.09	0.00813	0.152776272
GO:0006086	acetyl-CoA biosynthetic process from pyruvate	11	7	2.79	0.00824	0.152776272
GO:0035337	fatty-acyl-CoA metabolic process	11	7	2.79	0.00824	0.152776272
GO:0043471	regulation of cellular carbohydrate catabolic process	11	7	2.79	0.00824	0.152776272
GO:0006706	steroid catabolic process	16	9	4.06	0.00827	0.152776272
GO:0031998	regulation of fatty acid beta-oxidation	16	9	4.06	0.00827	0.152776272
GO:0042417	dopamine metabolic process	16	9	4.06	0.00827	0.152776272
GO:1902236	negative regulation of endoplasmic reticulum stress-induced intrinsic apoptotic signaling pathway	16	9	4.06	0.00827	0.152776272
GO:0019751	polyol metabolic process	82	31	20.82	0.00854	0.157367739
GO:0034620	cellular response to unfolded protein	63	25	15.99	0.00868	0.159546667
GO:0051260	protein homooligomerization	245	79	62.19	0.00879	0.16116465
GO:1905898	positive regulation of response to endoplasmic reticulum stress	30	14	7.62	0.00933	0.170638953
GO:2000378	negative regulation of reactive oxygen species metabolic process	51	21	12.95	0.00964	0.17587005
GO:0009636	response to toxic substance	211	69	53.56	0.00972	0.176889529
GO:0009067	aspartate family amino acid biosynthetic process	19	10	4.82	0.00991	0.179456642
GO:0033238	regulation of cellular amine metabolic process	19	10	4.82	0.00991	0.179456642
GO:0033619	membrane protein proteolysis	39	17	9.9	0.00998	0.180279113
GO:0015697	quaternary ammonium group transport	9	6	2.28	0.01079	0.191144589
GO:0016486	peptide hormone processing	9	6	2.28	0.01079	0.191144589
GO:0016559	peroxisome fission	9	6	2.28	0.01079	0.191144589
GO:0034214	protein hexamerization	9	6	2.28	0.01079	0.191144589
GO:0034384	high-density lipoprotein particle clearance	9	6	2.28	0.01079	0.191144589
GO:0046498	S-adenosylhomocysteine metabolic process	9	6	2.28	0.01079	0.191144589
GO:0048207	vesicle targeting, rough ER to cis-Golgi	9	6	2.28	0.01079	0.191144589
GO:0048208	COPII vesicle coating	9	6	2.28	0.01079	0.191144589
GO:0031348	negative regulation of defense response	133	46	33.76	0.01091	0.192804675
GO:0090114	COPII-coated vesicle budding	22	11	5.58	0.01116	0.196748654
GO:0000097	sulfur amino acid biosynthetic process	14	8	3.55	0.0113	0.196850831
GO:0009070	serine family amino acid biosynthetic process	14	8	3.55	0.0113	0.196850831
GO:0015936	coenzyme A metabolic process	14	8	3.55	0.0113	0.196850831
GO:0051043	regulation of membrane protein ectodomain proteolysis	14	8	3.55	0.0113	0.196850831
GO:1902176	negative regulation of oxidative stress-induced intrinsic apoptotic signaling pathway	14	8	3.55	0.0113	0.196850831
GO:0001914	regulation of T cell mediated cytotoxicity	25	12	6.35	0.01207	0.209766303
GO:0015908	fatty acid transport	52	21	13.2	0.01238	0.214138962
GO:0045454	cell redox homeostasis	52	21	13.2	0.01238	0.214138962

GO:1901616	organic hydroxy compound catabolic process	40	17	10.15	0.01333	0.224756882
GO:0009746	response to hexose	121	42	30.72	0.01361	0.224756882
GO:0002828	regulation of type 2 immune response	17	9	4.32	0.01365	0.224756882
GO:0009065	glutamine family amino acid catabolic process	17	9	4.32	0.01365	0.224756882
GO:0010867	positive regulation of triglyceride biosynthetic process	17	9	4.32	0.01365	0.224756882
GO:0051156	glucose 6-phosphate metabolic process	17	9	4.32	0.01365	0.224756882
GO:1901028	regulation of mitochondrial outer membrane permeabilization involved in apoptotic signaling pathway	17	9	4.32	0.01365	0.224756882
GO:0002713	negative regulation of B cell mediated immunity	7	5	1.78	0.01376	0.224756882
GO:0002890	negative regulation of immunoglobulin mediated immune response	7	5	1.78	0.01376	0.224756882
GO:0006105	succinate metabolic process	7	5	1.78	0.01376	0.224756882
GO:0010635	regulation of mitochondrial fusion	7	5	1.78	0.01376	0.224756882
GO:0030300	regulation of intestinal cholesterol absorption	7	5	1.78	0.01376	0.224756882
GO:0032372	negative regulation of sterol transport	7	5	1.78	0.01376	0.224756882
GO:0032375	negative regulation of cholesterol transport	7	5	1.78	0.01376	0.224756882
GO:0032790	ribosome disassembly	7	5	1.78	0.01376	0.224756882
GO:0034756	regulation of iron ion transport	7	5	1.78	0.01376	0.224756882
GO:0043102	amino acid salvage	7	5	1.78	0.01376	0.224756882
GO:0043252	sodium-independent organic anion transport	7	5	1.78	0.01376	0.224756882
GO:0048240	sperm capacitation	7	5	1.78	0.01376	0.224756882
GO:0061732	mitochondrial acetyl-CoA biosynthetic process from pyruvate	7	5	1.78	0.01376	0.224756882
GO:0070857	regulation of bile acid biosynthetic process	7	5	1.78	0.01376	0.224756882
GO:0071265	L-methionine biosynthetic process	7	5	1.78	0.01376	0.224756882
GO:0071267	L-methionine salvage	7	5	1.78	0.01376	0.224756882
GO:1903748	negative regulation of establishment of protein localization to mitochondrion	7	5	1.78	0.01376	0.224756882
GO:2000303	regulation of ceramide biosynthetic process	7	5	1.78	0.01376	0.224756882
GO:0031341	regulation of cell killing	59	23	14.98	0.01462	0.238273511
GO:0006644	phospholipid metabolic process	257	81	65.24	0.01475	0.239859202
GO:1901657	glycosyl compound metabolic process	95	34	24.12	0.01532	0.246805043
GO:0042092	type 2 immune response	20	10	5.08	0.01537	0.246805043
GO:0045922	negative regulation of fatty acid metabolic process	20	10	5.08	0.01537	0.246805043
GO:0006098	pentose-phosphate shunt	12	7	3.05	0.01548	0.246805043
GO:0009084	glutamine family amino acid biosynthetic process	12	7	3.05	0.01548	0.246805043
GO:0034497	protein localization to phagophore assembly site	12	7	3.05	0.01548	0.246805043
GO:0035376	sterol import	12	7	3.05	0.01548	0.246805043
GO:0043650	dicarboxylic acid biosynthetic process	12	7	3.05	0.01548	0.246805043
GO:0070508	cholesterol import	12	7	3.05	0.01548	0.246805043
GO:0034284	response to monosaccharide	122	42	30.97	0.01578	0.251042343
GO:0044262	cellular carbohydrate metabolic process	212	68	53.82	0.01612	0.253999329
GO:0070059	intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress	50	20	12.69	0.01622	0.253999329
GO:0006548	histidine catabolic process	5	4	1.27	0.01652	0.253999329
GO:0009403	toxin biosynthetic process	5	4	1.27	0.01652	0.253999329
GO:0009407	toxin catabolic process	5	4	1.27	0.01652	0.253999329
GO:0010815	bradykinin catabolic process	5	4	1.27	0.01652	0.253999329

GO:0015739	sialic acid transport	5	4	1.27	0.01652	0.253999329
GO:0019805	quinolinate biosynthetic process	5	4	1.27	0.01652	0.253999329
GO:0046292	formaldehyde metabolic process	5	4	1.27	0.01652	0.253999329
GO:0046826	negative regulation of protein export from nucleus	5	4	1.27	0.01652	0.253999329
GO:0051385	response to mineralocorticoid	5	4	1.27	0.01652	0.253999329
GO:0051412	response to corticosterone	5	4	1.27	0.01652	0.253999329
GO:0070813	hydrogen sulfide metabolic process	5	4	1.27	0.01652	0.253999329
GO:0090156	cellular sphingolipid homeostasis	5	4	1.27	0.01652	0.253999329
GO:1902031	regulation of NADP metabolic process	5	4	1.27	0.01652	0.253999329
GO:1903715	regulation of aerobic respiration	5	4	1.27	0.01652	0.253999329
GO:0019433	triglyceride catabolic process	23	11	5.84	0.01656	0.254081674
GO:0019882	antigen processing and presentation	66	25	16.75	0.01663	0.254623006
GO:0046173	polyol biosynthetic process	47	19	11.93	0.0167	0.254784075
GO:0043161	proteasome-mediated ubiquitin-dependent protein catabolic process	319	98	80.98	0.01671	0.254784075
GO:0072593	reactive oxygen species metabolic process	202	65	51.28	0.01707	0.259733154
GO:0007007	inner mitochondrial membrane organization	26	12	6.6	0.01733	0.262599628
GO:0090208	positive regulation of triglyceride metabolic process	26	12	6.6	0.01733	0.262599628
GO:0000041	transition metal ion transport	63	24	15.99	0.01738	0.262814268
GO:0010675	regulation of cellular carbohydrate metabolic process	116	40	29.45	0.01765	0.266347942
GO:0044275	cellular carbohydrate catabolic process	29	13	7.36	0.01778	0.267177769
GO:0034381	plasma lipoprotein particle clearance	32	14	8.12	0.01796	0.267177769
GO:0042398	cellular modified amino acid biosynthetic process	32	14	8.12	0.01796	0.267177769
GO:0042743	hydrogen peroxide metabolic process	32	14	8.12	0.01796	0.267177769
GO:0045981	positive regulation of nucleotide metabolic process	32	14	8.12	0.01796	0.267177769
GO:1900544	positive regulation of purine nucleotide metabolic process	32	14	8.12	0.01796	0.267177769
GO:1903580	positive regulation of ATP metabolic process	32	14	8.12	0.01796	0.267177769
GO:0032543	mitochondrial translation	73	27	18.53	0.01841	0.273317692
GO:0006390	mitochondrial transcription	15	8	3.81	0.0189	0.278338554
GO:0010039	response to iron ion	15	8	3.81	0.0189	0.278338554
GO:0010894	negative regulation of steroid biosynthetic process	15	8	3.81	0.0189	0.278338554
GO:0045939	negative regulation of steroid metabolic process	15	8	3.81	0.0189	0.278338554
GO:0009749	response to glucose	120	41	30.46	0.01928	0.283365772
GO:0006140	regulation of nucleotide metabolic process	80	29	20.31	0.01995	0.2926266

Supplemental Table 2

Table 2.S2. Enriched KEGG pathways from Cluster 2 from the k-means cluster analysis of genes expressed in the liver averaged from all groups (CV and GF, male and female, and days 1, 5, 10, 15, 25, 60, and 120).

GO.ID	Term	Annotated	Significant	Expected	result1	FDR
GO:0140053	mitochondrial gene expression	97	48	22.7	1.70E-08	1.25E-04
GO:0032543	mitochondrial translation	73	36	17.08	1.20E-06	4.40E-03
GO:0000963	mitochondrial RNA processing	13	11	3.04	5.50E-06	1.34E-02
GO:0065002	intracellular protein transmembrane transport	50	24	11.7	1.20E-04	1.34E-01
GO:1990542	mitochondrial transmembrane transport	62	28	14.51	1.30E-04	1.34E-01
GO:0034470	ncRNA processing	293	96	68.57	1.30E-04	1.34E-01
GO:0008033	tRNA processing	94	38	22	1.70E-04	1.34E-01
GO:0071806	protein transmembrane transport	51	24	11.93	1.80E-04	1.34E-01
GO:0006399	tRNA metabolic process	144	53	33.7	1.90E-04	1.34E-01
GO:0044743	protein transmembrane import into intracellular organelle	37	19	8.66	2.00E-04	1.34E-01
GO:0035456	response to interferon-beta	40	20	9.36	2.20E-04	1.34E-01
GO:0034660	ncRNA metabolic process	381	119	89.16	2.20E-04	1.34E-01
GO:0090646	mitochondrial tRNA processing	8	7	1.87	2.40E-04	1.35E-01
GO:0000959	mitochondrial RNA metabolic process	38	19	8.89	3.10E-04	1.61E-01
GO:0030150	protein import into mitochondrial matrix	17	11	3.98	3.30E-04	1.61E-01
GO:0070129	regulation of mitochondrial translation	25	14	5.85	4.40E-04	1.94E-01
GO:0045116	protein neddylation	15	10	3.51	4.50E-04	1.94E-01
GO:0070266	necroptotic process	28	15	6.55	5.20E-04	2.12E-01
GO:0035458	cellular response to interferon-beta	31	16	7.25	5.90E-04	2.28E-01
GO:0097300	programmed necrotic cell death	34	17	7.96	6.40E-04	2.35E-01
GO:1902600	proton transmembrane transport	52	23	12.17	7.20E-04	2.51E-01
GO:0017062	respiratory chain complex III assembly	9	7	2.11	8.70E-04	2.77E-01
GO:0034551	mitochondrial respiratory chain complex III assembly	9	7	2.11	8.70E-04	2.77E-01
GO:0042254	ribosome biogenesis	258	82	60.37	1.13E-03	3.45E-01
GO:0070265	necrotic cell death	39	18	9.13	1.48E-03	4.34E-01
GO:0070131	positive regulation of mitochondrial translation	17	10	3.98	1.80E-03	5.08E-01
GO:0007005	mitochondrion organization	379	113	88.69	2.01E-03	5.46E-01
GO:1905954	positive regulation of lipid localization	62	25	14.51	2.17E-03	5.68E-01
GO:2000322	regulation of glucocorticoid receptor signaling pathway	10	7	2.34	2.31E-03	5.84E-01
GO:0017004	cytochrome complex assembly	29	14	6.79	2.92E-03	7.08E-01
GO:0006400	tRNA modification	57	23	13.34	3.18E-03	7.08E-01
GO:0010884	positive regulation of lipid storage	18	10	4.21	3.21E-03	7.08E-01
GO:1990126	retrograde transport, endosome to plasma membrane	18	10	4.21	3.21E-03	7.08E-01
GO:0006457	protein folding	125	43	29.25	3.32E-03	7.08E-01
GO:0042776	mitochondrial ATP synthesis coupled proton transport	6	5	1.4	3.38E-03	7.08E-01
GO:0010885	regulation of cholesterol storage	13	8	3.04	3.62E-03	7.12E-01
GO:0071346	cellular response to interferon-gamma	74	28	17.32	3.67E-03	7.12E-01
GO:0032816	positive regulation of natural killer cell activation	21	11	4.91	3.69E-03	7.12E-01

GO:0006626	protein targeting to mitochondrion	61	24	14.27	3.86E-03	7.26E-01
GO:0006839	mitochondrial transport	141	47	33	4.45E-03	7.95E-01
GO:0003009	skeletal muscle contraction	16	9	3.74	4.64E-03	7.95E-01
GO:0007040	lysosome organization	52	21	12.17	4.66E-03	7.95E-01
GO:0080171	lytic vacuole organization	52	21	12.17	4.66E-03	7.95E-01
GO:0036295	cellular response to increased oxygen levels	11	7	2.57	5.08E-03	8.28E-01
GO:0045351	type I interferon biosynthetic process	11	7	2.57	5.08E-03	8.28E-01
GO:0006364	rRNA processing	175	56	40.95	5.47E-03	8.72E-01
GO:0032814	regulation of natural killer cell activation	25	12	5.85	6.11E-03	9.53E-01
GO:0001510	RNA methylation	50	20	11.7	6.45E-03	9.86E-01
GO:0015985	energy coupled proton transport, down electrochemical gradient	17	9	3.98	7.83E-03	1.00E+00
GO:0015986	ATP synthesis coupled proton transport	17	9	3.98	7.83E-03	1.00E+00
GO:0007034	vacuolar transport	113	38	26.44	8.42E-03	1.00E+00
GO:0002922	positive regulation of humoral immune response	7	5	1.64	9.54E-03	1.00E+00
GO:0046543	development of secondary female sexual characteristics	7	5	1.64	9.54E-03	1.00E+00
GO:0002098	tRNA wobble uridine modification	12	7	2.81	9.75E-03	1.00E+00
GO:0036296	response to increased oxygen levels	12	7	2.81	9.75E-03	1.00E+00
GO:0009206	purine ribonucleoside triphosphate biosynthetic process	107	36	25.04	1.01E-02	1.00E+00
GO:0007032	endosome organization	42	17	9.83	1.01E-02	1.00E+00
GO:0032481	positive regulation of type I interferon production	39	16	9.13	1.07E-02	1.00E+00
GO:0010575	positive regulation of vascular endothelial growth factor production	15	8	3.51	1.15E-02	1.00E+00
GO:0010878	cholesterol storage	15	8	3.51	1.15E-02	1.00E+00
GO:0045324	late endosome to vacuole transport	15	8	3.51	1.15E-02	1.00E+00
GO:0009145	purine nucleoside triphosphate biosynthetic process	108	36	25.27	1.18E-02	1.00E+00
GO:0000957	mitochondrial RNA catabolic process	5	4	1.17	1.22E-02	1.00E+00
GO:0000965	mitochondrial RNA 3'-end processing	5	4	1.17	1.22E-02	1.00E+00
GO:0006072	glycerol-3-phosphate metabolic process	5	4	1.17	1.22E-02	1.00E+00
GO:0045084	positive regulation of interleukin-12 biosynthetic process	5	4	1.17	1.22E-02	1.00E+00
GO:0032042	mitochondrial DNA metabolic process	21	10	4.91	1.29E-02	1.00E+00
GO:0032606	type I interferon production	63	23	14.74	1.30E-02	1.00E+00
GO:0030488	tRNA methylation	24	11	5.62	1.30E-02	1.00E+00
GO:0042026	protein refolding	24	11	5.62	1.30E-02	1.00E+00
GO:0006488	dolichol-linked oligosaccharide biosynthetic process	10	6	2.34	1.42E-02	1.00E+00
GO:0060340	positive regulation of type I interferon-mediated signaling pathway	10	6	2.34	1.42E-02	1.00E+00
GO:0009451	RNA modification	106	35	24.81	1.50E-02	1.00E+00
GO:0009201	ribonucleoside triphosphate biosynthetic process	110	36	25.74	1.59E-02	1.00E+00
GO:0140056	organelle localization by membrane tethering	54	20	12.64	1.67E-02	1.00E+00
GO:0008333	endosome to lysosome transport	44	17	10.3	1.69E-02	1.00E+00
GO:0030730	sequestering of triglyceride	13	7	3.04	1.69E-02	1.00E+00
GO:0042921	glucocorticoid receptor signaling pathway	13	7	3.04	1.69E-02	1.00E+00
GO:0034389	lipid droplet organization	16	8	3.74	1.83E-02	1.00E+00
GO:0010939	regulation of necrotic cell death	22	10	5.15	1.88E-02	1.00E+00
GO:0070585	protein localization to mitochondrion	79	27	18.49	1.92E-02	1.00E+00

GO:0007033	vacuole organization	141	44	33	2.00E-02	1.00E+00
GO:0044417	translocation of molecules into host	8	5	1.87	2.06E-02	1.00E+00
GO:0051836	translocation of molecules into other organism involved in symbiotic interaction	8	5	1.87	2.06E-02	1.00E+00
GO:0006941	striated muscle contraction	83	28	19.42	2.07E-02	1.00E+00
GO:0006605	protein targeting	202	60	47.27	2.21E-02	1.00E+00
GO:0061025	membrane fusion	87	29	20.36	2.22E-02	1.00E+00
GO:0071345	cellular response to cytokine stimulus	475	130	111.16	2.22E-02	1.00E+00
GO:0032479	regulation of type I interferon production	59	21	13.81	2.30E-02	1.00E+00
GO:0002755	MyD88-dependent toll-like receptor signaling pathway	11	6	2.57	2.50E-02	1.00E+00
GO:0006450	regulation of translational fidelity	11	6	2.57	2.50E-02	1.00E+00
GO:0006620	posttranslational protein targeting to endoplasmic reticulum membrane	11	6	2.57	2.50E-02	1.00E+00
GO:0010889	regulation of sequestering of triglyceride	11	6	2.57	2.50E-02	1.00E+00
GO:0046337	phosphatidylethanolamine metabolic process	11	6	2.57	2.50E-02	1.00E+00
GO:2000193	positive regulation of fatty acid transport	11	6	2.57	2.50E-02	1.00E+00
GO:2001014	regulation of skeletal muscle cell differentiation	11	6	2.57	2.50E-02	1.00E+00
GO:0030490	maturation of SSU-rRNA	39	15	9.13	2.51E-02	1.00E+00
GO:0022613	ribonucleoprotein complex biogenesis	386	107	90.33	2.53E-02	1.00E+00
GO:0009142	nucleoside triphosphate biosynthetic process	117	37	27.38	2.53E-02	1.00E+00
GO:0034341	response to interferon-gamma	88	29	20.59	2.59E-02	1.00E+00
GO:0006754	ATP biosynthetic process	99	32	23.17	2.64E-02	1.00E+00
GO:0007041	lysosomal transport	81	27	18.95	2.66E-02	1.00E+00
GO:0002097	tRNA wobble base modification	14	7	3.28	2.71E-02	1.00E+00
GO:0006264	mitochondrial DNA replication	14	7	3.28	2.71E-02	1.00E+00
GO:0031167	rRNA methylation	14	7	3.28	2.71E-02	1.00E+00
GO:0031958	corticosteroid receptor signaling pathway	14	7	3.28	2.71E-02	1.00E+00
GO:0044088	regulation of vacuole organization	36	14	8.42	2.72E-02	1.00E+00
GO:0006885	regulation of pH	53	19	12.4	2.75E-02	1.00E+00
GO:0060544	regulation of necrotic process	17	8	3.98	2.76E-02	1.00E+00
GO:0019730	antimicrobial humoral response	33	13	7.72	2.93E-02	1.00E+00
GO:0006228	UTP biosynthetic process	6	4	1.4	2.97E-02	1.00E+00
GO:0010887	negative regulation of cholesterol storage	6	4	1.4	2.97E-02	1.00E+00
GO:0010890	positive regulation of sequestering of triglyceride	6	4	1.4	2.97E-02	1.00E+00
GO:0017185	peptidyl-lysine hydroxylation	6	4	1.4	2.97E-02	1.00E+00
GO:0039533	regulation of MDA-5 signaling pathway	6	4	1.4	2.97E-02	1.00E+00
GO:0090158	endoplasmic reticulum membrane organization	6	4	1.4	2.97E-02	1.00E+00
GO:0097064	ncRNA export from nucleus	6	4	1.4	2.97E-02	1.00E+00
GO:1901533	negative regulation of hematopoietic progenitor cell differentiation	6	4	1.4	2.97E-02	1.00E+00
GO:1902035	positive regulation of hematopoietic stem cell proliferation	6	4	1.4	2.97E-02	1.00E+00
GO:2000018	regulation of male gonad development	6	4	1.4	2.97E-02	1.00E+00
GO:2000323	negative regulation of glucocorticoid receptor signaling pathway	6	4	1.4	2.97E-02	1.00E+00
GO:0033108	mitochondrial respiratory chain complex assembly	71	24	16.61	2.99E-02	1.00E+00
GO:0090174	organelle membrane fusion	71	24	16.61	2.99E-02	1.00E+00
GO:0031929	TOR signaling	89	29	20.83	3.00E-02	1.00E+00

GO:0070936	protein K48-linked ubiquitination	50	18	11.7	3.01E-02	1.00E+00
GO:0072599	establishment of protein localization to endoplasmic reticulum	30	12	7.02	3.15E-02	1.00E+00
GO:0016482	cytosolic transport	130	40	30.42	3.19E-02	1.00E+00
GO:0046939	nucleotide phosphorylation	75	25	17.55	3.21E-02	1.00E+00
GO:0009205	purine ribonucleoside triphosphate metabolic process	210	61	49.14	3.29E-02	1.00E+00
GO:0030641	regulation of cellular pH	47	17	11	3.30E-02	1.00E+00
GO:0050830	defense response to Gram-positive bacterium	47	17	11	3.30E-02	1.00E+00
GO:0032728	positive regulation of interferon-beta production	27	11	6.32	3.38E-02	1.00E+00
GO:0045047	protein targeting to ER	27	11	6.32	3.38E-02	1.00E+00
GO:0045851	pH reduction	27	11	6.32	3.38E-02	1.00E+00
GO:1903432	regulation of TORC1 signaling	27	11	6.32	3.38E-02	1.00E+00
GO:0010883	regulation of lipid storage	37	14	8.66	3.45E-02	1.00E+00
GO:0009144	purine nucleoside triphosphate metabolic process	215	62	50.31	3.65E-02	1.00E+00
GO:0009156	ribonucleoside monophosphate biosynthetic process	120	37	28.08	3.68E-02	1.00E+00
GO:0000338	protein deneddylation	9	5	2.11	3.75E-02	1.00E+00
GO:0002084	protein depalmitoylation	9	5	2.11	3.75E-02	1.00E+00
GO:0006013	mannose metabolic process	9	5	2.11	3.75E-02	1.00E+00
GO:0007042	lysosomal lumen acidification	9	5	2.11	3.75E-02	1.00E+00
GO:0032305	positive regulation of icosanoid secretion	9	5	2.11	3.75E-02	1.00E+00
GO:0034116	positive regulation of heterotypic cell-cell adhesion	9	5	2.11	3.75E-02	1.00E+00
GO:0035435	phosphate ion transmembrane transport	9	5	2.11	3.75E-02	1.00E+00
GO:0043649	dicarboxylic acid catabolic process	9	5	2.11	3.75E-02	1.00E+00
GO:0044539	long-chain fatty acid import	9	5	2.11	3.75E-02	1.00E+00
GO:0045136	development of secondary sexual characteristics	9	5	2.11	3.75E-02	1.00E+00
GO:0098734	macromolecule depalmitoylation	9	5	2.11	3.75E-02	1.00E+00
GO:0099638	endosome to plasma membrane protein transport	9	5	2.11	3.75E-02	1.00E+00
GO:0032006	regulation of TOR signaling	76	25	17.78	3.75E-02	1.00E+00
GO:0072655	establishment of protein localization to mitochondrion	76	25	17.78	3.75E-02	1.00E+00
GO:0016072	rRNA metabolic process	200	58	46.8	3.79E-02	1.00E+00
GO:0032607	interferon-alpha production	21	9	4.91	3.81E-02	1.00E+00
GO:0018126	protein hydroxylation	18	8	4.21	3.98E-02	1.00E+00
GO:0001731	formation of translation preinitiation complex	12	6	2.81	4.03E-02	1.00E+00
GO:0006490	oligosaccharide-lipid intermediate biosynthetic process	12	6	2.81	4.03E-02	1.00E+00
GO:0009220	pyrimidine ribonucleotide biosynthetic process	12	6	2.81	4.03E-02	1.00E+00
GO:0032823	regulation of natural killer cell differentiation	12	6	2.81	4.03E-02	1.00E+00
GO:0044406	adhesion of symbiont to host	12	6	2.81	4.03E-02	1.00E+00
GO:1904293	negative regulation of ERAD pathway	12	6	2.81	4.03E-02	1.00E+00
GO:0006896	Golgi to vacuole transport	15	7	3.51	4.07E-02	1.00E+00
GO:0046131	pyrimidine ribonucleoside metabolic process	15	7	3.51	4.07E-02	1.00E+00
GO:0046475	glycerophospholipid catabolic process	15	7	3.51	4.07E-02	1.00E+00
GO:0048490	anterograde synaptic vesicle transport	15	7	3.51	4.07E-02	1.00E+00
GO:0099514	synaptic vesicle cytoskeletal transport	15	7	3.51	4.07E-02	1.00E+00
GO:0099517	synaptic vesicle transport along microtubule	15	7	3.51	4.07E-02	1.00E+00

GO:0001961	positive regulation of cytokine-mediated signaling pathway	31	12	7.25	4.08E-02	1.00E+00
GO:0006904	vesicle docking involved in exocytosis	31	12	7.25	4.08E-02	1.00E+00
GO:0060337	type I interferon signaling pathway	31	12	7.25	4.08E-02	1.00E+00
GO:0071357	cellular response to type I interferon	31	12	7.25	4.08E-02	1.00E+00
GO:0009141	nucleoside triphosphate metabolic process	228	65	53.35	4.11E-02	1.00E+00
GO:0022406	membrane docking	59	20	13.81	4.36E-02	1.00E+00
GO:0050879	multicellular organismal movement	28	11	6.55	4.42E-02	1.00E+00
GO:0050881	musculoskeletal movement	28	11	6.55	4.42E-02	1.00E+00
GO:0032370	positive regulation of lipid transport	45	16	10.53	4.42E-02	1.00E+00
GO:0048278	vesicle docking	45	16	10.53	4.42E-02	1.00E+00
GO:0042089	cytokine biosynthetic process	70	23	16.38	4.52E-02	1.00E+00
GO:0042107	cytokine metabolic process	70	23	16.38	4.52E-02	1.00E+00
GO:0060048	cardiac muscle contraction	70	23	16.38	4.52E-02	1.00E+00
GO:0006511	ubiquitin-dependent protein catabolic process	463	124	108.35	4.58E-02	1.00E+00
GO:0045088	regulation of innate immune response	179	52	41.89	4.59E-02	1.00E+00
GO:0006888	ER to Golgi vesicle-mediated transport	107	33	25.04	4.65E-02	1.00E+00
GO:0009199	ribonucleoside triphosphate metabolic process	214	61	50.08	4.67E-02	1.00E+00
GO:0060760	positive regulation of response to cytokine stimulus	35	13	8.19	4.73E-02	1.00E+00
GO:1904292	regulation of ERAD pathway	25	10	5.85	4.78E-02	1.00E+00
GO:0008625	extrinsic apoptotic signaling pathway via death domain receptors	56	19	13.1	4.79E-02	1.00E+00
GO:0048284	organelle fusion	85	27	19.89	4.79E-02	1.00E+00
GO:0043632	modification-dependent macromolecule catabolic process	480	128	112.33	4.83E-02	1.00E+00
GO:0032648	regulation of interferon-beta production	42	15	9.83	4.86E-02	1.00E+00
GO:0051453	regulation of intracellular pH	42	15	9.83	4.86E-02	1.00E+00
GO:0009132	nucleoside diphosphate metabolic process	89	28	20.83	5.00E-02	1.00E+00
GO:0019941	modification-dependent protein catabolic process	473	126	110.69	5.12E-02	1.00E+00
GO:0002920	regulation of humoral immune response	22	9	5.15	5.14E-02	1.00E+00
GO:0010499	proteasomal ubiquitin-independent protein catabolic process	22	9	5.15	5.14E-02	1.00E+00
GO:0038202	TORC1 signaling	32	12	7.49	5.18E-02	1.00E+00
GO:0048641	regulation of skeletal muscle tissue development	32	12	7.49	5.18E-02	1.00E+00
GO:1905952	regulation of lipid localization	108	33	25.27	5.24E-02	1.00E+00
GO:0006413	translational initiation	97	30	22.7	5.38E-02	1.00E+00
GO:0007249	I-kappaB kinase/NF-kappaB signaling	131	39	30.66	5.44E-02	1.00E+00
GO:0010574	regulation of vascular endothelial growth factor production	19	8	4.45	5.50E-02	1.00E+00
GO:0000462	maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	29	11	6.79	5.66E-02	1.00E+00
GO:0006953	acute-phase response	29	11	6.79	5.66E-02	1.00E+00
GO:0002730	regulation of dendritic cell cytokine production	7	4	1.64	5.67E-02	1.00E+00
GO:0006241	CTP biosynthetic process	7	4	1.64	5.67E-02	1.00E+00
GO:0006646	phosphatidylethanolamine biosynthetic process	7	4	1.64	5.67E-02	1.00E+00
GO:0010886	positive regulation of cholesterol storage	7	4	1.64	5.67E-02	1.00E+00
GO:0014819	regulation of skeletal muscle contraction	7	4	1.64	5.67E-02	1.00E+00
GO:0017196	N-terminal peptidyl-methionine acetylation	7	4	1.64	5.67E-02	1.00E+00
GO:0018344	protein geranylgeranylation	7	4	1.64	5.67E-02	1.00E+00

GO:0021903	rostrocaudal neural tube patterning	7	4	1.64	5.67E-02	1.00E+00
GO:0030277	maintenance of gastrointestinal epithelium	7	4	1.64	5.67E-02	1.00E+00
GO:0031424	keratinization	7	4	1.64	5.67E-02	1.00E+00
GO:0032308	positive regulation of prostaglandin secretion	7	4	1.64	5.67E-02	1.00E+00
GO:0033683	nucleotide-excision repair, DNA incision	7	4	1.64	5.67E-02	1.00E+00
GO:0039530	MDA-5 signaling pathway	7	4	1.64	5.67E-02	1.00E+00
GO:0040016	embryonic cleavage	7	4	1.64	5.67E-02	1.00E+00
GO:0042090	interleukin-12 biosynthetic process	7	4	1.64	5.67E-02	1.00E+00
GO:0045075	regulation of interleukin-12 biosynthetic process	7	4	1.64	5.67E-02	1.00E+00
GO:0045086	positive regulation of interleukin-2 biosynthetic process	7	4	1.64	5.67E-02	1.00E+00
GO:0045350	interferon-beta biosynthetic process	7	4	1.64	5.67E-02	1.00E+00
GO:0045357	regulation of interferon-beta biosynthetic process	7	4	1.64	5.67E-02	1.00E+00
GO:0046036	CTP metabolic process	7	4	1.64	5.67E-02	1.00E+00
GO:0046051	UTP metabolic process	7	4	1.64	5.67E-02	1.00E+00
GO:0046784	viral mRNA export from host cell nucleus	7	4	1.64	5.67E-02	1.00E+00
GO:0051709	regulation of killing of cells of other organism	7	4	1.64	5.67E-02	1.00E+00
GO:0051712	positive regulation of killing of cells of other organism	7	4	1.64	5.67E-02	1.00E+00
GO:0052646	alditol phosphate metabolic process	7	4	1.64	5.67E-02	1.00E+00
GO:0060136	embryonic process involved in female pregnancy	7	4	1.64	5.67E-02	1.00E+00
GO:0072378	blood coagulation, fibrin clot formation	7	4	1.64	5.67E-02	1.00E+00
GO:0006613	cotranslational protein targeting to membrane	16	7	3.74	5.82E-02	1.00E+00
GO:0042451	purine nucleoside biosynthetic process	16	7	3.74	5.82E-02	1.00E+00
GO:0046129	purine ribonucleoside biosynthetic process	16	7	3.74	5.82E-02	1.00E+00
GO:0048643	positive regulation of skeletal muscle tissue development	16	7	3.74	5.82E-02	1.00E+00
GO:0071549	cellular response to dexamethasone stimulus	16	7	3.74	5.82E-02	1.00E+00
GO:0070972	protein localization to endoplasmic reticulum	50	17	11.7	5.82E-02	1.00E+00
GO:0032007	negative regulation of TOR signaling	36	13	8.42	5.87E-02	1.00E+00
GO:0032608	interferon-beta production	43	15	10.06	5.90E-02	1.00E+00
GO:0070613	regulation of protein processing	61	20	14.27	6.03E-02	1.00E+00
GO:0009218	pyrimidine ribonucleotide metabolic process	13	6	3.04	6.04E-02	1.00E+00
GO:0035751	regulation of lysosomal lumen pH	13	6	3.04	6.04E-02	1.00E+00
GO:0048305	immunoglobulin secretion	13	6	3.04	6.04E-02	1.00E+00
GO:0060546	negative regulation of necroptotic process	13	6	3.04	6.04E-02	1.00E+00
GO:0032825	positive regulation of natural killer cell differentiation	10	5	2.34	6.07E-02	1.00E+00
GO:0035461	vitamin transmembrane transport	10	5	2.34	6.07E-02	1.00E+00
GO:0036498	IRE1-mediated unfolded protein response	10	5	2.34	6.07E-02	1.00E+00
GO:0042033	chemokine biosynthetic process	10	5	2.34	6.07E-02	1.00E+00
GO:0045073	regulation of chemokine biosynthetic process	10	5	2.34	6.07E-02	1.00E+00
GO:0050755	chemokine metabolic process	10	5	2.34	6.07E-02	1.00E+00
GO:0051023	regulation of immunoglobulin secretion	10	5	2.34	6.07E-02	1.00E+00
GO:0051770	positive regulation of nitric-oxide synthase biosynthetic process	10	5	2.34	6.07E-02	1.00E+00
GO:0070071	proton-transporting two-sector ATPase complex assembly	10	5	2.34	6.07E-02	1.00E+00
GO:1905939	regulation of gonad development	10	5	2.34	6.07E-02	1.00E+00

GO:0009127	purine nucleoside monophosphate biosynthetic process	117	35	27.38	6.17E-02	1.00E+00
GO:0009168	purine ribonucleoside monophosphate biosynthetic process	117	35	27.38	6.17E-02	1.00E+00
GO:0007007	inner mitochondrial membrane organization	26	10	6.08	6.19E-02	1.00E+00
GO:0051452	intracellular pH reduction	26	10	6.08	6.19E-02	1.00E+00
GO:0006906	vesicle fusion	65	21	15.21	6.36E-02	1.00E+00
GO:0009124	nucleoside monophosphate biosynthetic process	125	37	29.25	6.44E-02	1.00E+00
GO:0042147	retrograde transport, endosome to Golgi	69	22	16.15	6.68E-02	1.00E+00
GO:0002753	cytoplasmic pattern recognition receptor signaling pathway	23	9	5.38	6.75E-02	1.00E+00
GO:0050999	regulation of nitric-oxide synthase activity	23	9	5.38	6.75E-02	1.00E+00
GO:0070536	protein K63-linked deubiquitination	23	9	5.38	6.75E-02	1.00E+00
GO:0045089	positive regulation of innate immune response	141	41	33	6.89E-02	1.00E+00
GO:0009161	ribonucleoside monophosphate metabolic process	211	59	49.38	6.89E-02	1.00E+00
GO:0006165	nucleoside diphosphate phosphorylation	73	23	17.08	6.97E-02	1.00E+00
GO:0046470	phosphatidylcholine metabolic process	30	11	7.02	7.12E-02	1.00E+00
GO:0046034	ATP metabolic process	188	53	43.99	7.17E-02	1.00E+00
GO:1901137	carbohydrate derivative biosynthetic process	399	106	93.37	7.31E-02	1.00E+00
GO:0051865	protein autoubiquitination	55	18	12.87	7.33E-02	1.00E+00
GO:0000154	rRNA modification	20	8	4.68	7.34E-02	1.00E+00
GO:0010573	vascular endothelial growth factor production	20	8	4.68	7.34E-02	1.00E+00
GO:0010954	positive regulation of protein processing	20	8	4.68	7.34E-02	1.00E+00
GO:0016578	histone deubiquitination	20	8	4.68	7.34E-02	1.00E+00
GO:0018345	protein palmitoylation	20	8	4.68	7.34E-02	1.00E+00
GO:0048193	Golgi vesicle transport	232	64	54.29	7.61E-02	1.00E+00
GO:0007029	endoplasmic reticulum organization	48	16	11.23	7.61E-02	1.00E+00
GO:0070646	protein modification by small protein removal	100	30	23.4	7.66E-02	1.00E+00
GO:0019915	lipid storage	59	19	13.81	7.73E-02	1.00E+00
GO:0016579	protein deubiquitination	85	26	19.89	7.74E-02	1.00E+00
GO:0045948	positive regulation of translational initiation	17	7	3.98	7.95E-02	1.00E+00
GO:0060333	interferon-gamma-mediated signaling pathway	17	7	3.98	7.95E-02	1.00E+00
GO:0019319	hexose biosynthetic process	63	20	14.74	8.10E-02	1.00E+00
GO:1903317	regulation of protein maturation	63	20	14.74	8.10E-02	1.00E+00
GO:0009260	ribonucleotide biosynthetic process	170	48	39.78	8.13E-02	1.00E+00
GO:0030101	natural killer cell activation	45	15	10.53	8.42E-02	1.00E+00
GO:0006474	N-terminal protein amino acid acetylation	14	6	3.28	8.55E-02	1.00E+00
GO:0008209	androgen metabolic process	14	6	3.28	8.55E-02	1.00E+00
GO:0030449	regulation of complement activation	14	6	3.28	8.55E-02	1.00E+00
GO:0032735	positive regulation of interleukin-12 production	14	6	3.28	8.55E-02	1.00E+00
GO:0071025	RNA surveillance	14	6	3.28	8.55E-02	1.00E+00
GO:0090151	establishment of protein localization to mitochondrial membrane	14	6	3.28	8.55E-02	1.00E+00
GO:0006891	intra-Golgi vesicle-mediated transport	24	9	5.62	8.64E-02	1.00E+00
GO:1901659	glycosyl compound biosynthetic process	24	9	5.62	8.64E-02	1.00E+00
GO:0009123	nucleoside monophosphate metabolic process	218	60	51.01	8.67E-02	1.00E+00
GO:1990928	response to amino acid starvation	38	13	8.89	8.67E-02	1.00E+00

GO:0002692	negative regulation of cellular extravasation	5	3	1.17	8.73E-02	1.00E+00
GO:0006104	succinyl-CoA metabolic process	5	3	1.17	8.73E-02	1.00E+00
GO:0006590	thyroid hormone generation	5	3	1.17	8.73E-02	1.00E+00
GO:0007253	cytoplasmic sequestering of NF-kappaB	5	3	1.17	8.73E-02	1.00E+00
GO:0019348	dolichol metabolic process	5	3	1.17	8.73E-02	1.00E+00
GO:0030238	male sex determination	5	3	1.17	8.73E-02	1.00E+00
GO:0030240	skeletal muscle thin filament assembly	5	3	1.17	8.73E-02	1.00E+00
GO:0031204	posttranslational protein targeting to membrane, translocation	5	3	1.17	8.73E-02	1.00E+00
GO:0031652	positive regulation of heat generation	5	3	1.17	8.73E-02	1.00E+00
GO:0032196	transposition	5	3	1.17	8.73E-02	1.00E+00
GO:0033860	regulation of NAD(P)H oxidase activity	5	3	1.17	8.73E-02	1.00E+00
GO:0034638	phosphatidylcholine catabolic process	5	3	1.17	8.73E-02	1.00E+00
GO:0035964	COPI-coated vesicle budding	5	3	1.17	8.73E-02	1.00E+00
GO:0042270	protection from natural killer cell mediated cytotoxicity	5	3	1.17	8.73E-02	1.00E+00
GO:0045006	DNA deamination	5	3	1.17	8.73E-02	1.00E+00
GO:0048630	skeletal muscle tissue growth	5	3	1.17	8.73E-02	1.00E+00
GO:0048631	regulation of skeletal muscle tissue growth	5	3	1.17	8.73E-02	1.00E+00
GO:0048633	positive regulation of skeletal muscle tissue growth	5	3	1.17	8.73E-02	1.00E+00
GO:0051029	rRNA transport	5	3	1.17	8.73E-02	1.00E+00
GO:0060059	embryonic retina morphogenesis in camera-type eye	5	3	1.17	8.73E-02	1.00E+00
GO:0060397	JAK-STAT cascade involved in growth hormone signaling pathway	5	3	1.17	8.73E-02	1.00E+00
GO:0070070	proton-transporting V-type ATPase complex assembly	5	3	1.17	8.73E-02	1.00E+00
GO:0070072	vacuolar proton-transporting V-type ATPase complex assembly	5	3	1.17	8.73E-02	1.00E+00
GO:0070309	lens fiber cell morphogenesis	5	3	1.17	8.73E-02	1.00E+00
GO:0071033	nuclear retention of pre-mRNA at the site of transcription	5	3	1.17	8.73E-02	1.00E+00
GO:0071372	cellular response to follicle-stimulating hormone stimulus	5	3	1.17	8.73E-02	1.00E+00
GO:0097152	mesenchymal cell apoptotic process	5	3	1.17	8.73E-02	1.00E+00
GO:0098887	neurotransmitter receptor transport, endosome to postsynaptic membrane	5	3	1.17	8.73E-02	1.00E+00
GO:1901858	regulation of mitochondrial DNA metabolic process	5	3	1.17	8.73E-02	1.00E+00
GO:1903265	positive regulation of tumor necrosis factor-mediated signaling pathway	5	3	1.17	8.73E-02	1.00E+00
GO:1903626	positive regulation of DNA catabolic process	5	3	1.17	8.73E-02	1.00E+00
GO:1903894	regulation of IRE1-mediated unfolded protein response	5	3	1.17	8.73E-02	1.00E+00
GO:1904415	regulation of xenophagy	5	3	1.17	8.73E-02	1.00E+00
GO:1904417	positive regulation of xenophagy	5	3	1.17	8.73E-02	1.00E+00
GO:2000002	negative regulation of DNA damage checkpoint	5	3	1.17	8.73E-02	1.00E+00
GO:2000020	positive regulation of male gonad development	5	3	1.17	8.73E-02	1.00E+00
GO:2000074	regulation of type B pancreatic cell development	5	3	1.17	8.73E-02	1.00E+00
GO:2000630	positive regulation of miRNA metabolic process	5	3	1.17	8.73E-02	1.00E+00
GO:2001016	positive regulation of skeletal muscle cell differentiation	5	3	1.17	8.73E-02	1.00E+00
GO:1901976	regulation of cell cycle checkpoint	31	11	7.25	8.79E-02	1.00E+00
GO:0006094	gluconeogenesis	60	19	14.04	8.93E-02	1.00E+00
GO:0042035	regulation of cytokine biosynthetic process	60	19	14.04	8.93E-02	1.00E+00
GO:0002043	blood vessel endothelial cell proliferation involved in sprouting angiogenesis	11	5	2.57	9.05E-02	1.00E+00

GO:0006517	protein deglycosylation	11	5	2.57	9.05E-02	1.00E+00
GO:0010669	epithelial structure maintenance	11	5	2.57	9.05E-02	1.00E+00
GO:0042454	ribonucleoside catabolic process	11	5	2.57	9.05E-02	1.00E+00
GO:0046132	pyrimidine ribonucleoside biosynthetic process	11	5	2.57	9.05E-02	1.00E+00
GO:0046134	pyrimidine nucleoside biosynthetic process	11	5	2.57	9.05E-02	1.00E+00
GO:0098969	neurotransmitter receptor transport to postsynaptic membrane	11	5	2.57	9.05E-02	1.00E+00
GO:1901070	guanosine-containing compound biosynthetic process	11	5	2.57	9.05E-02	1.00E+00
GO:1903540	establishment of protein localization to postsynaptic membrane	11	5	2.57	9.05E-02	1.00E+00
GO:1904263	positive regulation of TORC1 signaling	11	5	2.57	9.05E-02	1.00E+00
GO:0009100	glycoprotein metabolic process	211	58	49.38	9.26E-02	1.00E+00
GO:0002371	dendritic cell cytokine production	8	4	1.87	9.28E-02	1.00E+00
GO:0002756	MyD88-independent toll-like receptor signaling pathway	8	4	1.87	9.28E-02	1.00E+00
GO:0006183	GTP biosynthetic process	8	4	1.87	9.28E-02	1.00E+00
GO:0007530	sex determination	8	4	1.87	9.28E-02	1.00E+00
GO:0009125	nucleoside monophosphate catabolic process	8	4	1.87	9.28E-02	1.00E+00
GO:0009209	pyrimidine ribonucleoside triphosphate biosynthetic process	8	4	1.87	9.28E-02	1.00E+00
GO:0032511	late endosome to vacuole transport via multivesicular body sorting pathway	8	4	1.87	9.28E-02	1.00E+00
GO:0034058	endosomal vesicle fusion	8	4	1.87	9.28E-02	1.00E+00
GO:0039532	negative regulation of viral-induced cytoplasmic pattern recognition receptor signaling pathway	8	4	1.87	9.28E-02	1.00E+00
GO:0045039	protein import into mitochondrial inner membrane	8	4	1.87	9.28E-02	1.00E+00
GO:0045080	positive regulation of chemokine biosynthetic process	8	4	1.87	9.28E-02	1.00E+00
GO:0048537	mucosal-associated lymphoid tissue development	8	4	1.87	9.28E-02	1.00E+00
GO:0048541	Peyer's patch development	8	4	1.87	9.28E-02	1.00E+00
GO:0072321	chaperone-mediated protein transport	8	4	1.87	9.28E-02	1.00E+00
GO:0097049	motor neuron apoptotic process	8	4	1.87	9.28E-02	1.00E+00
GO:1902033	regulation of hematopoietic stem cell proliferation	8	4	1.87	9.28E-02	1.00E+00
GO:1903978	regulation of microglial cell activation	8	4	1.87	9.28E-02	1.00E+00
GO:1904153	negative regulation of retrograde protein transport, ER to cytosol	8	4	1.87	9.28E-02	1.00E+00
GO:1905146	lysosomal protein catabolic process	8	4	1.87	9.28E-02	1.00E+00
GO:0006213	pyrimidine nucleoside metabolic process	21	8	4.91	9.52E-02	1.00E+00
GO:0010866	regulation of triglyceride biosynthetic process	21	8	4.91	9.52E-02	1.00E+00
GO:0042832	defense response to protozoan	21	8	4.91	9.52E-02	1.00E+00
GO:0060338	regulation of type I interferon-mediated signaling pathway	21	8	4.91	9.52E-02	1.00E+00
GO:0090140	regulation of mitochondrial fission	21	8	4.91	9.52E-02	1.00E+00
GO:0046390	ribose phosphate biosynthetic process	176	49	41.19	9.61E-02	1.00E+00
GO:0034340	response to type I interferon	35	12	8.19	9.62E-02	1.00E+00
GO:0006458	'de novo' protein folding	28	10	6.55	9.74E-02	1.00E+00
GO:1901264	carbohydrate derivative transport	46	15	10.76	9.90E-02	1.00E+00
GO:0009126	purine nucleoside monophosphate metabolic process	208	57	48.67	9.91E-02	1.00E+00
GO:0009167	purine ribonucleoside monophosphate metabolic process	208	57	48.67	9.91E-02	1.00E+00
GO:0046031	ADP metabolic process	72	22	16.85	9.93E-02	1.00E+00
GO:0009135	purine nucleoside diphosphate metabolic process	76	23	17.78	1.02E-01	1.00E+00
GO:0009179	purine ribonucleoside diphosphate metabolic process	76	23	17.78	1.02E-01	1.00E+00

GO:0042274	ribosomal small subunit biogenesis	61	19	14.27	1.02E-01	1.00E+00
GO:0045022	early endosome to late endosome transport	39	13	9.13	1.03E-01	1.00E+00
GO:0009185	ribonucleoside diphosphate metabolic process	80	24	18.72	1.05E-01	1.00E+00
GO:0003016	respiratory system process	18	7	4.21	1.05E-01	1.00E+00
GO:0014912	negative regulation of smooth muscle cell migration	18	7	4.21	1.05E-01	1.00E+00
GO:0032770	positive regulation of monooxygenase activity	18	7	4.21	1.05E-01	1.00E+00
GO:0042730	fibrinolysis	18	7	4.21	1.05E-01	1.00E+00
GO:0048199	vesicle targeting, to, from or within Golgi	18	7	4.21	1.05E-01	1.00E+00
GO:0071548	response to dexamethasone	18	7	4.21	1.05E-01	1.00E+00
GO:0000002	mitochondrial genome maintenance	32	11	7.49	1.07E-01	1.00E+00
GO:0051646	mitochondrion localization	32	11	7.49	1.07E-01	1.00E+00
GO:1901068	guanosine-containing compound metabolic process	32	11	7.49	1.07E-01	1.00E+00
GO:0006091	generation of precursor metabolites and energy	281	75	65.76	1.07E-01	1.00E+00
GO:0006734	NADH metabolic process	25	9	5.85	1.08E-01	1.00E+00
GO:0032892	positive regulation of organic acid transport	25	9	5.85	1.08E-01	1.00E+00
GO:0044764	multi-organism cellular process	25	9	5.85	1.08E-01	1.00E+00
GO:0043122	regulation of I-kappaB kinase/NF-kappaB signaling	111	32	25.98	1.08E-01	1.00E+00
GO:0042866	pyruvate biosynthetic process	69	21	16.15	1.09E-01	1.00E+00
GO:0043039	tRNA aminoacylation	43	14	10.06	1.10E-01	1.00E+00
GO:0009152	purine ribonucleotide biosynthetic process	162	45	37.91	1.10E-01	1.00E+00
GO:0009119	ribonucleoside metabolic process	58	18	13.57	1.13E-01	1.00E+00
GO:0034404	nucleobase-containing small molecule biosynthetic process	131	37	30.66	1.14E-01	1.00E+00
GO:0002673	regulation of acute inflammatory response	36	12	8.42	1.15E-01	1.00E+00
GO:0006614	SRP-dependent cotranslational protein targeting to membrane	15	6	3.51	1.15E-01	1.00E+00
GO:0006744	ubiquinone biosynthetic process	15	6	3.51	1.15E-01	1.00E+00
GO:0006817	phosphate ion transport	15	6	3.51	1.15E-01	1.00E+00
GO:0035268	protein mannosylation	15	6	3.51	1.15E-01	1.00E+00
GO:0042159	lipoprotein catabolic process	15	6	3.51	1.15E-01	1.00E+00
GO:0051000	positive regulation of nitric-oxide synthase activity	15	6	3.51	1.15E-01	1.00E+00
GO:0060547	negative regulation of necrotic cell death	15	6	3.51	1.15E-01	1.00E+00
GO:1901663	quinone biosynthetic process	15	6	3.51	1.15E-01	1.00E+00
GO:1903358	regulation of Golgi organization	15	6	3.51	1.15E-01	1.00E+00
GO:2000010	positive regulation of protein localization to cell surface	15	6	3.51	1.15E-01	1.00E+00
GO:2000257	regulation of protein activation cascade	15	6	3.51	1.15E-01	1.00E+00
GO:0036503	ERAD pathway	81	24	18.95	1.17E-01	1.00E+00
GO:0019432	triglyceride biosynthetic process	29	10	6.79	1.19E-01	1.00E+00
GO:0016197	endosomal transport	167	46	39.08	1.19E-01	1.00E+00
GO:0001562	response to protozoan	22	8	5.15	1.20E-01	1.00E+00
GO:0001779	natural killer cell differentiation	22	8	5.15	1.20E-01	1.00E+00
GO:0002183	cytoplasmic translational initiation	22	8	5.15	1.20E-01	1.00E+00
GO:0009163	nucleoside biosynthetic process	22	8	5.15	1.20E-01	1.00E+00
GO:0042455	ribonucleoside biosynthetic process	22	8	5.15	1.20E-01	1.00E+00
GO:0070584	mitochondrion morphogenesis	22	8	5.15	1.20E-01	1.00E+00

GO:1902042	negative regulation of extrinsic apoptotic signaling pathway via death domain receptors	22	8	5.15	1.20E-01	1.00E+00
GO:1903319	positive regulation of protein maturation	22	8	5.15	1.20E-01	1.00E+00
GO:0016050	vesicle organization	187	51	43.76	1.21E-01	1.00E+00
GO:0006418	tRNA aminoacylation for protein translation	40	13	9.36	1.22E-01	1.00E+00
GO:0042157	lipoprotein metabolic process	97	28	22.7	1.25E-01	1.00E+00
GO:0002526	acute inflammatory response	74	22	17.32	1.26E-01	1.00E+00
GO:0046916	cellular transition metal ion homeostasis	74	22	17.32	1.26E-01	1.00E+00
GO:0000966	RNA 5'-end processing	12	5	2.81	1.26E-01	1.00E+00
GO:0008334	histone mRNA metabolic process	12	5	2.81	1.26E-01	1.00E+00
GO:0032303	regulation of eicosanoid secretion	12	5	2.81	1.26E-01	1.00E+00
GO:0044090	positive regulation of vacuole organization	12	5	2.81	1.26E-01	1.00E+00
GO:0051767	nitric-oxide synthase biosynthetic process	12	5	2.81	1.26E-01	1.00E+00
GO:0051769	regulation of nitric-oxide synthase biosynthetic process	12	5	2.81	1.26E-01	1.00E+00
GO:0071243	cellular response to arsenic-containing substance	12	5	2.81	1.26E-01	1.00E+00
GO:0071392	cellular response to estradiol stimulus	12	5	2.81	1.26E-01	1.00E+00
GO:0098877	neurotransmitter receptor transport to plasma membrane	12	5	2.81	1.26E-01	1.00E+00
GO:1990823	response to leukemia inhibitory factor	101	29	23.64	1.26E-01	1.00E+00
GO:1990830	cellular response to leukemia inhibitory factor	101	29	23.64	1.26E-01	1.00E+00
GO:0014068	positive regulation of phosphatidylinositol 3-kinase signaling	44	14	10.3	1.28E-01	1.00E+00
GO:0043038	amino acid activation	44	14	10.3	1.28E-01	1.00E+00
GO:0006513	protein monoubiquitination	59	18	13.81	1.29E-01	1.00E+00
GO:1901136	carbohydrate derivative catabolic process	82	24	19.19	1.30E-01	1.00E+00
GO:0086001	cardiac muscle cell action potential	26	9	6.08	1.33E-01	1.00E+00
GO:0045333	cellular respiration	129	36	30.19	1.34E-01	1.00E+00
GO:0007035	vacuolar acidification	19	7	4.45	1.34E-01	1.00E+00
GO:0014823	response to activity	19	7	4.45	1.34E-01	1.00E+00
GO:0032647	regulation of interferon-alpha production	19	7	4.45	1.34E-01	1.00E+00
GO:0032743	positive regulation of interleukin-2 production	19	7	4.45	1.34E-01	1.00E+00
GO:0051180	vitamin transport	19	7	4.45	1.34E-01	1.00E+00
GO:1903793	positive regulation of anion transport	37	12	8.66	1.36E-01	1.00E+00
GO:0001553	luteinization	9	4	2.11	1.37E-01	1.00E+00
GO:0002327	immature B cell differentiation	9	4	2.11	1.37E-01	1.00E+00
GO:0006991	response to sterol depletion	9	4	2.11	1.37E-01	1.00E+00
GO:0009208	pyrimidine ribonucleoside triphosphate metabolic process	9	4	2.11	1.37E-01	1.00E+00
GO:0009595	detection of biotic stimulus	9	4	2.11	1.37E-01	1.00E+00
GO:0018342	protein prenylation	9	4	2.11	1.37E-01	1.00E+00
GO:0019471	4-hydroxyproline metabolic process	9	4	2.11	1.37E-01	1.00E+00
GO:0032306	regulation of prostaglandin secretion	9	4	2.11	1.37E-01	1.00E+00
GO:0032933	SREBP signaling pathway	9	4	2.11	1.37E-01	1.00E+00
GO:0033131	regulation of glucokinase activity	9	4	2.11	1.37E-01	1.00E+00
GO:0033299	secretion of lysosomal enzymes	9	4	2.11	1.37E-01	1.00E+00
GO:0035437	maintenance of protein localization in endoplasmic reticulum	9	4	2.11	1.37E-01	1.00E+00
GO:0042761	very long-chain fatty acid biosynthetic process	9	4	2.11	1.37E-01	1.00E+00

GO:0045076	regulation of interleukin-2 biosynthetic process	9	4	2.11	1.37E-01	1.00E+00
GO:0060712	spongiotrophoblast layer development	9	4	2.11	1.37E-01	1.00E+00
GO:0060850	regulation of transcription involved in cell fate commitment	9	4	2.11	1.37E-01	1.00E+00
GO:0061436	establishment of skin barrier	9	4	2.11	1.37E-01	1.00E+00
GO:0070525	tRNA threonylcarbamoyladenosine metabolic process	9	4	2.11	1.37E-01	1.00E+00
GO:0070862	negative regulation of protein exit from endoplasmic reticulum	9	4	2.11	1.37E-01	1.00E+00
GO:0070986	left/right axis specification	9	4	2.11	1.37E-01	1.00E+00
GO:0071044	histone mRNA catabolic process	9	4	2.11	1.37E-01	1.00E+00
GO:0071501	cellular response to sterol depletion	9	4	2.11	1.37E-01	1.00E+00
GO:0097354	prenylation	9	4	2.11	1.37E-01	1.00E+00
GO:0043123	positive regulation of I-kappaB kinase/NF-kappaB signaling	71	21	16.61	1.38E-01	1.00E+00
GO:0072522	purine-containing compound biosynthetic process	173	47	40.48	1.38E-01	1.00E+00
GO:0019221	cytokine-mediated signaling pathway	197	53	46.1	1.39E-01	1.00E+00
GO:0035418	protein localization to synapse	41	13	9.59	1.42E-01	1.00E+00
GO:0042108	positive regulation of cytokine biosynthetic process	41	13	9.59	1.42E-01	1.00E+00
GO:0098927	vesicle-mediated transport between endosomal compartments	41	13	9.59	1.42E-01	1.00E+00
GO:0033059	cellular pigmentation	30	10	7.02	1.43E-01	1.00E+00
GO:0001660	fever generation	6	3	1.4	1.45E-01	1.00E+00
GO:0002372	myeloid dendritic cell cytokine production	6	3	1.4	1.45E-01	1.00E+00
GO:0002732	positive regulation of dendritic cell cytokine production	6	3	1.4	1.45E-01	1.00E+00
GO:0002733	regulation of myeloid dendritic cell cytokine production	6	3	1.4	1.45E-01	1.00E+00
GO:0002735	positive regulation of myeloid dendritic cell cytokine production	6	3	1.4	1.45E-01	1.00E+00
GO:0006102	isocitrate metabolic process	6	3	1.4	1.45E-01	1.00E+00
GO:0006607	NLS-bearing protein import into nucleus	6	3	1.4	1.45E-01	1.00E+00
GO:0006621	protein retention in ER lumen	6	3	1.4	1.45E-01	1.00E+00
GO:0009128	purine nucleoside monophosphate catabolic process	6	3	1.4	1.45E-01	1.00E+00
GO:0009158	ribonucleoside monophosphate catabolic process	6	3	1.4	1.45E-01	1.00E+00
GO:0009169	purine ribonucleoside monophosphate catabolic process	6	3	1.4	1.45E-01	1.00E+00
GO:0010807	regulation of synaptic vesicle priming	6	3	1.4	1.45E-01	1.00E+00
GO:0014841	skeletal muscle satellite cell proliferation	6	3	1.4	1.45E-01	1.00E+00
GO:0014842	regulation of skeletal muscle satellite cell proliferation	6	3	1.4	1.45E-01	1.00E+00
GO:0014866	skeletal myofibril assembly	6	3	1.4	1.45E-01	1.00E+00

Supplemental Table 3

Table 2.S3. Enriched KEGG pathways from Cluster 3 from the k-means cluster analysis of genes expressed in the liver averaged from all groups (CV and GF, male and female, and days 1, 5, 10, 15, 25, 60, and 120).

GO.ID	Term	Annotated	Significant	Expected	result1	FDR
GO:0048729	tissue morphogenesis	386	182	79.93	1.00E-30	7.33E-27
GO:0000904	cell morphogenesis involved in differentiation	454	199	94.01	4.00E-30	1.47E-26
GO:0001944	vasculature development	497	206	102.91	3.30E-27	8.07E-24
GO:0001568	blood vessel development	475	199	98.36	5.30E-27	9.72E-24
GO:0007507	heart development	391	173	80.96	8.30E-27	1.22E-23
GO:0048812	neuron projection morphogenesis	364	163	75.37	5.30E-26	6.48E-23
GO:0048858	cell projection morphogenesis	380	167	78.69	1.70E-25	1.78E-22
GO:0120039	plasma membrane bounded cell projection morphogenesis	376	165	77.86	4.10E-25	3.50E-22
GO:0048514	blood vessel morphogenesis	412	176	85.31	4.30E-25	3.50E-22
GO:0048667	cell morphogenesis involved in neuron differentiation	335	152	69.37	5.10E-25	3.74E-22
GO:0002009	morphogenesis of an epithelium	327	148	67.71	3.00E-24	2.00E-21
GO:0032990	cell part morphogenesis	399	170	82.62	4.20E-24	2.57E-21
GO:0070848	response to growth factor	386	164	79.93	4.00E-23	2.26E-20
GO:0060322	head development	372	158	77.03	2.80E-22	1.47E-19
GO:0003007	heart morphogenesis	162	89	33.54	5.00E-22	2.44E-19
GO:0060485	mesenchyme development	160	88	33.13	7.70E-22	3.53E-19
GO:0071363	cellular response to growth factor stimulus	375	157	77.65	2.20E-21	9.49E-19
GO:0030036	actin cytoskeleton organization	467	183	96.7	6.40E-21	2.61E-18
GO:0097435	supramolecular fiber organization	463	181	95.87	1.40E-20	5.40E-18
GO:0061564	axon development	262	120	54.25	2.30E-20	8.43E-18
GO:0048762	mesenchymal cell differentiation	134	76	27.75	4.20E-20	1.47E-17
GO:0001667	ameboidal-type cell migration	286	127	59.22	4.60E-20	1.53E-17
GO:0022604	regulation of cell morphogenesis	356	148	73.72	7.30E-20	2.33E-17
GO:0001525	angiogenesis	344	144	71.23	1.10E-19	3.36E-17
GO:0007409	axonogenesis	237	110	49.07	2.70E-19	7.90E-17
GO:0045664	regulation of neuron differentiation	453	175	93.8	2.80E-19	7.90E-17
GO:0090287	regulation of cellular response to growth factor stimulus	179	90	37.06	7.90E-19	2.15E-16
GO:0061061	muscle structure development	412	162	85.31	8.90E-19	2.33E-16
GO:0007417	central nervous system development	465	177	96.29	1.00E-18	2.53E-16
GO:0007420	brain development	335	139	69.37	1.30E-18	3.18E-16
GO:0060562	epithelial tube morphogenesis	235	108	48.66	1.40E-18	3.31E-16
GO:0030855	epithelial cell differentiation	333	138	68.95	1.90E-18	4.35E-16
GO:0040017	positive regulation of locomotion	395	156	81.79	2.50E-18	5.56E-16
GO:2000147	positive regulation of cell motility	374	149	77.44	6.00E-18	1.29E-15
GO:0030335	positive regulation of cell migration	368	147	76.2	7.60E-18	1.59E-15
GO:0051272	positive regulation of cellular component movement	383	151	79.31	1.10E-17	2.24E-15
GO:0031589	cell-substrate adhesion	235	106	48.66	1.60E-17	3.09E-15
GO:0120035	regulation of plasma membrane bounded cell projection organization	483	179	100.01	1.60E-17	3.09E-15

GO:0030155	regulation of cell adhesion	430	164	89.04	1.70E-17	3.20E-15
GO:0031344	regulation of cell projection organization	487	180	100.84	1.80E-17	3.30E-15
GO:0060541	respiratory system development	151	78	31.27	2.30E-17	4.11E-15
GO:0048598	embryonic morphogenesis	345	138	71.44	7.20E-17	1.26E-14
GO:0030198	extracellular matrix organization	142	74	29.4	8.20E-17	1.40E-14
GO:0051271	negative regulation of cellular component movement	193	91	39.96	1.00E-16	1.67E-14
GO:0030323	respiratory tube development	141	73	29.2	2.10E-16	3.42E-14
GO:0001501	skeletal system development	282	118	58.39	2.70E-16	4.21E-14
GO:0072001	renal system development	177	85	36.65	2.70E-16	4.21E-14
GO:0040013	negative regulation of locomotion	206	94	42.66	4.50E-16	6.88E-14
GO:0010975	regulation of neuron projection development	371	143	76.82	8.10E-16	1.21E-13
GO:0030324	lung development	138	71	28.58	8.40E-16	1.23E-13
GO:0007264	small GTPase mediated signal transduction	361	140	74.75	9.10E-16	1.31E-13
GO:0001822	kidney development	165	80	34.17	1.00E-15	1.41E-13
GO:0048568	embryonic organ development	257	109	53.22	1.20E-15	1.66E-13
GO:0007411	axon guidance	97	56	20.09	1.30E-15	1.70E-13
GO:0097485	neuron projection guidance	97	56	20.09	1.30E-15	1.70E-13
GO:0034330	cell junction organization	190	88	39.34	1.30E-15	1.70E-13
GO:0003205	cardiac chamber development	119	64	24.64	1.40E-15	1.80E-13
GO:0030900	forebrain development	194	89	40.17	1.80E-15	2.28E-13
GO:0003151	outflow tract morphogenesis	51	37	10.56	2.20E-15	2.73E-13
GO:0001655	urogenital system development	204	92	42.24	2.30E-15	2.81E-13
GO:0007160	cell-matrix adhesion	149	74	30.85	2.40E-15	2.89E-13
GO:0030336	negative regulation of cell migration	167	80	34.58	2.50E-15	2.91E-13
GO:0034329	cell junction assembly	155	76	32.1	2.50E-15	2.91E-13
GO:0030031	cell projection assembly	303	122	62.74	2.70E-15	3.09E-13
GO:0060840	artery development	70	45	14.49	2.90E-15	3.27E-13
GO:0007178	transmembrane receptor protein serine/threonine kinase signaling pathway	208	93	43.07	3.10E-15	3.44E-13
GO:2000146	negative regulation of cell motility	176	82	36.44	8.10E-15	8.87E-13
GO:0120031	plasma membrane bounded cell projection assembly	297	119	61.5	9.20E-15	9.92E-13
GO:0010810	regulation of cell-substrate adhesion	150	73	31.06	1.50E-14	1.59E-12
GO:0003279	cardiac septum development	86	50	17.81	2.90E-14	3.04E-12
GO:0001763	morphogenesis of a branching structure	140	69	28.99	3.50E-14	3.62E-12
GO:0060537	muscle tissue development	278	112	57.56	3.70E-14	3.77E-12
GO:0007265	Ras protein signal transduction	309	121	63.98	3.90E-14	3.92E-12
GO:0003206	cardiac chamber morphogenesis	84	49	17.39	4.40E-14	4.29E-12
GO:0010631	epithelial cell migration	209	91	43.28	4.50E-14	4.29E-12
GO:0090130	tissue migration	209	91	43.28	4.50E-14	4.29E-12
GO:0090132	epithelium migration	209	91	43.28	4.50E-14	4.29E-12
GO:0071559	response to transforming growth factor beta	156	74	32.3	5.30E-14	4.98E-12
GO:0006935	chemotaxis	294	116	60.88	7.20E-14	6.68E-12
GO:0007179	transforming growth factor beta receptor signaling pathway	125	63	25.88	1.20E-13	1.10E-11
GO:0042330	taxis	296	116	61.29	1.30E-13	1.16E-11

GO:0048844	artery morphogenesis	46	33	9.53	1.30E-13	1.16E-11
GO:0098609	cell-cell adhesion	396	144	82	1.60E-13	1.41E-11
GO:0048589	developmental growth	481	167	99.6	1.90E-13	1.66E-11
GO:0007389	pattern specification process	201	87	41.62	2.40E-13	2.07E-11
GO:0007423	sensory organ development	272	108	56.32	3.30E-13	2.81E-11
GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway	367	135	75.99	3.60E-13	3.03E-11
GO:0001837	epithelial to mesenchymal transition	93	51	19.26	3.80E-13	3.17E-11
GO:0050808	synapse organization	237	97	49.07	6.50E-13	5.36E-11
GO:0071560	cellular response to transforming growth factor beta stimulus	153	71	31.68	6.70E-13	5.46E-11
GO:0090092	regulation of transmembrane receptor protein serine/threonine kinase signaling pathway	138	66	28.58	7.60E-13	6.13E-11
GO:0045596	negative regulation of cell differentiation	448	156	92.77	9.40E-13	7.49E-11
GO:0001764	neuron migration	95	51	19.67	1.20E-12	9.36E-11
GO:0044089	positive regulation of cellular component biogenesis	365	133	75.58	1.20E-12	9.36E-11
GO:0051962	positive regulation of nervous system development	366	133	75.79	1.50E-12	1.16E-10
GO:1905114	cell surface receptor signaling pathway involved in cell-cell signaling	302	115	62.53	1.60E-12	1.21E-10
GO:0001654	eye development	187	81	38.72	1.60E-12	1.21E-10
GO:0014706	striated muscle tissue development	261	103	54.04	1.90E-12	1.42E-10
GO:0003231	cardiac ventricle development	85	47	17.6	2.20E-12	1.60E-10
GO:0051216	cartilage development	99	52	20.5	2.20E-12	1.60E-10
GO:0001503	ossification	211	88	43.69	2.20E-12	1.60E-10
GO:0060411	cardiac septum morphogenesis	54	35	11.18	2.50E-12	1.80E-10
GO:0048880	sensory system development	189	81	39.14	3.10E-12	2.19E-10
GO:0150063	visual system development	189	81	39.14	3.10E-12	2.19E-10
GO:0043062	extracellular structure organization	180	78	37.27	4.00E-12	2.79E-10
GO:0010769	regulation of cell morphogenesis involved in differentiation	220	90	45.55	4.60E-12	3.18E-10
GO:0016358	dendrite development	187	80	38.72	4.70E-12	3.22E-10
GO:0051493	regulation of cytoskeleton organization	387	137	80.13	6.40E-12	4.35E-10
GO:0061138	morphogenesis of a branching epithelium	128	61	26.5	6.90E-12	4.64E-10
GO:0071772	response to BMP	79	44	16.36	7.80E-12	5.15E-10
GO:0071773	cellular response to BMP stimulus	79	44	16.36	7.80E-12	5.15E-10
GO:0043010	camera-type eye development	166	73	34.37	8.30E-12	5.44E-10
GO:0050769	positive regulation of neurogenesis	346	125	71.65	1.20E-11	7.79E-10
GO:0090288	negative regulation of cellular response to growth factor stimulus	91	48	18.84	1.30E-11	8.36E-10
GO:0198738	cell-cell signaling by wnt	262	101	54.25	1.60E-11	1.02E-09
GO:0030510	regulation of BMP signaling pathway	54	34	11.18	1.70E-11	1.07E-09
GO:0072073	kidney epithelium development	75	42	15.53	1.80E-11	1.13E-09
GO:0010721	negative regulation of cell development	222	89	45.97	2.30E-11	1.43E-09
GO:0010720	positive regulation of cell development	397	138	82.21	2.40E-11	1.48E-09
GO:0048754	branching morphogenesis of an epithelial tube	107	53	22.16	2.60E-11	1.59E-09
GO:0007015	actin filament organization	282	106	58.39	3.00E-11	1.82E-09
GO:0016055	Wnt signaling pathway	261	100	54.04	3.10E-11	1.86E-09
GO:0002064	epithelial cell development	154	68	31.89	3.40E-11	2.03E-09
GO:0003281	ventricular septum development	55	34	11.39	3.70E-11	2.19E-09

GO:0002062	chondrocyte differentiation	63	37	13.05	4.40E-11	2.58E-09
GO:0001952	regulation of cell-matrix adhesion	88	46	18.22	5.00E-11	2.91E-09
GO:0003272	endocardial cushion formation	15	15	3.11	5.30E-11	3.06E-09
GO:0034332	adherens junction organization	97	49	20.09	5.90E-11	3.38E-09
GO:2000027	regulation of animal organ morphogenesis	109	53	22.57	6.30E-11	3.58E-09
GO:0003197	endocardial cushion development	30	23	6.21	7.50E-11	4.20E-09
GO:0072132	mesenchyme morphogenesis	30	23	6.21	7.50E-11	4.20E-09
GO:0048736	appendage development	95	48	19.67	9.20E-11	5.07E-09
GO:0060173	limb development	95	48	19.67	9.20E-11	5.07E-09
GO:0060560	developmental growth involved in morphogenesis	170	72	35.2	9.80E-11	5.36E-09
GO:0051961	negative regulation of nervous system development	207	83	42.86	1.10E-10	5.98E-09
GO:0042692	muscle cell differentiation	235	91	48.66	1.20E-10	6.47E-09
GO:0061448	connective tissue development	145	64	30.02	1.30E-10	6.96E-09
GO:0048705	skeletal system morphogenesis	117	55	24.23	1.40E-10	7.44E-09
GO:0031032	actomyosin structure organization	139	62	28.78	1.50E-10	7.86E-09
GO:0001570	vasculogenesis	65	37	13.46	1.50E-10	7.86E-09
GO:0030509	BMP signaling pathway	76	41	15.74	1.60E-10	8.32E-09
GO:0031346	positive regulation of cell projection organization	297	108	61.5	2.00E-10	1.03E-08
GO:0060070	canonical Wnt signaling pathway	169	71	34.99	2.00E-10	1.03E-08
GO:0060348	bone development	137	61	28.37	2.30E-10	1.17E-08
GO:0007266	Rho protein signal transduction	147	64	30.44	2.70E-10	1.37E-08
GO:0051056	regulation of small GTPase mediated signal transduction	190	77	39.34	2.80E-10	1.40E-08
GO:0045666	positive regulation of neuron differentiation	277	102	57.36	2.80E-10	1.40E-08
GO:0060828	regulation of canonical Wnt signaling pathway	141	62	29.2	3.10E-10	1.54E-08
GO:0003203	endocardial cushion morphogenesis	21	18	4.35	3.20E-10	1.58E-08
GO:0043542	endothelial cell migration	151	65	31.27	3.50E-10	1.70E-08
GO:0035909	aorta morphogenesis	23	19	4.76	3.50E-10	1.70E-08
GO:0010632	regulation of epithelial cell migration	168	70	34.79	4.30E-10	2.07E-08
GO:0045446	endothelial cell differentiation	81	42	16.77	4.90E-10	2.35E-08
GO:0003179	heart valve morphogenesis	34	24	7.04	5.10E-10	2.43E-08
GO:0035904	aorta development	46	29	9.53	5.20E-10	2.46E-08
GO:0003158	endothelium development	90	45	18.64	5.50E-10	2.59E-08
GO:0003013	circulatory system process	255	95	52.8	5.60E-10	2.62E-08
GO:0060412	ventricular septum morphogenesis	32	23	6.63	6.60E-10	3.06E-08
GO:0007517	muscle organ development	231	88	47.83	6.70E-10	3.09E-08
GO:0034333	adherens junction assembly	76	40	15.74	7.00E-10	3.21E-08
GO:0032970	regulation of actin filament-based process	274	100	56.74	7.40E-10	3.37E-08
GO:0048638	regulation of developmental growth	246	92	50.94	8.40E-10	3.80E-08
GO:0043087	regulation of GTPase activity	205	80	42.45	1.10E-09	4.95E-08
GO:0001649	osteoblast differentiation	110	51	22.78	1.20E-09	5.30E-08
GO:0048813	dendrite morphogenesis	110	51	22.78	1.20E-09	5.30E-08
GO:0030111	regulation of Wnt signaling pathway	185	74	38.31	1.20E-09	5.30E-08
GO:0050678	regulation of epithelial cell proliferation	223	85	46.18	1.30E-09	5.58E-08

GO:1901342	regulation of vasculature development	223	85	46.18	1.30E-09	5.58E-08
GO:0030278	regulation of ossification	129	57	26.71	1.30E-09	5.58E-08
GO:0050768	negative regulation of neurogenesis	192	76	39.76	1.30E-09	5.58E-08
GO:0003148	outflow tract septum morphogenesis	20	17	4.14	1.30E-09	5.58E-08
GO:0021537	telencephalon development	136	59	28.16	1.60E-09	6.82E-08
GO:0048732	gland development	278	100	57.56	1.80E-09	7.63E-08
GO:0001657	ureteric bud development	53	31	10.97	1.90E-09	7.92E-08
GO:0072163	mesonephric epithelium development	53	31	10.97	1.90E-09	7.92E-08
GO:0072164	mesonephric tubule development	53	31	10.97	1.90E-09	7.92E-08
GO:0023014	signal transduction by protein phosphorylation	494	157	102.29	2.10E-09	8.70E-08
GO:1902903	regulation of supramolecular fiber organization	250	92	51.77	2.20E-09	9.06E-08
GO:0000165	MAPK cascade	479	153	99.18	2.30E-09	9.37E-08
GO:0030199	collagen fibril organization	31	22	6.42	2.30E-09	9.37E-08
GO:0035265	organ growth	134	58	27.75	2.40E-09	9.72E-08
GO:0040008	regulation of growth	458	147	94.84	3.40E-09	1.36E-07
GO:0003170	heart valve development	41	26	8.49	3.40E-09	1.36E-07
GO:0045765	regulation of angiogenesis	206	79	42.66	3.50E-09	1.40E-07
GO:0001823	mesonephros development	54	31	11.18	3.60E-09	1.41E-07
GO:0008015	blood circulation	245	90	50.73	3.60E-09	1.41E-07
GO:0003002	regionalization	165	67	34.17	3.60E-09	1.41E-07
GO:0050770	regulation of axonogenesis	126	55	26.09	4.30E-09	1.68E-07
GO:0008285	negative regulation of cell proliferation	425	138	88	4.50E-09	1.75E-07
GO:0032956	regulation of actin cytoskeleton organization	247	90	51.15	5.80E-09	2.24E-07
GO:0050807	regulation of synapse organization	144	60	29.82	7.50E-09	2.88E-07
GO:0007162	negative regulation of cell adhesion	178	70	36.86	8.10E-09	3.09E-07
GO:0048562	embryonic organ morphogenesis	141	59	29.2	8.30E-09	3.15E-07
GO:0046578	regulation of Ras protein signal transduction	168	67	34.79	8.70E-09	3.29E-07
GO:0045165	cell fate commitment	106	48	21.95	9.80E-09	3.69E-07
GO:0048706	embryonic skeletal system development	53	30	10.97	1.00E-08	3.74E-07
GO:0045216	cell-cell junction organization	94	44	19.46	1.20E-08	4.47E-07
GO:0060976	coronary vasculature development	48	28	9.94	1.30E-08	4.82E-07
GO:0010718	positive regulation of epithelial to mesenchymal transition	33	22	6.83	1.40E-08	5.16E-07
GO:0031345	negative regulation of cell projection organization	133	56	27.54	1.50E-08	5.50E-07
GO:0048738	cardiac muscle tissue development	160	64	33.13	1.60E-08	5.84E-07
GO:0008360	regulation of cell shape	117	51	24.23	1.70E-08	6.11E-07
GO:0008544	epidermis development	150	61	31.06	1.70E-08	6.11E-07
GO:0048588	developmental cell growth	167	66	34.58	1.70E-08	6.11E-07
GO:0050803	regulation of synapse structure or activity	147	60	30.44	1.90E-08	6.80E-07
GO:0051017	actin filament bundle assembly	121	52	25.06	2.20E-08	7.79E-07
GO:0051145	smooth muscle cell differentiation	41	25	8.49	2.20E-08	7.79E-07
GO:0060317	cardiac epithelial to mesenchymal transition	18	15	3.73	2.30E-08	8.11E-07
GO:0010717	regulation of epithelial to mesenchymal transition	63	33	13.05	2.50E-08	8.77E-07
GO:0035107	appendage morphogenesis	84	40	17.39	2.90E-08	1.00E-06

GO:0035108	limb morphogenesis	84	40	17.39	2.90E-08	1.00E-06
GO:0045785	positive regulation of cell adhesion	258	91	53.42	2.90E-08	1.00E-06
GO:0007044	cell-substrate junction assembly	75	37	15.53	3.00E-08	1.02E-06
GO:0017015	regulation of transforming growth factor beta receptor signaling pathway	78	38	16.15	3.00E-08	1.02E-06
GO:0061572	actin filament bundle organization	122	52	25.26	3.00E-08	1.02E-06
GO:0043408	regulation of MAPK cascade	442	139	91.52	3.80E-08	1.29E-06
GO:0022612	gland morphogenesis	94	43	19.46	4.00E-08	1.35E-06
GO:0016049	cell growth	338	112	69.99	4.00E-08	1.35E-06
GO:0003012	muscle system process	216	79	44.73	4.10E-08	1.37E-06
GO:0010811	positive regulation of cell-substrate adhesion	91	42	18.84	4.20E-08	1.40E-06
GO:0007163	establishment or maintenance of cell polarity	160	63	33.13	4.30E-08	1.43E-06
GO:0007043	cell-cell junction assembly	67	34	13.87	4.40E-08	1.45E-06
GO:0002040	sprouting angiogenesis	82	39	16.98	4.60E-08	1.50E-06
GO:0045667	regulation of osteoblast differentiation	79	38	16.36	4.60E-08	1.50E-06
GO:1903844	regulation of cellular response to transforming growth factor beta stimulus	79	38	16.36	4.60E-08	1.50E-06
GO:0045995	regulation of embryonic development	76	37	15.74	4.70E-08	1.53E-06
GO:0050877	nervous system process	378	122	78.27	5.40E-08	1.74E-06
GO:0010976	positive regulation of neuron projection development	232	83	48.04	5.80E-08	1.87E-06
GO:0003208	cardiac ventricle morphogenesis	40	24	8.28	6.60E-08	2.11E-06
GO:0014031	mesenchymal cell development	48	27	9.94	6.70E-08	2.14E-06
GO:0050673	epithelial cell proliferation	266	92	55.08	7.20E-08	2.29E-06
GO:0002063	chondrocyte development	21	16	4.35	7.60E-08	2.40E-06
GO:0035329	hippo signaling	28	19	5.8	9.30E-08	2.93E-06
GO:0007186	G protein-coupled receptor signaling pathway	227	81	47	9.60E-08	3.00E-06
GO:0048863	stem cell differentiation	119	50	24.64	9.60E-08	3.00E-06
GO:0090101	negative regulation of transmembrane receptor protein serine/threonine kinase signaling pathway	66	33	13.67	1.10E-07	3.42E-06
GO:0014033	neural crest cell differentiation	49	27	10.15	1.20E-07	3.68E-06
GO:0048864	stem cell development	49	27	10.15	1.20E-07	3.68E-06
GO:0010959	regulation of metal ion transport	210	76	43.48	1.20E-07	3.68E-06
GO:0090066	regulation of anatomical structure size	349	113	72.27	1.40E-07	4.26E-06
GO:0044057	regulation of system process	292	98	60.46	1.40E-07	4.26E-06
GO:0035051	cardiocyte differentiation	117	49	24.23	1.50E-07	4.53E-06
GO:0050920	regulation of chemotaxis	117	49	24.23	1.50E-07	4.53E-06
GO:0045665	negative regulation of neuron differentiation	151	59	31.27	1.60E-07	4.75E-06
GO:0001738	morphogenesis of a polarized epithelium	44	25	9.11	1.60E-07	4.75E-06
GO:0048565	digestive tract development	58	30	12.01	1.60E-07	4.75E-06
GO:1901343	negative regulation of vasculature development	82	38	16.98	1.60E-07	4.75E-06
GO:0007045	cell-substrate adherens junction assembly	67	33	13.87	1.70E-07	5.01E-06
GO:0048041	focal adhesion assembly	67	33	13.87	1.70E-07	5.01E-06
GO:0060350	endochondral bone morphogenesis	39	23	8.08	1.90E-07	5.57E-06
GO:0021953	central nervous system neuron differentiation	92	41	19.05	2.00E-07	5.84E-06
GO:0008542	visual learning	29	19	6	2.20E-07	6.30E-06
GO:0010464	regulation of mesenchymal cell proliferation	29	19	6	2.20E-07	6.30E-06

GO:0010463	mesenchymal cell proliferation	34	21	7.04	2.20E-07	6.30E-06
GO:0055001	muscle cell development	115	48	23.81	2.20E-07	6.30E-06
GO:0006936	muscle contraction	142	56	29.4	2.20E-07	6.30E-06
GO:0060415	muscle tissue morphogenesis	42	24	8.7	2.40E-07	6.85E-06
GO:1903391	regulation of adherens junction organization	56	29	11.6	2.50E-07	7.11E-06
GO:2000181	negative regulation of blood vessel morphogenesis	77	36	15.94	2.60E-07	7.36E-06
GO:0055123	digestive system development	68	33	14.08	2.70E-07	7.62E-06
GO:0048013	ephrin receptor signaling pathway	20	15	4.14	2.80E-07	7.87E-06
GO:0003184	pulmonary valve morphogenesis	12	11	2.48	2.90E-07	8.12E-06
GO:0061005	cell differentiation involved in kidney development	27	18	5.59	3.10E-07	8.64E-06
GO:0003171	atrioventricular valve development	18	14	3.73	3.40E-07	9.45E-06
GO:0010977	negative regulation of neuron projection development	110	46	22.78	3.70E-07	1.02E-05
GO:0048286	lung alveolus development	40	23	8.28	3.70E-07	1.02E-05
GO:0003177	pulmonary valve development	16	13	3.31	3.70E-07	1.02E-05
GO:0110110	positive regulation of animal organ morphogenesis	54	28	11.18	3.80E-07	1.03E-05
GO:0030038	contractile actin filament bundle assembly	78	36	16.15	3.80E-07	1.03E-05
GO:0043149	stress fiber assembly	78	36	16.15	3.80E-07	1.03E-05
GO:0016525	negative regulation of angiogenesis	75	35	15.53	4.00E-07	1.08E-05
GO:1901888	regulation of cell junction assembly	69	33	14.29	4.20E-07	1.13E-05
GO:0007405	neuroblast proliferation	35	21	7.25	4.40E-07	1.18E-05
GO:0048675	axon extension	88	39	18.22	4.70E-07	1.26E-05
GO:1990138	neuron projection extension	121	49	25.06	5.00E-07	1.33E-05
GO:0014032	neural crest cell development	46	25	9.53	5.00E-07	1.33E-05
GO:0035050	embryonic heart tube development	49	26	10.15	5.50E-07	1.46E-05
GO:0002042	cell migration involved in sprouting angiogenesis	38	22	7.87	5.60E-07	1.47E-05
GO:1901379	regulation of potassium ion transmembrane transport	38	22	7.87	5.60E-07	1.47E-05
GO:0060349	bone morphogenesis	58	29	12.01	6.50E-07	1.69E-05
GO:0030326	embryonic limb morphogenesis	67	32	13.87	6.50E-07	1.69E-05
GO:0035113	embryonic appendage morphogenesis	67	32	13.87	6.50E-07	1.69E-05
GO:0110053	regulation of actin filament organization	185	67	38.31	6.60E-07	1.71E-05
GO:0003198	epithelial to mesenchymal transition involved in endocardial cushion formation	9	9	1.86	6.90E-07	1.78E-05
GO:0060977	coronary vasculature morphogenesis	21	15	4.35	8.00E-07	2.06E-05
GO:0060271	cilium assembly	154	58	31.89	8.50E-07	2.18E-05
GO:0048704	embryonic skeletal system morphogenesis	36	21	7.45	8.60E-07	2.20E-05
GO:0032231	regulation of actin filament bundle assembly	74	34	15.32	9.10E-07	2.31E-05
GO:0072006	nephron development	74	34	15.32	9.10E-07	2.31E-05
GO:0043266	regulation of potassium ion transport	50	26	10.35	9.20E-07	2.31E-05
GO:0051893	regulation of focal adhesion assembly	50	26	10.35	9.20E-07	2.31E-05
GO:0090109	regulation of cell-substrate junction assembly	50	26	10.35	9.20E-07	2.31E-05
GO:0007632	visual behavior	31	19	6.42	1.00E-06	2.46E-05
GO:0030513	positive regulation of BMP signaling pathway	26	17	5.38	1.00E-06	2.46E-05
GO:0060351	cartilage development involved in endochondral bone morphogenesis	26	17	5.38	1.00E-06	2.46E-05
GO:0001935	endothelial cell proliferation	100	42	20.71	1.00E-06	2.46E-05

GO:0001936	regulation of endothelial cell proliferation	87	38	18.01	1.00E-06	2.46E-05
GO:0048745	smooth muscle tissue development	19	14	3.93	1.00E-06	2.46E-05
GO:0044782	cilium organization	162	60	33.54	1.10E-06	2.70E-05
GO:0030512	negative regulation of transforming growth factor beta receptor signaling pathway	45	24	9.32	1.30E-06	3.15E-05
GO:0048644	muscle organ morphogenesis	45	24	9.32	1.30E-06	3.15E-05
GO:0098742	cell-cell adhesion via plasma-membrane adhesion molecules	91	39	18.84	1.30E-06	3.15E-05
GO:0110020	regulation of actomyosin structure organization	75	34	15.53	1.30E-06	3.15E-05
GO:0051146	striated muscle cell differentiation	181	65	37.48	1.40E-06	3.32E-05
GO:0050773	regulation of dendrite development	128	50	26.5	1.40E-06	3.32E-05
GO:0033002	muscle cell proliferation	156	58	32.3	1.40E-06	3.32E-05
GO:0030516	regulation of axon extension	72	33	14.91	1.40E-06	3.32E-05
GO:0030947	regulation of vascular endothelial growth factor receptor signaling pathway	24	16	4.97	1.40E-06	3.32E-05
GO:2000826	regulation of heart morphogenesis	24	16	4.97	1.40E-06	3.32E-05
GO:0030178	negative regulation of Wnt signaling pathway	88	38	18.22	1.50E-06	3.47E-05
GO:0003413	chondrocyte differentiation involved in endochondral bone morphogenesis	15	12	3.11	1.50E-06	3.47E-05
GO:0010092	specification of animal organ identity	15	12	3.11	1.50E-06	3.47E-05
GO:0010594	regulation of endothelial cell migration	118	47	24.43	1.50E-06	3.47E-05
GO:0001755	neural crest cell migration	29	18	6	1.50E-06	3.47E-05
GO:0060841	venous blood vessel development	13	11	2.69	1.50E-06	3.47E-05
GO:0061298	retina vasculature development in camera-type eye	13	11	2.69	1.50E-06	3.47E-05
GO:0009799	specification of symmetry	66	31	13.67	1.50E-06	3.47E-05
GO:0043534	blood vessel endothelial cell migration	85	37	17.6	1.60E-06	3.69E-05
GO:0008217	regulation of blood pressure	82	36	16.98	1.70E-06	3.91E-05
GO:0060389	pathway-restricted SMAD protein phosphorylation	40	22	8.28	1.90E-06	4.35E-05
GO:0021795	cerebral cortex cell migration	32	19	6.63	2.00E-06	4.56E-05
GO:0003416	endochondral bone growth	22	15	4.56	2.00E-06	4.56E-05
GO:0007156	homophilic cell adhesion via plasma membrane adhesion molecules	43	23	8.9	2.10E-06	4.75E-05
GO:0035023	regulation of Rho protein signal transduction	99	41	20.5	2.10E-06	4.75E-05
GO:0030514	negative regulation of BMP signaling pathway	27	17	5.59	2.20E-06	4.95E-05
GO:1903845	negative regulation of cellular response to transforming growth factor beta stimulus	46	24	9.53	2.20E-06	4.95E-05
GO:0043588	skin development	137	52	28.37	2.40E-06	5.37E-05
GO:0006939	smooth muscle contraction	49	25	10.15	2.40E-06	5.37E-05
GO:0060043	regulation of cardiac muscle cell proliferation	35	20	7.25	2.50E-06	5.54E-05
GO:0061383	trabecula morphogenesis	35	20	7.25	2.50E-06	5.54E-05
GO:0001704	formation of primary germ layer	55	27	11.39	2.50E-06	5.54E-05
GO:0061387	regulation of extent of cell growth	80	35	16.57	2.60E-06	5.74E-05
GO:0048640	negative regulation of developmental growth	77	34	15.94	2.80E-06	6.15E-05
GO:0021543	pallium development	90	38	18.64	2.80E-06	6.15E-05
GO:0022029	telencephalon cell migration	38	21	7.87	2.90E-06	6.35E-05
GO:0018209	peptidyl-serine modification	222	75	45.97	3.10E-06	6.77E-05
GO:0090090	negative regulation of canonical Wnt signaling pathway	71	32	14.7	3.20E-06	6.96E-05
GO:0018105	peptidyl-serine phosphorylation	211	72	43.69	3.30E-06	7.12E-05
GO:0048251	elastic fiber assembly	8	8	1.66	3.30E-06	7.12E-05

GO:0060947	cardiac vascular smooth muscle cell differentiation	8	8	1.66	3.30E-06	7.12E-05
GO:0021987	cerebral cortex development	68	31	14.08	3.40E-06	7.29E-05
GO:0032535	regulation of cellular component size	268	87	55.49	3.40E-06	7.29E-05
GO:0009855	determination of bilateral symmetry	65	30	13.46	3.50E-06	7.44E-05
GO:0051492	regulation of stress fiber assembly	65	30	13.46	3.50E-06	7.44E-05
GO:0043410	positive regulation of MAPK cascade	303	96	62.74	3.50E-06	7.44E-05
GO:0021954	central nervous system neuron development	47	24	9.73	3.70E-06	7.84E-05
GO:0032835	glomerulus development	33	19	6.83	3.80E-06	7.94E-05
GO:0048010	vascular endothelial growth factor receptor signaling pathway	33	19	6.83	3.80E-06	7.94E-05
GO:0048645	animal organ formation	33	19	6.83	3.80E-06	7.94E-05
GO:0007368	determination of left/right symmetry	59	28	12.22	3.80E-06	7.94E-05
GO:0050679	positive regulation of epithelial cell proliferation	118	46	24.43	3.80E-06	7.94E-05
GO:0061351	neural precursor cell proliferation	101	41	20.91	3.90E-06	8.13E-05
GO:0008361	regulation of cell size	136	51	28.16	4.40E-06	9.12E-05
GO:0021510	spinal cord development	36	20	7.45	4.40E-06	9.12E-05
GO:0090596	sensory organ morphogenesis	129	49	26.71	4.50E-06	9.27E-05
GO:1901016	regulation of potassium ion transmembrane transporter activity	28	17	5.8	4.50E-06	9.27E-05
GO:0098868	bone growth	23	15	4.76	4.70E-06	9.63E-05
GO:1902667	regulation of axon guidance	23	15	4.76	4.70E-06	9.63E-05
GO:0003181	atrioventricular valve morphogenesis	16	12	3.31	4.80E-06	9.81E-05
GO:0016331	morphogenesis of embryonic epithelium	112	44	23.19	4.90E-06	9.95E-05
GO:0032963	collagen metabolic process	69	31	14.29	4.90E-06	9.95E-05
GO:0001656	metanephros development	39	21	8.08	5.00E-06	1.01E-04
GO:0021885	forebrain cell migration	39	21	8.08	5.00E-06	1.01E-04
GO:0021915	neural tube development	126	48	26.09	5.10E-06	1.03E-04
GO:0043254	regulation of protein complex assembly	337	104	69.78	5.30E-06	1.06E-04
GO:0010812	negative regulation of cell-substrate adhesion	42	22	8.7	5.50E-06	1.10E-04
GO:0099537	trans-synaptic signaling	298	94	61.71	5.50E-06	1.10E-04
GO:0072089	stem cell proliferation	79	34	16.36	5.60E-06	1.12E-04
GO:0060993	kidney morphogenesis	45	23	9.32	5.80E-06	1.14E-04
GO:0001707	mesoderm formation	31	18	6.42	5.80E-06	1.14E-04
GO:0007528	neuromuscular junction development	31	18	6.42	5.80E-06	1.14E-04
GO:2001222	regulation of neuron migration	31	18	6.42	5.80E-06	1.14E-04
GO:0018212	peptidyl-tyrosine modification	203	69	42.03	6.10E-06	1.20E-04
GO:0060037	pharyngeal system development	12	10	2.48	6.20E-06	1.22E-04
GO:0045926	negative regulation of growth	170	60	35.2	6.40E-06	1.25E-04
GO:0099536	synaptic signaling	303	95	62.74	6.50E-06	1.27E-04
GO:0001569	branching involved in blood vessel morphogenesis	26	16	5.38	6.80E-06	1.32E-04
GO:1903522	regulation of blood circulation	110	43	22.78	7.20E-06	1.40E-04
GO:0060393	regulation of pathway-restricted SMAD protein phosphorylation	37	20	7.66	7.80E-06	1.51E-04
GO:0009612	response to mechanical stimulus	80	34	16.57	7.80E-06	1.51E-04
GO:0001942	hair follicle development	61	28	12.63	8.50E-06	1.64E-04
GO:0060038	cardiac muscle cell proliferation	43	22	8.9	9.00E-06	1.73E-04

GO:0061326	renal tubule development	55	26	11.39	9.10E-06	1.74E-04
GO:1904063	negative regulation of cation transmembrane transport	46	23	9.53	9.30E-06	1.77E-04
GO:0045778	positive regulation of ossification	52	25	10.77	9.30E-06	1.77E-04
GO:0048814	regulation of dendrite morphogenesis	74	32	15.32	9.30E-06	1.77E-04
GO:0035148	tube formation	111	43	22.98	9.50E-06	1.80E-04
GO:0048608	reproductive structure development	259	83	53.63	9.90E-06	1.87E-04
GO:0090100	positive regulation of transmembrane receptor protein serine/threonine kinase signaling pathway	71	31	14.7	1.00E-05	1.87E-04
GO:0048701	embryonic cranial skeleton morphogenesis	24	15	4.97	1.00E-05	1.87E-04
GO:0061384	heart trabecula morphogenesis	24	15	4.97	1.00E-05	1.87E-04
GO:0031334	positive regulation of protein complex assembly	187	64	38.72	1.00E-05	1.87E-04
GO:0046620	regulation of organ growth	81	34	16.77	1.10E-05	2.05E-04
GO:1990778	protein localization to cell periphery	233	76	48.25	1.20E-05	2.21E-04
GO:0072659	protein localization to plasma membrane	199	67	41.21	1.20E-05	2.21E-04
GO:0043547	positive regulation of GTPase activity	151	54	31.27	1.20E-05	2.21E-04
GO:0043269	regulation of ion transport	319	98	66.05	1.20E-05	2.21E-04
GO:0048332	mesoderm morphogenesis	35	19	7.25	1.20E-05	2.21E-04
GO:0098773	skin epidermis development	62	28	12.84	1.20E-05	2.21E-04
GO:0048483	autonomic nervous system development	17	12	3.52	1.30E-05	2.35E-04
GO:0085029	extracellular matrix assembly	17	12	3.52	1.30E-05	2.35E-04
GO:0007268	chemical synaptic transmission	292	91	60.46	1.30E-05	2.35E-04
GO:0098916	anterograde trans-synaptic signaling	292	91	60.46	1.30E-05	2.35E-04
GO:0055008	cardiac muscle tissue morphogenesis	38	20	7.87	1.30E-05	2.35E-04
GO:0061028	establishment of endothelial barrier	38	20	7.87	1.30E-05	2.35E-04
GO:0009952	anterior/posterior pattern specification	102	40	21.12	1.40E-05	2.50E-04
GO:0035924	cellular response to vascular endothelial growth factor stimulus	41	21	8.49	1.40E-05	2.50E-04
GO:0001885	endothelial cell development	53	25	10.97	1.40E-05	2.50E-04
GO:0018108	peptidyl-tyrosine phosphorylation	200	67	41.41	1.40E-05	2.50E-04
GO:0001954	positive regulation of cell-matrix adhesion	44	22	9.11	1.40E-05	2.50E-04
GO:0010761	fibroblast migration	44	22	9.11	1.40E-05	2.50E-04
GO:0008589	regulation of smoothened signaling pathway	47	23	9.73	1.50E-05	2.64E-04
GO:0009953	dorsal/ventral pattern formation	47	23	9.73	1.50E-05	2.64E-04
GO:0001558	regulation of cell growth	281	88	58.19	1.50E-05	2.64E-04
GO:1905314	semi-lunar valve development	22	14	4.56	1.50E-05	2.64E-04
GO:0072175	epithelial tube formation	99	39	20.5	1.50E-05	2.64E-04
GO:0014909	smooth muscle cell migration	69	30	14.29	1.50E-05	2.64E-04
GO:0010634	positive regulation of epithelial cell migration	106	41	21.95	1.60E-05	2.75E-04
GO:0061458	reproductive system development	262	83	54.25	1.60E-05	2.75E-04
GO:0003006	developmental process involved in reproduction	365	109	75.58	1.60E-05	2.75E-04
GO:0003348	cardiac endothelial cell differentiation	7	7	1.45	1.60E-05	2.75E-04
GO:0097084	vascular smooth muscle cell development	7	7	1.45	1.60E-05	2.75E-04
GO:1905651	regulation of artery morphogenesis	7	7	1.45	1.60E-05	2.75E-04
GO:1905653	positive regulation of artery morphogenesis	7	7	1.45	1.60E-05	2.75E-04
GO:0043271	negative regulation of ion transport	89	36	18.43	1.60E-05	2.75E-04

GO:0045601	regulation of endothelial cell differentiation	30	17	6.21	1.60E-05	2.75E-04
GO:0090257	regulation of muscle system process	138	50	28.58	1.70E-05	2.91E-04
GO:0097205	renal filtration	15	11	3.11	1.70E-05	2.91E-04
GO:0034765	regulation of ion transmembrane transport	205	68	42.45	1.80E-05	3.03E-04
GO:0007369	gastrulation	103	40	21.33	1.80E-05	3.03E-04
GO:0010927	cellular component assembly involved in morphogenesis	63	28	13.05	1.80E-05	3.03E-04
GO:0022404	molting cycle process	63	28	13.05	1.80E-05	3.03E-04
GO:0022405	hair cycle process	63	28	13.05	1.80E-05	3.03E-04
GO:0071804	cellular potassium ion transport	63	28	13.05	1.80E-05	3.03E-04
GO:0071805	potassium ion transmembrane transport	63	28	13.05	1.80E-05	3.03E-04
GO:0001701	in utero embryonic development	378	112	78.27	1.80E-05	3.03E-04
GO:1905330	regulation of morphogenesis of an epithelium	60	27	12.42	1.90E-05	3.19E-04
GO:0060491	regulation of cell projection assembly	128	47	26.5	2.00E-05	3.34E-04
GO:0050919	negative chemotaxis	25	15	5.18	2.00E-05	3.34E-04
GO:0072009	nephron epithelium development	54	25	11.18	2.10E-05	3.50E-04
GO:0010770	positive regulation of cell morphogenesis involved in differentiation	132	48	27.33	2.20E-05	3.59E-04
GO:0030048	actin filament-based movement	70	30	14.49	2.20E-05	3.59E-04
GO:0042303	molting cycle	70	30	14.49	2.20E-05	3.59E-04
GO:0042633	hair cycle	70	30	14.49	2.20E-05	3.59E-04
GO:0021535	cell migration in hindbrain	13	10	2.69	2.20E-05	3.59E-04
GO:0021952	central nervous system projection neuron axonogenesis	13	10	2.69	2.20E-05	3.59E-04
GO:0008306	associative learning	39	20	8.08	2.20E-05	3.59E-04
GO:0021799	cerebral cortex radially oriented cell migration	20	13	4.14	2.20E-05	3.59E-04
GO:0030501	positive regulation of bone mineralization	20	13	4.14	2.20E-05	3.59E-04
GO:0035886	vascular smooth muscle cell differentiation	20	13	4.14	2.20E-05	3.59E-04
GO:0043297	apical junction assembly	42	21	8.7	2.30E-05	3.71E-04
GO:0060425	lung morphogenesis	42	21	8.7	2.30E-05	3.71E-04
GO:0032233	positive regulation of actin filament bundle assembly	45	22	9.32	2.30E-05	3.71E-04
GO:0048015	phosphatidylinositol-mediated signaling	97	38	20.09	2.30E-05	3.71E-04
GO:0030010	establishment of cell polarity	104	40	21.53	2.30E-05	3.71E-04
GO:0010453	regulation of cell fate commitment	9	8	1.86	2.50E-05	3.98E-04
GO:0010644	cell communication by electrical coupling	9	8	1.86	2.50E-05	3.98E-04
GO:0003157	endocardium development	11	9	2.28	2.50E-05	3.98E-04
GO:0003174	mitral valve development	11	9	2.28	2.50E-05	3.98E-04
GO:0003418	growth plate cartilage chondrocyte differentiation	11	9	2.28	2.50E-05	3.98E-04
GO:0003433	chondrocyte development involved in endochondral bone morphogenesis	11	9	2.28	2.50E-05	3.98E-04
GO:0014910	regulation of smooth muscle cell migration	64	28	13.25	2.60E-05	4.13E-04
GO:0050804	modulation of chemical synaptic transmission	238	76	49.28	2.70E-05	4.27E-04
GO:0030856	regulation of epithelial cell differentiation	84	34	17.39	2.70E-05	4.27E-04
GO:0007498	mesoderm development	58	26	12.01	3.00E-05	4.70E-04
GO:0030001	metal ion transport	386	113	79.93	3.00E-05	4.70E-04
GO:0001838	embryonic epithelial tube formation	98	38	20.29	3.00E-05	4.70E-04
GO:0055002	striated muscle cell development	105	40	21.74	3.00E-05	4.70E-04

GO:0060419	heart growth	81	33	16.77	3.10E-05	4.81E-04
GO:0002053	positive regulation of mesenchymal cell proliferation	23	14	4.76	3.10E-05	4.81E-04
GO:0048546	digestive tract morphogenesis	23	14	4.76	3.10E-05	4.81E-04
GO:0099563	modification of synaptic structure	23	14	4.76	3.10E-05	4.81E-04
GO:0099177	regulation of trans-synaptic signaling	239	76	49.49	3.10E-05	4.81E-04
GO:0009611	response to wounding	298	91	61.71	3.20E-05	4.90E-04
GO:0003417	growth plate cartilage development	18	12	3.73	3.20E-05	4.90E-04
GO:0043267	negative regulation of potassium ion transport	18	12	3.73	3.20E-05	4.90E-04
GO:0048846	axon extension involved in axon guidance	18	12	3.73	3.20E-05	4.90E-04
GO:0099010	modification of postsynaptic structure	18	12	3.73	3.20E-05	4.90E-04
GO:1902284	neuron projection extension involved in neuron projection guidance	18	12	3.73	3.20E-05	4.90E-04
GO:0030902	hindbrain development	88	35	18.22	3.30E-05	5.04E-04
GO:0045859	regulation of protein kinase activity	461	131	95.46	3.40E-05	5.17E-04
GO:0072080	nephron tubule development	49	23	10.15	3.40E-05	5.17E-04
GO:0001666	response to hypoxia	145	51	30.02	3.50E-05	5.30E-04
GO:0072577	endothelial cell apoptotic process	40	20	8.28	3.50E-05	5.30E-04
GO:0120032	regulation of plasma membrane bounded cell projection assembly	127	46	26.3	3.60E-05	5.42E-04
GO:0090263	positive regulation of canonical Wnt signaling pathway	65	28	13.46	3.60E-05	5.42E-04
GO:0071900	regulation of protein serine/threonine kinase activity	299	91	61.91	3.60E-05	5.42E-04
GO:0055017	cardiac muscle tissue growth	75	31	15.53	3.80E-05	5.70E-04
GO:1900006	positive regulation of dendrite development	75	31	15.53	3.80E-05	5.70E-04
GO:0048017	inositol lipid-mediated signaling	99	38	20.5	3.90E-05	5.83E-04
GO:1902904	negative regulation of supramolecular fiber organization	99	38	20.5	3.90E-05	5.83E-04
GO:0034762	regulation of transmembrane transport	264	82	54.67	4.00E-05	5.95E-04
GO:0046777	protein autophosphorylation	183	61	37.89	4.00E-05	5.95E-04
GO:0055024	regulation of cardiac muscle tissue development	72	30	14.91	4.20E-05	6.24E-04
GO:0001938	positive regulation of endothelial cell proliferation	59	26	12.22	4.30E-05	6.35E-04
GO:0010563	negative regulation of phosphorus metabolic process	393	114	81.38	4.30E-05	6.35E-04
GO:0045936	negative regulation of phosphate metabolic process	393	114	81.38	4.30E-05	6.35E-04
GO:0000226	microtubule cytoskeleton organization	381	111	78.89	4.40E-05	6.47E-04
GO:1901861	regulation of muscle tissue development	110	41	22.78	4.40E-05	6.47E-04
GO:0021517	ventral spinal cord development	16	11	3.31	4.50E-05	6.60E-04

Supplemental Table 4

Table 2.S4. Enriched KEGG pathways from Cluster 4 from the k-means cluster analysis of genes expressed in the liver averaged from all groups (CV and GF, male and female, and days 1, 5, 10, 15, 25, 60, and 120).

GO.ID	Term	Annotated	Significant	Expected	result1	FDR
GO:0022613	ribonucleoprotein complex biogenesis	386	209	117.76	6.50E-23	4.77E-19
GO:0007059	chromosome segregation	229	139	69.86	1.30E-21	4.77E-18
GO:0000280	nuclear division	268	152	81.76	1.90E-19	4.64E-16
GO:0006397	mRNA processing	379	197	115.62	5.70E-19	1.05E-15
GO:0006260	DNA replication	206	123	62.85	2.00E-18	2.93E-15
GO:0006281	DNA repair	375	193	114.4	5.40E-18	5.97E-15
GO:0098813	nuclear chromosome segregation	181	111	55.22	5.70E-18	5.97E-15
GO:0033044	regulation of chromosome organization	289	156	88.17	3.40E-17	3.12E-14
GO:0000070	mitotic sister chromatid segregation	126	84	38.44	4.50E-17	3.67E-14
GO:0006261	DNA-dependent DNA replication	116	79	35.39	5.80E-17	4.25E-14
GO:0042254	ribosome biogenesis	258	141	78.71	3.00E-16	2.00E-13
GO:0000819	sister chromatid segregation	146	92	44.54	3.50E-16	2.14E-13
GO:0008380	RNA splicing	309	161	94.27	8.10E-16	4.57E-13
GO:0140014	mitotic nuclear division	204	117	62.23	9.60E-16	5.03E-13
GO:0044770	cell cycle phase transition	297	155	90.61	2.40E-15	1.17E-12
GO:0071103	DNA conformation change	127	80	38.74	3.10E-14	1.42E-11
GO:0044772	mitotic cell cycle phase transition	275	143	83.9	4.30E-14	1.86E-11
GO:0048285	organelle fission	306	155	93.35	6.60E-14	2.69E-11
GO:0051301	cell division	428	202	130.57	1.00E-13	3.86E-11
GO:0016072	rRNA metabolic process	200	111	61.01	1.20E-13	4.40E-11
GO:0051052	regulation of DNA metabolic process	326	161	99.45	3.40E-13	1.19E-10
GO:0022618	ribonucleoprotein complex assembly	195	107	59.49	8.60E-13	2.87E-10
GO:0000375	RNA splicing, via transesterification reactions	217	116	66.2	1.00E-12	2.93E-10
GO:0000377	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	217	116	66.2	1.00E-12	2.93E-10
GO:0000398	mRNA splicing, via spliceosome	217	116	66.2	1.00E-12	2.93E-10
GO:0010564	regulation of cell cycle process	418	195	127.52	1.10E-12	3.10E-10
GO:0034470	ncRNA processing	293	146	89.39	1.90E-12	5.16E-10
GO:0034502	protein localization to chromosome	73	51	22.27	4.40E-12	1.15E-09
GO:0006364	rRNA processing	175	97	53.39	4.60E-12	1.16E-09
GO:0051321	meiotic cell cycle	138	81	42.1	5.00E-12	1.22E-09
GO:0006310	DNA recombination	185	101	56.44	5.70E-12	1.35E-09
GO:0006323	DNA packaging	88	58	26.85	6.90E-12	1.58E-09
GO:0034660	ncRNA metabolic process	381	178	116.23	9.50E-12	2.11E-09
GO:1901987	regulation of cell cycle phase transition	212	111	64.68	1.80E-11	3.88E-09
GO:0071826	ribonucleoprotein complex subunit organization	205	108	62.54	2.00E-11	4.19E-09
GO:0071824	protein-DNA complex subunit organization	134	78	40.88	2.20E-11	4.48E-09
GO:2001251	negative regulation of chromosome organization	114	69	34.78	2.70E-11	5.35E-09
GO:0007346	regulation of mitotic cell cycle	372	173	113.49	3.00E-11	5.79E-09

GO:1901990	regulation of mitotic cell cycle phase transition	197	104	60.1	4.10E-11	7.71E-09
GO:0007051	spindle organization	133	77	40.57	4.30E-11	7.88E-09
GO:0000075	cell cycle checkpoint	150	84	45.76	6.10E-11	1.09E-08
GO:0002181	cytoplasmic translation	77	51	23.49	9.80E-11	1.71E-08
GO:0032200	telomere organization	112	67	34.17	1.10E-10	1.88E-08
GO:0042273	ribosomal large subunit biogenesis	69	47	21.05	1.20E-10	2.00E-08
GO:0051225	spindle assembly	84	54	25.63	1.50E-10	2.44E-08
GO:0065004	protein-DNA complex assembly	104	63	31.73	1.90E-10	3.03E-08
GO:0051304	chromosome separation	74	49	22.58	2.30E-10	3.59E-08
GO:0000723	telomere maintenance	110	65	33.56	4.30E-10	6.57E-08
GO:1903046	meiotic cell cycle process	97	59	29.59	5.60E-10	8.38E-08
GO:0006403	RNA localization	150	81	45.76	1.40E-09	2.05E-07
GO:0071897	DNA biosynthetic process	136	75	41.49	1.70E-09	2.44E-07
GO:0006302	double-strand break repair	170	89	51.86	1.80E-09	2.54E-07
GO:0140013	meiotic nuclear division	84	52	25.63	2.40E-09	3.32E-07
GO:0051054	positive regulation of DNA metabolic process	189	96	57.66	3.40E-09	4.62E-07
GO:0051983	regulation of chromosome segregation	87	53	26.54	3.90E-09	5.20E-07
GO:0051306	mitotic sister chromatid separation	53	37	16.17	4.10E-09	5.37E-07
GO:0002262	myeloid cell homeostasis	143	77	43.63	4.40E-09	5.66E-07
GO:0051236	establishment of RNA localization	129	71	39.35	5.10E-09	6.45E-07
GO:0034101	erythrocyte homeostasis	120	67	36.61	6.20E-09	7.71E-07
GO:0032204	regulation of telomere maintenance	75	47	22.88	8.10E-09	9.90E-07
GO:0000226	microtubule cytoskeleton organization	381	168	116.23	8.30E-09	9.98E-07
GO:1904356	regulation of telomere maintenance via telomere lengthening	56	38	17.08	8.70E-09	1.02E-06
GO:0044786	cell cycle DNA replication	42	31	12.81	8.80E-09	1.02E-06
GO:0050657	nucleic acid transport	126	69	38.44	1.10E-08	1.24E-06
GO:0050658	RNA transport	126	69	38.44	1.10E-08	1.24E-06
GO:0007093	mitotic cell cycle checkpoint	106	60	32.34	1.90E-08	2.11E-06
GO:0010833	telomere maintenance via telomere lengthening	70	44	21.36	2.10E-08	2.30E-06
GO:0007091	metaphase/anaphase transition of mitotic cell cycle	49	34	14.95	2.20E-08	2.37E-06
GO:1902850	microtubule cytoskeleton organization involved in mitosis	102	58	31.12	2.60E-08	2.76E-06
GO:2001020	regulation of response to DNA damage stimulus	165	84	50.34	2.80E-08	2.93E-06
GO:0022616	DNA strand elongation	23	20	7.02	3.00E-08	3.10E-06
GO:2001252	positive regulation of chromosome organization	153	79	46.68	3.30E-08	3.36E-06
GO:1905818	regulation of chromosome separation	54	36	16.47	4.40E-08	4.42E-06
GO:0050684	regulation of mRNA processing	115	63	35.08	4.70E-08	4.66E-06
GO:0010965	regulation of mitotic sister chromatid separation	50	34	15.25	4.90E-08	4.73E-06
GO:0044784	metaphase/anaphase transition of cell cycle	50	34	15.25	4.90E-08	4.73E-06
GO:0040029	regulation of gene expression, epigenetic	175	87	53.39	6.60E-08	6.29E-06
GO:0044839	cell cycle G2/M phase transition	95	54	28.98	8.10E-08	7.62E-06
GO:0033260	nuclear DNA replication	31	24	9.46	9.40E-08	8.51E-06
GO:0050686	negative regulation of mRNA processing	31	24	9.46	9.40E-08	8.51E-06
GO:0007098	centrosome cycle	100	56	30.51	9.40E-08	8.51E-06

GO:0045786	negative regulation of cell cycle	332	146	101.28	9.90E-08	8.84E-06
GO:0043414	macromolecule methylation	202	97	61.62	1.00E-07	8.84E-06
GO:0051028	mRNA transport	91	52	27.76	1.10E-07	9.60E-06
GO:0048024	regulation of mRNA splicing, via spliceosome	82	48	25.02	1.20E-07	1.04E-05
GO:0050000	chromosome localization	58	37	17.69	1.60E-07	1.36E-05
GO:1902749	regulation of cell cycle G2/M phase transition	69	42	21.05	1.70E-07	1.40E-05
GO:0006333	chromatin assembly or disassembly	78	46	23.8	1.70E-07	1.40E-05
GO:0030261	chromosome condensation	26	21	7.93	1.70E-07	1.40E-05
GO:1903311	regulation of mRNA metabolic process	196	94	59.79	1.80E-07	1.47E-05
GO:0006271	DNA strand elongation involved in DNA replication	16	15	4.88	2.10E-07	1.62E-05
GO:0000724	double-strand break repair via homologous recombination	97	54	29.59	2.10E-07	1.62E-05
GO:0000725	recombinational repair	97	54	29.59	2.10E-07	1.62E-05
GO:0090068	positive regulation of cell cycle process	176	86	53.69	2.10E-07	1.62E-05
GO:1903312	negative regulation of mRNA metabolic process	65	40	19.83	2.10E-07	1.62E-05
GO:0048872	homeostasis of number of cells	228	106	69.56	2.20E-07	1.68E-05
GO:0016570	histone modification	336	146	102.5	2.40E-07	1.80E-05
GO:0016569	covalent chromatin modification	347	150	105.86	2.40E-07	1.80E-05
GO:0000018	regulation of DNA recombination	61	38	18.61	2.70E-07	2.00E-05
GO:0030218	erythrocyte differentiation	110	59	33.56	3.30E-07	2.40E-05
GO:0043484	regulation of RNA splicing	110	59	33.56	3.30E-07	2.40E-05
GO:0051303	establishment of chromosome localization	57	36	17.39	3.40E-07	2.44E-05
GO:0048025	negative regulation of mRNA splicing, via spliceosome	23	19	7.02	3.50E-07	2.49E-05
GO:0061982	meiosis I cell cycle process	55	35	16.78	3.80E-07	2.68E-05
GO:0010638	positive regulation of organelle organization	453	187	138.2	4.30E-07	3.00E-05
GO:0031023	microtubule organizing center organization	106	57	32.34	4.70E-07	3.25E-05
GO:0042113	B cell activation	156	77	47.59	5.40E-07	3.70E-05
GO:0031497	chromatin assembly	60	37	18.3	5.70E-07	3.80E-05
GO:0033047	regulation of mitotic sister chromatid segregation	60	37	18.3	5.70E-07	3.80E-05
GO:0050792	regulation of viral process	141	71	43.02	5.70E-07	3.80E-05
GO:0033045	regulation of sister chromatid segregation	69	41	21.05	5.80E-07	3.83E-05
GO:0030071	regulation of mitotic metaphase/anaphase transition	45	30	13.73	5.90E-07	3.86E-05
GO:0007052	mitotic spindle organization	83	47	25.32	6.50E-07	4.22E-05
GO:0070192	chromosome organization involved in meiotic cell cycle	31	23	9.46	6.60E-07	4.24E-05
GO:0045814	negative regulation of gene expression, epigenetic	67	40	20.44	6.70E-07	4.24E-05
GO:0045787	positive regulation of cell cycle	251	113	76.57	6.70E-07	4.24E-05
GO:0045005	DNA-dependent DNA replication maintenance of fidelity	33	24	10.07	7.00E-07	4.39E-05
GO:1901988	negative regulation of cell cycle phase transition	117	61	35.69	7.70E-07	4.79E-05
GO:0010948	negative regulation of cell cycle process	165	80	50.34	8.30E-07	5.12E-05
GO:0007127	meiosis I	52	33	15.86	9.00E-07	5.50E-05
GO:0034728	nucleosome organization	70	41	21.36	1.00E-06	5.87E-05
GO:0042255	ribosome assembly	61	37	18.61	1.00E-06	5.87E-05
GO:0007017	microtubule-based process	492	199	150.1	1.00E-06	5.87E-05
GO:0000086	G2/M transition of mitotic cell cycle	84	47	25.63	1.00E-06	5.87E-05

GO:0051053	negative regulation of DNA metabolic process	108	57	32.95	1.00E-06	5.87E-05
GO:0006913	nucleocytoplasmic transport	229	104	69.86	1.10E-06	6.35E-05
GO:0051169	nuclear transport	229	104	69.86	1.10E-06	6.35E-05
GO:0010639	negative regulation of organelle organization	294	128	89.69	1.20E-06	6.72E-05
GO:0045132	meiotic chromosome segregation	46	30	14.03	1.20E-06	6.72E-05
GO:1902099	regulation of metaphase/anaphase transition of cell cycle	46	30	14.03	1.20E-06	6.72E-05
GO:1901991	negative regulation of mitotic cell cycle phase transition	106	56	32.34	1.20E-06	6.72E-05
GO:0006405	RNA export from nucleus	66	39	20.13	1.30E-06	7.22E-05
GO:0033119	negative regulation of RNA splicing	28	21	8.54	1.50E-06	8.27E-05
GO:0000027	ribosomal large subunit assembly	30	22	9.15	1.60E-06	8.76E-05
GO:0050853	B cell receptor signaling pathway	36	25	10.98	1.70E-06	9.03E-05
GO:0030099	myeloid cell differentiation	282	123	86.03	1.70E-06	9.03E-05
GO:0045841	negative regulation of mitotic metaphase/anaphase transition	34	24	10.37	1.70E-06	9.03E-05
GO:0015931	nucleobase-containing compound transport	152	74	46.37	1.70E-06	9.03E-05
GO:0032259	methylation	239	107	72.91	1.80E-06	9.50E-05
GO:2000278	regulation of DNA biosynthetic process	95	51	28.98	2.00E-06	1.05E-04
GO:0006401	RNA catabolic process	184	86	56.13	2.30E-06	1.19E-04
GO:0006338	chromatin remodeling	110	57	33.56	2.30E-06	1.19E-04
GO:0032210	regulation of telomere maintenance via telomerase	47	30	14.34	2.40E-06	1.22E-04
GO:0051310	metaphase plate congression	47	30	14.34	2.40E-06	1.22E-04
GO:0007088	regulation of mitotic nuclear division	123	62	37.52	2.80E-06	1.42E-04
GO:0006334	nucleosome assembly	43	28	13.12	2.90E-06	1.45E-04
GO:0045321	leukocyte activation	487	195	148.57	2.90E-06	1.45E-04
GO:0090329	regulation of DNA-dependent DNA replication	41	27	12.51	3.10E-06	1.54E-04
GO:0016458	gene silencing	144	70	43.93	3.50E-06	1.71E-04
GO:0031570	DNA integrity checkpoint	94	50	28.68	3.50E-06	1.71E-04
GO:0008608	attachment of spindle microtubules to kinetochore	25	19	7.63	3.60E-06	1.74E-04
GO:0016032	viral process	231	103	70.47	3.60E-06	1.74E-04
GO:0051783	regulation of nuclear division	134	66	40.88	3.80E-06	1.80E-04
GO:1902100	negative regulation of metaphase/anaphase transition of cell cycle	35	24	10.68	3.80E-06	1.80E-04
GO:2000816	negative regulation of mitotic sister chromatid separation	35	24	10.68	3.80E-06	1.80E-04
GO:0045930	negative regulation of mitotic cell cycle	173	81	52.78	4.10E-06	1.90E-04
GO:0010389	regulation of G2/M transition of mitotic cell cycle	59	35	18	4.10E-06	1.90E-04
GO:1903901	negative regulation of viral life cycle	59	35	18	4.10E-06	1.90E-04
GO:0006278	RNA-dependent DNA biosynthetic process	57	34	17.39	4.70E-06	2.15E-04
GO:0007004	telomere maintenance via telomerase	57	34	17.39	4.70E-06	2.15E-04
GO:0043903	regulation of symbiosis, encompassing mutualism through parasitism	153	73	46.68	5.20E-06	2.37E-04
GO:0071459	protein localization to chromosome, centromeric region	18	15	5.49	5.40E-06	2.44E-04
GO:0034508	centromere complex assembly	20	16	6.1	6.90E-06	3.10E-04
GO:0032943	mononuclear cell proliferation	165	77	50.34	8.10E-06	3.60E-04
GO:1905819	negative regulation of chromosome separation	36	24	10.98	8.10E-06	3.60E-04
GO:0042770	signal transduction in response to DNA damage	72	40	21.97	8.30E-06	3.67E-04
GO:0031577	spindle checkpoint	34	23	10.37	8.70E-06	3.82E-04

GO:0006270	DNA replication initiation	24	18	7.32	8.90E-06	3.89E-04
GO:0071174	mitotic spindle checkpoint	32	22	9.76	9.10E-06	3.95E-04
GO:0051168	nuclear export	114	57	34.78	9.50E-06	4.10E-04
GO:0006342	chromatin silencing	63	36	19.22	9.90E-06	4.25E-04
GO:0031123	RNA 3'-end processing	82	44	25.02	1.00E-05	4.26E-04
GO:0018205	peptidyl-lysine modification	266	114	81.15	1.10E-05	4.64E-04
GO:0072331	signal transduction by p53 class mediator	107	54	32.64	1.10E-05	4.64E-04
GO:0042274	ribosomal small subunit biogenesis	61	35	18.61	1.20E-05	5.03E-04
GO:0006479	protein methylation	128	62	39.05	1.40E-05	5.80E-04
GO:0008213	protein alkylation	128	62	39.05	1.40E-05	5.80E-04
GO:0034724	DNA replication-independent nucleosome organization	17	14	5.19	1.50E-05	6.18E-04
GO:0071166	ribonucleoprotein complex localization	57	33	17.39	1.60E-05	6.52E-04
GO:1903900	regulation of viral life cycle	108	54	32.95	1.60E-05	6.52E-04
GO:0070661	leukocyte proliferation	176	80	53.69	1.80E-05	7.29E-04
GO:0006275	regulation of DNA replication	86	45	26.24	1.90E-05	7.61E-04
GO:0000380	alternative mRNA splicing, via spliceosome	62	35	18.91	1.90E-05	7.61E-04
GO:0046651	lymphocyte proliferation	163	75	49.73	2.00E-05	7.97E-04
GO:0007094	mitotic spindle assembly checkpoint	31	21	9.46	2.10E-05	8.28E-04
GO:0071173	spindle assembly checkpoint	31	21	9.46	2.10E-05	8.28E-04
GO:1904357	negative regulation of telomere maintenance via telomere lengthening	23	17	7.02	2.20E-05	8.63E-04
GO:0070198	protein localization to chromosome, telomeric region	27	19	8.24	2.30E-05	8.92E-04
GO:0031297	replication fork processing	25	18	7.63	2.30E-05	8.92E-04
GO:0000381	regulation of alternative mRNA splicing, via spliceosome	51	30	15.56	2.50E-05	9.60E-04
GO:0006402	mRNA catabolic process	164	75	50.03	2.50E-05	9.60E-04
GO:0000077	DNA damage checkpoint	87	45	26.54	2.70E-05	1.03E-03
GO:0048525	negative regulation of viral process	70	38	21.36	2.80E-05	1.06E-03
GO:0071426	ribonucleoprotein complex export from nucleus	56	32	17.08	3.10E-05	1.17E-03
GO:0034504	protein localization to nucleus	216	94	65.9	3.10E-05	1.17E-03
GO:0033048	negative regulation of mitotic sister chromatid segregation	38	24	11.59	3.20E-05	1.20E-03
GO:0016571	histone methylation	100	50	30.51	3.30E-05	1.23E-03
GO:0032206	positive regulation of telomere maintenance	47	28	14.34	3.40E-05	1.26E-03
GO:0046649	lymphocyte activation	408	162	124.47	3.50E-05	1.29E-03
GO:0044419	interspecies interaction between organisms	286	119	87.25	3.60E-05	1.31E-03
GO:1901989	positive regulation of cell cycle phase transition	73	39	22.27	3.60E-05	1.31E-03
GO:0090307	mitotic spindle assembly	45	27	13.73	3.90E-05	1.41E-03
GO:0045576	mast cell activation	34	22	10.37	3.90E-05	1.41E-03
GO:0045069	regulation of viral genome replication	66	36	20.13	4.00E-05	1.43E-03
GO:0006336	DNA replication-independent nucleosome assembly	16	13	4.88	4.00E-05	1.43E-03
GO:0019080	viral gene expression	52	30	15.86	4.20E-05	1.50E-03
GO:0006611	protein export from nucleus	101	50	30.81	4.60E-05	1.63E-03
GO:0006284	base-excision repair	30	20	9.15	4.70E-05	1.66E-03
GO:0042100	B cell proliferation	50	29	15.25	4.90E-05	1.72E-03
GO:0051985	negative regulation of chromosome segregation	41	25	12.51	5.10E-05	1.78E-03

GO:0002252	immune effector process	466	181	142.16	5.40E-05	1.88E-03
GO:0035821	modification of morphology or physiology of other organism	84	43	25.63	5.70E-05	1.97E-03
GO:0033046	negative regulation of sister chromatid segregation	39	24	11.9	5.90E-05	2.03E-03
GO:0007080	mitotic metaphase plate congression	37	23	11.29	6.70E-05	2.29E-03
GO:0045071	negative regulation of viral genome replication	37	23	11.29	6.70E-05	2.29E-03
GO:0002429	immune response-activating cell surface receptor signaling pathway	110	53	33.56	7.00E-05	2.38E-03
GO:0044403	symbiont process	276	114	84.2	7.50E-05	2.53E-03
GO:0006305	DNA alkylation	44	26	13.42	7.80E-05	2.61E-03
GO:0006306	DNA methylation	44	26	13.42	7.80E-05	2.61E-03
GO:0032205	negative regulation of telomere maintenance	33	21	10.07	8.50E-05	2.81E-03
GO:0034501	protein localization to kinetochore	13	11	3.97	8.50E-05	2.81E-03
GO:1902969	mitotic DNA replication	13	11	3.97	8.50E-05	2.81E-03
GO:0006406	mRNA export from nucleus	42	25	12.81	9.00E-05	2.95E-03
GO:0071427	mRNA-containing ribonucleoprotein complex export from nucleus	42	25	12.81	9.00E-05	2.95E-03
GO:0044728	DNA methylation or demethylation	56	31	17.08	9.30E-05	3.03E-03
GO:0051784	negative regulation of nuclear division	49	28	14.95	9.50E-05	3.08E-03
GO:0006282	regulation of DNA repair	88	44	26.85	9.60E-05	3.10E-03
GO:0000082	G1/S transition of mitotic cell cycle	132	61	40.27	9.70E-05	3.12E-03
GO:0002274	myeloid leukocyte activation	119	56	36.3	1.00E-04	3.20E-03
GO:0045839	negative regulation of mitotic nuclear division	47	27	14.34	1.10E-04	3.49E-03
GO:0000956	nuclear-transcribed mRNA catabolic process	91	45	27.76	1.10E-04	3.49E-03
GO:0034968	histone lysine methylation	81	41	24.71	1.20E-04	3.78E-03
GO:0032211	negative regulation of telomere maintenance via telomerase	17	13	5.19	1.20E-04	3.78E-03
GO:2001235	positive regulation of apoptotic signaling pathway	141	64	43.02	1.30E-04	3.94E-03
GO:1901796	regulation of signal transduction by p53 class mediator	52	29	15.86	1.30E-04	3.94E-03
GO:0001783	B cell apoptotic process	21	15	6.41	1.30E-04	3.94E-03
GO:0002714	positive regulation of B cell mediated immunity	21	15	6.41	1.30E-04	3.94E-03
GO:0002891	positive regulation of immunoglobulin mediated immune response	21	15	6.41	1.30E-04	3.94E-03
GO:0043921	modulation by host of viral transcription	21	15	6.41	1.30E-04	3.94E-03
GO:0052312	modulation of transcription in other organism involved in symbiotic interaction	21	15	6.41	1.30E-04	3.94E-03
GO:0052472	modulation by host of symbiont transcription	21	15	6.41	1.30E-04	3.94E-03
GO:0008630	intrinsic apoptotic signaling pathway in response to DNA damage	89	44	27.15	1.30E-04	3.94E-03
GO:0032392	DNA geometric change	36	22	10.98	1.40E-04	4.23E-03
GO:0018022	peptidyl-lysine methylation	87	43	26.54	1.60E-04	4.73E-03
GO:1902275	regulation of chromatin organization	150	67	45.76	1.60E-04	4.73E-03
GO:1901992	positive regulation of mitotic cell cycle phase transition	67	35	20.44	1.60E-04	4.73E-03
GO:0006335	DNA replication-dependent nucleosome assembly	10	9	3.05	1.60E-04	4.73E-03
GO:0034723	DNA replication-dependent nucleosome organization	10	9	3.05	1.60E-04	4.73E-03
GO:2001022	positive regulation of response to DNA damage stimulus	77	39	23.49	1.70E-04	4.95E-03
GO:0002768	immune response-regulating cell surface receptor signaling pathway	113	53	34.47	1.70E-04	4.95E-03
GO:0051493	regulation of cytoskeleton organization	387	151	118.06	1.70E-04	4.95E-03
GO:0043967	histone H4 acetylation	55	30	16.78	1.70E-04	4.95E-03
GO:0006606	protein import into nucleus	116	54	35.39	1.90E-04	5.49E-03

GO:0044843	cell cycle G1/S phase transition	140	63	42.71	1.90E-04	5.49E-03
GO:0098542	defense response to other organism	273	111	83.28	2.00E-04	5.71E-03
GO:0051817	modification of morphology or physiology of other organism involved in symbiotic interaction	75	38	22.88	2.00E-04	5.71E-03
GO:0050685	positive regulation of mRNA processing	30	19	9.15	2.00E-04	5.71E-03
GO:0010212	response to ionizing radiation	106	50	32.34	2.20E-04	6.23E-03
GO:0051851	modification by host of symbiont morphology or physiology	58	31	17.69	2.20E-04	6.23E-03
GO:0070507	regulation of microtubule cytoskeleton organization	146	65	44.54	2.30E-04	6.37E-03
GO:0043900	regulation of multi-organism process	254	104	77.49	2.30E-04	6.37E-03
GO:0000387	spliceosomal snRNP assembly	28	18	8.54	2.30E-04	6.37E-03
GO:0051702	interaction with symbiont	63	33	19.22	2.30E-04	6.37E-03
GO:0061515	myeloid cell development	63	33	19.22	2.30E-04	6.37E-03
GO:0060249	anatomical structure homeostasis	257	105	78.4	2.30E-04	6.37E-03
GO:0090501	RNA phosphodiester bond hydrolysis	51	28	15.56	2.40E-04	6.50E-03
GO:0051653	spindle localization	37	22	11.29	2.40E-04	6.50E-03
GO:0061820	telomeric D-loop disassembly	7	7	2.14	2.40E-04	6.50E-03
GO:0090657	telomeric loop disassembly	7	7	2.14	2.40E-04	6.50E-03
GO:1900262	regulation of DNA-directed DNA polymerase activity	7	7	2.14	2.40E-04	6.50E-03
GO:1900264	positive regulation of DNA-directed DNA polymerase activity	7	7	2.14	2.40E-04	6.50E-03
GO:0040001	establishment of mitotic spindle localization	26	17	7.93	2.50E-04	6.69E-03
GO:0045292	mRNA cis splicing, via spliceosome	26	17	7.93	2.50E-04	6.69E-03
GO:0045911	positive regulation of DNA recombination	26	17	7.93	2.50E-04	6.69E-03
GO:0051170	import into nucleus	120	55	36.61	2.70E-04	7.20E-03
GO:0000470	maturation of LSU-rRNA	24	16	7.32	2.80E-04	7.36E-03
GO:0045143	homologous chromosome segregation	24	16	7.32	2.80E-04	7.36E-03
GO:0030219	megakaryocyte differentiation	35	21	10.68	2.80E-04	7.36E-03
GO:0033120	positive regulation of RNA splicing	35	21	10.68	2.80E-04	7.36E-03
GO:0002366	leukocyte activation involved in immune response	147	65	44.85	2.90E-04	7.49E-03
GO:0007076	mitotic chromosome condensation	14	11	4.27	2.90E-04	7.49E-03
GO:0045830	positive regulation of isotype switching	14	11	4.27	2.90E-04	7.49E-03
GO:0051383	kinetochore organization	14	11	4.27	2.90E-04	7.49E-03
GO:1904814	regulation of protein localization to chromosome, telomeric region	14	11	4.27	2.90E-04	7.49E-03
GO:1902751	positive regulation of cell cycle G2/M phase transition	22	15	6.71	3.00E-04	7.69E-03
GO:0030183	B cell differentiation	89	43	27.15	3.00E-04	7.69E-03
GO:0051298	centrosome duplication	54	29	16.47	3.10E-04	7.79E-03
GO:0007143	female meiotic nuclear division	20	14	6.1	3.10E-04	7.79E-03
GO:0048026	positive regulation of mRNA splicing, via spliceosome	20	14	6.1	3.10E-04	7.79E-03
GO:0032069	regulation of nuclease activity	16	12	4.88	3.10E-04	7.79E-03
GO:0043555	regulation of translation in response to stress	16	12	4.88	3.10E-04	7.79E-03
GO:1904666	regulation of ubiquitin protein ligase activity	16	12	4.88	3.10E-04	7.79E-03
GO:0000184	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	33	20	10.07	3.30E-04	8.09E-03
GO:0043489	RNA stabilization	33	20	10.07	3.30E-04	8.09E-03
GO:0048821	erythrocyte development	33	20	10.07	3.30E-04	8.09E-03
GO:0051293	establishment of spindle localization	33	20	10.07	3.30E-04	8.09E-03

GO:1904358	positive regulation of telomere maintenance via telomere lengthening	33	20	10.07	3.30E-04	8.09E-03
GO:0050864	regulation of B cell activation	79	39	24.1	3.30E-04	8.09E-03
GO:2000573	positive regulation of DNA biosynthetic process	59	31	18	3.30E-04	8.09E-03
GO:0010569	regulation of double-strand break repair via homologous recombination	31	19	9.46	3.80E-04	9.26E-03
GO:0006909	phagocytosis	151	66	46.07	3.80E-04	9.26E-03
GO:0050851	antigen receptor-mediated signaling pathway	95	45	28.98	3.90E-04	9.47E-03
GO:0050778	positive regulation of immune response	367	142	111.96	4.10E-04	9.92E-03
GO:0002440	production of molecular mediator of immune response	119	54	36.3	4.20E-04	1.01E-02
GO:0007099	centriole replication	29	18	8.85	4.30E-04	1.03E-02
GO:0098534	centriole assembly	29	18	8.85	4.30E-04	1.03E-02
GO:0034655	nucleobase-containing compound catabolic process	324	127	98.84	4.40E-04	1.05E-02
GO:0045931	positive regulation of mitotic cell cycle	130	58	39.66	4.50E-04	1.07E-02
GO:0007292	female gamete generation	70	35	21.36	4.80E-04	1.14E-02
GO:0046782	regulation of viral transcription	36	21	10.98	4.80E-04	1.14E-02
GO:0000727	double-strand break repair via break-induced replication	9	8	2.75	4.90E-04	1.14E-02
GO:0031055	chromatin remodeling at centromere	9	8	2.75	4.90E-04	1.14E-02
GO:0032070	regulation of deoxyribonuclease activity	9	8	2.75	4.90E-04	1.14E-02
GO:0051574	positive regulation of histone H3-K9 methylation	9	8	2.75	4.90E-04	1.14E-02
GO:0006897	endocytosis	430	163	131.18	4.90E-04	1.14E-02
GO:0031124	mRNA 3'-end processing	48	26	14.64	5.30E-04	1.23E-02
GO:0000731	DNA synthesis involved in DNA repair	25	16	7.63	5.50E-04	1.27E-02
GO:0045910	negative regulation of DNA recombination	25	16	7.63	5.50E-04	1.27E-02
GO:0002263	cell activation involved in immune response	150	65	45.76	5.60E-04	1.28E-02
GO:2000779	regulation of double-strand break repair	53	28	16.17	5.60E-04	1.28E-02
GO:0050776	regulation of immune response	458	172	139.72	5.80E-04	1.33E-02
GO:0010608	posttranscriptional regulation of gene expression	364	140	111.05	6.00E-04	1.37E-02
GO:0016180	snRNA processing	23	15	7.02	6.10E-04	1.39E-02
GO:0002700	regulation of production of molecular mediator of immune response	81	39	24.71	6.20E-04	1.40E-02
GO:0019079	viral genome replication	81	39	24.71	6.20E-04	1.40E-02
GO:0032886	regulation of microtubule-based process	159	68	48.51	6.60E-04	1.47E-02
GO:0002566	somatic diversification of immune receptors via somatic mutation	11	9	3.36	6.60E-04	1.47E-02
GO:0051299	centrosome separation	11	9	3.36	6.60E-04	1.47E-02
GO:1905214	regulation of RNA binding	11	9	3.36	6.60E-04	1.47E-02
GO:0007549	dosage compensation	21	14	6.41	6.80E-04	1.50E-02
GO:0009048	dosage compensation by inactivation of X chromosome	21	14	6.41	6.80E-04	1.50E-02
GO:0045943	positive regulation of transcription by RNA polymerase I	21	14	6.41	6.80E-04	1.50E-02
GO:0070199	establishment of protein localization to chromosome	21	14	6.41	6.80E-04	1.50E-02
GO:0006413	translational initiation	97	45	29.59	6.90E-04	1.52E-02
GO:0051258	protein polymerization	204	84	62.23	7.10E-04	1.55E-02
GO:0050434	positive regulation of viral transcription	19	13	5.8	7.30E-04	1.59E-02
GO:1904816	positive regulation of protein localization to chromosome, telomeric region	13	10	3.97	7.50E-04	1.63E-02
GO:0032508	DNA duplex unwinding	30	18	9.15	7.70E-04	1.67E-02
GO:0060561	apoptotic process involved in morphogenesis	17	12	5.19	7.70E-04	1.67E-02

GO:0043486	histone exchange	15	11	4.58	7.80E-04	1.68E-02
GO:0043923	positive regulation by host of viral transcription	15	11	4.58	7.80E-04	1.68E-02
GO:0009452	7-methylguanosine RNA capping	6	6	1.83	8.00E-04	1.68E-02
GO:0010032	meiotic chromosome condensation	6	6	1.83	8.00E-04	1.68E-02
GO:0032055	negative regulation of translation in response to stress	6	6	1.83	8.00E-04	1.68E-02
GO:0036260	RNA capping	6	6	1.83	8.00E-04	1.68E-02
GO:0044806	G-quadruplex DNA unwinding	6	6	1.83	8.00E-04	1.68E-02
GO:0048304	positive regulation of isotype switching to IgG isotypes	6	6	1.83	8.00E-04	1.68E-02
GO:0051231	spindle elongation	6	6	1.83	8.00E-04	1.68E-02
GO:1902255	positive regulation of intrinsic apoptotic signaling pathway by p53 class mediator	6	6	1.83	8.00E-04	1.68E-02
GO:1903313	positive regulation of mRNA metabolic process	69	34	21.05	8.10E-04	1.70E-02
GO:0031109	microtubule polymerization or depolymerization	82	39	25.02	8.40E-04	1.75E-02
GO:0098657	import into cell	485	180	147.96	8.40E-04	1.75E-02
GO:0051098	regulation of binding	291	114	88.78	8.70E-04	1.81E-02
GO:0090305	nucleic acid phosphodiester bond hydrolysis	90	42	27.46	8.80E-04	1.82E-02
GO:0018393	internal peptidyl-lysine acetylation	117	52	35.69	9.60E-04	1.98E-02
GO:0009314	response to radiation	275	108	83.9	1.06E-03	2.18E-02
GO:0016445	somatic diversification of immunoglobulins	40	22	12.2	1.07E-03	2.19E-02
GO:0051568	histone H3-K4 methylation	40	22	12.2	1.07E-03	2.19E-02
GO:0032944	regulation of mononuclear cell proliferation	123	54	37.52	1.10E-03	2.25E-02
GO:0016233	telomere capping	33	19	10.07	1.11E-03	2.26E-02
GO:0002200	somatic diversification of immune receptors	45	24	13.73	1.15E-03	2.34E-02
GO:0045639	positive regulation of myeloid cell differentiation	65	32	19.83	1.16E-03	2.34E-02
GO:0072332	intrinsic apoptotic signaling pathway by p53 class mediator	65	32	19.83	1.16E-03	2.34E-02
GO:0006301	postreplication repair	24	15	7.32	1.18E-03	2.36E-02
GO:0009303	rRNA transcription	24	15	7.32	1.18E-03	2.36E-02
GO:0051984	positive regulation of chromosome segregation	24	15	7.32	1.18E-03	2.36E-02
GO:1902253	regulation of intrinsic apoptotic signaling pathway by p53 class mediator	24	15	7.32	1.18E-03	2.36E-02
GO:0016073	snRNA metabolic process	38	21	11.59	1.28E-03	2.54E-02
GO:0042771	intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator	38	21	11.59	1.28E-03	2.54E-02
GO:0002712	regulation of B cell mediated immunity	31	18	9.46	1.31E-03	2.58E-02
GO:0002889	regulation of immunoglobulin mediated immune response	31	18	9.46	1.31E-03	2.58E-02
GO:0008156	negative regulation of DNA replication	31	18	9.46	1.31E-03	2.58E-02
GO:0006298	mismatch repair	22	14	6.71	1.34E-03	2.62E-02
GO:0046456	icosanoid biosynthetic process	22	14	6.71	1.34E-03	2.62E-02
GO:0016573	histone acetylation	113	50	34.47	1.34E-03	2.62E-02
GO:0009617	response to bacterium	356	135	108.61	1.42E-03	2.77E-02
GO:1904667	negative regulation of ubiquitin protein ligase activity	8	7	2.44	1.44E-03	2.80E-02
GO:0000466	maturation of 5.8S rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	20	13	6.1	1.50E-03	2.89E-02
GO:0010971	positive regulation of G2/M transition of mitotic cell cycle	20	13	6.1	1.50E-03	2.89E-02
GO:0045191	regulation of isotype switching	20	13	6.1	1.50E-03	2.89E-02
GO:1902742	apoptotic process involved in development	20	13	6.1	1.50E-03	2.89E-02
GO:0051567	histone H3-K9 methylation	29	17	8.85	1.54E-03	2.96E-02

GO:0044270	cellular nitrogen compound catabolic process	354	134	108	1.60E-03	3.06E-02
GO:0006304	DNA modification	66	32	20.13	1.60E-03	3.06E-02
GO:1902369	negative regulation of RNA catabolic process	41	22	12.51	1.65E-03	3.14E-02
GO:1902750	negative regulation of cell cycle G2/M phase transition	41	22	12.51	1.65E-03	3.14E-02
GO:0000028	ribosomal small subunit assembly	18	12	5.49	1.67E-03	3.16E-02
GO:2000104	negative regulation of DNA-dependent DNA replication	18	12	5.49	1.67E-03	3.16E-02
GO:0046605	regulation of centrosome cycle	51	26	15.56	1.74E-03	3.28E-02
GO:0000183	chromatin silencing at rDNA	10	8	3.05	1.79E-03	3.32E-02
GO:0002523	leukocyte migration involved in inflammatory response	10	8	3.05	1.79E-03	3.32E-02
GO:0007100	mitotic centrosome separation	10	8	3.05	1.79E-03	3.32E-02
GO:0016446	somatic hypermutation of immunoglobulin genes	10	8	3.05	1.79E-03	3.32E-02
GO:0061620	glycolytic process through glucose-6-phosphate	10	8	3.05	1.79E-03	3.32E-02
GO:0050867	positive regulation of cell activation	181	74	55.22	1.79E-03	3.32E-02
GO:0031057	negative regulation of histone modification	34	19	10.37	1.81E-03	3.32E-02
GO:0007129	synapsis	16	11	4.88	1.81E-03	3.32E-02
GO:0043968	histone H2A acetylation	16	11	4.88	1.81E-03	3.32E-02
GO:0070200	establishment of protein localization to telomere	16	11	4.88	1.81E-03	3.32E-02
GO:0018394	peptidyl-lysine acetylation	128	55	39.05	1.81E-03	3.32E-02
GO:0051099	positive regulation of binding	153	64	46.68	1.83E-03	3.35E-02
GO:0042742	defense response to bacterium	109	48	33.25	1.87E-03	3.41E-02
GO:0019081	viral translation	14	10	4.27	1.91E-03	3.44E-02
GO:0006475	internal protein amino acid acetylation	120	52	36.61	1.91E-03	3.44E-02
GO:0097530	granulocyte migration	77	36	23.49	1.92E-03	3.44E-02
GO:0006268	DNA unwinding involved in DNA replication	12	9	3.66	1.92E-03	3.44E-02
GO:0007096	regulation of exit from mitosis	12	9	3.66	1.92E-03	3.44E-02
GO:0051988	regulation of attachment of spindle microtubules to kinetochore	12	9	3.66	1.92E-03	3.44E-02
GO:0070202	regulation of establishment of protein localization to chromosome	12	9	3.66	1.92E-03	3.44E-02
GO:0019083	viral transcription	39	21	11.9	1.98E-03	3.53E-02
GO:0030888	regulation of B cell proliferation	39	21	11.9	1.98E-03	3.53E-02
GO:0050900	leukocyte migration	193	78	58.88	2.01E-03	3.57E-02
GO:0046785	microtubule polymerization	59	29	18	2.01E-03	3.57E-02
GO:0002250	adaptive immune response	239	94	72.91	2.05E-03	3.63E-02
GO:0002702	positive regulation of production of molecular mediator of immune response	54	27	16.47	2.07E-03	3.65E-02
GO:0070227	lymphocyte apoptotic process	54	27	16.47	2.07E-03	3.65E-02
GO:0006417	regulation of translation	271	105	82.67	2.08E-03	3.66E-02
GO:0030330	DNA damage response, signal transduction by p53 class mediator	49	25	14.95	2.09E-03	3.67E-02
GO:0033003	regulation of mast cell activation	25	15	7.63	2.12E-03	3.68E-02
GO:0043303	mast cell degranulation	25	15	7.63	2.12E-03	3.68E-02
GO:0072395	signal transduction involved in cell cycle checkpoint	25	15	7.63	2.12E-03	3.68E-02
GO:0072401	signal transduction involved in DNA integrity checkpoint	25	15	7.63	2.12E-03	3.68E-02
GO:0072422	signal transduction involved in DNA damage checkpoint	25	15	7.63	2.12E-03	3.68E-02
GO:0043044	ATP-dependent chromatin remodeling	32	18	9.76	2.15E-03	3.70E-02
GO:0043628	ncRNA 3'-end processing	32	18	9.76	2.15E-03	3.70E-02

GO:2000279	negative regulation of DNA biosynthetic process	32	18	9.76	2.15E-03	3.70E-02
GO:0071887	leukocyte apoptotic process	75	35	22.88	2.29E-03	3.93E-02
GO:0000245	spliceosomal complex assembly	37	20	11.29	2.37E-03	4.05E-02
GO:0050670	regulation of lymphocyte proliferation	121	52	36.91	2.37E-03	4.05E-02
GO:0000132	establishment of mitotic spindle orientation	23	14	7.02	2.46E-03	4.17E-02
GO:0010458	exit from mitosis	23	14	7.02	2.46E-03	4.17E-02
GO:0051570	regulation of histone H3-K9 methylation	23	14	7.02	2.46E-03	4.17E-02
GO:0071168	protein localization to chromatin	23	14	7.02	2.46E-03	4.17E-02
GO:0045646	regulation of erythrocyte differentiation	42	22	12.81	2.48E-03	4.18E-02
GO:2001244	positive regulation of intrinsic apoptotic signaling pathway	52	26	15.86	2.48E-03	4.18E-02
GO:0099024	plasma membrane invagination	47	24	14.34	2.51E-03	4.22E-02
GO:1902229	regulation of intrinsic apoptotic signaling pathway in response to DNA damage	30	17	9.15	2.56E-03	4.29E-02
GO:2000677	regulation of transcription regulatory region DNA binding	30	17	9.15	2.56E-03	4.29E-02
GO:0043901	negative regulation of multi-organism process	105	46	32.03	2.61E-03	4.31E-02
GO:0000022	mitotic spindle elongation	5	5	1.53	2.64E-03	4.31E-02
GO:0002227	innate immune response in mucosa	5	5	1.53	2.64E-03	4.31E-02
GO:0006370	7-methylguanosine mRNA capping	5	5	1.53	2.64E-03	4.31E-02
GO:0016321	female meiosis chromosome segregation	5	5	1.53	2.64E-03	4.31E-02
GO:0019370	leukotriene biosynthetic process	5	5	1.53	2.64E-03	4.31E-02
GO:0034080	CENP-A containing nucleosome assembly	5	5	1.53	2.64E-03	4.31E-02
GO:0048298	positive regulation of isotype switching to IgA isotypes	5	5	1.53	2.64E-03	4.31E-02
GO:0051255	spindle midzone assembly	5	5	1.53	2.64E-03	4.31E-02
GO:0061641	CENP-A containing chromatin organization	5	5	1.53	2.64E-03	4.31E-02
GO:0075525	viral translational termination-reinitiation	5	5	1.53	2.64E-03	4.31E-02
GO:0019058	viral life cycle	155	64	47.29	2.66E-03	4.34E-02
GO:0070665	positive regulation of leukocyte proliferation	73	34	22.27	2.73E-03	4.44E-02
GO:0070663	regulation of leukocyte proliferation	130	55	39.66	2.74E-03	4.45E-02
GO:0050865	regulation of cell activation	323	122	98.54	2.76E-03	4.47E-02
GO:0043304	regulation of mast cell degranulation	21	13	6.41	2.85E-03	4.60E-02
GO:0071478	cellular response to radiation	122	52	37.22	2.93E-03	4.72E-02
GO:0032946	positive regulation of mononuclear cell proliferation	68	32	20.74	2.94E-03	4.73E-02
GO:0006911	phagocytosis, engulfment	40	21	12.2	2.98E-03	4.74E-02
GO:1905268	negative regulation of chromatin organization	40	21	12.2	2.98E-03	4.74E-02
GO:0002275	myeloid cell activation involved in immune response	50	25	15.25	2.98E-03	4.74E-02
GO:0032272	negative regulation of protein polymerization	50	25	15.25	2.98E-03	4.74E-02
GO:0046824	positive regulation of nucleocytoplasmic transport	50	25	15.25	2.98E-03	4.74E-02
GO:0051294	establishment of spindle orientation	28	16	8.54	3.04E-03	4.83E-02
GO:0009566	fertilization	63	30	19.22	3.14E-03	4.97E-02
GO:0034250	positive regulation of cellular amide metabolic process	117	50	35.69	3.27E-03	5.14E-02
GO:0050707	regulation of cytokine secretion	106	46	32.34	3.27E-03	5.14E-02
GO:0031061	negative regulation of histone methylation	19	12	5.8	3.27E-03	5.14E-02
GO:0042119	neutrophil activation	19	12	5.8	3.27E-03	5.14E-02
GO:0032271	regulation of protein polymerization	159	65	48.51	3.30E-03	5.17E-02

GO:0051656	establishment of organelle localization	304	115	92.74	3.38E-03	5.29E-02
GO:0097327	response to antineoplastic agent	53	26	16.17	3.48E-03	5.42E-02
GO:2001021	negative regulation of response to DNA damage stimulus	53	26	16.17	3.48E-03	5.42E-02
GO:1905269	positive regulation of chromatin organization	90	40	27.46	3.52E-03	5.47E-02
GO:0002521	leukocyte differentiation	337	126	102.81	3.58E-03	5.54E-02
GO:0051972	regulation of telomerase activity	38	20	11.59	3.58E-03	5.54E-02
GO:0002312	B cell activation involved in immune response	48	24	14.64	3.59E-03	5.54E-02
GO:0002279	mast cell activation involved in immune response	26	15	7.93	3.60E-03	5.54E-02
GO:0002448	mast cell mediated immunity	26	15	7.93	3.60E-03	5.54E-02
GO:0002699	positive regulation of immune effector process	137	57	41.8	3.63E-03	5.56E-02
GO:0071156	regulation of cell cycle arrest	43	22	13.12	3.63E-03	5.56E-02
GO:0002697	regulation of immune effector process	243	94	74.13	3.64E-03	5.56E-02
GO:0046700	heterocycle catabolic process	358	133	109.22	3.66E-03	5.58E-02
GO:0034472	snRNA 3'-end processing	17	11	5.19	3.72E-03	5.66E-02
GO:0006367	transcription initiation from RNA polymerase II promoter	56	27	17.08	3.98E-03	6.04E-02
GO:0042098	T cell proliferation	118	50	36	4.02E-03	6.09E-02
GO:0002204	somatic recombination of immunoglobulin genes involved in immune response	31	17	9.46	4.07E-03	6.10E-02
GO:0002208	somatic diversification of immunoglobulins involved in immune response	31	17	9.46	4.07E-03	6.10E-02
GO:0045190	isotype switching	31	17	9.46	4.07E-03	6.10E-02
GO:0000055	ribosomal large subunit export from nucleus	7	6	2.14	4.16E-03	6.10E-02
GO:0006267	pre-replicative complex assembly involved in nuclear cell cycle DNA replication	7	6	2.14	4.16E-03	6.10E-02
GO:0006273	lagging strand elongation	7	6	2.14	4.16E-03	6.10E-02
GO:0006735	NADH regeneration	7	6	2.14	4.16E-03	6.10E-02
GO:0032071	regulation of endodeoxyribonuclease activity	7	6	2.14	4.16E-03	6.10E-02
GO:0036388	pre-replicative complex assembly	7	6	2.14	4.16E-03	6.10E-02
GO:0048302	regulation of isotype switching to IgG isotypes	7	6	2.14	4.16E-03	6.10E-02
GO:0061621	canonical glycolysis	7	6	2.14	4.16E-03	6.10E-02
GO:0061718	glucose catabolic process to pyruvate	7	6	2.14	4.16E-03	6.10E-02
GO:1902299	pre-replicative complex assembly involved in cell cycle DNA replication	7	6	2.14	4.16E-03	6.10E-02
GO:0002902	regulation of B cell apoptotic process	15	10	4.58	4.16E-03	6.10E-02
GO:0020027	hemoglobin metabolic process	15	10	4.58	4.16E-03	6.10E-02
GO:0030220	platelet formation	15	10	4.58	4.16E-03	6.10E-02

Supplemental Table 5

Table 2.S5. List of murine drug-processing genes, antioxidant components, nuclear receptors, and BA synthesis genes that were selected by the investigators based on current literature.

Gene symbol	Gene name	Synonym	Family	Category
Alb	albumin		Albumin	Antioxidant components
Alox12	arachidonate 12-lipoxygenase		Arachidonate lipoxygenase	Antioxidant components
Alox12b	arachidonate 12-lipoxygenase, 12R type		Arachidonate lipoxygenase	Antioxidant components
Alox12e	arachidonate lipoxygenase, epidermal		Arachidonate lipoxygenase	Antioxidant components
Alox15	arachidonate 15-lipoxygenase		Arachidonate lipoxygenase	Antioxidant components
Alox5	arachidonate 5-lipoxygenase		Arachidonate lipoxygenase	Antioxidant components
Alox5ap	arachidonate 5-lipoxygenase activating protein		Arachidonate lipoxygenase	Antioxidant components
Alox8	arachidonate 8-lipoxygenase		Arachidonate lipoxygenase	Antioxidant components
ApoE	apolipoprotein E			Antioxidant components
Atox1	antioxidant 1 copper chaperone			Antioxidant components
Bnip1	BCL2/adenovirus E1B interacting protein 1		BCL2 interacting protein	Antioxidant components
Bnip2	BCL2/adenovirus E1B interacting protein 2		BCL2 interacting protein	Antioxidant components
Bnip3	BCL2/adenovirus E1B interacting protein 3		BCL2 interacting protein	Antioxidant components
Cat	catalase			Antioxidant components
Ccl5	chemokine (C-C motif) ligand 5			Antioxidant components
Ccs	copper chaperone for superoxide dismutase			Antioxidant components
Cygb	cytoglobin		Cytoglobin	Antioxidant components
Dhcr24	24-dehydrocholesterol reductase			Antioxidant components
Duox1	dual oxidase 1			Antioxidant components
Duox2	dual oxidase 2			Antioxidant components
Duoxa1	dual oxidase maturation factor 1			Antioxidant components
Duoxa2	dual oxidase maturation factor 2			Antioxidant components
Dusp1	dual specificity phosphatase 1			Antioxidant components
Epx	eosinophil peroxidase			Antioxidant components
Foxm1	forkhead box M1			Antioxidant components
Fth1	ferritin heavy polypeptide 1			Antioxidant components
Glrx	glutaredoxin		Glutaredoxin	Antioxidant components
Glrx2	glutaredoxin 2 (thioltransferase)		Glutaredoxin	Antioxidant components
Glrx3	glutaredoxin 3		Glutaredoxin	Antioxidant components
Glrx5	glutaredoxin 5		Glutaredoxin	Antioxidant components
Gss	glutathione synthetase			Antioxidant components
Gtf2i	general transcription factor II I		Glutathione S-transferase	Antioxidant components
Hmox1	heme oxygenase 1		Heme oxygenase	Antioxidant components
Hspa1a	heat shock protein 1A			Antioxidant components
Krt1	keratin 1			Antioxidant components
Mb	myoglobin			Antioxidant components
Mbl2	mannose-binding lectin (protein C) 2			Antioxidant components
Mpv17	MpV17 mitochondrial inner membrane protein			Antioxidant components

Msra	methionine sulfoxide reductase A			Antioxidant components
Mt1	metallothionein 1		Metallothionein	Antioxidant components
Mt2	metallothionein 2		Metallothionein	Antioxidant components
Mt3	metallothionein 3		Metallothionein	Antioxidant components
Mt4	metallothionein 4		Metallothionein	Antioxidant components
Ncf1	neutrophil cytosolic factor 1		Neutrophil cytosolic factor	Antioxidant components
Ncf2	neutrophil cytosolic factor 2		Neutrophil cytosolic factor	Antioxidant components
Ncf4	neutrophil cytosolic factor 4		Neutrophil cytosolic factor	Antioxidant components
Nos1	nitric oxide synthase 1, neuronal		Nitric oxide synthase	Antioxidant components
Nos1ap	nitric oxide synthase 1 (neuronal) adaptor protein		Nitric oxide synthase	Antioxidant components
Nos2	nitric oxide synthase 2, inducible		Nitric oxide synthase	Antioxidant components
Nos3	nitric oxide synthase 3, endothelial cell		Nitric oxide synthase	Antioxidant components
Nosip	nitric oxide synthase interacting protein		Nitric oxide synthase	Antioxidant components
Nostrin	nitric oxide synthase trafficker		Nitric oxide synthase	Antioxidant components
Nox1	NADPH oxidase 1		NADPH oxidase	Antioxidant components
Nox3	NADPH oxidase 3		NADPH oxidase	Antioxidant components
Nox4	NADPH oxidase 4		NADPH oxidase	Antioxidant components
Noxa1	NADPH oxidase activator 1		NADPH oxidase	Antioxidant components
Noxo1	NADPH oxidase organizer 1		NADPH oxidase	Antioxidant components
Noxred1	NADP+ dependent oxidoreductase domain containing 1		NADPH oxidase	Antioxidant components
Nudt1	nudix (nucleoside diphosphate linked moiety X)-type motif 1			Antioxidant components
Oxr1	oxidation resistance 1			Antioxidant components
Oxsr1	oxidative-stress responsive 1			Antioxidant components
Pdlim1	PDZ and LIM domain 1 (elfin)			Antioxidant components
Pnkp	polynucleotide kinase 3'-phosphatase			Antioxidant components
Prdx1	peroxiredoxin 1		Peroxiredoxin	Antioxidant components
Prdx2	peroxiredoxin 2		Peroxiredoxin	Antioxidant components
Prdx3	peroxiredoxin 3		Peroxiredoxin	Antioxidant components
Prdx4	peroxiredoxin 4		Peroxiredoxin	Antioxidant components
Prdx5	peroxiredoxin 5		Peroxiredoxin	Antioxidant components
Prdx6	peroxiredoxin 6		Peroxiredoxin	Antioxidant components
Prdx6b	peroxiredoxin 6B		Peroxiredoxin	Antioxidant components
Prex1	phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 1		Phosphatidylinositol-3,4,5-trisphosphate dependent Rac exchange factor	Antioxidant components
Prex2	phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 2		Phosphatidylinositol-3,4,5-trisphosphate dependent Rac exchange factor	Antioxidant components
Prnp	prion protein			Antioxidant components
Pxdn	peroxidase			Antioxidant components
Rnf7	ring finger protein 7			Antioxidant components
Scara3	scavenger receptor class A, member 3			Antioxidant components
Selenop	selenoprotein P			Antioxidant components
Sftpd	surfactant associated protein D			Antioxidant components
Sirt2	sirtuin 2			Antioxidant components
Socs7	suppressor of cytokine signaling 7			Antioxidant components

Sod1	superoxide dismutase 1, soluble			Antioxidant components
Sod2	superoxide dismutase 2, mitochondrial			Antioxidant components
Srxn1	sulfiredoxin 1 homolog (<i>S. cerevisiae</i>)			Antioxidant components
Stk25	serine/threonine kinase 25 (yeast)			Antioxidant components
Ttn	titin			Antioxidant components
Txn1	thioredoxin 1			Antioxidant components
Txn2	thioredoxin 2			Antioxidant components
Txnrd1	thioredoxin reductase 1		Thioredoxin reductase	Antioxidant components
Txnrd2	thioredoxin reductase 2		Thioredoxin reductase	Antioxidant components
Txnrd3	thioredoxin reductase 3		Thioredoxin reductase	Antioxidant components
Ucp2	uncoupling protein 2 (mitochondrial, proton carrier)		Uncoupling protein	Antioxidant components
Acox2	acyl-Coenzyme A oxidase 2, branched chain		Other enzymes involved in primary bile acid synthesis	Bile acid synthesis
Akr1d1	aldo-keto reductase family 1, member D1		Aldo-keto reductase	Bile acid synthesis
Akr1c14	aldo-keto reductase family 1, member C14		Aldo-keto reductase	Bile acid synthesis
Amacr	alpha-methylacyl-CoA racemase		Other enzymes involved in primary bile acid synthesis	Bile acid synthesis
Baat	bile acid-Coenzyme A: amino acid N-acyltransferase	BAT	Bile acid conjugation, bile acid-Coenzyme A: amino acid N-acyltransferase	Bile acid synthesis
Crip2	cysteine rich protein 2		Transcriptional repressors as direct target gene of FXR	Bile acid synthesis
Cyp7a1	cytochrome P450, family 7, subfamily a, polypeptide 1		Cytochrome P450	Bile acid synthesis
Cyp7b1	cytochrome P450, family 7, subfamily b, polypeptide 1		Cytochrome P450	Bile acid synthesis
Cyp8b1	cytochrome P450, family 8, subfamily b, polypeptide 1		Cytochrome P450	Bile acid synthesis
Cyp27a1	cytochrome P450, family 27, subfamily a, polypeptide 1		Cytochrome P450	Bile acid synthesis
Cyp39a1	cytochrome P450, family 39, subfamily a, polypeptide 1		Cytochrome P450	Bile acid synthesis
Hmgcr	3-hydroxy-3-methylglutaryl-Coenzyme A reductase			Bile acid synthesis
Hsd3b7	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7			Bile acid synthesis
Hsd17b4	hydroxysteroid (17-beta) dehydrogenase 4			Bile acid synthesis
Mafg	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein G (avian)			Bile acid synthesis
Scp2	stem cell proliferation 2			Bile acid synthesis
Slc27a2	solute carrier family 27 (fatty acid transporter), member 2	BAL	Bile acid ligase, for bile acid conjugation reaction	Bile acid synthesis
Slc27a5	solute carrier family 27 (fatty acid transporter), member 5	Fatp5	Other enzymes involved in primary bile acid synthesis	Bile acid synthesis
Zfp385a	zinc finger protein 385A		Transcriptional repressors as direct target gene of FXR	Bile acid synthesis
Ar	androgen receptor			Nuclear receptor
Esr1	estrogen receptor 1 (alpha)	ER α		Nuclear receptor
Esr2	estrogen receptor 2 (beta)	ER β		Nuclear receptor
Esrra	estrogen related receptor, alpha	ERR α		Nuclear receptor
Esrrb	estrogen related receptor, beta	ERR β		Nuclear receptor
Esrrg	estrogen-related receptor gamma	ERR γ		Nuclear receptor
Hnf4a	hepatic nuclear factor 4, alpha	HNF4 α	Hepatocyte nuclear factor	Nuclear receptor
Hnf4g	hepatocyte nuclear factor 4, gamma	HNF4 γ	Hepatocyte nuclear factor	Nuclear receptor
Nr0b1	nuclear receptor subfamily 0, group B, member 1	DAX-1		Nuclear receptor
Nr0b2	nuclear receptor subfamily 0, group B, member 2	SHP		Nuclear receptor
Nr1d1	nuclear receptor subfamily 1, group D, member 1	REV-ERB α		Nuclear receptor
Nr1d2	nuclear receptor subfamily 1, group D, member 2	REV-ERB β		Nuclear receptor

Nr1h2	nuclear receptor subfamily 1, group H, member 2	LXR β	Cholesterol sensor	Nuclear receptor
Nr1h3	nuclear receptor subfamily 1, group H, member 3	LXR α	Cholesterol sensor	Nuclear receptor
Nr1h4	nuclear receptor subfamily 1, group H, member 4	FXR α	Bile acid receptor, nuclear receptor (farnesoid X receptor)	Nuclear receptor
Nr1h5	nuclear receptor subfamily 1, group H, member 5	FXR β		Nuclear receptor
Nr1i2	nuclear receptor subfamily 1, group I, member 2	PXR		Nuclear receptor
Nr1i3	nuclear receptor subfamily 1, group I, member 3	CAR		Nuclear receptor
Nr2c1	nuclear receptor subfamily 2, group C, member 1	TR2		Nuclear receptor
Nr2c2	nuclear receptor subfamily 2, group C, member 2	TR4		Nuclear receptor
Nr2e1	nuclear receptor subfamily 2, group E, member 1	TLX		Nuclear receptor
Nr2e3	nuclear receptor subfamily 2, group E, member 3	PNR		Nuclear receptor
Nr2f1	nuclear receptor subfamily 2, group F, member 1	COUP-TF α		Nuclear receptor
Nr2f2	nuclear receptor subfamily 2, group F, member 2	COUP-TF β		Nuclear receptor
Nr2f6	nuclear receptor subfamily 2, group F, member 6	COUP-TF γ		Nuclear receptor
Nr3c1	nuclear receptor subfamily 3, group C, member 1	GR		Nuclear receptor
Nr3c2	nuclear receptor subfamily 3, group C, member 2	MR		Nuclear receptor
Nr4a1	nuclear receptor subfamily 4, group A, member 1	NGF1-B		Nuclear receptor
Nr4a2	nuclear receptor subfamily 4, group A, member 2	NURR1		Nuclear receptor
Nr4a3	nuclear receptor subfamily 4, group A, member 3	NOR-1		Nuclear receptor
Nr5a1	nuclear receptor subfamily 5, group A, member 1	SF-1		Nuclear receptor
Nr5a2	nuclear receptor subfamily 5, group A, member 2	LRH-1		Nuclear receptor
Nr6a1	nuclear receptor subfamily 6, group A, member 1	GCMF		Nuclear receptor
Pgr	progesterone receptor	PR		Nuclear receptor
Ppara	peroxisome proliferator activated receptor alpha	PPAR α	Peroxisome proliferator activated receptor	Nuclear receptor
Ppard	peroxisome proliferator activator receptor delta	PPAR β / δ	Peroxisome proliferator activated receptor	Nuclear receptor
Pparg	peroxisome proliferator activated receptor gamma	PPAR γ	Peroxisome proliferator activated receptor	Nuclear receptor
Rara	retinoic acid receptor, alpha	RAR α	Retinoic acid receptor	Nuclear receptor
Rarb	retinoic acid receptor, beta	RAR β	Retinoic acid receptor	Nuclear receptor
Rarg	retinoic acid receptor, gamma	RAR γ	Retinoic acid receptor	Nuclear receptor
Rora	RAR-related orphan receptor alpha	ROR α	RAR-related orphan receptor	Nuclear receptor
Rorb	RAR-related orphan receptor beta	ROR β	RAR-related orphan receptor	Nuclear receptor
Rorc	RAR-related orphan receptor gamma	ROR γ	RAR-related orphan receptor	Nuclear receptor
Rxra	retinoid X receptor alpha	RXR α	Retinoid X receptor	Nuclear receptor
Rxrb	retinoid X receptor beta	RXR β	Retinoid X receptor	Nuclear receptor
Rxrg	retinoid X receptor gamma	RXR γ	Retinoid X receptor	Nuclear receptor
Thra	thyroid hormone receptor alpha	TR α		Nuclear receptor
Thrb	thyroid hormone receptor beta	TR β		Nuclear receptor
Vdr	vitamin D (1,25-dihydroxyvitamin D3) receptor	VDR	Nuclear receptor	Nuclear receptor
Aadac	arylacetamide deacetylase		Arylacetamide deacetylase	Phase I
Aadac12	arylacetamide deacetylase like 2		Arylacetamide deacetylase	Phase I
Aadac13	arylacetamide deacetylase like 3		Arylacetamide deacetylase	Phase I
Ache	acetylcholinesterase			Phase I
Adh1	alcohol dehydrogenase 1 (class I)		Alcohol dehydrogenase	Phase I

Adh4	alcohol dehydrogenase 4 (class II), pi polypeptide		Alcohol dehydrogenase	Phase I
Adh5	alcohol dehydrogenase 5 (class III), chi polypeptide		Alcohol dehydrogenase	Phase I
Adh6a	alcohol dehydrogenase 6A (class V)		Alcohol dehydrogenase	Phase I
Adh6-ps1	alcohol dehydrogenase 6 (class V), pseudogene 1		Alcohol dehydrogenase	Phase I
Adh7	alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide		Alcohol dehydrogenase	Phase I
Adhfe1	alcohol dehydrogenase, iron containing, 1		Alcohol dehydrogenase	Phase I
Akp1	alkaline phosphatase 1		Alkaline phosphatase	Phase I
Akp3	alkaline phosphatase 3, intestine, not Mn requiring		Alkaline phosphatase	Phase I
Akp4	alkaline phosphatase 4		Alkaline phosphatase	Phase I
Akp-ps1	alkaline phosphatase pseudogene 1		Alkaline phosphatase	Phase I
Akr1a1	aldo-keto reductase family 1, member A1 (aldehyde reductase)		Aldo-keto reductase	Phase I
Akr1b3	aldo-keto reductase family 1, member B3 (aldose reductase)		Aldo-keto reductase	Phase I
Akr1b7	aldo-keto reductase family 1, member B7		Aldo-keto reductase	Phase I
Akr1b8	aldo-keto reductase family 1, member B8		Aldo-keto reductase	Phase I
Akr1b10	aldo-keto reductase family 1, member B10 (aldose reductase)		Aldo-keto reductase	Phase I
Akr1c6	aldo-keto reductase family 1, member C6		Aldo-keto reductase	Phase I
Akr1c12	aldo-keto reductase family 1, member C12		Aldo-keto reductase	Phase I
Akr1c13	aldo-keto reductase family 1, member C13		Aldo-keto reductase	Phase I
Akr1c18	aldo-keto reductase family 1, member C18		Aldo-keto reductase	Phase I
Akr1c19	aldo-keto reductase family 1, member C19		Aldo-keto reductase	Phase I
Akr1c20	aldo-keto reductase family 1, member C20		Aldo-keto reductase	Phase I
Akr1c21	aldo-keto reductase family 1, member C21		Aldo-keto reductase	Phase I
Akr1e1	aldo-keto reductase family 1, member E1		Aldo-keto reductase	Phase I
Akr7a5	aldo-keto reductase family 7, member A5 (aflatoxin aldehyde reductase)		Aldo-keto reductase	Phase I
Aldh1a1	aldehyde dehydrogenase family 1, subfamily A1		Aldehyde dehydrogenase	Phase I
Aldh1a2	aldehyde dehydrogenase family 1, subfamily A2		Aldehyde dehydrogenase	Phase I
Aldh1a3	aldehyde dehydrogenase family 1, subfamily A3		Aldehyde dehydrogenase	Phase I
Aldh1a7	aldehyde dehydrogenase family 1, subfamily A7		Aldehyde dehydrogenase	Phase I
Aldh1b1	aldehyde dehydrogenase 1 family, member B1		Aldehyde dehydrogenase	Phase I
Aldh1l1	aldehyde dehydrogenase 1 family, member L1		Aldehyde dehydrogenase	Phase I
Aldh1l2	aldehyde dehydrogenase 1 family, member L2		Aldehyde dehydrogenase	Phase I
Aldh2	aldehyde dehydrogenase 2, mitochondrial		Aldehyde dehydrogenase	Phase I
Aldh3a1	aldehyde dehydrogenase family 3, subfamily A1		Aldehyde dehydrogenase	Phase I
Aldh3a2	aldehyde dehydrogenase family 3, subfamily A2		Aldehyde dehydrogenase	Phase I
Aldh3b1	aldehyde dehydrogenase 3 family, member B1		Aldehyde dehydrogenase	Phase I
Aldh3b2	aldehyde dehydrogenase 3 family, member B2		Aldehyde dehydrogenase	Phase I
Aldh4a1	aldehyde dehydrogenase 4 family, member A1		Aldehyde dehydrogenase	Phase I
Aldh5a1	aldehyde dehydrogenase family 5, subfamily A1		Aldehyde dehydrogenase	Phase I
Aldh6a1	aldehyde dehydrogenase family 6, subfamily A1		Aldehyde dehydrogenase	Phase I
Aldh7a1	aldehyde dehydrogenase family 7, member A1		Aldehyde dehydrogenase	Phase I
Aldh8a1	aldehyde dehydrogenase 8 family, member A1		Aldehyde dehydrogenase	Phase I
Aldh9a1	aldehyde dehydrogenase 9, subfamily A1		Aldehyde dehydrogenase	Phase I
Aldh16a1	aldehyde dehydrogenase 16 family, member A1		Aldehyde dehydrogenase	Phase I

Aldh18a1	aldehyde dehydrogenase 18 family, member A1		Aldehyde dehydrogenase	Phase I
Aloxe3	arachidonate lipoxygenase 3		Arachidonate lipoxygenase	Phase I
Alpi	alkaline phosphatase, intestinal			Phase I
Alpl	alkaline phosphatase, liver/bone/kidney			Phase I
Alpl2	alkaline phosphatase, placental-like 2			Phase I
Aoc1	amine oxidase, copper-containing 1			Phase I
Aoc2	amine oxidase, copper containing 2 (retina-specific)			Phase I
Aoc3	amine oxidase, copper containing 3			Phase I
Aox1	aldehyde oxidase 1			Phase I
Aox2	aldehyde oxidase 2			Phase I
Aox3	aldehyde oxidase 3			Phase I
Aox4	aldehyde oxidase 4			Phase I
Bche	butyrylcholinesterase			Phase I
Bphl	biphenyl hydrolase-like (serine hydrolase, breast epithelial mucin-associated antigen)			Phase I
Cbr1	carbonyl reductase 1		Carbonyl reductase	Phase I
Cbr2	carbonyl reductase 2		Carbonyl reductase	Phase I
Cbr3	carbonyl reductase 3		Carbonyl reductase	Phase I
Cbr4	carbonyl reductase 4		Carbonyl reductase	Phase I
Cda	cytidine deaminase			Phase I
Cel	carboxyl ester lipase			Phase I
Cela1	chymotrypsin-like elastase family, member 1		Chymotrypsin like elastase	Phase I
Cela2a	chymotrypsin-like elastase family, member 2A		Chymotrypsin like elastase	Phase I
Cela3b	chymotrypsin-like elastase family, member 3B		Chymotrypsin like elastase	Phase I
Celf1	CUGBP, Elav-like family member 1		CUGBP Elav-like family	Phase I
Celf2	CUGBP, Elav-like family member 2		CUGBP Elav-like family	Phase I
Celf3	CUGBP, Elav-like family member 3		CUGBP Elav-like family	Phase I
Celf4	CUGBP, Elav-like family member 4		CUGBP Elav-like family	Phase I
Celf5	CUGBP, Elav-like family member 5		CUGBP Elav-like family	Phase I
Celf6	CUGBP, Elav-like family member 6		CUGBP Elav-like family	Phase I
Celsr1	cadherin, EGF LAG seven-pass G-type receptor 1		Cadherin, EGF LAG seven-pass G-type receptor	Phase I
Celsr2	cadherin, EGF LAG seven-pass G-type receptor 2		Cadherin, EGF LAG seven-pass G-type receptor	Phase I
Celsr3	cadherin, EGF LAG seven-pass G-type receptor 3		Cadherin, EGF LAG seven-pass G-type receptor	Phase I
Ces1a	carboxylesterase 1A		Carboxylesterase	Phase I
Ces1b	carboxylesterase 1B		Carboxylesterase	Phase I
Ces1c	carboxylesterase 1C		Carboxylesterase	Phase I
Ces1d	carboxylesterase 1D		Carboxylesterase	Phase I
Ces1e	carboxylesterase 1E		Carboxylesterase	Phase I
Ces1f	carboxylesterase 1F		Carboxylesterase	Phase I
Ces1g	carboxylesterase 1G		Carboxylesterase	Phase I
Ces2a	carboxylesterase 2A		Carboxylesterase	Phase I
Ces2b	carboxylesterase 2B		Carboxylesterase	Phase I
Ces2c	carboxylesterase 2C		Carboxylesterase	Phase I
Ces2d-ps	carboxylesterase 2D, pseudogene		Carboxylesterase	Phase I

Ces2e	carboxylesterase 2E		Carboxylesterase	Phase I
Ces2f	carboxylesterase 2F		Carboxylesterase	Phase I
Ces2g	carboxylesterase 2G		Carboxylesterase	Phase I
Ces2h	carboxylesterase 2H		Carboxylesterase	Phase I
Ces3a	carboxylesterase 3A		Carboxylesterase	Phase I
Ces3b	carboxylesterase 3B		Carboxylesterase	Phase I
Ces4a	carboxylesterase 4A		Carboxylesterase	Phase I
Ces5a	carboxylesterase 5A		Carboxylesterase	Phase I
Clpb	ClpB caseinolytic peptidase B			Phase I
Cpb2	carboxypeptidase B2 (plasma)			Phase I
Cpe	carboxypeptidase E			Phase I
Cyb5a	cytochrome b5 type A		Cytochrome b5	Phase I
Cyb5b	cytochrome b5 type B		Cytochrome b5	Phase I
Cyb5d1	cytochrome b5 domain containing 1		Cytochrome b5	Phase I
Cyb5d2	cytochrome b5 domain containing 2		Cytochrome b5	Phase I
Cyb5r1	cytochrome b5 reductase 1		Cytochrome b5	Phase I
Cyb5r2	cytochrome b5 reductase 2		Cytochrome b5	Phase I
Cyb5r3	cytochrome b5 reductase 3		Cytochrome b5	Phase I
Cyb5r4	cytochrome b5 reductase 4		Cytochrome b5	Phase I
Cyb5rl	cytochrome b5 reductase-like		Cytochrome b5	Phase I
Cyp1a1	cytochrome P450, family 1, subfamily a, polypeptide 1		Cytochrome P450	Phase I
Cyp1a2	cytochrome P450, family 1, subfamily a, polypeptide 2		Cytochrome P450	Phase I
Cyp1b1	cytochrome P450, family 1, subfamily b, polypeptide 1		Cytochrome P450	Phase I
Cyp2a4	cytochrome P450, family 2, subfamily a, polypeptide 4		Cytochrome P450	Phase I
Cyp2a5	cytochrome P450, family 2, subfamily a, polypeptide 5		Cytochrome P450	Phase I
Cyp2a12	cytochrome P450, family 2, subfamily a, polypeptide 12		Cytochrome P450	Phase I
Cyp2a22	cytochrome P450, family 2, subfamily a, polypeptide 22		Cytochrome P450	Phase I
Cyp2ab1	cytochrome P450, family 2, subfamily ab, polypeptide 1		Cytochrome P450	Phase I
Cyp2b9	cytochrome P450, family 2, subfamily b, polypeptide 9		Cytochrome P450	Phase I
Cyp2b10	cytochrome P450, family 2, subfamily b, polypeptide 10		Cytochrome P450	Phase I
Cyp2b13	cytochrome P450, family 2, subfamily b, polypeptide 13		Cytochrome P450	Phase I
Cyp2b19	cytochrome P450, family 2, subfamily b, polypeptide 19		Cytochrome P450	Phase I
Cyp2b23	cytochrome P450, family 2, subfamily b, polypeptide 23		Cytochrome P450	Phase I
Cyp2c23	cytochrome P450, family 2, subfamily c, polypeptide 23		Cytochrome P450	Phase I
Cyp2c29	cytochrome P450, family 2, subfamily c, polypeptide 29		Cytochrome P450	Phase I
Cyp2c37	cytochrome P450, family 2, subfamily c, polypeptide 37		Cytochrome P450	Phase I
Cyp2c38	cytochrome P450, family 2, subfamily c, polypeptide 38		Cytochrome P450	Phase I
Cyp2c39	cytochrome P450, family 2, subfamily c, polypeptide 39		Cytochrome P450	Phase I
Cyp2c40	cytochrome P450, family 2, subfamily c, polypeptide 40		Cytochrome P450	Phase I
Cyp2c50	cytochrome P450, family 2, subfamily c, polypeptide 50		Cytochrome P450	Phase I
Cyp2c53-ps	cytochrome P450, family 2, subfamily c, polypeptide 53-ps		Cytochrome P450	Phase I
Cyp2c54	cytochrome P450, family 2, subfamily c, polypeptide 54		Cytochrome P450	Phase I
Cyp2c55	cytochrome P450, family 2, subfamily c, polypeptide 55		Cytochrome P450	Phase I

Cyp2c65	cytochrome P450, family 2, subfamily c, polypeptide 65		Cytochrome P450	Phase I
Cyp2c66	cytochrome P450, family 2, subfamily c, polypeptide 66		Cytochrome P450	Phase I
Cyp2c67	cytochrome P450, family 2, subfamily c, polypeptide 67		Cytochrome P450	Phase I
Cyp2c68	cytochrome P450, family 2, subfamily c, polypeptide 68		Cytochrome P450	Phase I
Cyp2c69	cytochrome P450, family 2, subfamily c, polypeptide 69		Cytochrome P450	Phase I
Cyp2c70	cytochrome P450, family 2, subfamily c, polypeptide 70		Cytochrome P450	Phase I
Cyp2d9	cytochrome P450, family 2, subfamily d, polypeptide 9		Cytochrome P450	Phase I
Cyp2d10	cytochrome P450, family 2, subfamily d, polypeptide 10		Cytochrome P450	Phase I
Cyp2d11	cytochrome P450, family 2, subfamily d, polypeptide 11		Cytochrome P450	Phase I
Cyp2d12	cytochrome P450, family 2, subfamily d, polypeptide 12		Cytochrome P450	Phase I
Cyp2d13	cytochrome P450, family 2, subfamily d, polypeptide 13		Cytochrome P450	Phase I
Cyp2d22	cytochrome P450, family 2, subfamily d, polypeptide 22		Cytochrome P450	Phase I
Cyp2d26	cytochrome P450, family 2, subfamily d, polypeptide 26		Cytochrome P450	Phase I
Cyp2d34	cytochrome P450, family 2, subfamily d, polypeptide 34		Cytochrome P450	Phase I
Cyp2d37-ps	cytochrome P450, family 2, subfamily d, polypeptide 37, pseudogene		Cytochrome P450	Phase I
Cyp2d40	cytochrome P450, family 2, subfamily d, polypeptide 40		Cytochrome P450	Phase I
Cyp2e1	cytochrome P450, family 2, subfamily e, polypeptide 1		Cytochrome P450	Phase I
Cyp2f2	cytochrome P450, family 2, subfamily f, polypeptide 2		Cytochrome P450	Phase I
Cyp2g1	cytochrome P450, family 2, subfamily g, polypeptide 1		Cytochrome P450	Phase I
Cyp2j5	cytochrome P450, family 2, subfamily j, polypeptide 5		Cytochrome P450	Phase I
Cyp2j6	cytochrome P450, family 2, subfamily j, polypeptide 6		Cytochrome P450	Phase I
Cyp2j7-ps1	cytochrome P450, family 2, subfamily j, polypeptide 6, pseudogene 1		Cytochrome P450	Phase I
Cyp2j7-ps2	cytochrome P450, family 2, subfamily j, polypeptide 6, pseudogene 2		Cytochrome P450	Phase I
Cyp2j7-ps3	cytochrome P450, family 2, subfamily j, polypeptide 6, pseudogene 3		Cytochrome P450	Phase I
Cyp2j8	cytochrome P450, family 2, subfamily j, polypeptide 8		Cytochrome P450	Phase I
Cyp2j9	cytochrome P450, family 2, subfamily j, polypeptide 9		Cytochrome P450	Phase I
Cyp2j11	cytochrome P450, family 2, subfamily j, polypeptide 11		Cytochrome P450	Phase I
Cyp2j12	cytochrome P450, family 2, subfamily j, polypeptide 12		Cytochrome P450	Phase I
Cyp2j13	cytochrome P450, family 2, subfamily j, polypeptide 13		Cytochrome P450	Phase I
Cyp2r1	cytochrome P450, family 2, subfamily r, polypeptide 1		Cytochrome P450	Phase I
Cyp2s1	cytochrome P450, family 2, subfamily s, polypeptide 1		Cytochrome P450	Phase I
Cyp2t4	cytochrome P450, family 2, subfamily t, polypeptide 4		Cytochrome P450	Phase I
Cyp2u1	cytochrome P450, family 2, subfamily u, polypeptide 1		Cytochrome P450	Phase I
Cyp2w1	cytochrome P450, family 2, subfamily w, polypeptide 1		Cytochrome P450	Phase I
Cyp3a11	cytochrome P450, family 3, subfamily a, polypeptide 11		Cytochrome P450	Phase I
Cyp3a13	cytochrome P450, family 3, subfamily a, polypeptide 13		Cytochrome P450	Phase I
Cyp3a16	cytochrome P450, family 3, subfamily a, polypeptide 16		Cytochrome P450	Phase I
Cyp3a25	cytochrome P450, family 3, subfamily a, polypeptide 25		Cytochrome P450	Phase I
Cyp3a41a	cytochrome P450, family 3, subfamily a, polypeptide 41A		Cytochrome P450	Phase I
Cyp3a44	cytochrome P450, family 3, subfamily a, polypeptide 44		Cytochrome P450	Phase I
Cyp3a57	cytochrome P450, family 3, subfamily a, polypeptide 57		Cytochrome P450	Phase I
Cyp3a59	cytochrome P450, family 3, subfamily a, polypeptide 59		Cytochrome P450	Phase I

Cyp4a10	cytochrome P450, family 4, subfamily a, polypeptide 10		Cytochrome P450	Phase I
Cyp4a12a	cytochrome P450, family 4, subfamily a, polypeptide 12a		Cytochrome P450	Phase I
Cyp4a12b	cytochrome P450, family 4, subfamily a, polypeptide 12B		Cytochrome P450	Phase I
Cyp4a14	cytochrome P450, family 4, subfamily a, polypeptide 14		Cytochrome P450	Phase I
Cyp4a30b	cytochrome P450, family 4, subfamily a, polypeptide 30b		Cytochrome P450	Phase I
Cyp4a30-ps	cytochrome P450, family 4, subfamily a, member 30, pseudogene		Cytochrome P450	Phase I
Cyp4a31	cytochrome P450, family 4, subfamily a, polypeptide 31		Cytochrome P450	Phase I
Cyp4a32	cytochrome P450, family 4, subfamily a, polypeptide 32		Cytochrome P450	Phase I
Cyp4b1	cytochrome P450, family 4, subfamily b, polypeptide 1		Cytochrome P450	Phase I
Cyp4f13	cytochrome P450, family 4, subfamily f, polypeptide 13		Cytochrome P450	Phase I
Cyp4f14	cytochrome P450, family 4, subfamily f, polypeptide 14		Cytochrome P450	Phase I
Cyp4f15	cytochrome P450, family 4, subfamily f, polypeptide 15		Cytochrome P450	Phase I
Cyp4f16	cytochrome P450, family 4, subfamily f, polypeptide 16		Cytochrome P450	Phase I
Cyp4f17	cytochrome P450, family 4, subfamily f, polypeptide 17		Cytochrome P450	Phase I
Cyp4f18	cytochrome P450, family 4, subfamily f, polypeptide 18		Cytochrome P450	Phase I
Cyp4f37	cytochrome P450, family 4, subfamily f, polypeptide 37		Cytochrome P450	Phase I
Cyp4f39	cytochrome P450, family 4, subfamily f, polypeptide 39		Cytochrome P450	Phase I
Cyp4f40	cytochrome P450, family 4, subfamily f, polypeptide 40		Cytochrome P450	Phase I
Cyp4f41-ps	cytochrome P450, family 4, subfamily f, polypeptide 41 pseudogene		Cytochrome P450	Phase I
Cyp4v3	cytochrome P450, family 4, subfamily v, polypeptide 3		Cytochrome P450	Phase I
Cyp4x1	cytochrome P450, family 4, subfamily x, polypeptide 1		Cytochrome P450	Phase I
Cyp11a1	cytochrome P450, family 11, subfamily a, polypeptide 1		Cytochrome P450	Phase I
Cyp11b1	cytochrome P450, family 11, subfamily b, polypeptide 1		Cytochrome P450	Phase I
Cyp11b2	cytochrome P450, family 11, subfamily b, polypeptide 2		Cytochrome P450	Phase I
Cyp17a1	cytochrome P450, family 17, subfamily a, polypeptide 1		Cytochrome P450	Phase I
Cyp19a1	cytochrome P450, family 19, subfamily a, polypeptide 1		Cytochrome P450	Phase I
Cyp20a1	cytochrome P450, family 20, subfamily a, polypeptide 1		Cytochrome P450	Phase I
Cyp21a1	cytochrome P450, family 21, subfamily a, polypeptide 1		Cytochrome P450	Phase I
Cyp24a1	cytochrome P450, family 24, subfamily a, polypeptide 1		Cytochrome P450	Phase I
Cyp26a1	cytochrome P450, family 26, subfamily a, polypeptide 1		Cytochrome P450	Phase I
Cyp26b1	cytochrome P450, family 26, subfamily b, polypeptide 1		Cytochrome P450	Phase I
Cyp26c1	cytochrome P450, family 26, subfamily c, polypeptide 1		Cytochrome P450	Phase I
Cyp27a1	cytochrome P450, family 27, subfamily a, polypeptide 1		Cytochrome P450	Phase I
Cyp27b1	cytochrome P450, family 27, subfamily b, polypeptide 1		Cytochrome P450	Phase I
Cyp39a1	cytochrome P450, family 39, subfamily a, polypeptide 1		Cytochrome P450	Phase I
Cyp46a1	cytochrome P450, family 46, subfamily a, polypeptide 1		Cytochrome P450	Phase I
Cyp51	cytochrome P450, family 51		Cytochrome P450	Phase I
Dao	D-amino acid oxidase			Phase I
Dhdh	dihydrodiol dehydrogenase (dimeric)			Phase I
Dhrs1	dehydrogenase/reductase (SDR family) member 1		Dehydrogenase/reductase	Phase I
Dhrs2	dehydrogenase/reductase member 2		Dehydrogenase/reductase	Phase I
Dhrs3	dehydrogenase/reductase (SDR family) member 3		Dehydrogenase/reductase	Phase I

Dhrs4	dehydrogenase/reductase (SDR family) member 4		Dehydrogenase/reductase	Phase I
Dhrs7	dehydrogenase/reductase (SDR family) member 7		Dehydrogenase/reductase	Phase I
Dhrs7b	dehydrogenase/reductase (SDR family) member 7B		Dehydrogenase/reductase	Phase I
Dhrs7c	dehydrogenase/reductase (SDR family) member 7C		Dehydrogenase/reductase	Phase I
Dhrs9	dehydrogenase/reductase (SDR family) member 9		Dehydrogenase/reductase	Phase I
Dhrs11	dehydrogenase/reductase (SDR family) member 11		Dehydrogenase/reductase	Phase I
Dhrs13	dehydrogenase/reductase (SDR family) member 13		Dehydrogenase/reductase	Phase I
Dhrsx	dehydrogenase/reductase (SDR family) X chromosome		Dehydrogenase/reductase	Phase I
Doxl2	diamine oxidase-like protein 2			Phase I
Dpyd	dihydropyrimidine dehydrogenase			Phase I
Ephx1	epoxide hydrolase 1, microsomal		Epoxide hydrolase	Phase I
Ephx2	epoxide hydrolase 2, cytoplasmic		Epoxide hydrolase	Phase I
Ephx3	epoxide hydrolase 3		Epoxide hydrolase	Phase I
Ephx4	epoxide hydrolase 4		Epoxide hydrolase	Phase I
Erap1	endoplasmic reticulum aminopeptidase 1			Phase I
Esd	esterase D/formylglutathione hydrolase			Phase I
Fmo1	flavin containing monooxygenase 1		Flavin containing dimethylaniline monooxygenase	Phase I
Fmo2	flavin containing monooxygenase 2		Flavin containing dimethylaniline monooxygenase	Phase I
Fmo3	flavin containing monooxygenase 3		Flavin containing dimethylaniline monooxygenase	Phase I
Fmo4	flavin containing monooxygenase 4		Flavin containing dimethylaniline monooxygenase	Phase I
Fmo5	flavin containing monooxygenase 5		Flavin containing dimethylaniline monooxygenase	Phase I
Fmo6	flavin containing monooxygenase 6		Flavin containing dimethylaniline monooxygenase	Phase I
Fmo9	flavin containing monooxygenase 9		Flavin containing dimethylaniline monooxygenase	Phase I
Gpx1	glutathione peroxidase 1		Glutathione peroxidase	Phase I
Gpx2	glutathione peroxidase 2		Glutathione peroxidase	Phase I
Gpx2-ps1	glutathione peroxidase 2, pseudogene 1		Glutathione peroxidase	Phase I
Gpx3	glutathione peroxidase 3		Glutathione peroxidase	Phase I
Gpx4	glutathione peroxidase 4		Glutathione peroxidase	Phase I
Gpx5	glutathione peroxidase 5		Glutathione peroxidase	Phase I
Gpx6	glutathione peroxidase 6		Glutathione peroxidase	Phase I
Gpx7	glutathione peroxidase 7		Glutathione peroxidase	Phase I
Gpx8	glutathione peroxidase 8 (putative)		Glutathione peroxidase	Phase I
Gsr	glutathione reductase			Phase I
Gusb	glucuronidase, beta			Phase I
Htra1	HtrA serine peptidase 1		Serine peptidase	Phase I
Htra2	HtrA serine peptidase 2		Serine peptidase	Phase I
Htra3	HtrA serine peptidase 3		Serine peptidase	Phase I
Htra4	HtrA serine peptidase 4		Serine peptidase	Phase I
Ky	kyphoscoliosis peptidase			Phase I
Kynu	kynureninase			Phase I
Lap3	leucine aminopeptidase 3			Phase I
Laptm4a	lysosomal-associated protein transmembrane 4A		Lysosomal-associated protein transmembrane	Phase I
Laptm4b	lysosomal-associated protein transmembrane 4B		Lysosomal-associated protein transmembrane	Phase I

Laptm5	lysosomal-associated protein transmembrane 5		Lysosomal-associated protein transmembrane	Phase I
Lox	lysyl oxidase			Phase I
Lpo	lactoperoxidase			Phase I
Lta4h	leukotriene A4 hydrolase			Phase I
Maoa	monoamine oxidase A			Phase I
Maob	monoamine oxidase B			Phase I
Marc1	mitochondrial amidoxime reducing component 1			Phase I
Marc2	mitochondrial amidoxime reducing component 2			Phase I
Mocos	molybdenum cofactor sulfurase			Phase I
Mocs1	molybdenum cofactor synthesis 1		Molybdenum cofactor synthesis	Phase I
Mocs2	molybdenum cofactor synthesis 2		Molybdenum cofactor synthesis	Phase I
Mocs3	molybdenum cofactor synthesis 3		Molybdenum cofactor synthesis	Phase I
Mpo	myeloperoxidase			Phase I
Nqo1	NAD(P)H dehydrogenase, quinone 1		NAD(P)H quinone dehydrogenase	Phase I
Nqo2	N-ribosyldihyronicotinamide quinone reductase 2		NAD(P)H quinone dehydrogenase	Phase I
Paox	polyamine oxidase (exo-N4-amino)			Phase I
Pepd	peptidase D			Phase I
Pon1	paraoxonase 1		Paraoxonase	Phase I
Pon2	paraoxonase 2		Paraoxonase	Phase I
Pon3	paraoxonase 3		Paraoxonase	Phase I
Por	P450 (cytochrome) oxidoreductase		Cytochrome p450 oxidoreductase	Phase I
Prdx6-ps2	peroxiredoxin 6 pseudogene 2		Peroxiredoxin	Phase I
Ptgs1	prostaglandin-endoperoxide synthase 1			Phase I
Ptgs2	prostaglandin-endoperoxide synthase 2			Phase I
Rnls	renalase, FAD-dependent amine oxidase			Phase I
Salpa1	serum alkaline phosphatase activity 1		Serum alkaline phosphatase	Phase I
Salpa2	serum alkaline phosphatase activity 2		Serum alkaline phosphatase	Phase I
Salpa3	serum alkaline phosphatase activity 3		Serum alkaline phosphatase	Phase I
Salpa4	serum alkaline phosphatase activity 4		Serum alkaline phosphatase	Phase I
Sdr9c7	4short chain dehydrogenase/reductase family 9C, member 7		Short chain dehydrogenase/reductase	Phase I
Sdr16c5	short chain dehydrogenase/reductase family 16C, member 5		Short chain dehydrogenase/reductase	Phase I
Sdr16c6	short chain dehydrogenase/reductase family 16C, member 6		Short chain dehydrogenase/reductase	Phase I
Sdr39u1	short chain dehydrogenase/reductase family 39U, member 1		Short chain dehydrogenase/reductase	Phase I
Sdr42e1	short chain dehydrogenase/reductase family 42E, member 1		Short chain dehydrogenase/reductase	Phase I
Smox	spermine oxidase			Phase I
Suox	sulfite oxidase		Sulfite oxidase	Phase I
Tpo	thyroid peroxidase			Phase I
Upp1	uridine phosphorylase 1			Phase I
Xdh	xanthine dehydrogenase			Phase I
Acsm1	acyl-CoA synthetase medium-chain family member 1			Phase II
Acsm2	acyl-CoA synthetase medium-chain family member 2			Phase II
Acsm3	acyl-CoA synthetase medium-chain family member 3			Phase II
Acsm4	acyl-CoA synthetase medium-chain family member 4			Phase II

Acsm5	acyl-CoA synthetase medium-chain family member 5			Phase II
As3mt	arsenic (+3 oxidation state) methyltransferase			Phase II
Baat	bile acid-Coenzyme A: amino acid N-acyltransferase			Phase II
Comt	catechol-O-methyltransferase			Phase II
Gamt	guanidinoacetate methyltransferase			Phase II
Gclc	glutamate-cysteine ligase, catalytic subunit			Phase II
Gclm	glutamate-cysteine ligase, modifier subunit			Phase II
Glyat	glycine-N-acyltransferase			Phase II
Glyatl3	glycine-N-acyltransferase-like 3			Phase II
Gnmt	glycine N-methyltransferase			Phase II
Gsta1	glutathione S-transferase, alpha 1 (Ya)		Glutathione S-transferase	Phase II
Gsta2	glutathione S-transferase, alpha 2 (Yc2)		Glutathione S-transferase	Phase II
Gsta3	glutathione S-transferase, alpha 3		Glutathione S-transferase	Phase II
Gsta4	glutathione S-transferase, alpha 4		Glutathione S-transferase	Phase II
Gstcd	glutathione S-transferase, C-terminal domain containing		Glutathione S-transferase	Phase II
Gstk1	glutathione S-transferase kappa 1		Glutathione S-transferase	Phase II
Gstm1	glutathione S-transferase, mu 1		Glutathione S-transferase	Phase II
Gstm2	glutathione S-transferase, mu 2		Glutathione S-transferase	Phase II
Gstm3	glutathione S-transferase, mu 3		Glutathione S-transferase	Phase II
Gstm4	glutathione S-transferase, mu 4		Glutathione S-transferase	Phase II
Gstm5	glutathione S-transferase, mu 5		Glutathione S-transferase	Phase II
Gstm6	glutathione S-transferase, mu 6		Glutathione S-transferase	Phase II
Gstm7	glutathione S-transferase, mu 7		Glutathione S-transferase	Phase II
Gsto1	glutathione S-transferase omega 1		Glutathione S-transferase	Phase II
Gsto2	glutathione S-transferase omega 2		Glutathione S-transferase	Phase II
Gstp1	glutathione S-transferase, pi 1		Glutathione S-transferase	Phase II
Gstp2	glutathione S-transferase, pi 2		Glutathione S-transferase	Phase II
Gstt1	glutathione S-transferase, theta 1		Glutathione S-transferase	Phase II
Gstt2	glutathione S-transferase, theta 2		Glutathione S-transferase	Phase II
Gstt3	glutathione S-transferase, theta 3		Glutathione S-transferase	Phase II
Gstt4	glutathione S-transferase, theta 4		Glutathione S-transferase	Phase II
Gstz1	glutathione transferase zeta 1 (maleylacetoacetate isomerase)		Glutathione S-transferase	Phase II
Hnmt	histamine N-methyltransferase			Phase II
Inmt	indolethylamine N-methyltransferase			Phase II
Lcat	lecithin cholesterol acyltransferase			Phase II
Lipa	lysosomal acid lipase A			Phase II
Mat1a	methionine adenosyltransferase I, alpha			Phase II
Mat2a	methionine adenosyltransferase II, alpha			Phase II
Mat2b	methionine adenosyltransferase II, beta			Phase II
Mgst1	microsomal glutathione S-transferase 1		Glutathione S-transferase	Phase II
Mgst2	microsomal glutathione S-transferase 2		Glutathione S-transferase	Phase II
Mgst3	microsomal glutathione S-transferase 3		Glutathione S-transferase	Phase II
Naa60	N(alpha)-acetyltransferase 60, NatF catalytic subunit		N-acetyltransferase	Phase II

Naa80	N(alpha)-acetyltransferase 80, catalytic subunit	NatH	N-acetyltransferase	Phase II
Nat1	N-acetyl transferase 1		N-acetyltransferase	Phase II
Nat2	N-acetyltransferase 2 (arylamine N-acetyltransferase)		N-acetyltransferase	Phase II
Nat3	N-acetyltransferase 3		N-acetyltransferase	Phase II
Nat8	N-acetyltransferase 8 (GCN5-related)		N-acetyltransferase	Phase II
Nat8l	N-acetyltransferase 8-like		N-acetyltransferase	Phase II
Nat9	N-acetyltransferase 9 (GCN5-related, putative)		N-acetyltransferase	Phase II
Nat10	N-acetyltransferase 10		N-acetyltransferase	Phase II
Nat14	N-acetyltransferase 14		N-acetyltransferase	Phase II
Nnmt	nicotinamide N-methyltransferase			Phase II
Papss1	3'-phosphoadenosine 5'-phosphosulfate synthase 1		3'-phosphoadenosine 5'-phosphosulfate synthase	Phase II
Papss2	3'-phosphoadenosine 5'-phosphosulfate synthase 2		3'-phosphoadenosine 5'-phosphosulfate synthase	Phase II
Pemt	phosphatidylethanolamine N-methyltransferase			Phase II
Pnmt	phenylethanolamine-N-methyltransferase			Phase II
Sars	seryl-aminoacyl-tRNA synthetase			Phase II
Sars2	seryl-aminoacyl-tRNA synthetase 2			Phase II
Soat1	sterol O-acyltransferase 1			Phase II
Soat2	sterol O-acyltransferase 2			Phase II
Sult1a1	sulfotransferase family 1A, phenol-preferring, member 1		Sulfotransferase	Phase II
Sult1b1	sulfotransferase family 1B, member 1		Sulfotransferase	Phase II
Sult1c1	sulfotransferase family, cytosolic, 1C, member 1		Sulfotransferase	Phase II
Sult1c2	sulfotransferase family, cytosolic, 1C, member 2		Sulfotransferase	Phase II
Sult1d1	sulfotransferase family 1D, member 1		Sulfotransferase	Phase II
Sult1e1	sulfotransferase family 1E, member 1		Sulfotransferase	Phase II
Sult2a1	sulfotransferase family 2A, dehydroepiandrosterone preferring, member 1		Sulfotransferase	Phase II
Sult2a2	sulfotransferase family 2A, dehydroepiandrosterone preferring, member 2		Sulfotransferase	Phase II
Sult2a3	sulfotransferase family 2A, dehydroepiandrosterone preferring, member 3		Sulfotransferase	Phase II
Sult2a4	sulfotransferase family 2A, dehydroepiandrosterone preferring, member 4		Sulfotransferase	Phase II
Sult2a5	sulfotransferase family 2A, dehydroepiandrosterone preferring, member 5		Sulfotransferase	Phase II
Sult2a6	sulfotransferase family 2A, dehydroepiandrosterone preferring, member 6		Sulfotransferase	Phase II
Sult2a7	sulfotransferase family 2A, dehydroepiandrosterone preferring, member 7		Sulfotransferase	Phase II
Sult2b1	sulfotransferase family, cytosolic, 2B, member 1		Sulfotransferase	Phase II
Sult3a1	sulfotransferase family 3A, member 1		Sulfotransferase	Phase II
Sult4a1	sulfotransferase family 4A, member 1		Sulfotransferase	Phase II
Sult5a1	sulfotransferase family 5A, member 1		Sulfotransferase	Phase II
Sult6b1	sulfotransferase family, cytosolic, 6B, member 1		Sulfotransferase	Phase II
Tpmt	thiopurine methyltransferase			Phase II
Ugdh	UDP-glucose dehydrogenase			Phase II
Ugp2	UDP-glucose pyrophosphorylase 2			Phase II

Ugt1a1	UDP glucuronosyltransferase 1 family, polypeptide A1		UDP glucuronosyltransferase	Phase II
Ugt1a2	UDP glucuronosyltransferase 1 family, polypeptide A2		UDP glucuronosyltransferase	Phase II
Ugt1a5	UDP glucuronosyltransferase 1 family, polypeptide A5		UDP glucuronosyltransferase	Phase II
Ugt1a6a	UDP glucuronosyltransferase 1 family, polypeptide A6A		UDP glucuronosyltransferase	Phase II
Ugt1a6b	UDP glucuronosyltransferase 1 family, polypeptide A6B		UDP glucuronosyltransferase	Phase II
Ugt1a7c	UDP glucuronosyltransferase 1 family, polypeptide A7C		UDP glucuronosyltransferase	Phase II
Ugt1a9	UDP glucuronosyltransferase 1 family, polypeptide A9		UDP glucuronosyltransferase	Phase II
Ugt1a10	UDP glycosyltransferase 1 family, polypeptide A10		UDP glucuronosyltransferase	Phase II
Ugt2a1	UDP glucuronosyltransferase 2 family, polypeptide A1		UDP glucuronosyltransferase	Phase II
Ugt2a2	UDP glucuronosyltransferase 2 family, polypeptide A2		UDP glucuronosyltransferase	Phase II
Ugt2a3	UDP glucuronosyltransferase 2 family, polypeptide A3		UDP glucuronosyltransferase	Phase II
Ugt2b1	UDP glucuronosyltransferase 2 family, polypeptide B1		UDP glucuronosyltransferase	Phase II
Ugt2b5	UDP glucuronosyltransferase 2 family, polypeptide B5		UDP glucuronosyltransferase	Phase II
Ugt2b34	UDP glucuronosyltransferase 2 family, polypeptide B34		UDP glucuronosyltransferase	Phase II
Ugt2b35	UDP glucuronosyltransferase 2 family, polypeptide B35		UDP glucuronosyltransferase	Phase II
Ugt2b36	UDP glucuronosyltransferase 2 family, polypeptide B36		UDP glucuronosyltransferase	Phase II
Ugt2b37	UDP glucuronosyltransferase 2 family, polypeptide B37		UDP glucuronosyltransferase	Phase II
Ugt2b38	UDP glucuronosyltransferase 2 family, polypeptide B38		UDP glucuronosyltransferase	Phase II
Ugt3a1	UDP glycosyltransferases 3 family, polypeptide A1		UDP glucuronosyltransferase	Phase II
Ugt3a2	UDP glycosyltransferases 3 family, polypeptide A2		UDP glucuronosyltransferase	Phase II
Ugt8a	UDP galactosyltransferase 8A		UDP glucuronosyltransferase	Phase II
AhR	aryl-hydrocarbon receptor		Transcription factor	Transcription factor
Hnf1a	HNF1 homeobox A		Hepatocyte nuclear factor	Transcription factor
Nfe2l2	nuclear factor, erythroid derived 2, like 2	Nrf2	Transcription factor	Transcription factor
Abca1	ATP-binding cassette, sub-family A (ABC1), member 1	Abca1	Efflux transporter; basolateral efflux transporter for cholesterol	Transporter
Abcb4	ATP-binding cassette, sub-family B (MDR/TAP), member 4	Mdr2	Efflux transporter; canicular efflux transporter for phospholipid	Transporter
Abcb11	ATP-binding cassette, sub-family B (MDR/TAP), member 11	Bsep	Efflux transporter; rate-limiting canicular efflux transporter for bile acids (deficiency causes cholestasis, PFIC II)	Transporter
Abcb1a	ATP-binding cassette, sub-family B (MDR/TAP), member 1A	Mdr1a	Efflux transporter; canicular efflux transporters that may contribute to bile acid efflux from the knockout studies of other bile acid transporters	Transporter
Abcb1b	ATP-binding cassette, sub-family B (MDR/TAP), member 1B	Mdr1b	Efflux transporter; canicular efflux transporters that may contribute to bile acid efflux from the knockout studies of other bile acid transporters	Transporter
Abcc1	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	Mrp1	Efflux transporter; basolateral efflux transporter	Transporter
Abcc2	ATP-binding cassette, sub-family C (CFTR/MRP), member 2	Mrp2	Efflux transporter; basolateral efflux transporter	Transporter
Abcc3	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	Mrp3	Efflux transporter; basolateral efflux transporter	Transporter
Abcc4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	Mrp4	Efflux transporter; basolateral efflux transporter	Transporter
Abcc5	ATP-binding cassette, sub-family C (CFTR/MRP), member 5	Mrp5	Efflux transporter; basolateral efflux transporter	Transporter
Abcc6	ATP-binding cassette, sub-family C (CFTR/MRP), member 6	Mrp6	Efflux transporter; basolateral efflux transporter	Transporter
Abcc8	ATP-binding cassette, sub-family C (CFTR/MRP), member 8	Mrp8, Sur	Efflux transporter; basolateral efflux transporter	Transporter
Abcc10	ATP-binding cassette, sub-family C (CFTR/MRP), member 10	Mrp7	Efflux transporter; basolateral efflux transporter	Transporter
Abcc12	ATP-binding cassette, sub-family C (CFTR/MRP), member 12	Mrp9	Efflux transporter; basolateral efflux transporter	Transporter
Abcg2	ATP binding cassette subfamily G member 2 (Junior blood group)		Efflux transporter	Transporter
Abcg5	ATP binding cassette subfamily G member 5		Efflux transporter; sterol efflux transporter	Transporter
Abcg8	ATP binding cassette subfamily G member 8		Efflux transporter; sterol efflux transporter	Transporter

Atp8b1	ATPase, class I, type 8B, member 1		Uptake transporter; canicular uptake transporter	Transporter
Slc10a1	solute carrier family 10 (sodium/bile acid cotransporter family), member 1	Ntcp	Uptake transporter	Transporter
Slc10a2	solute carrier family 10, member 2	Asbt	Uptake transporter	Transporter
Slc15a1	solute carrier family 15 (oligopeptide transporter), member 1	Pept1	Uptake transporter	Transporter
Slc15a2	solute carrier family 15 (H ⁺ /peptide transporter), member 2	Pept2	Uptake transporter	Transporter
Slc22a1	solute carrier family 22 (organic cation transporter), member 1	Oct1	Uptake transporter	Transporter
Slc22a2	solute carrier family 22 (organic cation transporter), member 2	Oct2	Uptake transporter	Transporter
Slc22a3	solute carrier family 22 (organic cation transporter), member 3	Oct3	Uptake transporter	Transporter
Slc22a4	solute carrier family 22 (organic cation transporter), member 4	Octn1	Uptake transporter	Transporter
Slc22a5	solute carrier family 22 (organic cation transporter), member 5	Octn2	Uptake transporter	Transporter
Slc22a6	solute carrier family 22 (organic anion transporter), member 6	Oat1	Uptake transporter	Transporter
Slc22a7	solute carrier family 22 (organic anion transporter), member 7	Oat2	Uptake transporter	Transporter
Slc22a8	solute carrier family 22 (organic anion transporter), member 8	Oat3	Uptake transporter	Transporter
Slc22a12	solute carrier family 22 (organic anion/cation transporter), member 12	Urat1, Oat4l	Uptake transporter	Transporter
Slc22a21	solute carrier family 22 (organic cation transporter), member 21	Octn3	Uptake transporter	Transporter
Slc28a1	solute carrier family 28 (sodium-coupled nucleoside transporter), member 1	Cnt1	Uptake transporter	Transporter
Slc28a2	solute carrier family 28 (sodium-coupled nucleoside transporter), member 2	Cnt2	Uptake transporter	Transporter
Slc28a3	solute carrier family 28 (sodium-coupled nucleoside transporter), member 3	Cnt3	Uptake transporter	Transporter
Slc29a1	solute carrier family 29 (nucleoside transporters), member 1	Ent1	Uptake/bidirectional transporter	Transporter
Slc29a2	solute carrier family 29 (nucleoside transporters), member 2	Ent2	Uptake/bidirectional transporter	Transporter
Slc29a3	solute carrier family 29 (nucleoside transporters), member 3	Ent3	Uptake/bidirectional transporter	Transporter
Slc29a4	solute carrier family 29 (nucleoside transporters), member 4	Ent4	Uptake/bidirectional transporter	Transporter
Slc47a1	solute carrier family 47, member 1	Mate1	Efflux transporter	Transporter
Slc47a2	solute carrier family 47, member 2	Mate2	Efflux transporter	Transporter
Slc51a	solute carrier family 51, alpha subunit	Ost α	Efflux transporter; basolateral efflux transporter for cholesterol	Transporter
Slc51b	solute carrier family 51, beta subunit	Ost β	Efflux transporter; basolateral efflux transporter for cholesterol	Transporter
Slco1a1	solute carrier organic anion transporter family, member 1a1	Oatp1a1	Uptake transporter	Transporter
Slco1a4	solute carrier organic anion transporter family, member 1a4	Asct1	Uptake transporter	Transporter
Slco1a5	solute carrier organic anion transporter family, member 1a5	Oatp3	Uptake transporter	Transporter
Slco1a6	solute carrier organic anion transporter family, member 1a6	Oatp5	Uptake transporter	Transporter
Slco1b2	solute carrier organic anion transporter family, member 1b2	Oatp1b2	Uptake transporter	Transporter
Slco1c1	solute carrier organic anion transporter family, member 1c1	OATP-14, OATP-F, Oatp2, Oatpf, Slc21a14	Uptake transporter	Transporter
Slco2a1	solute carrier organic anion transporter family, member 2a1	Pgt	Uptake transporter	Transporter
Slco2b1	solute carrier organic anion transporter family, member 2b1	Oatp2b1, OATP-B	Uptake transporter	Transporter
Slco3a1	solute carrier organic anion transporter family, member 3a1	Oatp3a1, OATP-D	Uptake transporter	Transporter
Slco4a1	solute carrier organic anion transporter family, member 4a1	Oatp4a1, OATP-E	Uptake transporter	Transporter
Slco4c1	solute carrier organic anion transporter family, member 4c1	Oatp4c1, OATP-H	Uptake transporter	Transporter
Slco5a1	solute carrier organic anion transporter family, member 5A1	Oatp5a1, OATP-J	Uptake transporter	Transporter
Slco6b1	solute carrier organic anion transporter family, member 6b1		Uptake transporter	Transporter
Slco6c1	solute carrier organic anion transporter family, member 6c1		Uptake transporter	Transporter
Slco6d1	solute carrier organic anion transporter family, member 6d1		Uptake transporter	Transporter

Chapter 3: Pharmacological Activation of PXR and CAR Down-regulates Distinct Bile Acid-metabolizing Intestinal Bacteria and Alters Bile Acid Homeostasis

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3.1 Introduction

Liver is a critical organ for xenobiotic biotransformation and nutrient homeostasis. The nuclear receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR) are well-known xenobiotic-sensing transcription factors with overlapping target genes involved in the Phase-I and Phase-II xenobiotic metabolism as well as transport (collectively called drug-processing genes [DPGs]) (Kliewer et al., 1998; Wei et al., 2000; Moore et al., 2003; Petrick and Klaassen, 2007; Hernandez et al., 2009; Cui and Klaassen, 2016).

PXR was first identified as a steroid-activating receptor before its function in xenobiotic metabolism was discovered (Kliewer et al., 1998; Kliewer et al., 2002). PXR is a promiscuous transcription factor that is activated by various xenobiotics, including therapeutic drugs such as dexamethasone, paclitaxel, and rifampicin (Moore et al., 2003), environmental toxicants such as the flame retardant polybrominated diphenyl ethers (PBDEs) (Pacyniak et al., 2007), some pesticides (Lemaire et al., 2006), and herbal supplements such as St. John's Wort (Moore et al., 2000). PXR is also activated by metabolites produced by the gut microbiome, such as the secondary bile acid (BA) lithocholic acid (LCA) and the tryptophan metabolite indole-3-propionic acid (Staudinger et al., 2001; Venkatesh et al., 2014). Pregnenolone-16 α -carbonitrile (PCN) is recognized as a specific PXR ligand in rodents, whereas rifampicin is specific to human PXR activation (Moore et al., 2003).

CAR was identified as a mediator of drug metabolism specifically for altering the sensitivity to barbiturates (Tzameli et al., 2000; Wei et al., 2000). Repressive endogenous ligands for constitutive activity of CAR include androstenol and androstanol (Forman et al., 1998), whereas endogenous activators of CAR are not well characterized. Phenobarbital and LCA can indirectly facilitate the nuclear localization of CAR and up-regulation of CAR-target genes (Swales and Negishi, 2004; Beilke et al., 2009a; Beilke et al., 2009b). A potent and selective CAR activator in mice is 1,4-bis-[2-(3,5-dichloropyridyloxy)]benzene, 3,3',5,5'-tetrachloro-1,4-bis(pyridyloxy)benzene (TCPOBOP) (Tzameli et al., 2000), whereas 6-(4-

chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime (CITCO) is a selective CAR activator in humans (Swales and Negishi, 2004). The flame retardant BDE-47 is an activator of both mouse and human CAR (Sueyoshi et al., 2014).

The gut microbiome is increasingly recognized as a key modifier of host xenobiotic biotransformation through direct and indirect mechanisms (Spanogiannopoulos et al., 2016; Koppel et al., 2017). For direct mechanisms, intestinal bacteria use their own enzymes that are distinct from the host to metabolize ingested compounds, such as dietary components, pharmaceuticals, and environmental chemicals (Spanogiannopoulos et al., 2016; Koppel et al., 2017). These microbial modifications, many of which remain poorly characterized, can either increase or decrease the toxicity or efficacy of drugs. The PharmacoMicrobiomics database has identified over 90 drugs in the literature that are modified by the gut microbiome, but only 28 have identifiable interactions with a specific bacterial species. For example, the first drug-bug interaction was reported by Drs. Lindenbaum and Saha for the inactivation of digoxin by *Eggerthella lenta* (Lindenbaum et al., 1981; Saha et al., 1983). Characterization of these microbial enzymes has led to better understanding of the pharmacokinetics of digoxin as well as improved efficacy and safety (Haiser et al., 2013; Haiser et al., 2014; Koppel et al., 2018). Furthermore, microbial β -glucuronidases deconjugate host Phase-II glucuronidation metabolites and modulate the potential toxicity of circulating endogenous and xenobiotic compounds (Wallace et al., 2015; Pollet et al., 2017; Little et al., 2018).

For indirect mechanisms, the gut microbiome can modify host metabolites that are subsequently absorbed into systemic circulation and enter various host organs to interact with host receptors involved in xenobiotic biotransformation (Spanogiannopoulos et al., 2016; Fu and Cui, 2017). The presence of the gut microbiome is necessary in regulating the constitutive expression of many drug processing genes (DPGs), evidenced from altered expression of DPGs in mice lacking a gut microbiome (i.e. germ-free [GF] mice) (Bjorkholm et al., 2009; Toda et al., 2009c; Selwyn et al., 2015a; Selwyn et al., 2015c). For example, the expression of the major

Phase-I oxidation enzyme cytochrome P450, family 3, subfamily a, polypeptide 11 (Cyp3a11)—a PXR-target gene and the mouse ortholog to human CYP3A4—was decreased in GF mice compared to CV mice. This effect was also observed in CV mice treated with antibiotics and was correlated with decreased bacteria in the feces that produce the PXR-activating BA LCA (Toda et al., 2009b; Toda et al., 2009a). Therefore, modulation of the gut microbiome and the bacterial metabolites can alter xenobiotic biotransformation of the host.

BAs are amphipathic molecules synthesized in liver *de novo* from cholesterol to primary BAs through two distinct pathways: 1) the classic pathway with the overall rate-limiting BA synthesis enzyme Cyp7a1 and 2) the alternative pathway initiated by Cyp27a1. Primary BAs are conjugated with taurine, glycine, or other co-substrates and undergo enterohepatic circulation by secretion into the bile and re-absorption from the intestine, facilitating the uptake of dietary fats, steroids, drugs, and lipophilic vitamins (Hofmann, 2009; Li and Chiang, 2014). In the intestine, bacteria further metabolize primary BAs to secondary BAs through deconjugation, oxidation, and epimerization reactions; this, in general, increases the hydrophobicity of the BA pool (Ridlon et al., 2006; Hofmann, 2009; Li and Chiang, 2014). Primary and secondary BAs also include their conjugate forms. In humans, the majority of BAs are conjugated with taurine, glycine, or 3'-phosphoadenosine-5'-phosphosulfate, whereas in mice, the majority of BAs are conjugated with taurine (Thakare et al., 2018). Primary BAs all have a 7-hydroxy substituent (from Cyp7a1), and in humans, the majority of primary BAs are chenodeoxycholic acid (CDCA) and cholic acid (CA). Cholic acid (CA) is the only primary BA from the classic pathway in liver and is metabolized by intestinal bacteria to the secondary BA deoxycholic acid (DCA). CDCA is produced from the alternative pathway of BA synthesis, and in mice, CDCA is metabolized to alpha-muricholic acid (α MCA), β MCA, and ursodeoxycholic acid (UDCA) (Hofmann, 2009). UDCA has been found increased in both genders of GF compared to CV mice, suggesting that a host mechanism exists to produce UDCA (Zhang and Klaassen, 2010; Selwyn et al., 2015b; Wahlstrom et al., 2017). There is no known host enzyme for the synthesis of UDCA in mice and humans. Secondary BAs

produced from the alternative pathway of BA synthesis include ωMCA, LCA, hyocholic acid (HCA), and hyodeoxycholic (HDCA). These BA biotransformation pathways create diverse sets of BAs with different physiological functions and nuclear receptor affinities (Li and Chiang, 2013).

Several studies have shown the importance of PXR and CAR in BA homeostasis. Pharmacological activation of PXR by PCN represses hepatic Cyp7a1 mRNA and activity in mice (Li and Chiang, 2013; Li and Chiang, 2014), and endogenous activation of PXR by the secondary BA LCA protects the liver against toxicity (Staudinger et al., 2001). In intestine/enterocytes, PXR activation induces fibroblast growth factor 19 (FGF19; Fgf15 in mice), an intestinally secreted hormone that inhibits BA synthesis by repressing the transcription of Cyp7a1 in liver, and the promoter of *FGF19* contains a PXR response element (Wistuba et al., 2007; Li and Chiang, 2013). Activation of CAR has also been shown to repress the expression of *CYP7A1* in human hepatocytes (Miao et al., 2006); yet, BAs are not known to serve as ligands of CAR (Li and Chiang, 2013). In a study investigating BA profiles in CV mice following CAR activation, TCPOBOP decreased total BAs in both males and females, largely due to a decrease in the primary BA taurocholic acid (T-CA), and this was associated with an increase in the mRNA expression of Cyp7a1 in liver to restore liver BAs to physiological concentrations (Lickteig et al., 2016). The BA ligase solute carrier family 27 member 5 (Slc27a5/Bal), which regulates BA conjugation, was decreased following TCPOBOP exposure (Cui and Klaassen, 2016), possibly contributes to the improved metabolic health following CAR activation (Dong et al., 2009; Gao et al., 2009).

Transporters are important to the distribution of BAs throughout the enterohepatic organs. The basolateral uptake transporter sodium taurocholic transporting polypeptide (Ntcp/Slc10a1) is responsible for the uptake of the majority of conjugated BAs from the portal blood into hepatocytes, whereas the organic anion transporting polypeptide (Oatp) 1b2 was thought to transport unconjugated BAs into hepatocytes (Klaassen and Aleksunes, 2010; Csanaky et al., 2011). The canalicular bile salt efflux pump (Bsep/Abcb11) is the rate-limiting transporter for BA biliary excretion, whereas on the basolateral membrane, the solute carriers Slc51a/Osta and

Slc51b/Ost β , as well as efflux transporter multidrug resistance-associated protein ATP binding cassette subfamily C member 3 (Abcc3/Mrp3) and Abcc4/Mrp4, transport BAs into blood as a compensatory mechanism during cholestasis (Halilbasic et al., 2013). Transporters regulated by PXR and CAR play an important role in the disposition of BAs between different compartments, such as liver, blood, and intestinal lumen. For example, early studies showed that biliary excretion of ouabain was increased by pre-treatment with PCN, demonstrating that the biliary clearance could be mediated by pharmacological activation of PXR (Russell and Klaassen, 1972; Thompson and Klaassen, 1995). PXR activators are known to increase the expression of Abcc3/Mrp3 (Zelcer et al., 2003; Cui and Klaassen, 2016); Mrp3 plays a prominent role in protecting the liver from BA overload by lowering the BA concentration in the liver and preventing apoptosis or necrosis (Hirohashi et al., 2000; Teng and Piquette-Miller, 2007; Dawson et al., 2009). TCPOBOP activation of CAR in mice increased Abcc2/Mrp2, Abcc3/Mrp3, and Abcc4/Mrp4. Similar to Mrp3, Mrp4 is a basolateral efflux transporter of BAs, reduced glutathione, and protects the liver from BA-induced injuries during cholestasis (Rius et al., 2006; Dawson et al., 2009). Mrp2 is a canalicular efflux transporter that contributes to the bile flow of sulfated and tetra-hydroxylated BAs (Akita et al., 2001; Nies and Keppler, 2007). Regarding uptake transporters, the promoter of Oatp1a4 has a PXR binding site but was not found to be important in BA uptake, but did modulate secondary BA metabolism in male mice (Cui et al., 2010; Zhang et al., 2013).

It is known that activation of PXR and CAR can modulate BA homeostasis through the regulation of host BA synthesis enzymes and transporters. However, little is known regarding whether activation of the host nuclear receptors modulates the gut microbiome and the subsequent microbial functions, including BA metabolism. Because primary BAs are metabolized by the gut microbiome into secondary BAs, the host regulation of primary BA synthesis is poorly understood. In addition, little is known regarding the characterization of BA profiles following PXR activation, and there is no comparison of the roles for PXR versus CAR activation on BA homeostasis. Because GF mice have no intestinal bacteria, they do not have secondary BAs,

allowing for the investigation of specific regulation of primary BAs by pharmacological interventions. Therefore, the goal of this study was to determine the effect of PXR and CAR activation on gut microbiome composition and the extent that the gut microbiome modulates primary and secondary BA homeostasis.

3.2 Materials and Methods

Chemicals. The mouse CAR ligand TCPOBOP (PubChem CID: 5382) and the mouse PXR ligand PCN (PubChem CID: 15032) were purchased from Sigma-Aldrich (St Louis, MO). For BA analysis, acetonitrile (PubChem CID: 6342), methanol (PubChem CID: 887), ammonium acetate (PubChem CID: 12432), and acetic acid (PubChem CID: 176; all LC-MS grade) were purchased from Fisher Scientific (Pittsburgh, PA). The 56 BA standard compounds (Supplemental Table 1) were purchased from Sigma-Aldrich (Saint Louis, MO) and Steraloids, Inc (Newport, RI). Stable isotope-labeled internal standards (IS), cholic acid-2,2,4,4-D4 (CA-D4) (PubChem CID of CA: 221493), lithocholic acid-2,2,4,4-D4 (LCA-D4) (PubChem CID of LCA: 9903), deoxycholic acid-2,2,4,4-D4 (DCA-D4) (PubChem CID of DCA: 222528), glycocholic acid-2,2,4,4-D4 (G-CA-D4) (PubChem CID of GCA: 10140), and glycochenodeoxycholic acid-2,2,4,4-D4 (G-CDCA-D4) (PubChem CID of GCDCA: 12544), were purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA). The purities of non-labeled standards were above 95-99%, and the purities of the five isotope-labeled compounds were above 98%.

Animals. C57BL/6J CV mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Age-matched male GF mice in C57BL/6 background were provided by the University of Washington Gnotobiotic Animal Core Facility (the initial GF breeding colony was established with mice purchased from the National Gnotobiotic Rodent Resource Center (University of North Carolina, Chapel Hill)). All mice were housed according to the Association for Assessment and Accreditation of Laboratory Animal Care International guidelines, and studies were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Washington. The CV mice were exposed to the same diet (laboratory autoclaved rodent diet #5010), water (non-acidified autoclaved water), and bedding (autoclaved Enrich-N'Pure) as the GF mice for four weeks prior to chemical exposure. All chemical solutions were sterilized using a Steriflip Vacuum-driven Filtration System with a 0.22 μ m Millipore Express Plus Membrane (EMD Millipore,

Temecula, CA). All gavage needles and syringes were sterilized by autoclave. At nine weeks of age, CV (n=5 per group) and GF mice (n=4-5 per group) were orally gavaged with vehicle (corn oil, 10 mL/kg), PCN (75 mg/kg), or TCPOBOP (3 mg/kg) once daily for four days. Doses for activating PXR and CAR with PCN and TCPOBOP, respectively, were selected based on previous studies (Cui and Klaassen, 2016; Li et al., 2016). On the 5th day, livers were collected and immediately frozen in liquid nitrogen. Blood was collected via cardiac puncture, and serum was obtained after centrifugation at 4 °C in a MiniCollect blood collection tube (Greiner bio-one, Monroe NC). The small and large intestinal contents (SIC and LIC) were flushed using ice-cold PBS containing 10 mM dithiothreitol and centrifuged at 20,000 g for 1h at 4 °C to collect pellets. The ileum tissue was also collected and immediately frozen in liquid nitrogen. All samples were stored at -80 °C until further analysis.

Bacterial 16S rDNA Sequencing. Bacterial DNA was isolated from LIC pellet of CV mice using an OMEGA E.Z.N.A. Stool DNA Kit (OMEGA Biotech Inc., Norcross, GA) per the manufacturer's protocol. The concentration of DNA was determined using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA). Bacterial 16S rDNA V4 amplicon sequencing was performed using an Illumina HiSeq 2500 sequencing system (250bp paired-end; n=3 per group; Beijing Genome Institute Americans Corporation, Cambridge, MA).

De-multiplexed and quality-filtered FASTQ files were analyzed using various python scripts in QIIME (Caporaso et al., 2010). Briefly, paired-end reads of the same sample were joined using *join_paired_ends.py*. Joined FASTQ files of all samples were each labeled with a unique identifier and then merged into one FASTA file using *split_libraries_fastq.py* for downstream analysis. Chimera sequences generated from PCR amplification artifacts were removed using *identify_chimeric_seqs.py*, and reference-based chimera detection was performed using usearch61 against the Greengenes 13_8 operational taxonomic units (OTUs)

database (99_otus.fasta) (http://qiime.org/home_static/dataFiles.html). OTUs were first picked using *pick_open_reference_otus.py* against the reference database followed by *de novo* OTU-picking of unaligned sequences, and both forward and reverse strands were considered. OTU tables were then sorted, format-converted, and taxonomy-summarized from level 2 (L2, phylum level) to L7 (species level) using various python scripts in QIIME. The α - and β -diversities were determined using *alpha_rarefaction.py* and *jackknifed_beta_diversity.py*, respectively. Predictive functional profiling of 16S rDNA data at the class level (L3) was performed using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST) (Langille et al., 2013) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathways.

qPCR validation of microbial bile acid-metabolizing enzymes. The DNA encoding selected microbial enzymes—the BA inducible operons (*bai*) CD and *baiJ*—that are known to convert primary BAs to secondary BAs as well as the BA-deconjugation enzyme bile salt hydrolase (*bsh*) were validated by quantitative polymerase chain reaction (qPCR) using a BioRad CFX384 Real-Time PCR Detection System (Bio-Rad, Hercules, CA). For each qPCR reaction, 30 ng of DNA reaction was loaded, and data were normalized to universal bacterial 16S rDNA. The bacterial DNA qPCR primers are shown in Supplemental Table 2.

Bile acid extraction. BAs from liver, serum, as well as SIC and LIC pellets were extracted using a similar method described previously (Zhang and Klaassen, 2010) with modifications. Briefly, for liver and intestinal content pellets, 5 μ L of ice-cold analytical grade H₂O were added per mg of sample, homogenized in a glass Dounce homogenizer until uniform, and 250 μ L of homogenate was removed and mixed with 10 μ L of the following 5 ISs: CA-d4, DCA-d4, GCA-d4, G-CDCA-d4, and LCA-d4. Samples were then precipitated in acetonitrile with 5% NH₄OH and centrifuged to obtain pellets. Pellets were re-suspended in methanol, centrifuged, and the supernatant was removed and dried under vacuum. For serum, 50 μ L of serum was mixed with 10 μ L of IS,

precipitated with ice-cold methanol, centrifuged, and the supernatant was dried under vacuum. The precipitates were reconstituted in 100 μ L 50% methanol water solution prior to LC-MS.

LC-MS quantification of bile acids. LC-MS analysis was performed on a Waters Acquity I-Class UPLC TQS-micro MS (Waters, Milford, MA) system. Five μ L of each sample were injected for analysis using negative ionization mode. Chromatographic separation was performed on a Waters XSelect HSS T3 column (2.5 μ m, 2.1 $\times$ 150 mm). The flow rate was 0.3 mL/min, auto-sampler temperature was kept at 4 $^{\circ}$ C, and the column compartment was set at 40 $^{\circ}$ C. The mobile phase was composed of Solvents A (5 mM ammonium acetate in H₂O with 0.1% acetic acid) and B (acetonitrile with 0.1% acetic acid). After the initial 1 min isocratic elution of 75% A, the percentage of Solvent A progressively decreased to 5% at t=15 min. The composition of Solvent A was maintained at 5% for 10 min (t=25 min), and then the percentage of A returned to 75% at t=25 for 10 min to prepare for the next injection. The total experimental time for each injection was 40min. The mass spectrometer was equipped with an electrospray ionization (ESI) source. Targeted data acquisition was performed in multiple-reaction-monitoring (MRM) mode. A total of 56 BAs (Supplemental Table 1) and 5 IS MRM transitions were monitored in the negative mode; tauro- α muricholic acid (T- α MCA) and T- β MCA could not be separated by chromatography and were analyzed together as T- α/β MCA. The metabolite identities were confirmed by spiking mixtures of standard compounds. The extracted MRM peaks were integrated using the TargetLynx software (Waters, Milford, MA).

RNA isolation and RT-qPCR of host bile acid-processing genes. Total RNA was isolated from liver and ileum tissue using RNeasy lysis reagent per manufacturer's protocol (Qiagen, Crawfordsville, IN). RNA concentration was determined using a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Waltham, MA) at 260 nm. Integrity of total RNA samples was confirmed by gel electrophoresis with visualization of 28S and 18S rRNA bands under

ultraviolet light. Reverse transcription was performed using a High Capacity cDNA Synthesis Kit (Applied Biosystems, Foster City, CA). The cDNAs were amplified by PCR using SsoAdvanced Universal SYBR Green Supermix in a BioRad CFX384 Real-Time PCR Detection System (BioRad, Hercules, CA). The qPCR primers targeting the cDNAs of the host BA-processing genes are shown in Supplemental Table 3. Data were normalized to the geometric means of the ddCq values of two housekeeping genes, namely glyceraldehyde 3-phosphate dehydrogenase (Gapdh) and β -actin.

Data analysis. For CV and GF mice exposed to chemicals or vehicle, statistical differences were determined using two-way ANOVA (exposure and enterotype) followed by Tukey's post hoc test (adjusted p -value < 0.05) in R. Two-way hierarchical clustering dendrograms of BAs were generated using the native function of R. For CV mice only, differentially regulated taxa were determined using one-way ANOVA followed by Duncan's post hoc test ($p < 0.05$). The R package corplot was used for Pearson's correlation and visualization between differentially regulated bacteria at the species level (L7) and the pooled sum of 14 BAs from all four compartments that were statistically significant among exposure groups ($p < 0.05$).

3.3 Results

Composition and predicted functions of bacteria in the large intestine

Overall, following PCN or TCPOBOP, we did not observe an apparent increase in body weight of CV or GF mice. As expected, TCPOBOP-exposed mice had increased liver weight due to increased cell proliferation. To determine the effect of pharmacological activation of PXR and CAR on the composition and predicted functions of the gut microbiome in CV mice, DNA was isolated from LIC and 16S rDNA sequencing was performed as described in Materials and Methods. The alpha diversity between corn oil, PCN, and TCPOBOP was determined using the Chao1 index, and no differences were observed in species richness and evenness (Supplemental Figure 3.S1A). The principle coordinates analysis plot of beta diversity as calculated by weighted UniFrac showed that mice of the same exposure had similar taxa abundance and phylogeny (Figure 3.1A).

At the class level, PCN and/or TCPOBOP differentially regulated the percentage of OTU of three taxa: Actinobacteria (decreased by PCN and TCPOBOP), Firmicutes Other (decreased by PCN), and Gammaproteobacteria (increased by PCN) (Figure 3.1B). Specifically, the PCN and TCPOBOP-mediated decrease in the relative abundance of Actinobacteria was attributed to decreased *Bifidobacterium* spp. (Figure 3.1C). *Bifidobacterium* spp. are known to express the BA deconjugation enzyme bsh (Ridlon et al., 2006). Consistent with reduced relative abundance of *Bifidobacterium* spp., qPCR analysis of the LIC DNA showed a decrease in the abundance of *bsh* genes (Figure 3.1D). Furthermore, the *baiCD* and *baiJ*, which metabolize primary BAs to secondary BAs, tended to decrease, but were not statistically significant (Figures 3.2D-G); *baiCD* and *baiJ* are known to be present in certain *Clostridia* species (Ridlon et al., 2006). This data suggests that PCN and TCPOBOP exposure may reduce secondary BA synthesis.

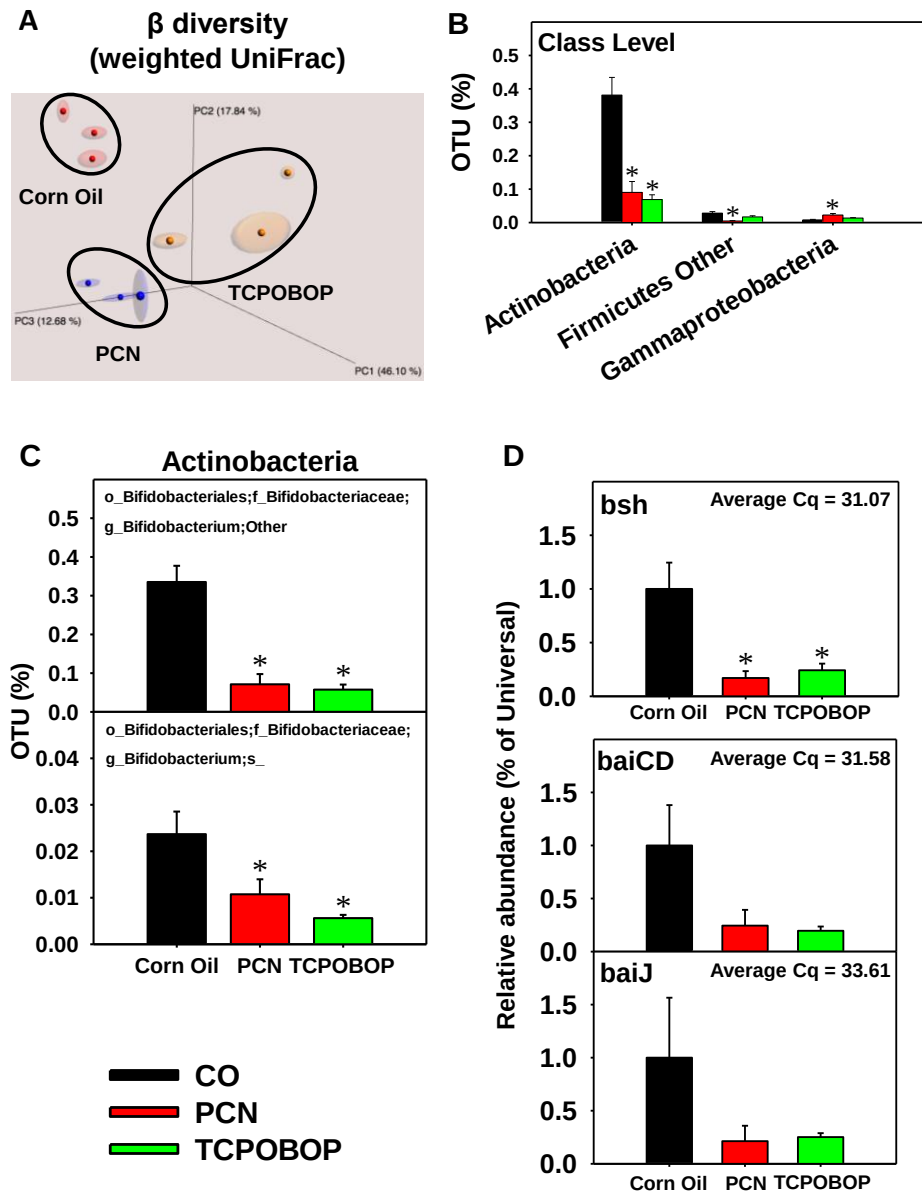


Figure 3.1. **A.** Beta diversity of LIC microbiota in CV mice orally exposed to CO, PCN, or TCPOBOP (n=3 per group). The 16S rDNA sequencing data were analyzed using QIIME as described in Materials and Methods. **B.** Differentially regulated intestinal bacteria at the class level (L3). **C.** Differentially regulated bacteria at the species level (L7) from the class Actinobacteria. **D.** Abundance of intestinal microbial DNA encoding bsh, which is known to be present in certain Bifidobacterium spp. (Ridlon et al., 2006), as well as BA inducible operons (bai) CD and J, which are known to be present in certain Clostridia species (Ridlon et al., 2006), from CV mice exposed to corn oil, PCN, or TCPOBOP (n=5 per group) (qPCR). Asterisks (*) represent statistically significant differences as compared to corn oil exposed group (one-way ANOVA followed by Duncan's post hoc test; statistical significance was considered at $p < 0.05$).

As shown in Figure 3.2, several other taxa were differentially regulated by PCN and/or TCPOBOP exposure. For example, PCN increased the *Ruminococcus* genus in the Clostridia class (Figure 3.2F). TCPOBOP increased a taxon of the Lactobacillales order in the Bacilli class, and this taxon was undetected in vehicle and PCN-exposed groups (Figure 3.2C). Both PCN and TCPOBOP reduced the relative abundance of the Actinobacteria class, *Anaeroplasma* spp. in the Mollicutes class (Figure 3.2B), two taxa in the *Dorea* genus (Figure 3.2D and E), as well as a taxon in the Peptococcaceae family (Figure 3.2G).

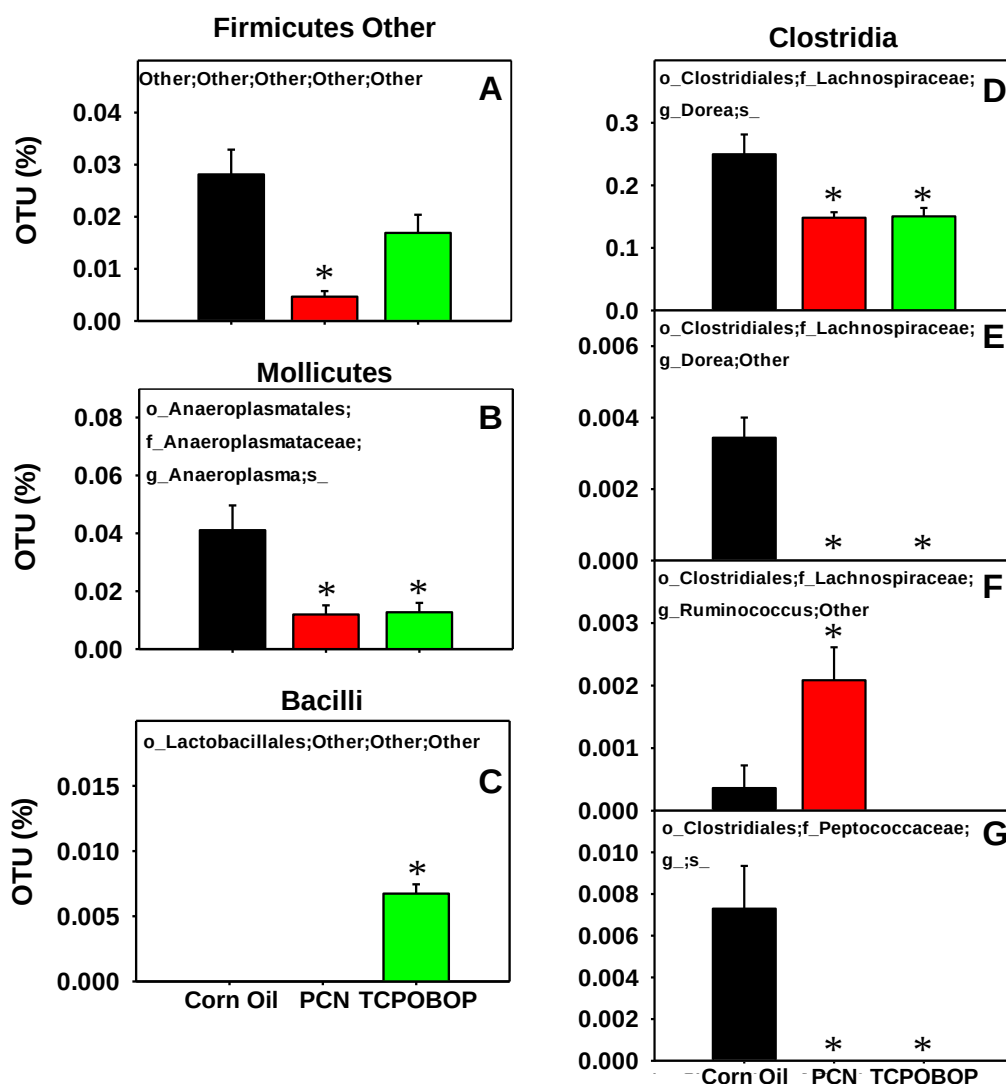


Figure 3.2. Differentially regulated intestinal bacteria at the species level (L7) within classes Firmicutes Other (**A**), Mollicutes (**B**), Bacilli (**C**), and Clostridia (**D**) from LIC of CV mice orally exposed to CO, PCN, or TCPOBOP (n=3 per group) (16S rDNA sequencing). Data were analyzed using QIIME as described in Materials and Methods. Asterisks (*) represent statistically significant differences as compared to corn oil exposed group (one-way ANOVA followed by Duncan's post hoc test; statistical significance was considered at $p < 0.05$).

PICRUSt was used to predict the abundance of gene functions of 274 identified gene families that form distinct KEGG pathways. PCN did not affect any of the KEGG pathways of the gut microbiome. However, TCPOBOP increased the gene family counts of three KEGG pathways: mineral absorption, ether lipid metabolism, and phosphotransferase system (Supplemental Figure 3.S3.1B). The KEGG pathway for secondary BA synthesis was present, but not significantly altered by PCN or TCPOBOP exposure.

Bile acids in liver, serum, SIC, and LIC

To determine how oral exposure to PCN and TCPOBOP affects BA homeostasis and to test our hypothesis that the decrease in BA-metabolizing bacteria and BA-metabolizing enzymes (Figure 3.1C and 1D) leads to reduced formation of secondary BAs, targeted metabolomics of 56 BAs in serum, liver, SIC, and LIC of CV mice given corn oil, PCN, or TCPOBOP. Individual BAs were classified into seven major categories as described in Supplemental Table 4: total, primary, secondary, conjugated, unconjugated, 6-OH, and 12-OH BAs. Interestingly, in all compartments tested, we detected low but significant levels of total secondary BAs. Because the GF mice were confirmed for the sterile conditions by bacterial culture and 16S rRNA qPCR, the presence of secondary BAs is not likely due to contamination. It is possible that certain BAs that were defined as secondary BAs may undergo host synthesis/repair mechanisms in GF conditions.

In liver, 21 BAs were above the limit of detection, and 18 of them were differentially regulated by either chemicals or enterotype. Under basal conditions, GF mice had higher total BAs than CV mice, and this was attributed to higher primary, conjugated, and unconjugated BAs (Figure 3.3A). In addition, there was a basal increase in 6-OH BAs but not 12-OH BAs in the absence of gut microbiota. Following chemical exposure, consistent with an apparent decrease in BA-metabolizing microbial enzymes (*bsh*, *baiCD*, and *baiJ*; Figure 3.1C and 1D), both PCN and TCPOBOP decreased total secondary BAs in liver in a microbiota-dependent manner (Figure 3.3A). PCN and TCPOBOP did not alter BAs from other categories in livers of CV mice. In livers

of GF mice, PCN also had minimal effect on any of the seven BA categories. In contrast, lack of gut microbiota augmented the TCPOBOP-mediated hepatic increase in total BAs, which was attributed to an increase in primary, conjugated, and 6-OH BAs, as well as an apparent increase in 12-OH BAs, but not unconjugated BAs (Figure 3.3A).

A two-way hierarchical clustering dendrogram of the relative concentrations of individual BAs in livers of CV and GF mice is shown in Supplemental Figure 3.S2, and examples of individual differentially regulated BAs are shown in Figure 3.3B. Under basal conditions, a total of two BAs (β MCA and T- α/β MCA) were higher and 11 BAs (UDCA, HDCA, CDCA, T-LCA, 12-DHCA, G-CA, T-DCA, ω MCA, 5 β -cholanic acid-3 α ,6 α -diol-7-one, T-CA, and T-CDCA) were lower in livers of GF mice compared to CV mice. In livers of CV mice, PCN reduced CDCA, α MCA, 12-DHCA, T-DCA, and 5 β -cholanic acid-3 α ,6 α -diol-7-one, all in a gut microbiota-dependent manner; whereas TCPOBOP reduced UDCA, HDCA, MCA, T-LCA, α MCA, 12-DHCA, T-DCA, 5 β -cholanic acid-3 α ,6 α -diol-7-one, and CA in a gut microbiota-dependent manner except for UDCA. Conversely, TCPOBOP increased T-CDCA in livers of CV mice, and such increase was further augmented in livers of GF mice. In addition, lack of gut microbiota potentiated the TCPOBOP-mediated increase in T- α/β MCA. T-HDCA was not altered by chemical exposure or lack of microbiota but was a major contributor to secondary BAs in GF mice.

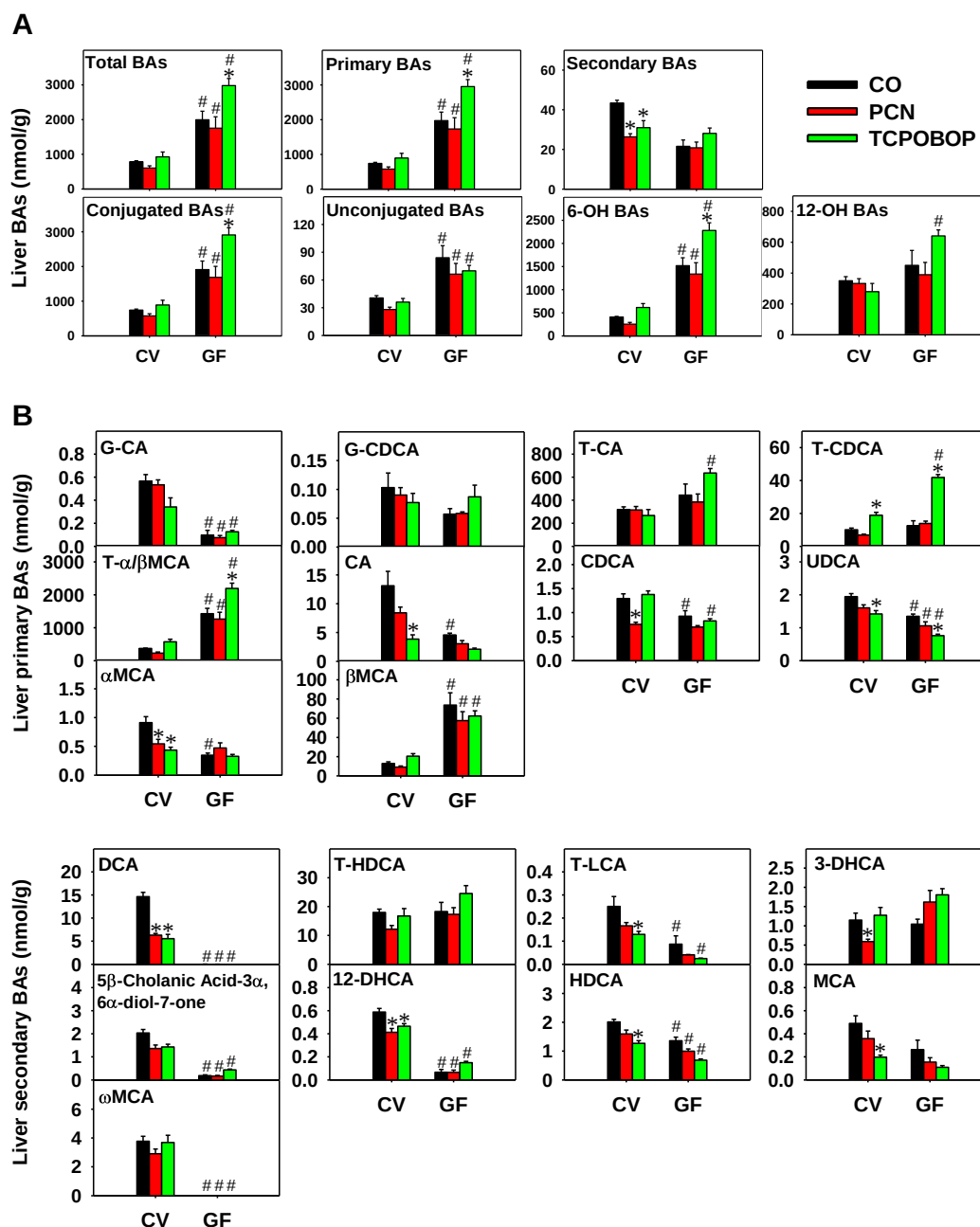


Figure 3.3. A. LC-MS quantification of total, primary, secondary, conjugated, unconjugated, 6-OH, and 12-OH BAs in livers of CV and GF mice exposed to corn oil, PCN, or TCPOBOP (n=4-5 per group) as described in Materials and Methods. **B.** Individual differentially regulated primary BAs (top panels) and secondary BAs (bottom panels) in livers of CV and GF mice exposed to corn oil, PCN, and TCPOBOP. Asterisks (*) represent statistically significant differences as compared to corn oil-exposed group of the same enterotype; pounds (#) represent statistically significant differences between CV and GF mice exposed to the same chemical (two-way ANOVA followed by Tukey's post hoc test; statistical significance was considered at adjusted *p*-value < 0.05).

In serum, 13 BAs were above the LOD, and 8 were differentially regulated by either chemicals or enterotype. As shown in Supplemental Figure 3.S3.3A, control GF mice had higher primary, conjugated, and 6-OH BAs in serum than control CV mice. In serum of GF mice, PCN appeared to lower the serum primary, conjugated and 6-OH BAs, whereas TCPOBOP did not have a major effect on these BAs.

The regulation of individual BAs in serum of CV and GF mice is shown in Supplemental Figure 3.S3B. Under basal conditions, two BAs (i.e. the secondary BAs DCA and T-DCA) were decreased, whereas three BAs (T- α / β MCA, T-HDCA, and T-UDCA) were increased in serum of GF mice relative to CV mice. PCN and TCPOBOP in general did not alter these individual BAs in serum, except for a marked increase in T-CDCA by TCPOBP in serum of GF mice. Serum of PCN-exposed GF mice had lower T-DCA and CA as compared to serum of PCN-exposed CV mice. Serum of TCPOBOP-exposed CV mice also had lower T-DCA as compared to serum of TCPOBOP-exposed CV mice, but had higher T- α / β MCA, T-CA, T-HDCA, and T-UDCA. These data are summarized as a two-way hierarchical clustering dendrogram of the relative concentrations of individual BAs as shown in Supplemental Figure 3.S3.3C.

For intestinal content, a systematic comparison between BA profiles in SIC and LIC of CV and GF mice exposed to corn oil, PCN, or TCPOBPOP was performed as shown in Figure 3.5-3.6 and Figure S4-5.

In SIC, 32 BAs were detected above the LOD, and they were all differentially regulated by either chemical exposure or enterotype. As shown in Figure 3.4A, control GF mice, as expected, had higher total BAs compared to CV mice, and this was attributed to primary BAs, whereas secondary BAs were decreased. The basal levels of conjugated BAs were higher and unconjugated BAs were lower in GF control compared to CV control mice. BAs from the alternative pathway (6-OH BAs) but not the classic pathway (12-OH BAs) were preferably increased in the absence of gut microbiota. The increase in total, primary, conjugated, and 6-OH BAs and the decrease in unconjugated BAs was maintained in GF mice following PCN and

TCPOBOP exposure. Interestingly, there was a decrease in secondary BAs by PCN exposure. In contrast, lack of gut microbiome potentiated the TCPOBOP-mediated increase in total, primary, conjugated, and 6-OH BAs in SIC. In LIC, 35 BAs were detected above the LOD. As shown in Figure 3.4B, control GF mice had higher primary and conjugated BAs than control CV mice, but there was no statistical increase in total BAs (Figure 3.5A). The basal levels of secondary and unconjugated BAs were markedly lower in GF mice, and the decreased levels of secondary and unconjugated BAs was remained significant following PCN and TCPOBOP exposure. There were no PCN- or TCPOBOP-mediated differences among the BA categories listed in Figure 3.4B.

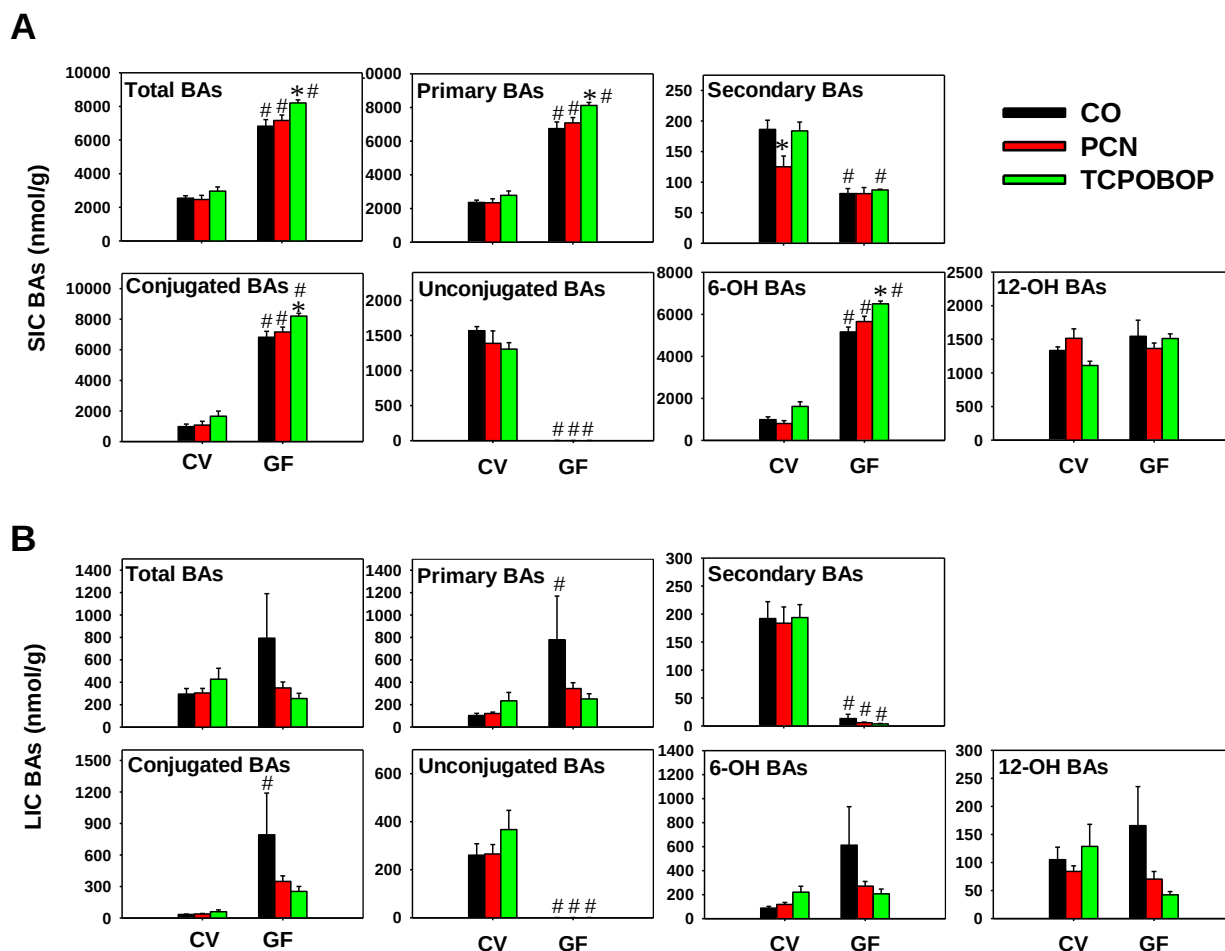


Figure 3.4. A. LC-MS quantification of total, primary, secondary, conjugated, unconjugated, 6-OH, and 12-OH BAs in SIC of CV and GF mice exposed to corn oil, PCN, or TCPOBOP (n=4-5 per group) as described in Materials and Methods. **B.** Individual differentially regulated primary BAs (top panels) and secondary BAs (bottom panels) in SIC of CV and GF mice exposed to corn oil, PCN, and TCPOBOP. Asterisks (*) represent statistically significant differences as compared to corn oil-exposed group of the same enterotype; pounds (#) represent statistically significant differences between CV and GF mice exposed to the same chemical (two-way ANOVA followed by Tukey's post hoc test; statistical significance was considered at adjusted p -value < 0.05).

Regarding the regulation of individual BAs in SIC of CV and GF mice, as shown in Figure 3.5A and Figure S4, under basal conditions, a total of 17 BAs (UDCA, HDCA, 5 β -Cholanic Acid-3 α , 6 α -diol-7-one, CA, β MCA, α MCA, CDCA, DCA, G-HDCA, G-DCA, T-HDCA, T-UDCA, T- α / β MCA, T-HCA, T-CA, T- ω MCA, and T-CDCA) were increased and ten BAs (8(14),(5 β)-cholonic acid-3 α , 12 α -diol, 3 α -OH-7 KetoLCA, 5 α -cholanic Acid-3 α -ol-6-one, 12-DHCA, ω MCA, 3-DHCA, LCA, MCA, 3 α -OH-12 KetoLCA, and T-CDCA) were decreased in SIC of GF mice. In livers of CV mice, PCN decreased UDCA, HDCA, 8(14),(5 β)-cholonic acid-3 α , 12 α -diol, 3 α -OH-7 KetoLCA, 5 α -cholanic acid-3 α -ol-6-one, and MCA. TCPOBOP also decreased MCA, but increased UDCA, HDCA, 8(14),(5 β)-cholonic acid-3 α , 12 α -diol, 5 α -cholanic acid-3 α -ol-6-one in SIC of CV mice. In SIC of GF mice, lack of gut microbiota potentiated the TCPOBOP-mediated increase in T- α / β MCA, T-HCA, and T-CDCA and the PCN-mediated increase in G-HDCA.

Regarding the regulation of individual BAs in LIC of CV and GF mice, as shown in Figure 3.6 and Figure S5, the lack of gut microbiota had a greater regulatory effect on BA levels in LIC than did chemical exposure. In LIC of control GF mice, a total of four BAs (T- α / β MCA, T- ω MCA, T-UDCA, and T-CA) were increased and 20 BAs were decreased (3,12-diketocholanic acid, 3,7-diketocholanic acid, 5 α -cholanic acid-3, 6-dione, 3,6-diketocholanic acid, 3 α -OH-12 KetoLCA, 3-ketocholanic acid, 3 α -OH-7 KetoLCA, 5 α -cholanic acid-3 α -ol-6-one, 8(14),(5 β)-cholonic acid-3 α , 12 α -diol, HDCA, UDCA, MCA, DCA, LCA, IsoLCA, 3-DHCA, 5 β -cholanic acid-3 α , 6 α -diol-7-one, ω MCA, HCA, and AlloLCA). In LIC of CV mice, PCN reduced AlloLCA, whereas TCPOBOP increased AlloLCA, HCA and ω MCA in a gut microbiome-dependent manner. Neither PCN nor TCPOBOP altered LIC BAs in GF conditions.

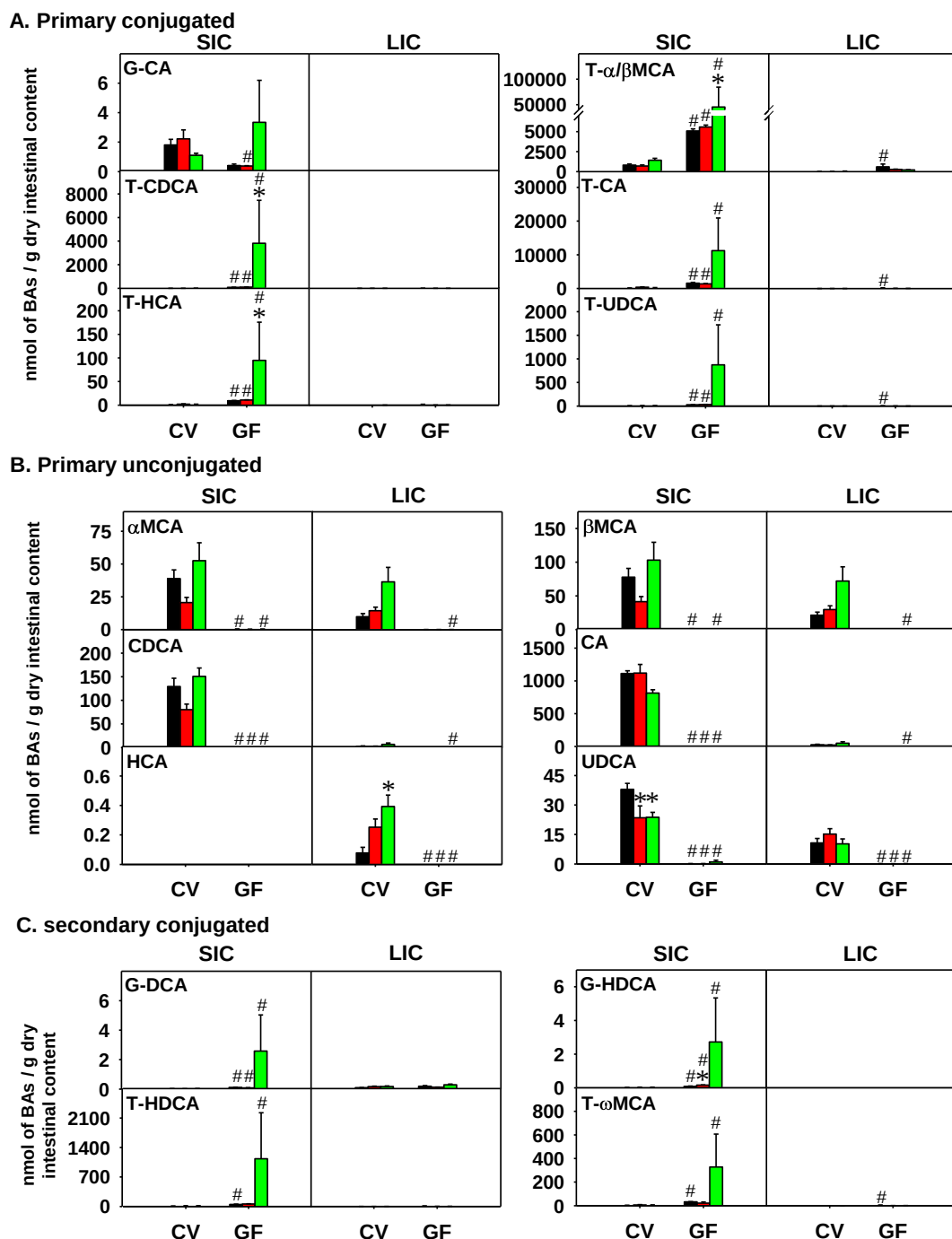


Figure 3.5. LC-MS quantification of primary conjugated BAs (**A**), primary unconjugated BAs (**B**), and secondary conjugated BAs (**C**) in SIC and LIC of CV and GF mice exposed to corn oil, PCN, or TCPOBOP (n=4-5 per group) as described in Materials and Methods. Asterisks (*) represent statistically significant differences as compared to corn oil-exposed group of the same enterotype; pounds (#) represent statistically significant differences between CV and GF mice exposed to the same chemical (two-way ANOVA followed by Tukey's post hoc test; statistical significance was considered at adjusted *p*-value < 0.05).

Pearson correlation analysis of LIC bacteria and bile acids

To determine the relationship between BAs and gut microbiota, BAs that were differentially regulated in serum (Figure S6A) and LIC (Figure S6B) were correlated against the 11 differentially regulated bacteria in LIC. In serum, the primary conjugated BAs T- α/β MCA and T-CDCA were negatively correlated with seven taxa (two *Bifidobacterium spp.*, two *Dorea spp.*, *Peptococcaceae*, *Anaeroplasma spp.*, and *Ruminococcus*). In contrast, the majority of these taxa (except for *Ruminococcus*) were positive correlated with the secondary unconjugated BA DCA. The taxa *Lactobacillales*, *Bradyrhizobiaceae*, and *Anaerotruncus spp.* were positively correlated with T- α/β MCA and T-CDCA but negatively correlated with DCA. In LIC, *Bifidobacterium spp.*, *Dorea spp.*, *Peptococcaceae*, and *Anaeroplasma spp.* were positively correlated with the conjugated primary BA T-UDCA, but negatively correlated with the unconjugated BAs (HCA, α MCA, β MCA, AlloLCA, ω MCA, 3-DHCA). Conversely, *Lactobacillales*, *Bradyrhizobiaceae*, and *Anaerotruncus spp.* were positively correlated with these unconjugated BAs, but negatively correlated with T-UDCA.

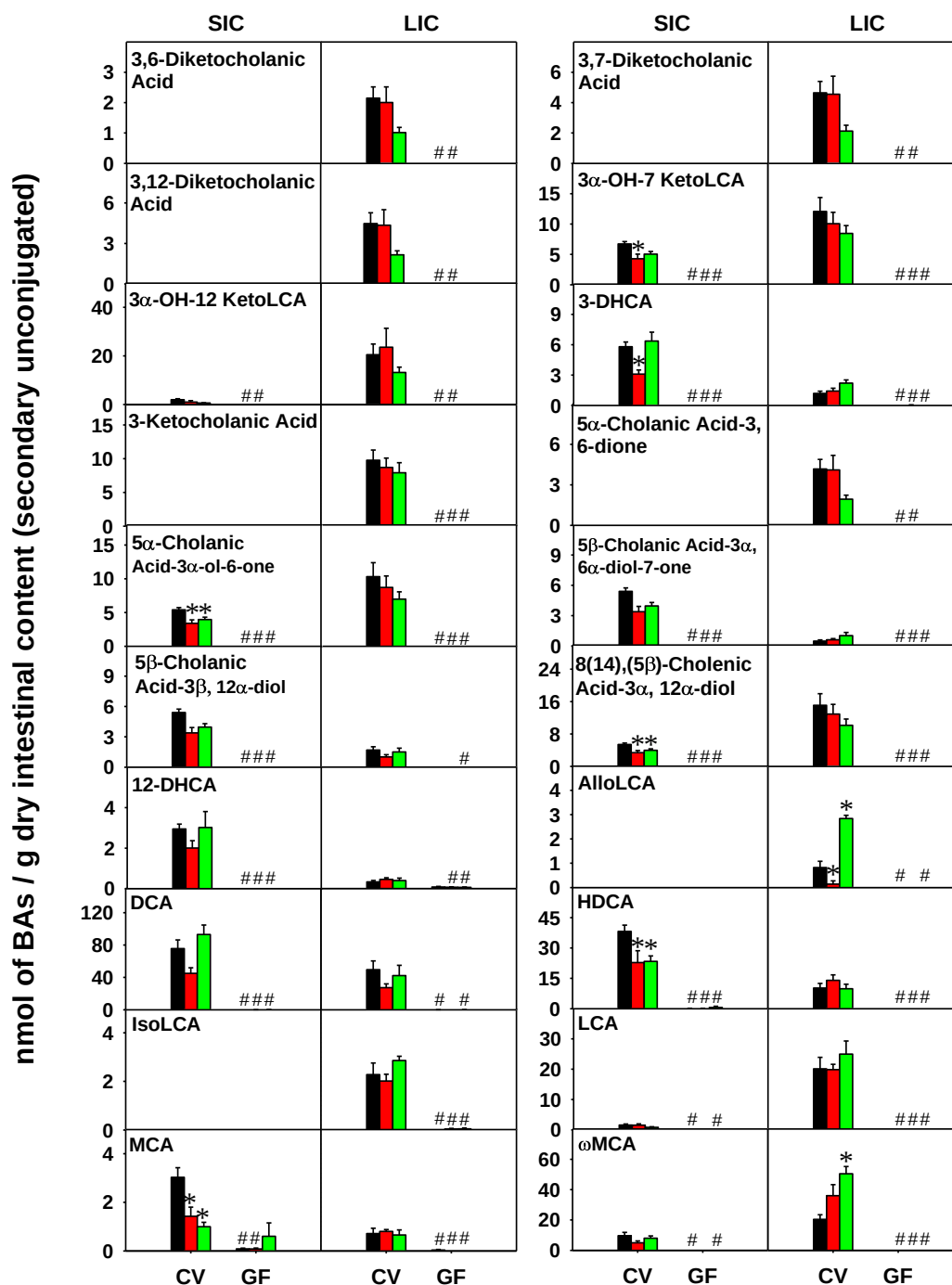


Figure 3.6. Individual differentially regulated BAs in SIC and LIC of CV and GF mice exposed to corn oil, PCN, or TCPOBOP (n=4-5 per group) as described in Materials and Methods. Asterisks (*) represent statistically significant differences as compared to corn oil-exposed group of the same enterotype; pounds (#) represent statistically significant differences between CV and GF mice exposed to the same chemical (two-way ANOVA followed by Tukey's post hoc test; statistical significance was considered at adjusted *p*-value < 0.05).

Hepatic Gene Expression

Xenobiotic receptor-target genes. The mRNA expression of five prototypical target genes of major xenobiotic-sensing transcription factors were determined to confirm pharmacological activation of PXR by PCN or CAR by TCPOBOP (Figure 3.7A). Regarding PXR activation, PCN increased the expression of Cyp3a11 in GF mice and tended to increase expression in CV mice, suggesting that lack of gut microbiota potentiates PXR activation, a similar observation as we reported previously (Cui and Klaassen, 2016). CAR activation by TCPOBOP increased the expression of Cyp2b10 as expected (Cui and Klaassen, 2016). Lack of gut microbiota sensitized the TCPOBOP-mediated increase in the expression of Cyp1a2, which has been reported to carry a CAR binding motif in the promoter region (Yoshinari et al., 2010). Both PXR and CAR activation decreased the peroxisome proliferator-activated receptor α (PPAR α) target gene Cyp4a14, and this was consistent with our previous reports (Cui and Klaassen, 2016; Li et al., 2016). No changes in gene expression were observed in CV or GF mice for the nuclear factor, erythroid derived 2, like 2 (Nfe2l2/Nrf2) target gene NAD(P)H dehydrogenase, quinone 1 (Nqo1).

Hepatic bile acid synthesis genes. The mRNA expression for six major BA-processing genes was determined (Figure 3.7B). Interestingly, for the classic pathway of BA synthesis, lack of gut microbiota potentiated the TCPOBOP-mediated increase in the mRNA of the rate-limiting BA-synthesizing enzyme Cyp7a1 in liver, and this corresponded with an increase in total and primary BAs in liver of GF mice but not in CV mice (Figure 3.3A). In livers of CV mice, TCPOBOP decreased the expression of Cyp8b1, which produces CA and determines the ratio between CA (classic pathway) and CDCA (alternative pathway). For the alternative pathway of BA synthesis, in CV mice, PCN and TCPOBOP exposure decreased the expression of the rate-limiting enzyme Cyp27a1 and the downstream enzyme Cyp7b1. Regarding BA conjugation, TCPOBOP decreased Slc27a5/Bal in a gut microbiome dependent manner; however, expression of the BA-

amino acid conjugating enzyme BA-CoA:amino acid N-acyltransferase Baat remained unchanged.

Hepatic basolateral uptake transporters. In general, neither chemical exposure nor enterotype altered the mRNA expression of hepatic basolateral uptake transporters examined (Figure 3.7C). TCPOBOP exposure tended to decrease the expression of several transporters (Oatp1a1, 1b2, 2b1), but statistical significance was not achieved.

Hepatic basolateral efflux transporters. PCN and TCPOBOP had no effect on basolateral efflux transporters examined in the livers of CV mice (Figure 3.7D). However, lack of gut microbiota potentiated the TCPOBOP-mediated increase in the multidrug resistance-associated protein ATP-binding cassette, sub-family C (CFTR/MRP), member 3 (Abcc3/Mrp3) and Abcc4/Mrp4.

Hepatic canalicular efflux transporters for bile acids, lipids, and cholesterol. For BA canalicular efflux transporters in CV and GF mice, TCPOBOP exposure decreased the expression of the phospholipid transporter Abcb4/Mdr2, whereas lack of gut microbiota and PCN downregulated mRNA expression of Mdr2 (Figure 3.7E). In livers of GF mice, the basal expression of the cholesterol efflux dimer Abcg5/g8 was higher than in livers of CV mice in control conditions, but PCN and TCPOBOP exposure decreased their expression towards CV levels. In liver of CV mice, the mRNA of the canalicular membrane transporter ATPase, class I, type 8B, member 1 (Atp8b1), which translocates phosphatidylserine and phosphatidylethanolamine from the outer to the inner leaflet bilayer (Klaassen and Aleksunes, 2010), was decreased by TCPOBOP exposure and tended to decrease by TCPOBOP in GF mice (Figure 3.7F).

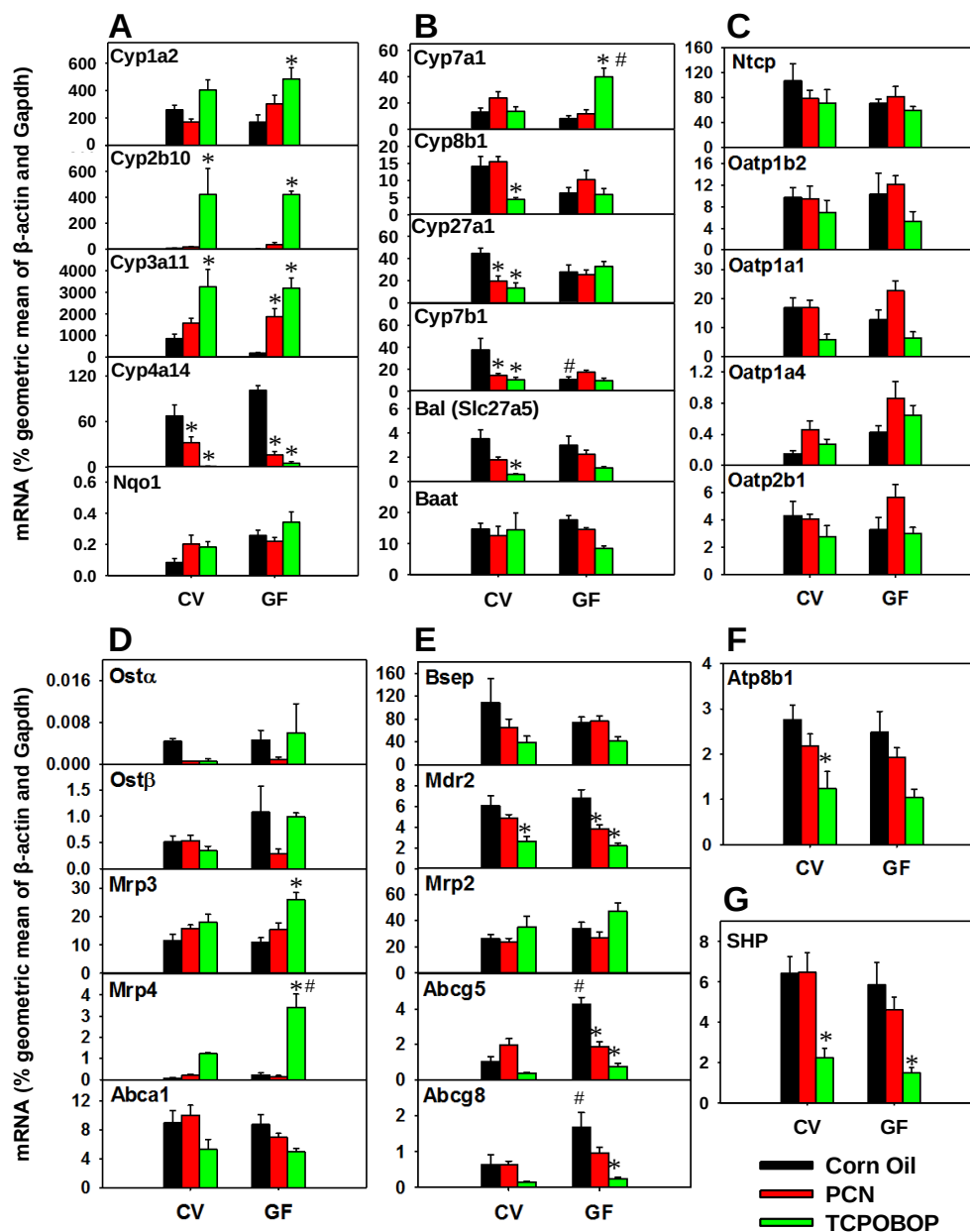


Figure 3.7. RT-qPCR quantification of mRNAs in livers of CV and GF mice exposed to corn oil, PCN, and TCPOBOP (n=4-5 per group) for prototypical PXR- and CAR-regulated target genes (A), primary BA synthesis (B), hepatic BA uptake transporters (C), basolateral efflux transporters for BAs and cholesterol (D), canalicular efflux transporters for BAs, lipids, and cholesterol (E and F), and the repressive nuclear receptor Shp (G). The mRNA was calculated by dividing the ddCq for the gene of interest by the ddCq for the geometric mean of β -actin and Gapdh. Asterisks (*) represent statistically significant differences as compared to corn oil-exposed group of the same enterotype; pounds (#) represent statistically significant differences between CV and GF mice exposed to the same chemical (two-way ANOVA followed by Tukey's post hoc test with adjusted *p*-value < 0.05).

Hepatic small heterodimer partner (SHP) and ileal bile acid-processing gene expression.

Hepatic expression of the repressive nuclear hormone nuclear receptor subfamily 0, group B, member 2 (Nr0b2/SHP) was decreased by TCPOBOP exposure in both CV and GF mice, but not by PCN exposure (Figure 3.7G). Overall, there were no significant PCN- or TCPOBOP-mediated effects on the expression of BA transporters in CV or GF mice (Figure 3.8). The mRNA expression of the cholesterol efflux transporter *Abca1* was higher in vehicle-exposed GF mice than CV, and the enterotype effect was attenuated by PCN and TCPOBOP in GF mice (Figure 3.8A). Similarly, the BA signaling hormone *Fgf15* was increase in GF corn oil mice compared to CV corn oil mice, but the enterotype difference was attenuated following exposure to PCN or TCPOBOP (Figure 3.8B).

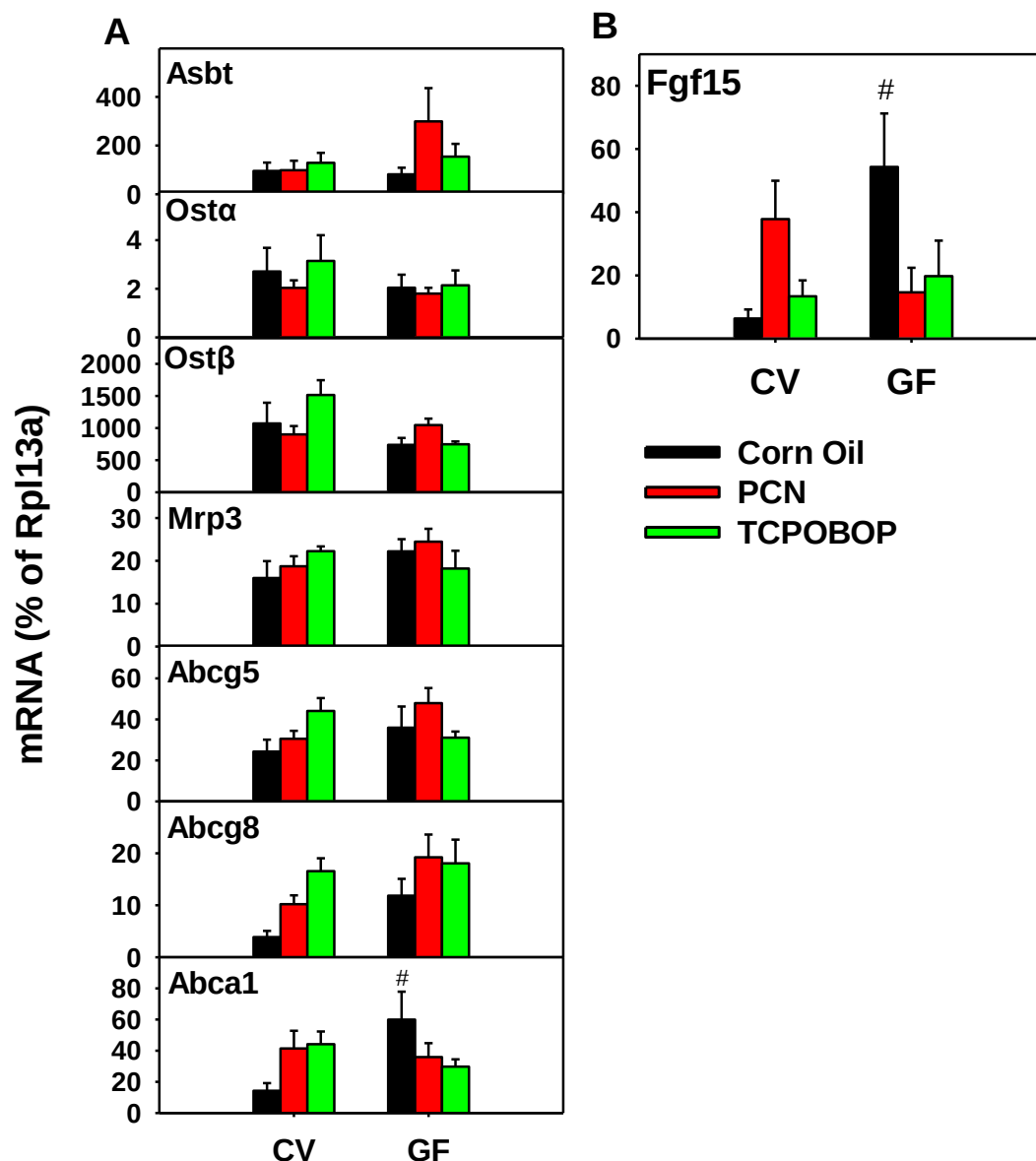


Figure 3.8. RT-qPCR quantification of mRNAs in ileum of CV and GF mice exposed to corn oil, PCN, and TCPOBOP (n=4-5 per group) for intestinal BA transporters (A) and the murine BA synthesis regulating hormone Fgf15 (B). The mRNA was calculated by dividing the ddCq for the gene of interest by the ddCq for the geometric mean of β -actin and Gapdh. Asterisks (*) represent statistically significant differences as compared to corn oil-exposed group of the same enterotype; pounds (#) represent statistically significant differences between CV and GF mice exposed to the same chemical (two-way ANOVA followed by Tukey's post hoc test with adjusted p -value < 0.05).

Hepatic muricholic acid synthesis genes

Recently, it was shown that in mice, Cyp2c70 is responsible for the species difference in mice regarding the synthesis of α MCA and β MCA from CDCA and UDCA, (Takahashi et al., 2016), but not Cyp3a11 (Wahlstrom et al., 2017). We hypothesize that other Cyp2c members may also contribute to muricholic acid-synthesis in liver. Therefore, the mRNA of Cyp2c70 and other Cyp2c members were quantified by RT-qPCR as shown in Figure 3.9.

There were no differences in basal expression of Cyp2c genes between CV and GF mice, and PCN did not alter the Cyp2c gene expression in CV or GF mice. In contrast, TCPOBOP increased the mRNAs of Cyp2c29, 2c37, 2c38, 2c39, Cyp2c50, 2c55, 2c65, and 2c66 in both CV and GF mice. The TCPOBOP-mediated increase in the expression of Cyp2c54 was potentiated in livers of GF mice. Conversely, Cyp2c44 mRNA was decreased by TCPOBOP in both CV and GF mice. The expression of Cyp2c67, 2c68, 2c69, and 2c70 was not altered by any exposure conditions.

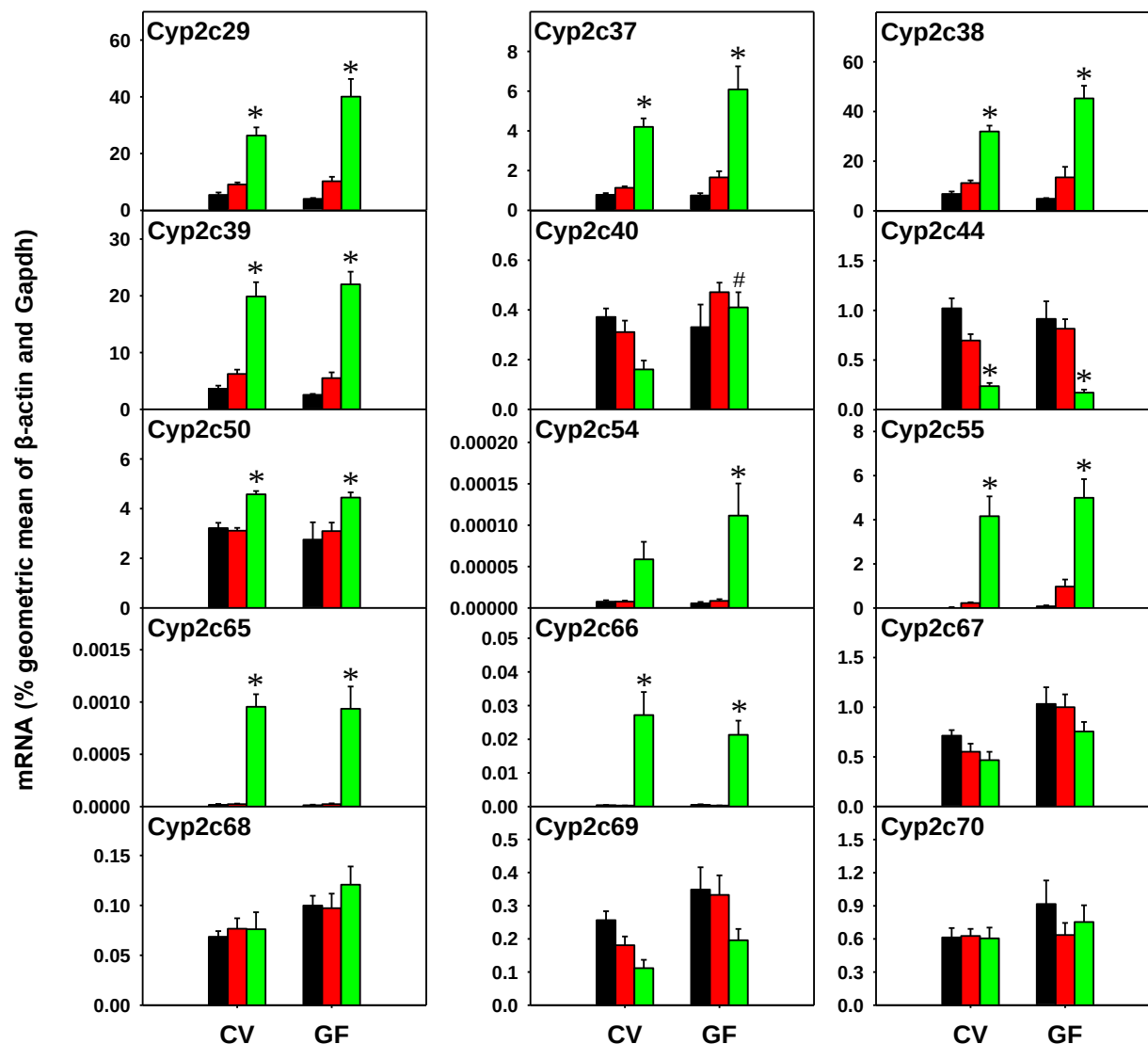


Figure 3.9. RT-qPCR quantification of mRNAs of Cyp2c genes in liver of CV and GF mice exposed to corn oil, PCN, and TCPOBOP (n=4-5 per group). The mRNA was calculated by dividing the ddCq for the gene of interest by the ddCq for the housekeeping gene Rpl13a. Asterisks (*) represent statistically significant differences as compared to corn oil-exposed group of the same enterotype; pounds (#) represent statistically significant differences between CV and GF mice exposed to the same chemical (two-way ANOVA followed by Tukey's post hoc test with adjusted p -value < 0.05).

3.4 Discussion

Taken together, the present study used 16S rDNA sequencing, BA targeted metabolomics, bioinformatics, and RT-qPCR analysis, to determine the gut microbiota-dependent regulation of BA homeostasis in response to pharmacological activation of PXR or CAR in mice. Although there is a breadth of research demonstrating how PXR and CAR activators and repressors modulate host gene expression in the liver, there is limited information regarding how bacteria in the gut are regulated by host receptor activation. The present study provides evidence of the effect of pharmacological activation of the host xenobiotic sensing nuclear receptors PXR and CAR on the gut microbiome composition and functions with a primary focus on BA homeostasis. Prior to our work, Dr. Curtis Klaassen's laboratory has systematically characterized the effect of pharmacological activation of PXR, aryl hydrocarbon receptor (AhR), and peroxisome proliferator-activated receptor α (PPAR α) on BA homeostasis and the regulation of host BA processing genes in CV mice (Lickteig et al., 2016; Zhang et al., 2017; Csanaky et al., 2018; Zhang et al., 2018). As a follow-up, the present study adds the knowledge to this field by 1) examining the effect of pharmacological activation of PXR and CAR in both CV and GF mice; and 2) unveiling the regulation of gut microbiota by these receptors. The rationale for using GF mice was that they allowed for the investigation of primary BA regulation independently from secondary BAs. Investigation of the bacterial composition of LIC enabled the identification of taxonomic shift associated with host xenobiotic-sensing nuclear receptor activation and BA changes. Targeted metabolomics of BAs in four major compartments (liver, serum, SIC, and LIC) unveiled gut microbiota-dependent and xenobiotic-mediated effects on BA homeostasis.

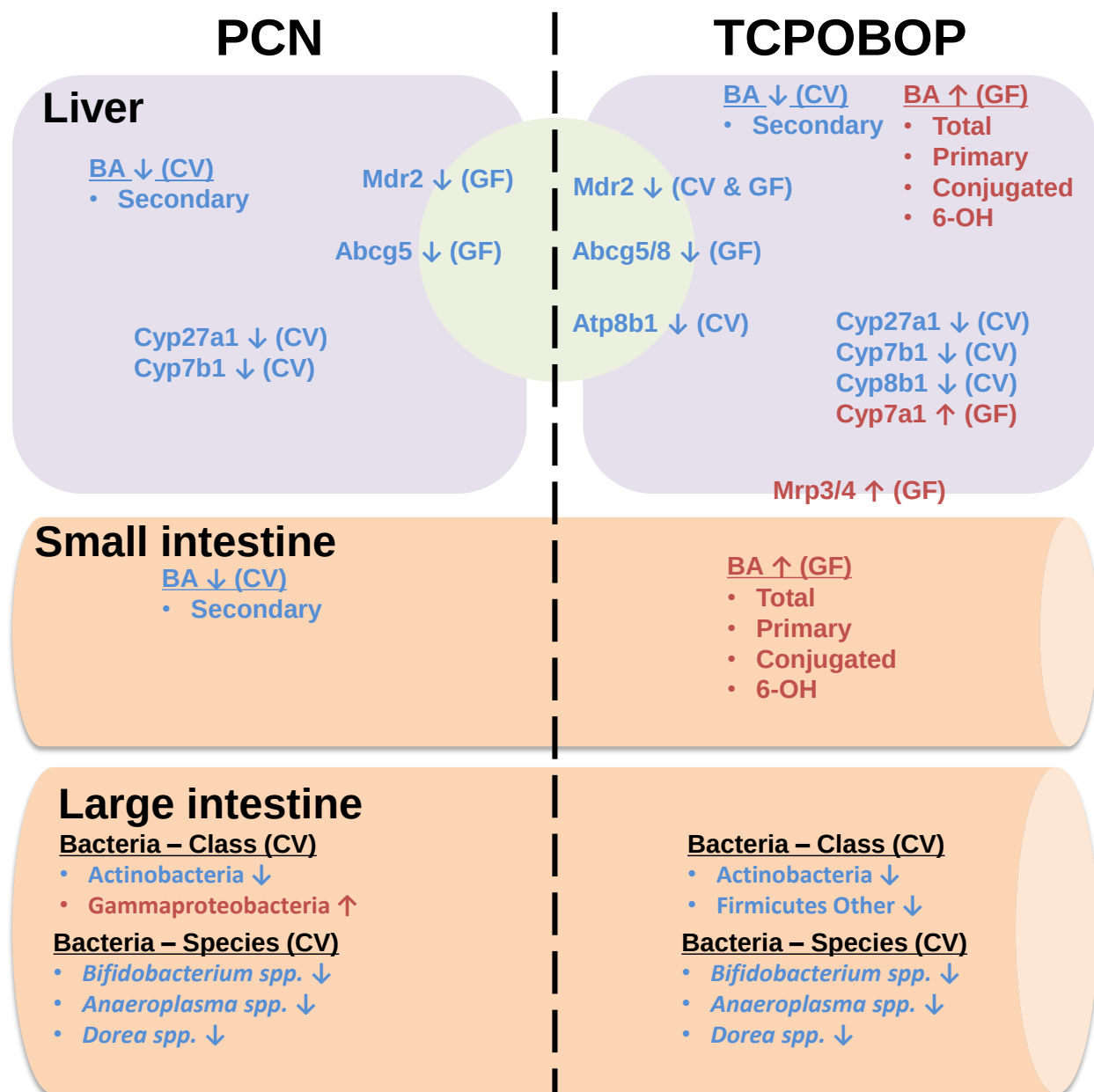


Figure 3.10. A diagram illustrating the major findings of the present study. Briefly, 1) PCN and TCPOBOP exposure decreased *Bifidobacterium spp.*, *Anaeroplasma spp.*, and *Dorea spp.*; 2) lack of gut microbiota potentiated an increase in total, primary, conjugated and 6-OH BAs in SIC and liver, whereas secondary BAs were increased in CV mice by PCN exposure; and 3) expression of the genes in the alternative BA synthesis were decreased by PCN and TCPOBOP exposure in CV and GF mice, whereas Cyp7a1 was increased in GF mice by TCPOBOP.

Regarding PXR, several studies have established a role between PXR activation, anti-inflammation, and the microbiome. For example, the microbial metabolite indole-3-propionic acid is a PXR activator in intestine that promotes PXR to interact with Toll-like receptor 4 to protect the epithelial barrier integrity and reduce inflammation (Venkatesh et al., 2014). PXR activation is also known to suppress inflammatory responses in hepatocytes, and PXR activation was found to be lower in patients with inflammatory bowel disease (Wallace et al., 2010; Sun et al., 2015). In the present study, we demonstrated that pharmacological activation of PXR reduced the relative abundance of *Bifidobacterium* spp., which have been shown to protect the intestinal epithelial barrier in inflammatory models (Figure 3.1 and summarized in Figure 3.10) (Wang et al., 2014; Guo et al., 2017). Previously, it was reported that PXR activation by the cholesterol-lowering drugs pravastatin and atorvastatin also decreased the abundance of *Bifidobacterium* spp. in a female mouse model (Caparros-Martin et al., 2017). Our study using PCN as a prototypical PXR activator showed consistent findings with the previous report using statins as PXR activators. Together, these studies suggest that the anti-inflammatory properties of PXR may decrease the necessity for immune modulation by *Bifidobacterium* spp. in the gut. For other bacteria, *Ruminococcus* spp., which was increased by pharmacological activation of PXR (Figure 3.2 and summarized in Figure 3.10), is considered a beneficial taxon that facilitates the digestion of complex carbohydrates from high fiber food and has been negatively associated with diabetes and colon cancer (Lagier et al., 2012; Ze et al., 2012). PCN also decreased a non-specific taxon in the Firmicutes phylum, which contains the class Clostridiales and are capable of producing toxic secondary BAs (Ridlon et al., 2006). Therefore, the benefits of host PXR activation may act in part through increasing the percentage of beneficial bacteria in the gut.

There are limited studies examining the interaction between CAR activation and the gut microbiome. The mRNA of CAR was found higher in livers of mice lacking a gut microbiome (Lundin et al., 2008). The present study showed that pharmacological activation of CAR increased the Lactobacillales order, which is known to decompose plant and milk products

through carbohydrate fermentation, producing lactic acid as the major metabolic product (Figure 3.2) (Kandler, 1983). *Aneroplasma* spp. was decreased by both CAR and PXR activation and is associated with fiber digestibility (Niu et al., 2015); *Dorea* spp., which colonizes mucosal regions where sialic acid is abundant, was also decreased by both PXR and CAR and has been shown to an increase in IBS patients, suggesting that *Dorea* spp. may not act positively on the host (Rajilic-Stojanovic et al., 2011; Leclercq et al., 2014; Tailford et al., 2015). CAR activation also decreased *Bifidobacterium* spp., which has been shown to protect the intestinal epithelial barrier in inflammatory models of post-infectious irritable bowel syndrome model in mice and in the human epithelial colorectal adenocarcinoma Caco-2 cell line (Wang et al., 2014; Guo et al., 2017). Activation of CAR has long been known to reduce blood-glucose and improve insulin sensitivity in diabetic patients (Lahtela et al., 1985; Sotaniemi and Karvonen, 1989) as well as suppress the expression of gluconeogenic genes in rat primary hepatocytes (Argaud et al., 1991) and mouse liver (Ueda et al., 2002). Later, in both leptin-deficient ob/ob mice and wild-type mice fed a high-fat diet, it was shown that CAR agonists decreased fasting serum-glucose levels and hepatic glucose production to decrease hyperglycemia and improve insulin sensitivity (Dong et al., 2009; Gao et al., 2009). On the other hand, it was also shown in a choline-deficient non-alcoholic steatohepatitis (NASH) mouse model that CAR activation caused lipid peroxidation and increased fibrosis (Yamazaki et al., 2007). Certain compounds from the gut microbiome, such as BAs and short-chain fatty acids from fiber, act as signaling molecules to host organs to regulate metabolic health, including obesity, type II diabetes, non-alcoholic fatty liver disease, and NASH (Schroeder and Backhed, 2016; Makki et al., 2018). More research is needed to understand how CAR may interact with the gut microbiome to improve metabolic health.

The present study found that several bacterial genes involved in secondary BA synthesis were decreased or appeared to be down-regulated after PXR or CAR activation (Figure 3.1). In particular, the PCN- and TCPOBOP-mediated decrease in *Bifidobacterium* spp. was associated with a decrease in the DNA encoding the bacterial BA deconjugation enzyme gene *bsh*. Because

Bifidobacterium is known to carry *bsh* activity, the decrease in this taxa explains the decrease in the DNA encoding the *bsh* enzyme (Jarocki et al., 2014). In addition, there was an apparent decrease in the DNA encoding *baiCD* and *baiJ*, which are responsible for secondary BA synthesis. This corresponded with a decrease in total secondary BAs in liver following PCN and TCPOBOP exposure. Conjugated BAs are considered less toxic than unconjugated BAs and do not undergo passive diffusion due to increased size and ionization in the small intestine (Hofmann, 2009). However, overall, the decreased *bsh* genes did not significantly change the pool of conjugated or unconjugated BA in any compartment of CV mice, suggesting that the expression of *bsh* genes was maintained despite a decrease in gene number or additional genes may act similarly to *bsh*, but they have not been identified. Because the total BAs and primary BAs did not change due to treatment in CV mice, it is possible that PXR and CAR activation diminished the synthesis and/or increased the excretion of secondary BAs.

There is no evidence demonstrating that BAs can directly function as ligands of CAR, but the CAR activators phenobarbital and TCPOBOP have been shown to repress *Cyp7a1* and may protect against BA toxicity similar to the functions of farnesoid X receptor (FXR/Nr1h4) and PXR (Guo et al., 2003; Miao et al., 2006; Li and Chiang, 2014). Following exposure to TCPOBOP, there was no change in the expression of *Cyp7a1* in CV mice. However, there was a marked increase in GF mice that was potentiated by the lack of gut microbiome. This corresponded with an increase in total, primary, conjugated and 6-OH BAs in liver and SIC of GF mice exposed to TCPOBOP (summarized in Figure 3.10). Very little is known regarding the molecular mechanisms underlying the potentiated CAR ligand-mediated increase in *Cyp7a1* mRNA in germ free conditions. It has been shown that during a lithogenic diet CAR increases *Cyp7a1* expression to promote the metabolism of cholesterol into BAs, as a compensatory mechanism (Cheng et al., 2017). It is known that livers of GF mice have higher cholesterol content (Mistry et al., 2017), thus it is possible that germ free conditions triggered the same signaling pathway for *Cyp7a1* mRNA up-regulation. Dr. Curtis Omiecinski's laboratory recently demonstrated identified a

positive CAR-DNA binding site with over 30-fold of enrichment near the promoter of mouse *Cyp7a1* gene following TCPOBOP exposure (Niu et al., 2018) (https://genome.ucsc.edu/cgi-bin/hgTracks?hgS_doOtherUser=submit&hgS_otherUserName=nxb939&hgS_otherUserSessionName=ChIP_exo_CAR). In summary, these observations from the literature and our data suggest that the increase in *Cyp7a1* mRNA by CAR activation is sensitized by cholesterol overload and likely a result of an increase in direct CAR-binding.

Regarding individual BAs, the TCPOBOP-mediated, gut microbiome-dependent increase in BAs in liver is attributable to T- α / β MCA and T-CDCA as well as β MCA and T-CA, whereas the increase in SIC this was attributed to T- α / β MCA, T-CDCA, and T-HCA, as well as T-UDCA, T-CA, and T- ω MCA. The majority of these BAs are derivatives of CDCA an alternative pathway BA, which is converted to α / β MCA in mice by *Cyp2c* (Takahashi et al., 2016). To note, β MCA is an antagonist of FXR (Sayin et al., 2013). Because activation of FXR inhibits the expression of *Cyp7a1*, it is possible that potent CAR activation increases muricholic acids, which in turn blocks the FXR-mediated inhibition of BA synthesis. In a mouse bile duct ligation model, total BAs in serum increased less in CAR-null mice than PXR-null mice; in particular, this was due to a lesser increase in T-CA, T-CDCA, and T- β MCA, suggesting that constitutive activation of CAR is important in the synthesis of these BAs during conditions of toxicity (Stedman et al., 2005). Despite several studies showing indirect associations between CAR and FXR, there are no known direct interactions such that CAR and FXR co-regulate BA homeostasis (Guo et al., 2003; Saini et al., 2004; Stedman et al., 2005; Beilke et al., 2009b). The present research shows that lack of gut microbiome potentiates BA synthesis following CAR activation and that the regulation of BA homeostasis by activation of CAR in mice may be microbiome dependent.

In a study investigating the basal regulation of BAs between CV and GF mice, in liver, Selwyn et al. found increased total, primary, and conjugated BA species in GF mice compared to CV mice (Selwyn et al., 2015b), which is comparable to the present study, and this was most likely attributable to an increase in T- α / β MCA (Figure 3.3). Under basal conditions, the

concentrations of total, primary, conjugated, and 6-OH BAs were higher in GF compared to CV mice in multiple bio-compartments (liver, SIC, and LIC); in addition, unconjugated BAs were also higher in the liver of GF mice. It has been suggested that the increased BA pool in GF mice may be due to increased reabsorption of BAs and decreased excretion in the feces (Gustafsson et al., 1957; Riottot and Sacquet, 1985; Sayin et al., 2013). Consistent with Sayin et al., muricholic acids (T- α / β MCA and β MCA), which are derivatives of CDCA, were also increased in GF mice (Figures 3.3, 3.5, and 3.S3), but was not previously reported in Swiss Webster mice (Sayin et al., 2013) and could reflect a strain difference with C57BL/6J mice. The increased BA pool may also be contributed by FXR antagonism due to increased T- α / β MCAs, which are known FXR antagonists (Sayin et al., 2013). FXR antagonism in intestine may subsequently suppress the intestinal Fgf15-mediated down-regulation of the hepatic BA synthetic enzyme Cyp7a1. The lack of this FXR-Fgf15-Cyp7a1 negative feedback loop may explain the higher basal levels of BAs in GF mice. The increased T- α / β MCAs may be due to decreased expression of Cyp8b1, which affects the ratio of CA (12-OH) to CDCA (6-OH). Alternatively, bacteria could preferentially metabolize muricholic acids over CA, resulting in the large increase in GF mice. Furthermore, the increased BA pool in GF mice may be due to the FXR antagonistic activity of T- α / β MCA in liver. T- α / β MCA had the highest concentration in GF mice in the current study; following TCPOBOP in GF mice, both T- α / β MCA and Cyp7b1 increased, which is consistent with known FXR antagonism by T- β MCA.

Sayin et al. (2013) quantified 16 BAs between CV and GF mice, among which six were secondary BAs (DCA, LCA, ω MCA, and their taurine conjugates). In their GF mice, these secondary BAs were not detected in liver, small intestine, or large intestine, and DCA and ω MCA were minimally detected in serum (Sayin et al., 2013). Quantification of DCA, LCA, ω MCA, and their taurine conjugates in the present study, matched Sayin et al., with the exception of T- ω MCA in SIC. It was highly abundant in SIC, especially following exposure to TCPOBOP, suggesting that there may be an unknown host mechanism be capable of producing T- ω MCA that could be

CAR-dependent. Other BAs traditionally considered secondary BAs monitored in the current study were identified in germ-free mice. In particular, T-HDCA has similar concentration levels in liver between CV and GF mice and was markedly increased in GF SIC following TCPOBOP exposure, suggesting that there is a host mechanism for T-HDCA production that is CAR-dependent. Indeed, HDCA was previously identified in GF pigs (Haslewood, 1971). Overall, the quantification of some traditional secondary BAs in GF mice suggests that the gut microbiota may mask host synthesis of these BAs.

In a previous study, CAR was activated by TCPOBOP (3 mg/kg) in 12-15 week old CV male mice for four days by intraperitoneal (i.p.) route and BAs were quantified in serum and liver and BA synthesis genes in liver and ileum were measured by qPCR (Lickteig et al., 2016). In serum, Lickteig et al. observed increased T- α/β MCA, T-UDCA, T-HDCA, and α MCA as well as decreased T-DCA and DCA following TCPOBOP exposure; whereas the present study found no changes in BA categories in serum (Supplemental Figure 3.S2). In liver, Lickteig et al. found that TCPOBOP exposure decreased total, primary, secondary, conjugated, unconjugated, and 12-OH BAs; regarding specific BAs, the primary BA T-CA was markedly decreased while the secondary BAs T-DCA, DCA, and T-LCA were decreased. The present study only found a decrease in livers of CV mice of secondary BAs due to the minor BAs HDCA, MCA, and T-LCA, whereas T-DCA increased. Comparison of the BA concentrations between the two studies suggest that the route of exposure (i.p. versus oral) or age can influence individual BA species in serum. In addition, because the studies were performed at two different facilities, the different housing conditions (food, bedding, water, etc.) could have led to differing responses to CAR activation and microbiome composition. There were also different responses seen in hepatic BA synthesis gene expressions. Lickteig et al. found increased hepatic expression of Cyp7a1 and Cyp27a1 and decreased Cyp7b1 and Cyp8b1, which could be a compensatory response to decreased concentrations of BAs in liver. Whereas the present study found no change in Cyp7a1 and decreased Cyp27a1, Cyp7b1, Cyp8b1, which could explain why total BAs were unchanged while

the concentration of CA, CDCA, and their derivatives tended to decrease. Overall, both studies have demonstrated that CAR is an important regulator of BA homeostasis.

Pearson correlation was used to identify relationships between the differentially regulated taxa and BAs in serum and LIC (Figure 3.6). *Bifidobacterium* spp. and *Lactobacillus* spp. express the BA deconjugating enzyme bsh (Ridlon et al., 2006), so they were anticipated to be positively correlated with deconjugated BAs. In serum, *Bifidobacterium* spp. was positively correlated with deconjugated BAs; however, in LIC, *Bifidobacterium* spp. was positively correlated with conjugated BAs. It is possible that the deconjugation reactions by *Bifidobacterium* spp. are BA-specific. Consistent with the literature, Dorea was positively correlated with DCA in serum (Martin et al., 2018); *Ruminococcus* was also reported to be positively associated with deconjugation of TCA (Martin et al., 2018). The BA metabolism of *Bradyrhizobiaceae* is not known, however Gram-negatives are thought to have a higher BA tolerance than Gram-positive bacteria (Ridlon et al., 2016). The unknown taxa in the phylum Firmicutes may have a competitive advantage following changes in BA homeostasis as rodents fed BAs or high fat diets tend to have increased Firmicutes (Hildebrandt et al., 2009; Ridlon et al., 2016). More research is needed to understand the specificity of BA-bacteria interactions and the selectivity of bacterial BA metabolizing enzymes.

CAR activation by TCPOBOP decreased the cholesterol canalicular efflux transporters Abcg5 and Abcg8 in livers of GF mice but not CV mice. The decreased hepatic expression of these transporters predicts a build-up of cholesterol and oxysterols in hepatocytes in the absence of the gut microbiome. The liver X receptors (LXRs) coordinately regulate sterol catabolism, storage, efflux, and elimination, including Abcg5 and Abcg8 (Berge et al., 2000; Repa et al., 2002), and mice lacking either gene accumulate cholesterol in the liver (Yu et al., 2002). CAR and LXR have distinct functions for coordinating a response to xenobiotics and promoting lipogenesis. *In vivo* CAR activation inhibited the expression of LXR target genes and LXR-induced lipogenesis, demonstrating the presence of a crosstalk between CAR and LXR (Gnerre et al., 2005). Therefore, it is plausible that CAR activation inhibited LXR regulated genes, such as Abcg5 and

Abcg8. Additionally, both LXR and CAR interact with peroxisome proliferator-activated receptors (PPARs), and these interactions have been associated with atherosclerosis and obesity (Xu et al., 2018). Understanding the interactions between CAR, LXR, and PPARs may reveal new therapeutic targets for metabolic diseases and provide insight for the evaluation of BA-dependent toxicological responses. Similarly, in this study, SHP was decreased by CAR activation. It is known that SHP can interact with CAR to inhibit CAR-target gene expression; however, more research is needed to identify co-regulatory networks between SHP, CAR, and BA synthesis (Bae et al., 2004; Li and Chiang, 2013).

The present study investigated how pharmacological activation of PXR and CAR can modulate the gut microbiome as well as primary and secondary BA homeostasis. The gut microbiome was sequenced from the LIC by 16S rDNA. Because this method uses primers, the identified taxa may be a biased representation of the DNA that was amplified during library preparation. Additionally, resolution of 16S rDNA data was limited to the mapping database and therefore PXR or CAR activation could not reliably be associated with bacteria at the species level. Furthermore, there was weak evidence correlating the significantly differentiated bacteria with altered BAs. Active BA uptake occurs in the ileum, but some BAs are passively absorbed in the colon. In this study, we did not sequence the bacterial DNA from the small intestine. However, two major reactions occur in the large intestine, namely 7-dehydroxylation and epimerization reactions of hydroxyl moieties (Hofmann, 2009), and we were able to capture the bacteria performing these important BA modifications. While we measured BAs in four compartments (blood, liver, SIC, and LIC), we did not quantify BAs in the gall bladder or other peripheral tissues that may also be affected by BA signaling, such as brown adipose tissue (Broeders et al., 2015). Overall, this study showed that PXR and CAR activation altered the gut microbiome composition. Lack of gut microbiota potentiated an increase in total, primary, conjugated, and 6-OH BAs in SIC and liver following TCPOBOP exposure; in CV mice, PCN exposure decreased secondary BAs in liver and SIC, whereas TCPOBOP only decreased secondary BAs in liver (summarized in

Figure 3.10). In addition, because the turnover rate for BAs is about 5% per day (Chiang, 2013), a new steady-state for BA homeostasis following PXR or CAR activation was not achieved; although the microbiome can change rapidly following a dietary exposure (David et al., 2014), the lack of a new BA steady-state will affect the gut microbiota composition, particularly BA metabolizing bacteria. Therefore, the results should be interpreted as the effect of an acute exposure activating these nuclear receptors on BAs and gut microbiota. Expression of genes in the alternative pathway of BA synthesis was decreased by PCN and TCPOBOP exposure in CV and GF mice, whereas Cyp7a1 mRNA was increased in GF mice by TCPOBOP. Future studies should focus on the metabolic and mechanistic effects of BAs modulated by PXR and CAR activation to identify new therapeutic methods to treat metabolic diseases.

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3.8 Supplemental Figures

Supplemental Figure 1

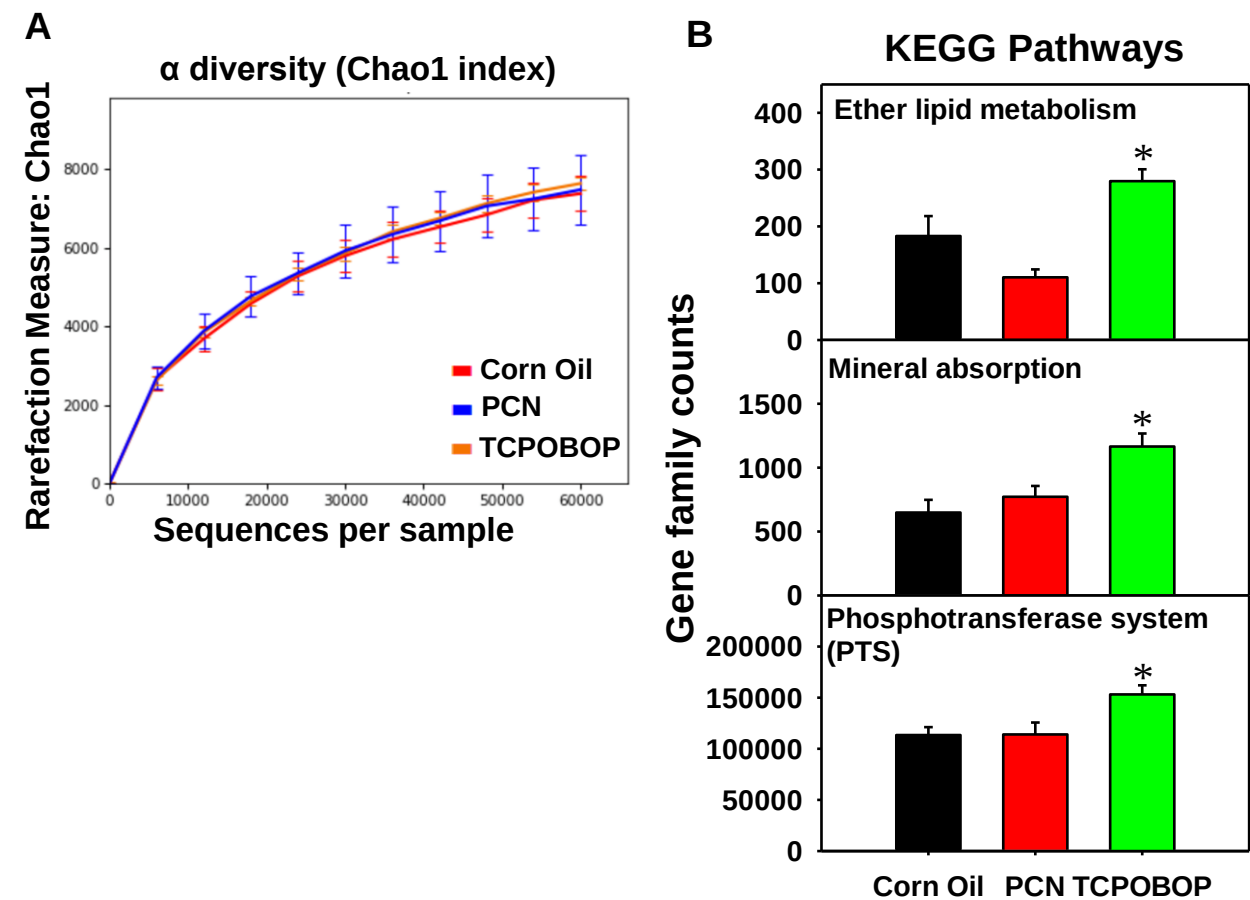


Figure 3.S1. A. α diversity (Chao1 index) of gut microbiome in corn oil, PCN, and TCPOBOP exposed CV mice. Data were analyzed using QIIME as described in Materials and Methods. B. Predicted KEGG Pathways of differentially regulated taxa using PICRUST as described in Materials and Methods.

Supplemental Figure 2

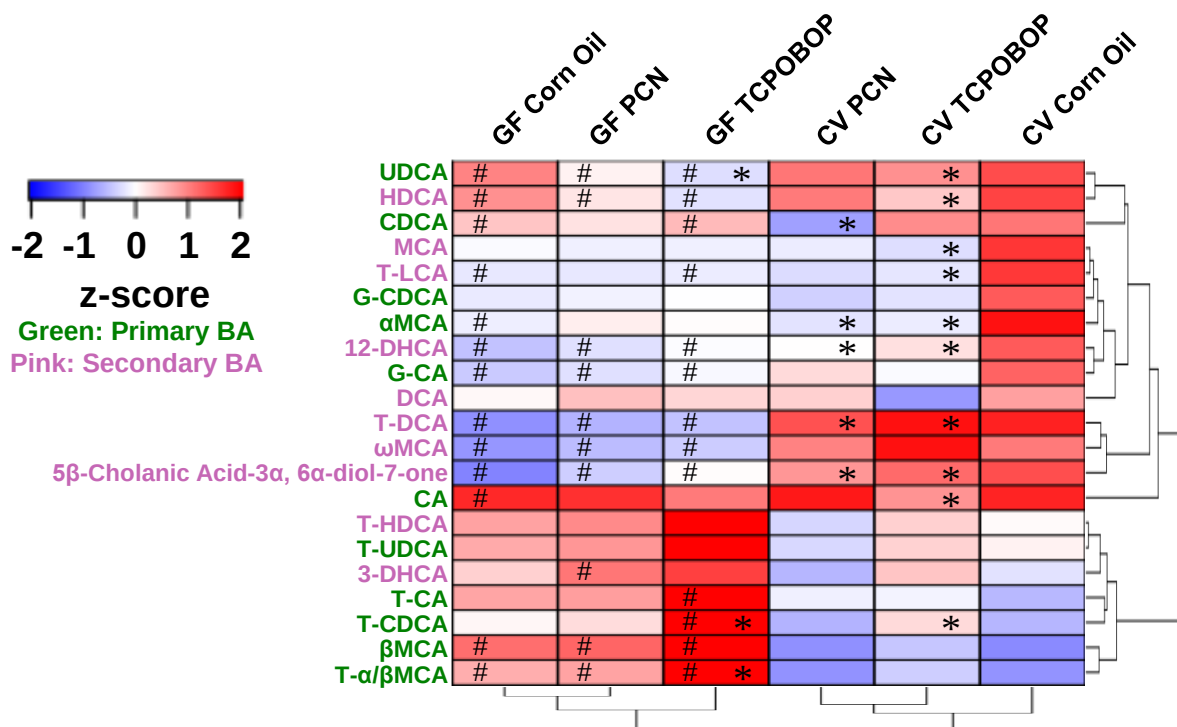


Figure 3.S2. A two-way hierarchical clustering dendrogram of primary BAs (green) and secondary BAs (pink) in liver of CV and GF mice exposed to corn oil, PCN, or TCPOBOP. Data were plotted using R. Asterisks (*) represent statistically significant differences between corn oil and chemicals (PCN or TCPOBOP); pounds (#) represent statistically significant differences between CV and GF mice of the same exposure (two-way ANOVA followed by Tukey's post hoc test, adjusted p -value < 0.05).

Supplemental Figure 3

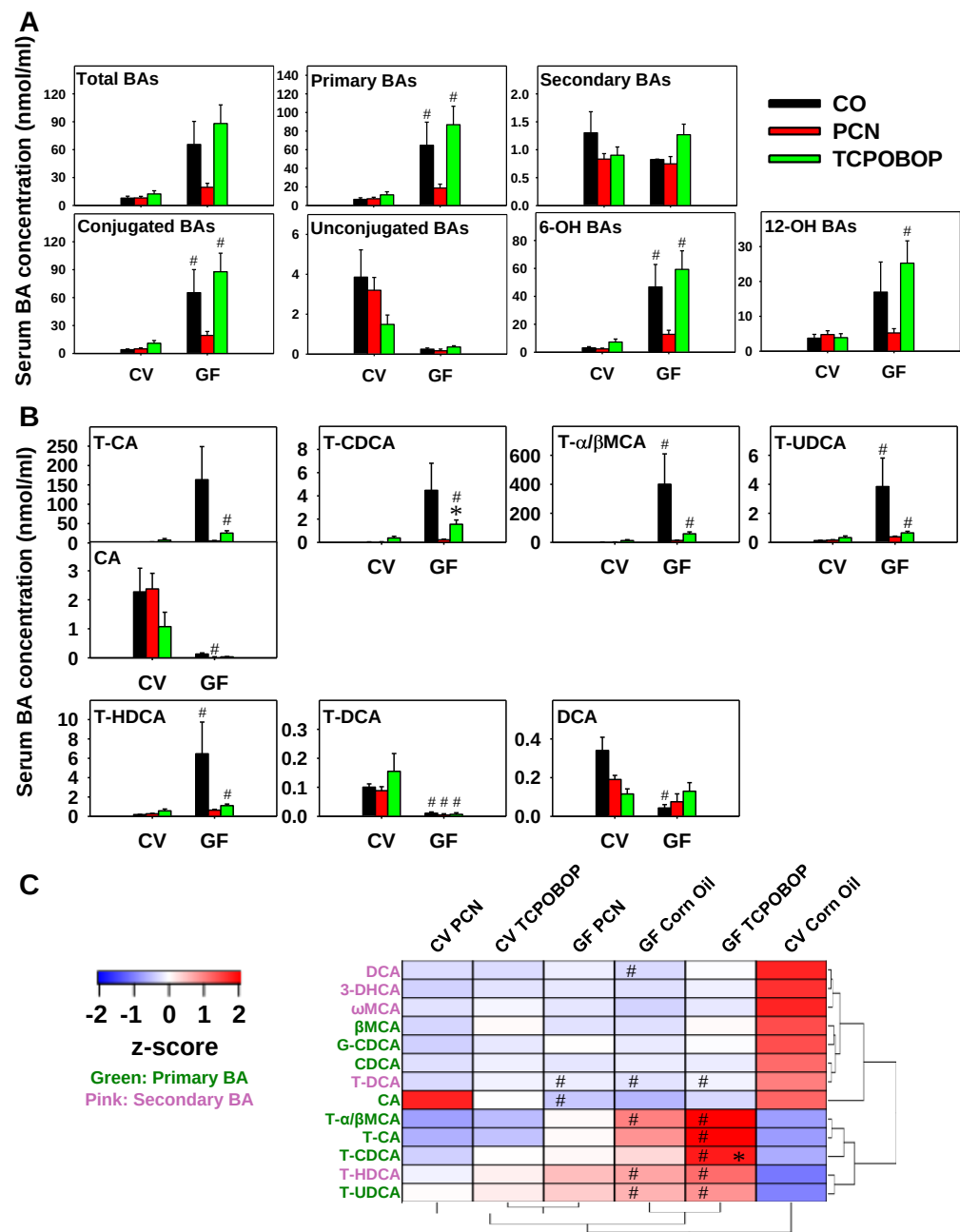


Figure 3.S3. A. Total, primary, secondary, conjugated, unconjugated, 6-OH, and 12-OH BA categories in serum of CV and GF mice exposed to corn oil, PCN, or TCPOBOP. **B.** Individual primary BAs (top panel) and secondary BAs (bottom panel) that were differentially regulated by chemicals and/or enterotype in serum. **C.** A two-way hierarchical clustering dendrogram of primary BAs (green) and secondary BAs (pink) in serum of CV and GF mice exposed to corn oil, PCN, or TCPOBOP. For **A-C**, asterisks (*) represent statistically significant differences between corn oil and chemicals (PCN or TCPOBOP); pounds (#) represent statistically significant differences between CV and GF mice of the same exposure (two-way ANOVA followed by Tukey's post hoc test, adjusted *p*-value < 0.05).

Supplemental Figure 4

A

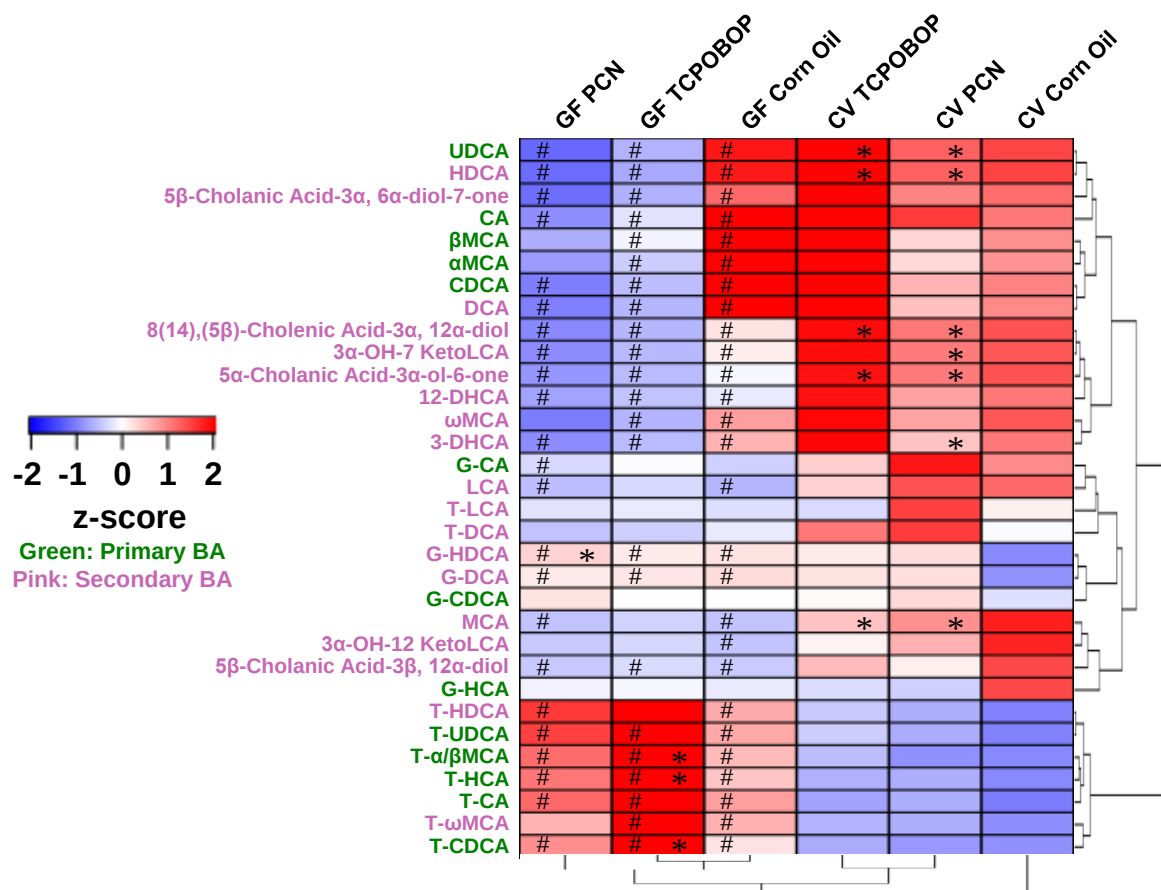


Figure 3.S4. A two-way hierarchical clustering dendrogram of primary BAs (green) and secondary BAs (pink) in SIC of CV and GF mice exposed to corn oil, PCN, or TCPOBOP. Asterisks (*) represent statistically significant differences between corn oil and chemicals (PCN or TCPOBOP); pounds (#) represent statistically significant differences between CV and GF mice of the same exposure (two-way ANOVA followed by Tukey's post hoc test, adjusted p -value < 0.05).

Supplemental Figure 5

A

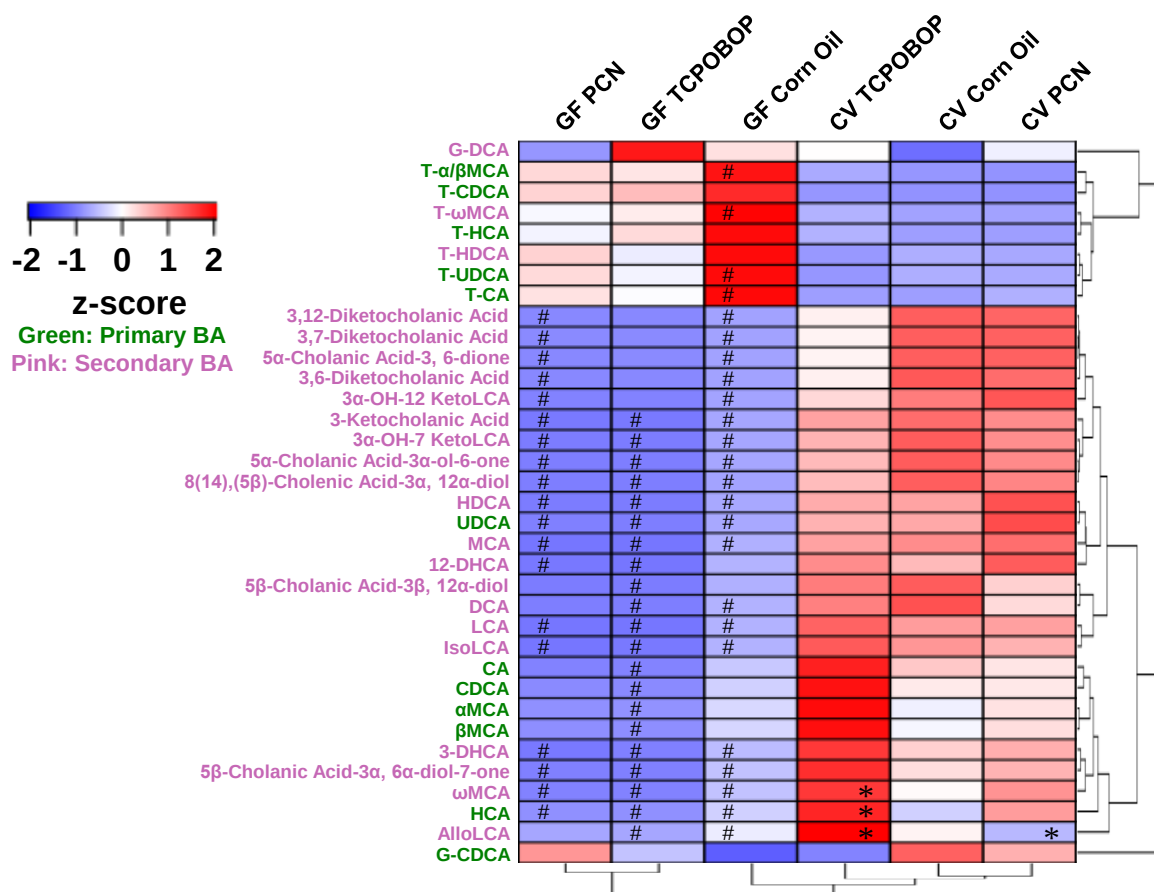


Figure 3.S5. A two-way hierarchical clustering dendrogram of primary BAs (green) and secondary BAs (pink) in LIC of CV and GF mice exposed to corn oil, PCN, or TCPOBOP. Asterisks (*) represent statistically significant differences between corn oil and chemicals (PCN or TCPOBOP); pounds (#) represent statistically significant differences between CV and GF mice of the same exposure (two-way ANOVA followed by Tukey's post hoc test, adjusted p -value < 0.05).

3.9 Supplemental Tables

Supplemental Table 1

Table 3.S1. List of BAs quantified by LS-MS with the corresponding abbreviation, compartment detected, and the internal standard (IS) used for normalization.

Bile acid names	Abbreviations	Compartment quantified	IS used for normalization
12-dehydrocholic acid	12-DHCA	Liver, SIC, LIC	CA-D4
3,12-diketocholanic acid		LIC	DCA-D4
3,6-diketocholanic acid		LIC	DCA-D4
3,7-diketocholanic acid		LIC	DCA-D4
3-dehydrocholic acid	3-DHCA	Serum, Liver, SIC, LIC	CA-D4
3-ketocholanic acid		LIC	LCA-D4
3 α ,12 α ,23-nordeoxycholic acid			DCA-D4
3 α -hydroxy-12 ketolithocholic acid	3 α -OH-12 KetoLCA	SIC, LIC	LCA-D4
3 α -hydroxy-6,7-diketocholanic acid			CA-D4
3 α -Hydroxy-7 ketolithocholic acid	3 α -OH-7 KetoLCA	SIC, LIC	DCA-D4
3 α -hydroxy-7,12-diketocholanic acid			CA-D4
5-cholenic acid-3 β -ol			LCA-D4
5 α -cholanic acid-3, 6-dione		LIC	DCA-D4
5 α -cholanic acid-3 α -ol-6-one		SIC, LIC	DCA-D4
5 β -cholanic acid-3 α , 6 α -diol-7-one		Liver, SIC, LIC	DCA-D4
5 β -cholanic acid-3 β , 12 α -diol		SIC, LIC	DCA-D4
8(14),(5 β)-cholenic acid-3 α , 12 α -diol		SIC, LIC	DCA-D4
9(11), (5 β)-cholenic acid-3 α -ol-12-one			DCA-D4
Allolithocholic acid	AlloLCA	LIC	LCA-D4
Chenodeoxycholic acid	CDCA	Serum, Liver, SIC, LIC	DCA-D4
Cholic acid	CA	Serum, Liver, SIC, LIC	CA-D4
Dehydrocholic acid	DHCA		CA-D4
Deoxycholic acid	DCA	Serum, Liver, SIC, LIC	DCA-D4
Glycochenodeoxycholic acid	G-CDCA	Serum, Liver, SIC, LIC	GCDCA-D4
Glycocholic acid	G-CA	Liver, SIC	GCA-D4
Glycodehydrocholic acid	G-DHCA		GCA-D4
Glycodeoxycholic acid	G-DCA	SIC, LIC	GCDCA-D4
Glycohyocholic acid	G-HCA	SIC	GCA-D4
Glycodyoxycholic acid	G-HDCA	SIC	GCDCA-D4
Glycolithocholic acid	G-LCA		LCA-D4
Glyco-ursocholanic acid			GCDCA-D4
Glycoursodeoxycholic acid	G-UDCA		GCDCA-D4
Hyochoic acid	HCA	LIC	CA-D4
Hyodeoxycholic acid	HDCA	Liver, SIC, LIC	DCA-D4
Isooxycholic acid			DCA-D4

Isolithocholic acid	IsoLCA	LIC	LCA-D4
Lithocholenic acid			LCA-D4
Lithocholic acid	LCA	SIC, LIC	LCA-D4
Murocholic acid	MCA	Liver, SIC, LIC	DCA-D4
Taurochenodeoxycholic acid	T-CDCA	Serum, Liver, SIC, LIC	GCDCA-D4
Taurocholic acid	T-CA	Serum, Liver, SIC, LIC	GCA-D4
Taurodehydrocholic acid	T-DHCA		GCA-D4
Taurodeoxycholic acid	T-DCA	Serum, Liver, SIC,	GCDCA-D4
Taurohyocholic acid	T-HCA	SIC, LIC	GCA-D4
Taurohyodeoxycholic acid	T-HDCA	Serum, Liver, SIC, LIC	GCDCA-D4
Taurolithocholic acid	T-LCA	Liver, SIC,	LCA-D4
Tauro-ursocholanic acid			GCA-D4
Tauroursodeoxycholic acid	T-UDCA	Serum, Liver, SIC, LIC	GCDCA-D4
Tauro- α +Tauro- β muricholic acid	T- α/β MCA	Serum, Liver, SIC, LIC	GCA-D4
Tauro- ω muricholic acid	T- ω MCA	SIC, LIC	GCA-D4
Ursocholanic acid			LCA-D4
Ursodeoxycholic acid	UDCA	Liver, SIC, LIC	DCA-D4
α -muricholic acid	α MCA	Liver, SIC, LIC	CA-D4
β -muricholic acid	β MCA	Serum, Liver, SIC, LIC	CA-D4
ω -muricholic acid	ω MCA	Serum, Liver, SIC, LIC	CA-D4

Supplemental Table 2**Table 3.S2.** List of primers used to quantify bacterial gene abundance.

Bacteria gene qPCR primers	Forward	Reverse	Source
<i>bsh</i>	ATGGGCGGACTAGGATTACC	TGCCACTCTCTGTCTGCATC	(1)
<i>baiCD</i>	CAGCCRCAGATGTTCTTTG	GCATGGAATTCWACTGCYTC	(2)
<i>baiJ</i>	TCAGGACGTGGAGGCGATCCA	TACRTGATACTGGTAGCTCCA	(3)

Supplemental Table 3

Table 3.S3. List of primers used to quantify host gene expression (mRNA).

Host qPCR primers	Forward	Reverse
Abca1	CCAGACAGTTGTGGATGTGG	GACCTCGCTCTTCCTTCCTT
Abcg5	TGCATCTTAGGCAGCTCAGG	GGCTGCTCAGAAAAACGTCG
Abcg8	CACCTTCCACATGTCTCCT	TTCAGGTCCATGGCACTGAT
Asbt (Slc10a2)	ACAGCCTGGGTTTCTTCCTG	GGGGGAGAAGGAGAGCTGTA
Atp8b1	GGCTGAAGAGCAGTGGAAC	GGAGATGAGGTCTGCGTAGC
Baat	TAGAGCACACCACGTTCTCTG	GCACAGGCTCATCAACAAGA
Bal (Slc27a5)	TGTGTGTGAAGGAACCTGGA	ACCCGGACAACCTTTGTGAAG
Bsep	CCACCAGAACATGACAAACG	CCCAGTGATGACCCATAACC
Cyp1a2	GACATGGCCTAACGTGCAG	GGTCAGAAAGCCGTGGTTG
Cyp27a1	GAGAGTGAATCAGGGGACCA	TCAGGAATGGAGGGTTTCAG
Cyp2b10	AAGGAGAAGTCCAACCAGCA	CTCTGCAACATGGGGGTACT
Cyp2c29	ATTCATCGACCTCCTCCCA	AAAGTGCCAGGGTCAAACA
Cyp2c37	TCCTGGGCTGTGCTCCTTGC	TGCAAATCTGCAACCAAGGGCTG
Cyp2c38	CTACCCCATGCAGTGACCTG	TGGCCAGGGTCAAACATCTC
Cyp2c39	AAGCCTATGGCCCTGTGTTC	AATTCGCAGGGTCGTGAGT
Cyp2c40	TCTACAGGAAAGGGCATTGG	GACCCTTGTGGCTTTCCATA
Cyp2c44	CCTGTGTTGGCGAGGGTTT	CACCAGAGCCTTTAGGGAAAAT
Cyp2c50	CTCATTGGATTGGCCTCAGT	AGGAATGAAGCAGAGCTGGA
Cyp2c54	TTGACCATGGGGATGTCTTT	ATCCAATGCCCATTCATTA
Cyp2c55	TGGCCACTGTAACCGACATA	TCGATCTCTGCCTGGACTTT
Cyp2c65	CGGGGTTGTTTTCAGCAGTG	CAATGCTCCTCCTCCCATC
Cyp2c66	GGAACCTTTCCAGTGGCTGA	CTCGGTCTCAATGCTCCTC
Cyp2c67	ACACCGCAGCCCCTGTATGC	TGCACCAAATAATTGGGCCAAGGT
Cyp2c68	TGGAGTGCCTGATCATACCA	CCACAGTGACAGGAGAAGCA
Cyp2c69	GTGCTTCATCCCTGTGGAAT	TGGGACTGGGTAAATGAGG
Cyp2c70	TGGCTTTCTCAGCAGGAAGAA	AACTGGCTTGGTGTGATGT
Cyp3a11	ACAAACAAGCAGGGATGGAC	GGTAGAGGAGCACCAAGCTG
Cyp4a14	CTGGGTGATGGAACCTCTGT	CATCTGGGAAGGTGACAGGT
Cyp7a1	CAACGGGTTGATTCCATACC	ATTTCCCATCAGTTTGCAG
Cyp7b1	CCTGCAGTCAACAGGTCAAA	GAGCACAGCCTCAGAACCTC
Cyp8b1	AGTTGCAGCGTCTCTTCCAT	CCTTGCTCCCTCAGAACTG
Fgf15	AGAACAGCTCCAGGACCAGA	TCCATGCTGTCACTCTCCAG
Mdr2	GGATCTTGAGGCAGCGAGAA	CCTGCCAGTCAGAGTATCGG
Mrp2	AACCGGAAGGTCAAGTTCT	CAGAGTCACTGGTCCAAGCA
Mrp3	CCAGACCTCACACCCTGTTT	CGTCTTGAGCCTGGATAAGC
Mrp4	ACCAGGATGCCGACATCTAC	CGTGCAAAGTCTGGCAGATA
Nqo1	TATCCTTCCGAGTCATCTCTAGCA	TCTGCAGCTTCCAGCTTCTTG
Ntcp	GGTGCCCTACAAAGGCATTA	ACAGCCACAGAGAGGGAGAA
Oatp1a1	ATCCAGTGTGTGGGGACAAT	GCAGCTGCAATTTTGAAACA
Oatp1a4	GGAAGATTGGACACGCATCT	GGCATTGTGACTGAAGCAGA
Oatp1b2 (Slco1b2)	TCGCCAGCCTAGGCCAAATGC	TGGGCAGCTTTGCTTGATGTT
Oatp2b1	CTAGGCCAAATGCCAGAAAG	TTGCTTGGATGCTGAGTTTG
Osta	GTTGCCATTTTTCTGGAGGA	GACCAAAGCAGCAGAACACA
Ostβ	GTTCTGGCAGTCCTGGTGAT	CCAGGCTGCTGTTTTCTTTC
SHP	CTCATGGCCTCTACCCTCAA	GGTCACCTCAGCAAAGCAT

Supplemental Table 4

Table 3.S4. Categorization of BAs for grouping of overall quantifications for primary, secondary, classic pathway (12-OH), and alternative pathway (6-OH).

Primary	Secondary	6-OH	12-OH
CA	12-DHCA	HDCA	AlloLCA
CDCA	3,12-diketocholanic acid	MCA	CA
G-CA	3,6-diketocholanic acid	T-HDCA	DCA
G-CDCA	3,7-diketocholanic acid	T- α/β MCA	G-CA
G-HCA	3-DCA	T- ω MCA	G-DCA
HCA	3-DHCA	α MCA	IsoLCA
T-CA	3-ketocholanic acid	β MCA	LCA
T-CDCA	3 α -OH-12 KetoLCA	ω MCA	T-CA
T-HCA	3 α -OH-7 KetoLCA		T-DCA
T-UDCA	5 α -cholanic acid-3, 6-dione		T-LCA
T- α/β MCA	5 α -cholanic acid-3 α -ol-6-one		
UDCA	5 α -cholanic acid-3 α -ol-6-one		
α MCA	5 β -cholanic acid-3 α , 6 α -diol-7-one		
β MCA	5 β -cholanic acid-3 β , 12 α -diol		
	8(14),(5 β)-cholenic acid-3 α , 12 α -diol		
	AlloLCA		
	DCA		
	G-DCA		
	G-HDCA		
	HDCA		
	IsoLCA		
	LCA		
	MCA		
	T-DCA		
	T-HDCA		
	T-LCA		
	T- ω MCA		
	ω MCA		

Chapter 4: Regulation of Hepatic Long Non-coding RNAs by PXR and CAR Agonists in Mouse Liver

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4.1 Introduction

Long non-coding RNAs (lncRNAs) are functional transcripts over 200 nucleotides in length whose genes are estimated to comprise at least 62-75% of the human genome (Djebali et al., 2012; St Laurent et al., 2015). According to the ENCODE Project, lncRNAs represent approximately 80% of functional sequences in the human DNA and are the predominant non-ribosomal and non-mitochondrial RNA species in human cells (Kapranov et al., 2010; Consortium, 2012). lncRNAs transcribed proximally or distally to protein-coding genes (PCGs) can modulate a wide spectrum of biological events, including chromatin epigenetic remodeling, transcription factor assembly, alternative splicing, mRNA stability, and protein translation efficiency (Geisler and Coller, 2013; Karlsson and Baccarelli, 2016; Dempsey and Cui, 2017). Growing evidence in the literature suggests that lncRNAs are novel biomarkers and/or key contributors during physiological, pharmacological, and toxicological responses, including complex human diseases, developmental disorders, as well as xenobiotic-induced adverse outcomes (Dempsey and Cui, 2017).

Many lncRNAs are highly expressed in liver, which is a major organ for xenobiotic biotransformation and nutrient homeostasis. A stringent computational pipeline identified 15,558 lncRNAs that are expressed in mouse liver, based on an analysis of 186 mouse liver RNA-Seq datasets ranging over 30 biological conditions (Yu et al., 2017). Interestingly, Yu et al. also demonstrated greater inter-species conservations within DNA sequences and higher frequency of proximal binding by hepatic transcription factors in the liver-expressed lncRNA gene promoters as compared to protein-coding gene (PCG) promoters (Yu et al., 2017). The liver-enriched lncRNAs were predicted to be metabolically sensitive regulators with diverse functions in physiological homeostasis, especially energy metabolism (Yang et al., 2016). Regulation of genes involved in energy metabolism and nutrient homeostasis by lncRNAs found in the literature include gluconeogenesis (Goyal et al., 2017), cholesterol and bile acid (BA) homeostasis (Ananthanarayanan, 2016; Lan et al., 2016; Yu et al., 2017; Zhang et al., 2017), lipid metabolism

(Li et al., 2015; Chen, 2016; Yang et al., 2016; Li et al., 2017; Zhao et al., 2017), and non-alcoholic fatty liver disease (Chen et al., 2017).

In addition to modulating intermediary metabolism in liver, lncRNAs are suggested to modulate hepatic xenobiotic biotransformation. For example, two lncRNAs that are transcribed from the anti-sense strands of the DNA encoding the hepatocyte nuclear factor 1 α (HNF1 α) and HNF4 α are important in regulating the major drug-metabolizing cytochrome P450s (CyPs) in human liver cancer-derived HepaRG cells (Chen et al., 2018). Specifically, short hairpin RNA (shRNA) knockdown of the lncRNA gene *HNF1 α antisense 1 (AS-1)* decreased the mRNA expression of PXR and CAR, as well as the expression of seven major P450s (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1, and CYP3A4) in HepaRG cells. In addition, small interfering RNA (siRNA) knockdown of the lncRNA gene *HNF4 α -AS1* increased the mRNA expression of PXR as well as the expression of six P450s (CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2E1, and CYP3A4). This indicated that there is a complex network between transcription factors and lncRNAs that regulate the expression of P450s (Chen et al., 2018).

During postnatal liver maturation, the developmental expression patterns of various lncRNAs have been unveiled at 12 developmental ages in mice (Peng et al., 2014), and the potential role of lncRNAs in regulating the ontogenic expression of the xenobiotic-metabolizing CyPs was proposed (Ingelman-Sundberg et al., 2013). lncRNAs have also been suggested to confer drug resistance through modulating the stability and translation of mRNAs that produce proteins involved in cell survival, proliferation, and drug metabolism (Pan et al., 2015). However, very little is known regarding to what extent the hepatic lncRNAs are regulated by exposure to drugs and other xenobiotics.

Regarding xenobiotic biotransformation, in liver, the nuclear receptors pregnane X receptor (PXR/Nr1i2) and constitutive androstane receptor (CAR/Nr1i3) are major xenobiotic-sensing nuclear receptors that can be activated by a wide spectrum of therapeutic drugs, environmental toxicants, dietary factors, and endogenous chemicals (Kliewer et al., 2002; Willson

and Kliewer, 2002; Moore et al., 2003; Pacyniak et al., 2007). Upon activation, PXR and CAR play critical roles in xenobiotic bio-activation and detoxification (Handschin and Meyer, 2003). In addition, recent studies have unveiled novel functions of these drug receptors in various intermediary metabolism pathways, such as lipid and glucose metabolism (Poulin-Dubois and Shultz, 1990; Wada et al., 2009; Gao and Xie, 2010; Mackowiak et al., 2018; Pu et al., 2018). Our research group and others have extensively characterized the effect of pharmacological activation of PXR and CAR on the regulation of various protein-coding genes, especially the drug-processing genes in liver (Cheng et al., 2005b; Maher et al., 2005; Kiyosawa et al., 2008; Pratt-Hyatt et al., 2013; Oshida et al., 2015; Cui and Klaassen, 2016).

There has been some investigation on effect of the CAR ligand 1, 4-bis[2-(3, 5-dichloropyridyloxy)]benzene (TCPOBOP) on the hepatic chromatin assembly and certain lncRNAs that contribute to liver tumor promotion (Lempiainen et al., 2013; Lodato et al., 2018). TCPOBOP differentially regulated 166 lncRNAs in liver that are produced from intragenic or anti-sense strand relative to PCGs that encode CAR-regulated drug-metabolizing enzymes, suggesting that an efficient co-regulatory mechanism may exist (Yu et al., 2017). In addition, two studies have investigated the effect of activating the xenobiotic-sensing transcription factor aryl hydrocarbon receptor (AhR) on the hepatic expression of lncRNAs (Recio et al., 2013; Grimaldi et al., 2018). However, there have been no systematic studies characterizing the effect of PXR activation on the regulation of liver-enriched lncRNAs and how PXR activation differs from CAR activation regarding lncRNA expression profiles.

Therefore, the purpose of the present study was to 1) determine the effect of pharmacological activation of PXR on the hepatic expression of lncRNAs; 2) to identify direct PXR-binding sites to the lncRNA gene loci and associated epigenetic signatures under basal and PXR activated conditions; 3) to compare the similarities and differences between PXR and CAR targeted lncRNAs; and 4) to predict the lncRNA-regulated protein-coding gene networks following PXR/CAR activation in mouse liver.

4.2 Materials and Methods

Chemicals

Corn oil (vehicle), the mouse PXR ligand pregnenolone-16 α -carbonitrile (PCN; $\geq$ 97% purity; CAS Number 1434-54-4), and the mouse CAR ligand 1,4-*bis*-[2-(3,5-dichloropyridyloxy)]benzene,3,3',5,5'-tetrachloro-1,4-*bis*(pyridyloxy)benzene (TCPOBOP; $\geq$ 98% purity; CAS Number 76150-91-9) were purchased from Sigma-Aldrich (St. Louis, MO).

Animals and procedures

As described previously (Cui and Klaassen, 2016), 12-week-old adult male C57BL/6 wild-type mice were purchased from Charles River Laboratories (Wilmington, MA) and were housed in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited facility (Animal Care and Use Program number 2011-1969) at the University of Kansas Medical Center (KUMC) with *ad libitum* access to the Laboratory Rodent Chow 8604 (Harlan, Madison, WI) and drinking water. The housing conditions were temperature- and humidity-controlled with a 14-hour light and 10-hour dark cycle in a temperature- and humidity-controlled environment. Mice were acclimated for at least one week within the animal facilities prior to experiments. Mice were administered PCN (200 mg/kg, i.p.), TCPOBOP (3 mg/kg, i.p.), or vehicle (corn oil, 5 ml/kg, i.p.) once daily for four consecutive days (n=5 per group). Twenty-four hours after the final dose, livers were collected, immediately frozen in liquid nitrogen, and stored in a -80 °C freezer. All animal experiments were approved by the IACUC at KUMC.

RNA isolation

Total RNA was isolated from mouse liver using RNA-Bee reagent (Tel-Test Inc., Friendswood, TX) per the manufacturer's protocol and quantified using a Nanodrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE). RNA integrity was confirmed using gel electrophoresis and a dual Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA).

RNA samples used for cDNA library construction and RNA sequencing had RNA integrity (RIN) values between 7.0 and 10.0.

cDNA library construction and RNA-Seq data analysis

The cDNA library preparation from poly-A selection was performed in the KUMC Genome Sequencing Facility, as described previously (Cui and Klaassen, 2016). Samples for sequencing were randomly selected (n=3 per group) from all biological replicates (n=5) for all studies. Sequencing was performed on an Illumina HiSeq2000 sequencer using 100 bp paired-end multiplexing strategy. FASTQ files and analyzed data are available at NCBI GEO database (GSE104734), and data were re-analyzed for the present study. Briefly, FASTQ files containing paired-end sequence reads were mapped to the mouse reference genome (GRCm38/mm10) using HISAT2 (Hierarchical Indexing for Spliced Alignment of Transcripts) (version 2.0.5). The output SAM (sequencing alignment/map) files were converted to BAM (binary alignment/map) files and sorted using SAMtools (version 1.3.1). The transcript abundance for lncRNAs and PCGs was estimated by Cufflinks (version 2.2.1) using the NONCODE 2016 lncRNA and UCSC mm10 PCG reference databases, respectively. The mRNA abundance was expressed as fragments per kilobase of exon per million reads mapped (FPKM). lncRNAs and PCGs with an average FPKM above 1 in at least one sample were considered expressed. Differential analysis was performed using Cuffdiff, and transcripts with $p < 0.05$ were considered differentially regulated by chemical exposure. Data were expressed as mean FPKM $\pm$ S.E., and asterisks (*) represent statistically significant differences between vehicle and chemical exposure. Two-way hierarchical clustering dendrograms of differentially regulated lncRNAs were generated using the native function of R.

Genomic annotation of lncRNAs and proximal lncRNA-PCG pair identification

To annotate and visualize the genomic location of lncRNAs relative to the closest PCGs, the web-based tool peak annotation and visualization (PAVIS,

<https://manticore.niehs.nih.gov/pavis2/>) was used to identify lncRNAs proximal to PCGs, including 5 kb upstream of the transcription start site (TSS), intronic, exonic, 5'-untranslated region (UTR), 3'-UTR, and up to 1 kb downstream of the transcriptional termination site (TTS). A lncRNA and PCG are considered paired if 1) the lncRNA overlaps with or is within 5 kb upstream of the TSS or 1 kb downstream of TTS of any PCG and 2) both the lncRNA and the proximal PCG were differentially expressed between vehicle and chemical exposed mice (FPKM > 1 at least one sample and $p < 0.05$). Gene structure and relative genomic location of the lncRNA-PCG pairs were visualized using Integrated Genome Viewer (Broad Institute, Cambridge, MA). Pathways that are associated with PCGs paired with lncRNAs were shown using Ingenuity Pathway Analysis (IPA) software.

PXR ChIP-Seq and motif analysis for PXR-DNA binding near lncRNA gene loci

The PXR ChIP-Seq was performed in livers of corn oil or PCN exposed adult male C57BL/6 mice as described previously (Cui et al., 2010b), and data were re-analyzed for the present study. The chromosome coordinates of positive PXR DNA binding peaks in either corn oil or PCN exposed conditions were retrieved. The chromosome coordinates for the enrichment locations were adjusted to match mm10 using Galaxy to be consistent with the reference genome used in the RNA-Seq dataset. The lncRNA genes differentially regulated by PCN were examined for positive enrichment of PXR-DNA binding peaks within 10 kb upstream of the TSS and 10 kb downstream of the TTS. The Motif analysis of PXR binding sites near lncRNA gene loci was performed using Homer (findMotifsGenome.pl). DNA sequences associated with the PXR-DNA binding sites were retrieved using the mouse mm10 genome. The size of the analyzed DNA regions was set as 200 bp, and the motif lengths for *de novo* motif search were set at 8, 10, and 12bp.

Epigenetic marks near PXR-regulated lncRNAs

The positive enrichment of the active gene transcription mark histone H3 lysine 4 dimethylation (H3K4me2) as well as the gene silencing marks H3K27me3 and DNA methylation (5MeC) on chromosomes 5, 12, and 15 in livers of adult C57BL/6 male mice was determined by CHIP-on-chip as described before (Cui et al., 2009; Li et al., 2009; Choudhuri et al., 2010; Cui et al., 2010a). The chromosome coordinates for the epigenetic mark locations that were originally generated were lifted-over to mm10 using Galaxy to be consistent with the reference genomes used in the PXR CHIP-Seq and RNA-Seq datasets. The potential co-localizations of PXR and the three epigenetic marks within ± 10 kb of the lncRNA gene loci were determined.

4.3 Results

Overall comparison of PCN- and TCPOBOP-regulated liver-expressed lncRNAs

As shown in Figure 4.1, the majority (>120,000) of the lncRNAs were below the detection limit in mouse livers under any exposure condition, whereas approximately 4,000 lncRNAs were expressed (average FPKM > 1 in at least one group). Among these liver-expressed lncRNAs, most of them showed stable expression and were not altered following PCN (96%) or TCPOBOP (86%) exposure. PCN up-regulated approximately 2% and down-regulated 2% of the liver-expressed lncRNAs, whereas TCPOBOP up-regulated 7% and down-regulated approximately 7% of the liver-expressed lncRNAs.

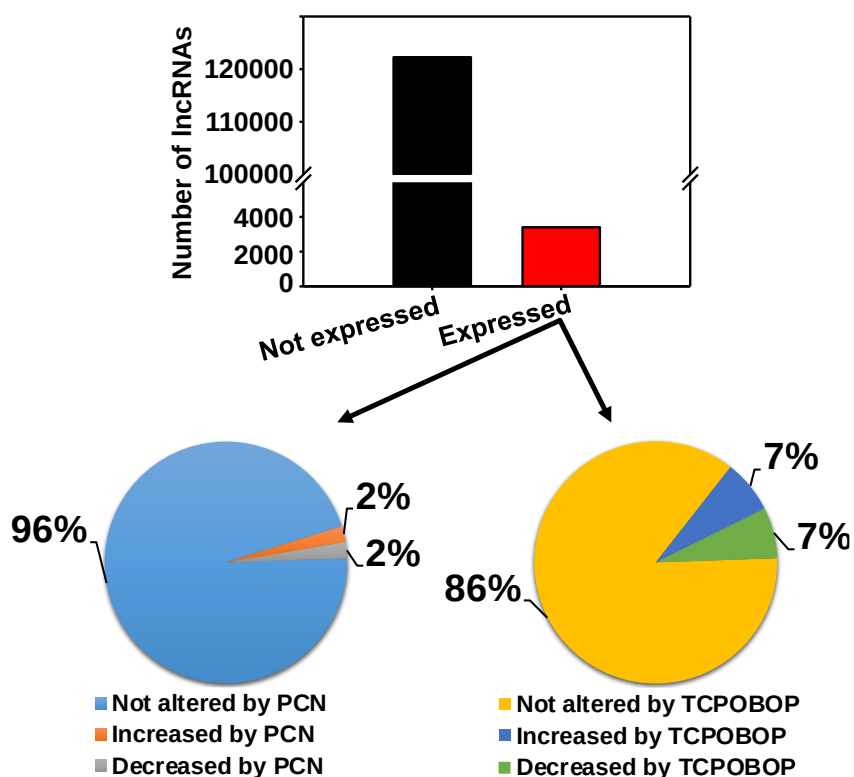


Figure 4.1. Number of lncRNAs that were not expressed (or not detected using the poly-A selection method) and expressed in liver. A lncRNA is considered to be expressed in liver if the average FPKM is above 1 in at least one exposure groups (corn oil, PCN, or TCPOBOP). Among the liver-expressed lncRNAs, the percentages of lncRNAs that were not differentially regulated, increased, or decreased by chemical exposure were calculated and displayed as two pie charts (PCN and TCPOBOP). Differential expression was considered at $p < 0.05$ (Cuffdiff).

As shown in Figure 4.2A, TCPOBOP in general had a more prominent effect than PCN in differentially regulating the liver expressed lncRNAs in that 193 lncRNAs were altered by PCN exposure as compared to 625 that were altered by TCPOBOP exposure. There were 81 lncRNAs that were commonly regulated by both PCN and TCPOBOP, suggesting that PXR and CAR have unique lncRNA gene targets in liver. A two-way hierarchical clustering dendrogram showed that biological replicates from the same exposure groups clustered together for the lncRNA gene expression (Figure 4.2B) and confirmed that TCPOBOP had a more profound effect in both up- and down-regulation of lncRNAs. This may be due to TCPOBOP being a highly potent activator of CAR, whereas the potency of PCN is less towards PXR activation.

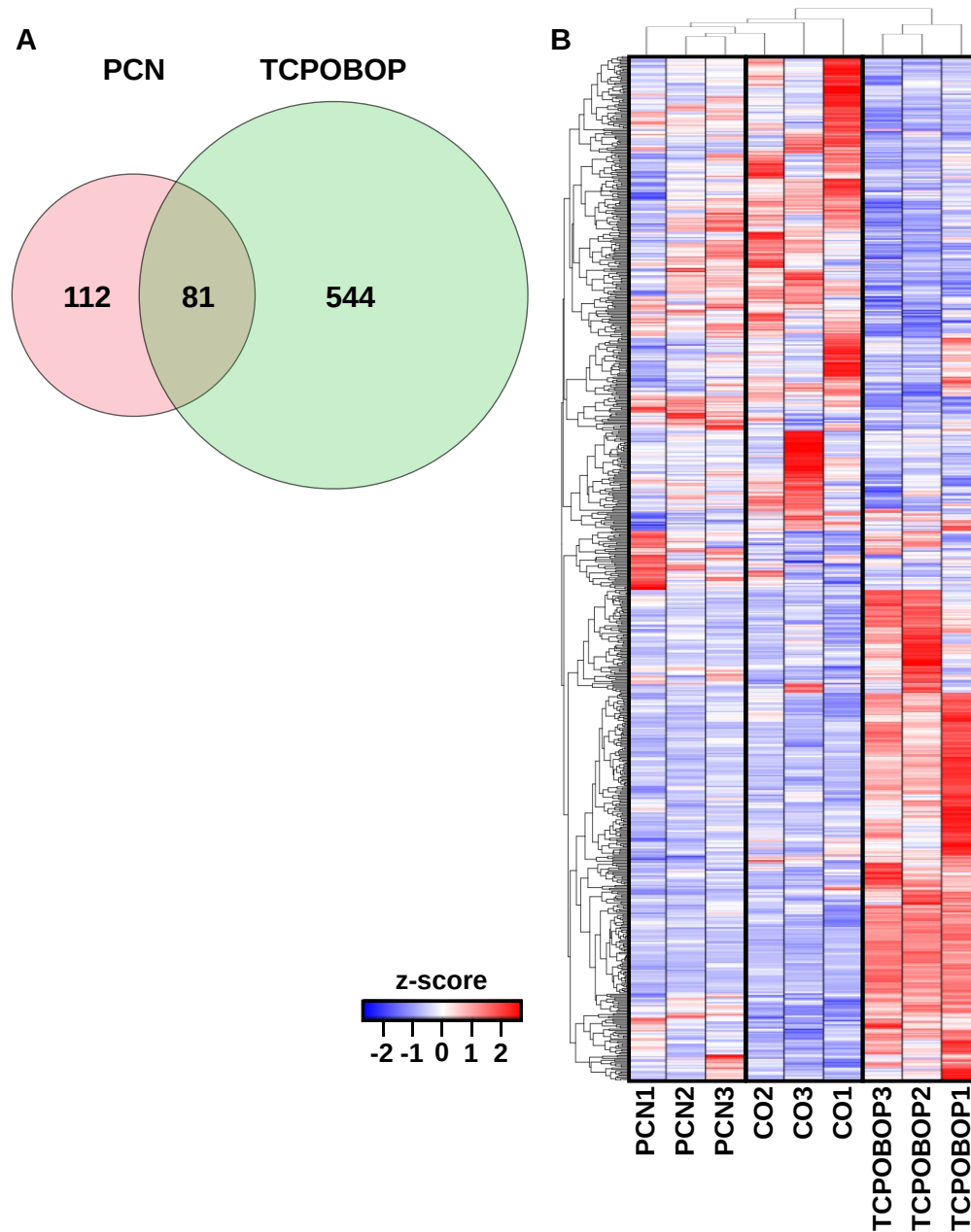


Figure 4.2. **A.** Common and unique lncRNA targets following PCN and TCPOBOP exposure in mouse liver ($p < 0.05$ as determined by Cuffdiff). **B.** A hierarchical clustering dendrogram showing the relative expression patterns of lncRNAs in liver in corn oil, PCN, and TCPOBOP exposed conditions. Data were standardized and are expressed as z-scores.

Genomic annotation of PCN- and TCPOBOP-regulated liver-expressed lncRNAs relative to PCGs and predicted gene networks

As a first step to predict the function of lncRNAs with regards to influencing the transcriptional output of PCGs, the genomic locations of PCN- and TCPOBOP-regulated lncRNAs relative to the PCGs were determined using PAVIS, and the lncRNA-PCG gene pairs were defined if they met all of the following criteria: 1) the lncRNA gene overlaps with or is within 5 kb upstream of TSS or 1 kb downstream of TTS of any PCG and 2) both the lncRNA and the proximal PCG were differentially expressed by PCN or TCPOBOP exposure (average FPKM > 1 and $p < 0.05$). These criteria were set based on the assumption that although exceptions may exist, lncRNAs produced locally around the neighboring PCGs have a more spatial advantage in influencing the transcriptional output of these PCGs as compared to lncRNAs produced in distal regions (Orom et al., 2010; Kim et al., 2012; Villegas and Zaphiropoulos, 2015; Engreitz et al., 2016; Chen et al., 2018).

As shown in Figure 4.3A and Supplemental Table 1, following PCN exposure, 141 (73.1%) of 193 lncRNAs differentially regulated by PCN paired with distinct PCGs. The remaining 52 (26.9%) were produced from the intergenic regions and did not pair with any PCGs. As shown in Figure 4.3B, among the lncRNAs that paired with PCGs, the majority of them were produced from the intronic regions of PCGs (43%), followed by the 3'-untranslated regions (3'-UTRs) (14.5%), exonic regions (6.7%), downstream of TTS (5.2%), and upstream of TSS (3.6%). There were no lncRNAs that were co-differentially regulated by PCN and were produced within the 5'-UTR of PCGs that were differentially regulated by PCN. The PCGs that paired with lncRNAs formed distinct signaling networks including lipid metabolism, molecular transport, and small molecular biochemistry centering around ERK1/2, which is a MAP kinase that catalyzes the phosphorylation of many cytoplasmic and nuclear substrates (Supplemental Figure 4.S1), as well as cellular growth and proliferation (Supplemental Figure 4.S2). For example, HDL as well as its regulator

serum amyloid A1 (SAA1) was down-regulated, whereas AMPK, which activates glucose and fatty acid uptake and oxidation, was up-regulated.

Following TCPOBOP exposure, additional lncRNA-PCG pairs were discovered. As shown in Figure 4.3C and Supplemental Table 2, 359 (73%) lncRNAs were paired with PCGs, whereas 134 were not paired (27.1%). Similar to the PCN exposure conditions, the majority of the paired lncRNAs were produced from the intronic (34%) and 3'-UTR (21.5%) of the PCGs, followed by downstream (6.4%), upstream (6.1%), and exonic (5.3%) regions, whereas the mapping to 5'-UTR was minimal (0.2%) (Figure 4.3D). Also similar to PCN exposure, the PCGs that paired with lncRNAs following TCPOBOP exposure were also important for lipid metabolism and molecular transport (Supplemental Figure 4.S3). For example, Apolipoprotein A4 (ApoA4), which is a lipid transporter, was up-regulated, whereas SAA1, which plays an important role in HDL metabolism and cholesterol homeostasis, was down-regulated (Supplemental Figure 4.S3). In addition, the carcinogenesis network was enriched in TCPOBOP-regulated PCGs that paired with lncRNAs (Supplemental Figure 4.S4).

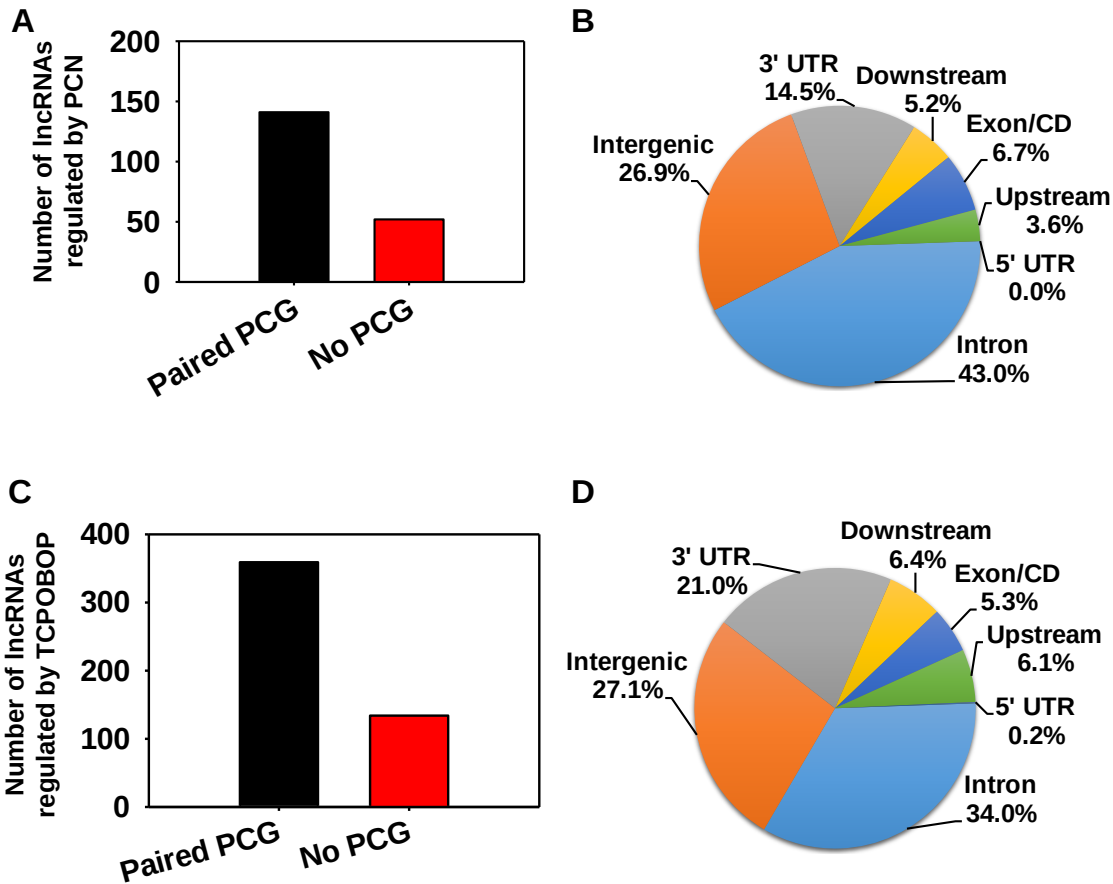
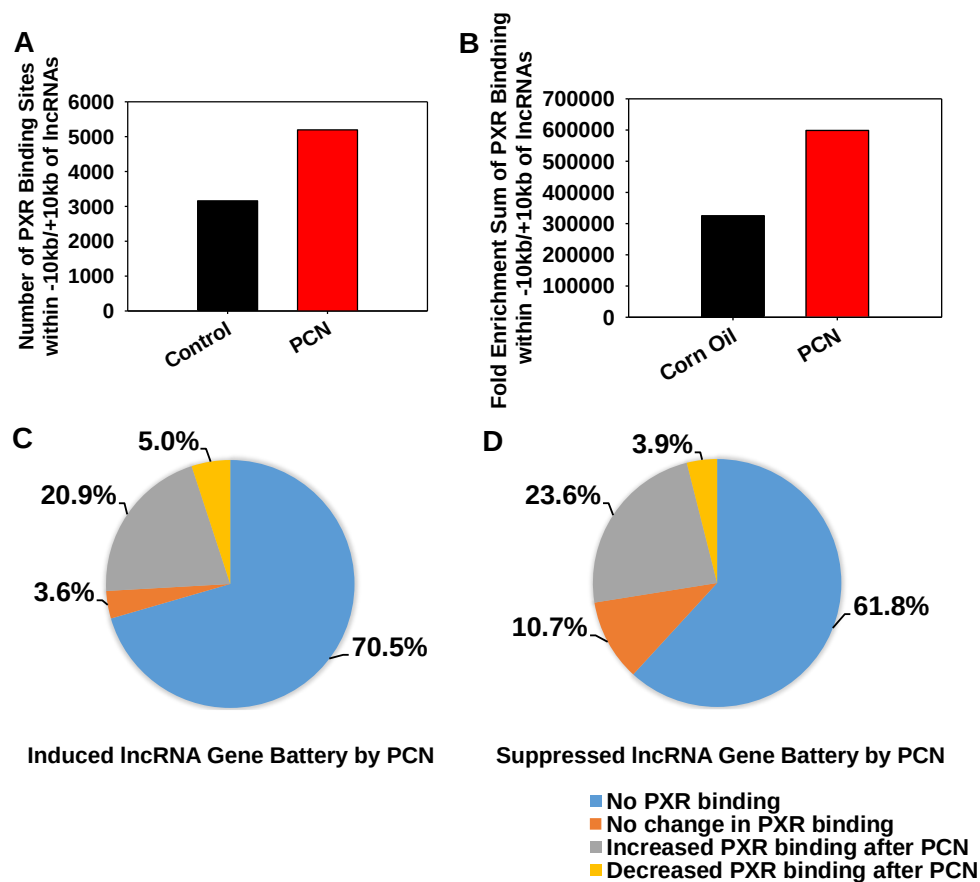


Figure 4.3. A. Number of lncRNAs that paired with PCGs or not paired with PCGs following PCN exposure. A lncRNA-PCG gene pair is defined as: 1) the lncRNA gene overlaps with or is within 5 kb upstream of transcription start sites (TSS) or 1 kb downstream of transcription termination sites (TTS) of any PCG and 2) both the lncRNA and the proximal PCG were differentially expressed by PCN exposure (average FPKM > 1 and $p < 0.05$). **B.** Genomic annotation of PCN-regulated lncRNAs ($p < 0.05$) relative to the PCGs. Data were analyzed using PAVIS. **C.** Number of lncRNAs that paired with PCGs or not paired with PCGs following TCPOBOP exposure. A lncRNA-PCG gene pair is defined as: 1) the lncRNA gene overlaps with or is within 5 kb upstream of transcription start sites (TSS) or 1 kb downstream of transcription termination sites (TTS) of any PCG and 2) both the lncRNA and the proximal PCG were differentially expressed by PCN exposure (average FPKM > 1 and $p < 0.05$). **D.** Genomic annotation of TCPOBOP-regulated lncRNAs ($p < 0.05$) relative to the PCGs. Data were analyzed using PAVIS.

PXR-DNA binding to PCN-regulated hepatic lncRNAs and motif analysis

As shown in Figure 4.4, both the total numbers of PXR-DNA binding sites and the cumulative PXR-DNA binding fold enrichment near the lncRNA gene loci (± 10 kb) increased following PCN exposure. Approximately 70% of the differentially regulated lncRNAs had no PXR binding. Interestingly, among the direct PXR-targeted lncRNA genes, both the up-regulated and the down-regulated lncRNAs had increased PXR-DNA binding. This indicates that PXR has dual functions in the transcription of these lncRNAs—cis-activation and cis-suppression (Figure 4.4C and 4D)—likely due to a functional switch of other transcription factors that co-localize with PXR at these sites. To address this, *de novo* motif analysis was performed in lncRNA gene-associated PXR-DNA binding peaks in corn oil and PCN exposed conditions independently (Figure 4.4E). Interestingly, in control conditions, HNNRNPA2B1 and RAR α were significantly enriched in PXR-DNA binding sites of the lncRNA genes, whereas Nr5a2/LRH-1 was enriched by PCN. The molecular switches of these transcription factors may alter the fate of transcriptional output of PXR-targeted lncRNA genes. Other transcription factors associated with known DNA binding motifs within the PXR-DNA binding intervals around the differentially regulated lncRNA gene loci by PCN are shown in Supplemental Figure 4.S5A.



E. Homer *de novo* Motif Analysis

Corn Oil

Rank	Motif	P-value	log P-value	% of Targets	% of Background	STD(Bg STD)	Best Match/Details
1	GGGCGTCCCTA	1e-13	-3.001e+01	13.04%	0.23%	55.3bp (54.3bp)	hNRNPA2B1(RRM)/Homo_sapiens-RNCMP00024-PBM/HughesRNA (0.746) More Information Similar Motifs Found
2	AGAACAGTTC	1e-12	-2.801e+01	34.78%	5.97%	50.2bp (64.4bp)	RARa(NR)/K562-RARa-ChIP-Seq(Encode)/Homer(0.662) More Information Similar Motifs Found

PCN

Rank	Motif	P-value	log P-value	% of Targets	% of Background	STD(Bg STD)	Best Match/Details
1	ITGGCCCTTGGAC	1e-13	-3.120e+01	10.00%	0.15%	57.5bp (65.4bp)	Nr5a2/MA0505.1/Jaspar(0.811) More Information Similar Motifs Found

Figure 4.4. A. Number of PXR-DNA binding sites within ± 10 kb of lncRNA gene loci in corn oil and PCN exposed conditions. **B.** Cumulatively PXR-DNA binding fold enrichment within ± 10 kb of lncRNA gene loci in corn oil and PCN exposed conditions. **C.** Percentages of induced lncRNA gene battery by PCN that had no PXR binding, no change in PXR binding, increase in PXR binding, and decreased PXR binding following PCN exposure. **D.** Percentages of decreased lncRNA gene battery by PCN that had no PXR binding, no change in PXR binding, increase in PXR binding, and decreased PXR binding following PCN exposure. **E.** Homer *de novo* motif analysis of PXR-DNA binding intervals in corn oil and PCN exposed groups using findMotifsGenome.pl. Data were re-analyzed from the PXR ChIP-Seq dataset as described in Materials and Methods.

Determining the potential co-localization of epigenetic factors and PXR near lncRNA gene loci

Distinct PCN exposure associated permissive and repressive epigenetic marks have previously been identified on mouse chromosomes 5, 12, and 15 (see Materials and Methods). In a preliminary investigation into the role of epigenetic marks and PXR binding on lncRNA expression, we searched for co-localization of PXR-DNA binding, specific epigenetic marks (H3K4me2, H3K27me3, and 5MeC) near lncRNA loci on the same chromosomes. The majority of the lncRNAs associated with these epigenetic marks or PXR were not expressed or not detected on the three chromosomes, likely due to the presence of other suppressive marks that were not investigated in the present study, or were not detected due to the method of RNA selection in the cDNA library preparation procedure (i.e. only poly-A tailed lncRNAs were captured) (Supplemental Table 3). Among the liver-expressed lncRNAs on these three chromosomes, the majority of stably expressed lncRNAs had positive enrichment of the active chromatin epigenetic mark H3K4me2 and PXR. The overlap between H3K4me2 and PXR was also the most in the stably expressed lncRNA gene category. This indicates that H3K4me2 and PXR may act in concert to maintain the constitutive expression of liver-enriched lncRNAs. Interestingly, DNA methylation (5MeC), commonly thought to silence gene expression, was another epigenetic mark that co-localized with stably expressed lncRNA gene loci and overlapped a moderately with PXR binding sites. Paradoxically, although 5MeC is considered a gene silencing mark, our observation is consistent with the more recent literature report that many actively transcribed gene bodies are marked with 5MeC. This correlates with transcriptional activity rather than repression, that the basal function of gene body methylation facilitates the establishment of their constitutive expression, and that cytosine-specific methylation may regulate gene expression (Coleman-Derr and Zilberman, 2012; Vyhldal et al., 2016). Following PXR activation, a moderate number of PCN-regulated lncRNAs (either up- or down-regulated) had positive enrichment of H3K4me2, and most of these lncRNAs had direct PXR-DNA binding sites.

In contrast, almost none of the PCN-regulated lncRNAs had positive enrichment of H3K27me3 or 5MeC, and there was also no overlap between these epigenetic marks and PXR at these lncRNA gene loci (Supplemental Table 3).

Two examples of lncRNAs that had co-localization of PXR and H3K4me2 are shown in Figure 4.5A (a lncRNA that paired with a PCG encoding P450 reductase [Por] that is important for cytochrome P450-mediated hepatic drug metabolism) and Figure 4.5B (an intergenic lncRNA) with the gene annotation shown in Supplemental Figure 4.S6. As shown in Figure 4.5A, the lncRNA NONMMUG034025.2 and the neighboring PCG Por were co-up-regulated by PCN, and this was associated with increased PXR-DNA binding fold-enrichment around the lncRNA-PCG loci. In addition, this region was marked with the active gene transcription mark H3K4me2, but not H3K27me3 or 5MeC. As shown in Figure 4.5B, the PCN-mediated up-regulation of the lncRNA NONMMUG014541.1 was also positively associated with increased PXR-DNA binding and positive enrichment of H3K4me2 but was independent of PCGs.

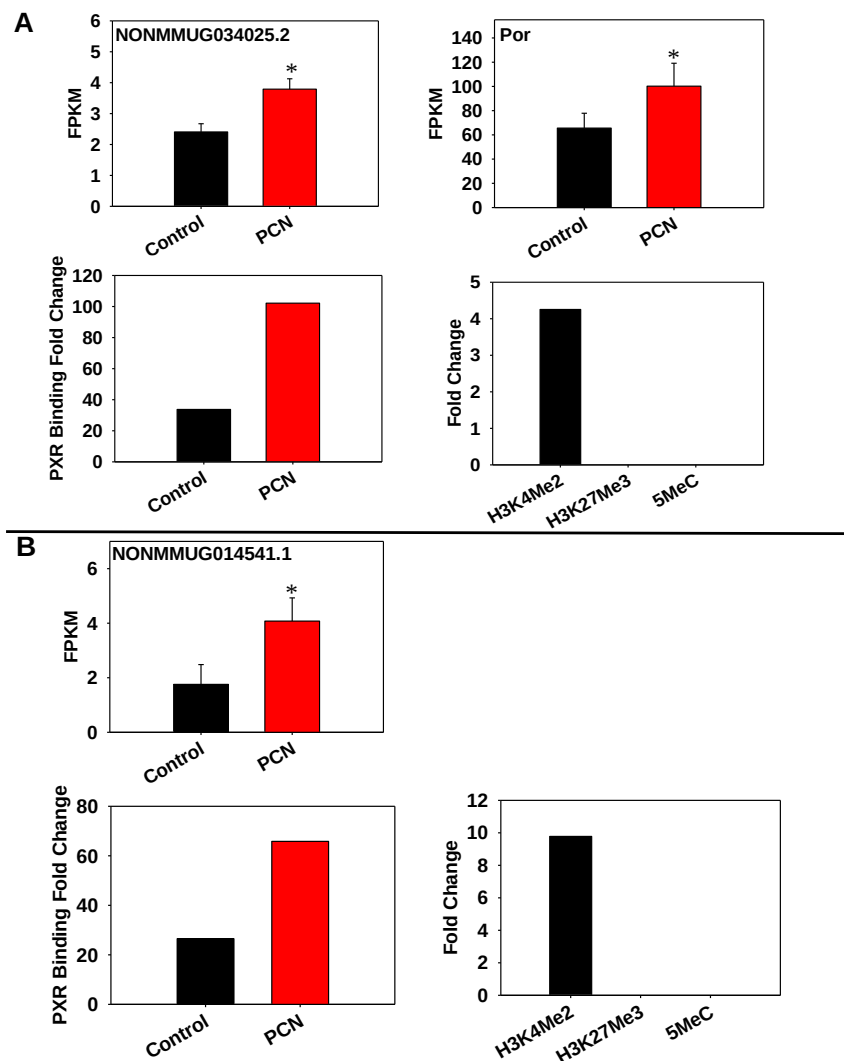


Figure 4.5. A. Co-localization of PXR and H3K4me2 around the lncRNA NONMMUG034025.2 and the paired PCG *Por* gene loci. The genomic locations of lncRNA and PCG are visualized by in Supplemental Figure 4.S6A. Asterisks represent statistically significant differences as compared to corn oil control group ($p < 0.05$, Cuffdiff). FPKM: Fragments Per Kilobase of transcript per Million mapped reads, which is a unit to express RNA abundance from the RNA-Seq data. Data from RNA-Seq (corn oil and PCN exposed groups), ChIP-Seq (for PXR-DNA binding in corn oil and PCN exposed conditions), and ChIP-on-chip (for H3K4me2, H3K27me3, and 5MeC on mouse chromosomes 5, 12, and 15) were integrated as described in Materials and Methods. **B.** Co-localization of PXR and H3K4me2 around the intergenic lncRNA NONMMUG014541.1 gene locus. The genomic location of lncRNA and PCG is visualized by in Supplemental Figure 4.S6B. Asterisks represent statistically significant differences as compared to corn oil control group ($p < 0.05$, Cuffdiff). Data from RNA-Seq (corn oil and PCN exposed groups), ChIP-Seq (for PXR-DNA binding in corn oil and PCN exposed conditions), and ChIP-on-chip (for H3K4me2, H3K27me3, and 5MeC on mouse chromosomes 5, 12, and 15) were integrated as described in Materials and Methods.

Confirmation of the literature-reported CAR-targeted lncRNAs in liver following TCPOBOP exposure and comparison with the effect of PCN exposure

Consistent with the recently published study regarding the effect of the CAR ligand TCPOBOP on the hepatic lncRNA gene expression (Lodato et al., 2018), the present study confirmed the TCPOBOP-mediated increase in 6 lncRNAs (NONMMUG002974.2, NONMMUG017205.2, NONMMUG020358.2, NONMMUG021206.2, NONMMUG026099.2, and NONMMUG036870.2) (Figure 4.6A), as well as the TCPOBOP-mediated decrease in 5 lncRNAs (NONMMUG005073.2, NONMMUG009893.2, NONMMUG015071.2, NONMMUG028068.2, and NONMMUG041315.2) (Figure 4.6B). In comparison, at the given dose, the PXR ligand PCN up-regulated 3 of the same lncRNAs (NONMMUG021206.2, NONMMUG026099.2, and NONMMUG036870.2) as TCPOBOP, albeit to a lesser extent. PCN had only minimal effects on the other TCPOBOP up-regulated lncRNAs.

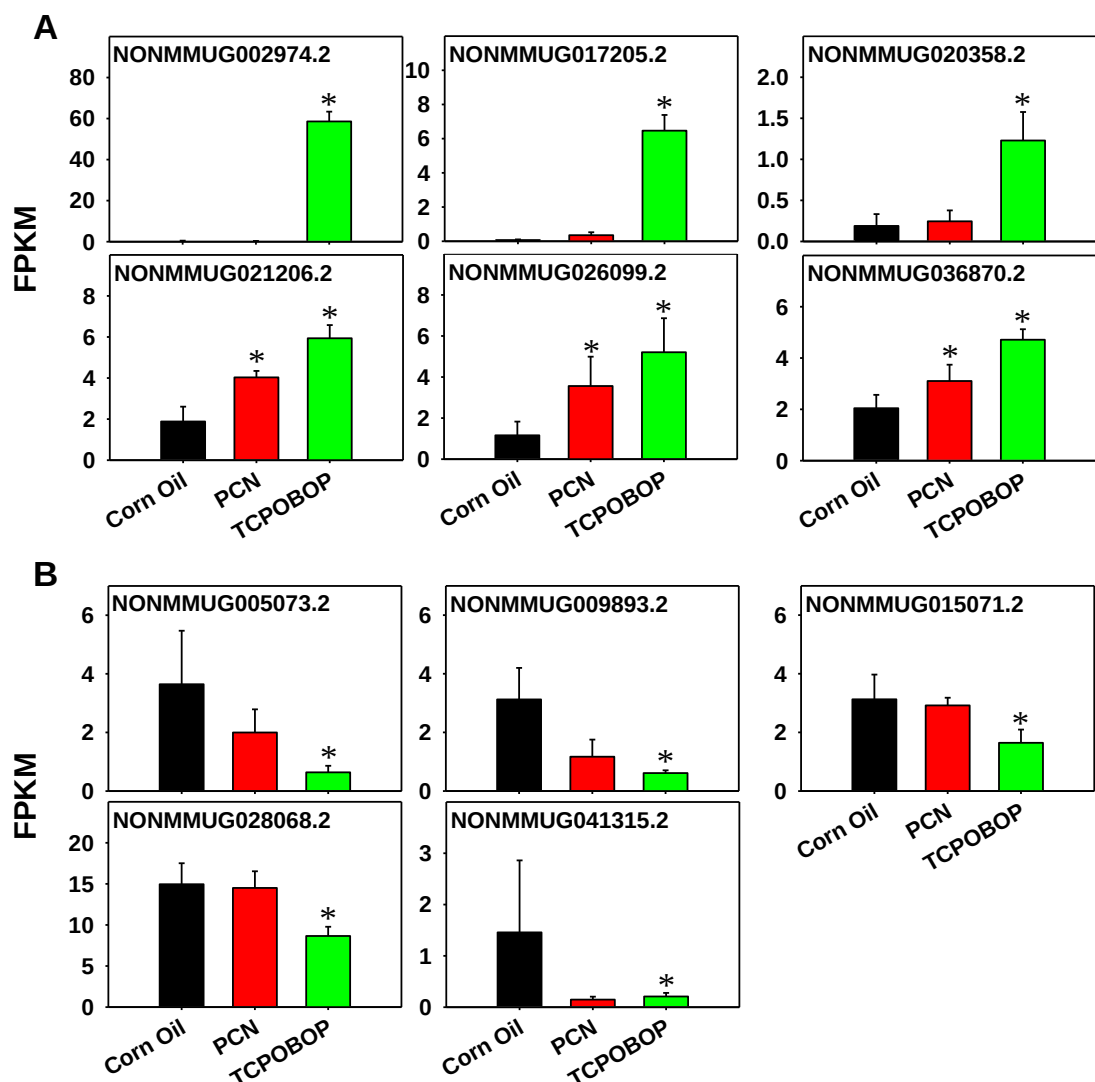


Figure 4.6. Examples of hepatic lncRNAs that were up-regulated (**A**) or down-regulated (**B**) by TCPOBOP (which are consistent with the literature report (Lodato et al., 2018)). The effect of PCN on these CAR-targeted lncRNAs is also shown. Asterisks represent statistically significant differences as compared to the corn oil group ($p < 0.05$, Cuffdiff). FPKM: Fragments Per Kilobase of transcript per Million mapped reads, which is a unit to express RNA abundance from the RNA-Seq data.

4.4 Discussion

The key findings of the present study are 1) at the given doses for the nuclear receptor agonists, CAR activation affects the expression of more lncRNAs than PXR activation in liver, and both commonly and uniquely regulated lncRNAs are observed for the two receptors; 2) the majority of the differentially regulated lncRNAs are present in introns, intergenic, and 3'-UTRs relative to PCGs; 3) for both receptors, the top differentially regulated PCGs paired with lncRNAs are involved in lipid metabolism and molecular transport, highlighting the potential importance of lncRNAs in fine-tuning this signaling pathway in liver; 4) increased PXR-binding by PCN exposure may lead to either trans-activation or trans-suppression of direct lncRNA targets, and motif analysis suggests that this context-specific duality may be due to a molecular switch of co-localized transcription factors; 5) the epigenetic activation mark H3K4me2 may facilitate the PXR-recruitment to the genomic regions proximal to lncRNA gene loci for activation of gene transcription.

One technical limitation of this study was the inability to detect non-polyadenylated lncRNAs, which may also be important for liver functions, due to the use of poly-A tail selection in RNA-Seq library construction. This likely explains why a previous study identified more liver-enriched lncRNAs than the present study in mice (Figure 4.4.1) (Yu et al., 2017). It has been shown that the non-polyadenylated lncRNAs are generally expressed at lower levels than the non-polyadenylated mRNAs and are prevalent in the nucleus, suggesting that they may be involved in the transcriptional regulation of target genes (Cheng et al., 2005a; Furuno et al., 2006). At this time, the ratio between polyadenylated lncRNAs and non-polyadenylated lncRNAs in liver is not known and most of the well characterized lncRNAs are produced using the same machinery as the PCGs (i.e. transcribed by RNA Pol-II, polyadenylated, and spliced) (Kung et al., 2013). Future studies using whole transcriptome analysis could use ribosomal depletion as an alternative library construction option, although the trade-off appears to be lower signal per transcript at the same read depth.

An experimental limitation of the present study is the lack of validations of the findings using PXR and CAR knockout mice. Using these knockout mice would demonstrate the extent which the regulation of lncRNAs are dependent on PXR and CAR under basal conditions. Previously, one study examined the species differences in gene regulation using human CAR transgenic mice as well as CAR-null mice (Cheng et al., 2017). This study showed that expression of Cyp3a11 and Cyp2b10 was up-regulated by TCPOBOP only in livers of WT mice but not in CAR-null mice. In a separate study, α -tocopherol was shown to be a PXR activator using wild-type and PXR-null mice. PXR-null compared to wild-type mice did not differ greatly in the regulated gene expression of the major P450s (Johnson et al., 2013). The purpose of the current study is to investigate those lncRNAs that are regulated by the pharmacological activation of PXR or CAR (similar to a toxicological response) as a first step. In future directions it will be important to determine the necessity of the nuclear receptors in modulating the constitutive lncRNA gene expression using knockout mice.

Another interesting finding is that the majority of the differentially regulated lncRNAs are produced within the intronic region of the PCGs (43%), followed by the intergenic regions (26.9%) and 3'-UTRs (14.5%) (Figure 4.3B). Because only the mature mRNAs that are poly-A tailed are enriched, the intronic transcripts should be separate lncRNA fragments instead of nascent mRNA transcripts. This suggests that the mammalian transcription machinery is highly efficient in that both lncRNAs and PCGs may share the same transcription machinery due to the relative genomic distributions. Intronic lncRNAs are known to initiate their transcription inside introns of PCGs in either direction and terminate without overlapping exons (Rinn and Chang, 2012). Little is known regarding the specific functions of intronic lncRNAs, although their potential involvement in cancer has been suggested (Tahira et al., 2011). Intergenic lncRNAs are encoded completely within intergenic regions between PCG loci and have been suggested to be more stable than intronic lncRNAs (Rinn and Chang, 2012). The differential regulation of intergenic lncRNAs by PXR and CAR ligands indicates the importance of PXR and CAR genomic binding to the intergenic regions.

Indeed, we have previously demonstrated that approximately 30% of PXR-genomic DNA binding are present in the intergenic regions (Cui et al., 2010b). CAR ChIP-Seq experiments are challenging due to lack of a good antibody targeting endogenous CAR protein, although one could overcome this technical challenge using an adenovirus-based system to direct the expression of YFP-CAR fusion constructs in transgenic mice (Niu et al., 2018). However, it seems reasonable to speculate that a substantial number of differentially regulated intergenic lncRNAs by TCPOBOP may be a result of direct CAR-binding to the intergenic regions. Last, lncRNAs produced from the 3'-UTR regions relative to PCGs may be important in protecting the mRNAs from miRNA-mediated degradation and/or inhibition of protein synthesis (Karapetyan et al., 2013; Dempsey and Cui, 2017). Although lncRNAs produced in distal regions may migrate to the 3'-UTRs of PCGs and perform similar functions, the local production of lncRNAs may have a special advantage in this process.

Regarding the potency of the two nuclear receptors, at the given dose, CAR activation by TCPOBOP differentially regulated more lncRNAs in liver than did PXR activation by PCN (Figure 4.1). Similarly, as we observed before, CAR activation also differentially regulated more PCGs than PXR activation (Cui and Klaassen, 2016). Therefore, it appears that TCPOBOP-mediated CAR activation is a highly potent stressor leading to an overall greater change in the mouse transcriptome. Among all of the differentially regulated PCGs that paired with lncRNAs, two pathways appeared to be affected the most by PXR and CAR activation, namely lipid metabolism (Figures 4.3C and 4.4C) and cell proliferation/cancer (Supplemental Figures 4.S1 and 4.S2). Both nuclear receptors and certain lncRNAs have been implicated in these two pathways independently (Mo et al., 2016; Schmitt and Chang, 2016; Kong and Guo, 2018), whereas the present study is among the first to show that the biological outcomes (changes in lipid metabolism and cell proliferation status) mediated by PXR/CAR may be co-regulated by lncRNAs. The PXR-mediated change in the lipid metabolism pathway is further supported by the motif analysis in that the PXR-targeted ChIP DNA from PCN-treated mouse livers had enrichment in the LRH-1 DNA

binding motifs (Figure 4.4E). LRH-1 is an important orphan nuclear receptor that regulates cholesterol, BA, and steroid hormone synthesis (Lee et al., 2008). The PXR activation by PCN has also been shown to be important for regulating BA synthesis (Staudinger et al., 2001). Under basal conditions, there was no enrichment in LRH-1 DNA binding motifs; instead, the DNA-binding motifs for hnRNP2B1, which is involved in alternative splicing of pre-mRNAs, as well as RAR α , which is a receptor for retinoic acid, were enriched (Figure 4.5E).

Traditionally, PXR is considered a transcriptional activator for various drug metabolizing enzymes and efflux transporters. However, recent studies using ChIP-Seq and microarrays have suggested that increased PXR binding may result in either trans-activation or trans-suppression of *bona fide* PXR-targeted protein coding genes (Cui et al., 2010). The present study adds to the existing literature showing that the PXR-targeted lncRNA genes can also be further divided into inducible vs. suppressive gene batteries upon increased PXR binding. We propose two potential mechanisms for this phenomenon: 1) the co-regulators that interact with PXR may ultimately determine the fate of PXR-target gene transcription. There is evidence in the literature showing that human PXR may interact with either a co-activator (e.g. SRC-1) or a co-repressor (e.g. SMRT or NCoR) (Navaratnarajah et al., 2012). Human PXR activity is repressed by the co-repressor silencing mediator of retinoid and thyroid hormone receptors (SMRT) (Johnson et al., 2006). 2) Another possibility is that PXR may compete with other trans-activators that are more important in the transcription of the target genes. Previous studies showed that PXR activation inhibits cAMP responsive element binding protein (CREB) and subsequently down-regulates the expression of rate-limiting enzymes for glucose homeostasis, such as glucose-6-phosphatase catalytic subunit (G6Pase) and phosphoenolpyruvate carboxykinase 1 (PEPCK1) (Kodama et al., 2007; Oh et al., 2013). In the present study, *in silico* analysis suggests that pharmacological activation of PXR is associated with increased nuclear occupancy of LRH-1 but decreased nuclear occupancy of RAR α (Figure 4.4E). This molecular switch may also contribute to the transcriptional silencing of a subset of PXR-targeted lncRNA genes. Additional studies using GST

pull down assays as well as overlay between PXR cistrome and ChIP-Seq data of other transcription factors will verify our hypotheses.

Regarding the human relevance, it is known that both PXR and CAR both have species differences between mice and humans, especially their contribution in regulating cell proliferation (Kong and Guo, 2018; Niu et al., 2018). However, the regulation of lncRNAs may be more conserved between the two species, because it has been shown that lncRNA promoters are more conserved than lncRNA exons and almost as conserved as those of PCGs (Carninci et al., 2005; Guttman et al., 2009). In addition, there are greater species conservations and higher frequency of proximal binding by hepatic transcription factors in the liver-enriched lncRNA gene promoters than PCG promoters (Yu et al., 2017). Therefore, it is reasonable to speculate that similar regulatory patterns of the lncRNAs may also be present in human livers following PXR and CAR activation.

The present study unveiled PCG-lncRNA pairs based on the positive associations of the neighboring PCG and lncRNAs following chemical exposure. Further mechanistic investigations are needed using lncRNA knockdown approach to validate the dependency of lncRNAs in the transcriptional/translational output of the paired PCGs. In humans, a recent study demonstrated that knocking down the neighboring lncRNAs produced from the antisense strand of the transcription factors HNF1 α and HNF4 α affected the expression of P450s in HepaRG cells (Chen et al., 2018). These observations have demonstrated the critical role of lncRNAs may play in modulating the PCG expression.

The rationale for the selection of H3K4me2, H3K27me3, and 5MeC over other epigenetic marks was that these marks were shown to associate with the regulation of important drug-processing genes in liver, as well as the focus of the present study on xenobiotic-sensing nuclear receptors (PXR and CAR). For example, the age-specific enrichment in H3K4me2 around the *Cyp3a* gene loci positively associates with the age-specific expression of the *Cyp3a* gene isoforms in mouse liver (Li et al., 2009). In addition, adult-specific enrichment of H3K4me2

positively associated with the adult-specific mRNA expression of other drug-processing genes including *glutathione S-transferase zeta 1* (*Gstz1*) (Cui et al., 2010a), *UDP glucuronosyltransferase 2 and 3* (*Ugt2* and *Ugt3*) (Choudhuri et al., 2010), as well as *AhR* gene locus (Cui et al., 2009). Conversely, the presence of H3K27me3 was associated with the down-regulation of CYP1A2 mRNA in human embryonic stem cell-derived hepatocytes (hESC-Hep) and primary human hepatocytes (Park et al., 2015). Regarding 5MeC, an investigation of DNA methylation in human liver samples demonstrated variable CpG hyper-methylation of the *CYP3A4* promoter region in adults, as well as in other CCAAT-enhancer-binding proteins (C/EBP) and HNF4 α binding sites (Kacevska et al., 2012), indicating DNA methylation contributes to the regulation of CYP3A4 expression and the subsequent modifications in xenobiotic metabolism. It has also been suggested that methylation of gene bodies can serve as a novel therapeutic target for cancer treatment (Yang et al., 2014). Because many drug-processing genes are known PXR and CAR targets, and the present study identified that many lncRNAs are also regulated by these drug receptors, the primary goal of the present study was to determine the interactions between these liver genes and the enrichment of these three epigenetic marks related to xenobiotic biotransformation. Many other epigenetic marks have been identified in the literature (Tan et al., 2011; Rivera and Ren, 2013), and it is important to investigate the involvement of these other marks in PXR- and CAR-mediated regulation of PCGs and lncRNAs in future studies.

Previously, it was demonstrated that the majority of lncRNAs share similar epigenetic marks at the promoter regions, such as H3K4me3 and RNA Pol-II binding sites as PCGs. However, a certain fraction of lncRNAs display a high prevalence of H3K4me1, which marks the enhancer region (Kashi et al., 2016). The present study adds to the evidence showing that a subset of mouse chromosomes (5, 12, and 15), H3K4me2, which marks the enhancers and actively transcribed gene bodies, is co-localized with PXR-DNA binding near the PCG-lncRNA gene loci, leading to active gene transcription. H3K4me2 may provide a permissive chromatin

environment for PXR-binding and the subsequent gene transcription and may serve as an important mechanism for the co-expression of the PCG-lncRNA pairs.

Taken together, the present study is among the first to systemically compare the PXR- and CAR-targeted lncRNA profiles in liver, and has provided novel insights into the molecular mechanisms underlying the lncRNA gene transcriptional regulation and potential biological outcomes in liver, and lay the foundation for further decoding the mechanism of the regulation of the gene transcription and the lncRNA-PCG networks *in vivo*.

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4.8 Supplemental Figures

Supplemental Figure 1

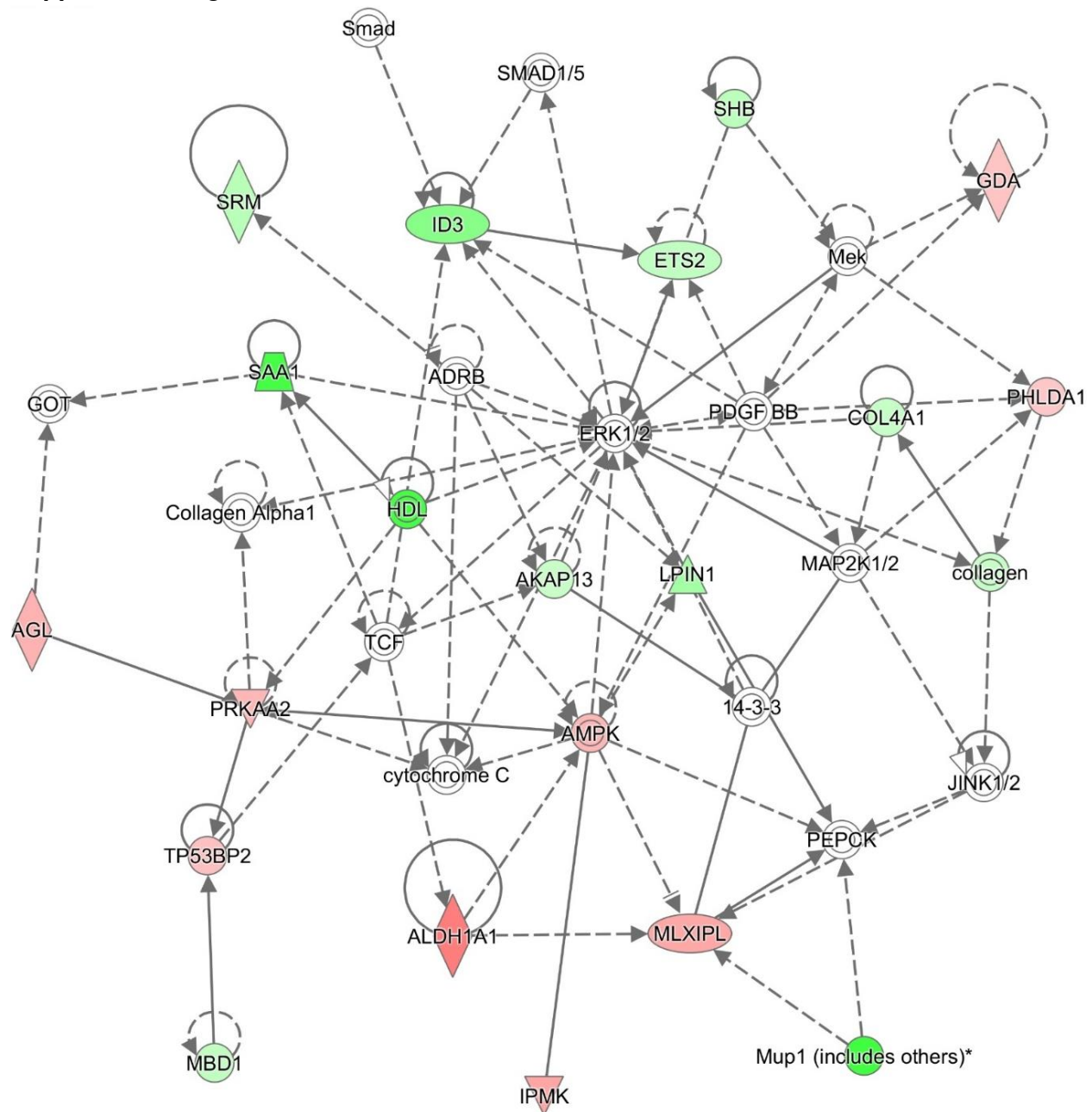


Figure 4.S1. Additional network associated with PCGs that paired with lncRNAs following PCN exposure: lipid metabolism, molecular transport, and small molecular biochemistry. Data were analyzed using Ingenuity Pathway Analysis (IPA). Red represents up-regulated genes; green represents down-regulated genes.

Supplemental Figure 2

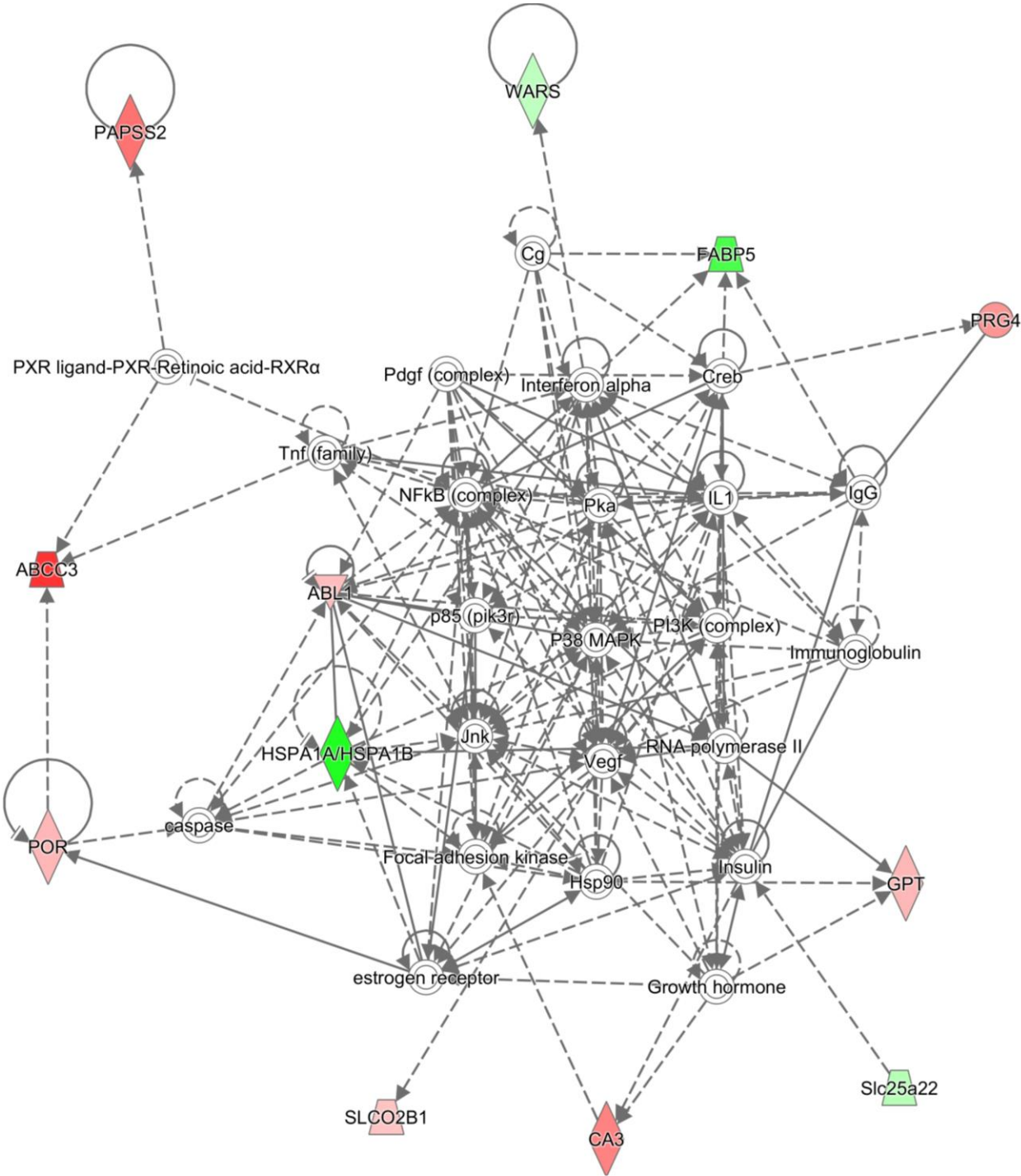


Figure 4.S2. Additional network associated with PCGs that paired with lncRNAs following PCN exposure: cellular growth and proliferation. Data were analyzed using Ingenuity Pathway Analysis (IPA). Red represents up-regulated genes; green represents down-regulated genes.

Supplemental 3

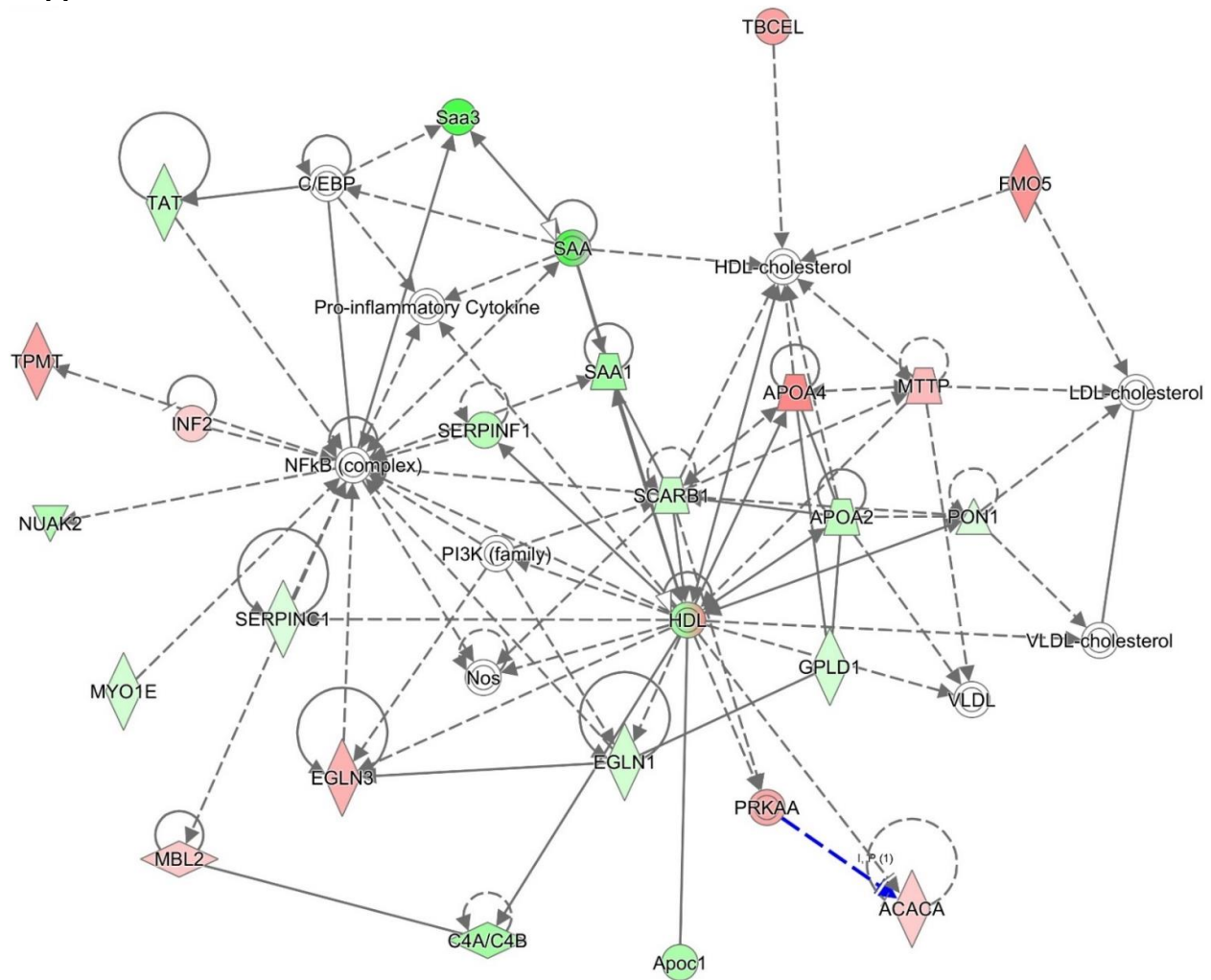


Figure 4.S3. Additional network associated with PCGs that paired with lncRNAs following TCPOBOP exposure: lipid metabolism and molecular transport. Data were analyzed using Ingenuity Pathway Analysis (IPA). Red represents up-regulated genes; green represents down-regulated genes.

Supplemental Figure 4

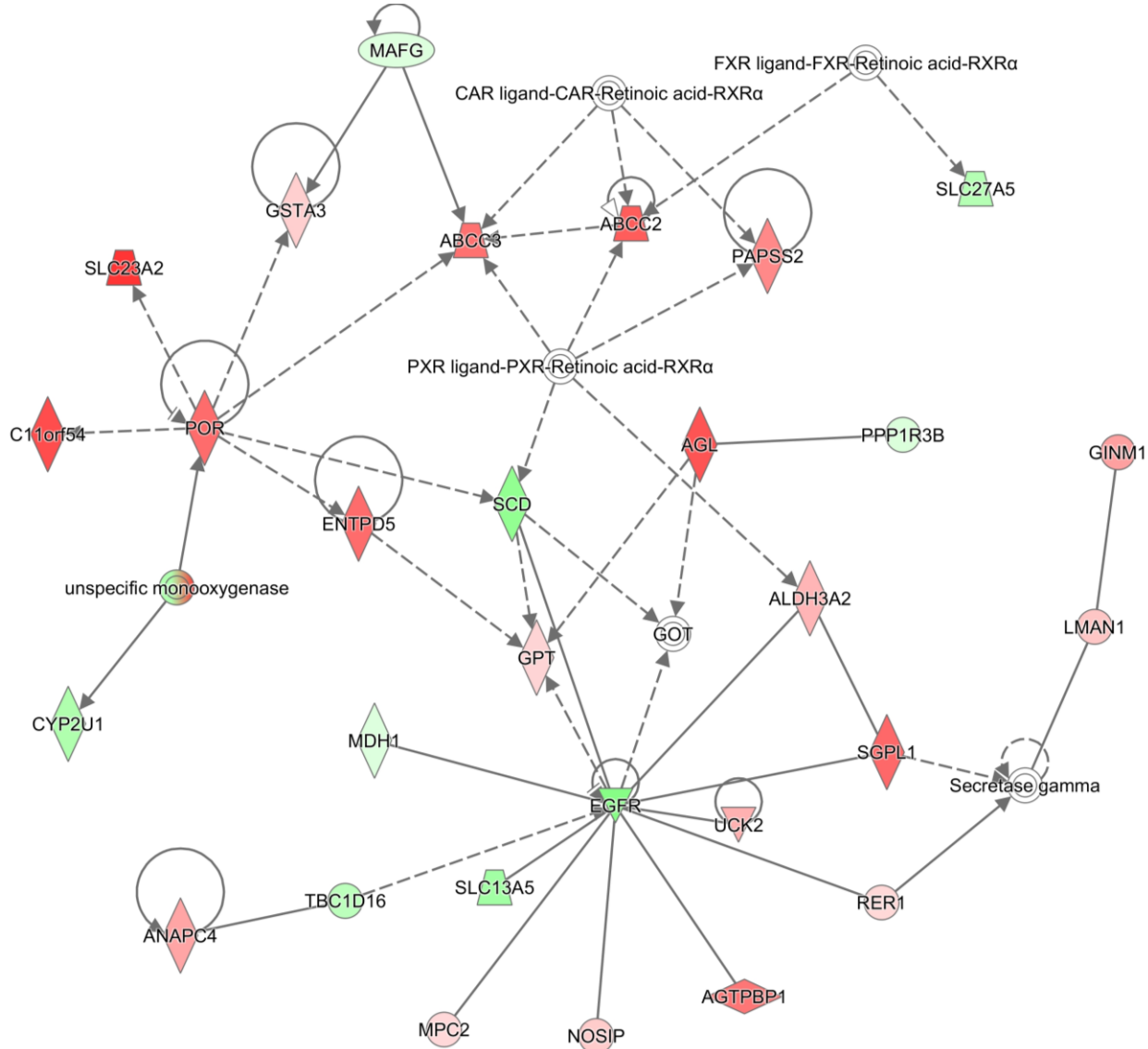


Figure 4.S4. Additional network associated with PCGs that paired with lncRNAs following TCPOBOP exposure: cancer injury. Data were analyzed using Ingenuity Pathway Analysis (IPA). Red represents up-regulated genes; green represents down-regulated genes.

Supplemental Figure 5

Corn Oil

Rank	Motif	Name	P-value	log P-value	q-value (Benjamini)	# Target Sequences with Motif	% of Targets Sequences with Motif
1		COUP-TFII(NR) K562-NR2F1-ChIP-Seq(Encode) Homer	1e-10	-2.321e+01	0.0000	33.0	47.83%
2		RARa(NR) K562-RARa-ChIP-Seq(Encode) Homer	1e-9	-2.289e+01	0.0000	46.0	66.67%
3		PPARa(NR) DR1/Liver-Ppara-ChIP-Seq(GSE47954) Homer	1e-8	-1.922e+01	0.0000	26.0	37.68%
4		COUP-TFII(NR) Artia-Nr2f1-ChIP-Seq(GSE46497) Homer	1e-8	-1.908e+01	0.0000	33.0	47.83%
5		CEBP(bZIP) ThioMac-CEBPb-ChIP-Seq(GSE21512) Homer	1e-7	-1.620e+01	0.0000	16.0	23.19%
6		THRB(NR) Liver-NR1A2-ChIP-Seq(GSE52613) Homer	1e-6	-1.572e+01	0.0000	50.0	72.46%
7		EAR2(NR) K562-NR2F6-ChIP-Seq(Encode) Homer	1e-6	-1.537e+01	0.0000	27.0	39.13%
8		PPARE(NR) DR1/3T3L1-Pparg-ChIP-Seq(GSE13511) Homer	1e-6	-1.494e+01	0.0000	22.0	31.88%
9		RXR(NR) DR1/3T3L1-RXR-ChIP-Seq(GSE13511) Homer	1e-6	-1.467e+01	0.0000	24.0	34.78%
10		Erra(NR) HepG2-Erra-ChIP-Seq(GSE31477) Homer	1e-6	-1.398e+01	0.0001	35.0	50.72%
11		THRB(NR) HepG2-THRB-Flag-ChIP-Seq(Encode) Homer	1e-5	-1.371e+01	0.0001	16.0	23.19%
12		HNFA(NR) DR1/HepG2-HNFA-ChIP-Seq(GSE25021) Homer	1e-5	-1.365e+01	0.0001	15.0	21.74%
13		VDR(NR) DR3/GM10855-VDR-vitD-ChIP-Seq(GSE22484) Homer	1e-4	-1.035e+01	0.0024	8.0	11.59%
14		HLF(bZIP) HSC-HLF-Flag-ChIP-Seq(GSE69817) Homer	1e-4	-9.959e+00	0.0033	13.0	18.84%
15		FOXA1(Forkhead) LNCAP-FOXA1-ChIP-Seq(GSE27824) Homer	1e-3	-9.154e+00	0.0069	17.0	24.64%
16		FoxEbox(Forkhead)HLH/Panc1-Foxa1-ChIP-Seq(GSE47459) Homer	1e-3	-9.040e+00	0.0072	17.0	24.64%
17		NPAS2(bHLH) Liver-NPAS2-ChIP-Seq(GSE39860) Homer	1e-3	-8.897e+00	0.0079	18.0	26.09%

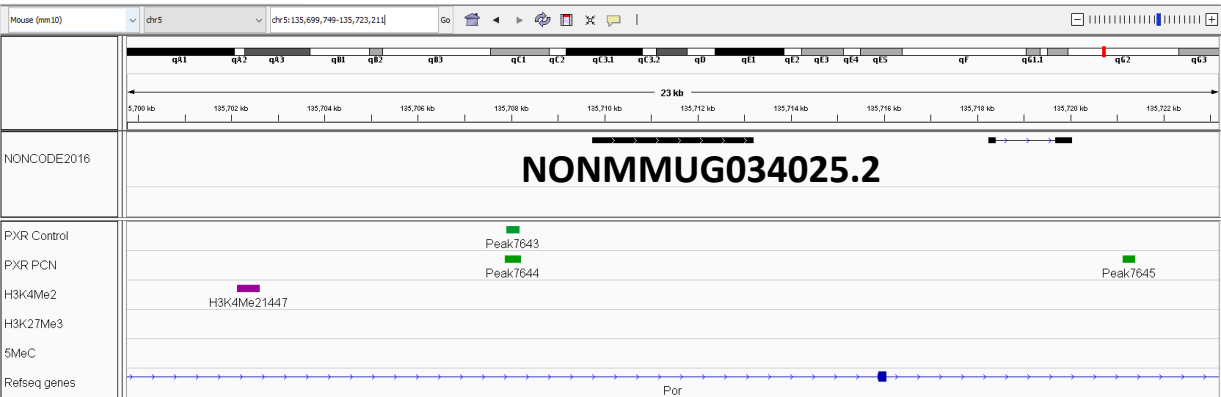
PCN

Rank	Motif	Name	P-value	log P-value	q-value (Benjamini)	# Target Sequences with Motif	% of Targets Sequences with Motif
1		RARa(NR) K562-RARa-ChIP-Seq(Encode) Homer	1e-11	-2.679e+01	0.0000	39.0	65.58%
2		CEBP(bZIP) ThioMac-CEBPb-ChIP-Seq(GSE21512) Homer	1e-9	-2.292e+01	0.0000	23.0	25.58%
3		CEBP AP1(bZIP) ThioMac-CEBPb-ChIP-Seq(GSE21512) Homer	1e-9	-2.085e+01	0.0000	24.0	26.67%
4		Erra(NR) HepG2-Erra-ChIP-Seq(GSE31477) Homer	1e-7	-1.653e+01	0.0000	44.0	48.89%
5		THRB(NR) Liver-NR1A2-ChIP-Seq(GSE52613) Homer	1e-6	-1.580e+01	0.0000	62.0	68.89%
6		PPARa(NR) DR1/Liver-Ppara-ChIP-Seq(GSE47954) Homer	1e-6	-1.479e+01	0.0001	27.0	30.00%
7		Atf4(bZIP) MEF-Atf4-ChIP-Seq(GSE35681) Homer	1e-5	-1.373e+01	0.0002	11.0	12.22%
8		COUP-TFII(NR) K562-NR2F1-ChIP-Seq(Encode) Homer	1e-5	-1.360e+01	0.0002	32.0	35.58%
9		HNFA(NR) DR1/HepG2-HNFA-ChIP-Seq(GSE25021) Homer	1e-5	-1.360e+01	0.0002	17.0	18.89%
10		PPARE(NR) DR1/3T3L1-Pparg-ChIP-Seq(GSE13511) Homer	1e-5	-1.287e+01	0.0003	24.0	26.67%
11		HLF(bZIP) HSC-HLF-Flag-ChIP-Seq(GSE69817) Homer	1e-5	-1.287e+01	0.0003	18.0	20.00%
12		Chop(bZIP) MEF-Chop-ChIP-Seq(GSE35681) Homer	1e-5	-1.214e+01	0.0004	9.0	10.00%
13		EAR2(NR) K562-NR2F6-ChIP-Seq(Encode) Homer	1e-5	-1.194e+01	0.0005	29.0	32.22%
14		FOXM1(Forkhead) MCF7-FOXM1-ChIP-Seq(GSE72977) Homer	1e-4	-1.130e+01	0.0009	21.0	23.33%
15		THRB(NR) HepG2-THRB-Flag-ChIP-Seq(Encode) Homer	1e-4	-1.115e+01	0.0009	16.0	17.78%
16		RXR(NR) DR1/3T3L1-RXR-ChIP-Seq(GSE13511) Homer	1e-4	-1.117e+01	0.0009	25.0	27.78%
17		FOXA1(Forkhead) MCF7-FOXA1-ChIP-Seq(GSE26831) Homer	1e-4	-1.030e+01	0.0019	19.0	21.11%
18		VDR(NR) DR3/GM10855-VDR-vitD-ChIP-Seq(GSE22484) Homer	1e-4	-9.711e+00	0.0038	9.0	10.00%
19		COUP-TFII(NR) Artia-Nr2f1-ChIP-Seq(GSE46497) Homer	1e-4	-9.334e+00	0.0045	31.0	34.44%
20		GRE(NR) DR3/RAW264.7-GRE-ChIP-Seq(Unpublished) Homer	1e-4	-9.239e+00	0.0047	9.0	10.00%
21		bZIP18(bZIP) col-4ZIP18-DAP-Seq(GSE60143) Homer	1e-3	-9.134e+00	0.0050	8.0	8.89%
22		FOXA1(Forkhead) LNCAP-FOXA1-ChIP-Seq(GSE27824) Homer	1e-3	-9.127e+00	0.0050	21.0	23.33%
23		Nr2a2(NR) Pancras-LRH1-ChIP-Seq(GSE34295) Homer	1e-3	-8.987e+00	0.0053	18.0	20.00%
24		USF1(bHLH) GM12878-UAF1-ChIP-Seq(GSE32465) Homer	1e-3	-8.812e+00	0.0061	12.0	13.33%
25		ABF1(bZIP) Arabidopsis-ABF1-ChIP-Seq(GSE50564) Homer	1e-3	-8.405e+00	0.0087	16.0	17.78%
26		THRB(NR) C17-2-THRB-ChIP-Seq(GSE3347) Homer	1e-3	-8.405e+00	0.0087	12.0	13.33%
27		NFIL3(bZIP) HepG2-NFIL3-ChIP-Seq(Encode) Homer	1e-3	-8.362e+00	0.0087	12.0	13.33%
28		ABF1(bZIP) col-4ZIP18-DAP-Seq(GSE60143) Homer	1e-3	-8.359e+00	0.0087	7.0	7.78%
29		AREB3(bZIP) col-AREB3-DAP-Seq(GSE60143) Homer	1e-3	-8.174e+00	0.0093	8.0	8.89%

Figure 4.S5. Homer known motif enrichment analysis of PXR-DNA binding intervals in corn oil and PCN exposed groups using findMotifsGenome.pl. Data were re-analyzed from the PXR ChIP-Seq dataset as described in Materials and Methods.

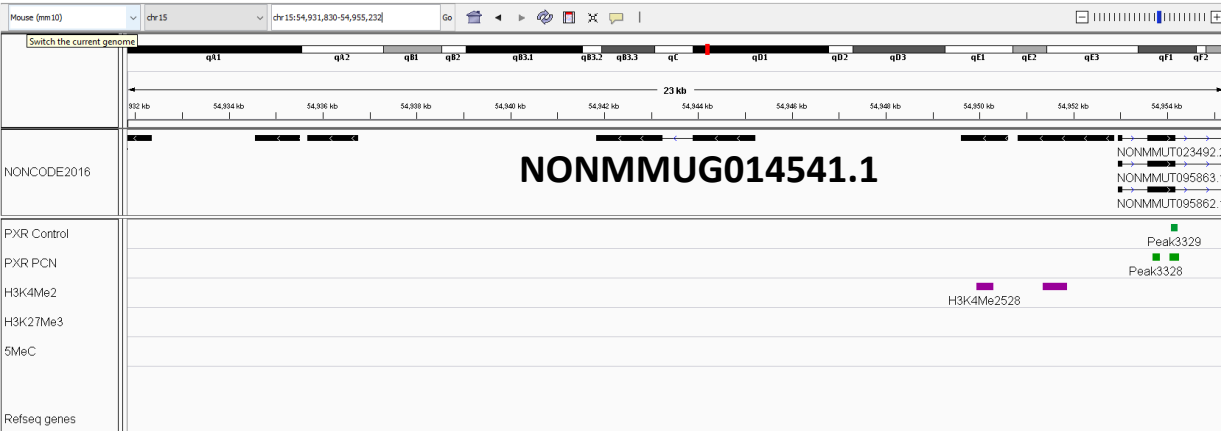
Supplemental Figure 6

A



Green: PXR-DNA binding peaks;
Purple: H3K4me2 peaks

B



Green: PXR-DNA binding peaks;
Purple: H3K4me2 peaks

Figure 4.S6. Examples of co-localization between PXR-binding and the epigenetic mark H3K4me2 around lncRNA gene loci. Integrated Genome Viewer (IGV) is a high performance visualization tool for displaying and exploring large datasets including RNA-Seq data, and can be accessed at <http://software.broadinstitute.org/software/igv/>.

4.9 Supplemental Tables

Supplemental Table 1

Table 4.S1. Liver-expressed lncRNAs that were differentially regulated by PCN and their genomic distance to PCGs.

lncRNA gene ID	lncRNA gene locus	lncRNA CO_1 FPKM	lncRNA CO_2 FPKM	lncRNA CO_3 FPKM	lncRNA POL_1 FPKM	lncRNA POL_2 FPKM	lncRNA POL_3 FPKM	CovPOL_3_wt		lncRNA location relative to PCGs	Paired PCGs	Distance of lncRNA genes to TSS of PCGs	PCG CO_1 FPKM	PCG CO_2 FPKM	PCG CO_3 FPKM	PCG POL_1 FPKM	PCG POL_2 FPKM	PCG POL_3 FPKM	CovPOL_3_wt	
NONMMUG00732	chr11:94511745-94511896	2.1946	5.66007	0.78276	4.19238	4.26151	3.76641	0.00005	Intron	Abcc3		36256	17.677	28.282	25.2963	80.2146	82.4349	72.337	0.00005	
NONMMUG00235	chr7:33499010-33499024	0.2474	0.66714	0.60841	2.95808	0.98839	1.50422	0.01095	Intron	Abi1		44706	1.49249	1.24059	1.45447	2.46179	1.94625	1.6105	0.0176	
NONMMUG00676	chr11:76416743-76417888	2.1364	1.16438	1.44947	0.07060	1.13357	0.61811	0.01415	3'UTR	Abi1		92114								
NONMMUG00342	chr11:11935940-11935929	6.6162	4.92301	4.46913	6.19944	5.44062	16.0158	0.00745	Intron	Adipor2		22611	42.6571	53.2479	46.7868	63.4445	52.0181	56.967	0.1663	
NONMMUG00277	chr8:116740500-116741571	2.3912	6.25286	2.46344	3.49466	4.17472	4.44429	0.01055	3'UTR	Agf1		60481	1.16884	1.76279	2.00008	2.50178	2.52004	2.4302	0.0024	
NONMMUG003840	chr7:75707884-75707918	0.9683	6.65816	2.1106	1.24831	1.23219	2.71277	0.0206	Intron	Akap13		6516	2.39133	1.79906	2.34956	1.26484	1.64207	1.7404	0.03345	
NONMMUG00296	chr19:20567044-20641341	1.0393	1.6988	0.91976	2.33009	3.82216	3.22088	0.00275	Intron	Albhl1		2232	197.448	243.027	255.234	422.236	522.39	525.58	0.00005	
NONMMUG005167	chr10:128252944-128254893	34.686	14.2385	61.7898	15.0662	23.6325	14.7326	0.00375	Upstream	Apon		-218	236.266	132.805	195.554	120.27	147.962	117.84	0.0537	
NONMMUG002704	chr3:87618348-87619420	0.4755	2.10023	0.65629	3.91179	3.70999	0.76321	0.03379	Intron	Arhgef11		785	1.8801	2.37945	2.53484	2.88828	2.27384	2.1371	0.4808	
NONMMUG001147	chr13:80883480-80883524	0.2091	1.8152	0.55317	2.95371	2.27868	3.53826	0.00025	Intron	Arnd3		1117	8.69192	14.9651	3.11141	19.0149	12.4928	17.886	0.0001	
NONMMUG002127	chr1:87776937-87777209	5.3332	1.5191	0	0	0	0	0.0005	Intron	Argl1		1508	3.48037	4.70257	4.35744	3.39209	3.5598	3.6653	0.41645	
NONMMUG001326	chr11:11935940-18123049	1.0903	1.94133	15.5068	3.491	2.48956	2.21282	0.0035	3'UTR	Blgap1		3628	0.90500	1.37351	13.9452	2.30508	2.31692	1.4906	0.00005	
NONMMUG002113	chr2:152823117-152827892	3.5489	5.83948	1.69847	0.84096	1.54737	2.47291	0.0207	Intron	Bcl2l1		6124	9.57479	9.31551	26.1386	14.2111	9.08495	30.077	0.61085	
NONMMUG002053	chr1:44119967-44127660	0.5440	1.05144	0.82337	1.65435	1.8038	1.84466	0.0203	Intron	Bim		3814	2.51186	2.92538	2.00274	2.55573	3.01944	3.7490	0.13905	
NONMMUG002523	chr11:8005988-8007021	13.274	37.3823	71.3675	17.6557	14.3342	18.819	0.0085	Intron	Camk2b		58944								
NONMMUG002610	chr1:14861558-14862123	13.75	6.59408	0.83579	4.79254	23.0204	22.2725	0.00025	Exon/CD	Car3		832	173.488	994.958	435.343	841.522	975.076	1440.4	0.00115	
NONMMUG003576	chr6:83103042-83103980	2.4822	1.41469	2.32111	0.81387	2	1.24006	1.56385	0.0212	Upstream	Cdc34c		-590							
NONMMUG003546	chr1:14861558-41556533	1.8073	0.77817	1.47058	6.04788	3.67319	3.1634	0.02505	Intron	Cdc162		51438								
NONMMUG002971	chr4:83537825-83540840	0.9375	2.96759	1.57532	1.99705	4.83418	2.39869	0.0122	Intron	Cdc171		13697								
NONMMUG003046	chr11:19337724-119336414	2.0861	0.81278	3.78228	1.10067	1.13916	0.67826	0.0271	Intron	Cdc30		25625								
NONMMUG003094	chr4:134118794-134144827	0.1272	57.3642	152.506	26.3804	21.6361	47.7507	0.02945	Intron	Cag8b		24872	2.38932	3.82534	5.65837	4.07916	4.84617	3.7463	0.53365	
NONMMUG004144	chr10:104344-10495626	10.344	20.7611	12.3523	27.5827	24.0649	23.7011	0.01325	3'UTR	Cag2g		7629	15.076	27.7203	13.0237	38.5569	34.3147	38.461	0.00005	
NONMMUG001489	chr1:118975935-118997845	0.8282	1.90277	1.90728	0.83888	1.46824	3.95512	0.0985	Intron	Cbr2		7140	40.5805	80.8394	81.2031	27.5854	53.895	46.488	0.01865	
NONMMUG002302	chr5:10053255-10071501	3.1419	2.38209	1.12411	0.61233	1.61816	1.26332	0.0326	3'UTR	Cnot6l		91022	1.172	1.76889	1.8734	1.69144	1.65846	1.7577	0.5173	
NONMMUG004045	chr10:77085077-77086299	10.274	0.91677	3.41513	1.37338	4.69557	3.35512	0.0117	Intron	Ctcf1a1		80860	63.2202	81.4785	80.2522	94.5658	81.8539	75.115	0.3956	
NONMMUG002886	chr10:11218085-11218680	0.8641	2.18906	4.43626	1.0389	0.85707	0.88884	0.02255	Intron	Ctcf1a1		98226	2.39603	3.45459	7.5635	3.05175	3.44352	2.4752	0.03295	
NONMMUG002571	chr2:17682783-17713844	6.7418	6.26423	7.85795	1.64222	2.7789	1.33474	0.01010	Intron	Cxcr1		8962								
NONMMUG002795	chr11:11788446-112189178	2.1163	11.1812	1.48818	2.46161	3.74777	7.09238	0.0007	3'UTR	Cyp2u1		14412	2.81912	2.61965	1.24749	1.01706	1.42735	1.3757	0.0082	
NONMMUG002937	chr4:57905096-57911480	2.2844	1.34617	2.13378	4.07791	3.10374	2.36779	0.0091	3'UTR	DEADENYLATION		7299	2.35238	1.62842	2.44684	5.57019	3.68663	2.6855	0.00045	
NONMMUG001752	chr17:14195126-14198301	4.1421	2.82799	2.98543	6.44864	4.57299	3.36209	0.0329	Exon/CD	Dact1		7073	4.027	2.98134	3.33843	6.35403	4.59981	3.225	0.03685	
NONMMUG002750	chr3:105802865-10588440	1.8884	1.10553	1.51859	0.28546	0.91275	7	0.01315	Intron	Dctnb1b		3726								
NONMMUG002785	chr11:10676255-10678104	9.1820	11.6093	14.6842	3.08121	7.94568	9.06528	0.0035	3'UTR	Dda5		7833	10.7122	14.8745	27.4681	8.72496	12.7617	13.207	0.01545	
NONMMUG01454	chr11:105163111-105163111	0.9031	0.94805	0.90791	1.86783	0.65644	1.77385	0.04805	Intron	Deaf1		91335	18.286	17.9641	12.3108	14.217	14.6779	17.978	0.8818	
NONMMUG003880	chr1:8517455-85179128	29.814	8.78712	5.37637	10.763	4.25552	10.8276	0.044	Intron	Dgat2		4922	131.094	115.624	158.948	120.392	109.927	109.63	0.37585	
NONMMUG000840	chr12:374114-1781398	4.6545	1.96878	1.14303	0.65239	1.25374	2.69439	0.0296	Intron	Dmb		3331	5.3405	5.86242	6.97374	5.53874	5.965	6.1112	0.8079	
NONMMUG002445	chr3:122279346-122280339	0	0.11965	0.03952	28	0	3.21889	2.4701	0.00195	3'UTR	Dmb2		18373							
NONMMUG002766	chr11:10174562-10174562	5.4915	1.79875	11.0763	1.55466	2.12231	2.00618	0.0303	3'UTR	Dusp8		8779	6.13823	7.04532	7.68131	5.62068	7.61205	6.2422	0.7785	
NONMMUG04410	chr5:114713516-114714315	12.482	8.3825	9.34948	2.95049	2.6934	3.54143	0.0008	Intron	Dync1l1		35325	3.48063	2.53778	3.54548	2.46146	3.32884	2.5492	0.10405	
NONMMUG005335	chr11:14752320-14752320	6.6030	3	18.3274	16.9222	4.77066	5.70939	4.5178	0.002	Intron	Egfr		1795	93.2258	102.678	137.34	52.4981	77.0274	69.383	0.01225
NONMMUG006741	chr2:120102219-12020143	0	0	0	0	6.52604	6.27386	0	0.61785	Exon/CD	Eno4		52281	1.30583	2.16344	1.86591	1.78513	2.15773	1.5959	0.68395
NONMMUG002897	chr4:41638275-41638291	20.128	8.79385	34.2953	3.26274	5.99412	7.62382	0.00005	3'UTR	Eno5		1728	13.5608	6.31299	18.0383	2.2948	4.1579	5.1727	0.00005	
NONMMUG001836	chr17:440085139-44008534	1.4875	1.96181	1.21454	1.87217	3.09404	2.3878	0.0126	Intron	Engp5		2847	6.67907	7.25383	5.48346	6.02849	9.55077	7.7309	8.2347	
NONMMUG006222	chr16:11125232-11125443	0	0	0	0	16.2084	0	0.008	Exon/CD	Erc4		13543								
NONMMUG001725	chr16:95717733-95721045	3.7805	2.40495	4.60994	1.92572	2.71983	3.30891	0.0361	Downstream	Eno2		3535	3.99373	4.80279	4.50413	2.78027	3.67764	3.5399	0.0167	
NONMMUG002605	chr3:1001204-1001203	5.0496	1.32559	1.13733	0.94881	0.98582	0.87762	0.00005	Exon/CD	Fabp5		1864	102.697	25.3802	31.9057	17.5794	17.1452	21.223	0.00005	
NONMMUG001755	chr17:15421797-15434948	1.0738	2.6487	0.75040	4.12021	2.86887	2.4783	0.00745	Intron	Fam120b		32126	1.59275	1.75946	2.4741	0.90824	2.13884	2.4026	0.908	
NONMMUG002905	chr4:43663948-43664009	54.384	49.0149	65.7617	41.881	40.61	30.8235	0.01055	Intron	Fam221b		4963								
NONMMUG04532	chr8:124740257-124741144	0.8699	3.39815	0.30965	3.33252	2.70664	3.60825	0.0369	3'UTR	Fam87a		11189	0.56666	0.76522	0.28230	0.93193	2.3309	1.4970	0.0048	
NONMMUG003795	chr7:45102343-45102376	12.354	17.0624	57.6507	13.1931	14.5276	14.994	0.00235	Intron	Fcgr1		508	44.1915	44.5173	55.6952	31.5547	41.3439	38.168	0.14715	
NONMMUG002736	chr9:97637164-97641000	1.0151	0.94031	0.93812	0.75231	1.21051	1.38554	0.0209	Downstream	Fmnl5		10199	89.6305	92.1122	78.5294	93.6325	82.3672	79.568	0.9641	
NONMMUG00096	chr1:71609772-71609772	0.9135	1.96081	3.23649	3.97031	3.81951	1.51838	0.0134	Exon/CD	Fnl1		46410	136.683	117.137	176.526	153.185	112.63	116.675	0.96475	
NONMMUG001280	chr14:7188025-71895526	1.3101	5.6629	7.80554	2.459	2.66533	3.72843	0.04005	Upstream	Gdfr1		-2352	23.8319	25.4127	21.2893	16.7468	22.7474	23.193	0.43335	
NONMMUG002097	chr12:1191341-1191341	4.0323	3.90287	5.65626	5.86116	8.45113	5.59725	0.04555	Intron	Gda		46126	1.97395	2.06026	3.90077	3.02301	4.01885	3.2003	0.0286	
NONMMUG001394	chr15:3535112-3535580	0.4722	2.26217	1.80547	4.18224</															

NONMMUG03102	chr6:136143824 1.1	5.7502	2.97	12.7793	3.02358	2.93555	2.67777	0.00005	Intron	l69	598	46.2346	22.1525	75.2861	28.9543	16.2489	26.357	0.00005	
NONMMUG03207	chr19:8896647-8897923 7.2	4.4851	4.04061	10.055	1.66821	4.21216	2.99065	0.00375	Exon/CD	en55	4298	3.8322	3.33636	7.61345	2.08992	3.59535	2.6956	0.0012	
NONMMUG03036	chr10:11821866 7.2	4.3249	4.40367	3.48623	6.15907	6.19299	6.82978	0.00314	Intron	lpmk	2375	3.91083	4.05237	3.86225	7.38611	6.03626	6.0112	0.00086	
NONMMUG03164	chr11:95870776 4.1	3.7112	2.76	3.81317	1.22765	1.12095	2.30864	0.01555	Intron	ngag2	50839	8.22646	15.9727	20.5739	16.1921	17.5173	15.9102	0.30835	
NONMMUG03406	chr18:20583661 6.1	14.807	17.3032	28.4888	11.5579	14.0462	10.0562	0.02435	Intron	knq1	10781	1060.12	1089.95	1181.65	1092.24	963.814	1205.3	0.95056	
NONMMUG03056	chr12:16536032 5.2	27.336	16.0991	55.6272	25.0204	4.89542	14.5268	0.0215	3'UTR	lpm1	53341	8.19611	6.6636	17.2593	9.85951	2.06211	5.0354	0.00125	
NONMMUG03103	chr12:111574476 6.2	0.6436	1.8823	1.11257	2.86453	1.55982	1.9823	0.03795	Intron	Mark3	28727	4.21638	4.07317	5.79329	2.48429	3.62343	4.2847	0.07555	
NONMMUG03032	chr18:74267807 6.2	2.1338	1.47612	13.4271	1.00948	3.46121	3.87469	0.0055	Intron	Mbd1	388	7.11088	5.9521	21.9866	3.35294	5.21657	10.256	0.02855	
NONMMUG03182	chr18:44426882 4.2	0.6440	1.11051	0.61214	4	1.98891	1.20027	1.24982	0.00555	3'UTR	Mcc	38408	-	-	-	-	-	-	
NONMMUG03074	chr9:1613025-1613282 4.1	10.110	6.90596	5.17953	1.44811	6.11432	3.79719	0.0458	Intron	Mosln3	1446	11.1109	8.54269	9.57619	7.14979	9.47819	8.1505	0.3426	
NONMMUG03148	chr15:100111186 7.2	18.283	24.7327	20.7205	23.8697	38.186	40.4431	0.0092	Intron	Ment7a3	1359	-	-	-	-	-	-	-	
NONMMUG03399	chr5:15113331 8.1	1.0974	0.4437	0.76745	1.85839	1.00544	1.16111	0.0291	Intron	Mwgl	7337	10.5913	12.3921	6.94744	21.1918	13.877	11.982	0.0032	
NONMMUG04264	chr9:42051403 5.2	4.1052	5.022	2.87423	7.08746	4.13228	7.63243	0.0117	3'UTR	Mpl2	10958	5.93744	7.81365	4.98723	10.7679	6.90563	11.965	0.0015	
NONMMUG03446	chr15:59052770 9.2	6.1115	4.15577	2.20944	2.39048	1.04475	3.89964	0.02065	Intron	Mmci1	27949	9.67407	12.5727	10.0423	8.36236	8	12.235	0.0922	
NONMMUG03206	chr10:138103915 4.2	1.7571	1.66952	1.30404	1.36781	2.35945	1.30366	0.04825	Intron	Mtbp	21347	60.5453	65.0624	58.1117	64.5718	86.9369	67.255	0.2799	
NONMMUG03246	chr4:62090088 1.1	425.96	611.953	256.595	113.363	277.807	369.698	0.0001	3'UTR	Mup20	3857	1702.6	2285.14	1367.07	264.334	991.227	768.31	0.00005	
NONMMUG03724	chr4:62083475 4.1	14582	3540.42	2185.94	884.768	1319.72	1022.35	0.00095	Intron	Mup3	3262	1314.73	2610.43	2663.25	616.017	1142.01	1262.9	0.00015	
NONMMUG04124	chr9:70241300 0.2	4.7994	2.99415	1.17732	1.30654	1.92333	1.82756	0.0233	Intron	Myp1e	47736	8.99006	10.535	9.57121	8.69113	8.40588	8.1781	0.4046	
NONMMUG03219	chr7:22581032 2.2	7.1115	6.15838	2.5641	9.99091	7.19452	6.3348	0.02005	Intron	Myo1a	9133	-	-	-	-	-	-	-	
NONMMUG03188	chr5:21661878 5.2	1.7140	2.83654	1.47946	3.22448	3.20029	4.0394	0.04565	Downstream	Nagep16	39152	-	-	-	-	-	-	-	
NONMMUG03805	chr7:4891468 5.4	0.2813	0.25953	0.67508	3.53994	2.54537	0.45379	0.00065	Intron	Nau2	6920	0.84554	1.3565	1.86577	1.75263	1.64252	1.8142	0.0503	
NONMMUG03252	chr2:15538956 1.2	15.658	20.0261	15.7042	9.76327	10.36	9.57471	0.02355	Downstream	Ncac6	51309	1.23875	1.27705	2.50249	1.95991	2.6794	1.6386	0.29035	
NONMMUG03012	chr18:18518861 8.2	3.7175	2.27154	0.88522	3	0.42708	1.57	0.0042	Intron	Nedaf1	14562	5	2.91921	2.63642	6.41952	2.82566	2.84608	3.3817	0.1639
NONMMUG03036	chr12:68361384 3.2	85.942	128.385	175.659	250.646	149.236	66.5482	0.0294	Upstream	Nemf	4136	1.89568	1.97975	2.20805	1.86466	1.44879	2.098	0.197	
NONMMUG03106	chr11:13993326 1.2	1.0788	6.82177	0.18673	2.15147	1.11018	0.76783	0.0091	Intron	Nep2	1986	6.15669	10.3866	8.1369	10.8074	9.88831	10.582	0.1226	
NONMMUG04138	chr8:12836715 7.2	1.8240	0.57547	1.04989	3.38073	2	2.68611	0.05861	Intron	Nep3	9879	8.96689	7.22934	6.08204	5.66033	7.26311	7.0873	0.52975	
NONMMUG04134	chr9:7494782 7.2	3.5751	5.68226	6.85302	3.76882	3.92303	4.86328	0.03025	Intron	Onccu1	3244	3.22742	2.06249	4.61195	3.94366	1.89409	1.0541	0.25415	
NONMMUG03120	chr19:13266284 9.2	16.239	51.6205	36.405	66.9572	69.8379	72.0529	0.0004	3'UTR	Pap20	46731	10.8287	36.6177	26.4775	63.0405	53.5132	45.599	0.00005	
NONMMUG03120	chr19:13266284 6.1	1.1317	0.83897	0.97274	7	2.48326	2.27383	1.1366	0.01565	Intron	Pap22	7771	10.8287	36.6177	26.4775	63.0405	53.5132	45.599	0.00005
NONMMUG04049	chr10:111506485 2.2	22.324	14.2268	13.9176	36.7799	12.6953	22.0548	0.04135	Exon/CD	Phd21	1276	26.1091	16.618	19.5524	44.2008	15.2527	23.201	0.04925	
NONMMUG03040	chr10:111506485 2.2	0.5328	3.47862	2.20572	8	0.81814	1.06124	0.04265	Intron	Pln	5651	-	-	-	-	-	-	-	
NONMMUG03014	chr4:108084381 1.2	0.4054	0.76388	1.34986	1.30467	0.77822	2.59857	0.0108	Intron	Pp4b	10703	-	-	-	-	-	-	-	
NONMMUG03402	chr19:1091949 5.2	2.8010	2.51652	1.90305	4.35306	3.82822	3.192	0.11	Intron	Ppr	23232	87.6467	45.3194	63.8356	104.518	130.627	68.426	0.00925	
NONMMUG03036	chr4:105213178 4.2	11.8338	25.8334	11.0702	27.7281	21.6494	24.2228	0.02722	3'UTR	Ppaz2b	74903	13.1541	21.4793	13.8055	27.4157	25.4432	26.179	0.00445	
NONMMUG03030	chr12:76513174 6.2	2.1873	1.98805	1.87715	0.86348	0.80138	1.2078	0.0086	Intron	Ppp2r5e	40499	4.76226	5.64397	6.72309	4.28138	4.75423	7.4295	0.95035	
NONMMUG03000	chr11:150453959 2.2	19.191	6.93856	21.1399	39.2077	22.9359	16.9935	0.0044	Intron	Pp4a	10420	21.9194	8.84356	14.6871	36.0659	23.7124	21.076	0.00395	
NONMMUG03065	chr4:105025065 9.2	2.5470	2.81343	2.1021	4.52604	3.82542	4.24868	0.01095	3'UTR	Ppkaz1	79714	0.59011	1.50222	2.05932	2.26657	2.38486	2.4061	0.0061	
NONMMUG03187	chr2:6308874-6310513 2.2	2.4719	2.6946	1.04142	0.74608	3	1.34577	0.0452	Intron	Proser2	20541	5.0251	4.6296	2.06889	2.48661	4.55213	3.9782	0.6497	
NONMMUG03068	chr6:128493347 4.2	2.1046	3.17083	9.12329	7.8971	8.47946	6.53808	0.0232	Intron	Pp3	32435	441.947	492.725	734.839	499.211	569.262	464.18	0.8376	
NONMMUG03090	chr9:7962518 4.2	1.4093	3.10961	2.2406	1.20613	0.69514	6	0.04784	3'UTR	Ragapf2	81604	-	-	-	-	-	-	-	
NONMMUG03027	chr8:13931366 6.2	6.8035	6.10594	1.78858	0.32533	3.43462	1.87768	0.0294	3'UTR	Rbm1	21214	8	1.36638	1.53299	2.11819	1.95692	1.56511	1.7203	0.69645
NONMMUG03238	chr2:3051562 6.1	5.9399	6.27089	1.89581	1.08104	2.22097	0	0.047	3'UTR	Rbp4	10516	3.44588	3.12624	2.28098	1.79395	2.74455	1.9926	0.10145	
NONMMUG03262	chr4:13970079 5.2	3.0363	0.73165	1.18025	0.74756	0.56074	0.59817	0.0474	Intron	Rend1	213	4.57436	5.53837	3.71704	4.69428	5.64737	5.1116	0.48735	
NONMMUG03177	chr17:2517453 6.2	2.2084	1.38021	4.97656	1.40314	1.59796	1.86383	0.02705	Intron	Rp25	27905.7	8244	-	-	-	-	-	-	
NONMMUG04076	chr6:7089328 1.1	27.659	12.1868	15.8143	7.92358	28.7035	28.7572	0.00595	Exon/CD	Rpl18a	1460	329.665	264.963	297.093	276.106	361.357	375.55	0.40158	
NONMMUG03048	chr11:97781340 9.2	7.7527	15.1818	8.41711	19.7969	14.8368	10.9205	0.02995	Intron	Rpl23	639	56.2689	54.4832	42.7621	49.7969	67.8425	68.673	0.2505	
NONMMUG03182	chr7:46171672 0.1	329.26	7.06081	219.321	107.283	31.1303	20.6078	0.0011	Intron	Saa2	1818	676.004	164.387	644.679	277.81	729.383	552.48	0.00005	
NONMMUG03060	chr17:56958052 7.1	3.5866	4.76877	5.54057	0.95564	8	1.09402	1.49717	0.0198	Intron	Saf22	14236	1.95448	2.57689	3.3705	3.29111	2.66849	2.1628	0.62556
NONMMUG03014	chr4:108117009 3.2	0.4646	0.81957	6	1.08275	0.48019	8	0.00425	Intron	Scp2	789	180.385	295.238	288.369	117.268	235.435	209.08	0.121	
NONMMUG03015	chr4:108107190 5.1	6.2971	6.20598	5.52487	4.22468	5.01578	23.0276	0.00855	Intron	Scp2	10979	180.385	295.238	288.369	117.268	235.435	209.08	0.121	
NONMMUG03955	chr7:144526592 4.1	1.5840	0.58911	0.04513	0.96131	2.02961	3.18495	0.00665	Intron	Shank2	42700	-	-	-	-	-	-	-	
NONMMUG03012	chr4:45334442 5.2	15.883	6.99309	7.09627	4.49628	7.74943	7.97217	0.0232	Intron	Shb	7579	7.42293	6.75095	15.0378	6.17127	3.84667	6.4392	0.0097	
NONMMUG03012	chr4:45334442 6.2	4.2355	2.15817	3.62845	1.81548	2.54451	1.97893	0.0356	Intron	Shb	10893	7.42293	6.75095	15.0378	6.17127	3.84667	6.4392	0.0097	
NONMMUG03060	chr7:140871040 6.2	5.1593	1.6292	0.60960	0.51965	1.22098	2.44461	0.0331	Intron	Shc3	6426	23.1638	21.0821	15.9993	19.3614	15.3409	21.305	0.76795	
NONMMUG03963	chr7:141429751 9.2	10.612	9.90621	12.143	8.54037	6.76284	5.41722	0.04135	Intron	Sh2a22	302	26.6521	26.2429	36.388	22.6101	18.4998	13.984	0.00445	
NONMMUG03012	chr4:45334442 7.2	15.837	6.70395	13.976	4.27722	5.16147	6.08263	0.0002	Intron	Sh2a61	2063	4.34897	2.90089	8.02493	2.95449	3.83943			

NONMMUG04610	chr9:151088486-151088488	6.9397	7.39763	6.07626	1.02931	2.53315	4.76027	0.0212	-	3'UTR	Tur2	6878	1.32407	1.06497	1.26503	0.93881	0.77634	1.0197	0.2179
NONMMUG05057	chr18:47405037-47405038	3.5771	4	9.21124	6.07605	2.73608	2.40456	1.96261	0.01615	Exon/CD	TubB6	11297	3.77039	3.5382	8.17797	3.40873	2.73463	2.9976	0.0034
NONMMUG05084	chr11:116438621-116438623	8.8617	2	6.06622	6.90388	1.81638	4.16776	5.42764	0.03235	Downstream	Uba42	4338	-	-	-	-	-	-	-
NONMMUG05052	chr11:21504564-2336308	3.0900	1.27787	0.90822	0.46668	0.65540	0.94529	4	0.0225	Upstream	Up34	-1659	0.88673	1.15475	1.29151	1.04452	0.88621	1.2976	0.95155
NONMMUG03366	chr6:72375309-72375310	4.7789	0.62386	0.49496	0.03815	0.11603	1	1.28877	0.00595	Intron	Vamp6	4011	2.29055	2.18883	1.73825	1.03199	1.38582	1.6723	0.07256
NONMMUG05094	chr12:10896036-108861428	4.2362	9	5.7195	4.88862	0.95629	4	3.33952	5.36657	3'UTR	Wars	32466	4.15193	4.10005	8.5404	2.62409	4.24001	4.0292	6.0246
NONMMUG03424	chr5:143329246-14333250	0.3012	1.4911	0.71801	4.4876	1.91737	1.73554	0.03945	0.0087	Upstream	Zdhc4	-2096	12.6361	11.1194	8.20969	11.7455	12.6655	12.184	0.34165
NONMMUG04483	chr6:48202685-4820666	0.7378	0.84284	1.24049	2.60425	2.00724	1.59229	0.00085	-	Intron	Zdhc9	1310	36.412	36.1528	26.5072	29.9811	30.4816	25.738	6.4562
NONMMUG02225	chr2:24895465-24902967	2.9023	2.15814	2.48708	0.25052	1.6888	1.72233	0.0087	-	Downstream	Zmynd19	10006	0.94334	0.96904	2.34066	0.81876	1.63652	1.3824	0.8331
NONMMUG03199	chr5:25004884-25010291	3.4386	0.94534	0.61934	0.19515	0.48225	0.58206	4	0.00005	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG02081	chr12:104070500-104081483	4.1608	0.95338	1	3.30314	0.42136	0.05662	1	0.00005	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG01404	chr15:54941830-5494532	0.9157	8.20862	1.15517	5.25937	2.42687	4.5443	-	0.00025	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG04991	chr10:12238850-12242298	1.2198	4.39145	2.62386	5.43024	5.50139	7.71012	0.00045	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG02476	chr2:133024711-133027821	1.6790	2.39466	2.29834	3.91213	3.74984	3.36963	0.00225	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG00030	chr1:278484010-27849027	1.6973	2.24438	1.79558	4.08064	4.5869	0.0026	-	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG02059	chr2:167973516-167980013	4.9260	2.77969	10.548	2.6189	3.02044	3.34797	0.00475	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG02224	chr2:24349367-24350531	1.9856	1.48997	28.7038	4.61241	4.38728	2.28132	0.00505	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG02214	chr9:143131440-14313105	1.6430	5.16225	1.42047	4.64255	2.68026	3.98895	0.00585	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG07388	chr1:148415148-148417175	0.7111	1.30847	1.08471	1.71398	2.06251	2.15642	0.0075	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG01944	chr18:2098630-20987349	5.788	25.6696	13.7863	26.3811	29.0866	26.1367	0.0073	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG04280	chr5:13437121-13438703	0.7243	1.02095	0.71290	2.18305	1.1843	1.32665	0.0096	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG02120	chr19:32543317-32563256	2.3922	1.75074	0.69470	1	4.08815	3.46275	4.50514	0.00075	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG01796	chr1:71152751-71163252	1.7591	1.53187	1.54749	3.53495	2.76542	2.60403	0.01145	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG05434	chr12:72605914-72606891	27.899	1	21.7112	8.3636	37.3312	25.6302	38.0205	0.01205	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG00035	chr15:100976108-100977000	3.8805	1.83227	0.81811	0	0.97709	1.30088	0.0134	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG00983	chr12:104394714-104396140	2.3811	2.25029	29.2169	5.04021	7.72326	5.33875	0.01405	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG00701	chr11:18472375-183236005	9.9875	269257	2.13809	1.193	2.44251	3.27743	0.01625	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG03327	chr5:10844734-108518236	2.0191	4.18522	4.13489	4.91203	5.21125	3.08962	0.01665	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG01010	chr10:12812686-12812686	4.0778	6.1865	6.03227	4.97744	4.11267	4.02271	0.0173	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG04131	chr6:9436796-94400115	2.4005	5.2253	0.92086	0.32989	0.51232	0.74471	5	0.0109	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG01260	chr15:76794305-33796130	1.55	0.44852	7	0.33843	2.64487	2.54732	1.38881	0.02145	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG02922	chr4:49907412-49911933	4.8503	0.57404	3.69317	0.73602	1.4551	0.93918	8	0.0201	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG03490	chr14:14414376-144247805	0.5173	2.71841	2.71631	4.83712	2.17727	2.5548	0.01995	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG02034	chr1:106815101-106815484	3.2209	1.57051	3.68805	7.94087	5.37794	6.66395	0.0207	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG00385	chr11:149137615-149138152	13.816	34.169	31.3848	9.29072	19.7759	19.0308	0.02005	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG00327	chr10:27936241-27927484	1.0393	1.47544	1.01389	1.50213	2.87321	2.5435	0.0231	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG02091	chr1:67062395-67136774	1.0085	2.3243	1.25889	0.55959	0.75893	0.51367	1	0.0247	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG03687	chr6:14386356-143878021	1.7196	3.96255	1.93035	2.76706	4.33399	2.2163	0.0257	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG02093	chr10:5003619-5005415	5.0870	4.93214	4.26333	6.60621	6.78194	7.89139	0.0251	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG02133	chr19:40472774-404748140	2.9935	5	1.25397	0.97702	0.86277	0.8939	1.13302	0.02789	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG02009	chr5:14847195-14852247	0	2.31714	1.17002	0.91950	5	3.93605	5.83313	0.0278	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG01005	chr12:111943981-111944483	2.7637	1.41486	0.91362	2	3.07279	3.51234	2.85052	0.02740	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG02518	chr5:145891607-145894275	21.272	36.3749	7.90989	23.0461	34.785	35.1773	0.02785	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG02090	chr15:16384253-163845915	0.9501	1.99328	1.34098	2.29188	1.95998	3.00866	0.0294	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG04362	chr9:44664519-44667358	0.5971	1.86658	0.59523	0.26944	0.55976	1.33022	0.03195	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG07158	chr5:12963232-12963343	0	0	0	0	4.10538	2.15249	0.0301	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG01366	chr14:99870849-99873629	0.5600	1.34463	1.17376	1.58181	1.89678	1.45493	0.0333	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG00020	chr11:5554203-5557753	1.7409	2.1477	4.83795	0.70790	2	1.6871	1.70004	0.0312	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG04162	chr8:134147539-141451487	2.3235	2.70977	3.7446	4.45955	2.89438	6.67898	0.0311	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG02595	chr2:181551140-181552880	7.9409	10.4761	14.2716	4.59443	6.96402	9.63105	0.03315	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG00714	chr11:86811827-86816129	3.3492	4.96473	10.8053	3.89071	3.9571	4.25941	0.0374	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG07388	chr4:149434400-149452371	0.8913	4.82843	1.21327	3.63041	4.28243	3.93426	0.0384	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG03988	chr6:11834234-11835204	3.9718	6.10903	3.30975	7.37521	6.12881	2.95858	0.03795	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG02672	chr3:6246089-62462217	10.048	7.79723	25.4146	8.056	7.18343	12.6482	0.04175	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG00876	chr12:33129591-33330467	0.8845	0.62515	0.79534	1	1.30994	1.30028	2.1795	0.04225	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG00279	chr1:190370295-19024119	4.1499	4.4624	2.24538	0.84406	1.78697	2.70397	0.0429	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG00836	chr11:121306662-121306884	7.7862	6.61619	12.3846	5.52656	6.69524	6.92895	0.0442	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG01250	chr14:28649675-28706470	1.3730	0.71661	0.73711	3.2144	1.56268	1.61631	0.0477	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG09411	chr5:138367718-13838287	15.719	0.24056	19.6704	17.2867	13.0768	17.7961	0.04635	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG00889	chr12:41129419-41121301	0.4064	2.42401	1.46111	1.35509	1.60728	4.03961	0.04875	-	RNA	RNA	RNA	-	-	-	-	-	-	-
NONMMUG03782	chr7:39585317-39591680	0.9725	0.83368	0.93286	1.90527	1.35882	0.92302	1	0.0495	RNA	RNA	RNA	-	-	-	-	-	-	-

Supplemental Table 2

Table 4.S2. Liver-expressed lncRNAs that were differentially regulated by TCPOBOP and their genomic distance to PCGs.

lncRNA gene ID	lncRNA gene locus	CO_1 FPKM	CO_2 FPKM	CO_3 FPKM	TCPOBOP_1 FPKM	TCPOBOP_2 FPKM	TCPOBOP_3 FPKM	TCPOBOP_lncRNA_ p_value	Paired PCGs	Distance of lncRNA genes to TSS of PCGs	Distance of lncRNA genes to TSS of PCGs	PCG CO_1 FPKM	PCG CO_2 FPKM	PCG CO_3 FPKM	PCG TCPOBOP_1 FPKM	PCG TCPOBOP_2 FPKM	PCG TCPOBOP_3 FPKM	CountTCPOBOP_p _value
NONMMUG02 4772.2	chr2:132094431- 132094434	2.4687	5.0872	5.1465	1.2518	0.814957	4.63487	0.0489	111003462 BRK	Intron	6194	2.4453 5	1.6034 8	1.513 6	1.85403	1.39692	1.46578	0.10765
NONMMUG03 0058.2	chr4:509215989- 509216003	0.84031	1.55373	1.26624	6.90484	4.51666	6.25768	0.00005	170202481 BRK	Downstr eam	1034 87	0.8206 59	1.2618 7	1.251 6	5.61746	6.03141	6.70586	0.00005
NONMMUG01 0351.1	chr18:22098725- 22098749	0.84822	0.59314	0.36111	2.39525	0.616064	1.32389	0.0434	170011280 BRK	Intron	7975 6	0.1372 4	0.2158 98	0	2.0723	1.90789	1.96562	0.13095
NONMMUG04 5655.2	chr6:50775761- 507758481	0.54381	1.09163	1.2776	2.23776	2.14556	2.80613	0.00875	261000254 DNRK	UTR	5253 8	0.8227 08	1.1819 17	1.750 1	1.55017	2.29894	2.18752	0.08865
NONMMUG00 60373.2	chr10:60346906- 603732097	0.82235	1.21697	1.64062	0.662857	1.27374	0.521255	0.0431	463242890 SRK	Intron	1028 5	0.8951 02	1.2606 5	2.213 63	0.94225 1	1.54455	0.69369 1	0.0068
NONMMUG00 3953.2	chr10:70524623- 70524662	3.83646	1.25297	0.77520 6	1.37055	0.788081	0.59553	0.045	459353361 BRK	Intron	5568 3	-	-	-	-	-	-	-
NONMMUG00 7252.2	chr11:94351745- 94351806	2.19468	1.66007	0.78276	8.74223	9.13866	5.27087	0.00005	Abcc3	Intron	3625 6	17.677 4	28.218 9	25.29 19	70.7303	140.693	124.685	0.00005
NONMMUG02 7793.2	chr3:121730808- 121730864	34.0178	40.3284	20.0249	57.9651	23.2123	33.5056	0.03105	Abcc3	Intron	5139 1	33.097 9	70.656 9	44.42 26	86.7385	114.303	95.384	0.0033
NONMMUG08 5332.1	chr5:106431380- 106431919	102.058	97.965	69.6464	70.0062	45.6337	54.007	0.001	Abhd14b	Downstr eam	3835 0	56.636 3	56.472 9	58.62 24	35.4714	40.8842	27.8233	0.00001
NONMMUG02 1688.2	chr15:97534805- 97537712	2.17581	2.0294	1.33871	2.96799	4.81626	4.82478	0.00345	Abhm1	UTR	1799 74	1.6381 2	2.3433 4	1.256 21	3.38992	4.98535	4.206	0.00025
NONMMUG00 6743.1	chr11:76436743- 76437658	2.13644	1.16418	1.44847	0.719512	1.15871	0.929981	0.0355	Abi	UTR	9211 0	-	-	-	-	-	-	-
NONMMUG00 7052.2	chr11:84210524- 84220078	0	0.75736	0.50389	1.55383	2.90364	1.07706	0.00005	Acaca	Intron	2286 3	2.9543 3	4.0371 6	4.566 87	4.73485	11.3808	7.18779	0.0027
NONMMUG00 6743.1	chr11:84210524- 84220078	2.72083	4.58301	3.45993	6.88823	5.94849	6.04959	0.0467	Acpi1	UTR	1615 3	3.2801 3	4.799 19	8.6153	8.39245	8.7216	0.001	
NONMMUG02 1643.2	chr15:52297636- 52297634	1.84631	8.3252	2.3508	3.86679	1.83337	2.7025	0.0258	Accl5	Downstr eam	4353 3	44.185 6	70.082 5	59.761	60.2196	61.2275	0.4768	
NONMMUG02 1643.2	chr15:52297636- 52297634	17.5224	17.5084	9.85132	10.8959	7.75347	7.33088	0.0011	Adh7	UTR	2403 0	0.9802 46	0.9102 63	0.837 683	1.49566	1.41446	1.73495	0.2316
NONMMUG02 5329.2	chr2:12684637- 12684685	1.37281	1.76276	2.19373	2.72243	3.8526	3.36488	0.0468	Adig	Upstream	4853 1	-	-	-	-	-	-	-
NONMMUG03 6428.2	chr6:129353156- 129353161	39.6895	34.8138	21.8807	31.8874	19.3542	16.5735	0.01325	Adipor2	UTR	6431 1	42.657 68	53.247 1	46.78 68	33.6565	48.4418	37.0576	0.0146
NONMMUG02 7736.2	chr3:116740000- 116740371	2.39126	2.25286	2.68364	35.3988	14.9309	15.027	0.00005	Ag1	UTR	6548 1	1.1888 4	1.7627 9	2.000 58	6.34644	12.1029	12.1634	0.00005
NONMMUG02 1784.2	chr11:183138208- 183138266	0.82765	1.59991	0.84716	2.28491	1.68003	2.13575	0.0315	Ada	Upstream	4811 22	0.8449 55	1.7309 8	1.481 55	2.5881	2.48203	2.80601	0.00985
NONMMUG00 6330.1	chr11:61265546- 61267633	2.33738	1.69003	3.32088	2.26305	1.59122	5.02885	0.00005	Adh5a2	Intron	5906 1	27.846 1	35.466 1	33.71 36	59.3768	83.7644	74.1803	0.00005
NONMMUG04 6018.2	chr5:12646457- 126465028	1.01886	0.78694	0.48846	1.39343	2.71533	1.91399	0.0026	Amot	UTR	3877 1	-	-	-	-	-	-	-
NONMMUG02 2477.2	chr5:52809512- 52809588	0.65441	0.50479	0.52988	2.61859	1.62381	1.70588	0.03525	Anapc4	Intron	2250 8	1.0987 6	2.5431 4	1.955 91	4.40446	5.9097	5.47204	0.00005
NONMMUG01 81705.1	chr5:83088773- 83088793	1.11731	2.18684	1.80591	4.01775	3.09124	3.40794	0.01865	Ankrd28	Intron	1507 32	0.8456 6	1.3562 8	1.264 23	0.76368	2.82512	2.46146	0.09285
NONMMUG01 4229.2	chr15:31709083- 31709093	4.1739	2.7776	4.49758	8.82339	5.85137	7.61538	0.0393	Ankrd33b	Intron	7592 6	1.9953 7	1.7820 5	1.063 5	2.85488	4.64437	3.58055	0.20475
NONMMUG00 7232.2	chr12:96134438- 96134507	17.9711	17.464	32.4531	30.6235	63.1869	37.1883	0.03815	Ankrd40	Downstr eam	1219 2	12.037 2	14.527 1	15.33 51	20.7042	30.8958	23.4844	0.0384
NONMMUG04 7619.1	chr11:71212053- 71212639	3.64584	9.88951	9.80954	2.29894	6.83447	2.96063	0.02785	Apoa2	Exon/CD	625 5	13875 5	12703 6	10316 6	719427	6138.14	4881.65	0.00005
NONMMUG04 5685.2	chr5:60626919- 60626930	42.9567	35.8422	34.8839	136.289	150.106	171.134	0.00005	Apoa4	Exon/CD	2378 2	48.624 6	59.589 6	59.79 53	143.416	191.823	253.623	0.00005
NONMMUG04 5684.2	chr9:46218940- 46240032	1.79859	2.11513	1.94021	6.86526	6.85996	6.85996	0.00005	Apoa4	UTR	240 2	48.624 2	59.589 6	59.79 53	143.416	191.823	253.623	0.00005
NONMMUG01 7360.1	chr7:15683138- 15683827	1.24136	3.48954	0.60653	1.37065	0.424214	0.717638	0.0167	Apoa1	Downstr eam	5585 1	64302 8	72624 1	3753 27	3349.75	2463.93	1387.13	0.00005
NONMMUG04 5434.2	chr5:98120175- 98120212	0.48033	0.85573	0.75003	2.14401	1.16339	1.67341	0.01275	Ar	UTR	1719 72	-	-	-	-	-	-	-
NONMMUG02 6726.2	chr15:94337523- 94337523	0.70376	0.63139	6.20998	0.1915	1.09901	1.62734	0.0886	Arhgef56	Exon/CD	53 9	5.3325 9	4.2665 68	17.11 68	2.21137	2.88106	4.11894	0.00005
NONMMUG02 7189.2	chr2:91671876- 91671866	2.59771	2.30825	3.39901	2.35001	2.24847	1.85021	0.03695	Arg13	Downstr eam	3680 8	8.7360 8	11.808 8	16.16 78	7.96741	11.9071	11.2288	0.0181
NONMMUG03 6312.2	chr6:13746673- 13746813	0.29533	0.23349	0.12108	8.56711	7.10841	6.54374	0.00005	Atip3a2	UTR	2951 00	0.2090 44	0.2896 93	0.114 78	3.75632	5.73965	4.08085	0.00005
NONMMUG00 7676.2	chr11:102289300- 102289393	6.6347	4.76192	4.52459	4.21629	4.03833	4.02717	0.0457	Atip3a2	Exon/CD	3248 5	4.9964 8	35.306 4	4.325 8	2.59529	3.42984	3.381	0.0197
NONMMUG06 3265.2	chr2:288125951- 28812592	1.09005	1.94113	15.5068	1.47296	2.22967	1.8645	0.00005	Atip3a1	Intron	3626 08	0.9056 1	1.3755 52	13.94 7	0.91202	2.02679	1.58402	0.00005
NONMMUG06 6137.1	chr15:5346632- 5346636	18.4224	16.4523	15.8026	40.3002	30.1575	30.8823	0.01536	Banp1	Intron	559 5	22.761 5	19.378 45	18.66 45	48.0071	38.2621	37.2947	0.0265
NONMMUG07 1543.1	chr5:124524442- 124524900	1.04745	0.21804	0.53708	0.762487	5.9611	4.36059	0.0026	Bcar5	Intron	2792 8	4.5867 7	5.0096 9	2.606 46	5.77111	8.40456	6.7475	0.09485
NONMMUG03 7997.2	chr7:45362791- 45363049	63.115	52.0363	40.2852	39.3794	36.7548	27.8446	0.00985	Bcat2	Intron	9051 8	4.6134 6	3.5455 2	2.080 42	3.23603	2.53207	2.43636	0.00475
NONMMUG01 1596.1	chr13:9381423- 93815166	9.03424	6.50765	5.57479	0.371942	0.053316	0.405403	0.00005	Btlnr1	Intron	8232 3	99.10 3	125.53 91	79.13 91	149.811	156.969	75.9788	0.00005
NONMMUG03 5461.2	chr15:184574269- 184574269	3.32719	6.45139	6.03913	18.4799	12.4432	13.3637	0.0001	C1gat1	UTR	8013 76	2.3131 4	3.2812 76	4.613 76	4.21802	11.42	9.53664	0.0001
NONMMUG03 4560.2	chr6:7871535- 7874028	1.55035	3.14312	3.39624	4.62851	8.02697	6.94222	0.0007	C1gat2	UTR	2782 4	2.3131 4	3.2812 76	4.613 76	4.21802	11.42	9.53664	0.0001
NONMMUG03 5541.2	chr6:124530930- 124530931	71.8581	74.2608	78.2867	38.1257	41.8508	18.8119	0.00005	C1i1	Intron	5711 90	60.49 90	83.776 90	89.50 90	76.5249	38.2498	22.2594	0.00005
NONMMUG03 9745.1	chr5:5567587- 5567587	0.22573	0.54605	0.11373	1.44884	1.25846	2.44109	0.0271	C13002123 SRK	Intron	5294 63	0.9904 3	1.2502 315	0.573 315	2.85885	2.33453	2.22022	0.0012
NONMMUG01 81261	chr17:34734504- 34734519	11.5706	8.77077	18.8375	4.85852	9.29936	3.1478	0.0004	Cab	Exon/CD	1109 82	159.20 1	159.72 82	267.3 82	42.6009	90.0502	54.961	0.00005
NONMMUG02 1798.2	chr15:60524730- 60524739	3.27808	5.17951	6.25355	5.583	8.14006	11.0893	0.0173	Calu1	UTR	5487 2	4.3240 8	5.9397 2	9.067 98	8.44371	11.4282	12.691	0.01025
NONMMUG03 7327.2	chr7:16915381- 16916232	11.2668	11.151	10.1156	30.3727	21.2793	19.4544	0.00735	Caln3	UTR	8263 8	10.015 8	12.383 8	10.99 18	28.375	29.164	25.5311	0.00005
NONMMUG00 5332.1	chr11:6007088- 6007091	13.274	17.8823	71.9675	11.1052	4.93983	6.8251	0.00005	Camk2b	Intron	5894 4	-	-	-	-	-	-	-
NONMMUG03 10912.2	chr4:18457014- 18457023	27.5719	25.6618	27.01	29.9829	15.0968	19.8697	0.0489	Camk2n1	UTR	3004 8	15.735 9	11.252 9	12.75	16.1957	11.7827	10.1762	0.10985
NONMMUG03 3739.2	chr5:122731255- 122731247	1.82675	1.71995	1.2154	1.38747	0.951308	0.846002	0.03055	Camk2c	UTR	4713 1	-	-	-	-	-		

NONMMUG00	01212104763639	8.62324	3.46105	2.48051	1.99653	1.66209	1.58224	0.0007	Clim	FUTR	9717	5.5574	5.1860	4.519	3.35933	4.63086	3.81566	0.0017
NONMMUG03	01212104763639	104.591	110.351	102.201	75.139	48.5003	45.0251	0.00005	Cmi1	Intron	4635	113.27	123.01	136.3	85.3664	60.2318	55.5785	0.00005
NONMMUG04	01212104763639	4.66886	3.92079	2.51354	2.86057	3.50429	1.45132	0.0247	Cmi18	Upstream	452	39.341	33.822	22.26	33.3994	24.9603	18.48	0.0079
NONMMUG00	01212104763639	1.49328	1.62976	1.89922	3.69966	3.76009	3.53639	0.00605	Cmi18	FUTR	9947	1.3463	1.6227	2.738	1.40974	3.98161	3.37912	0.0018
NONMMUG00	01212104763639	10.742	3.91477	3.41513	5.20589	1.67602	2.8942	0.00135	Cmi18A1	Intron	8086	68.220	81.478	80.32	28.4541	55.2195	31.6019	0.00005
NONMMUG03	01212104763639	0.85432	2.18906	4.35265	0.55402	1.14633	1.56835	0.0195	Cmi41	Intron	9822	2.3960	3.4545	7.563	2.94762	5.55315	3.62333	0.0615
NONMMUG01	01212104763639	1.33572	2.50792	1.94524	4.7536	4.33211	4.25531	0.0118	Cmi43b3p	FUTR	9682	0.5324	1.4713	1.451	2.12773	2.96085	2.58863	0.0006
NONMMUG03	01212104763639	1.21001	1.53786	1.85133	5.20119	6.94322	4.54599	0.01735	Cmi1c	FUTR	6584	3.8131	4.4361	5.295	5.50405	8.83243	7.24518	0.0724
NONMMUG00	01212104763639	885.004	1427.11	820.641	948.202	619.444	611.132	0.00945	Cmi1	FUTR	1080	223.60	437.26	318.7	169.651	270.645	234.89	0.0045
NONMMUG00	01212104763639	31.6072	26.1504	12.7274	25.4077	14.874	15.4675	0.00125	Cmi2	Upstream	1702	1.3611	1.9211	2.058	1.90533	2.1488	2.15248	0.8304
NONMMUG06	01212104763639	0.82156	2.3713	1.47433	0.608361	0.858286	1.62705	0.04725	Cmi1a	Downstr	6323	30.251	45.609	47.25	21.5131	38.1223	29.3997	0.00121
NONMMUG03	01212104763639	2.96809	1.01322	0.49482	0.820154	0.567329	0.519043	0.0095	Cmi2	Intron	4493	30.171	25.768	24.87	22.4308	33.9188	22.7742	0.10095
NONMMUG00	01212104763639	1.13981	0.18389	0.46095	2.48366	1.0171	1.13475	0.0149	Cmi1	Intron	2540	221.99	171.23	302.8	206.166	269.146	232.15	0.26265
NONMMUG03	01212104763639	2.76435	2.58728	3.85021	2.20669	0.500439	0.0886	Cmi1c	FUTR	1019	1.2802	1.7972	1.973	0.83366	1.58207	1.11435	0.00085	
NONMMUG02	01212104763639	6.74185	6.26423	7.85795	2.81698	1.48743	2.119	0.0001	Cmi1c	Intron	8962	-	-	-	-	-	-	-
NONMMUG02	01212104763639	1.56151	1.57439	0.48421	2.28409	2.27158	1.0230	0.02405	Cmi1	Intron	2900	53.245	89.158	59.98	95.8007	123.675	103.438	0.10445
NONMMUG02	01212104763639	2.57636	1.0071	1.113	1.12481	0.450507	0.561895	0.00595	Cmi1	Intron	2275	1.7229	1.6412	4.065	1.41643	2.95626	2.05515	0.04155
NONMMUG01	01212104763639	0.47491	0.31315	0.03801	4.39561	5.91313	0.00009	Cmi2b3	Intron	3286	2.6286	3.5063	1.515	511.462	947.071	604.473	0.00009	
NONMMUG01	01212104763639	2.63076	2.2475	9.07436	2.08364	4.37716	3.38551	0.03385	Cmi2a22	FUTR	9702	52.909	65.075	50.99	62.9749	76.4126	53.0752	0.49525
NONMMUG02	01212104763639	21.163	11.1812	1.46818	5.89836	2.10088	0.40078	0.00009	Cmi2a1	FUTR	1441	2.8191	2.6194	1.247	0.75662	1.14684	0.74666	0.00005
NONMMUG03	01212104763639	67.7294	40.8812	12.9334	7.02319	3.98349	2.85681	0.00035	Cmi2a12a	Intron	3193	348.44	210.90	96.11	97.079	133.519	50.9058	0.00005
NONMMUG02	01212104763639	2.28441	1.34637	2.33038	3.67302	3.73646	6.36336	0.00055	Cmi2b3b3a	Intron	7299	2.3523	2.6284	2.646	3.93943	4.50294	7.3267	0.00015
NONMMUG02	01212104763639	0.52764	1.41458	1.12007	2.78675	3.09496	1.71082	0.04175	Cmi2b3	FUTR	2028	0.4872	0.6488	0.812	1.05182	2.17578	1.31486	0.0015
NONMMUG04	01212104763639	4.1421	2.82789	2.88543	5.18872	6.85623	6.83801	0.028	Cmi2b3	Exon/CD	7073	4.027	2.9823	5.338	5.10064	7.63602	6.81245	0.0204
NONMMUG04	01212104763639	3.12226	2.26666	3.0054	1.9902	1.73999	1.59424	0.0079	Cmi2b3	Intron	5102	7.9765	9.6633	7.504	13.8886	10.7862	8.78624	0.99815
NONMMUG01	01212104763639	13.8433	13.3151	10.53	12.3961	6.87776	7.48038	0.0007	Cmi2b3	Downstr	945	18.344	50.13	51.10	42.635	39.7285	33.2396	0.00001
NONMMUG01	01212104763639	3.90916	4.71598	2.93461	5.97136	8.64091	8.89038	0.01166	Cmi1	FUTR	4464	3.4882	4.4929	2.409	5.73963	8.55722	6.87419	0.00345
NONMMUG01	01212104763639	19.446	15.474	17.7148	12.0412	11.2618	10.9579	0.0104	Cmi2b3	Intron	7171	43.537	30.272	41.26	31.802	34.6673	31.5381	0.7244
NONMMUG01	01212104763639	1.33368	1.56725	1.3973	1.96891	0.999487	0.845931	0.00835	Cmi2b3	FUTR	6765	1.9933	1.5406	2.472	0.91079	1.57718	1.2546	0.00005
NONMMUG01	01212104763639	5.49155	1.79675	1.0765	1.37497	6.88502	6.88502	0.0032	Cmi2b3	FUTR	8779	6.1382	7.0453	6.581	11.7321	18.8486	16.4411	0.00015
NONMMUG01	01212104763639	0.77306	0.63530	0.53975	1.60301	1.59627	1.48104	0.01735	Cmi2b3	FUTR	7755	0.8062	0.8024	0.731	1.41453	1.99974	1.79884	0.004
NONMMUG00	01212104763639	6.60803	7.81874	16.9222	0.882531	5.89196	1.22305	0.00009	Cmi2b3	Intron	1796	93.225	102.67	137.3	16.8964	39.8661	15.8931	0.00009
NONMMUG04	01212104763639	4.23777	4.44421	4.22685	3.53365	2.98443	2.44308	0.0161	Cmi2b3	FUTR	9974	3.8575	4.1409	4.377	3.19423	2.85182	1.97299	0.0003
NONMMUG01	01212104763639	2.22522	0.65872	3.26011	3.60358	3.12886	0.0331	Cmi2b3	Intron	1356	4.2635	5.8363	3.206	11.6536	12.1242	10.0533	0.00009	
NONMMUG02	01212104763639	4.65368	1.52734	0.64301	2.87351	0.439365	1.01538	0.04985	Cmi2b3	Intron	5470	1.6219	1.5406	1.544	5.15965	2.18203	1.49333	0.2573
NONMMUG00	01212104763639	4.26184	2.90694	1.56109	1.56301	1.46357	1.63252	0.0001	Cmi2b3	Intron	3722	28.657	36.557	46.71	19.4592	42.7549	37.2199	0.03575
NONMMUG01	01212104763639	2.34663	4.48762	2.42529	3.09261	1.49966	1.85942	0.07705	Cmi2b3	Downstr	2020	17.411	27.118	18.01	21.2407	30.9449	29.5662	0.1562
NONMMUG02	01212104763639	2.22125	2.04276	1.25067	0.982925	0.337827	1.93303	0.039	Cmi2b3	FUTR	6825	-	-	-	-	-	-	-
NONMMUG02	01212104763639	20.184	8.79385	34.2853	5.11346	7.1212	2.67998	0.00005	Cmi2b3	FUTR	1728	13.560	63.129	18.05	1.04664	3.53204	7.03264	0.00005
NONMMUG01	01212104763639	3.59542	1.64089	0.53212	10.0342	2.74833	6.48428	0.0031	Cmi2b3	Intron	7057	0.3944	0.7790	0.648	1.37767	1.90394	1.53687	0.0001
NONMMUG01	01212104763639	0.30234	0.14214	2	1.66071	2.57636	0.94005	0.04005	Cmi2b3	Exon/CD	8853	0.6182	0.7500	0.529	2.41669	4.1187	3.13061	0.00005
NONMMUG02	01212104763639	5.04962	1.32559	1.13733	0.544709	0.308996	0.179019	0.00009	Cmi2b3	Exon/CD	1864	102.69	25.380	31.90	10.0283	6.20476	4.68018	0.00009
NONMMUG07	01212104763639	0.70355	1.57602	2.36261	1.16385	0.83887	5.56277	0.01975	Cmi2b3	Intron	8666	2.5534	5.0294	4.682	7.55386	13.0887	9.50766	0.00005
NONMMUG03	01212104763639	0.70229	0.18179	0.66782	4.42844	3.50915	3.31426	0.0002	Cmi2b3	Intron	1121	-	-	-	-	-	-	-
NONMMUG03	01212104763639	2.84558	2.34554	2.28624	2.09782	1.55105	1.01215	0.01265	Cmi2b3	FUTR	1405	3.9352	4.0780	5.133	4.30461	5.67438	3.44883	0.25075
NONMMUG04	01212104763639	2.23464	1.43445	0.72136	1.1462	0.738477	0.588183	0.00275	Cmi2b3	Intron	4489	7.7086	6.3156	3.86	5.21225	6.82701	4.98441	0.1003
NONMMUG00	01212104763639	7.68546	11.9341	5.1396	31.9655	13.5629	10.2469	0.0045	Cmi2b3	FUTR	1754	11.233	17.568	11.64	25.7055	51.4002	25.3638	0.00005
NONMMUG04	01212104763639	0.68191	1.00671	0.78079	4.08997	2.7512	2.95488	0.00005	Cmi2b3	Intron	1908	17.161	17.943	20.85	45.2748	58.0334	28.1398	0.0001
NONMMUG02	01212104763639	0.56846	0.53446	0.90217	1.87879	1.55069	1.27536	0.0174	Cmi2b3	Exon/CD	3793	0.8900	0.8683	1.522	2.90728	2.54303	1.95969	0.00045
NONMMUG01	01212104763639	0.77413	0.72571	0.62889	2.15625	2.58677	2.87309	0.00015	Cmi2b3	Intron	1670	6.7771	8.1535	6.455	18.6316	18.861	16.4684	0.00025
NONMMUG02	01212104763639	1.03138	3.94331	0.93612	4.33355	4.14778	5.9394	0.0033	Cmi2b3	Downstr	1019	89.630	92.112	76.52	238.292	333.705	315.527	0.00005
NONMMUG01	01212104763639	0.91358	1.96581	3.23649	1.16085	1.35867	1.8365	0.0389	Cmi2b3	Intron	4641	136.68	117.13	214.1	60.9149	170.514	108.091	0.0067
NONMMUG02	01212104763639	0.85194	0.77979	0.39529	2.77358	2.03755	1.61789	0.00185	Cmi2b3	FUTR	1115	0.7594	0.9136	1.435	2.2279	2.81147	2.82308	0.00065
NONMMUG03	01212104763639	269.539	305.337	378.318	248.671	313.912	114.677	0.00175	Cmi2b3	Intron	2452	312.12	314.35	316.6	228.622	250.404	129.472	0.00005
NONMMUG03	01212104763639	19.0512	15.2813	16.7088	41.2662	25.6328	34.9089	0.0493	Cmi2b3	Exon/CD	2228	6.4965	3.8947	6.603	9.15835	11.955	8.37538	0.0464
NONMMUG01	01212104763639	11.1512	12.1863	13.8078	11.456	10.0931	7.81968	0.032	Cmi2b3	FUTR	1406	15.886	19.300	21.87	16.8428	19.8033	15.8641	0.0644
NONMMUG02	01212104763639	0.15726	0.29745	0.61330	60.5706	65.741	49.4884	0.00005	Cmi2b3	Exon/CD	120	22.787	15.24					

NONMMUG01	015157696791	18.3203	22.6764	17.843	35.125	41.2444	34.765	0.0195	Egt	Inten	1438	54.317	64.943	45.96	97.7201	113.547	96.1755	0.0108
NONMMUG06	8853.2	43.2031	100.529	27.5525	46.9176	23.0385	23.2371	0.0004	Gss	Downstr	2983	14.039	11.852	15.44	24.72	29.823	27.6541	0.00245
NONMMUG00	155564114	0.45075	0.99598	0.12185	2.36318	0.656415	1.57392	0.0044	Gtata3	Inten	4368	498.11	781.89	865.6	1495	1054.2	1150.29	0.01801
NONMMUG00	01211	0	0	0	0	0	0	0.00795	Gtfa5	Upstream	4627	4.9908	5.5174	0.098	6.52426	4.95402	5.44606	0.1455
NONMMUG03	8440.2	0	1.09358	0.43120	2.31554	0.922673	1.31985	0.0365	Gtys2	Inten	9521	18.914	19.806	11.51	17.6296	24.7535	15.7473	0.92635
NONMMUG01	84881.1	20.9531	15.8205	12.7518	60.8044	53.941	60.2729	0.00005	Hdac7	Downstr	3968	9	9	9	9	9	9	0.00005
NONMMUG00	5388.2	39.994	41.1213	38.3252	34.4414	31.7219	29.9083	0.0316	Hdlbp	FUTR	7246	50.971	59.253	62.07	52.2027	97.1258	88.0747	0.5253
NONMMUG02	4402.2	33.0733	25.3236	13.8357	70.7779	43.3722	56.9328	0.0016	Hfe2	FUTR	3670	15.945	15.068	9.336	35.4289	25.9533	32.7588	0.00005
NONMMUG01	7343.1	24.0228	25.89	11.1542	18.3984	9.73545	11.5059	0.0066	Hgd	Inten	5171	254.40	275.16	222.4	239.01	269.238	248.652	0.20605
NONMMUG02	64181	9.7769	5.93333	7.3114	5.79917	7.22383	5.2025	0.0095	HistH2aa1	Upstream	533	48.892	20.067	29.89	21.9102	38.8196	12.1302	0.00005
NONMMUG01	7313.2	1.66991	1.81323	1.55551	6.49397	3.97081	5.29824	0.00025	Hmgcr	Downstr	2231	4.5619	5.9297	9.468	12.6056	20.2171	19.4576	0.00001
NONMMUG03	1566.2	53.7966	51.0085	40.0213	36.8594	30.622	27.7224	0.00245	Hud17b11	Inten	8048	67.875	71.754	61.92	52.335	56.0331	36.8433	0.00035
NONMMUG02	1177.2	3.92048	2.87007	0.96237	1.78752	1.14337	0.505368	0.0022	Hud3b3	Inten	3635	50.158	73.873	34.73	18.264	29.8821	13.1712	0.00005
NONMMUG01	7387.2	5.09692	7.1805	2.85122	2.47371	3.60952	2.21426	0.0128	Htpa1b	Exon/CD	520	15.823	22.186	6.084	10.3882	6.13641	2.23739	0.00005
NONMMUG03	8155.2	5.75028	2.97	12.7793	3.16246	3.18034	1.77958	0.00005	Id3	Inten	598	46.234	22.152	75.23	21.9301	19.6563	12.1667	0.00005
NONMMUG00	1021.2	14.76	28.666	28.3088	14.4344	11.1383	8.4343	0.0001	Igf1	Downstr	7051	82.186	74.443	86.33	40.4205	47.9547	25.5813	0.0002
NONMMUG00	4339.1	12.87	785.941	905.217	734.11	551.356	582.522	0.0009	Igfbp4	Downstr	4796	716.74	491.89	632.5	412.876	412.466	352.351	0.00007
NONMMUG00	7554.2	1.26007	4.30805	1.61323	1.87627	0.960644	2.78991	0.0091	Ighfb5	FUTR	1592	1.7100	2.7741	1.027	0.86178	0.73746	2.23739	0.00017
NONMMUG02	9991.2	4.03026	9.65197	7.79575	18.0726	14.858	17.7871	0.0059	Impa21	FUTR	2829	0.5789	1.5057	1.801	1.45337	3.87275	3.30461	0.00095
NONMMUG00	6403.2	23.6433	55.4383	28.6328	36.7151	86.035	72.8309	0.00075	Insu2	FUTR	2329	26.765	55.385	11.96	79.3102	95.4238	84.4839	0.00015
NONMMUG02	1607.2	4.48518	4.04061	10.055	3.90023	4.97193	3.07468	0.01095	Ints5	Exon/CD	4298	3.8322	3.3363	7.631	2.41709	3.62465	3.44918	0.00025
NONMMUG03	0771.2	1.79808	1.68523	1.63826	1.59288	1.08051	1.07451	0.0383	Irbp1	FUTR	2110	1.0584	2.1303	1.100	0.79406	1.5357	1.20888	0.02895
NONMMUG00	7502.2	2.50381	2.9646	2.37463	1.00899	1.01958	5.757896	0.0472	Ivms1abp	Upstream	2060	8.6438	9.1715	14.02	11.8604	15.0043	14.9533	0.59675
NONMMUG02	5376.2	0.06130	0.12636	0.14688	3.12114	1.87746	1.43186	0.00005	Izaf1	Downstr	9732	0.0888	0.0248	0.186	3.51882	2.87036	2.17849	0.00005
NONMMUG04	1305.2	0.62351	1	0.91496	1.36688	2.53268	3.17771	1.97268	Ikbic2	FUTR	1711	0.9834	0.9507	1.005	1.84728	2.11064	1.82517	0.0104
NONMMUG01	9602.2	4.49133	2.00949	2.54652	1.76431	1.87146	1.04428	0.0095	Ikbic2	FUTR	3086	12.576	12.671	14.22	9.65845	17.1101	13.1528	0.02035
NONMMUG01	85047	2.15548	0.95539	3.46692	0.620803	0.177851	0.58061	0.0007	Ikbic2	Inten	2031	5.929	4.7798	4.962	4.56036	5.21761	4.66109	0.0629
NONMMUG02	10212.2	5.71284	13.7672	13.7893	9.8048	6.697	6.22603	0.00835	Ikbic2	Downstr	2638	14.915	16.297	14.25	11.8572	9.88609	8.09576	0.00005
NONMMUG04	0717.2	0.85012	1.75098	1.72679	1.09914	0.854527	1.07274	0.0348	Ikbic2	Inten	1086	2.4515	3.0750	3.566	1.9208	3.00572	2.72478	0.00055
NONMMUG01	6069.1	14.9074	17.3052	28.4698	5.76464	1.98465	0.00005	Ikbic2	Inten	1078	1080.1	1088.9	1161	101.047	502.708	474.305	0.00005	
NONMMUG02	6825.2	1.1003	0.78872	1.73952	8.38055	3.16029	4.3775	0.0272	Ikbic2	Inten	1183	1.6191	4.0209	3.779	3.17505	4.91476	4.63337	0.5302
NONMMUG00	7466.1	8.7294	9.23192	14.309	19.451	17.387	0.0081	Ikbic2	Inten	2508	8.6285	11.496	11.80	15.1961	23.625	19.4668	0.0105	
NONMMUG01	7700.2	2.57225	2.28975	1.63804	1.6761	1.78028	1.36401	0.04065	Ikbic2	Downstr	2825	2	2	2	2	2	2	0.7269
NONMMUG04	12681	1.84305	0.53027	0.48406	6.22461	1.37089	1.44258	0.0082	Ikbic2	Inten	5150	23.463	20.595	20.48	23.3805	37.4594	24.8905	0.00005
NONMMUG02	0154.2	34.2485	45.3572	33.0551	6	73.6998	74.106	0.01245	Ikbic2	FUTR	2077	25.736	36.49	25.44	54.8033	84.9742	65.5495	0.0007
NONMMUG01	5380.2	1.99214	1.95461	1.71622	3.89817	3.82617	3.3361	0.02795	Ikbic2	Inten	3342	5.9575	4.7391	6.075	9.02564	15.0665	10.6124	0.0001
NONMMUG04	8609.1	2.54477	0.67801	0.73274	2.99833	2.47095	3.26751	0.02685	Ikbic2	Inten	1166	15.454	15.476	15.55	36.6778	49.5514	52.5466	0.00005
NONMMUG02	1164.2	12.2281	8.44831	5.13866	10.9214	3.49883	4.5025	0.0209	Ikbic2	Inten	4587	39.223	49.111	37.00	45.5842	46.8795	41.574	0.4255
NONMMUG00	8545.2	27.3364	16.0851	35.6272	9.54944	7.91595	7.14426	0.00005	Ikbic2	FUTR	5334	8.1361	6.6508	17.25	2.89242	5.05102	3.21719	0.00005
NONMMUG02	0719.2	78.1697	110.437	53.345	30.7359	15.0907	23.0306	0.0178	Ikbic2	Inten	1973	7.1462	15.458	10.57	5.13762	5.62303	4.04994	0.00005
NONMMUG01	8320.2	5.33862	3.1394	2.61095	2.2964	2.58558	2.21342	0.0016	Ikbic2	Inten	6657	7.5055	5.0090	1.239	5.0142	4.40434	4.25883	0.0478
NONMMUG02	4532.2	10.7429	16.7458	13.0442	42.8584	42.295	40.5797	0.00005	Ikbic2	FUTR	3043	7.4649	12.472	10.14	30.6234	32.9905	30.1383	0.00005
NONMMUG00	3650.2	0.34441	1.25495	0.65831	3.36651	1.20412	1.2652	0.0109	Ikbic2	Inten	6727	12.497	20.186	27.77	21.1862	36.1247	32.8624	0.1924
NONMMUG02	0719.2	0.77032	1.6354	2.00123	0.292861	1.19523	1.00255	0.00825	Ikbic2	Inten	1271	20.590	25.001	25.38	12.0766	15.3608	12.822	0.0019
NONMMUG01	0336.2	2.15381	1.47621	1.42717	2.0397	4.30325	3.36172	0.00875	Ikbic2	Inten	388	7.1108	5.5521	21.99	7.9085	7.48955	10.528	0.0591
NONMMUG02	826.1	47.7382	44.7823	48.8368	115.562	72.2667	79.6057	0.02305	Ikbic2	Downstr	8821	144.29	117.84	165.9	332.015	273.993	245.234	0.00235
NONMMUG03	826.1	0.35979	0.45172	0.73633	2.13729	1.35629	1.89373	0.00025	Ikbic2	Inten	7318	0.5535	1.2894	2.196	2.20469	5.3362	4.1172	0.00005
NONMMUG00	5472.2	1.13751	1.96556	3.17054	3.7714	6.77761	5.61323	0.00095	Ikbic2	FUTR	2271	0.5535	1.2894	2.196	2.20469	5.3362	4.1172	0.00005
NONMMUG02	9549.2	0.45440	0.76893	1.98255	0.862764	2.90113	2.44244	0.04675	Ikbic2	FUTR	1022	0.3382	0.6093	2.004	0.50533	3.52366	2.73888	0.00455
NONMMUG01	5487.2	18.2839	24.7327	20.7205	44.3534	55.5398	41.122	0.00235	Ikbic2	Inten	1359	81.501	127.78	92.83	93.2994	86.7838	71.1167	0.0168
NONMMUG02	9549.2	0.45440	0.76893	1.98255	0.862764	2.90113	2.44244	0.04675	Ikbic2	FUTR	1022	0.3382	0.6093	2.004	0.50533	3.52366	2.73888	0.00455
NONMMUG01	5487.2	18.2839	24.7327	20.7205	44.3534	55.5398	41.122	0.00235	Ikbic2	Inten	1359	81.501	127.78	92.83	93.2994	86.7838	71.1167	0.0168
NONMMUG03	9549.2	0.45440	0.76893	1.98255	0.862764	2.90113	2.44244	0.04675	Ikbic2	FUTR	1022	0.3382	0.6093	2.004	0.50533	3.52366	2.73888	0.00455
NONMMUG02	9549.2	0.45440	0.76893	1.98255	0.862764	2.90113	2.44244	0.04675	Ikbic2	FUTR	1022	0.3382	0.6093	2.004	0.50533	3.52366	2.73888	0.00455
NONMMUG01	9549.2	0.45440	0.76893	1.98255	0.862764	2.90113	2.44244	0.04675	Ikbic2	FUTR	1022	0.3382	0.6093	2.004	0.50533	3.52366	2.73888	0.00455
NONMMUG02	9549.2	0.45440	0.76893	1.98255	0.862764	2.90113	2.44244	0.04675	Ikbic2	FUTR	1022	0.3382	0.6093	2.004	0.50533	3.52366	2.73888	0.00455
NONMMUG03	9549.2	0.45440	0.76893	1.98255	0.862764	2.90113	2.44244	0.04675	Ikbic2	FUTR	1022	0.3382	0.6093	2.004	0.50533	3.52366	2.73888	0.00455
NONMMUG01	9549.2	0.45440	0.76893	1.98255	0.862764	2.90113	2.44244	0.04675	Ikbic2	FUTR	1022	0.3382	0.6093	2.004	0.50533	3.52366	2.73888	0.00455
NONMMUG02	9549.2	0.45440	0.76893	1.98255	0.862764	2.90113	2.44244	0.04675	Ikbic2	FUTR	1022	0.3382	0.6093	2.004	0.50533	3.523		

NONMMUG01	chr13:1311029-1291248	1.24828	4.3993	1.72974	13.1189	12.8747	13.9846	0.00045	Nid1	UTR	7363	0.9922	2.1095	1.902	5.66468	9.38661	9.53845	0.00005
NONMMUG03	chr7:40077525-7954.2	4.545	6.85056	5.60851	11.2861	11.5867	11.7208	0.02	Nesip	Downstr	1355	5.4252	2	5.990	6.88597	8.54815	7.54372	0.00365
NONMMUG01	chr11:13986501-11987814	3.75841	6.87979	4.73476	13.3462	15.1837	13.2125	0.00006	Nuq1	UTR	2249	6.3566	10.186	8.136	19.4566	20.1787	22.1426	0.00021
NONMMUG01	chr13:17965326-13967964	1.07882	0.62177	0.31675	1.6979	1.59975	2.64566	0.0027	Nuq2	intron	1986	6.3566	10.186	8.136	19.4566	20.1787	22.1426	0.00025
NONMMUG04	chr9:12843635-12849699	22.4668	31.5524	37.3469	14.8434	13.4175	10.6807	0.0017	Nup1	intron	1033	8.9668	7.2293	6.082	6.89405	8.00605	7.517	0.1778
NONMMUG04	chr7:72954577-5105.2	1.50389	1.12561	1.57457	1.55509	0.225259	0.453604	0.0007	Nuq2	intron	4614	43.294	36.681	45.16	36.8408	21.125	31.5652	0.00005
NONMMUG00	chr11:132139400-17081	2.70136	1.26601	2.07071	1.16558	0.571464	0.799782	0.00075	Nuak2	Upstream	451	4.8768	3.0991	5.701	1.87565	2.11716	1.76151	0.00005
NONMMUG04	chr9:19847982-3347.2	3.57513	5.68226	6.85202	1.08572	1.57575	1.55071	0.01005	Onco1	intron	3244	3.2274	2.0624	4.611	0.61372	1.34456	0.59888	0.00005
NONMMUG02	chr19:13266294-1209.2	16.2395	51.6205	34.405	157.641	102.086	141.024	0.00005	Pap2a	UTR	4673	10.828	36.617	26.47	73.362	95.8759	104.025	0.00005
NONMMUG02	chr19:13217807-1206.1	1.11173	0.81897	0.97274	1.91139	2.48906	5.41075	0.0032	Pap2a	intron	7775	10.828	36.617	26.47	73.362	95.8759	104.025	0.00005
NONMMUG01	chr14:5607260-9031.2	2.15222	3.50765	3.20479	1.44121	1.47871	1.7367	0.01745	Parp4	intron	3831	-	-	-	-	-	-	-
NONMMUG02	chr10:43037253-3814.1	2.5513	2.42027	0.81140	1.61889	0.614675	0.570962	0.0006	PRG2	intron	1830	19.735	20.653	19.93	13.7448	12.8249	8.81929	0.00005
NONMMUG02	chr10:43038099-9315.1	4.19527	4.64753	3.28628	3.71723	2.10528	1.55407	0.00115	PRG2	Upstream	1559	19.735	20.653	19.93	13.7448	12.8249	8.81929	0.00005
NONMMUG02	chr11:130532638-5541.1	0.26328	0.85128	0.68187	2.45698	0.705043	0.01395	Prx13	intron	1225	4.7359	5.7270	6.145	7.29866	9.67544	8.94445	0.1606	
NONMMUG00	chr11:51596594-4050.2	0.61267	0.80856	1.00417	2.17906	1.58223	2.10497	0.0085	Phylp1	Upstream	1263	4.9749	3.0743	3.609	4.24364	3.57461	3.135	0.7502
NONMMUG02	chr3:94958620-7241.2	0.84890	1.84419	0.85338	2.17122	3.61023	3.34172	0.0413	PI4D	Upstream	4488	5.8663	5.7136	6.430	6.66183	5.70857	6.72074	0.545
NONMMUG03	chr7:92027694-8664.2	9.78295	15.8924	16.9608	58.2907	50.4421	55.6786	0.00005	Pic4m	UTR	7803	5.1019	8.6157	11.51	24.1854	36.8567	32.2277	0.00005
NONMMUG02	chr11:84239913-1169.2	2.77244	2.719	2.96111	2.74803	1.74803	0.0111	Pid1	intron	2357	15.840	19.931	89.95	21.5402	26.8627	24.632	0.1679	
NONMMUG05	chr11:75599645-1244.1	167.32	125.568	102.063	172.565	137.89	83.6394	0.0211	Pitpna	intron	1177	8.3525	9.7299	10.06	11.0679	11.6681	11.0008	0.918
NONMMUG03	chr7:116281511-9125.2	0.45818	0.14336	0.21137	3.78175	0.98219	1.75505	0.02115	Plekha7	intron	2604	-	-	-	-	-	-	-
NONMMUG03	chr4:141625734-1184.2	5.19731	3.37529	4.49606	3.0987	2.84616	2.32542	0.0313	Plekha7	UTR	3807	1.2667	1.3122	1.974	1.03973	1.99579	1.35173	0.1592
NONMMUG03	chr4:130804381-10141.2	0.40544	0.76386	0.88221	1.98594	2.96394	1.2325	0.00905	Pod1	intron	1070	0.4277	0.5450	0.463	2.52809	3.54564	2.22981	0.00005
NONMMUG03	chr6:1166257-6314.1	31.9697	45.5612	16.0686	30.5552	15.3229	25.8117	0.0075	Pon1	Exon/CD	2557	251.93	309.17	220.4	201.038	174.331	233.864	0.00735
NONMMUG03	chr5:13577097-10101.1	1.70865	0.24322	4.58664	7.70824	5.17376	5.38541	0.00005	Por	Upstream	442	87.646	45.329	63.85	261.871	418.472	320.26	0.00005
NONMMUG03	chr5:13577097-10101.1	2.80101	2.51652	1.90305	12.3889	4.1085	15.0401	0.00005	Por	intron	2312	87.646	45.329	63.85	261.871	418.472	320.26	0.00005
NONMMUG03	chr5:13577097-10101.1	0.22774	0.13081	0.86221	0.889235	2.62135	4.21262	0.00195	Por	intron	9432	87.646	45.329	63.85	261.871	418.472	320.26	0.00005
NONMMUG04	chr6:130804381-10141.2	26.0997	44.9943	13.9543	29.179	15.5553	18.4303	0.0196	Ppp1r3b	UTR	1122	20.315	36.265	11.17	19.9278	14.9202	15.8639	0.00165
NONMMUG03	chr19:46063458-14901.1	0.42148	0.61833	2.90664	0.405123	1.30775	0.706065	0.02855	Pprc1	Exon/CD	805	1.0736	0.9931	1.432	0.66316	1.47141	1.05551	0.0001
NONMMUG03	chr4:141625734-1229.2	5.72661	3.06457	3.09555	2.29719	1.79187	2.35333	0.0014	Prdm2	UTR	1046	5.0674	2.5162	3.295	1.52806	2.79244	2.15669	0.0008
NONMMUG01	chr18:41875802-7990.2	0	0	0	13.1498	16.8858	5.97344	0.0002	Prdm2	UTR	17	1	6	48	5	6	48	0.00005
NONMMUG03	chr4:130529105-10501.187	2.54703	2.81143	2.1021	6.70888	6.17851	5.63234	0.0016	Prkna2	UTR	7971	0.9301	1.9322	2.059	2.78629	6.11863	4.36487	0.00005
NONMMUG03	chr4:130529105-10501.187	0.62993	2.00999	3.78137	1.4878	0.84569	5.4993	0.01095	Prkna2	UTR	7525	0.9301	1.9322	2.059	2.78629	6.11863	4.36487	0.00005
NONMMUG03	chr4:130529105-10501.187	18.122	19.122	22.4632	79.4481	99.5195	99.8025	0.00005	Prkna2	Upstream	4805	-	-	-	-	-	-	-
NONMMUG01	chr15:10261115-4054.1	0.63902	0.57460	2.71314	7.47993	0.887613	3.73726	0.01445	Prx	intron	1538	10.795	12.545	24.49	12.8034	13.41	24.7084	0.6752
NONMMUG02	chr2:12923513-1892.1	2.47196	1.6946	1.04142	1.99548	0.454469	1.22989	0.0039	Prx2a	intron	2054	5.0251	4.6796	2.068	3.95835	3.99113	3.15059	0.0438
NONMMUG02	chr2:12923513-1892.1	5.6252	4.29511	3.12927	4.21319	1.65277	1.67638	0.00235	Prx	intron	265	7.2886	7.9036	7.889	8.72519	11.5938	9.2536	0.8785
NONMMUG02	chr2:12923513-1892.1	0.30176	1.08021	0.81741	1.21719	2.30995	1.94405	0.02175	Prgin	intron	6953	-	-	-	-	-	-	-
NONMMUG02	chr2:12923513-1892.1	0.28374	0.66458	1.01488	0.515804	3.15004	2.01684	0.0087	Pripn1	Exon/CD	4571	1.1541	1.4230	1.474	2.24876	2.88008	3.61123	0.00085
NONMMUG04	chr5:13044195-2892.2	1.92345	1.66465	2.07086	1.05013	1.27221	0.867502	0.02475	Pripn	intron	6311	2.3458	5.0173	4.018	4.29563	5.47446	6.11871	0.0261
NONMMUG03	chr7:19723440-7362.1	5.45169	4.8072	3.0045	3.87858	2.6992	2.68637	0.0114	Prp2	intron	2509	14.241	15.403	12.26	10.8904	12.4409	7.85044	0.00115
NONMMUG03	chr19:71571024-4070.2	1.46764	1.40588	1.33053	1.0785	0.989604	0.765504	0.0219	Prp2	UTR	1368	2.0557	1.9414	1.867	1.32762	1.61836	1.63889	0.01115
NONMMUG03	chr6:128524201-5647.1	5.50496	2.70966	2.18377	4.13463	1.53071	1.46193	0.0208	Prp	intron	1221	443.94	492.72	774.8	203.179	352.571	287.431	0.00005
NONMMUG02	chr13:10716483-5420.2	1.94745	4.55111	2.47538	10.8471	5.3106	4.62081	0.0225	Rab1	intron	1528	14.446	22.018	26.44	31.0873	29.8253	27.7433	0.40955
NONMMUG02	chr2:173701564-5756.2	14.0025	7.34998	9.91189	8.65906	6.78157	6.08977	0.02245	Rab22a	Downstr	4201	5.5611	3.8052	8.958	4.55012	6.05735	4.61267	0.1044
NONMMUG03	chr4:137508755-1068.2	0	0	0	1.87035	2.59745	2.59745	0.00005	Rap1a	intron	1437	0.2358	0.5338	0.172	2.01096	2.51775	2.55059	0.00005
NONMMUG01	chr14:13430655-2260.2	0.27404	0.49680	0.59127	3.09116	3.0477	3.88076	0.00005	Rarb	UTR	1438	0.4285	0.4963	0.507	3.7419	4.37324	4.98301	0.00005
NONMMUG03	chr4:130274133-1566.2	14.0247	17.1076	14.9838	33.2913	36.379	24.9966	0.04360	Rer1	intron	1003	29.728	38.193	32.13	62.0573	37.4008	55.5239	0.0172
NONMMUG00	chr19:8501041-65040137	1.20915	1.44392	1.20094	1.20258	0.606373	0.835127	0.02135	RicBb	Downstr	9903	0.9479	1.4485	1.180	0.89857	0.79387	0.99960	0.0065
NONMMUG02	chr13:17965326-1794.2	2.05087	2.82431	1.62046	8.81853	5.03898	8.44951	0.00005	Ripk3	Downstr	4555	1.7672	1.4991	1.818	2.60796	1.50972	1.59505	0.8183
NONMMUG02	chr19:4382259-5594.1	1.22383	3.28841	0.50617	0.201021	0.525883	0.74158	0.0388	Rps68b2	intron	494	5.9052	3.8721	4.090	5.38683	6.73822	4.68997	0.7628
NONMMUG01	chr13:17965326-1794.2	2.99003	1.06003	1.0513	1.10575	0.593687	2.06049	0.0217	Rrebb1	Exon/CD	1089	2.3735	2.7025	3.071	2.51823	4.3887	2.72024	0.7638
NONMMUG04	chr5:102986200-6052.2	16.1123	12.1473	16.1351	4.93057	9.38039	6.29944	0.00115	Rp3	intron	2711	28.075	15.380	16.88	10.9568	11.4244	8.37255	0.00005
NONMMUG02	chr13:130431309-7634.2	1.41804	1.26074	1.7028	0.794999	0.936601	1.19484	0.0338	Rrnc1	Exon/CD	9144	2.1510	2.3889	3.015	1.42527	1.91274	2.00076	0.0039
NONMMUG02	chr13:13710402-7718.1	4.88734	4.69265	6.40137	6.80734	1.74611	1.52347	0.03125	S1pr1	Upstream	3644	20.070	20.765	26.68	15.045	10.9573	11.8382	0.00005
NONMMUG03	chr7:46751872-7830.1	129.269	7.06081	218.321	111.798	48.9954	52.5099	0.0002	Saa2	intron	1838	6736.0	164.39	6447	1899.62	1020.04	1128.38	0.00005
NONMMUG03	chr7:46751872-7830.1	51.7024	18.7651	103.57	11.7518	12.6769	11.1829	0.00005	Saa3	intron	3160	25.066	27.078	293.0	13.816	8.08449	7.27573	0.00005

NONMMUG01	chr17:1329709-134811	1.43945	1.26102	1.34622	6.12425	1.79395	1.89361	0.0385	Sk22a1	Intron	9762	146.85	125.45	135.3	214.87	233.917	199.881	0.1297
NONMMUG02	chr7:12065740-130067876	0.22693	0.81571	0.34475	2.58917	2.127	2.67017	0.00005	Sk23a2	Exon/CD	232	0.2117	0.5128	1.083	3.16662	8.16657	7.12627	0.00005
NONMMUG03	chr7:141429751-141438141	10.6128	9.90621	12.163	4.73851	4.94737	3.83899	0.00006	Sk25a22	Intron	302	26.652	26.242	36.38	12.2279	14.564	10.6035	0.00001
NONMMUG07	chr7:12961123-12967029	21.4903	25.8136	13.4094	10.3984	11.3568	9.41545	0.01155	Sk27a6	Intron	1274	265.64	313.09	275.7	111.489	145.651	95.618	0.00005
NONMMUG00	chr1:185466810-185468190	3.88542	7.58961	6.73652	4.49983	4.05129	2.12181	0.00155	Sk30a10	UTR	1298	3.3735	6.2862	6.228	9.12167	4.16034	2.33423	0.00005
NONMMUG04	chr8:12477997-72481809	3.48274	3.64145	6.05758	2.86234	2.75421	2.60232	0.0021	Sk35a1	Downstr	1271	5.4342	5.5356	5.222	3.12571	5.42661	4.25577	0.00005
NONMMUG04	chr9:10745724-10762242	300.352	243.282	254.471	158.843	130.699	116.941	0.00005	Sk38a3	Downstr	9645	290.16	261.08	359.5	143.959	200.305	146.034	0.00005
NONMMUG00	chr10:82120840-83223717	6.69237	5.24504	20.2885	7.19457	5.90921	10.117	0.01695	Sk41a2	UTR	1056	4.4462	3.378	15.27	3.69024	4.662	6.61895	0.001
NONMMUG03	chr6:143191077-143192192	0	0	0	10.2391	0	2.45683	0.0423	Skc1a1	Intron	3162	12.507	33.923	28.02	1.04365	2.16825	0.42579	0.00005
NONMMUG04	chr9:103088218-103090448	3.63339	1.64117	1.60493	1.87139	1.47403	1.59621	0.03385	Skc2a1	Intron	8083	24.318	21.047	20.88	13.9082	22.1448	15.4088	0.001
NONMMUG02	chr19:26712331-30720438	0.54805	0.98060	1.48875	4.10516	1.58484	2.46037	0.01805	Smara2	Intron	1093	8.9680	8.9113	5.560	16.4787	11.5501	13.3983	0.1228
NONMMUG00	chr10:128488399-128490587	12.7022	13.2188	11.1167	11.4297	8.30763	9.10389	0.02815	Smara2	UTR	8065	8.6964	10.279	12.45	6.3384	10.7703	8.7602	0.0094
NONMMUG00	chr11:160787252-160788198	3.3949	4.4842	6.11246	2.40192	2.881	3.18288	0.01145	Smcra	UTR	1024	2.2180	2.9230	5.028	1.40139	2.61301	2.45086	0.00075
NONMMUG01	chr14:31112841-11128905	44.2074	22.0425	27.7673	17.9402	11.311	15.8471	0.0057	Smcra	Exon/CD	120	17.504	15.388	14.16	7.59216	7.62624	8.11531	0.11755
NONMMUG02	chr19:40239889-402399407	0.76816	1.92923	1.61578	2.96301	4.1141	3.64363	0.03905	Sorba1	Downstr	1075	2.9418	4.3277	3.536	4.20455	7.21551	5.51898	0.1442
NONMMUG01	chr15:99211075-99211214	2.20154	1.75557	2.1817	5.81903	3.51204	3.94764	0.03735	Spa2a	Exon/CD	8556	0.7826	1.015	1.768	2.40081	3.39973	2.65273	0.0025
NONMMUG00	chr12:14645404-16464119	9.33183	18.3882	12.4409	35.4242	18.4894	21.2846	0.04505	Spta5a	UTR	1080	6.2668	12.329	8.691	19.797	14.3284	13.4496	0.02805
NONMMUG02	chr2:181203452-181229688	5.51967	5.60877	4.54389	3.85663	3.63175	3.63175	0.04115	Srma	UTR	1390	-	-	-	-	-	-	-
NONMMUG04	chr9:15118338-16123034	0.39505	0.64337	1.34884	2.69371	1.31955	1.76481	0.02455	Srpr	Intron	4547	75.094	55.421	74.58	68.4792	88.7458	79.6792	0.66805
NONMMUG02	chr3:63739663-63761765	58.7087	63.9314	58.5996	137.38	143.466	133.396	0.001	Su3	UTR	1190	47.745	55.277	53.79	111.726	134.105	137.39	0.00005
NONMMUG03	chr4:117975035-11798071	0	0	0	4.46756	5.44638	5.30811	0.00005	Sdga3	Intron	1503	17.648	16.426	26.48	12.7405	18.7419	9.45418	0.0001
NONMMUG03	chr7:15413089-14411717	36.3767	90.9624	65.0683	6.53691	3.62135	3.62135	0.00005	Su2a2a	UTR	2734	32.784	83.303	70.59	6.75767	7.71236	6.58203	0.00005
NONMMUG00	chr11:18285225-18289639	11.4348	9.77981	13.1757	0.717017	0.908127	0.31992	0.00005	Sud4a	Intron	1295	8.1100	7.613	13.03	0.36059	1.31333	0.21149	0.00005
NONMMUG03	chr10:108979633-10898186	1.40514	3.21493	2.03866	6.03213	7.60699	6.39176	0.0001	Sy1	UTR	5124	0.8521	1.8052	1.302	3.12023	4.64286	3.76938	0.00005
NONMMUG04	chr10:10898495-15200541	3.43611	2.12605	1.49546	2.00782	1.72517	0.22907	0.0212	Tacc1	Intron	1431	3.9872	4.3420	4.099	3.58125	3.90925	3.57894	0.0215
NONMMUG03	chr7:13006262-13008345	2.12447	1.37501	1.30639	1.23215	1.16003	1.34176	0.04745	Tacc2a	Intron	1098	4.8113	2.9131	15.88	2.89025	5.44189	3.17354	0.00005
NONMMUG00	chr10:109002676-12006844	2.15998	1.17814	0.83489	0.88884	0.750544	0.59644	0.0217	Tacc1	UTR	101	4.3186	5.1725	4.706	3.16883	6.4843	4.16473	0.1737
NONMMUG03	chr5:117130322-11713248	2.70151	10.1999	0.50320	1.38448	0.495106	0.657046	0.0106	Taxk3	Intron	1484	3.9508	4.5299	5.796	4.88702	6.88849	5.00043	0.7728
NONMMUG03	chr4:15614535-148516058	6.75219	6.08007	3.63558	2.61678	1.51126	2.44906	0.0001	Tardap	Intron	1146	2.1041	2.9505	2.214	3.01164	2.54337	2.31651	0.83335
NONMMUG04	chr8:109886251-109986755	5.25378	0	57.4704	0	0	0	0.00005	Tat	UTR	4034	407.44	304.62	320.4	179.968	199.103	186.804	0.00005
NONMMUG00	chr13:121453447-119145945	2.84027	2.57744	1.27423	1.34289	1.17531	0.63348	0.00025	Tbc1d16	UTR	8405	-	-	-	-	-	-	-
NONMMUG04	chr9:90202962-90209162	4.90881	4.48031	4.86297	4.03395	2.69074	3.43097	0.018	Tbc1d26	UTR	6766	2.7821	2.5136	3.379	1.85072	2.62518	2.4498	0.00685
NONMMUG04	chr9:94423943-42435131	10.4771	7.9322	5.79076	23.3778	21.9912	23.961	0.00005	Tbccl	UTR	4807	4.8889	4.0022	2.891	11.0004	11.7447	12.4694	0.00005
NONMMUG03	chr5:175815117-13794980	1.06133	1.15988	1.00347	0.988243	1.13767	1.02446	0.0002	Tb2	UTR	57	78.891	60.257	75.81	28.0447	41.2211	26.6743	0.00005
NONMMUG03	chr6:7113974-7113973	1.1291	0.71292	0.40247	2.17093	2.70179	2.32139	0.00035	Tbnc1	Intron	9893	13.331	11.445	25.75	36.3682	42.6954	35.5059	0.00005
NONMMUG01	chr14:12199345-12921486	0.37470	1.37504	0.28759	2.43365	1.16325	1.68717	0.0387	Tbnc23	Intron	1953	32.925	36.067	39.48	46.3159	46.2225	32.9656	0.5499
NONMMUG04	chr5:16044295-35186930	5.64365	4.24557	2.52406	3.88251	2.23764	3.57687	0.00325	Tbap	UTR	1462	4.6770	4.0065	6.151	3.75464	3.00626	2.74719	0.00005
NONMMUG03	chr15:95048053-39941048	509.072	461.134	393.052	401.156	351.549	282.597	0.0353	Tbnc1a	Downstr	9475	379.90	375.37	338.4	288.763	307.012	237.425	0.0065
NONMMUG02	chr10:10870863-10871074	5.58687	4.51755	3.57469	11.2572	9.2766	11.6799	0.0264	Tbnc129	UTR	1113	7.4035	6.5103	5.138	9.30572	11.8509	11.3579	0.00945
NONMMUG03	chr14:10873551-10873751	0	0	0	0	18.818	9.13175	0.0397	Tbnc130	Downstr	8299	2.1195	1.6499	1.730	2.10173	1.87925	1.29426	0.223
NONMMUG02	chr19:10891127-10860508	1.09185	2.07039	2.67802	0.780206	1.39983	0.83051	0.01095	Tbnc112a	Intron	1011	6	4	25	-	-	-	-
NONMMUG02	chr2:167426127-167494189	6.88739	2.49241	3.01142	1.20393	2.17562	1.01763	0.00319	Tbnc139	UTR	1864	4.9166	4.3598	5.094	1.43439	2.48328	2.04405	0.00005
NONMMUG03	chr6:15042125-10452139	1.36703	1.02096	0.85383	2.66951	2.53006	2.45451	0.0269	Tbnc129	Intron	2791	1.9942	1.3829	1.037	2.6959	4.56795	4.44642	0.00025
NONMMUG02	chr13:12120212-12120782	3.79411	8.61849	5.05405	15.1052	11.6562	10.1717	0.00735	Tbnc156	Intron	8023	2.8977	7.4429	6.165	10.2781	16.7277	11.8323	0.00005
NONMMUG03	chr4:14151850-141616000	0.85021	0.50791	0.67437	2.58452	0.556515	0.0334	Tbnc162	Intron	8704	3.4268	3.4238	4.405	5.38334	6.24623	4.47129	0.36615	
NONMMUG00	chr10:11100418-11168002	1.64991	1.93105	1.1445	4.90955	3.12617	5.08489	0.00255	Tbnc1	Intron	1055	3.0055	3.4020	3.454	8.26224	10.6099	10.1413	0.00005
NONMMUG01	chr15:79438745-78439301	0.57236	0.96730	2.20221	3.30871	4.08879	2.74467	0.03365	Tbnc1a6	Downstr	2963	56.212	62.537	78.97	23.6465	48.3704	21.7379	0.00005
NONMMUG03	chr4:156010843-156013614	3.11111	3.67985	3.87776	2.66351	2.36743	2.09138	0.05045	Tbnc1a4	UTR	1540	-	-	-	-	-	-	-
NONMMUG00	chr1:156010613-156007303	6.06312	6.45432	3.21728	4.79116	6.83007	4.9889	0.0303	Tbnc1a5	UTR	2895	5.7310	6.5997	9.806	5.28127	8.27745	6.50418	0.1747
NONMMUG01	chr13:14702825-47021286	5.79665	7.80699	5.0214	19.0909	16.2589	13.4351	0.00015	Tjnc1	Exon/CD	1775	6.7001	9.1342	6.641	23.0398	21.014	20.3004	0.00005
NONMMUG04	chr9:14665868-14667735	5.62472	4.02569	2.55195	8.31489	7.08499	8.42783	0.04435	Tlnc1	UTR	2194	1.9420	1.0336	0.789	1.11969	1.64147	1.53205	0.5961
NONMMUG03	chr4:46023958-46024012	0.79603	1.10759	0.82371	2.83775	1.21482	1.71657	0.02505	Trin14	Intron	9435	10.343	12.295	8.098	14.1399	19.6452	16.516	0.0829
NONMMUG00	chr11:18657473-18678051	0.04861	1.5028	0.43293	5.42415	1.84544	1.2206	0.01965	Trin17	UTR	8097	-	-	-	-	-	-	-
NONMMUG00	chr11:18724855-18725224	0	0	0	0	4.62273	4.52395	0.0004	Uck2	UTR	6023	1.8178	1.7579	2.430	3.38737	6.84474	5.4278	0.00085
NONMMUG04	chr9:10810976-1081177	1.01167	1.79621	1.74405	4.01495	2.19797	3.16082	0.0392	Utpa4	UTR	7571	1.5756	2.5833	2.625	3.88386	4.53651	4.12973	0.01063
NONMMUG02	chr9:140539536-14661248	27.7026	24.439	8.44424	9.53005	4.58	5.91361	0.00005	Txc210	Exon/CD	2591	22.534	21.					

NORMAMUG0	chv17-0362321-7952.2	4.89713	1.24757	0.65204 9	2.48856	0.066217	8.78805	0.0177	Zmnd9	Intron	1585 61	6.7522 4	9.9308	11.76 11	9.11463	16.1359	7.12028	0.79185
NORMAMUG0	chv2-170109050-1702047.2	1.16672	1.54719	2.73893	6.90325	4.67795	5.48692	0.0381	Zfp217	FUTR	2196	0.9633	0.8383	1.239	1.3739	1.62796	1.13103	0.2947
NORMAMUG0	chv10-76040610-4013.2	6.60410	0.54356	1.2507	1.66379	2.12668	-	0.01015	Zfp308b	FUTR	9045	0.9623	0.7514	1.09	1.07756	1.90193	1.77254	0.0546
NORMAMUG0	chv1-734139849-4039077.1	6.72341	4.3586	1.47199	3.11201	2.85934	2.55888	0.021	Zfp395	FUTR	3923	3.9296	2.8401	1.224	1.81358	2.31671	2.23451	0.00635
NORMAMUG0	chv6-20505916-0186.2	3.54611	3.43621	4.64805	1.92979	2.85021	2.25092	0.0489	Zfp703	FUTR	3852	2.9832	1.7505	2.931	1.22388	1.8836	1.26689	0.00005
NORMAMUG0	chv5-22430341-22430341.0	1.15802	1.95597	3.64454	4.78114	3.74421	4.33046	0.0399	Zfp80b	Intron	1450	0.1031	4.733	6.441	8.99235	7.97876	6.46899	0.7622
NORMAMUG0	chv6-12681846-0459.2	0.81043	1.15485	0.82757	2.06275	2.04282	4.45701	0.01035	Zmyr4	FUTR	1450	0.6059	0.6000	0.626	0.94284	1.75096	1.72677	0.0484
NORMAMUG0	chv2-2495945-2495945.0	2.90239	2.15814	2.48708	1.56192	1.74993	1.28409	0.0113	Zmyrd19	Downtst acc	1000	0.9453	0.9690	46	1.52696	1.72465	0.82825	0.40335
NORMAMUG0	chv1-130808030-1376.2	112.248	140.399	107.88	38.744	33.0995	23.0381	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-114487061-806.2	22.8413	19.8939	10.769	52.4441	49.7853	57.403	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv11-109011639-7938.2	13.1307	33.0204	10.5799	14.0905	53.7604	28.1119	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-140135767-3358.2	13.8568	34.189	31.3848	4.39676	3.92906	1.86749	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv17-31132791-7989.2	1.57912	1.53187	1.54749	25.291	28.4795	22.9988	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv6-128802057-2951.1	15.8335	12.3651	19.0347	2.72989	9.76432	4.57279	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv2-3736669-3044.2	2.06327	6.74156	5.89738	14.8214	16.3125	15.066	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-6240685-6729.2	10.0487	7.79723	25.4146	5.57469	4.58326	7.11899	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv17-34722609-8127.1	20.9419	7.71229	15.849	2.73235	2.43226	1.60981	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv6-118142754-9880.2	9.3783	3.10903	3.50975	11.8491	12.6779	14.8338	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-8208111-5082.2	8.966	12.8819	11.0806	5.31634	5.54542	2.99511	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv12-10594714-9817.2	2.38113	2.26225	29.2189	2.88774	3.49465	3.41295	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv2-10734327-3079.2	6.36153	5.99605	10.7005	2.64316	2.85085	2.77787	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv7-124534907-124539061	1.38212	2.89037	2.3972	8.57656	8.1692	7.73024	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv2-72224887-72224887.0	1.6053	1.97009	2.7503	8.06513	7.22619	7.22194	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv18-46623890-9866.2	5.46905	8.38633	7.9306	0.144153	0.44443	1.08019	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-93058446-3614.2	13.2427	4.31591	2.72809	0.905038	0.189953	0.0476285	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv14-65763205-3246.2	1.61502	2.02054	11.8446	0.753182	1.12378	0.438921	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-109635215-7943.1	2.75973	8.01112	2.2949	2.40142	1.89712	0.917778	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-804842139-6997.2	0	0	0	0	3.81783	4.54909	4.56279	0.00005	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-128911187-5073.2	7.25993	1.91127	1.7142	0.911689	0.193703	0.81209	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv10-127878178-5084.1	5.96672	2.27158	1.23395	0	0	0	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv6-10736117-1520.2	0.20204	0.64109	0.35413 5	2.46416	2.24013	3.56111	0.00005	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-830803106-1515.2	15.2058	17.5071	13.6154	5.20717	11.2526	7.78208	0.0001	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv2-167979316-5595.1	4.93088	2.77569	10.548	2.65961	3.44003	2.28031	0.0001	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv2-133247111-4705.2	1.67907	2.39466	2.39814	3.25783	7.60606	7.10227	0.0001	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv6-124574539-1623.1	2.32369	2.70977	3.7448	1.5799	1.20452	0.646992	0.0001	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv2-72221625-72221625.0	0.37174	0.72755	1.1398	1.29715	4.031	3.43437	0.0001	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv17-32502616-8074.1	5.38413	7.03226	5.42708	3.47133	4.32307	1.51025	0.00015	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-101466160-5987.2	4.13379	3.08311	1.84509	1.32303	0.95811	1.35052	0.00015	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv14-32117999-7760.1	7.95785	11.8237	19.4902	5.35687	5.12443	6.71197	0.0002	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-879736070-4363.1	5.3113	8.44411	8.8943	6.04012	3.46643	1.77022	0.0002	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv9-11047611-6191.1	52.6373	62.8293	78.7007	50.6468	31.176	25.9807	0.00025	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv7-79963807-8439.2	17.3951	24.0294	21.8552	51.2766	48.69	53.6588	0.00025	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv15-75427605-0873.1	8.73008	3.97591	6.57837	23.1836	19.1386	14.8921	0.00025	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-136114037-8069.2	0.17976	0.45135 3	0.79972	2.46395	2.55676	2.33899	0.00025	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-16808791-5382.1	1.69089	2.89738	2.88975	0.560552	0.748189	0.939884	0.0003	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-209070500-9814.1	4.16089	0.95538	1.30314	0.059475	0.0769378	0.0418798	0.0003	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv6-18801607-0052.1	0.10666	0.97706	0.22964	2.79805	1.80623	1.94598	0.0004	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv4-53355-36294-4358.2	2578.69	2186.15	1638.66	1051.63	807.27	761.362	0.00045	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv15-77384941-4884.2	6.28103	3.73293	1.25121	0.504393	0.516084	0.222074	0.0008	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv6-94596798-1326.2	2.60057	1.2253	0.32096	0.10993	0.113747	0.237381	0.0009	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv2-26136778-2610559	55.7832	38.1617	26.9775	28.1188	24.6978	21.7584	0.001	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv10-93076657-4122.2	3.35506	3.73422	1.50803	13.5842	7.1064	8.09446	0.0016	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-79718547-376.1	0	0	0	0	2.40345	3.80001	0	0.001	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv7-48877948-8051.2	9.14992	11.4905	17.1395	8.2923	5.77724	7.35195	0.00105	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-21125160-945.1	8.75862	3.83693	12.622	4.8979	4.32764	4.71418	0.00115	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-31113294-2018.2	8.34749	3.48802	4.14148	2.02978	2.13398	0.830207	0.0015	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-43719001-1419.1	6.7491	7.18387	2.0088	27.3156	6.89664	17.6425	0.00125	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-129633232-1589.1	0	0	0	0	2.34885	4.93835	14.514	0.00145	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-21876654-1325.1	6.30366	5.19147	3.1034	16.2003	7.54033	14.1646	0.0016	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-45311440-2140.2	1.64305	3.16225	1.42047	6.84189	4.57664	5.00392	0.0017	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv15-26129213-1324.2	0.23707	0.09289	0.02382 36	5.17664	4.95571	4.08405	0.0017	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv10-111339050-4891.2	0.99901	0.76302	5.4894	6.74501	2.81808	1.97638	0.0018	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-138149675-2502.2	1.37305	0.71651	0.77711	6.60806	16.2113	8.8628	0.00185	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-131930883-1595.2	4.64791	12.2164	8.83903	18.0174	21.7382	17.9732	0.00195	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv13-40596413-0775.2	3.86411	3.91627	4.02024	1.75484	2.91506	1.85977	0.00195	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv1-13046468-13046468.0	0.35994	1.64272	1.91059	5.15479	3.64548	2.83841	0.00245	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv7-35137253-7743.2	4.86084	3.26624	2.58812	1.26271	1.03355	0.554115	0.00245	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0	chv5-13564234-4012.2	3.388	2.04106	2.99921	4.77189	9.38609	5.60337	0.00265	#N/A	#N/A	#N/A	-	-	-	-	-	-	-
NORMAMUG0																		

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Supplemental Table 3**Table 4.S3.** Overlay between PXR-DNA binding and epigenetic marks around lncRNA gene loci on chromosomes 5, 12, and 15.

Epigenetic marks	# lncRNAs not expressed	# lncRNAs stably expressed	# lncRNAs increased by PCN	# lncRNAs decreased by PCN
H3K4me2	1680	174	9	13
H3K27me3	303	1	0	0
5MeC	1234	67	2	0
PXR	8257	753	45	70
PXR & H3K4me2	500	62	6	9
PXR & H3K27me3	11	0	0	0
PXR & 5MeC	121	9	0	0

Chapter 5: Conclusions and Future Directions

5.1 Ontogenic Mechanisms for the Regulation of Hepatic Drug Metabolism Through Host- and Gut Microbiome-Derived Bile Acids

Liver is a critical organ for nutrient homeostasis and xenobiotic biotransformation. The gut microbiome regulates important host metabolic pathways in liver including xenobiotic metabolism and intermediary metabolism, such as the conversion of primary bile acids (BAs) into secondary BAs and is an important factor of healthy development. However, little is known regarding the age specificity of secondary BA metabolism or other microbial metabolites. To test the hypothesis that age-specific bacteria produce unique patterns of secondary BAs during development that may regulate xenobiotic metabolism, various tissues were isolated from male and female conventional (CV) and germ-free (GF) mice at various ages: days 1, 5, 10, 15, 25, 60, and 120. 16S rRNA gene sequencing was performed on large intestinal DNA. BAs were quantified by LC-MS in serum, liver, ileum, and large intestine, and short-chain fatty acids (SCFAs) were quantified in liver by GC-MS. Expression of hepatic genes was quantified by RNA-Seq of total RNA. With increasing age, the gut microbiome in mice become more diverse and had increased functional capacity to produce secondary BAs, including the family *S24-7*. Twenty-seven bacteria, primarily present in day 120 mice, were positively associated with secondary unconjugated, deoxy, and keto BAs with 9 of these BAs only produced in CV mice. T-DCA and ω MCA were detected only in the liver of CV mice, suggesting they may act as a feedback signaling molecule from bacteria. SCFAs were also only found in livers of CV mice with six SCFAs increasing with age. Global analysis of hepatic RNA expression showed an age-specific enrichment of KEGG pathways, including xenobiotic metabolism for adult CV and GF mice (days 25, 60, and 120). Differentially expressed drug-metabolizing enzymes and transporters exhibited age and sex specific expression patterns with more genes differentially expressed with increasing age. Overall, the age-specific gut microbiome impacts host xenobiotic metabolism likely through circulation of metabolites (e.g. BAs and SCFAs).

5.2 Pharmacological Activation of PXR and CAR Down-Regulates Distinct Bile Acid-Metabolizing Intestinal Bacteria and Alters Bile Acid Homeostasis

The gut microbiome regulates important host metabolic pathways including xenobiotic metabolism and intermediary metabolism, such as the conversion of primary BAs into secondary BAs. The nuclear receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR) are well-known regulators for xenobiotic biotransformation in liver. However, little is known regarding the potential effects of PXR and CAR on the composition and function of the gut microbiome. To test our hypothesis that activation of PXR and CAR regulates gut microbiota and secondary BA synthesis, nine-week-old male conventional (CV) and germ-free (GF) mice were orally gavaged with corn oil, PXR agonist PCN (75 mg/kg), or CAR agonist TCPOBOP (3 mg/kg) once daily for four days. PCN and TCPOBOP decreased two taxa in the *Bifidobacterium* genus, which corresponded with decreased gene abundance of the BA-deconjugating enzyme bile salt hydrolase. In liver and small intestinal content (SIC) of GF mice, there was a TCPOBOP-mediated increase in total, primary, and conjugated BAs corresponding with increased Cyp7a1 mRNA. *Bifidobacterium*, *Dorea*, *Peptococcaceae*, *Anaeroplasma*, and *Ruminococcus* positively correlated with T-UDCA in LIC, but negatively correlated with T-CDCA in serum. In conclusion, PXR and CAR activation down-regulates BA-metabolizing bacteria in the intestine and modulates BA homeostasis in a gut microbiota-dependent manner.

5.3 Regulation of Hepatic Long Non-coding RNAs by PXR and CAR Agonists in Mouse Liver

Altered expression of lncRNAs by environmental chemicals modulates the expression of xenobiotic biotransformation related genes and may serve as therapeutic targets and novel biomarkers of exposure. The pregnane X receptor (PXR/NR1I2) is a critical xenobiotic-sensing nuclear receptor that regulates the expression of many drug-processing genes, and it has similar

target gene profiles and DNA binding motifs with another xenobiotic-sensing nuclear receptor, namely constitutive androstane receptor (CAR/Nr1i3). To test our hypothesis that lncRNAs are regulated by PXR in concert with protein-coding genes (PCGs) and to compare the PXR-targeted lncRNAs with CAR-targeted lncRNAs, RNA-Seq was performed from livers of adult male C57BL/6 mice treated with corn oil, the PXR agonist PCN, or the CAR agonist TCPOBOP. Among 125,680 known lncRNAs, 3,843 were expressed in liver and 193 were differentially regulated by PXR (among which 40% were also regulated by CAR). The majority of PXR- or CAR-regulated lncRNAs were mapped to the introns and 3'-UTRs of PCGs as well as intergenic regions. Combining the RNA-Seq data with a published PXR ChIP-Seq dataset (Cui et al., 2010b), we identified 774 expressed lncRNAs with direct PXR-DNA binding sites, and 26.8% of differentially expressed lncRNAs had changes in PXR-DNA binding following PCN exposure. *De novo* motif analysis identified co-localization of PXR with LRH-1, which regulates BA synthesis, following PCN exposure. There was limited overlap of PXR binding with an epigenetic mark for transcriptional activation (histone-H3K4-di-methylation, H3K4me2), but no overlap with epigenetic marks for transcriptional silencing (H3K27me3 and DNA methylation). Among differentially expressed lncRNAs, 264 were in proximity of PCGs, and the lncRNA-PCG pairs displayed a high co-regulatory pattern by PXR and CAR activation. This study was among the first to demonstrate that lncRNAs are regulated by PXR and CAR activation and that they may be important regulators of PCGs involved xenobiotic metabolism.

5.4 Summary and Future Directions

These three studies showed that 1) an age-specific gut microbiome impacts host xenobiotic metabolism through BAs; 2) activation of host xenobiotic-sensing nuclear receptors PXR and CAR modulate BA homeostasis and BA metabolizing bacteria; and 3) PXR and CAR activation can regulate lncRNAs that may be important in the regulation of some xenobiotic metabolizing enzymes and transporters. In these works, my central hypothesis was that the age-

dependent interaction of the gut-liver axis is governed by BA homeostasis in a circulatory pattern that ultimately modulates the expression of hepatic drug processing genes (Figure 5.1). Primary BAs produced in the liver modulated the gut microbiome. This was demonstrated by acute activation of PXR and CAR in the liver altering the gut microbiome composition and its function (decreased *bsh*). In turn, the secondary bile acids produced by the gut microbiome altered signaling in the liver, affecting bile acid synthesis. Together, both the liver and gut microbiome are modulated by host development as demonstrated by the change in gut microbiome composition with age which corresponded to differences in age-specific bile acids between CV and GF mice. Because bile acids can interact with nuclear receptors and transcription factors that regulate DPGs, altered bile acid homeostasis with age in part regulated expression of DPGs.

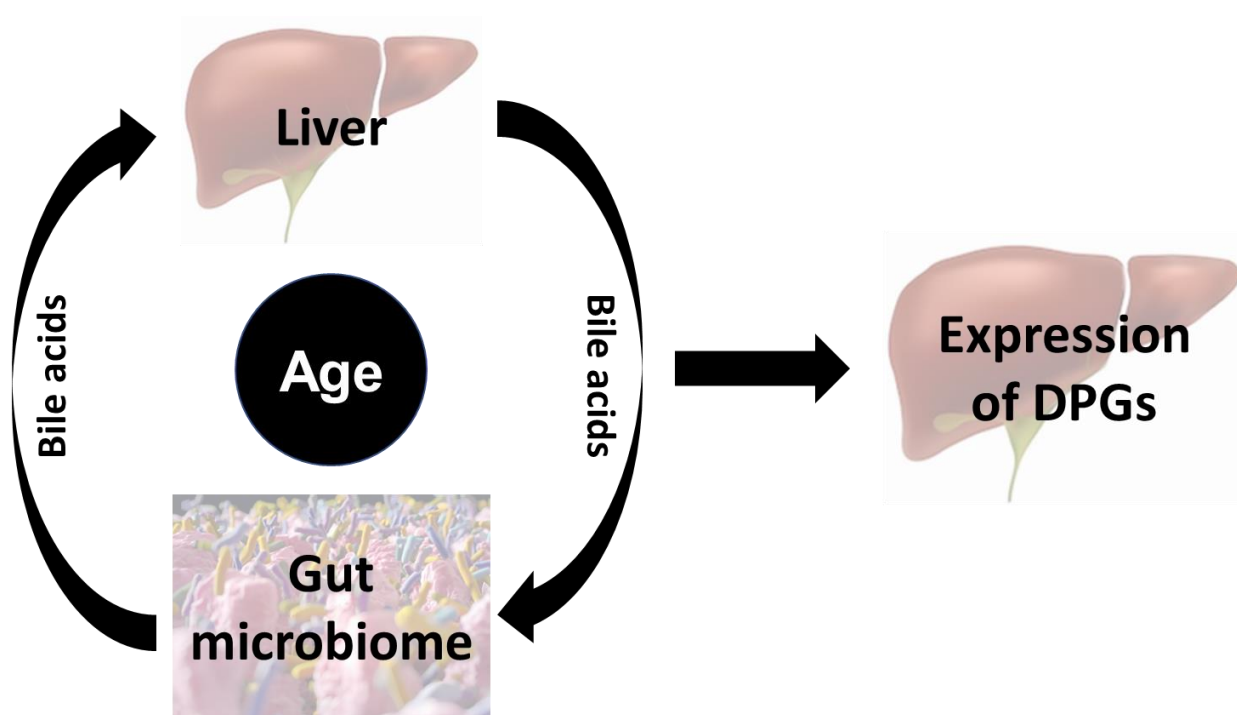


Figure 5.1. A diagram illustrating the central hypothesis for these studies.

There are several limitations to the conclusion that the expression of DPGs is regulated by an age-dependent gut-liver axis and BA homeostasis. Metabolites other than BAs may be age- and microbiome-dependent and interact with both the host liver, intestine, and microbiota. The gut microbiome produces SCFAs, which may indirectly affect xenobiotic metabolism. SCFAs act

as ligands for GPR41 and GPR43 and modify histone acetylation that impacts signaling of the transcription factor AhR (Boffa et al., 1978; Sealy and Chalkley, 1978; Siavoshian et al., 2000). However, SCFAs are produced from fiber in the diet and do not occur as part of an endogenous interactions, which differs from BAs. Further research is needed to investigate other key families of metabolites produced by the host and metabolized by bacteria that affect the regulation of DPGs.

It remains unclear how alterations in BA profiles by bacteria may affect the expression of DPGs. Feeding of BAs to CV mice changed signaling profiles that regulated BA homeostasis (Song et al., 2015). LCA metabolites and its derivatives can activate PXR (Staudinger et al., 2001; Ishii et al., 2012), indicating that a modified BA pool would impact drug metabolism and disposition. Therefore, increased the production of LCA or other bile acids that alter the expression of DPGs could occur through manipulation of the microbiome. In this study, the family *S24-7* was predicted to be a driver in secondary bile acid synthesis in adult mice, yet there are no known BA metabolizing enzymes in the *S24-7* genome. This suggests that bacteria may have unknown genes that metabolize bacteria. Culturing and exposing bacteria to primary BAs (conjugated and unconjugated) followed by metabolomics of BAs will identify which predicted bacteria have BA metabolizing capabilities. In addition, metatranscriptomics of naïve cultures compared to BA-exposed cultures would show which microbial genes are regulated in response to bile acid and could be further tested for BA metabolizing capabilities.

Changes in the expression of DPGs was dependent on both BAs and the microbiome, but it is unclear to what extent those changes may be dependent on the microbiome only. It is necessary to understand the extent the microbiome modifies the regulation of DPGs at each age. To investigate how the microbiome at a specific age of development modifies the expression of DPGs, adult GF mice could be exposed to an age-specific microbiome—feces or intestinal contents of CV mice or humans at development ages—and allowed to form a homeostasis before

investigating the regulation of gene expression. This will begin to show how a neonatal microbiome modifies hepatic DPGs differently from an adult microbiome.

The multi-omics (metagenomics, metabolomics, and transcriptomics) approach used to investigate the gut microbiome-mediated and age-dependent effects that modulate xenobiotic metabolism were effective. One caveat was that multi-omics studies require follow-up experiments to verify the mechanisms found in these studies. For example, functional analysis of the family *S24-7* is suggested to metabolize BAs, yet there are no known genes within the current *S24-7* genome that metabolize BAs. *S24-7* was positively associated with secondary unconjugated, deoxy, and keto BAs and is known to produce SCFAs. However, *S24-7* is highly present in the adult murine gut and will positively associate with any metabolite that occurs primarily at an adult age. Regardless, because *S24-7* is present in humans and is a dominant family in the murine gut, it is necessary to understand the possible functions of *S24-7* species – individually and within the gut microbiome community. This will provide insight into the health effects of the gut microbiome and if *S24-7* may be used as a bacteria to stabilize an unhealthy microbiome to a healthy state.

PXR and CAR activation altered the gut microbiome composition, yet these nuclear receptors are also important in the regulation of many DPGs. In addition, activation of PXR and CAR altered the BA homeostasis, although a new steady state for BA homeostasis was not achieved. This demonstrates that even an acute exposure that activates PXR or CAR can alter BAs and gut microbiota. However, it is unclear how modulation of gut microbiome and BAs which are known to affect energy homeostasis, may impact the metabolic system. Future studies should focus on the metabolic and mechanistic effects of BAs modulated by PXR and CAR to identify new therapeutic methods to treat metabolic diseases.

LncRNAs are a method of epigenetic regulation due to their ability to regulate transcription and translation. Activation of PXR and CAR modulated the expression of lncRNAs in the liver, among which some lncRNAs may impact the regulation of DPGs. Yet, it is unknown if the

lncRNAs paired with DPGs can independently regulate transcription and translation of DPGs or if the regulation of the lncRNAs by PXR or CAR is independent of protein-coding genes. It is necessary to do mechanistic studies that independently regulate individual lncRNAs to identify their impact on DPGs. This may provide alternative gene therapy to modulate DPGs to prevent toxicity upon exposure to xenobiotics.

In these studies, it was shown that the expression and function of drug metabolizing enzymes and transporters in the liver are impacted by the gut microbiome through metabolites. However, the mechanisms regulating DPGs by the gut microbiome is not well characterized. For example, the gut microbiome regulates DPGs through metabolites interacting with xenobiotic sensing nuclear receptors. The expression of Cyp3a11—regulated by PXR—is greatly reduced in adult GF mice (Toda et al., 2009b; Toda et al., 2009a). Supplementation of the secondary BA, microbial metabolite, and PXR activator LCA to GF mice can increase and recapitulate, to an extent, the expression of Cyp3a11 to CV levels (Staudinger et al., 2001; Ishii et al., 2012). However, the concentrations of LCA are much higher than physiological levels. Furthermore, GF mice treated with the probiotic VSL3 did not restore the expression of Cyp3a11, but housing GF mice in a conventional setting did restore Cyp3a11 expression (Selwyn et al., 2016). This indicates that a subset of bacteria produce PXR activating metabolites and that activation of PXR to regulate DPGs requires a mixture of microbial metabolites. To investigate which commensal bacteria produce metabolites that regulate xenobiotic metabolism, the media from cultures of single bacteria should be applied to receptor activation assays. Positive cultures could be followed by metabolomics to identify the metabolites. This could be applied to the common DPG regulators (AhR, CAR, PXR, PPAR α , etc.) as well as any other nuclear receptors of interest.

In addition to nuclear receptors, the interaction of the gut microbiome and epigenetics may regulate DPGs, including lncRNAs. Here, it was shown that the expression of some hepatic lncRNAs regulated by PXR and CAR in liver and are associated with DPGs. In HepaRG cells, lncRNAs can control the basal and induced expression of P450s (Chen et al., 2018). The

lncRNAs hepatic nuclear factor (HNF) 1 α anti-sense 1 (HNF1 α -AS1) and HNF4 α -AS1 regulate the expression of some P450s and modify drug induced liver injury (Chen et al., 2018; Chen et al., 2020a; Chen et al., 2020b), suggesting that some lncRNAs may act as intermediaries between nuclear receptors and P450s to regulate DPGs. Because of the regulatory capacity of lncRNAs, it is suggested that lncRNAs be used as a pharmacological target as a therapy for epigenetic diseases (Prabhakar et al., 2017). In addition, the expression of lncRNAs in liver, intestine, fat, and muscle are regulated by the microbiome, in part, when comparing between CV and GF mice (Dempsey et al., 2018). It is possible that microbiome is a cofactor in the regulation of lncRNAs and DPGs as a modifier of an epigenetic mechanism. Studies should continue to examine lncRNAs associated with DPGs and gut microbiome for co-regulatory networks, defining which DPGs and lncRNAs are co-regulated by the microbiome.

Gut microbiome may also affect epigenetic programming during development as well as transgenerational epigenetic programming through epimutations. It is generally accepted that phenotypic diversity can occur in the same genome during development due to exogenous stimuli, such as drugs, food, and other xenobiotics, particularly early in life (Nilsson and Skinner, 2015; Skinner, 2015; Blanco Rodriguez and Camprubi Sanchez, 2019; Bordoni et al., 2019; Gabbianelli et al., 2020). A prominent example is maternal nutrition during gestational development. For humans, *in utero* exposure to famine increased obesity in adult males and in adult females resulted in increased glucose intolerance (Ravelli et al., 1976; Ravelli et al., 1998; Heard and Martienssen, 2014). Alternatively, maternal obesity was related to variations in newborn blood DNA methylation in humans, although it is unclear how this may impact the liver (Sharp et al., 2017). The gut microbiome, which is essential for proper nutrition and development, can be easily modulated due to method of birth, antibiotics, diet, and other xenobiotic exposures. Associations between early life health and microbiome are known, yet the causes or the epigenetic mechanisms have not been identified. At this time, there are no definitive studies demonstrating that the microbiome causes epigenetic programming. Modifying the early life microbiome of mice

and conducting epigenome wide association studies (EWAS) or ChIP-seq could demonstrate this effect (Flanagan, 2015; Birney et al., 2016). It is possible that only a subset of bacteria and their metabolites modify the host epigenome. Metabolomics—both targeted and untargeted techniques—could help identify the metabolites that are unique to microbial species and cause epigenetic modifications. Confirmation of epigenetic modifying capabilities by the microbiome could be explored by exposing GF mice or mice raised with a defined microbiome that are previously naïve to the metabolites of interest. Comparing these treated mice to CV or GF mice will demonstrate which epigenetic sites are malleable to the gut microbiome.

In humans, retrospective cohort studies regarding method of birth, antibiotics, and other known xenobiotic exposures compared to health outcomes could demonstrate the persistent health effects of an altered microbiome, implying epigenetics are programmed by the microbiome (Peng and Zhong, 2015; Wang et al., 2020). Because these studies could demonstrate the epigenetic programming capability of the microbiome through signaling molecules in both humans and mice and affect the xenobiotic metabolism and health, it is hypothesized that probiotics could be used to program a health epigenome (Morovic and Budinoff, 2020). This also has implications for epimutation studies. To promote the health of plants, it has been proposed to identify the soil microbiome around the roots of plants that promote positive epimutations that persist across generations (Corbin et al., 2020). By applying this theory to the gut microbiome in mice, positive, healthy bacteria that regulate epigenetic traits could be used therapeutically. Overall, there needs to be more research into the signaling and epigenetic mechanisms of the host that is partially regulated by the gut microbiome.

This work expanded the current literature regarding the regulation of hepatic xenobiotic metabolism and transport through the interaction of the gut microbiome and the liver. Specifically, these works showed in mice how development of the liver or activation of the xenobiotic sensing nuclear receptors PXR and or CAR modify metabolome signaling gut-liver axis. However, xenobiotic metabolism and transport is not exclusive to the liver; oral exposures first interact with

the metabolic capabilities of the intestines and gut microbiome before being absorbed or transported into the blood for first pass metabolism by liver. For oral exposures, including diet and orally administered drugs, metabolism by the intestines and the gut microbiome are the first barrier before systemic circulation in the body. It is increasingly recognized that the gut microbiome has a significant impact on the metabolism and efficacy of drugs (Clarke et al., 2019). Inhibition of microbial β -glucuronidases improved efficacy and decreased toxicity of cancer drugs, including irinotecan (Wallace et al., 2015; Bhatt et al., 2020). Levodopa for Parkinson's disease and the cardiac drug digoxin can be metabolized by at least *Eggerthella lenta* (Haiser et al., 2013; Maini Rekdal et al., 2019). Beyond drugs, diet affects intestinal drug absorption and inflammation (Ang et al., 2020; Zou et al., 2020). Therefore, it is critical to understand how the gut microbiome metabolizes xenobiotic exposures and how it regulates intestinal xenobiotic metabolism and transport.

Like characterizing the interaction of the gut microbiome and liver during development, the basal interaction of the gut microbiome and intestines needs to be determined. In the intestines of mice, the expression of DPGs varies by location (Fu et al., 2016), and proteomics of the human intestinal sections of non-P450 enzymes demonstrated expression differences by location (Zhang et al., 2020). However, it is unknown to what extent the expression and metabolic capacity of the intestine site is dependent on the proximal bacteria. Similar to expression of DPGs in the intestine, the microbiota are specific to each section of the intestines as characterized in mice (Suzuki and Nachman, 2016), pigs (Crespo-Piazuelo et al., 2018), and humans (Leite et al., 2020). The expression of DPGs in duodenum, jejunum, ileum, and colon is augmented different between CV and GF mice (Fu et al., 2017). In addition, the localization of bacteria along the intestinal tract corresponds to localized metabolomics that interact with the specific intestinal site as shown in non-human primates (Yuan et al., 2020). Therefore, it is important to investigate xenobiotic metabolism and transport in the intestine in combination with bacteria. Animal models

could first be used to understand the localization of bacteria and xenobiotic metabolism capacity followed by application in humans.

To investigate the bacteria-intestine interaction in xenobiotic metabolism and transport, future research must identify those bacteria and microbial genes with the capacity to metabolize xenobiotics (Bisanz et al., 2018; Clarke et al., 2019). Not only is it important to investigate the microbial metabolism of parent xenobiotics, but also host metabolites that are excreted through the bile and intestines. Products of host Phase I and Phase II metabolism can be re-metabolized by the microbiome. For drugs, this could potentially cause re-activation of the metabolite, such as removal of glucuronides from active irinotecan by microbial β -glucuronidases (Wallace et al., 2015; Bhatt et al., 2020). Cultures of single isolates of 76 human gut bacteria exposed to 271 orally administered drugs showed significant metabolism and affected intestinal and system drug metabolism in mice (Zimmermann et al., 2019). Because of the large metagenome and metabolic capacity of the gut microbiome, it may become standard microbial metabolism assays in drug development. These studies could use a high-throughput method similar to Zimmermann et al. (2019), combining metabolomics with single colony isolates in 96 well plates exposed to drug candidates as well as any potentially toxic xenobiotics. Activity-based protein profiling will identify specific microbial enzymes that metabolize a xenobiotic of interest (Jariwala et al., 2020). Because this method targets the enzyme, not the bacterium, it is independent of horizontal genes transfer. This technique may be helpful for the development of microbial enzyme inhibitors to improve drug efficacy or prevent toxicity.

The goal for studying the intestinal localization of microbial metabolism is increased knowledge for pharmacokinetic modeling. For example, regional proteomics in the intestines have improved pharmacokinetic modeling (Zhang et al., 2020), which can help prevent drug toxicities. This could be applied to the localized affect and metabolic capacity of the human microbiome. By identifying DPGs in the gut microbiome and their intestinal preferences, a

pharmacokinetic model could include the gut microbiome as a modifier for better predictability of drug metabolism.

Studying the bacteria-intestine interaction in humans will require new, non-invasive technologies to sample bacteria (and metabolites in the local environment) from different sections of the intestines. Currently, the Brisbane Aseptic Biopsy Device uses a clean, forceps system to sample through an endoscopy, which is an invasive technique (Shanahan et al., 2016; Tang et al., 2020). Intelligence caps are activated to aspirate fluid by changes in pH, temperature, or salt, making it specific to each intestinal section (Koziolek et al., 2015; Nejad et al., 2019). These samples can be applied to intestine-on-a-chip models that include bacteria (Jalili-Firoozinezhad et al., 2019). Other *ex vivo* models to characterize the human bacteria-intestine interaction include InTESTine, Ussing chamber, tissue engineering of intestinal sections, and co-culture of polarized intestinal cells models (e.g. Caco-2, HT-29, T84, IEC-6, and IEC-18) and bacteria (Pearce et al., 2018). Improved methods for sampling bacteria in the intestine along with more efficient *in vitro* culturing methods will demonstrate the interactions between bacteria and human intestines.

The role of the gut microbiome in pharmacology and toxicology remains an understudied area of research. This is in contrast to the extent the microbiome has been characterized as a mechanism or cofactor of disease, including metabolic syndrome, cancers, and neurological diseases. Using a multi-omics approach, the objective of this began to show how the gut microbiome and age modulate hepatic xenobiotic metabolism and transport related genes. Expanding research to understand the interactions of the gut microbiome and intestines will provide a better understanding of how xenobiotics are metabolized in the liver. Further research focused on improved biological tools and bioinformatic techniques to integrate microbiome sequencing and metabolomics data are expected to expand the understanding of the gut-liver axis in pharmacology and toxicology. This will lead to achieving the long-term goal to identify key regulatory mechanisms of the gut-microbiome-liver axis that regulate host xenobiotic metabolism

and disposition and nutrient homeostasis, with the purpose of developing novel probiotic therapies that protect vulnerable populations from diseases and toxicities.

5.5 References

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