Examining the effects of endogenous sex steroids and the xenoestrogen contaminant 17α-ethinylestradiol on previtellogenic coho salmon ovarian growth and function

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Abstract

Examining the effects of endogenous sex steroids and the xenoestrogen contaminant 17α-ethinylestradiol on previtellogenic coho salmon ovarian growth and function.

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In teleost fish, as in other oviparous vertebrates, the production of a fertilizable egg is a complex process driven by the specific spatiotemporal coordination of endocrine and paracrine factors in multiple tissues. Across the brain-pituitary-ovary-liver (BPOL) axis, this includes the synthesis and release of pituitary gonadrotopins, the ovarian production and release of sex steroids, and the hepatic synthesis and release of egg yolk protein precursors, as well as numerous factors that mediate these processes. The factors controlling the earliest stages of oogenesis, the loading of yolk proteins (vitellogenesis), and the final maturational stage are fairly well understood, but the control of the intermediate growth stages (primary and early secondary growth) and the developmental point analogous to the onset of mammalian puberty, the transition from primary to secondary growth, are less clear. The research described in this dissertation addresses the regulation of the ovarian transcriptome by sex steroids during the primary and early secondary

growth stages in coho salmon (*Oncorhynchus kisutch*), and the potential disruption of normal ovarian processes during early secondary growth by a potent synthetic steroidal xenoestrogen. In the first chapter, the dramatic alterations of the primary growth ovarian transcriptome, and ovarian follicle growth following exposure to a natural steroidal androgen, 11-ketotestosterone (11-KT), are described. Potential alterations in gonadotropin signaling, growth factor signaling, and the extracellular matrix (ECM) were identified. The study described in the second chapter examines the effects of both 11-KT and the natural steroidal estrogen, estradiol-17β (E2), on the early secondary growth ovarian transcriptome. As in the study on primary growth, 11-KT dramatically altered the expression of genes involved in gonadotropin or growth factor signaling, the ECM, lipid incorporation, as well as steroidogenesis. E2 had a relatively smaller effect, but did alter the expression of nearly 100 genes. The third chapter examines the effects of the synthetic steroidal xenoestrogen 17α-ethinylestradiol (EE2) on the previtellogenic coho ovarian transcriptome. After a 1-week exposure, EE2 dramatically altered the ovarian transcriptome, impacting expression of genes involved in steroidogenesis, cell signaling, and protein synthesis/metabolism. After a 6-week exposure, the effects were less pronounced. Overall, this research identifies hundreds of ovarian transcripts and associated processes regulated by sex steroids during primary and early secondary growth, providing a basis for further targeted approaches to understand ovarian development during the onset of puberty. This research also demonstrates that EE2 can potentially disrupt normal endocrine function in fish by altering the potential for the synthesis of endogenous sex steroids.

Dedication

For Devrim and Nehir, who make every day a little bit brighter, and for my wife and parents, whose support has always been unwavering.

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REVIEW OF CURRENT KNOWLEDGE AND STUDY OBJECTIVES

Introduction

The production of a fertilizable egg involves a complex series of developmental events, collectively known as oogenesis, which transforms a relatively unspecialized cell, the oogonium into a full-grown oocyte provisioned with an array of maternal factors that support early embryonic development before the zygote genome becomes activated. In vertebrates, these events are regulated by the brain-pituitary-ovary axis. The vast majority of teleost fish are oviparous, producing eggs containing yolk that are released from the female and fertilized externally, although viviparous species also exist [1]. There are three main categories of female reproductive development among oviparous species: synchronous, in which all oocytes share the same developmental trajectory, group synchronous, in which developmentally distinct populations of oocytes are present in the ovary, and asynchronous, in which oocytes at all developmental stages are present. Regardless, the process of oocyte development follows the same general path towards the production of a fertilizable egg. To provide context for the specific research undertaken in this dissertation, below is an overview of: (*i*) the main events occurring during oogenesis; (*ii*) a summary of current understanding of the major endocrine factors regulating oocyte development; and (*iii*) the potential for contaminants within the aquatic environments to disrupt normal reproductive endocrine signaling.

i. Oogenesis

At the beginning of the female vertebrate reproductive cycle, primordial germ cells transition into small female diploid cells (oogonia) that undergo a series of mitotic divisions resulting in relatively large haploid cells (primary oocyte). These cells arrest in prophase I of the first

meiosis, where they begin to accumulate maternal RNAs, proteins, lipids, and other factors essential to embryonic survival and development [2,3]. After a significant increase in size, and following the resumption of meiosis, the mature oocyte is released (ovulated) from the somatic follicle cells. This process, oogenesis, is known to be regulated by a number of systemic hormones and by numerous locally-derived ovarian factors.

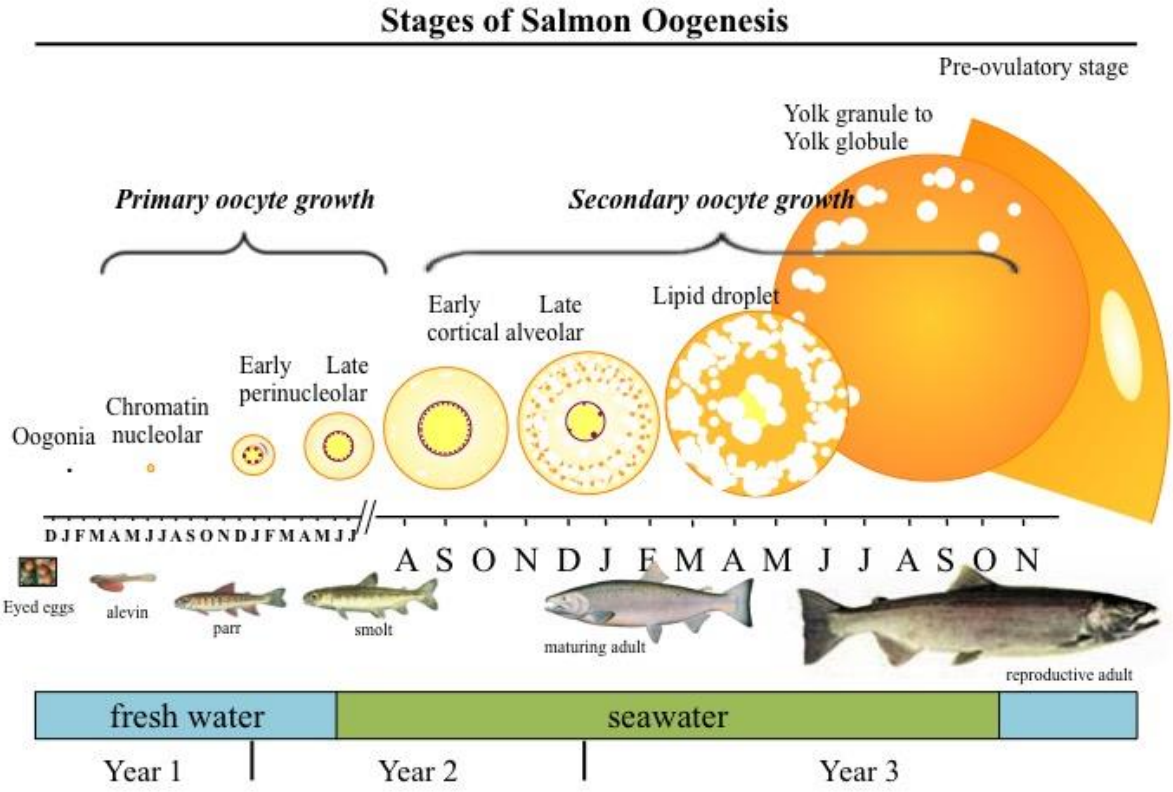

Figure 0.1. Stages of oogenesis in coho salmon relative to life history, age, and habitat. Modified from Forsgren, 2010, I. Nakamura (unpubl.), P. Swanson (unpubl.), and Campbell et al., 2006.

The process of oogenesis in teleosts is generally divided into three stages, primary growth, secondary growth, and the maturation/pre-ovulatory stage [4], which are illustrated in Figure 0.1 relative to the life history of coho salmon. Primary growth commences with the onset of meiosis

in which oogonia develop into chomatin nucleolar oocytes that are arrested in meiosis prophase I. Formation of an inner granulosa layer and an outer theca layer that eventually surrounds the oocyte commences at this time. As this ovarian follicle (oocyte and surrounding follicle cells) develops, ribosome-producing nucleoli appear around the periphery of the nucleus and maternal RNAs begin to be deposited. During this primary growth phase, the oocyte begins to undergo a substantial increase in size that continues in secondary growth. The appearance of glycoprotein-rich cortical alveoli signifies the initiation of secondary growth. Following this, or concurrently depending on the species, lipids are deposited in the perinuclear area and subsequently hepatically-derived yolk proteins accumulate in a process termed vitellogenesis [5]. During this time, the number and/or size of oocytes is adjusted depending on available energy resources [6]. Completion of vitellogenesis marks the end of secondary growth. During the maturational stage, the oocyte is released from meiotic arrest, marked morphologically by the migration of the oocyte's nucleus, also known as the germinal vesicle, to the periphery where it breaks down. Ovulation, the expulsion of the mature oocyte from the follicle, and the completion of oogenesis, then occurs. The first meiotic division is completed at ovulation while the second is completed at fertilization.

ii. Endocrine regulation of oogenesis: the brain-pituitary-ovary axis

The reproductive endocrine system functions to initiate and coordinate development of the ovarian follicle. In vertebrates, environmental information is received and integrated in the brain to influence hypothalamic neurohormones, the most important of which is gonadotropin-releasing hormone (GnRH). This hormone regulates the synthesis and secretion of two pituitary gonadotropin hormones, whose main targets are the somatic follicle cells surrounding the oocyte

[7–10]. These follicle cells produce a number of hormones and growth factors that act locally and/or systemically. Three classes of steroid hormones produced by the follicle cells, estrogens, progestins, and androgens play major roles at distinct stages of oogenesis.

Gonadotropins. The pituitary gland of most vertebrates produces two large glycoprotein gonadotropins (GTHs), follicle-stimulating hormone (Fsh) and luteinizing hormone (Lh). Each gonadotropin consists of a common alpha subunit, and a hormone-specific beta subunit. These gonadotropins act on the gonads to directly stimulate gonadal steroid synthesis. Sex steroids in turn participate in regulating gonadotropin secretion via negative or positive feedback on the brain and pituitary. Other ovarian hormones and growth factors, such as inhibin or activin also act at higher levels of the brain-pituitary-gonad axis to regulate gonadotropin production.

In most teleosts fishes, the pattern of circulating levels of gonadotropins during ovarian development varies between Fsh and Lh. The elevated levels of Fsh during secondary growth (characterized by accumulation of cortical alveoli, lipids, and yolk protein incorporation) [11–14] and experimental studies strongly suggest that this gonadotropin regulates secondary growth [5,15,16]. Lh levels in most species remain low throughout primary and secondary growth but increase just prior to final oocyte maturation and ovulation [5,17–20]. Experimental studies have shown that Lh promotes oocyte maturation and ovulation [21]. The roles of the gonadotropins prior to secondary growth and maturation are much less well defined. Studies utilizing the removal of the pituitary (hypophysectomy) implied that pituitary gonadotropins are not necessary for completion of primary growth, since primary follicles of hypophysectomized females develop and arrest in the late perinucleolar (LPN) or very early cortical alveolus (ECA)

stage [22], suggesting that early ovarian development is GTH-independent [23]. This idea is not compatible with recent zebrafish *fshβ* and *fsh*-receptor (*fshr*) deletion studies [23,24] in which primary growth was retarded in the absence of the Fsh-beta subunit and was completely arrested in the absence of Fshr at the very early perinucleolar stage. The deletion of *lh-beta* did not affect primary growth in females, but did prevent oocyte maturation and ovulation. In contrast, *lh*-receptor (*lhcgr*) deficient zebrafish were fertile [24,25]. Further studies on other species will be necessary before any general conclusions can be made.

Role of Sex Steroids in Vitellogenesis and Maturation/Ovulation. The onset of vitellogenesis is characterized by increases in plasma Fsh and expression of ovarian *fshr* [11–14,26]. Fsh, as well as Lh stimulate the production of E2 (and also testosterone) in the ovary [27–31] which regulates the production of vitellogenins (Vtg - yolk protein precursors) in the liver. Fsh has also been shown to increase ovarian uptake of Vtgs in vitro [32]. Vtgs are incorporated into the oocyte through Vtg-receptor mediated endocytosis. The end of vitellogenesis and the resumption of meiosis (oocyte maturation) are driven by a dramatic increase in plasma Lh levels and ovarian *lhcgr* expression. This is accompanied by a decrease in cytochrome p450 family 19 subfamily a member 1 (*cyp19a1*) expression, and a switch from predominantly E2 production to maturation inducing steroids (MIS), 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P), and/or in some species 17,20β,21-trihydroxy-4-pregnen-3-one [21]. The ability to synthesize these MIS is known as maturational competency. MIS are produced by the follicle cell layers following a switch in synthesis of precursor steroids, from favoring testosterone – the precursor of E2, to 17α-hydroxyprogesterone, the precursor of 17,20β-P, and a switch in expression of steroidogenic enzymes, from Cyp19a1 to 20β-hydroxysteroid dehydrogenase. The site of MIS action is on the

surface of the oocyte via membrane-bound progestin receptors (mPRs), which when bound to MIS, initiate a non-genomic cascade ultimately resulting in the production of maturation promoting factor (a complex of cyclin dependent kinase 1 and cyclin B proteins). This factor brings about morphological changes in the oocyte such as germinal vesicle breakdown and chromosome condensation [33] indicative of final maturation. At this point, the mature egg arrests in metaphase II of the second meiosis until fertilization. Ovulation follows due to the binding of 17,20β-P to nuclear progesterone receptors and is thought to be controlled by genomic actions which result in the rupture of the follicle cell layers [34], but the precise biochemical and molecular events are still under investigation.

Emerging Roles of Androgens and Estrogens in Promoting Previtellogenic Growth of Follicles. Recent studies on several teleost models indicate that estrogens and androgens play stage-specific roles in regulating development of previtellogenic follicles. Until fairly recently, the significance of androgens during oogenesis in vertebrates has been regarded simply as serving as a precursors for E2 synthesis. However, androgens have been implicated in regulating aspects of early oogenesis [35,36] in several mammalian models. Androgens promote growth and increase the number of preantral (those in primary and secondary growth phases) and early antral (those in maturational growth phases) follicles [37–40], enhance Fsh-stimulated growth and granulosa cell differentiation [40–42], and depending on follicle stage, both inhibit [43] and increase atresia [44].

A role for androgens in regulating primary growth of teleost ovarian follicles has emerged from studies on several teleost models. Most of these studies have used 11-ketotestosterone (11-KT)

rather than testosterone (T), since T can be readily aromatized to E2. 11-KT is the major androgen in male teleosts [45] and has been generally considered to be male specific. However, in anguillid eels relatively high circulating levels are found females at the initiation of secondary ovarian follicle growth [45–47]. Studies on a range of female teleosts show that in most it is present at relatively low levels (<2 ng/ml) in blood plasma. 11-KT is non-aromatizable; unlike testosterone, it cannot be converted into E2 by Cyp19a1a (the ovarian form of the enzyme known as aromatase). Studies on several species of teleosts indicate that 11-KT promotes primary and/or early secondary ovarian follicle growth, depending on the model used. Using long-term culture of explants of the shortfinned eel ovary, containing follicles that had completed primary growth, Lokman et al. [48] found that 11-KT, but not E2, increased follicle size. Subsequent studies on this and other eel species showed that 11-KT stimulates processes that lead to lipid uptake and the development of oil droplets in the ooplasm, resulting in a previtellogenic secondary follicle phenotype [48–52]. A similar approach reported that extremely high concentrations of 11-KT modestly stimulated primary follicle growth in cod ovarian explants containing a mixture of follicles at various stages of primary development [53].

Forsgren and Young [54], using a coho salmon ovarian explant model, showed that androgens promote growth of the ovarian follicle in the LPN stage of primary growth, just prior to entry into secondary growth. Although E2 was without effect on growth at this stage, E2-treated oocytes contained cortical alveoli. Androgens and E2 both stimulated growth of ECA stage follicles but E2 caused a far more substantial increase in abundance of cortical alveoli. Thus, the transition from primary to secondary growth is characterized by the follicle becoming sensitive to E2. Previous studies have shown a strong correlation between development of the cortical

alveolus stage follicle and increasing plasma E2 levels [55,56], and E2 treatment of hypohysectomized goldfish resulted in the formation of cortical alveoli [22]. Together these studies suggest that E2 plays an important role in regulating the transition into the cortical alveolus stage.

Although the promotion of previtellogenic follicle development by 11-KT and E2 has been demonstrated, their precise actions and the underlying mechanisms have not been explored in any detail, aside from the effects of 11-KT on lipid incorporation into eel follicles [47,49,50,52]. The action of these steroids requires binding to specific steroid hormone receptors, which then act as transcription factors that regulate the expression of specific genes.

Androgens play essential roles in vertebrate sex differentiation, maturation, and behavior, and these actions are mediated through the androgen receptors. Two distinct nuclear androgen receptors (Ara and Arb) are present in teleost fish with different tissue distributions. Ara is found in the brain and Arb is found both in the brain and gonad [48,57–59]. The binding affinities of these two receptors has been characterized in Atlantic croaker, *Micropogonias undulates* [57] and kelp bass, *Paralabrax clathratus* [58]. In general, Arb has a much broader specificity for androgens and a higher binding affinity for androgens with general modifications to the basic structure of testosterone (e.g., 11-KT). Ara has a higher binding affinity for T and dihydrotestosterone (DHT). This suggests that the receptors potentially mediate the actions of different androgens in different tissues.

The primary (genomic) mode of action of androgens is binding to the nuclear receptors, which act as ligand bound transcription factors, binding to cognate response elements and influencing gene transcription. In addition to the genomic actions of nuclear androgen receptors, recent work has identified a membrane-bound protein (Zip9) that exclusively binds testosterone [60], modulates intracellular zinc levels, and induces apoptosis.

Like androgens, estrogens act through both nuclear and membrane receptors. Genomic actions of E2 are mediated through its ability to bind nuclear estrogen receptors (Esr), forming a complex which act as a ligand-bound transcription factor, regulating the transcription of target genes [61] by activating specific estrogen response elements. The *esr* family in salmonids is composed of two isoforms each of *esr1* (*esr1a*, *esr1b*; often referred to as *Erα1* and *Erα2*) and *esr2* (*esr2a*, *esr2b*; often referred to as *Erβ1* and *Erβ2*) [62] and expression varies among tissues and temporally throughout development. The predominantly studied *esr* subtype, in terms of biological function in teleosts, is *esr1*, expressed in the liver during vitellogenesis [63–66] where it is involved in the expression of *vtg* transcripts, and comparatively little is known about the expression and function of *esr2b* and *esr2a*. In mammals, the number of primary ovarian follicles is reduced by ESR2 antagonists [67], indicating a role for Esr2 in early follicle development.

There is increasing evidence that fast non-genomic responses to estrogens lead to rapid activation of intracellular signaling pathways [68–70]. Nuclear receptors expressed near the cell surface appear to be responsible for some of these actions [70], but there is evidence that the orphan G-protein coupled receptor, Gper (formerly G-protein coupled receptor 30 [Gpr30]) functions as a specific membrane estrogen receptor that promotes rapid cellular responses

[71,72]. Homologs of this receptor have been identified in several teleosts such as Atlantic croaker [73], zebrafish [74], common carp [75], and European eel [76].

iii. Potential Disruption of Ovarian Follicle Function and Development by Endocrine-Disrupting Chemicals.

A multitude of environmental contaminants are now known to interfere with hormonal signaling in animals. Endocrine disruption is not a toxicological endpoint, but a functional change that can lead to adverse effects. Many endocrine disrupting chemicals (EDCs) are known to affect the reproductive endocrine system but the reproductive health consequences of exposure are difficult to predict and monitor due to the intricate nature of endocrine signaling and the complexity of reproductive development. These contaminants may mimic or antagonize the actions of hormones, and/or interfere with their biosynthesis, secretion, transport, receptor binding, or metabolism [77,78].

A growing list of chemicals are known EDCs and include human-made plasticizers (phthalates), plastics (bisphenol A), industrial solvents and lubricants (polychlorinated biphenyls, dioxins), pesticides (such as vinclozilin), and pharmaceuticals (such as 17α-ethinylestradiol, EE2), as well as naturally occurring phytoestrogens (genistein and daidzein), and research has shown that even low levels of environmental exposure to these chemicals can have deleterious effects on endocrine-controlled processes.

Of particular concern is EE2, a potent synthetic steroidal estrogen that is nearly 10 times more bioactive (estrogen receptor affinity) than endogenous estrogens [79], and is the active estrogen

in oral contraceptives. It is common in wastewater treatment plant (WWTP) effluent and is present in aquatic habitats with levels potentially up to 273 ng/L [80]. EE2 has been identified as the likely agent causing feminization of male fish downstream of WWTPs [81], where male fish express *vtg* [82–84] and show signs of intersex [85]. EE2 is persistent in the environment, is rapidly absorbed, and bioaccumulates in fish [86].

Fish and other aquatic animals are commonly used as sentinel species when assessing the effects of EDCs. Due to the potential impact of EE2 on fish reproduction, a number of experiments have attempted to quantify the effects of EE2. Environmentally relevant concentrations of EE2 caused significant increases in hepatic *vtg* expression in male fathead minnow [87,88] and altered reproductive capacity in females [89–91]. Additionally, chronic exposures caused complete reproductive failure in zebrafish [92] and complete feminization in a population of fathead minnow [93]. Attempts to identify transcriptional impacts of estrogen exposure in the brain [94,95], pituitary [96], liver [97–100], and testis [101–103] of fish are fairly extensive, but less information exists on the ovary. Santos et al. [90] found that in maturing female zebrafish ovaries, EE2 disrupted the expression of genes involved in cell cycle progression and mitochondrial function, and Vang et al. [104] found that high concentrations of EE2 transiently modulates steroidogenic acute regulatory protein (*star*) and P450 side chain cleavage (*cyp11a1*) expression in previtellogenic Atlantic salmon ovaries cultured in vitro. Although differential effects of EE2 and endogenous E2 on gene expression have been reported [105], since E2 appears to be essential to development of secondary previtellogenic and vitellogenic follicles, and also exerts negative feedback to suppress Fsh secretion, EE2 could potentially impact several

sites in the brain-pituitary ovary axis and disrupt reproductive processes, including steroidogenesis.

Model

Several studies have used long-term culture of either follicles or ovarian fragments in teleosts, based on methods developed by Miura et al. [106]. Lokman et al. [48] used this culture system to show androgens and insulin-like growth factor 1 (Igf1) promote growth of primary follicles in the freshwater eel, *Anguilla australis*. Kortner et al. [53] showed androgens modulate growth of Atlantic cod, *Gadus morhua*, previtellogenic oocytes. However, these model systems have some limitations. Many ovarian follicles in the eel culture model become atretic and follicles of some individuals are unresponsive to treatments. The Atlantic cod ovarian explant contains a mixture of follicle stages which complicates analysis and interpretation.

The semelparous nature (reproducing once in a lifetime) of Pacific salmon, *Oncorhynchus spp.*, provides a major advantage in the study of early oogenesis. Unlike mammalian and other fish models (such as medaka and zebrafish), only a single clutch of follicles is produced by a female and they develop synchronously. The ovaries of juvenile salmon are relatively large, and may contain up to 10,000 follicles at exactly the same developmental stage, and this development is predictable based on season and growth rate [56]. The coho salmon (*Oncorhynchus kisutch)* ovary model allows extensive experimental replication, using follicles from the same individual, or between individuals, all of which are at the same developmental stage. Importantly, gene expression studies are not confounded by extraction of RNA from a mixed population of follicles or limited by the numbers of follicles. *Thus, use of the coho salmon offers significant*

advantages in the study of ovarian follicle development compared to mammalian and other teleost models.

Objectives

I. Determine the effects of endogenous sex steroids on the ovarian follicle.

Previous in vitro studies have demonstrated stage-specific effects of steroids on development of previtellogenic ovarian follicles of coho salmon. In vitro treatment with 11-KT at low concentrations promoted growth of mid-late perinucleolar follicles but E2 had no growth-promoting effect. However, both steroids stimulated growth of ECA stage follicles, but with E2 having a much more potent effect on increasing the number of cortical alveoli present in each oocyte [54]. These data indicate that androgens promote the completion of perinucleolar follicle development. However, once follicles develop to the ECA stage, they become highly sensitive to E2, which has potent growth-promoting effects, and stimulates cortical alveoli accumulation at very low concentrations [107]. 11-KT may also participate in the regulation of previtellogenic secondary follicle since it also stimulates growth of ECA stage follicles in vitro [54].

Similar results were obtained after long-term in vivo treatment of females with LPN and ECA follicles with these steroids but with some notable differences: 11-KT but not E2 treatment for 10 days resulted in growth of LPN follicles, but unlike the in vitro results, prolonged E2 exposure for 20 days also stimulated growth. These differences may be due to these steroids having actions on other sites in the brain-pituitary-ovary axis when applied in vivo.

The expression of a number of genes encoding steroidogenic enzymes, various oocyte specific components such as cortical alveoli, regulators of ovarian development such as transforming growth factor beta (Tgf-β) superfamily members, and several hormone receptors were measured in these in vivo studies. However, these data were not very informative on potential mechanisms underlying the morphological effects of these steroids, most likely because significant growth had already occurred at the first time-point (10 days) that tissue was collected for gene expression analysis. Gaining insight into the transcriptional mechanisms and gene networks altered that lead to steroid-induced ovarian follicle development at these stages, requires elucidation of the changes in the ovarian transcriptome prior to any alterations in follicle phenotype.

This research was aimed at describing the transcriptional mechanisms by which 11-KT and E2 mediate growth and development of the ovarian follicle during primary growth stages (perinucleolar stages) and the transition to the initial secondary growth (cortical alveolus) stage. Based on preliminary results and previous *in vivo* and *in vitro* studies, the overall hypothesis that was addressed was: *Androgens stimulate growth of the primary oocyte during the mid- and late-perinucleolar stages and the transition to secondary growth, while priming the follicle cells for E2-stimulated secondary growth beginning at the early cortical alveolus stages*. Experiments to address this were conducted to:

1. Investigate androgen-mediated effects on the primary ovarian follicle transcriptome that precede ovarian follicle growth. Specifically, an RNA-Seq approach was used to identify ovarian processes under the control or influence of androgen signaling that promote ovarian follicle stage progression.

2. Investigate both androgen- and estrogen-mediated effects on the previtellogenic secondary ovarian follicle transcriptome, using the same approach as above.

II. Determine the effects of a synthetic xenoestrogen contaminant, 17α-ethinylestradiol (EE2), on the ovarian transcriptome in vivo and on the ability of the ovarian follicle to produce E2 in vitro. Control of female reproduction in fish is based on hypothalamic and pituitary hormones, gonadal steroidogenic enzymes, bioactive sex steroids, and other endocrine factors across the brain-pituitary-ovary-liver (BPOL) axis. Briefly, environmental cues are received by the brain [15,18,19,108,109] and translated into the synthesis and release of hypothalamic GnRH. GnRH stimulates the production and/or release of the pituitary GTHs Fsh or Lh [19], which stimulate somatic follicle cell layers in the ovary to produce bioactive sex steroids, such as E2. Estradiol plays multiple central roles in reproduction in female teleosts, and is especially critical during secondary ovarian follicle growth [3]. This critical phase of development is when the oocyte acquires the biochemical machinery in the form of maternal RNAs and proteins, and also energy stores in the form of neutral and polar lipids and yolk proteins, necessary to support later embryonic development. The BPOL axis must be coordinated to produce the appropriate endocrine factors at the appropriate stage. Disruption at any point across this axis may negatively impact reproductive fitness.

In vivo models have shown impacts on E2 production from EE2 exposure [110], but it remains unclear at which level of the BOPL axis inhibition is occurring. Several studies suggest direct effects on steroid production through the inhibition of steroidogenic proteins [111,112]. This objective addresses the potential mechanism of action of EE2 on ovarian function.

While Objective I was pursued to determine the mechanisms by which the ovarian follicle responds to endogenous sex steroids, Objective II was to identify deleterious impacts of estrogenic contaminants on reproductive development. The overall hypothesis that was addressed by this research was: *The synthetic estrogen, EE2, can impact normal reproductive development by altering the expression of genes in the developing ovary and reducing the capacity of the ovarian follicle to produce endogenous sex steroids.* Experiments to address this hypothesis were conducted to identify the effects of EE2 on the previtellogenic secondary ovarian transcriptome. Specifically, an RNA-Seq approach was used to identify ovarian processes affected by in vivo xenoestrogen exposure at environmentally relevant levels.

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CHAPTER 1: A teleost androgen promotes development of primary ovarian follicles in coho salmon and rapidly alters the ovarian transcriptome

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Supplementary figures and tables are included.

Abstract

Recent studies using several teleost models have revealed that androgens increase the size of previtellogenic (primary and/or early secondary) ovarian follicles. To explore our hypothesis that androgens drive the development of primary follicles into early secondary follicles, and to determine the mechanisms underlying these androgenic effects, we exposed juvenile coho salmon to near-physiological and relatively sustained levels of the non-aromatizable androgen 11-ketotestosterone (11-KT). This resulted in significant growth of primary ovarian follicles after 10 and 20 days, with follicles after 20 days displaying a morphological phenotype characteristic of early secondary follicles (presence of cortical alveoli). Utilizing the same experimental approach, we then analyzed how 11-KT rapidly altered the ovarian transcriptome after 1 and 3 days of treatment. RNA-Seq analysis revealed that 69 (day 1) and 1022 (day 3) contiguous sequences (contigs) were differentially expressed relative to controls. The differentially expressed contigs mapped to genes including those encoding proteins involved in gonadotropin, steroid hormone, and growth factor signaling, and in cell and ovarian development, including genes with putative androgen-response elements. Biological functions and canonical pathways identified as potentially altered by 11-KT include those involved in

ovarian development, tissue differentiation and remodeling, and lipid metabolism. We conclude that androgens play a major role in stimulating primary ovarian follicle development and the transition into secondary growth.

Introduction

In oviparous vertebrates, the previtellogenic primary growth stage of oocyte development is characterized by intense synthesis of maternal RNAs, proteins, and lipids that are used to both facilitate maturation and fertilization, and to support early development of the zygote before zygotic genes begin to be expressed [1–3]. Growth of the primary oocyte is accompanied by the proliferation and functional differentiation of the surrounding somatic cell layers that together with the oocyte form the ovarian follicle. All components of the ovarian follicle participate in sending and receiving hormonal signals that coordinate ovarian follicle development (reviewed by Hsueh et al. [4]).

Primary growth of ovarian follicles of teleost fish encompasses the chromatin nucleolus and perinucleolar stages, the former being a relatively transitory stage in many species. Perinucleolar follicles early in development are characterized by the presence of Balbiani bodies. These structures (or "nuages") are a dense mass of mitochondria, endoplasmic reticulum, germinal granules, germplasm RNAs and RNA-binding proteins. In the late perinucleolar stage (LPN), the Balbiani body disassembles and transcripts become associated with the vegetal pole of the follicle (review by Lubzens et al. [3]). Completion of primary ovarian follicle development is often marked by the appearance of a few cortical alveoli (equivalent to the cortical granules of

other species). Previtellogenic secondary follicle growth is characterized by the often massive accumulation of cortical alveoli, accompanied or followed by deposition of neutral and/or basic lipids, after which gonadotropin-mediated vitellogenesis commences [5–9].

The genes encoding the beta polypeptides subunits of the pituitary gonadotropin (GTHs), follicle stimulating hormone (Fsh) and luteinizing hormone (Lh), are differentially expressed during secondary ovarian follicle development in teleosts [10–12] and the roles of the gonadotropins in regulating vitellogenesis and maturation of ovarian follicles are reasonably well-established. Plasma levels of Fsh increase during development of secondary ovarian follicles of teleosts, and this GTH stimulates follicular steroidogenesis, resulting in increased estradiol 17β (E2) production. E2 stimulates the synthesis of hepatic vitellogenin [5] which is then incorporated into the ooplasm by a receptor mediated process. In most species, Lh levels remain low throughout primary and secondary growth, but increase just prior to final oocyte maturation and ovulation [13]. Numerous experimental studies in a variety of teleost models confirm that the major actions of Lh are to stimulate follicular production of maturation-inducing progestins and prostaglandins, resulting in maturation of the oocyte and ovulation [5,6].

The endocrine regulation of primary growth of ovarian follicles and their transition into previtellogenic secondary follicle development is relatively less well understood. Early work showing that ovarian follicles of several hypophysectomized teleost species completed primary growth [14,15] led to the concept that development of the primary follicle was “gonadotropin-independent” [16]. This idea has been challenged by recent zebrafish studies in which primary

ovarian follicle growth was retarded in the absence of the *follicle stimulating hormone-beta subunit* [17], and completely was arrested at the very early perinucleolar stage in the absence of the *follicle stimulating hormone receptor* (*fshr*) [18]. These phenotypes differ from those in mouse models, in which knockout studies suggest that local regulators largely drive early follicle development, with Fsh playing a facilitatory role [19].

Irrespective of the precise roles of gonadotropins, studies on several species of teleosts indicate that androgens participate in promoting primary and/or early secondary ovarian follicle growth, depending on the model used. Androgens have been implicated in regulating development of an analogous stage, the pre-antral follicle, in mammals [20–23]. Some of the earliest teleost studies used freshwater eels (*Anguilla spp.)* as a model. Anguillid eels undergo a significant physiological change prior to their seaward spawning migration, known as silvering. This change coincides with the transition to secondary growth and vitellogenesis in the ovary, and with remarkably high plasma concentrations of the teleost-specific non-aromatizable androgen, 11-ketotestosterone (11-KT) [24]. Androgen-induced ovarian follicle development and accumulation of lipids arrested at the completion of primary growth, in vivo [25] and in vitro [26], respectively; E2 was without effect in vitro. Subsequent studies on eels showed that 11-KT stimulates cellular mechanisms that lead to lipid uptake and the accumulation of oil droplets in the ooplasm, resulting in a previtellogenic secondary follicle phenotype [9,26–29]. In cod with ovaries containing a mixture of follicles at various stages of primary development, androgens stimulated primary ovarian follicle development in vivo and in vitro [30,31], but only at pharmacological concentrations in vitro.

More recently, we have developed an in vitro model that exploits the advantages of using ovarian explants of coho salmon, *Oncorhynchus kisutch* [32]. Unlike many other teleosts species whose ovaries contain two and often more distinct clutches of ovarian follicles at different stages of development, coho salmon are semelparous, and females produce only a single clutch of oocytes before death. Because the coho salmon ovary displays highly synchronous ovarian follicle development with only a single class of follicles present at any time, histological analyses and interpretation of gene expression data is not confounded by the presence of multiple stages of primary and secondary follicles. We have demonstrated stage-specific effects of steroids on development of previtellogenic coho salmon follicles. At low concentrations in vitro, 11-KT promoted growth and development of mid-late perinucleolar follicles. At this stage, E2 had no growth-promoting effects. Conversely, the early cortical alveolus stage follicle was highly sensitive to E2. Both E2 and 11-KT at low concentrations caused marked increases in size of previtellogenic secondary oocytes (early cortical alveolus stage follicles), but E2 had a more substantial effect on the abundance of cortical alveoli [32]. Based on these in vitro data, we hypothesized that in coho salmon, androgens are the primary steroids driving the development of primary ovarian follicles through the transition into the early secondary growth stage, a stage in which E2 promotes both growth and synthesis of the stage-characteristic cortical alveoli.

This study was designed to provide further understanding of the role of androgens in regulating primary ovarian follicle development. We developed an in vivo coho salmon model using sustained-release 11-KT implants that demonstrated that this steroid promotes perinucleolar stage follicle development, which after 20 days of exposure, resulted in an early cortical alveolus stage phenotype similar to that seen in the in vitro studies described above. To reveal the

molecular events that precede androgen-induced morphological changes, we used transcriptome sequencing to show that short-term in vivo treatment with 11-KT alters the expression of hundreds of genes associated with hormonal signaling pathways, steroidogenesis, tissue remodeling, lipid metabolism, and ovarian growth and development.

Methods

Chemicals

11-Ketotestosterone (11-KT) was purchased from Steraloids (Newport, RI). Cholesterol was purchased from Sigma-Aldrich (St. Louis, MO). Lebovitz (L-15), hematoxylin, eosin, and diethyl ether were purchased from Thermo Fisher Scientific (Waltham, MA). Bouin fixative was purchased from Ricca Chemical Company (Arlington, TX).

Animals and Tissue and Blood Sampling

All fish were maintained and treated following an approved protocol (4078-02) according to guidelines established by the Institutional Animal Care and Use Committee, University of Washington. For both studies described below, juvenile coho salmon (Issaquah Hatchery stock, Issaquah, WA) were reared from eyed embryos to 22-23 months of age at the hatchery facilities of the Northwest Fisheries Science Center, Seattle, WA under simulated natural photoperiod in re-circulated 10-11°C fresh water. Fish were fed twice daily with a commercial feed, BioDiet (Bio-Oregon, Longview, WA) according to the manufacturer's guidelines. Either mixed sex fish, or an all-female stock were used. Since gender cannot be determined by body morphology at this

age in coho salmon, fish were implanted with passive-integrated transponder (PIT) tags and genetic sex was determined from DNA extracted from a small piece of fin tissue using a validated genetic marker for males [33].

At the termination of experiments, fish were anesthetized in buffered 0.05% tricaine methanesulfonate until movement of the gill operculum ceased. Fork length and body weight were measured. Blood was collected from the caudal vein using 21-gauge needles and heparinized 1 ml syringes and immediately transferred to microcentrifuge tubes and placed on ice. Blood plasma was separated by centrifugation at 1200 × g for 15 minutes. Fish were euthanized by decapitation and tissues were removed and weighed, and then either snap frozen in liquid nitrogen, or fixed in Bouin fixative for histological analysis. Gonadosomatic index (GSI) was calculated (ovary weight/bodyweight × 100).

Sex steroid assays

Steroids were double extracted from 250 µl of plasma using diethyl ether (1.5 ml x 2). Extracts were evaporated under nitrogen gas, and re-suspended in assay buffer. Plasma 11-KT levels were measured by enzyme-linked immunoassay [34] using tracer and secondary antibody coated plates from Cayman Chemicals (Ann Arbor, MI) and primary antibody donated by David Kime (University of Sheffield, UK). Plasma E2 was measured by radioimmunoassay, as described by Sower and Schreck [35], and modified by Fitzpatrick et al. [36]. Validation and characteristics of these assays have been reported previously [34,35,37].

Histological analysis

Fixed tissues were washed with 70% ethanol, dehydrated in increasing concentrations of ethanol and xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut and mounted on microscope slides and stained with hematoxylin and eosin. Average ovarian follicle volume was calculated from at least 15 follicles per sample that were sectioned through the nucleus of the oocyte, using an image analysis system (NIS-elements, Nikon, USA), as described previously [32]. Oocytes were scored for stage based on previously published criteria [32,38].

Experimental Procedures

i. Effects of long-term treatment with 11-KT on perinucleolar stage follicle morphology and steroid levels. The goal of this study was to determine if exposure to 11-KT in vivo resulted in promotion of ovarian follicle development similar to that reported for in vitro exposure [32]. At a time when females displayed mid-late perinucleolar stage ovarian follicles, coho salmon (mixed sex, 22-23 months old, approximately 77 g body weight), received peritoneal implants of either vehicle or vehicle containing 11-KT. Implants consisted of 1 µl/g body weight molten vegetable oil/vegetable shortening mixture with or without 2.5 mg/ml 11-KT, as described previously [39,40]. Examination of the release characteristics in vitro showed a sustained rate of release of 11-KT for the first 12 days followed by a gradual decline thereafter [41]. Immediately prior to implantation, or after 10 or 20 days of exposure, fish were euthanized, and blood was collected. Upon dissection, males were discarded and ovaries from the first five females sampled in both control and 11-KT-treated groups were dissected out and fragments were fixed and processed for histological analyses. Ovarian follicle volume was determined as described above

and data from controls and 11-KT-treated fish on day 10 and day 20 were compared using Students *t*-tests. Morphological characteristics of the oocytes were also recorded (e.g. appearance, cortical alveoli, loss of Balbiani bodies).

ii. Alterations in the ovarian transcriptome after short-term 11-KT treatment. The goal of this study was to characterize the changes in the ovarian transcriptome that are initiated by 11-KT prior to pronounced morphological changes in the follicle. Female juvenile coho salmon, in the late perinucleolar stage of ovarian development (86.5±4.8 g, 22-23 months of age), were implanted with either blank cholesterol pellets or pellets containing 11-KT. Preliminary studies demonstrated that this delivery system had similar release characteristics to the lipid-based delivery system described above, but with less individual variability in plasma levels of 11-KT attained. Cylindrical pellets (approximately 2 × 6 mm) of 30 mg [27,42,43], with or without 5 µg 11-KT, were produced using a custom-made pellet press, and implanted into the peritoneal cavity (n=10 females/treatment) using a 10-gauge needle and implanting syringe. Preliminary studies showed that pellets initially released high levels of 11-KT within the first 24 hours, and then, as in the first experiment, a fairly constant rate of release through day 7. To temper the initial release of 11-KT, pellets were incubated for 24 hours in sterile L-15 media (Thermo Fisher Scientific) and were then washed with L-15 prior to implantation. Fish were lethally sampled immediately prior to implantation or at one and three days after implantation. Fork length, body weight, and gonad weight were measured, and blood and ovaries were collected. After histological screening, any female with overtly asynchronous ovarian stages or with ovaries that were not at the mid-late perinucleolar stage, were eliminated from further analyses. Frozen ovarian samples from three control and three implanted females at day 1 and day 3 were

selected from remaining samples for RNA-Seq analysis. Plasma levels of 11-KT and E2 were also measured in females samples seven days after implantation in order to compare with levels achieved in the first study.

RNA Extraction

Total RNA was extracted using Qiagen RNEasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA pellets were re-suspended in DNAse/RNAse free water (Sigma-Aldrich). Total RNA concentrations in extracts were determined using a NanoDrop ND-100 (NanoDrop Technologies, Wilmington, DE).

RNA-Seq and pathway analysis of alteration in the ovarian transcriptome

i. *Sample preparation:* Total ovarian RNA (200 ng) was submitted to the University of Washington High Throughput Genomics Unit for quality checking, library preparation (poly A selected), and 36 base pair, paired-end sequencing (three samples per lane) on the Illumina© HiSeq 2000 platform. A total of 12 samples were analyzed over 4 lanes.

ii. RNA-Seq Analysis: Bioinformatics procedures were performed according to a method modified from Harding et al. [44]. Sequences were quality trimmed using Trim Galore v0.4.0 [45]. Trimmed reads were assembled into a de novo backbone with Drap v1.8 [46] and Oases v0.2.09 [47] using the kmer values of 19, 23, 25, 27, 31, and 35. There were 60,838 contiguous sequences (contigs) that had FPKM (fragments per kilobase of transcript per million mapped

reads) greater than 1 and had sequence lengths greater than 200 bp. The contigs were annotated using BlastX against the NCBI non-redundant protein database (nr) and partially non-redundant nucleotide database (nt); only sequences with an E-value ≤-05 were retained. Gene level count estimates were made using RSEM v.1.2.31 [48] and bowtie2 v2.2.6 [49] and differential expression was determined using DESeq2 [50]. Contigs with a P-adjusted (P-adj) value ≤0.1 were considered significantly altered between control and 11-KT treatment. To control for sequencing errors and differences in sequencing depth leading to misidentification of differential expression of contigs with low read counts, basemean ≤10 were excluded from further analysis. Gene clustering at 1 and 3 days was performed using cluster::agnes package in R with the Spearman method [51]; data were $\log_2$ transformed and centered on a mean expression value to improve visualization of expression differences.

iii. Pathway Analysis: Ingenuity® Systems Pathway Analysis (IPA) software (Qiagen) was used to conduct pathway and network analyses and estimate upstream and downstream effects of 11-KT treatment. Contigs were initially mapped to zebrafish orthologs using BLASTN against the Ensembl *Danio rerio* gene database (v.Zv9.72). However, some zebrafish genes have not been mapped to mammalian orthologs, so the remaining contigs were mapped to the *Homo sapiens* transcript database (v.GRCH37.72) for inclusion in IPA. If more than one contig (P≤0.05) mapped to the same gene, the average expression value of those contigs was used as the gene expression value in further analyses. Zebrafish and human orthologs were compared to the Ingenuity Knowledge database to estimate altered canonical pathways and biological functions (Fisher exact test P≤0.05 [$-\log_{10}$ P-value ≥1.3]). This program generates networks that maximize the connectivity of genes with significantly altered expression based on known functional

interactions [52], predicts alterations in biological function, and predicts both upstream and downstream regulators given the direction of expression differences in given gene sets. A positive or negative z-score value indicates that a function is predicted to be increased or decreased in 11-KT treated samples relative to controls. Zebrafish nomenclature is used throughout, although for the use of this software, human gene names are used in places where annotation to the zebrafish database was not possible.

Quantitative PCR assays

Genes related to steroidogenesis and ovarian function, as well as several differentially expressed genes identified from RNA-Seq analysis, were selected for quantitative PCR (qPCR) and compared with RNA-Seq expression data. Some of the genes were selected because their expression has been shown to be differentially regulated between late perinucleolar and mid-cortical alveolus stage follicles [53] (*anti mullerian hormone* [*amh*], *fshr*), or regulated in mid-cortical alveolus stage follicles by Fsh [54,55] (*amh, decorin* [*dcn*], *fibronectin 1* [*fn1*], *hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2* [*hsd3b2*]). Total RNA (1 µg) was reverse transcribed using Superscript IV reverse transcriptase (Thermo Fisher Scientific), random primers and RNasin RNase inhibitor (Promega). Primers (Table S1.1) designed with Primer3plus software [56] using contigs derived from transcriptome sequencing, or from previously published primer sequences [37,53], were purchased from Integrated DNA Technologies (Coralville, IA).

Total volume for qPCR was 25 µl, and consisted of 12.5 µl Power SYBR Green Master Mix (ABI/Invitrogen, Foster City, CA), 150 nM of gene specific forward and reverse primers, and 0.2 ng cDNA template based on total RNA loaded into the RT reaction. Assays were run on an ABI 7500 Sequence Detector in 96-well plates using standard cycling conditions: 50°C for 2 mins, 95°C for 10 mins, followed by 40 cycles consisting of 95°C for 15 secs, and 60°C for 1 min. A standard curve constructed from five concentrations of serially diluted cDNA synthesized from pooled ovary RNA (ranging from 0.004 to 4 ng) was included in each assay. No cDNA template controls (NTC) and no amplification controls (NAC, reverse transcriptase excluded from RT reaction) were included in each assay and showed no amplification over 40 cycles of PCR. Melt curve analyses showed single signals for all genes and no peaks were observed in NTC or NAC wells. Data were expressed relative to the expression of *eukaryotic elongation factor 1 alpha* (*eef1a*), which we have validated previously for use as a normalizer for coho salmon ovarian tissue [53] and which was stably expressed across treatments according to RNA-Seq analysis. The mean expression level of each gene transcript was compared between control and 11-KT samples at each time point using Student unpaired *t*-test with significance accepted at $P < 0.05$. Data were $\log_2$ transformed to meet parametric test criteria. Mean transcript expression determined by qPCR was plotted against expression values determined by RNA-Seq and data were analyzed using Spearman correlation coefficient.

Results

Effects of long-term treatment with 11-KT on perinucleolar stage follicle morphology and steroid levels.

The mean ovarian follicle volume in 11-KT implanted fish was 31% ($P<0.001$) and 56% ($P<0.001$) higher than that of controls at day 10 and day 20, respectively (Fig. 1.1 A). After 20 days of 11-KT treatment, approximately half of the ovarian follicles of 11-KT treated fish displayed a few cortical alveoli, which were absent in controls and not observed in samples from females treated with 11-KT at day 10 (Fig. 1.1 B-E). Sex steroid levels were altered by 11-KT implants at both 10 and 20 days. Implants increased plasma 11-KT (Fig. 1.1 F) significantly from 0.03-0.09 ng/ml to 1.0 ng/ml (10 days; $P<0.05$) and to 0.3 ng/ml (20 days; $P<0.05$). Levels of plasma 11-KT (0.03±0.01 ng/ml) or E2 (0.12±0.02 ng/ml) in fish collected immediately prior to implantation were not significantly different from controls at day 10. Plasma E2 levels (Fig. 1.1 G) in 11-KT implanted fish (6.4 ng/ml) were 13-fold higher than controls (0.05 ng/ml) at day 20 ($P<0.0001$). Levels of plasma 11-KT in controls decreased ($P<0.05$) from 0.09 ng/ml on day 10 to 0.03 ng/ml on day 20 (Fig. 1.1F).

Alterations in the ovarian transcriptome after short-term 11-KT treatment

Morphometrics and sex steroid levels. Fork length, body weight, GSI and ovarian follicle volume did not differ significantly between control and treated females at either day 1 or day 3 (all $P>0.8$). Plasma levels of 11-KT (Fig. 1.2 A) in control fish at day 1 (0.04±0.01 ng/ml) and day 3 (0.09±0.05 ng/ml) were significantly lower than those in 11-KT treated fish at day 1 (15.5±5.6 ng/ml; $P<0.001$) and day 3 (7.0±2.1 ng/ml; $P<0.001$).). At day 7, 11-KT levels in controls (0.06±0.003 ng/ml) were significantly lower ($P<0.01$) than those in 11-KT implanted females (3.56±0.68 ng/ml). Plasma 11-KT (0.08±0.13 ng/ml) and E2 (0.11±0.00 ng/ml) levels in fish collected prior to implanting were not significantly different from control levels. Plasma E2 levels (Fig. 1.2 B) in 11-KT implanted fish (0.15 ng/ml) were 2.2 fold higher than controls (0.07

ng/ml) at day 1 ($P<0.05$) and 2.4 fold higher (0.14 ng/ml) than controls (0.06 ng/ml) at day 3 ($P<0.05$). At day 7, although mean plasma E2 levels in 11-KT-treated fish (0.412±0.32 ng/ml were 3-4 times those of controls (0.128±0.03 ng/ml), the difference was not significant. There was no observable morphological differences between follicles sampled from control and 11-KT-treated females, and histologically, these follicles were indistinguishable from the control follicles in the long-term exposure study, illustrated in Fig. 1.1 B-C.

RNA-Seq and bioinformatics. Sequencing using the Illumina® HiSeq platform resulted in 1,611,376,806 total reads (805,688,403 matched paired end reads). Quality trimming resulted in 100% retained. Using these reads, de novo assembly generated 113,160 transcripts that were collapsed into 60,838 gene-cluster contigs of between 201 and 12,999 bases in length, with a mean of 1,207 bases. A total of 52,005 contigs were annotated and used in the creation of the backbone. Summary statistics for each sample can be found in Table S1.2.

A total of 69 contigs (30 upregulated and 39 downregulated) at day 1 and 1022 contigs (680 upregulated and 342 downregulated) at day 3 were identified as differentially expressed between control and 11-KT treatment by RNA-Seq analysis (DESeq2, P-adj$\leq$0.1). Cluster analysis demonstrated consistent differences between control and treatment groups at both time points and considerable homogeneity among each of the three samples from control and 11-KT treated fish at day 1 (Fig. 1.3 A) and day 3 (Fig. 1.3 B).

Contigs were annotated to zebrafish and human orthologs and uploaded into Ingenuity® Pathway Analysis software (IPA) to determine the biological processes potentially regulated by 11-KT in the ovary. A total of 33,899 out of 52,005 contigs (65%) that were used to create the backbone were annotated to zebrafish or human orthologs, and mapped to IPA. Duplicate contigs were collapsed to the gene-ID level using the average expression value relative to controls, resulting in 27 and 411 genes whose expression was significantly altered by 11-KT at day 1 and day 3 respectively (DESeq2, P-adj $\leq$0.1). Detailed lists of differentially expressed genes (DEGs) with P-adj values of <0.1 and fold change 1.5$\leq$ or $\geq$-1.5 are found in Supplementary Table S1.3 (day 1) and Supplementary Table S1.4 (day 3). There was very little overlap in DEGs at day 1 and day 3 (Fig. 1.4).

Ovarian DEGs associated with steroidogenesis and steroid action. The expression of the following genes involved in steroid synthesis was altered (DESeq2, P-adj <0.05) by 11-KT in the ovary at day 3: *hsd3b2* (1.51 fold upregulated), and *cytochrome P450 family 19 subfamily A member 1* (*cyp19a1*, 2.09 fold upregulated). In our dataset, the contig that mapped to zebrafish *hsd3b2* shares 99.2% identity with rainbow trout *hsd3b* (E-value 0.0, accession: S72665.1) over 1363 bp. The contig that mapped to zebrafish *cyp19a1* shares 99.8% identity with coho salmon gonadal *aromatase* (*cyp19a1a*, E-value 0.0, accession: HQ184096.1) partial coding sequence [54] over 687 bp. The expression of the nuclear steroid receptors *androgen receptor beta* (*ar*, 1.75 fold upregulated), and *estrogen receptor beta* (*esr2*, 2.05 fold upregulated) was significantly altered (DESeq2, P-adj <0.05) by 11-KT. In salmonids, there are two isoforms of *esr2* (*estrogen receptor beta 1* and *beta 2*) and three contigs that mapped to either salmonid *estrogen receptor*

beta isoforms share high sequence identity with zebrafish *esr2*. The expression of *fshr* was also increased (1.66-fold, P-adj = 0.064).

DEGs associated with signaling and ovarian processes

At day 3, the expression of *apolipoprotein* (*apoo*) and *lipoprotein lipase* (*lpl*) was increased by 11-KT 1.62 and 1.71-fold, respectively (P-adj <0.05). The expression of *transforming growth factor beta* (*tgf-beta*) superfamily members, *anti mullerian hormone* (*amh*) (1.70 fold), and *inhibin alpha* (*inha*) (1.61 fold) was increased at day 3 (P-adj <0.05).

Interrogation of the DEGs from the coho salmon ovary from day 3 using a published list of genes with putative androgen response elements (AREs) and/or estrogen response elements (EREs) from the testis of rainbow trout (*O. mykiss*) [57], a closely related species within the same genus as coho salmon, identified eighteen genes (Table 1.1). Expression of putative ERE-containing genes *annexin a11* (*anx11*), *amh*, *FK506 binding protein 10* (*fkb10*), *hsd3b2*, *inhibitor of DNA binding 1* (*id1*), *interleukin 13 receptor subunit alpha 2* (*il13ra2), pleckstrin homology domain containing B2* (*plekhb2*), and *serpin family F member 1* (*serpinf1*) was upregulated (>1.3 fold, P-adj <0.1). The expression of putative ARE-containing genes *H1 histone family, member 0* (*h1f0*) and *myosin IC* (*myo1c*) was upregulated (>1.4 fold, P-adj <0.01) by 11-KT. Expression of genes containing both AREs and EREs, including *atpase H+ transporting V1 subunit F* (*atp6v1f*), *collagen type I alpha 1* (*col1a1*), *collagen type VI alpha 2* (*col6a2*), *fatty acid binding protein 2* (*fabp2*), *G-protein signaling modulator 2* (*gpsm2*), *hemoglobin subunit epsilon 1* (*hbe1*), *retinol*

binding protein 4 (*rbp4*), and *ubiquitin protein ligase E3 component n-recognin 7* (*ubr7*) was upregulated >1.3 fold (P-adj <0.05).

Comparison of qPCR and RNA-Seq data

Expression of ten DEGs from the RNA-Seq of day 3 samples were analyzed by qPCR: *amh*, *amine oxidase, copper containing 3* (*aoc3*), *ar*, *cyp19a1*, *dcn*, *esr2*, *fn1*, *fshr* and *hsd3b2* were identified by both qPCR assays and RNA-Seq as upregulated by 11-KT, while both methods indicated that *protein tyrosine phosphatase, non-receptor type 11* (*ptpn11*) was downregulated. The changes in expression of target genes assayed by qPCR were similar to the changes in expression derived by RNA-Seq data (Fig. S1.6) when plotted against each other (Spearman Rho R=0.81, P <0.01), although the slope and intercept suggests a slight bias towards estimations of larger expression differences for negative fold-changes with RNA-Seq and estimations of larger expression differences for positive fold-changes with qPCR.

Canonical pathways and biological functions

Treatment with 11-KT altered 6 and 32 canonical pathways (curated by IPA) at days 1 and 3, respectively (Table 1.2). Hepatic fibrosis/stellate cell activation was the top altered pathway at day 3, and the seventh at day 1, by P-value ($-\text{Log}_{10}$ P = 1.58 at day 1 and 7.05 at day 3). This pathway is represented by two genes at day 1, *collagen type XXIV alpha 1* (*col24a1*) and *collagen XXVIII alpha 1* (*col28a1*), and 16 genes at day 3, *collagen type I alpha I* (*col1a1*), *collagen type I alpha 2* (*col1a2*), *collagen II alpha 1* (*col2a1*), *collagen type IV alpha 2* (*col4a2*), *collagen type IV alpha 6* (*col4a6*), *collagen type VI alpha 1* (*col6a1*), *collagen type VI alpha 2*

(*col6a2*), *collagen VI alpha 3* (*col16a3*), *collagen 16 alpha 1* (*col16a1*), *endothelin converting enzyme 1* (*ece1*) *fibronectin 1* (*fn1*), laminin *subunit alpha 1* (*lama1*), *myosin heavy chain 4* (*myh4*), *myosin light chain 9* (*myl9*), *serpine1, tgfb2, tumor necrosis factor superfamily member 10* (*tnfsf10*). Additionally, other pathways altered in the ovarian follicles of 11-KT treated females include retinoic acid receptor activation ($-Log_{10}$ P = 3.40; 11 genes), aryl hydrocarbon signaling ($-Log_{10}$ P = 3.23; 9 genes), fatty acid beta-oxidation III ($-Log_{10}$ P = 2.78; 2 genes), and clathrin-mediated endocytosis signaling ($-Log_{10}$ P = 1.73; 8 genes). The full list of canonical pathways can be found in Table 1.2 and genes associated with canonical pathways are shown in Table S1.5. Treatment with 11-KT was predicted to alter 8 biological functions (Z-score ≥1.5, Fig. 1.5A) associated with reproductive development in the ovary at day 3, in categories of reproductive system development and function, organ morphology, tissue morphology, and organismal development. Genes associated with these biological functions are listed in figure 5B. For the other top ten biological function identified as altered by 11-KT, the associated genes are identified in Table S1.6.

Discussion

Our previous study showed that 11-KT at low concentrations induces growth of primary perinucleolar stage follicles of coho salmon in vitro [32]. This study developed an in vivo model for androgen exposure to first test whether the in vitro results could be replicated in vivo, and second, to determine the androgen-induced changes in the ovarian transcriptome that precede the transformation of primary follicles into the cortical alveolus stage of secondary growth [38,53]. Exposure to 11-KT for 20 days resulted in an early secondary follicle phenotype, and short-term exposure to 11-KT induced rapid and widespread changes in the ovarian transcriptome with the

expression of genes linked to cell, tissue, organ morphology and development, and pathways associated with extracellular matrix. This study is the first to comprehensively analyze the transcriptomic changes underlying promotion of primary follicle development by androgens in teleost fish.

11-KT induces growth of the coho previtellogenic ovarian follicle.

In vivo 11-KT treatment for 10 or 20 days resulted in plasma 11-KT concentrations within the same range that are effective in vitro [32], and induced highly significant increases in ovarian follicle volume at both sampling points. By day 20, approximately half of the ovarian follicles contained cortical alveoli that are characteristic of the completion of primary follicle growth in Pacific salmon [38]. These changes were accompanied by a highly significant 13-fold increase in plasma E2 levels by day 20 in 11-KT treated females. These growth effects are generally similar to those seen in vitro, where 11-KT concentrations as low as 0.03 ng/ml promoted significant increase in follicle volume, but differ in the effect of 11-KT on the appearance of cortical alveoli [32]. By day 20 of in vivo treatment, approximately half of the follicles contained peripheral cortical alveoli. In vitro, the highest concentration of 11-KT (30 ng/ml) had a minor but significant impact on the number of follicles (7%) containing cortical alveoli [32]. The difference in the effects on 11-KT on the appearance of cortical alveoli between the two models may be due to the 11-KT-induced increase in plasma E2 in vivo. In vitro, E2 did not alter size of perinucleolar follicles, but had a much greater impact than 11-KT on the presence and number of cortical alveoli even at the lowest concentration tested (0.03 ng/ml) [32].

The growth promoting effect of 11-KT in vitro is mediated via androgen receptors since the androgen receptor antagonist flutamide abolished, the growth promoting effects of 11-KT at the perinucleolar follicle stage [32]. Incubation with flutamide alone led to widespread atresia, evidence that endogenous androgens are essential survival factors. Testosterone had relatively modest growth-promoting effects in vitro on perinucleolar follicles. Blocking conversion of testosterone to E2 with an aromatase inhibitor (AI) resulted in testosterone having a similar growth-promoting potency as 11-KT on perinucleolar follicles [32]. Since the AI abolishes follicular E2 production, these results provide evidence that in vitro, none of 11-KT's actions in promoting the completion of primary follicle development are mediated by E2.

The 11-KT induced elevation in plasma E2 levels in vivo suggests the development of an early cortical alveolus stage follicle phenotype by day 20 is likely due to a combination of early androgenic and later estrogenic effects. An androgen-induced elevation in plasma E2 was also seen at day 1 and 3 in the short-term study (discussed later), and similar effects have been reported to accompany the in vivo growth-promoting effects of 11-KT on previtellogenic ovarian follicle development of shortfinned eels [58].

The levels of plasma 11-KT decreased in the 11-KT implanted fish from day 10 to day 20, reflecting the gradual decrease in release rate (and possibly altered clearance rates) of the implants over time, but levels were still significantly higher than control levels. For the controls, the reduction from ~0.09 ng/ml at day 10 to ~0.03 ng/ml at day 20 may reflect a stage-dependent decline in 11-KT levels, and/or could be due to mild chronic stress, although females behaved

and fed normally during the experimental period. Plasma levels of 11-KT in females across numerous teleosts species range widely from 0.1 to 42 ng/ml [59]. In salmonids levels in females of up to 1-18 ng/ml have been reported [60], well within the range of concentrations that effectively promote follicle growth in vitro. Other non-aromatizable androgens, such as 5α-dihydrotestosterone may participate in increasing the follicular capacity for E2 biosynthesis [61].

Acute effects of 11-KT on the ovarian transcriptome

To identify the potential underlying mechanisms associated with the growth-promoting effects of 11-KT on primary follicles, we examined the effects of short-term elevation of plasma 11-KT on the ovarian transcriptome. In order to identify the early response to 11-KT before follicle growth was apparent (day 7 in vitro [32], day 10 in the present study), females were sampled after 1 and 3 days of 11-KT treatment. Implants generated plasma 11-KT concentrations similar to those previously shown to promote follicle growth in vitro [32] and close to or within the physiological range reported for salmonids [59,60]. The levels achieved in females implanted for seven days (~3.5 ng/ml) are within the same range as those achieved at day 10 in the long-term exposure study (~1.0 ng/ml), indicating that the release profiles/levels achieved in both studies were similar.

The developing oocyte carries a vast pool of gene transcripts that are translated later in, and contribute to, oocyte development or early embryonic development. Previous studies in teleosts show the ovarian transcriptome [62–69] is dominated by abundant oocyte RNAs. To identify relatively rare transcripts in the oocyte and surrounding somatic follicle cells that are regulated

by 11-KT, we adopted a strategy to maximize the coverage and the total number of unique sequence reads. The number of sequence reads has been shown to be more important than read length for increasing the accuracy of transcript abundance estimates [48,64]. We obtained a high read count (over 805 million paired end reds) and large breadth of coverage of the coho ovary transcriptome, resulting in over 60,000 unique contigs meeting thresholds of ≥201 base pairs and basemean >10. We also identified numerous DEGs associated with ovarian follicle cells, such as those encoding extracellular matrix (ECM) associated proteins and proteins involved in steroidogenesis and steroid signaling, and genes that were previously reported [70] to be expressed in follicle-cell enriched mRNAs. The response of the ovarian transcriptome was very distinct between treatment days and cluster analysis demonstrated clear and consistent transcriptional differences between control and 11-KT treated ovaries. Only seven genes were differentially expressed at both day 1 and day 3, indicating a rapid and dynamic response to 11-KT.

Altered expression of genes associated with secondary follicle growth

Increases in expression at day 3 of transcripts encoding genes involved in steroidogenesis (*fshr, hsd3b2, cyp19a1*) and steroid action (*ar, esr2*) indicate that 11-KT potentially alters the ability of the ovarian follicle to produce and respond to sex steroids. Both *hsd3b2* and *cyp19a1* encode steroidogenic enzymes essential for the synthesis of E2. To our knowledge, in salmonids, only a single *hsd3b* gene is expressed in the gonad [71]. The expression of ovarian *hsd3b* in previtellogenic coho salmon [53] and *hsd3b* and *cyp19a1a* in early vitellogenic rainbow trout [72] was significantly increased in vitro by Fsh. Thus, the increase in transcript levels for these

two steroidogenic enzymes we observed in response to 11-KT could be due to enhanced Fsh signaling, as suggested by the increase in *fshr* transcripts.

The upregulation of *hsd3b* and *cyp19a1* transcripts after short-term treatment with 11-KT is presumably part of the mechanism that resulted in an elevation in plasma E2 levels after both short- and long-term treatment of coho salmon with 11-KT. Elevations in plasma E2 also occur in 11-KT treated eels [58]. Increasing E2 levels are a characteristic of the transition into early secondary follicle growth in coho salmon [38], and are associated with increases in plasma and pituitary Fsh, and in ovarian *fshr*, *steroidogenic acute regulatory protein* (*star*), and *hsd3b* transcripts [37,38].

A variety of studies using coho salmon to model the transition from primary growth to secondary growth have shown increased Fsh signaling to the ovary, via increases in *fshr* transcripts and plasma Fsh. Fsh upregulates expression of several steroidogenic enzyme transcripts in teleost follicles in vitro [37,38,54,55]. Consequently, plasma E2 levels progressively increase during early secondary growth and through vitellogenesis. The increase in plasma E2 and increased *hsd3b*, *cy19a1*, *ar* and *esr2* transcripts in 11-KT treated fish support the hypothesis that a major androgen action during previtellogenic follicle development is to prepare the follicle for production of and response to E2 at the start of secondary growth. Whether this is a direct action on the ovary, or indirectly, mediated by Fsh or other non-ovarian factors remains to be established. Similarly, depending on follicle stage, the elevated plasma E2 levels in female eels treated with 11-KT was accompanied by increased ovarian *fshr* and *cyp19a1* transcripts [58].

Early previtellogenic secondary growth of the coho salmon ovarian follicle is characterized by the massive accumulation of cortical alveoli which eventually occupy most of the ooplasm and function in fertilization as a slow block to polyspermy [73]. No transcripts encoding cortical alveoli components were differentially expressed after short-term 11-KT treatment, but the presence of cortical alveoli after 20 days of 11-KT treatment suggests that the observed effects on cortical alveoli abundance are driven partly via increases in E2 synthesis as described above.

Lipid deposition and the accumulation of lipid droplets occurs both during and after the cortical alveolus stage, depending on species [74], followed by the onset of vitellogenesis. At day 3, 11-KT increased expression of genes involved in lipid transport/uptake (*apoo*, *lpl*), and absorption (*fabp2*), and decreased the expression of genes involved in fatty acid oxidation (*enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase* and *enoyl-CoA delta isomerase 1*) at day 3, possibly reflecting increased storage through reduced metabolism. Genes identified by suppression subtractive hybridization as more highly expressed in mid-cortical alveolus stage coho salmon follicles, compared to those at late perinucleolar stage [53] included genes involved in lipid uptake and processing (*apolipoprotein e* and *lpl*). In eels, the 11-KT-induced increase in lipid droplet abundance is accompanied by an increase in follicle transcripts encoding lipoprotein lipase [27]. Thus, lipid uptake and processing in the previtellogenic follicle may be partially regulated by androgens in two teleost models.

RNA-Seq analysis also showed that treatment with 11-KT altered expression of a number of genes in the tgf-beta superfamily. Expression of *tgfb2, inha,* and *amh* increased at day 3. Although changes in expression of many of these genes have been described during teleost ovarian follicle development [3,5], information on the function of the encoded proteins in the ovary of teleosts is scarce. In previous studies, ovarian *amh* expression in coho salmon was considerably upregulated from the late perinucleolar stage to the mid-cortical alveolus stage [53]. *Anti mullerian hormone* is expressed in GCs of developing mammalian follicles until they reach FSH-mediated growth phases [75,76]; AMH may inhibit the growth-promoting effects of FSH [77]. During secondary growth of coho salmon ovarian follicles, plasma Fsh and ovarian *fshr* expression increase as the ovarian follicle begins to display increased steroidogenic capacity [37,78]. The increase in expression of *inha* and *follistatin* after 11-KT exposure may reflect the ovarian follicle response to, and/or preparation for, Fsh signaling.

Prior to undertaking the RNA-Seq study, we undertook a limited targeted gene approach (qPCR) on ovarian samples at day 10 and 20. Transcripts for the only differentially-expressed genes in the RNA-Seq dataset that were previously measured at day 10 or 20 were *hsd3b*, *cyp19a1*, *amh*, *inha*, and *fshr*: transcript levels for these genes did not differ between follicles from control and 11-KT-treated females, aside from cyp19a1 [41]. This is not surprising since an early secondary follicle (early cortical alveolus stage) phenotype was apparent after 20 days of treatment with 11-KT, and volumes of follicles from 11-KT-treated females were ~25-30% greater than controls at both day 10 and day 20. These non-informative data prompted the RNA-Seq-based examination of transcriptional changes preceding histologically observable changes in follicle size or morphology.

Effect of 11-KT on expression of genes associated with the extracellular matrix and apoptosis.

Many transcripts linked to the ECM, including those encoding numerous forms of collagen and laminin, were altered by 11-KT at day 3. The ECM is integral to ovarian follicle development in vertebrates. During reproductive development, the ECM is continually reorganized to support extensive cell proliferation and differentiation [79–81]. Interactions between the ECM and follicular cells influence gene regulation, cell differentiation, and cell growth [82,83]. The ECM regulates GC survival and proliferation in mammals [84], and changes in the ECM composition may regulate growth factor and hormone access to the developing oocyte [81]. Some ECM-associated genes (*fn1, dcn, clusterin*) that are upregulated in mid-cortical alveolus stage follicles by Fsh in vitro [55] were also upregulated after 11-KT treatment.

Depending on species and follicle stage, androgens may inhibit [20,85] or increase atresia in mammalian ovaries [86,87]. Recent work shows that androgens suppress expression of pro-apoptotic proteins and increase *fshr* expression in mouse preantral granulosa cells, decreasing atresia and stimulating preantral follicle growth [22]. However, testosterone, but not DHT, promotes apoptosis of secondary Atlantic croaker ovarian follicles via a recently-discovered membrane androgen receptor that shows high specificity for testosterone [88]. As in mammals (e.g., [89–91]), E2 appears to also act as an anti-apoptotic factor for teleost ovarian follicles [83,92]. Taken together, the present results support the hypothesis that 11-KT causes extensive ECM and tissue remodeling that supports cellular proliferation, differentiation, and intraovarian communication, and may have direct or indirect (via E2) suppressive effects on apoptosis.

Canonical pathways and biological functions altered by 11-KT

The expression of genes with putative roles in canonical pathways and specific biological functions in reproductive development was altered by 11-KT treatment at day 1 and day 3. The benefits of using transcriptome sequencing combined with RNA-Seq and pathway analysis software is that the transcriptional processes occurring at a particular time point can be identified on a global scale and altered transcriptional pathways and activated biological processes defined. The top ranked canonical pathway, at day 3 post implant affected by 11-KT was hepatic fibrosis/hepatic stellate cell activation. In the context of the ovary, this likely indicates changes in ECM composition of the follicle cell layers, which during late primary and early secondary growth, must undergo extensive remodeling to continue to provide both structural and hormonal support to developing oocytes. The genes included in this pathway with altered expression by 11-KT primarily encode ECM proteins (e.g. collagen isoforms, *fn1, lama1*). Retinoic acid receptor activation is the third ranked canonical pathway and clathrin-mediated endocytosis signaling is the 21st ranked canonical pathway altered by 11-KT at day 3, further implicating 11-KT in regulating biological processes in primary growth ovarian follicles. Retinals are known to be important throughout development [93–95] and are associated with lipid droplets in salmonid oocytes [96]. A number of genes involved in retinoid metabolism (including *rbp4*, 2.10 fold upregulated by 11-KT at day 3) were found to be expressed in rainbow trout ovaries throughout development [97]. Retinoic acid receptor alpha expression was also altered by DHT in maturing fathead minnow ovaries after 9-hours of treatment in vitro [98], in a study that also found that DHT alters cell processes relating to lipids and triglyceride metabolism. Vitellogenins synthesized in the liver in response to E2 are the principal carriers of retinoids and are

sequestered into developing oocytes by receptor-mediated endocytosis. Recently, clathrins have been implicated in the receptor-mediated endocytosis of lipids and vitellogenins in a teleost [99]

Additionally, aryl hydrocarbon receptor signaling was the third ranked canonical pathway in terms of predicted and observed regulated genes. In mammals, the aryl hydrocarbon receptor is involved in regulating the growth of pre-antral and antral follicles by increasing GC proliferation [100] and in regulation of E2 production [101]. Results from this study suggest that androgen-driven changes in the ovarian follicle layers provide hormonal and functional support needed for transition into secondary growth.

The IPA® Pathway analysis also performs causal analyses that uses previously reported cause-effect relationships from the IPA® knowledgebase and the direction of effects within a data set to predict downstream biological consequences [102]. Using this application, a number of biological functions within the category of reproductive development are predicted to be activated in ovary as a result of 11-KT treatment, including *fertility*, the *quantity of ovarian follicle*, *quantity of germ cell*, and *quantity of gonad*. These predictions support the hypothesis that the early transcriptional events described above are driving the morphological changes observed at day 10 and day 20.

Ovarian actions of androgens in mammals: comparison with teleost models

Common themes that have emerged from studies on androgenic regulation of previtellogenic ovarian follicle development of freshwater eels and coho salmon include androgen-driven increased expression of *cyp19a1*, increased plasma levels of E2 in vivo, and increased expression of ovarian *ar, esr* (coho salmon) and *fshr*. Some of these themes are also shared with several mammalian models in which androgens promote pre-antral ovarian follicle growth and survival, stimulate GC proliferation and functional differentiation and increase sensitivity of pre-antral follicles to FSH [23,103–105]; see reviews by Prizant et al. [106], Walters [107], and Lebbe and Woodruff [108]. In mice, these effects are mediated by the AR: GC-specific AR knock out mice were sub-fertile, with ovaries that contained more pre-antral and atretic follicles and fewer antral follicles compared to controls [109]. A positive correlation exists in several mammalian species between *AR* and *FSHR* mRNA, and in primate [110,111] and bovine [112] studies, androgens increased *FSHR* transcripts, an action that was blocked by specific AR antagonists [22,112]. Additionally, in rats, androgens acting through the AR directly modulate FSH-induced estrogen synthesis by increasing *Fshr* and *Cyp19a1* expression [113]. Thus, androgens synergize with FSH in the promotion of pre-antral follicle growth [22,105]. Conversely, androgens are also implicated in *AMH* expression in hyperandrogenism [113] and a positive correlation between *AMH* and *AR* expression is observed in patients with polycystic ovary syndrome [115]. Neuron-specific androgen signaling has also been implicated as part of the mechanism underlying polycystic ovary syndrome, due to androgen excess [116]. In mice, AMH decreases the sensitivity of the ovarian follicle to FSH [77] and decreases FSH-mediated *Cyp19a1* expression [117]. Thus, it is hypothesized that androgens, by increasing expression of their own receptor, amplify their effects locally and by increasing the expression of *Amh*, are fine-tuning the ovarian follicle's sensitivity and response to FSH. In mammals, this may protect the preantral and early

antral follicle pool from early selection for maturation [107], although it is unclear how this may translate to a semelparous species such as coho salmon.

Conclusions

This study presents a workflow in a non-model organism using RNA-Seq and pathway analysis to describe transcriptional events underlying 11-KT-induced growth of the late primary growth ovarian follicle. Previous in vitro and in vivo studies of late perinucleolar stage ovarian follicles in teleosts [25,26,30–32] have shown that 11-KT has strong growth-promoting effects at certain stages of ovarian development, roughly analogous to mammalian puberty. Since 11-KT is a non-aromatizable androgen, previous and current studies indicate that the effects are androgen-induced and not due to the conversion of androgens to estrogens.

In early secondary growth stage coho salmon, both 11-KT and E2 promoted ovarian follicle growth in vitro [32], although E2 was much more effective. In this study, E2 was increased in late perinucleolar stage coho treated with 11-KT after 3 days and cortical alveoli were present after 10 days, signifying a secondary growth stage follicle phenotype. An examination of the transcriptional changes in the ovary associated with both E2 and 11-KT at the beginning of secondary growth (ECA stage) would further define stage-specific responses to these steroids and provide a basis for mechanistic studies of the ovarian follicle stage transition.

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Table 1.1. The expression of ovarian genes with putative androgen and/or estrogen response elements after treatment of females for three days with 11-KT. Ovarian contigs identified as altered by 11-KT were mapped to genes with putative androgen (ARE) and/or estrogen response (ERE) elements- in rainbow trout [110], a closely-related species within the same genus. Data are expressed as fold-change relative to controls.

Ovarian genes of coho salmon with putative AREs and/or EREs regulated by 11-KT after 3 days.

Gene ID	Gene Name	ARE	ERE	**Fold change**
amh	anti mullerian hormone		X	**1.70**
anxa11	annexin A11		X	**1.63**
atp6v1f	ATPase, H+ transporting, lysosomal, V1 subunit F	X	X	**1.32**
col1a1	collagen, type I, alpha 1	X	X	**1.92**
col6a2	collagen, type VI, alpha 2	X	X	**1.95**
fabp2	fatty acid binding protein 2, intestinal	X	X	**1.83**
fkbp10	FK506 binding protein 10		X	**1.66**
gpsm2	G-protein signaling modulator 2	X	X	**-1.53**
h1f0	H1 histone family, member 0	X		**2.03**
hbe1	hemoglobin beta embryonic-1	X	X	**1.67**
hsd3b2	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2		X	**1.51**
id1	inhibitor of DNA binding 1		X	**1.60**
il13ra2	interleukin 13 receptor, alpha 2		X	**1.49**
myo1c	myosin Ic	X		**1.42**
plekhb2	pleckstrin homology domain containing B2		X	**1.37**
rbp4	retinol binding protein 4, plasma	X	X	**1.89**
serpinf1	serpin peptidase inhibitor, clade F, member 1		X	**1.86**
ubr7	ubiquitin protein ligase E3 component n-recognin 7	X	X	**-1.40**

Table 1.2. Canonical pathways significantly altered in ovaries of females treated with 11-KT for one and three days, identified by Ingenuity® Pathway Analysis software. Pathway, -Log_{10} P-value, number of genes represented in data set from total genes reported in pathway.

Ingenuity Canonical Pathways altered by 11-KT at day 1	-log(p-value)	number of genes
Tetrahydrobiopterin Biosynthesis I	2.47	1
Tetrahydrobiopterin Biosynthesis II	2.47	1
Superoxide Radicals Degradation	2.04	1
Mitochondrial Dysfunction	1.79	2
Hepatic Fibrosis / Hepatic Stellate Cell Activation	1.74	2
Glutathione Redox Reactions I	1.67	1
Gluconeogenesis I	1.55	1
Ethanol Degradation IV	1.55	1
Serotonin Receptor Signaling	1.32	1

Ingenuity Canonical Pathways altered by 11-KT at day 3	-log(p-value)	number of genes
Hepatic Fibrosis / Hepatic Stellate Cell Activation	7.05	16
EIF2 Signaling	5.96	15
RAR Activation	3.40	11
Aryl Hydrocarbon Receptor Signaling	3.21	9
Regulation of eIF4 and p70S6K Signaling	2.85	9
Atherosclerosis Signaling	2.85	8
Fatty Acid β-oxidation III (Unsaturated, Odd Number)	2.78	2
Acute Phase Response Signaling	2.63	9
IL-12 Signaling and Production in Macrophages	2.47	8
TGF-β Signaling	2.45	6
Regulation of Actin-based Motility by Rho	2.36	6
Actin Cytoskeleton Signaling	2.26	10
Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis	2.21	10
mTOR Signaling	2.17	9
Estrogen-Dependent Breast Cancer Signaling	2.01	5
Hereditary Breast Cancer Signaling	1.98	7
Intrinsic Prothrombin Activation Pathway	1.90	3
Agranulocyte Adhesion and Diapedesis	1.82	8
Role of NANOG in Mammalian Embryonic Stem Cell Pluripotency	1.76	6
ILK Signaling	1.74	8
Clathrin-mediated Endocytosis Signaling	1.73	8
IL-6 Signaling	1.68	6
Nucleotide Excision Repair Pathway	1.68	3

PCP pathway	1.67	4
Mitochondrial Dysfunction	1.58	7
RANK Signaling in Osteoclasts	1.55	5
Glutamate Biosynthesis II	1.48	1
Glutamate Degradation X	1.48	1
IGF-1 Signaling	1.47	5
Oxidative Phosphorylation	1.43	5
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	1.43	10
Epithelial Adherens Junction Signaling	1.43	6
Glutathione Redox Reactions I	1.40	2
Regulation of the Epithelial-Mesenchymal Transition Pathway	1.38	7
Role of Oct4 in Mammalian Embryonic Stem Cell Pluripotency	1.37	3
CD40 Signaling	1.37	4
Rac Signaling	1.32	5
S-adenosyl-L-methionine Biosynthesis	1.30	1

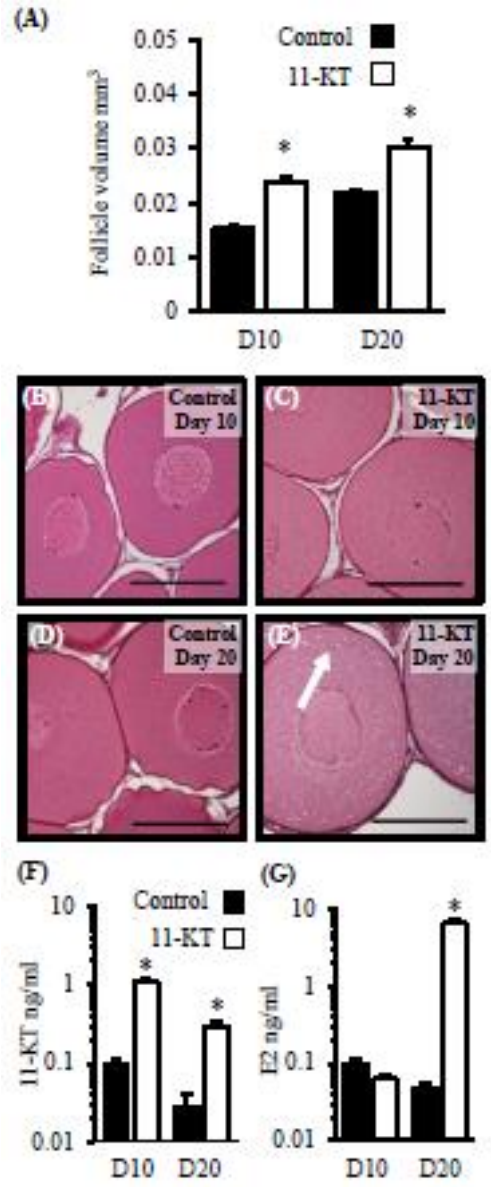


Figure 1.1. Effects of treatment of females with 11-KT for 10 and 20-days on ovarian follicle volume and morphology and on plasma sex steroid levels. A: Ovarian follicle volume (mean ± SEM) was significantly greater after treatment of females with 11-KT (white bars) for 10 days (D10) or 20 days (D20). (*P<0.05, Student *t*-test). B-E: Representative sections of ovarian follicles from control fish at 10 days (B), after 10 days exposure to 11-KT (C), from control fish at 20 days (D), and after 20 days exposure to 11-KT (E). Cortical alveoli (white arrow) are visible in ovarian follicles from 11-KT treated fish at day 20 (E). Scale bars = 200 µm. F-G: Plasma levels of 11-KT (F) and plasma E2 (G) in females treated with 11-KT (white bars) for 10 days (D10) or 20 days (D20). 11-KT treated fish had significantly elevated plasma levels of both steroids (*P<0.001).

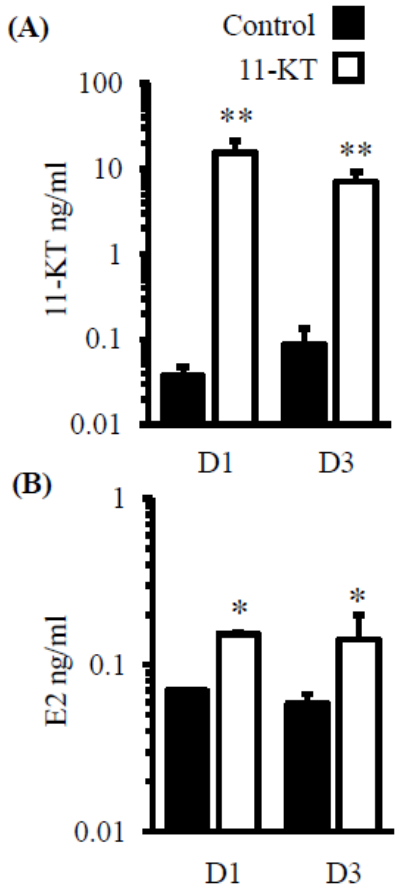


Figure 1.2. Effects of short-term treatment of females with 11-KT for one and three days on plasma sex steroid levels. Plasma 11-KT (A) and E2 (B) levels (mean ± SEM) were significantly higher in females treated with 11-KT for one day (D1) and three days (D3) compared to control levels (* $P<0.05$, ** $P<0.001$, Student *t*-test).

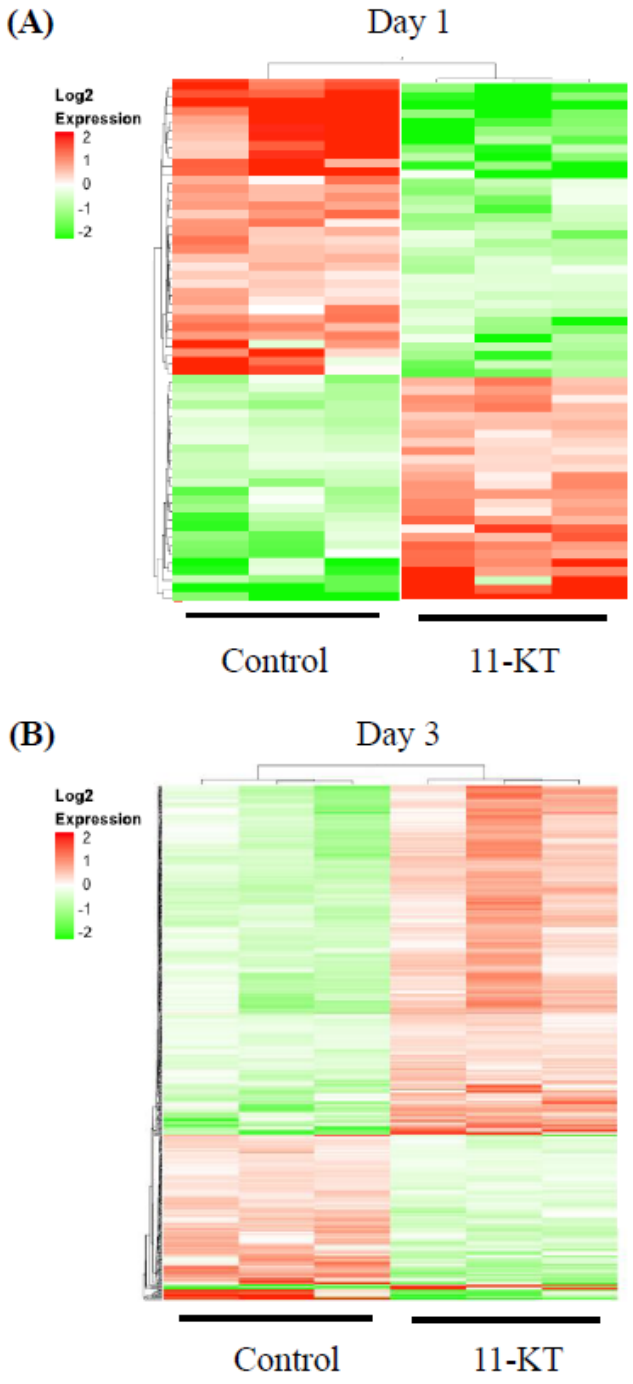


Figure 1.3. Cluster analysis of differentially expressed contigs after short-term treatment of females with 11-KT. Cluster analysis (DESeq2) of differentially expressed contigs (DESeq2, basemean>10, P-adj<0.05) in ovaries from females treated with 11-KT for one day (A, Day 1) and three days (B, Day 3). Expression of contigs (rows) is displayed for three independent samples (columns), with red representing up-regulation and green representing down-regulation from the mean expression value of each contig (white). Each column represents data from ovaries of a single individual.

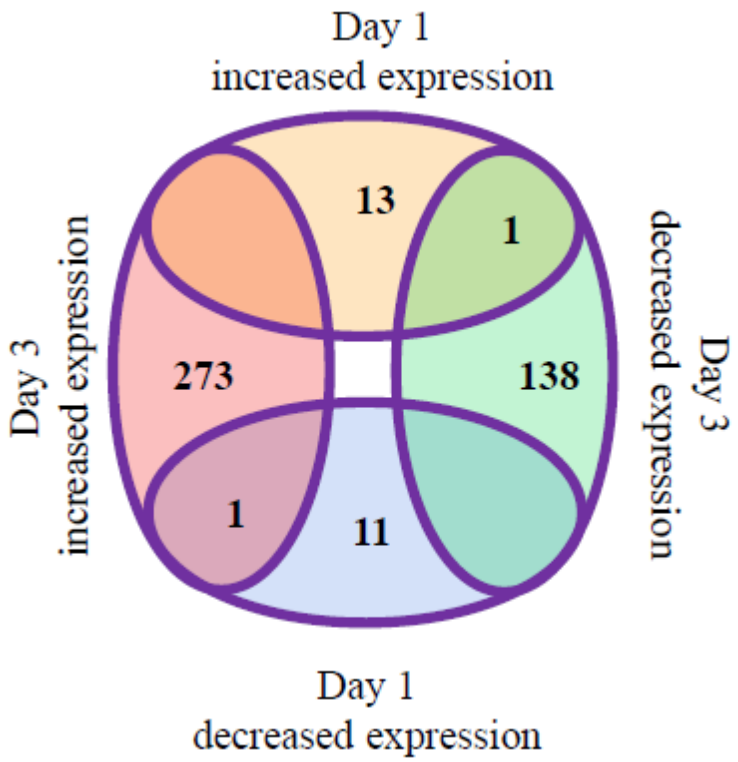


Figure 1.4. Comparison of genes whose expression was altered after short-term treatment of females with 11-KT. After treatment of females with 11-KT for one day or three days, 381 genes, annotated to zebrafish or human genomes, were identified as regulated by 11-KT (DESeq2, $P \leq 0.05$). Only two genes were identified as being regulated by 11-KT at both day 1 and day 3: expression of *dnl-type zinc finger* (*dnlz*) was upregulated at day 1 and downregulated at day 3, and *laminin subunit beta 1* (*lamb1*) was downregulated at day 1 and upregulated at day 3.

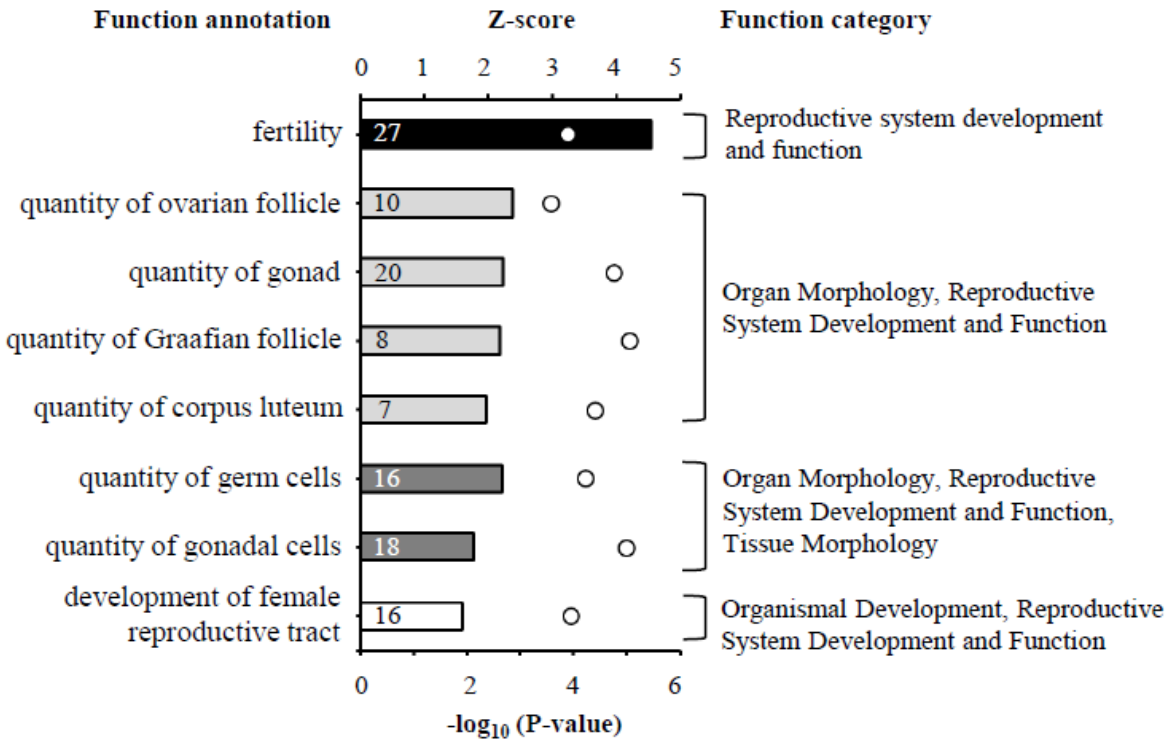


Figure 1.5. Biological functions related to reproductive development in the ovary. Pathway analysis of DEGs was performed based on two metrics: Z-score and P-value. The biological functions in the ovary that are expected to be increased or decreased by 11-KT given gene expression in our dataset were identified using the IPA regulation Z-score algorithm. Only functions (annotations) with a Z-score ≥1.5 or ≤−1.5 are shown. Functions with a Z-score ≥2.0 (fertility, quantity of ovarian follicle, quantity of gonad, quantity of Graafian follicle, quantity of germ cell) are considered to be significantly predictive. The P-value (circles; Fisher exact test) reflects the likelihood that the association between gene expression in our dataset and a related biological function is significant (P ≤0.05 [i.e., $-\text{Log}_{10}$ P ≥1.3]). Numbers within bars indicate total number of genes represented in each biological function. Bar colors indicate biological functions within the same category.

Supplementary Table 1.S1. Ovarian genes analyzed by qPCR, regulation of expression by 11-KT, qPCR primer sequences, and PCR product size.

Gene name	Symbol	Regulation by 11-KT at day 3	Forward primer	Reverse primer	Product size (bp)
Reference genes					
eukaryotic elongation factor 1 alpha	*eef1a*	No effect	cccctggacacagagatttcatc	agagtcacaccgttggcgttac	409
Extracellular matrix components					
decorin	*dcn*	2.01 fold	gaccacaagtacatccaggtga	aacacacagcggaaggtgat	176
fibronectin 1	*fn1*	2.31 fold	gctcttcagaatgtccagagaa	aggccgttgttacctactactg	167
Steroidogenesis related genes					
aromatase	*cyp19a1*	2.19 fold	cgcatgcacagatcagagtt	accgtgacaaaagcgtaacc	175
betahydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2	*hsd3b2*	1.52 fold	ccttcatctacaccagcagcatc	tacaacacatccccgttccg	283
TGF-beta superfamily					
amh	*amh*	1.75 fold	tcactttcaccagtcactctctgc	cacttcttgttccgtcaccaatc	204
Steroid receptors					
androgen receptor	*ar*	1.88 fold	gtcaaactgacccagacagaa	cagcatatttgtcctccattgt	87
estrogen receptor 2	*esr2*	1.98 fold	acagcaggagcaattggact	ttggacagcattgggataca	220
Highly regulated contigs					
amine oxidase, copper containing 3	*aoc3*	3.57 fold	aacgcgtctctttggagtgt	tttaccctgcgcgtaaaact	195
protein tyrosine phosphatase, non-receptor type 22	*ptpn22*	-2.78 fold	aaggccttcccaatgttttt	tccaagcaaagagttcactg	175
Genotyping primers			ctacagagtgcagttggcctc	cctggatgacaatgactctca	

Supplementary Table 1.S2. Summary statistics of the RNA-Seq pipeline showing number of paired reads, number after quality trimming, number mapped, and percent of reads mapped.

	Sample	Total Reads	Paired Reads	Trimmed	Mapped	% Mapped
Control Day 1	1	134,274,870	69,919,205	66,777,658	50,619,323	75.8
	2	149,342,160	77,784,173	74,639,188	56,905,344	76.2
	3	127,623,802	66,492,600	63,858,191	48,345,700	75.7
Control Day 3	4	214,695,172	112,052,096	107,274,490	82,214,706	76.6
	5	122,674,568	64,052,917	61,391,835	47,224,636	76.9
	6	123,061,360	64,226,174	61,586,159	47,468,167	77.1
11-KT Day 1	7	66,071,732	34,011,299	32,895,513	24,769,132	75.3
	8	136,119,546	70,084,843	67,938,192	51,667,084	76.1
	9	158,102,928	81,431,856	79,241,721	60,875,915	76.8
11-KT Day 3	10	131,360,380	67,908,150	65,644,219	50,237,671	76.5
	11	127,435,480	65,946,058	63,248,968	48,044,064	76.0
	12	120,614,808	62,332,734	60,151,965	46,504,879	77.3
	Average	**134,281,401**	**69,686,842**	**67,054,008**	**51,239,718**	**76.4**
	Total	1,611,376,806	836,242,105	804,648,099	614,876,621	

Supplementary Table 1.S3. Changes in expression of ovarian genes after treatment of females with 11-KT for one day. The table lists ovarian contigs regulated by 11-KT after one day, identified by DESeq2 (base mean >10, fold change ≥1.5 or -1.5≤, P-adj <0.1), showing fold change in expression relative to control values.

Most significantly altered ovary contigs 1 day after 11-KT implant in juvenile coho salmon

Top contigs (base mean >10, fold change ≥1.5 or ≤1.5, p-adjusted ≤0.1

Gene Symbol	Gene title (up-regulated)	**Fold change**	P-adj value
camsap2	calmodulin regulated spectrin associated protein family member 2	**3.02**	0.00E+00
dok7	docking protein 7	**2.28**	0.00E+00
slc25a38	solute carrier family 25 member 38	**2.22**	0.00E+00
kctd12b	potassium channel tetramerisation domain containing 12b	**2.05**	6.00E-03
sp5	Sp5 transcription factor	**2.05**	6.00E-03
cdh4	cadherin 4	**2.01**	0.00E+00
gcom1	GRINL1A complex locus 1	**2.00**	1.50E-02
dyrk3	dual specificity tyrosine phosphorylation regulated kinase 3	**1.91**	2.30E-02
malt1	MALT1 paracaspase	**1.87**	1.80E-02
casr	calcium sensing receptor	**1.85**	6.40E-02
dnlz	DNL-type zinc finger	**1.82**	0.00E+00
col28a1	collagen type XXVIII alpha 1	**1.77**	3.70E-02
grb14	growth factor receptor bound protein 14	**1.65**	2.00E-02
me2	malic enzyme 2	**1.63**	3.80E-02

Gene Symbol	Gene title (down-regulated)	**Fold change**	P-adj value
emilin1	elastin microfibril interfacer 1	**-4.72**	0.00E+00
cox6b1	cytochrome c oxidase subunit 6B1	**-2.49**	0.00E+00
urgcp	upregulator of cell proliferation	**-2.15**	0.00E+00
lamb1	laminin subunit beta 1	**-2.11**	1.00E-03
gpx7	glutathione peroxidase 7	**-1.99**	0.00E+00
asap2	ArfGAP with SH3 domain, ankyrin repeat and PH domain 2	**-1.95**	9.00E-03
prdm5	PR domain 5	**-1.94**	0.00E+00
gulp1	GULP, engulfment adaptor PTB domain containing 1	**-1.91**	4.00E-03
f3	coagulation factor III, tissue factor	**-1.88**	5.70E-02
col24a1	collagen type XXIV alpha 1	**-1.82**	2.70E-02
gpr137b	G protein-coupled receptor 137B	**-1.75**	0.00E+00
tmsb10/tmsb4x	thymosin beta 10	**-1.57**	1.80E-02

Supplementary Table 1.S4. Changes in expression of ovarian genes after treatment of females with 11-KT for three days. The table lists ovarian contigs regulated by 11-KT after three days, identified by DESeq2 (base mean >10, fold change ≥1.5 or -1.5≤, P-adj <0.1), showing fold change in expression relative to control values.

Mosty significantly altered ovary contigs 3 days after 11-KT implant in juvenile coho salmon

Top contigs (base mean >10, fold change ≥1.5 or ≤1.5, p-adjusted ≤0.1

Gene Symbol	Gene title (up-regulated)	**Fold change**	p-value
aoc3	amine oxidase, copper containing 3	**3.13**	0.00E+00
ercc1	excision repair cross-complementation group 1	**2.91**	0.00E+00
fn1	fibronectin 1	**2.48**	0.00E+00
col6a3	collagen type VI alpha 3	**2.28**	0.00E+00
col6a1	collagen type VI alpha 1	**2.19**	0.00E+00
dcn	decorin	**2.18**	0.00E+00
hmgb3	high mobility group box 3	**2.18**	0.00E+00
atp1b3	ATPase Na+/K+ transporting subunit beta 3	**2.17**	0.00E+00
ppp6c	protein phosphatase 6 catalytic subunit	**2.14**	0.00E+00
nr2f2	nuclear receptor subfamily 2 group F member 2	**2.14**	0.00E+00
lyz	lysozyme	**2.13**	1.00E-03
col16a1	collagen type XVI alpha 1	**2.13**	0.00E+00
c6	complement component 6	**2.13**	1.00E-03
nr2f1	nuclear receptor subfamily 2 group F member 1	**2.12**	0.00E+00
cpxm2	carboxypeptidase X (M14 family), member 2	**2.09**	0.00E+00
cyp19a1	cytochrome P450 family 19 subfamily A member 1	**2.09**	0.00E+00
krt15	keratin 15	**2.09**	0.00E+00
spon1	spondin 1	**2.06**	0.00E+00
esr2	estrogen receptor 2	**2.05**	0.00E+00
ndnf	neuron-derived neurotrophic factor	**2.04**	0.00E+00
mxra8	matrix-remodelling associated 8	**2.04**	0.00E+00
h1f0	H1 histone family, member 0	**2.03**	1.00E-03
paip2b	poly(A) binding protein interacting protein 2B	**2.01**	0.00E+00
minos1-nbl1/nbl1	neuroblastoma 1, DAN family BMP antagonist	**2.00**	0.00E+00
oard1	O-acyl-ADP-ribose deacylase 1	**1.99**	0.00E+00
fam110b	family with sequence similarity 110 member B	**1.99**	0.00E+00
coro1a	coronin 1A	**1.99**	1.00E-03
hrh1	histamine receptor H1	**1.99**	2.00E-03
sepp1	selenoprotein P, plasma, 1	**1.98**	1.00E-03
ctsk	cathepsin K	**1.98**	0.00E+00
tg	thyroglobulin	**1.98**	3.00E-03
nid2	nidogen 2	**1.96**	2.00E-03
c7	complement component 7	**1.96**	2.00E-03
clu	clusterin	**1.96**	0.00E+00
podxl	podocalyxin like	**1.96**	0.00E+00
col6a2	collagen type VI alpha 2	**1.95**	1.00E-03
dpep3	dipeptidase 3	**1.95**	1.00E-03

c1rl	complement C1r subcomponent like	**1.95**	2.00E-03
ptrf	polymerase I and transcript release factor	**1.95**	0.00E+00
wt1	Wilms tumor 1	**1.94**	1.00E-03
il12rb2	interleukin 12 receptor subunit beta 2	**1.93**	1.00E-03
col1a1	collagen type I alpha 1	**1.92**	1.00E-03
ahnak	AHNAK nucleoprotein	**1.92**	4.00E-03
naga	N-acetylgalactosaminidase, alpha-	**1.91**	2.00E-03
mxra5	matrix-remodelling associated 5	**1.90**	6.00E-03
podn	podocan	**1.90**	2.00E-03
slco2b1	solute carrier organic anion transporter family member 2B1	**1.90**	1.00E-03
ccnd2	cyclin D2	**1.89**	3.00E-03
art1	ADP-ribosyltransferase 1	**1.89**	5.00E-03
fam69a	family with sequence similarity 69 member A	**1.89**	6.00E-03
clec4m	C-type lectin domain family 4 member M	**1.89**	4.00E-03
rbp4	retinol binding protein 4	**1.89**	7.00E-03
krt8	keratin 8	**1.88**	0.00E+00
fam83h	family with sequence similarity 83 member H	**1.88**	2.00E-03
slc4a11	solute carrier family 4 member 11	**1.88**	1.00E-03
cckbr	cholecystokinin B receptor	**1.88**	5.00E-03
crim1	cysteine rich transmembrane BMP regulator 1 (chordin-like)	**1.87**	3.00E-03
tcf23	transcription factor 23	**1.87**	9.00E-03
col4a6	collagen type IV alpha 6	**1.86**	1.00E-03
itsn2	intersectin 2	**1.86**	5.00E-03
rgs14	regulator of G-protein signaling 14	**1.86**	0.00E+00
serpinf1	serpin family F member 1	**1.86**	8.00E-03
pcolce	procollagen C-endopeptidase enhancer	**1.85**	0.00E+00
tinagl1	tubulointerstitial nephritis antigen like 1	**1.85**	0.00E+00
cd82	CD82 molecule	**1.85**	8.00E-03
sparc	secreted protein acidic and cysteine rich	**1.84**	0.00E+00
tgfb2	transforming growth factor beta 2	**1.83**	8.00E-03
fabp2	fatty acid binding protein 2	**1.83**	1.40E-02
cbx5	chromobox 5	**1.82**	2.00E-03
gata6	GATA binding protein 6	**1.82**	1.00E-03
rgs7bp	regulator of G-protein signaling 7 binding protein	**1.81**	5.00E-03
sdpr	serum deprivation response	**1.81**	4.00E-03
vwa1	von Willebrand factor A domain containing 1	**1.80**	0.00E+00
mdk	midkine (neurite growth-promoting factor 2)	**1.80**	5.00E-03
lamb1	laminin subunit beta 1	**1.80**	1.20E-02
lhx9	LIM homeobox 9	**1.79**	1.40E-02
ctss	cathepsin S	**1.79**	4.00E-03
atp1b2	ATPase Na+/K+ transporting subunit beta 2	**1.79**	1.00E-02
hsd11b2	hydroxysteroid (11-beta) dehydrogenase 2	**1.77**	5.00E-03
tgm2	transglutaminase 2	**1.77**	1.10E-02
glud1	glutamate dehydrogenase 1	**1.77**	2.50E-02
trim2	tripartite motif containing 2	**1.77**	2.30E-02
col4a2	collagen type IV alpha 2	**1.76**	1.70E-02
entpd1	ectonucleoside triphosphate diphosphohydrolase 1	**1.76**	2.10E-02
agrn	agrin	**1.76**	4.00E-03
mtpn	myotrophin	**1.76**	2.50E-02
scarb2	scavenger receptor class B member 2	**1.76**	9.00E-03

sla	Src-like-adaptor	**1.76**	2.70E-02
serpine1	serpin family E member 1	**1.76**	2.90E-02
fgl2	fibrinogen like 2	**1.75**	1.50E-02
ar	androgen receptor	**1.75**	2.90E-02
slit1	slit guidance ligand 1	**1.75**	1.40E-02
krt18	keratin 18	**1.75**	2.20E-02
foxl2	forkhead box L2	**1.75**	1.40E-02
pank1	pantothenate kinase 1	**1.75**	2.30E-02
spag7	sperm associated antigen 7	**1.75**	1.80E-02
sulf2	sulfatase 2	**1.74**	1.70E-02
col4a5	collagen, type IV, alpha 5	**1.74**	0.00E+00
notch1	notch 1	**1.74**	1.30E-02
ampd2	adenosine monophosphate deaminase 2	**1.74**	3.00E-03
caskin2	CASK interacting protein 2	**1.74**	1.00E-03
gsn	gelsolin	**1.73**	1.50E-02
cebpd	CCAAT/enhancer binding protein delta	**1.73**	2.60E-02
ehd2	EH domain containing 2	**1.73**	1.40E-02
lgals3bp	lectin, galactoside binding soluble 3 binding protein	**1.73**	3.00E-02
fam132a	family with sequence similarity 132 member A	**1.73**	1.00E-03
dag1	dystroglycan 1	**1.72**	2.50E-02
aldoa	aldolase, fructose-bisphosphate A	**1.72**	1.50E-02
ror2	receptor tyrosine kinase like orphan receptor 2	**1.72**	1.70E-02
angptl4	angiopoietin like 4	**1.71**	3.90E-02
lpl	lipoprotein lipase	**1.71**	1.20E-02
chst12	carbohydrate (chondroitin 4) sulfotransferase 12	**1.71**	3.90E-02
col1a2	collagen type I alpha 2	**1.70**	4.20E-02
prelp	proline/arginine-rich end leucine-rich repeat protein	**1.70**	4.00E-03
nfix	nuclear factor I X	**1.70**	1.00E-02
amh	anti-Mullerian hormone	**1.70**	2.20E-02
gabarapl2	GABA type A receptor associated protein like 2	**1.69**	2.70E-02
tbxas1	thromboxane A synthase 1	**1.69**	1.00E-02
dkk1	dickkopf WNT signaling pathway inhibitor 1	**1.69**	4.70E-02
ugt1a1	UDP glucuronosyltransferase family 1 member A1	**1.69**	1.90E-02
nudt8	nudix hydrolase 8	**1.68**	4.00E-02
lama1	laminin subunit alpha 1	**1.68**	0.00E+00
lxn	latexin	**1.68**	0.00E+00
slc43a3	solute carrier family 43 member 3	**1.68**	1.40E-02
hsd17b1	hydroxysteroid (17-beta) dehydrogenase 1	**1.68**	1.00E-03
slc38a2	solute carrier family 38 member 2	**1.67**	3.00E-02
dnase1l3	deoxyribonuclease I like 3	**1.67**	3.20E-02
mafb	v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog B	**1.67**	4.30E-02
mmp14	matrix metallopeptidase 14	**1.67**	2.80E-02
kcnk4	potassium two pore domain channel subfamily K member 4	**1.67**	3.70E-02
npr2	natriuretic peptide receptor 2	**1.67**	4.90E-02
lama4	laminin subunit alpha 4	**1.67**	1.60E-02
hbe1	hemoglobin subunit epsilon 1	**1.67**	2.40E-02
ldhb	lactate dehydrogenase B	**1.66**	0.00E+00
fkbp10	FK506 binding protein 10	**1.66**	6.00E-03
aldh1a2	aldehyde dehydrogenase 1 family member A2	**1.66**	2.50E-02
phyh	phytanoyl-CoA 2-hydroxylase	**1.66**	4.20E-02

fshr	follicle stimulating hormone receptor	**1.66**	6.40E-02
il1r1	interleukin 1 receptor type 1	**1.66**	6.50E-02
hla-b	major histocompatibility complex, class I, B	**1.65**	3.20E-02
scara3	scavenger receptor class A member 3	**1.64**	4.00E-03
mis12	MIS12 kinetochore complex component	**1.64**	5.00E-02
ece1	endothelin converting enzyme 1	**1.64**	2.60E-02
pld5	phospholipase D family member 5	**1.64**	2.90E-02
tfap4	transcription factor AP-4 (activating enhancer binding protein 4)	**1.64**	5.50E-02
col2a1	collagen type II alpha 1	**1.63**	2.10E-02
eva1b	eva-1 homolog B (C. elegans)	**1.63**	4.10E-02
cav1	caveolin 1	**1.63**	5.40E-02
anxa11	annexin A11	**1.63**	3.50E-02
rnf41	ring finger protein 41	**1.63**	3.00E-03
apoo	apolipoprotein O	**1.62**	3.00E-03
arpc1a	actin related protein 2/3 complex subunit 1A	**1.62**	1.70E-02
serpinh1	serpin family H member 1	**1.62**	4.30E-02
rplp2	ribosomal protein lateral stalk subunit P2	**1.62**	1.10E-02
ephx2	epoxide hydrolase 2	**1.62**	4.30E-02
prkd2	protein kinase D2	**1.62**	8.70E-02
inha	inhibin alpha	**1.61**	0.00E+00
cldn19	claudin 19	**1.61**	5.00E-03
arrdc3	arrestin domain containing 3	**1.61**	7.70E-02
serpind1	serpin family D member 1	**1.61**	2.60E-02
nfkbia	NFKB inhibitor alpha	**1.61**	8.80E-02
hcn3	hyperpolarization activated cyclic nucleotide gated potassium channel 3	**1.60**	3.60E-02
sgcd	sarcoglycan delta	**1.60**	6.80E-02
ephb1	EPH receptor B1	**1.60**	7.50E-02
p3h3	prolyl 3-hydroxylase 3	**1.60**	4.50E-02
bcam	basal cell adhesion molecule (Lutheran blood group)	**1.60**	2.50E-02
id1	inhibitor of DNA binding 1, HLH protein	**1.60**	9.90E-02
antxr1	anthrax toxin receptor 1	**1.60**	4.80E-02
il6st	interleukin 6 signal transducer	**1.60**	3.00E-03
h2afy2	H2A histone family member Y2	**1.60**	1.50E-02
cemip	cell migration inducing hyaluronan binding protein	**1.59**	0.00E+00
s100a4	S100 calcium binding protein A4	**1.59**	8.70E-02
sumo3	small ubiquitin-like modifier 3	**1.59**	4.00E-03
nit2	nitrilase family member 2	**1.59**	2.00E-02
lpgat1	lysophosphatidylglycerol acyltransferase 1	**1.58**	1.50E-02
lims1	LIM zinc finger domain containing 1	**1.58**	7.90E-02
sash1	SAM and SH3 domain containing 1	**1.58**	1.00E-03
actr2	ARP2 actin-related protein 2 homolog (yeast)	**1.58**	4.10E-02
jam3	junctional adhesion molecule 3	**1.58**	5.80E-02
ripk2	receptor interacting serine/threonine kinase 2	**1.57**	1.70E-02
gpx3	glutathione peroxidase 3	**1.57**	5.00E-03
elmo2	engulfment and cell motility 2	**1.57**	1.00E-02
mxd1	MAX dimerization protein 1	**1.57**	3.30E-02
mt-co1	cytochrome c oxidase subunit I	**1.57**	3.00E-03
slc9a3r1	SLC9A3 regulator 1	**1.55**	1.80E-02
tmem173	transmembrane protein 173	**1.55**	3.00E-03
plp1	proteolipid protein 1	**1.55**	3.00E-03

dlx2	distal-less homeobox 2	**1.55**	4.00E-03
traf5	TNF receptor associated factor 5	**1.55**	4.10E-02
plekhh1	pleckstrin homology, MyTH4 and FERM domain containing H1	**1.55**	4.00E-02
app	amyloid beta precursor protein	**1.55**	9.40E-02
gpcpd1	glycerophosphocholine phosphodiesterase 1	**1.55**	4.00E-02
tnfsf10	tumor necrosis factor superfamily member 10	**1.54**	8.70E-02
rdh16	retinol dehydrogenase 16 (all-trans)	**1.54**	0.00E+00
itgav	integrin subunit alpha V	**1.53**	5.80E-02
rbpj	recombination signal binding protein for immunoglobulin kappa J region	**1.53**	3.00E-03
rnf122	ring finger protein 122	**1.53**	3.00E-03
ndfip1	Nedd4 family interacting protein 1	**1.53**	4.90E-02
rpl37	ribosomal protein L37	**1.53**	4.20E-02
nfe2l2	nuclear factor, erythroid 2 like 2	**1.52**	5.20E-02
rps27	ribosomal protein S27	**1.52**	5.00E-03
tmem14c	transmembrane protein 14C	**1.52**	2.00E-03
rpl39	ribosomal protein L39	**1.52**	5.00E-02
hist2h2ab	histone cluster 2, H2ab	**1.52**	7.00E-03
hsd3b2	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2	**1.51**	7.00E-03
rbx1	ring-box 1	**1.51**	8.40E-02
gpx6	glutathione peroxidase 6	**1.51**	2.00E-02
cryzl1	crystallin zeta like 1	**1.51**	1.00E-03
rps4x	ribosomal protein S4, X-linked	**1.50**	0.00E+00
c2orf68	chromosome 2 open reading frame 68	**1.50**	1.40E-02
igfbp7	insulin like growth factor binding protein 7	**1.50**	4.00E-03

Gene Symbol	Gene title (down-regulated)	**Fold change**	p-value
ptpn11	protein tyrosine phosphatase, non-receptor type 11	**-3.52**	0.00E+00
dnlz	DNL-type zinc finger	**-3.04**	0.00E+00
znf598	zinc finger protein 598	**-2.10**	0.00E+00
ddb2	damage specific DNA binding protein 2	**-2.08**	0.00E+00
tbx6	T-box 6	**-2.07**	1.00E-03
tnfaip2	TNF alpha induced protein 2	**-1.99**	0.00E+00
atp6v0a4	ATPase H+ transporting V0 subunit a4	**-1.98**	1.00E-03
fam204a	family with sequence similarity 204 member A	**-1.97**	3.00E-03
fos	FBJ murine osteosarcoma viral oncogene homolog	**-1.96**	1.00E-03
pip4k2c	phosphatidylinositol-5-phosphate 4-kinase type 2 gamma	**-1.94**	4.00E-03
naa15	N(alpha)-acetyltransferase 15, NatA auxiliary subunit	**-1.93**	4.00E-03
ssbp2	single stranded DNA binding protein 2	**-1.92**	0.00E+00
chga	chromogranin A	**-1.88**	1.00E-03
gdpd5	glycerophosphodiester phosphodiesterase domain containing 5	**-1.87**	3.00E-03
sh2b2	SH2B adaptor protein 2	**-1.84**	2.00E-03
rerg	RAS like estrogen regulated growth inhibitor	**-1.83**	5.00E-03
mei1	meiotic double-stranded break formation protein 1	**-1.80**	5.00E-03
ppdpf	pancreatic progenitor cell differentiation and proliferation factor	**-1.79**	0.00E+00
fam101b	family with sequence similarity 101 member B	**-1.79**	2.10E-02
cdx2	caudal type homeobox 2	**-1.78**	1.60E-02
sh3pxd2b	SH3 and PX domains 2B	**-1.78**	2.30E-02
gnat1	G protein subunit alpha transducin 1	**-1.76**	5.00E-03
itpka	inositol-trisphosphate 3-kinase A	**-1.76**	4.00E-03

myh4	myosin, heavy chain 4, skeletal muscle	**-1.75**	1.00E-03
hdac6	histone deacetylase 6	**-1.73**	2.40E-02
gsc	goosecoid homeobox	**-1.72**	8.00E-03
c1qtnf9b	C1q and tumor necrosis factor related protein 9B	**-1.72**	4.10E-02
ehhadh	enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase	**-1.71**	3.20E-02
ercc5	excision repair cross-complementation group 5	**-1.71**	2.00E-03
ston2	stonin 2	**-1.69**	1.00E-03
ccdc151	coiled-coil domain containing 151	**-1.69**	1.00E-03
trappc3	trafficking protein particle complex 3	**-1.68**	3.30E-02
b4galnt1	beta-1,4-N-acetyl-galactosaminyltransferase 1	**-1.67**	5.20E-02
ppl	periplakin	**-1.65**	4.30E-02
cfap58	cilia and flagella associated protein 58	**-1.65**	2.00E-02
eif4a2	eukaryotic translation initiation factor 4A2	**-1.64**	2.90E-02
tgm1	transglutaminase 1	**-1.64**	5.60E-02
bcl9l	B-cell CLL/lymphoma 9-like	**-1.64**	2.90E-02
march2	membrane associated ring-CH-type finger 2	**-1.63**	4.80E-02
id3	inhibitor of DNA binding 3, HLH protein	**-1.63**	8.50E-02
camkv	CaM kinase like vesicle associated	**-1.63**	0.00E+00
dynll2	dynein light chain LC8-type 2	**-1.62**	2.30E-02
wnt3	Wnt family member 3	**-1.62**	1.50E-02
ctnnal1	catenin alpha like 1	**-1.62**	8.70E-02
avil	advillin	**-1.61**	1.00E-03
eci1	enoyl-CoA delta isomerase 1	**-1.61**	1.00E-03
golga3	golgin A3	**-1.61**	4.00E-02
csnk1g2	casein kinase 1 gamma 2	**-1.60**	7.20E-02
grb10	growth factor receptor bound protein 10	**-1.60**	3.90E-02
rsad1	radical S-adenosyl methionine domain containing 1	**-1.59**	3.40E-02
pmp22	peripheral myelin protein 22	**-1.59**	2.00E-03
jag1	jagged 1	**-1.58**	5.40E-02
ovol1	ovo like zinc finger 1	**-1.58**	2.70E-02
pik3cg	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma	**-1.57**	1.20E-02
zfyve1	zinc finger FYVE-type containing 1	**-1.57**	1.40E-02
casp8	caspase 8	**-1.56**	6.10E-02
mepce	methylphosphate capping enzyme	**-1.56**	1.00E-03
rnf220	ring finger protein 220	**-1.56**	6.00E-03
foxi1	forkhead box I1	**-1.55**	9.00E-03
atxn7l2	ataxin 7 like 2	**-1.54**	1.70E-02
tnrc6b	trinucleotide repeat containing 6B	**-1.54**	2.90E-02
gaa	glucosidase alpha, acid	**-1.53**	2.20E-02
gpsm2	G-protein signaling modulator 2	**-1.53**	0.00E+00
rtn2	reticulon 2	**-1.50**	1.00E-03
smyd4	SET and MYND domain containing 4	**-1.50**	1.10E-02
thumpd1	THUMP domain containing 1	**-1.50**	4.10E-02

Supplementary Table 1.S5. Genes from RNA-seq data represented in canonical pathway analyses. Top canonical pathways altered by 11-KT at day 1 and day 3; Ingenuity canonical pathway, gene IDs. See figure Table S3 and S4 for full gene names.

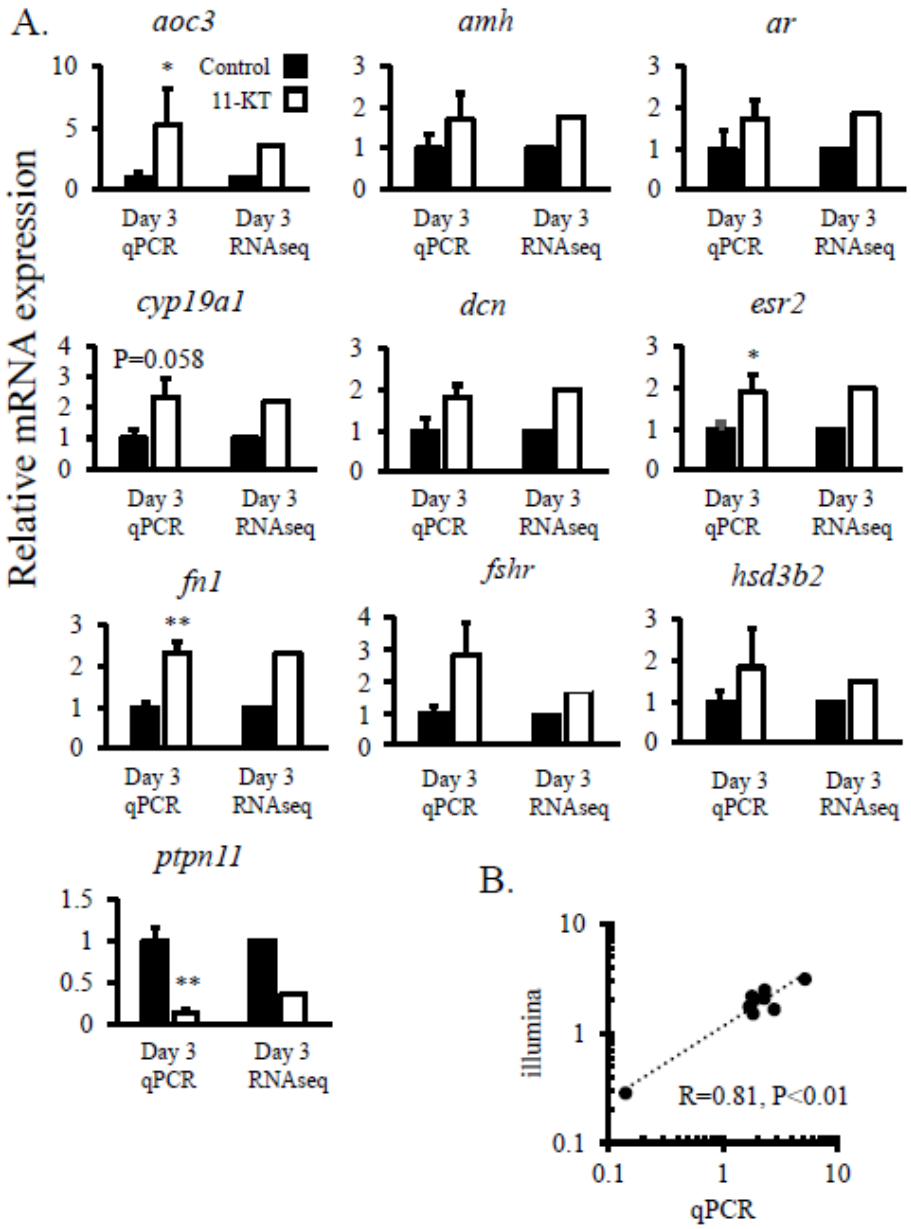
Ingenuity Canonical Pathways altered by 11-KT at day 1	gene IDs
Tetrahydrobiopterin Biosynthesis I	*spr*
Tetrahydrobiopterin Biosynthesis II	*spr*
Superoxide Radicals Degradation	*gpx7*
Mitochondrial Dysfunction	*cox6b1, gpx7*
Hepatic Fibrosis / Hepatic Stellate Cell Activation	*col24a1, col28a1*
Glutathione Redox Reactions I	*gpx7*
Gluconeogenesis I	*me2*
Ethanol Degradation IV	*gpx7*
Serotonin Receptor Signaling	*spr*

Ingenuity Canonical Pathways altered by 11-KT at day 3	gene IDs
Hepatic Fibrosis / Hepatic Stellate Cell Activation	*myh4, fn1, col6a2, col4a6, col2a1, col4a2, col1a2, col16a1, myl9, col1a1, col6a1, col6a3, lama1, tgfb2, ece1, serpine1*
EIF2 Signaling	*rps27, rplp2, rpl39, eif4a2, rps11, rpl7, rps4x, fau, rps7, ptpn11, rps13, pik3cg, rpl37, rpl31, rpl18*
RAR Activation	*nr2f1, fos, smarca2, pik3cg, aldh1a2, nr2f2, rdh16, tgfb2, carm1, smarca4, rbp4*
Aryl Hydrocarbon Receptor Signaling	*tgm2, nr2f1, fos, nfix, ccnd2, aldh1a2, tgfb2, esr2, smarca4*
Regulation of eIF4 and p70S6K Signaling	*rps7, ptpn11, rps27, rps13, pik3cg, eif4a2, rps11, rps4x, fau*
Atherosclerosis Signaling	*col1a2, col1a1, lyz, lpl, col2a1, rarres3, clu, rbp4*
Fatty Acid β-oxidation III (Unsaturated, Odd Number)	*ehhadh, eci1*
Acute Phase Response Signaling	*il6st, fos, fn1, ptpn11, pik3cg, serpinf1, serpine1, rbp4, serpind1*
IL-12 Signaling and Production in Macrophages	*fos, lyz, ptpn11, pik3cg, tgfb2, il12rb2, clu, rbp4*
TGF-β Signaling	*fos, inha, gsc, amh, tgfb2, serpine1*
Regulation of Actin-based Motility by Rho	*myl9, actr2, arpc1a, pfn2, gsn, pip4k2c*
Actin Cytoskeleton Signaling	*myl9, myh4, actr2, fn1, arpc1a, ptpn11, pik3cg, pfn2, gsn, pip4k2c*

Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis	*col1a1, fos, ctsk, ptpn11, wnt3, mmp14, pik3cg, traf5, dkk1, gsn*
mTOR Signaling	*rps7, ptpn11, rps27, rps13, pik3cg, eif4a2, rps11, rps4x, fau*
Estrogen-Dependent Breast Cancer Signaling	*fos, ptpn11, cyp19a1, pik3cg, hsd17b1*
Hereditary Breast Cancer Signaling	*polr2g, hdac6, ptpn11, smarca2, pik3cg, ddb2, smarca4*
Intrinsic Prothrombin Activation Pathway	*col1a2, col1a1, col2a1*
Agranulocyte Adhesion and Diapedesis	*aoc3, myl9, myh4, hrh1, fn1, cldn19, mmp14, podxl*
Role of NANOG in Mammalian Embryonic Stem Cell Pluripotency	*il6st, ptpn11, wnt3, pik3cg, cdx2, gata6*
ILK Signaling	*myl9, myh4, fos, fn1, ptpn11, pik3cg, sh2b2, krt18*
Clathrin-mediated Endocytosis Signaling	*actr2, lyz, arpc1a, ston2, ptpn11, pik3cg, clu, rbp4*

Supplementary Table 1.S6. Genes associated with biological functions related to reproductive system development and function. Biological function, activation state, gene IDs. See tables S4 for full gene names.

Biological Function	Predicted activation state	Genes altered in function
fertility	Increased	*ace, amh, app, ar, cav1, cyp19a1, dcn, entpd1, ercc1, esr2, fshr, hsd17b1, il6st, inha, krt8, lamb2, mmel1, nr2f2, ovol1, sec23ip, sepp1, slc6a6, slc9a3r1, sulf2, vdac3, wt1, zp3*
quantity of ovarian follicle	Increased	*akt2, amh, ar, cyp19a1, esr2, fshr, hsd17b1, il6st, inha, zp3*
quantity of gonad	Increased	*akt2, amh, ar, b4galnt1, cyp19a1, entpd1, esr2, fos, fshr, hsd17b1, il6st, inha, ovol1, paip2b, ror2, sepp1, tgfb2, wnt3, wt1, zp3*
quantity of Graafian follicle	Increased	*akt2, ar, cyp19a1, esr2, fshr, hsd17b1, inha, zp3*
quantity of corpus luteum		*akt2, ar, cyp19a1, esr2, fshr, hsd17b1, inha*
quantity of germ cells	Increased	*ar, cyp19a1, entpd1, esr2, fos, fshr, il6st, inha, ovol1, paip2b, ror2, sepp1, tgfb2, wnt3, wt1, zp3*
quantity of gonadal cells		*akt2, ar, b4galnt1, cyp19a1, entpd1, esr2, fos, fshr, il6st, inha, ovol1, paip2b, ror2, sepp1, tgfb2, wnt3, wt1, zp3*
development of female reproductive tract		*amh, ar, ccnd2, cyp19a1, esr2, foxl2, fshr, gata6, inha, lhx9, npr2, rbp4, serpine1, trim25, wnt9b, zp3*



Supplementary Figure 1.S1. Ovarian genes identified by RNA-Seq as regulated 11-KT, analyzed by qPCR. A: Comparison of the relative change in expression of individual genes from ovarian samples from females treated for three days (Day 3) with 11-KT, analyzed by qPCR and RNAseq. Genes were chosen for targeted qPCR analysis based on either the magnitude of difference from controls (*aoc3*, *ptpn11*) determined by DESeq2, or on genes known to be involved in regulation of ovarian growth/function (*amh, ar, cyp19a1, dcn, esr2, fn1, hsd3b1)*. Values (mean ± SEM) that are statistically different from controls are marked by asterisks (*P<0.05; **P<0.01, n=3; Student t-test). B: All qPCR and RNA-Seq expression values for the ten genes shown in Fig. S1A were plotted and their linear relationship analyzed. All values were highly correlated (Spearman correlation coefficient, R=0.81, P<0.01).

CHAPTER 2: In vivo treatment with sex steroids rapidly alters the ovarian transcriptome of previtellogenic secondary growth coho salmon (*Onchorhynchus kisutch*).

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Introduction

The development of a competent oocyte is divided into three general stages: primary growth, secondary growth, and maturation (reviewed in [1,2]). Primary growth commences with the onset of meiosis in which the relatively small non-specialized oogonia develop into chomatin nucleolar oocytes that arrest in prophase I. An inner granulosa layer and an outer theca layer of cells begins to form at this time and eventually surrounds the oocyte, providing the oocyte with mechanical and biochemical support. Together, these somatic layers with the oocyte make up the ovarian follicle. As this ovarian follicle develops, ribosome-producing nucleoli appear around the periphery of the nucleus and maternal RNAs begin to be deposited. This is known as the perinucleolar stage of primary growth, and is further characterized by the appearance and later disassembly of the Balbiani body, a mitochondrial rich nuage of cellular organelles, maternal RNAs, and RNA-binding proteins. The appearance of glycoprotein-rich cortical alveoli in the oocyte periphery is a marker of the initiation of the early cortical alveolous stage, and the beginning of secondary growth. During secondary growth, the ovarian follicle undergoes a massive size increase due to the sequential incorporation of lipids and then vitellogenin (Vtg) during vitellogenesis [1,3,4]. Vitellogenesis is mediated primarily by pituitary-derived follicle stimulating hormone (Fsh) stimulating the production of ovarian estradiol-17β (E2) which stimulates the hepatic synthesis and release of vitellogenin proteins [1] and vitellogenein uptake [5]. These proteins bind to specific vitellogenin receptors on the oocyte surface and are endocytosed, cleaved into yolk proteins, and centripetally incorporated into the oocyte.

The processes described above are under the control of numerous endocrine and paracrine factors [1], and recent studies using several teleost models indicate that estrogenic and androgenic steroids play stage-specific roles in regulating development [2]. During the perinucleolar stage of primary growth in vitro exposure to a non-aromatizable androgen, 11-ketotestosterone (11-KT), but not E2, resulted in a significant increase in the volume of ovarian follicles [6]. Increased size of previtellogenic follicles after exposure to 11-KT has also been reported for shortfinned eel [7] and cod [8]. In vivo exposure to 11-KT for 10 days also increased the volume of perinucleolar follicles of coho salmon [9], and a secondary follicle phenotype had developed after 20 days of treatment, while 20 days of exposure to E2 was required before a significant increase in volume occurred [10]. This suggest that androgen signaling may be a primary driver of growth at this stage.

Using deep transcriptome sequencing, we identified hundreds of ovarian follicle transcripts in which expression levels were altered by in vivo 11-KT treatment, prior to development of the secondary follicle phenotype [9]. These included transcripts encoding proteins involved in steroidogenesis and steroid action, growth factor signaling, and the extracellular matrix. Using pathway analysis software, we identified biological functions and canonical pathways that were potentially altered, including ovarian development, tissue differentiation and remodeling, and lipid metabolism. Plasma E2 levels were also increased by this treatment, as well as *fsh* transcript levels, both hallmarks of entry into secondary growth [11,12]. Together these results suggest that

androgens promote both primary ovarian follicle development, and the transition into secondary ovarian follicle growth.

Early hypophsectomy studies suggested that ovarian follicle development prior to secondary growth is gonadotropin independent [13,14], but more recent studies in zebrafish have challenged that assumption [15]. Nonetheless, during early (previtellogenic) secondary growth, sex steroids appear to be important regulators of development. Treatment of perinucleolar stage follicles with E2 promoted the formation of cortical alveoli in vivo [10,13] and in vitro [6] and increased ovarian follicle volume in vivo [10]. 11-KT was as effective as E2 in promoting ovarian follicle growth at this stage [6], but not the formation of cortical alveoli. In order to identify the mechanisms underlying the growth-promoting actions of these steroids, we implanted female coho salmon containing ovaries at the early secondary (cortical alveolus) stage with sustained release pellets containing either E2 or 11-KT for three days and determined changes in the ovarian follicle transcriptome using RNA-Seq followed by pathway analysis.

Methods

Chemicals and general animal procedures

11-Ketotestosterone was purchased from Steraloids (Newport, RI). Cholesterol and E2 were purchased from Sigma-Aldrich (St. Louis, MO). L-15, Hematoxylin, eosin, and diethyl ether were purchased from Thermo Fisher Scientific (Waltham, MA). Bouin fixative was purchased from Ricca Chemical Company (Arlington, TX).

Juvenile coho salmon (Issaquah Hatchery stock, Issaquah, WA) stock were reared from eyed embryos to 22-23 months of age at the hatchery facilities of the Northwest Fisheries Science Center, Seattle, WA under simulated natural photoperiod in re-circulated 10-11°C fresh water, under an approved protocol according to guidelines established by the Institutional Animal Care and Use Committee, University of Washington (protocol #4078-04). Fish were fed twice daily with a commercial feed (BioDiet, Bio-Oregon, Longview, WA) according to the manufacturer's guidelines.

Genetic sex was determined using an established sex marker in tagged fish, which were sorted to generate an all-female stock, that was reared as previously described [9]. At the termination of experiments, fish were anesthetized in buffered 0.05% tricaine methanesulfonate until movement of the gill operculum ceased. Fork length and body weight were measured. Blood was collected from the caudal vein and immediately transferred to heparinized microcentrifuge tubes and placed on ice. Blood plasma was separated by centrifugation at 1200 × g for 15 minutes. After decapitation, ovaries were removed and weighed and then either snap frozen in liquid nitrogen, or fixed in Bouin fixative for histological analysis.

Experimental procedures

Female juvenile coho (121±2.8 g, 2-years of age) were implanted with either blank cholesterol pellets or cholesterol pellets containing 10 µg 11-KT or 100 µg E2 (produced as described in

Monson et al. [9]). The amount of steroid included in the pellets was determined in preliminary experiments to result in significant, but physiologically relevant increases in plasma steroid levels. As in our previous study [9], pellets containing 11-KT were incubated for 24 hours in sterile L-15 media (Thermo Fisher Scientific) and were then washed with L-15 prior to implantation to temper the initial release rate. Fish were lethally sampled after 3 days. At that time, fork length, body weight, and gonad weight were measured, and blood and ovaries were collected as described above. Histological screening eliminated any female that displayed overtly asynchronous ovarian stages or were not at the cortical alveolus stage, and frozen ovarian samples from control, 11-KT, and E2 treated females (N=3 per group) were selected for RNA-Seq analysis.

Sex steroid assays

Steroids were double extracted from 250 µl of plasma using diethyl ether (1.5 ml x 2) and extracts were evaporated under a nitrogen gas stream, then re-suspended in appropriate buffers. Plasma 11-KT levels were measured by enzyme-linked immunoassay [16] using tracer and secondary antibody coated plates from Cayman Chemicals (Ann Arbor, MI) and primary antibody donated by David Kime (University of Sheffield, UK). Plasma E2 was measured by radioimmunoassay, as described by Sower and Schreck [17], and modified by Fitzpatrick et al. [18]. Validation and characteristics of these assays have been reported previously [16,19].

Histological analysis

Fixed ovarian tissues were washed with 70% ethanol, dehydrated in increasing concentrations of ethanol and xylene, and embedded in paraffin wax. Sections with a thickness of 5 µm were cut

and mounted on microscope slides and stained with hematoxylin and eosin. Average ovarian follicle volume was calculated from at least 15 follicles per sample that were sectioned through the nucleus of the oocyte, using an image analysis system (NIS-elements, Nikon, USA), as described previously [6], and oocytes were scored for stage based on previously published criteria [6,11].

RNA Extraction

Total RNA was extracted using Qiagen RNEasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's guidelines. RNA pellets were re-suspended in DNAse/RNAse free water (Sigma-Aldrich). Total RNA concentrations in extracts were determined using a NanoDrop ND-100 (NanoDrop Technologies, Wilmington, DE).

RNA-Seq and pathway analysis of alteration in the ovarian transcriptome

i. *Sample preparation:* Total ovarian RNA (200 ng) was submitted to Omega Bio-Tek Inc (Norcross, GA) for quality checking, library preparation (poly A selected), and 100 base pair, paired-end sequencing.

ii. RNA-seq Analysis: Bioinformatic analyses were performed using the DRAP pipeline as described in Cabau et al. [20] and Monson et al. [9]. Briefly, sequences were quality trimmed using Trim Galore v0.4.0 [21] and assembled into a de novo backbone with Drap v1.8 [20] and Oases v0.2.09 [22] using the kmer values of 19, 23, 25, 27, 31, and 35. Contiguous sequences

(contigs) that had FPKM (fragments per kilobase of transcript per million mapped reads) greater than 1 and had sequence lengths greater than 200 bp were retained. These contigs were annotated using BlastX against the NCBI non-redundant protein database (nr) and partially non-redundant nucleotide database (nt); only sequences with an E-value ≤-05 were retained. Gene level count estimates were made using RSEM v.1.2.31 [23] and bowtie2 v2.2.6 [24] and differential expression was determined using DESeq2 [25]. Contigs with a P-adjusted (P-adj) value ≤0.1 were considered significantly altered between control and 11-KT or E2 treatment. To control for sequencing errors and differences in sequencing depth leading to misidentification of differential expression of contigs with low read counts, those contigs with a basemean ≤10 were excluded from further analysis. Gene clustering was performed using cluster::agnes package in R with the Spearman method [26]; data were $\log_2$ transformed and centered on a mean expression value to improve visualization of expression differences.

iii. Pathway Analysis: Ingenuity Pathway Analysis® (IPA) software was used to conduct pathway and network analyses and predict the effects of steroid treatment on biological functions. Contigs were initially mapped to zebrafish orthologs using BLASTN against the Ensembl *Danio rerio* gene database (v.Zv9.72). However, some zebrafish genes have not been mapped to mammalian orthologs, so the remaining contigs were mapped to the *Homo sapien* transcript database (v.GRCH37.72) for inclusion in IPA. If more than one contig (P≤0.05) mapped to the same gene, the average expression value of those contigs was used as the gene expression value in further analyses. The expression patterns of the zebrafish and human gene orthologs were compared to the IPA database to estimate altered canonical pathways and biological functions (Fisher exact test P≤0.05 [$-\log_{10}$ P-value ≥1.3]). This program generates

networks that maximize the connectivity of genes with significantly altered expression based on known functional interactions [27], predicts alterations in biological function, and predicts both upstream and downstream regulators given the direction of expression differences in given gene sets. A z-score was calculated to identify predicted increases or decreases in biological functions in treated samples relative to controls. The z-score is a statistical measure of the match between expected and observed gene expression direction. Zebrafish nomenclature is used throughout when referring to fish species, although due to the use of this software, human gene names are used in places where annotation to the zebrafish database was not possible.

Results

Morphometrics and sex steroid levels.

No significant treatment effects on fish selected for analysis were observed between control and treated samples with regard to fork length (21.6±0.2 cm), body weight (121.6±2.8 g), GSI (0.4±0.02), ovarian follicle volume (0.044±0.004 mm^2), or gross ovarian follicle morphology. All samples selected for sequencing displayed an early to mid-cortical alveolus stage phenotype (Fig. 2.1 A-C). Plasma 11-KT and E2 levels in control fish were 0.12±0.04 and 0.18±0.02 ng/ml ng/ml respectively. In samples selected for RNA-Seq, treatment with 11-KT for 3 days significantly increased plasma 11-KT levels to 18.5±3.7 ng/ml (Fig. 2.1 D), but did not alter E2 levels (0.2±0.1 ng/ml) and treatment with E2 for 3 days significantly increased plasma E2 to 15.3±3.4 ng/ml (Fig. 2.1 E), and did not alter plasma 11-KT levels (0.05±0.02 ng/ml).

RNA-seq

Sequencing resulted in 1.6 billion total reads from 9 samples (Table 2.1). Following quality trimming and pairing, greater than 99.7% of reads were retained. De novo assembly generated 63,423 contigs between 201 bp and 15,353 bp with a mean contig length of 1,673 bp. Eighty-one percent of reads were mapped to the denovo backbone. A total of 63,048 of these contigs (99.4%) were annotatable.

RNA-seq analysis identified 8,707 and 139 contigs that were differentially expressed (DESeq2, P-adjusted ≤0.1) from controls in 11-KT and E2 treated samples, respectively (Fig. 2.2A-B). Cluster analysis of differentially expressed contigs in 11-KT treated samples (Fig. 2.2C) and in E2 treated samples (Fig. 2.2D) demonstrated distinct differences in expression patterns between control and treatment groups.

Alterations in the ovarian transcriptome from acute sex steroid exposure

Of the 63,423 contigs generated, 43,820 (69%) were annotated to zebrafish or human gene orthologs and mapped to IPA. Duplicate contigs were collapsed to the gene-ID level using the average expression value relative to controls, resulting in 3,853 (Table 2.2) and 100 genes (Table 2.3) with expression altered by 11-KT or E2, respectively (P-adjusted ≤0.1).

In the 11-KT treated samples, the expression of genes that encode proteins involved in steroid synthesis or metabolism (Fig. 2.3A), proteins involved in vitellogenin and lipid uptake and

processing (Fig. 2.3B), proteins that mediate Fsh signaling or expression (Fig. 2.3C), extracellular matrix proteins (Fig. 2.3D), and growth factors (Fig. 2.3E) was altered by 11-KT. Treatment with E2 resulted in alteration of the expression of relatively fewer genes, and nearly half of these (48/100) were also altered by 11-KT (Fig. 2.4).

Pathway analysis

IPA software was used to identify canonical pathways and biological functions altered by 3 days of 11-KT or E2 exposure. A total of 263 and 12 canonical pathways were significantly associated ($-Log_{10}$ P ≥ 1.31) with 11-KT (Table 2.4) and E2 (Table 2.5) treatment, respectively. A total of 49 canonical pathways were predicted to be significantly altered by the IPA z-score algorithm following 11-KT treatment. Of these, two of the pathways most significantly associated with our dataset that had significant z-scores are involved in cell adhesion to the extracellular matrix: integrin signaling ($-Log_{10}$ P =13.00, z-score =3.53) and actin cytoskeleton signaling ($-Log_{10}$ P =8.87, z-score =2.26). Additional pathways significantly associated with 11-KT treatment included insulin receptor signaling ($-Log_{10}$ p =9.40), estrogen receptor signaling ($-Log_{10}$ P=7.75), androgen signaling ($-Log_{10}$ P=7.12), GnRH signaling ($-Log_{10}$ P =5.05), and clathrin-mediated endocytosis signaling ($-Log_{10}$ P=6.89). No canonical pathways associated with E2 treatment contained a significant z-score.

IPA was used to predict changes in biological functions following 11-KT or E2 exposure. 11-KT treatment led to the predicted significant alteration ($|z| \geq 2$) of 26 biological functions in the ovary. Many of these biological functions in the ovary of 11-KT treated fish were in categories

related to cellular processes (Fig. 2.5), but also to lipid metabolism and organismal survival. No biological functions were significantly changed by E2 treatment. Biological functions predicted from E2 treatment, with a $|z| \geq 1.5$, are shown in Fig. 2.6.

Discussion

Our previous studies on coho salmon have shown that low concentrations of 11-KT induces growth and development of primary ovarian follicles in vitro, and in vivo [10], dramatically alters the ovarian transcriptome [9]. Both E2 and 11-KT are potent stimulators of early secondary follicle growth [6,10]. In the present study, we used the previously described in vivo steroid exposure model to undertake deep transcriptome sequencing of ovarian tissue in order to identify early transcriptional changes resulting from 11-KT or E2 exposure during previtellogenic secondary growth.

After three days of sex steroid exposure, 11-KT dramatically altered the ovarian transcriptome, to a much greater degree than E2. These widespread transcriptomic changes induced by 11-KT are consistent with a role for 11-KT in lipid and vitellogenin uptake and processing, and in Fsh signaling, which are hallmarks of secondary growth, as well as cellular development and other cellular processes, and changes in the extracellular matrix.

Early secondary ovarian follicle transcriptome

During primary and early secondary ovarian follicle growth, many maternal gene products are deposited in the developing oocyte and play roles in later oocyte and zygote development. To also identify rarer transcripts associated with the somatic follicle cells in addition to the abundant oocyte RNAs, we used a deep sequencing approach, maximizing coverage and the number of unique sequence reads. Sequencing resulted in an average of over 88 million paired reads per sample, similar to that attained in our previous study [9].

The assembly of a de novo ovary backbone resulted in 63,422 unique contigs and included multiple contigs mapped to portions of the same gene. Following annotation, contigs were mapped to 13,917 unique human or zebrafish orthologous genes, similar to the number of genes reported to be expressed in embryonic and adult mouse ovary [28]. Since many of the annotated genes are known to be expressed in the follicle/interstitial cells [29], we are confident that our sequencing depth was sufficient to identify differential expression of relatively modestly expressed ovarian genes.

The effects of 11-KT on early secondary ovarian follicle transcriptome

The expression of 3,252 genes in the ovary was altered by in vivo treatment with 11-KT by more than 1.5 fold. Sixty eight genes were highly differentially expressed (>10 fold or <-10 fold), including 14 transcripts that were altered >100 fold or <-100 fold.

In our previous study [9], we exposed female coho salmon in the late perinucleolar stage of primary growth to 11-KT for 1 and 3 days, and performed RNA-Seq and pathway analyses on ovarian tissue. We identified 69 and 1022 differentially expressed contigs, and after mapping to zebrafish or human genomes, identified 26 and 411 differentially expressed genes between control and treated samples at day 1 and day 3, respectively. The expression of many of these genes was increased and a number of these genes encode proteins involved in steroidogenesis and steroid action, including hydroxy-delta-5-steroid dehydrogenase, 3beta- and steroid delta-isomerase 2 (*hsd3b2*), cytochrome P450 family 19 subfamily A member 1 (*cyp19a1*), estrogen receptor 2(b) (*esr2*), androgen receptor (*ar*), and follicle stimulating hormone receptor (*fshr*). These results implicate androgens in processes that prepare the ovarian follicle for secondary growth (i.e. Fsh-mediated E2 synthesis). Additionally, we identified canonical pathways that indicated potential modifications to the extracellular matrix and potential alterations in biological functions involved in reproductive development. These results led to the hypothesis that 11-KT plays a major role in primary growth, enhancing the potential for Fsh- and E2-mediated signaling in secondary growth. Consistent with the potent growth-promoting effects of 11-KT on early secondary follicles in vitro [6], the large number of contigs (8,707) and corresponding genes (3,853) that were differentially expressed, and the magnitude of the fold change in many genes after 3 days of 11-KT treatment in the current study indicate that the early secondary ovarian follicle is more sensitive to androgens.

The fundamental difference between the two RNA-Seq studies was the ovarian follicle stage, as identified by histological indices (absence/presence of cortical alveoli). The ovarian follicles in the present study contained peripheral cortical alveoli consistent with the morphology of the

cortical alveolus stage of early secondary growth. However, common themes emerged from the biological functions predicted to be altered after 3-days of 11-KT treatment in the two studies (Fig. 2.7). The IPA biological functions analysis predicts biological consequences by leveraging the known interactions of genes in a given dataset with the direction of differential expression of those genes. The direction and degree of the activation (or inhibition) state is predicted by the z-score algorithm, a statistical measure of the match between expected relationship direction and observed gene expression. Biological functions in categories of cell-to-cell signaling and interaction, cellular development, and cellular movement were activated (z-score >2) in both studies. The biological functions of morbidity and mortality and organismal death were predicted to be significantly inhibited (z-score <-2) in both studies. These predictions point towards 11-KT playing a similar role in regulating basic cellular processes and cell survival at these stages.

Abolishing androgen signaling in perinucleolar follicles of coho salmon in vitro led to widespread atresia of primary follicles [6], indicating that androgens are essential survival factors, at least at the primary follicle stage. Notable follicle stage-associated differences in the transcriptomic response to 11-KT include genes encoding proteins involved in steroid synthesis, and in particular, the synthesis of E2 (*hsd3b*, *cyp19a1*), even though very similar 11-KT levels were achieved with implants in each study. The expression of these genes was significantly increased by 11-KT in primary follicles [9], but was significantly decreased by 11-KT in early secondary follicles. The products of these genes both act to catalyze the production of sex steroids, and *cyp19a1* encodes aromatase, the enzyme responsible for the conversion of testosterone (T) to E2. Decreases in expression indicate that the potential for production of E2 by previtellogenic secondary follicles was reduced by 11-KT. Teleosts express two isoforms of

cyp19a1, *cyp19a1a* in the gonads and *cyp19a1b* in the brain. In our dataset, two contigs were annotated to zebrafish *cyp19a1*, and the contig sequences shared 100% identity with a predicted coho salmon aromatase-like genomic sequence, and 99% identity with a published partial coding sequence for coho *cyp19a1a* [30]. Thus, we are confident that the contigs annotated to *cyp19a1* are in fact the gonadal form of aromatase, *cyp19a1a*.

Additionally in early secondary follicles, the expression of hydroxysteroid 17-beta dehydrogenase 4 (*hsd17b4*), hydroxysteroid 17-beta dehydrogenase 12 (*hsd17b12*), hydroxysteroid 17-beta dehydrogenase 14 (*hsd17b14*) and steroid 5 alpha-reductase 3 (*srd5a3*) was increased, while hydroxysteroid 17-beta dehydrogenase 8 (*hsd17b8*) was decreased. The precise functions of the various Hsd17b isoforms are not completely understood in fish. Isoforms of *hsd17b*s are conserved and mammalian orthologs are generally understood to be involved in steroid synthesis and metabolism. The hydroxysteroid (17β) dehydrogenase activity catalyzes the interconversion of high-activity, 17β-hydroxyl, and low-activity, 17-keto, forms of C-19 (androgen) and C18 (estrogen) steroids (see review by Saloniemi et al. [31]). However, the oxidation or reduction activity of these enzymes may be dependent on cofactors present [32] and thus steroidogenic outcomes may be difficult to predict based on mRNA expression.

In mammals, there are at least 15 Hsd17b isoforms and at least 13 are selective for androgens or estrogens [31]. The enzymatic activity of the mammalian orthologs of the isoforms differentially expressed by 11-KT in coho salmon have been fairly well documented. The isoforms HSD17B4 and HSD17B8 have been implicated in the oxidation of E2 to estrone (E1), thus reducing

estrogenic activity. HSD17B4 may also catalyze the reduction of E1 to E2 [33], and mediate the conversion of Δ5-androstene-3β,17β-diol (Δ5-diol) to dehydroepiandrosterone (DHEA) [34]. HSD17B8 may also catalyze the oxidation (and inactivation) of dihydrotestosterone (DHT) to androstanedione or from testosterone (T) to Δ4-androstenedione (A4) [33]. HSD17B12 may also convert E1 to E2 [35] due to its sequence similarity to Hsd17b3 [31]. Conversely, HSD17B14 was able to oxidize E2 and T to estrone and A4, respectively [36].

Functions similar to mammalian HSD17Bs are assumed for teleost Hsd17b isoforms, but there are currently few functional studies available. In tilapia, both Hsd17b1 and Hsd17b8 could catalyze the interconversion between E1 and E2 [37]. Hsd17b1 was also able to convert A4 to T, while conversely the Hsd17b8 isoform was able to convert T to A4. In Japanese eel, Hsd17b1 [38], and in zebrafish, Hsd17b3 [39] display similar catalytic activity as their mammalian isoforms, in the synthesis of estrogens and androgens, respectively. However, zebrafish Hsd17b3 lacks the ability to convert androsterone to androstanediol, a reaction that is readily catalyzed by human HSD17B3 [39]. The steroidogenic functions of teleost Hsd17b12 have not been identified, and contrary to our results the expression of ovarian *hsd17b12a* in the fathead minnow ovary was decreased by in vivo exposure to the synthetic androgen 17β-trenbolone [40].

Transcripts encoding Srd5a3 were upregulated 5.48–fold after 11-KT exposure. The 5α-reductase activity of Srd5a3 catalyzes the conversion from T to DHT [41] or potentially promotes conversion of 11-KT to other potent non-aromatizable androgens [42]. Taken together, these results suggest that in early secondary growth, 11-KT alters steroidogenic capacity of the

ovarian follicle, and in particular, increases the potential for the production of potent androgens (*srd5a3*). Several studies have implicated DHT as a bioactive androgen in female teleosts [43–45].

Given the abundance of differentially expressed genes following 11-KT treatment, we focused further analyses on genes and pathways potentially involved in processes characteristic of early secondary follicle development: (i) vitellogenin (Vtg) and lipid uptake, (ii) Fsh signaling, (iii) structural changes in the ovarian follicle, and (iv) changes in growth factor signaling.

i. 11-KT effects on vitellogenesis and lipid uptake

After 3 days of treatment with 11-KT, the expression of very low density lipoprotein receptor/vitellogenin receptor (*vldlr/vtgr*), cathepsin d (*ctsd*), cathepsin z (*ctsz*), and clathrin light chain a (*clta*), clathrin light chain b (*cltb*), and clathrin heavy chain (*cltc*) was increased. The proteins encoded by these genes play fundamental roles in oocyte development, controlling the uptake of lipids and Vtg during vitellogenesis [2,46]. The process of vitellogenesis is the hepatic production and ovarian uptake the Vtg molecule, the large lipid transfer protein and the major precursor of egg yolk proteins in fish. The production of Vtg in the liver is known to be under the regulation of ovarian E2, the synthesis of which is controlled at least partially by the pituitary-derived Fsh. Our data indicate that androgens may be crucial to several components of lipid and Vtg uptake and processing.

Most of what is known about the effects of androgens on vitellogenesis or Vtg production is from studies linking androgen exposure to increases [47,48] or decreases [40] in hepatic Vtg production. The expression of *vtgr* has been reported for oocytes of several species and these studies indicate collectively that expression peaks during primary growth [49–53] and declines thereafter [54]. Transcripts can be stored and later translated during vitellogenesis [49]. Conversely, the endocrine or paracrine control of *vtgr* expression by sex steroids has only been described in two species, largemouth bass [50] and medaka [55]. E2 was shown to repress *vtgr* expression [55] and insulin (Ins) increased *vtgr* expression in previtellogenic follicles [50], although co-exposure with Ins and either 11-KT or E2 reduced the Ins-induced expression in vitro [50], suggesting that androgen and estrogen receptor signaling may regulate insulin-mediated pathways [2]. The latter study [48] used very high concentrations of 11-KT or E2 (500 nM), which may explain the differences in expression following 11-KT exposure in our model. Interestingly, in the present study, the expression of both ovarian *ins* and *insulin receptor* (*insr*) was reduced with 11-KT treatment. Insulin also stimulated Vtg uptake in rainbow trout oocytes denuded of follicle cells [56], indicating the action of insulin is not follicle-cell dependent. Caution should also be taken when making claims about the biological consequences of *vtgr* expression as protein levels of the gene do not always correlate well with transcript levels, and the protein itself is internalized and then may be recycled back to the membrane during vitellogenesis [49,57], and thus even if *vtgr* transcripts are reduced, as vitellogenesis commences the Vtgr protein may be enough to support vitellogenesis. Signaling through Esr1 and Esr2a, but not Esr2b, was able to repress transcriptional activity of the *vtgr* promoter region [58]. Thus, since *vtgr* transcripts are expressed prior to, and rapidly decline at the onset of vitellogenesis, the rise in E2 that induces Vtg production during secondary growth may also act to repress *vtgr*

mRNA levels. Nonetheless, androgens likely play a role in regulating several aspects of vitellogenesis prior to hepatic Vtg production. More studies are needed to further elucidate the endocrine/paracrine regulation of *vtgr* expression, and functional studies are needed to better understand the roles of androgen signaling in the ovary.

Teleost *vtgr* is a splice variant of *vldlr*, only lacking an *O*-linked sugar domain and the sequence of the contig mapped to *vtgr*/*vldlr* in our dataset does not cover that domain. The contig mapped with 98% identity to rainbow trout *vtgr* (LOC100136065). In rainbow trout, a closely related species in the same genus as coho salmon, Vtgr appears to specifically bind Vtg [5], whereas additional somatic lipoprotein receptors bind very low-density lipoprotein (Vldl), and low-density lipoprotein (Ldl).

The Vtgr protein is active at the oocyte cell surface during vitellogenesis, associated with endocytotic clathrin-coated pits. A recent study [46] identified *cltc* isoforms expressed during late primary and previtellogenic early secondary growth in cutthroat trout, and Cltc proteins localized near the oocyte membrane as the follicle entered the lipid droplet stage. Translocation of Vtgr and Cltc proteins to the oocyte surface occurred at similar times in this study, and Cltc was localized at the oolema throughout vitellogenesis. Clathrins are actively involved in the endocytosis of large molecules such as Vtg, but also lipids.

Following endocytosis of the Vtg-Vtgr complex, Vtg is then cleaved into component yolk proteins by lysosomal cathepsins (Cts), which recognize particular amino acid sequences in the Vtg protein. Transcripts for *ctsz* were expressed in early vitellogenic follicles in mummichog [59] and throughout vitellogenesis in carp [60]. In carp, the Ctsz protein remained associated with yolk granules into embryogenesis. In teleosts, Ctsd has been implicated in the cleavage of Vtg into the three primary yolk components, lipovitellin, phosvitin, and the β'-component [61] and in *Xenopus* it was identified as catalyzing Vtg processing within oocytes [62]. Additional Cts isoforms, Ctsb and Ctsl, are implicated in the maturational degradation of yolk proteins [61]. In coho salmon ovarian follicles, the expression of *ctsb* was inversely correlated with the transition to secondary growth, whereas the expression of *ctsd* and *ctsz* was unchanged between late primary and early secondary growth [52]. However, ovarian capthesins likely undergo post-transcriptional regulation, and thus transcript levels may not correlate well with enzymatic activity [61].

A significant characteristic of the transition to secondary growth is accumulation of neutral lipids in the ooplasm of the oocyte. Two pathways have been proposed for the ovarian uptake of neutral lipids in teleosts. Lipoproteins are either incorporated into the oocyte by receptor-mediated endocytosis and later processed into free fatty acids, as described above for Vtg, or are processed by endothelial or somatic cells in the ovarian follicle by lipoprotein lipase (Lpl), resulting in free fatty acids that enter the oocyte [63]. Lipid deposition and the accumulation of lipid droplets occurs both during and after the cortical alveolus stage in early secondary growth, depending on species [64], prior to the onset of vitellogenesis. In Japanese eels at the primary growth stage, 11-KT induced ovarian apolipoprotein b (*apob*) expression [65], and both

increased lipid droplet abundance and *lpl* expression [66]. Apob is a member of the large lipid transfer protein superfamily that includes Vtg. The protein encoded by *apob* is the primary protein component of Vldl and Ldl molecules. Likewise, in primary growth coho salmon, 11-KT increased apolipoprotein o (*apoo*) and *lpl* expression, as well as decreased the expression genes involved in fatty acid oxidation [9], which may indicate that 11-KT increases lipid storage through reduced metabolism at the late primary growth stage.

In the present study, 11-KT also altered the expression of lipid transfer genes in secondary stage follicles. But, in contrast to primary stage follicles, 11-KT decreased expression of *apob*, a member of the large lipid transfer protein superfamily that includes Vtg. The protein encoded by *apob* is the primary protein component of Vldl and Ldl molecules.

The expression of Ldl receptor-related proteins *lrp1* (decreased 4.04 fold), *lrp1b* (increased 2.21 fold), *lrp2* (increased 2.11 fold), *lrp5* (decreased 1.62 fold), and *lrp10* (increased 2.59 fold), and Ldl receptor-related protein associated protein 1 (*lrpap1*, increased 1.83 fold) was also altered by 11-KT. These genes encode conserved proteins that are related to the cell surface Ldl receptor, exhibit similar endocytosis functions, but also have fundamental roles in a diverse range of intercellular signal transduction pathways [67], interacting with multiple diverse ligands. In fish a novel *lrp* isoform, *lrp13*, was recently described that shares some functional characteristics with Vtgr [68].

Pathway analysis also identified several potential canonical pathways and biological functions that further implicate 11-KT treatment with alterations in aspects of ovarian preparation for vitellogenesis and lipid uptake. The canonical pathways *insulin signaling* and *clathrin-mediated endocytosis signaling* were significantly associated with 11-KT treatment, supporting the previously discussed results. The biological function *concentration of lipids*, identified from the differential expression of 267 genes in our dataset, was predicted to be activated in 11-KT treated samples in comparison to controls, further supporting the hypothesis that 11-KT modulates lipid incorporation in the ovarian follicle. Similar results were observed from 11-KT treatment at the LPN stage.

ii. 11-KT effects on Fsh signaling in the ovary

The expression of several genes with known effects on Fsh synthesis or action was altered after three days of 11-KT treatment, including reduced expression of *inha, inhbb, cyp19a1,* and *amh*, and increased expression of *amhr2*. During secondary growth, Fsh secretion [11,69–71] and the ovarian response to Fsh stimulation increases [30,72], peaking during vitellogenesis. Ovarian *fshr* expression follows this pattern [12,52,73]. Fsh signaling through the Fshr modulates the expression of a number of genes during early secondary growth [19,71]. We previously showed that in primary follicles, 11-KT increases both *fshr* expression as well as a major downstream target of Fshr signaling, *cyp19a1* [9], which encodes the enzyme that converts T to E2. Additionally, plasma E2 was increased, and we hypothesized that 11-KT functions to prepare the ovarian follicle for Fsh mediated effects in secondary growth. Expression of *cyp19a1* is generally relatively low prior to vitellogenesis, and in vitro effects of Fsh on *cyp19a1* expression in

previtellogenic salmon have not been observed, but Fsh increased *cyp19a1a* expression in vitellogenic follicles of rainbow trout in vitro [74] and the temporal pattern of plasma E2 and ovarian *fshr* transcripts are well correlated [19]. But, in contrast to results from our studies in primary follicles, 11-KT had no effect on expression of *fshr*, and *cyp19a1* expression was reduced after 3-days of 11-KT treatment in early secondary follicles. The reason for these dissimilar effects on expression between these stages are unclear, but are perhaps due to stage-dependent changes in endocrine or paracrine feedback of 11-KT on the ovary, or alterations in intracellular pathways mediating the effects of 11-KT.

Levels of two transcripts for *inhibin alpha* subunit (*inha*) and *inhibin beta b* subunit (*inhbb*), which encode monomers of the heterodimeric inhibin B protein complex were decreased following 11-KT treatment. In mammals, inhibin B is produced in granulosa cells of the ovarian follicle and may suppress the synthesis and secretion of Fsh in the pituitary via the proposed ovary-pituitary inhibin feedback loop [75]. In rainbow trout, *inha* transcripts peaked during vitellogenesis in concert with Fsh levels [76], and Fsh treatment increased *inha* mRNA levels in zebrafish [75]. However, in vitro treatment of secondary coho salmon follicles with Fsh had no effect on *inha* transcripts [30]. The expression of *inhbb*, and *inhibin beta a* (*inhba*) was decreased by treatment of previtellogenic shortfinned eel ovarian fragments with 11-KT, but *inha* was unchanged [77]. Expression patterns of inhibin subunits (which also encode the dimeric activin protein complex consisting of two *inhibin beta* subunits) tend to be higher during earlier stages of ovarian follicle development [75,78], while the *alpha* subunit, and thus mature inhibins, increase in response to Fsh later in development This suggests mature activins are produced earlier, potentially regulating early follicular development [79] while inhibins begin to play a

role as follicles shift to Fsh-responsiveness. . Interpreting the impact of inhibins and activins is challenging because of complexity of their structure and limited information in fishes. Homo or heterodimers of either Inhba and/or Inhbb form activins that can stimulate Fsh, whereas heterodimers of Inha with Inhba or Inhbb form inhibins that inhibit Fsh. In non-teleosts *Inha* and *Inhbb* subunits appear to be estrogen responsive [80,81], and although E2 levels were not significantly increased by 11-KT in this study, paracrine actions of estrogens on *inha* and *inhbb* expression cannot be dismissed.

The expression of another factor expressed in granulosa cells, *wilms tumor 1* (*wt1*), that may regulate *fshr* expression, was increased in response to 11-KT. Immature rat granulosa cells transfected with WT1 showed a decrease Fshr expression [82]. Additionally, in vitro FSH treatment reduces Wt1 expression (Roh et al., 2009), likely by direct promoter antagonism [82], suggesting that WT1 is a direct regulator of Fshr expression in response to FSH in mammals. The same may also hold true in fish. The expression of a related factor, *wilms tumor protein 2-like* (*wtl2*), in salmon ovaries, is positively correlated with *fshr* expression [19], and is stimulated by Fsh in vitro [71].

The expression of *amhr* was significantly increased while the expression of *amh* was significantly decreased by 11-KT in the current study, indicating androgenic modulation of the Amh signal in early secondary growth ovarian follicles. In female fish, the role of Amh is not clear, and although it has been linked to early ovarian development, very little experimental information exists regarding its specific actions. It is unknown if Amh serves a similar function

in teleost ovarian follicle progression to that in mammals, where it is also expressed in ovarian granulosa cells and functions in limiting the progression of follicle development [83], and maintaining the primordial follicle pool reserve by repressing the FSH signal. Levels of mammalian AMH have been recognized as an indicator of the remaining ovarian follicle pool [84]. Expression of *amh* in female teleosts has been detected in the ovary of multiple species (see review by Pfennig et al.[85]), albeit at a lower level than in testis. Expression is primarily restricted to granulosa cells and in general the gene is expressed in primary growth follicles, and expression increases in early secondary growth and during vitellogenesis. In Atlantic salmon, the Amh protein localizes to cortical alveoli [86]. So far, the most relevant functional data on Amh action has come from medaka *amh* and *amhr2* null mutants, called hotei [87], or by using recombinant zebrafish Amh (rAmh), or eel Amh antibodies. In homozygous hotei females, ovarian follicles arrest during vitellogenesis. Germ cell proliferation was inhibited by using *amh* and *amhr2* siRNAs in medaka [88], a phenotype that was rescued with the addition of eel rAmh in the *amh* knockdown animals, but not in the *amhr2* knockdown, indicating that the receptor is necessary for Amh action.

In mammals, both androgens and estrogens inhibit *Amh* expression [89]. However there is no consistent action of sex steroids on *amh* expression in fish [85]. In zebrafish, the *amh* promoter has estrogen response elements (EREs), and responds to E2 treatment, while EREs are absent in the medaka *amh* promoter region and therefore expression may be independent of E2 [90]. Expression of *amh* is increased in primary ovarian follicles of coho salmon in response to 11-KT treatment in vivo [9], contrary to what was observed in secondary ovarian follicles in the present

study. However, the increased expression of *amhr* in the present study may indicate that 11-KT continues to modulate the *amh* signal in early secondary growth ovarian follicles.

AMH can also be regulated by Fsh. In mammals, ovarian *Amh* expression is inhibited by Fsh. In several studies, *amh* mRNA was reduced in Fsh-treated testis, but in early secondary growth coho salmon ovaries, *amh* transcript levels were not altered by Fsh [30]. Clearly, further work is needed to understand the role and regulation of Amh in female fish.

iii. Structural changes in the ovarian follicle following 11-KT exposure

Alterations in expression of several genes encoding extracellular matrix (ECM) proteins in response to 11-KT indicates that 11-KT may be involved in regulating the structure of the ovarian follicle, as we have reported for primary follicles [9]. As oocytes increase in volume during the progression through primary and secondary growth stages, the ovarian follicle layers also undergo numerous changes to maintain structural and biochemical support, and to increase communication between the components of the ovarian follicle. The ECM is a network of extracellular molecules that functions to provide structural support and facilitate cell-to-cell interactions. It is comprised primarily of collagen isoforms that form both filament-like structures and the basement membrane, fibronectins which facilitate cell movement through the ECM, and web-like laminins that provide overall tensile-strength. The ECM interacts with ovarian follicle cells to regulate gene expression, cell differentiation, and cellular growth [91,92], and changes in composition may alter growth factor or hormone access to the developing oocyte [92].

The expression of several collagen type IV isoforms was decreased while the expression of type VI isoforms was increased by 11-KT treatment. Collagen type IVs are primarily basement membrane components [93], while type VIs perform various cytoprotective functions in the ECM [94], including interaction with various membrane receptors involved in intracellular signaling. The basement membrane, between the stromal and epithelial compartments of the ovary, maintains cellular organization of ovarian follicle layers [95]. This expression pattern suggests that 11-KT plays a role in reorganization of the ECM. The expression of another gene, *prolyl 4-hydroxylase, transmembrane* (*p4htm*) which is involved in basement membrane development was dramatically increased (>300-fold). The protein encoded by *p4htm* is a collagen P4h, which participates in post-translational folding of collagen polypeptides. Morpholino-induced depletion of *p4htm* led to basement membrane defects in multiple tissues in embryonic zebrafish [96]. Likewise, the expression of decorin (*dcn*) was increased by 11-KT. Dcn binds type-I collagens and plays a role in ECM assembly, but also cell cycle regulation and apoptosis [97]. The role of Dcn in the fish ovary is not well described, although transcript levels correlate with *fshr* expression, peak during vitellogenesis in coho salmon [19], and are regulated by Fsh [30].

Facilitating cell-to-cell signaling is a major function of the ECM, and biological functions in the category of cell-to-cell signaling were significantly activated by 11-KT in both primary [9] and secondary ovarian follicles. Additionally, the expression of a number of genes encoding gap junction and tight junction associated proteins, including claudin isoforms, which coordinate cell

signaling and membrane trafficking [98] was altered by 11-KT in the present study. In mammals, androgens are involved in regulating the expression of tight junction protein encoding genes in reproductive tissues [99,100] (see review by Firestone and Kapadia [101]). This suggests that 11-KT, by altering the expression of claudins, may be involved in modulating cell signaling and membrane trafficking in the ovarian follicle cell layers. In our previous study, 11-KT altered many transcripts linked to the ECM, including those encoding numerous forms of collagen and laminin [9]. Together with the present study, this provides compelling evidence that 11-KT modulates the structure of the ECM and enhances intrafollicular communication.

iv. Changes in growth factor signaling. The expression of several growth factor ligands and receptors was altered by 11-K: *tgf-beta* superfamily member ligands *bmp7*, *gdf3*, and *tgfb3* transcript levels were decreased while receptors *acvr2a*, *bmpr1a (alk3)*, *tgfbr2*, and *tgfbr3 (betaglycan)* were increased. The increase in receptor expression implies an increase in Tgf-beta signaling potential. Much of what is known about the signaling through Tgf-beta superfamily receptors comes studies on mammals, although the functional mechanisms appear to be well conserved [102]. Extracellular Tgf-beta superfamily ligands signal by binding type 2 receptors (i.e. Acvr2a and Tgfbr2) which form heterotetramers with type 1 receptors (e.g., Bmpr1a). This activates receptor kinase activity, inducing signaling via Smad complexes which translocate to the nucleus and participate in activating or inhibiting transcription of various target genes [103]. The expression of *smad1*, which encodes a transcription factor that is activated by Bmpr1a, was also increased by 11-KT. Tgf-beta superfamily ligands also bind to Tgfbr3, which serves as a ligand reservoir, and may enhance or suppress type 2 receptor binding depending on expression levels and localization [103]. Signaling through these receptors is known to control a wide range

of cell processes and tissue homeostasis. The breadth of Tgf-beta superfamily member genes with altered expression following 11-KT treatment provides further evidence that androgens may be involved in mediating many cellular processes in the ovary: IPA® analysis identified numerous cellular process pathways in the ovary containing Tgf-beta superfamily ligands and receptors that were predicted to be significantly activated by 11-KT treatment.

The expression of growth factor receptors *igf1r* and *igf2r* was increased by 11-KT, implicating androgens in the modulation of intraovarian Igf signaling. Igfs have various effects on the teleost ovary, although the majority of studies have focused on the role Igf1 and Igf2 in secondary follicle steroid production and oocyte maturation [104] or the teleost specific gonadal Igf3 [105]. Both Igf1 and Igf2 bind Igf1r, and only Igf1r has been shown to activate signaling pathways [106]. As in mammals, Igf2r may function to attenuate signaling by binding and internalizing the Igf2 peptides which are trafficked to lysosomes for degradation [107]. The addition of Igf binding proteins further regulates the bioavailability of Igfs. In fish, the primary endocrine source of Igf1 and Igf2 is the liver, which synthesizes and releases them into circulation, although *igf1* mRNA has been detected in oocytes and ovarian follicle cells in previtellogenic carp [108].. The IgfI protein has been localized to ovarian granulosa cells of previtellogenic oocytes in several species [108–110], where it binds cognate receptors and has been implicated in paracrine/autocrine regulation in the ovary [111]. In eel, in vitro culture of previtellogenic ovaries with Igf1 resulted in increased follicle diameter [7] and in sterlet, *igf1* and *igf1r* were more highly expressed in previtellogenic oocytes and vitellogenic ovaries in females that underwent maturation, than ones that did not mature that year [111], implicating Igf1r signaling in the control of primary and secondary ovarian follicle development. Further evidence of Igf

involvement in ovarian function was shown in tilapia, where Igf3 increased the expression of genes involved in steroidogenesis [23]. Androgen treatment has been shown to stimulate plasma IgfI levels [112], thus the effects of 11-KT on Igf signaling could involve both endocrine paracrine/autocrine mechanisms.

The effects of E2 on the early secondary ovarian follicle transcriptome

Treatment with E2 for 3 days produced only modest changes on the ovarian transcriptome, relative to the effects of 11-KT. E2 treatment altered the expression of only 140 contigs, which were annotated to 100 zebrafish or human genes. This represents just 3% of the number of genes regulated by 11-KT at 3-days. Nearly half of the genes regulated by E2 (26 genes upregulated and 17 downregulated) were also regulated by 11-KT. Although similar steroid levels were achieved with the two treatments, the differences in the dynamics of action between 11-KT and E2 may underlie the observed differences in transcriptomic response. These differences in dynamics may be partially due to differences in the type of receptors that steroid ligands bind to, and to relative differences in the abundance of androgen and estrogen receptors.

Sex steroid receptors signal through two primary mechanisms, genomic and non-genomic pathways. In genomic signaling, the steroid ligand diffuses through the plasma membrane and binds an intracellular receptor in the nucleus and/or cytoplasm, which causes activation of the receptor's transcriptional domain. The receptor protein then can either bind directly to response elements in gene promoter regions, or act indirectly with promoter regions along with other DNA-binding transcription factors, and influence gene transcription. Rapid non-genomic

signaling occurs following plasma membrane-associated receptors binding sex steroids and activating non-genomic signaling cascades [113]. However, membrane bound receptors also mediate genomic actions. There are also differences between receptor isoforms in receptor binding characteristics for specific steroids as well as differences in transcriptional properties [114].

In salmonids, there are four nuclear estrogen receptor and two nuclear androgen receptor isoforms that potentially have different expression profiles, different ligand specificity, and different transcriptional consequences, although this is largely unexplored. In rainbow trout, the four nuclear *esr* isoforms show drastically different tissue expression patterns, with the beta 1 and beta 2 isoforms more highly expressed in the ovary [115]. Membrane estrogen signaling can have rapid effects on gene transcription, and these effects may be cell-type specific [116].

Ovarian expression of *ar* isoforms varies during development, although both expressed isoforms are present in previtellogenic ovarian follicles of European sea bass [73]. The salmonid *ar* isoforms appear to be duplicated *arβ*s, with the *arα* isoform lost following the salmonid tetraploidization event [117]. However, differences in signaling through the various isoforms has not been determined. Signaling through a recently identified membrane androgen receptor (Zip9), that has high specificity for testosterone, also presents a route for androgens to influence ovarian physiology [2,118]. DHT, a non-aromatizable androgen, has similar effects to 11-KT in promoting primary ovarian follicle growth in vitro. Membrane impermeable DHT-bovine serum

albumin conjugate had the same growth promoting effects on primary ovarian follicles of coho salmon as the unconjugated form, indicating the presence of a membrane bound Ar [10].

In the present study, contigs that mapped to zebrafish *ar* (originally annotated to *Salmo salar, androgen receptor beta 2*) or zebrafish *esr1* (originally annotated to *Oncorhynchus mykiss, estrogen receptor beta 2*) were identified in the secondary follicle transcriptome. No other *ar* or *esr* isoforms were identified. The contig mapped to *ar* had a control average basemean count of 2110 and while the *esr1* contig had a control average basemean count of 527. The basemean is the normalized mean expression level of a contig, and thus in control samples is an indication of steady state expression levels. The difference between the basemean of *ar* and *esr1* indicates that the expression level of *ar* is potentially 4x greater than *esr1*, and while expression does not necessarily equate to protein levels, the potential for androgen receptor signaling may be greater than estrogen receptor signaling at this stage of ovarian follicle development and explain why the effects of E2 on the secondary follicle transcriptome was less profound thant that of 11-KT.

Genes with highly altered expression after E2 treatment. Although the expression of relatively fewer genes was altered by E2 compared to 11-KT, the level of differential expression of several genes involved in protein synthesis and folding during cellular stress conditions was dramatic. The expression of *protein phosphatase 1 regulatory subunit 15B* (*ppp1r15b*, also known as *CReP* in mamals) and *heat shock protein 90 beta family member 1* (*hsp90b1*, also known as *grp94*) was decreased by >100-fold and >450-fold, respectively. Both of these genes are highly conserved and expressed in a wide range of tissues. The protein encoded by *ppp1r15b* regulates

translation by dephosphorylating eukaryotic translation initiation factor 2a, and promotes survival during cellular stress [119]. Hsp90b1 is an immune chaperone that plays a role in maintenance of homeostasis and suppression of apoptosis during stress conditions by binding misfolded proteins. E2 has been shown to act as an anti-apoptotic factor for teleost ovarian follicles [120,121]. Hsp90s are also known interactors with steroid receptors, associating with Esr in multiprotein complexes [122,123]. Esr requires Hsp90 for efficient ligand binding and subsequent transcriptional induction of Esr target genes [124]. Thus, the dramatic repression of ovarian *hsp90b1* by E2 may represent a local negative (short-loop) feedback on ovarian E2 production. There are at least six *hsp90* paralogs in salmonids, including *hsp90b1a* and *hsp90b1b*, but functional studies on these genes are limited to their role in skeletal muscle [125] and liver [126].

Canonical pathways and biological functions in the ovary associated with E2 treatment. The top canonical pathways associated with E2 treatment are involved in cellular metabolism. NAD salvage pathway II and III, and both spermine and spermidine biosynthesis had $-\text{Log}_{10}$ P-values of >1.5. The NAD-related pathways results were driven by the expression of *nmnat3* and *acp5* which were modestly downregulated (-1.29 fold, and -3.99 fold respectively, P-adj <0.05). The sole gene in our dataset related to spermine or spermidine biosynthesis was *amd1*, which was upregulated 2.78 fold (P-adj <0.05). A z-score could not be calculated for any canonical pathways associated with E2 treatment due to the low number of genes represented in our data. E2 treatment related biological functions were identified in several categories, although no functions were predicted to be significantly altered based on the activation z-score. The most

highly altered pathways were metastasis (z-score =-1.96) and apoptosis of sarcoma cell lines (z-score =1.93).

The relative scarcity of effects of E2 on the ovarian transcriptome may also be due to the 3-day exposure time frame, or relative levels of the steroid reaching the gonad. In vitro exposures to concentrations as low as 3.0 pg/ml of E2 increased growth of early secondary ovarian follicles by nearly 2-fold after 7 days, and increased cortical alveoli abundance [10] suggesting that the secondary follicle is sensitive to E2. Co-exposure with the estrogen receptor antagonist tamoxifen abolished this effect. We hypothesized that 3 days of in vivo exposure would allow us to identify the transcriptomic changes underlying these rapid and pronounced morphological effects. However, there is a well-known negative feedback mechanism of E2 on pituitary gonadotropin secretion [127] that may have been activated with our treatment, and in previous studies ovarian expression of steroidogenic enzymes was repressed after in vitro [10] or in vivo [128] treatment with E2 .

Conclusions

In this study, we provide further evidence that androgens play important roles in previtellogenic ovarian follicle development. A non-aromatizable androgen (e.g. 11-KT) cannot be converted to E2. Thus, the 11-KT induced alterations in the ovarian transcriptome are due to androgen signaling, either directly or indirectly. The expression of thousands of genes was altered by 11-KT treatment, across a variety of cellular processes. Importantly, specific increases in expression

of vitellogenic machinery and alterations in Fsh signaling indicate that androgens control important aspects of the early secondary ovarian follicle phenotype.

Although E2 treatment resulted in a smaller alteration to the ovarian transcriptome, since this exposure was in vivo, the response to E2 may have been muted by negative feedback mechanisms. Our exposure resulted in a significant increase in plasma E2 levels, similar to those used in vitro to induce growth of coho salmon ovarian follicles. The implants used in this study have previously been shown to have a high initial release rate that gradually reduces to relatively sustained levels of E2 [9]. Therefore, it is possible that greater transcriptomic effects of E2 would be observed at a later time point.

The potency and relatively low circulating levels of 11-KT at this stage raises the question of the precise androgen signaling mechanisms in the ovary, particularly the identity of the endogenous androgen ligand. Ar isoforms in several fish species display differences in affinity for various androgens [129–131], and may mediate different physiological processes. In order to better understand androgen mediated effects on ovarian development, a comprehensive analysis of the ovarian and circulating levels of androgens (including 5α-reduced metabolites) and their receptor binding characteristics is necessary, as well as an analysis of the proteomic changes associated with androgen treatment. The potency of the non-aromatizable androgen 11-KT in the ovary at this stage in concert with the lack of appreciable plasma levels suggests an autocrine/paracrine androgen signaling mechanism.

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Table 2.1. Summary statistics of the RNA-Seq pipeline showing the number of raw reads, number of paired reads, number after quality trimming, number mapped and percent of reads mapped.

Treatment	Sample	Raw reads	Paired Reads	Trimmed	Mapped reads	percent mapping
	1	164,464,526	82,232,263	82,071,323	66,591,651	81.14
Control	2	214,106,184	107,053,092	106,790,936	86,663,462	81.15
	3	170,010,428	85,005,214	84,830,356	67,743,146	79.86
	Average	***182,860,379***	***91,430,190***	***91,230,872***	***77,203,304***	***80.72***
	1	190,527,516	95,263,758	95,048,317	76,010,066	79.97
E2 treated	2	168,924,010	84,462,005	84,209,920	68,399,236	81.22
	3	175,561,102	87,780,551	87,606,079	71,701,627	81.85
	Average	***178,337,543***	***89,168,771***	***88,954,772***	***72,036,976***	***81.01***
	1	174,052,222	87,026,111	86,854,547	71,104,279	81.87
11-KT treated	2	170,960,500	85,480,250	85,297,177	69,699,048	81.71
	3	168,526,044	84,263,022	84,100,132	67,897,057	80.73
	Average	***171,179,589***	***85,589,794***	***85,417,285***	***69,566,795***	***81.44***
	Total	**1,597,132,532**	**798,566,266**	**796,808,787**	**579,217,921**	**Average = 81.06**

Table 2.2 Changes in expression of ovarian genes after treatment of females with 11-KT for three days. The table lists ovarian contigs regulated by 11-KT after three days, identified by DESeq2 (base mean >10, fold change ≥1.5 or -1.5≤, P-adj <0.1), showing fold change in expression relative to control values.

Most significantly altered ovary contigs 3 days after 11-KT treatment

Top contigs (Basemean >10, fold change ≥1.5 or ≤-1.5, P-adjusted ≤0.1)

Gene symbol	Gene name (upregulated)	**Fold change**	P-adjusted value
p4htm	prolyl 4-hydroxylase, transmembrane	**312.35**	1.08E-59
bsdc1	BSD domain containing 1	**272.29**	9.44E-53
psmb2	proteasome subunit beta 2	**165.65**	4.83E-41
c7orf57	chromosome 7 open reading frame 57	**146.63**	6.05E-39
srsf9	serine and arginine rich splicing factor 9	**143.31**	5.92E-38
set	SET nuclear proto-oncogene	**132.24**	4.96E-38
rnf24	ring finger protein 24	**125.37**	5.12E-34
arih1	ariadne RBR E3 ubiquitin protein ligase 1	**122.19**	3.00E-34
hsdl1	hydroxysteroid dehydrogenase like 1	**118.52**	4.53E-34
b4galnt2	beta-1,4-N-acetyl-galactosaminyltransferase 2	**116.16**	4.04E-33
slc25a44	solute carrier family 25 member 44	**68.78**	3.00E-24
snapc1	small nuclear RNA activating complex polypeptide 1	**67.37**	2.01E-33
cdk10	cyclin dependent kinase 10	**64.40**	3.02E-22
atl2	atlastin GTPase 2	**62.08**	1.25E-21
cdo1	cysteine dioxygenase type 1	**60.72**	1.52E-26
tspan3	tetraspanin 3	**47.74**	5.39E-13
gsta4	glutathione S-transferase, alpha 4	**34.01**	1.73E-30
traf3ip1	TRAF3 interacting protein 1	**33.66**	3.37E-15
ddx19a	DEAD-box helicase 19A	**31.58**	9.84E-14
pygm	glycogen phosphorylase, muscle associated	**30.44**	6.38E-14
clcc1	chloride channel CLIC like 1	**28.40**	1.74E-12
ccdc69	coiled-coil domain containing 69	**28.34**	1.33E-12
bbs1	Bardet-Biedl syndrome 1	**27.82**	1.79E-12
lamc2	laminin subunit gamma 2	**26.56**	8.50E-16
fam122a	family with sequence similarity 122A	**25.46**	3.82E-02
cdk2ap2	cyclin dependent kinase 2 associated protein 2	**23.49**	7.27E-11
slc7a8	solute carrier family 7 member 8	**21.39**	2.27E-10
surf2	surfeit 2	**20.81**	3.62E-11
plau	plasminogen activator, urokinase	**20.74**	3.61E-10
cbwd1	COBW domain containing 1	**20.22**	6.75E-10
kctd9	potassium channel tetramerization domain containing 9	**18.26**	5.83E-09
pip4k2c	phosphatidylinositol-5-phosphate 4-kinase type 2 gamma	**17.32**	1.85E-02
hhat	hedgehog acyltransferase	**16.23**	4.31E-08
hsd17b4	hydroxysteroid 17-beta dehydrogenase 4	**16.16**	8.62E-16
ddost	dolichyl-diphosphooligosaccharide--protein glycosyltransferase non-catalytic subunit	**15.20**	2.29E-02
fyb	FYN binding protein	**14.99**	1.55E-15
insig2	insulin induced gene 2	**14.62**	2.32E-07
trappc13	trafficking protein particle complex 13	**14.26**	2.46E-07
prdm5	PR/SET domain 5	**13.60**	2.26E-02
fam96b	family with sequence similarity 96 member B	**12.41**	4.03E-26
tbce	tubulin folding cofactor E	**12.03**	2.87E-06
stn1	STN1, CST complex subunit	**11.97**	3.82E-06
abcf1	ATP binding cassette subfamily F member 1	**11.42**	8.62E-07
kpna7	karyopherin subunit alpha 7	**11.38**	5.95E-06
brk1	BRICK1, SCAR/WAVE actin nucleating complex subunit	**11.30**	2.59E-07
isyna1	inositol-3-phosphate synthase 1	**11.05**	7.87E-06
yrdc	yrdC N6-threonylcarbamoyltransferase domain containing	**10.90**	9.15E-07
ufsp2	UFM1 specific peptidase 2	**10.54**	1.70E-05
ajap1	adherens junctions associated protein 1	**9.40**	2.21E-05
irf6	interferon regulatory factor 6	**9.30**	1.34E-02
ctsd	cathepsin D	**9.16**	2.93E-06
mob1a	MOB kinase activator 1A	**9.08**	1.46E-05
prkch	protein kinase C eta	**8.99**	2.66E-02
dpm1	dolichyl-phosphate mannosyltransferase subunit 1, catalytic	**8.63**	9.75E-05
fkbp10	FK506 binding protein 10	**8.54**	4.08E-09
myo5b	myosin VB	**8.44**	1.84E-04
aldh9a1	aldehyde dehydrogenase 9 family member A1	**8.33**	5.06E-05
mitf	melanogenesis associated transcription factor	**8.31**	2.11E-04
vegfa	vascular endothelial growth factor A	**7.46**	7.03E-06
ddx21	DExD-box helicase 21	**6.84**	2.18E-17
ca7	carbonic anhydrase 7	**6.56**	1.72E-03
rab1a	RAB1A, member RAS oncogene family	**6.47**	2.57E-02
abca3	ATP binding cassette subfamily A member 3	**6.30**	1.89E-02
tbc1d20	TBC1 domain family member 20	**6.27**	2.41E-03
marco	macrophage receptor with collagenous structure	**6.13**	2.30E-05
optn	optineurin	**6.09**	2.83E-02
aldh1a3	aldehyde dehydrogenase 1 family member A3	**6.08**	5.99E-05
tmem184a	transmembrane protein 184A	**6.05**	8.01E-04
ovos2	ovostatin 2	**6.04**	8.22E-04
cnn2	calponin 2	**5.93**	2.20E-03
pak5	p21 (RAC1) activated kinase 5	**5.93**	1.94E-03
farsb	phenylalanyl-tRNA synthetase beta subunit	**5.87**	8.27E-03
fam72a	family with sequence similarity 72 member A	**5.84**	8.36E-04
ccdc149	coiled-coil domain containing 149	**5.83**	3.33E-02
fuca2	fucosidase, alpha-L- 2, plasma	**5.66**	3.96E-03
cpt2	carnitine palmitoyltransferase 2	**5.53**	5.62E-03
nadk2	NAD kinase 2, mitochondrial	**5.49**	2.44E-03
srd5a3	steroid 5 alpha-reductase 3	**5.48**	8.36E-04
slc16a5	solute carrier family 16 member 5	**5.39**	5.99E-03
wdr5	WD repeat domain 5	**5.27**	3.80E-02
samhd1	SAM and HD domain containing deoxynucleoside triphosphate triphosphohydrolase 1	**5.20**	4.84E-02
syf2	SYF2 pre-mRNA splicing factor	**5.17**	8.15E-03
rnf4	ring finger protein 4	**5.12**	1.32E-03
znf432	zinc finger protein 432	**5.12**	2.07E-05
kiaa0907	KIAA0907	**5.11**	2.05E-03
pdcl3	phosducin like 3	**5.08**	2.75E-08
morn2	MORN repeat containing 2	**5.01**	6.64E-03
alas2	5'-aminolevulinate synthase 2	**4.98**	1.50E-05
sp1	Sp1 transcription factor	**4.97**	8.12E-03
rtl1	retrotransposon-like 1	**4.81**	5.26E-09
sar1b	secretion associated Ras related GTPase 1B	**4.79**	8.45E-04
kdm4a	lysine demethylase 4A	**4.76**	3.93E-02
pdpk1	3-phosphoinositide dependent protein kinase 1	**4.72**	3.25E-02
zcchc7	zinc finger CCHC-type containing 7	**4.67**	9.22E-04
calb1	calbindin 1	**4.66**	1.00E-02
grap2	GRB2-related adaptor protein 2	**4.65**	7.49E-03
csnk2b	casein kinase 2 beta	**4.65**	9.97E-03
sult1c3	sulfotransferase family 1C member 3	**4.59**	5.45E-04
spin1	spindlin 1	**4.58**	1.08E-02
gnptab	N-acetylglucosamine-1-phosphate transferase alpha and beta subunits	**4.54**	4.60E-03
rce1	Ras converting CAAX endopeptidase 1	**4.53**	4.47E-03
klhl18	kelch like family member 18	**4.49**	5.27E-03
tspan4	tetraspanin 4	**4.49**	6.57E-04
herpud1	homocysteine inducible ER protein with ubiquitin like domain 1	**4.48**	3.91E-03
ankzf1	ankyrin repeat and zinc finger domain containing 1	**4.45**	1.04E-02
mboat7	membrane bound O-acyltransferase domain containing 7	**4.42**	1.93E-02
cldn4	claudin 4	**4.41**	5.75E-04
adam9	ADAM metallopeptidase domain 9	**4.40**	9.44E-03
kdm5b	lysine demethylase 5B	**4.30**	2.86E-02
pfkfb1	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1	**4.30**	1.49E-03
cd55	CD55 molecule (Cromer blood group)	**4.29**	8.02E-04
erlin2	ER lipid raft associated 2	**4.28**	1.50E-03
rad23b	RAD23 homolog B, nucleotide excision repair protein	**4.28**	1.03E-02
cyp2g1	cytochrome P450, family 2, subfamily g, polypeptide 1	**4.24**	2.40E-02

spint1	serine peptidase inhibitor, Kunitz type 1	**4.20**	1.70E-02
pnliprp1	pancreatic lipase related protein 1	**4.18**	1.08E-07
hirip3	HIRA interacting protein 3	**4.16**	1.30E-08
atxn2	ataxin 2	**4.14**	1.37E-02
twf1	twinfilin actin binding protein 1	**4.14**	3.52E-02
ckap2	cytoskeleton associated protein 2	**4.13**	1.57E-02
ptprg	protein tyrosine phosphatase, receptor type G	**4.10**	3.88E-03
c12orf49	chromosome 12 open reading frame 49	**4.06**	1.13E-03
cpt1a	carnitine palmitoyltransferase 1A	**4.06**	2.91E-03
cul2	cullin 2	**4.05**	9.45E-08
ncl	nucleolin	**4.05**	1.84E-03
rnf144a	ring finger protein 144A	**4.05**	2.31E-02
smg7	SMG7, nonsense mediated mRNA decay factor	**4.03**	2.99E-02
cwf19l1	CWF19-like 1, cell cycle control (S. pombe)	**4.02**	3.03E-02
spire1	spire type actin nucleation factor 1	**3.99**	2.99E-02
fam227b	family with sequence similarity 227 member B	**3.96**	3.06E-02
trim69	tripartite motif containing 69	**3.94**	4.25E-03
limd2	LIM domain containing 2	**3.94**	1.93E-02
slc39a11	solute carrier family 39 member 11	**3.94**	9.44E-03
slc25a35	solute carrier family 25 member 35	**3.91**	2.21E-03
dhps	deoxyhypusine synthase	**3.90**	1.60E-03
trim55	tripartite motif containing 55	**3.89**	9.44E-03
ndufv1	NADH:ubiquinone oxidoreductase core subunit V1	**3.89**	2.30E-02
borcs8	BLOC-1 related complex subunit 8	**3.87**	9.66E-04
gpi	glucose-6-phosphate isomerase	**3.86**	3.35E-02
bcl2l14	BCL2 like 14	**3.86**	3.63E-04
prkci	protein kinase C iota	**3.86**	2.85E-02
gabrg3	gamma-aminobutyric acid type A receptor gamma3 subunit	**3.85**	1.75E-02
myof	myoferlin	**3.83**	3.12E-03
decr1	2,4-dienoyl-CoA reductase 1, mitochondrial	**3.82**	1.07E-04
blzf1	basic leucine zipper nuclear factor 1	**3.80**	5.69E-06
hoxc13	homeobox C13	**3.80**	3.27E-02
scarb1	scavenger receptor class B member 1	**3.79**	4.33E-03
pdik1l	PDLIM1 interacting kinase 1 like	**3.79**	6.32E-09
ints8	integrator complex subunit 8	**3.79**	4.49E-03
fam63a	family with sequence similarity 63 member A	**3.79**	2.46E-06
agps	alkylglycerone phosphate synthase	**3.78**	8.23E-03
upf3a	UPF3 regulator of nonsense transcripts homolog A (yeast)	**3.78**	3.81E-02
cpxm2	carboxypeptidase X, M14 family member 2	**3.77**	1.11E-03
atg16l1	autophagy related 16 like 1	**3.76**	2.12E-02
ttf1	transcription termination factor, RNA polymerase I	**3.74**	2.26E-02
man1a2	mannosidase alpha class 1A member 2	**3.74**	7.97E-13
cbr1	carbonyl reductase 1	**3.74**	2.38E-03
gpr4	G protein-coupled receptor 4	**3.74**	2.86E-02
il17re	interleukin 17 receptor E	**3.74**	2.98E-02
tmem39a	transmembrane protein 39A	**3.73**	9.00E-03
foxred1	FAD dependent oxidoreductase domain containing 1	**3.72**	4.06E-02
cdc7	cell division cycle 7	**3.69**	2.42E-02
dsn1	DSN1 homolog, MIS12 kinetochore complex component	**3.68**	3.25E-02
atp1b2	ATPase Na+/K+ transporting subunit beta 2	**3.67**	3.69E-06
sorbs3	sorbin and SH3 domain containing 3	**3.67**	2.22E-03
vldlr	very low density lipoprotein receptor	**3.66**	2.32E-02
imp4	IMP4 homolog, U3 small nucleolar ribonucleoprotein	**3.66**	4.12E-02
mettl5	methyltransferase like 5	**3.64**	3.70E-03
gpr107	G protein-coupled receptor 107	**3.64**	4.39E-02
gpr137	G protein-coupled receptor 137	**3.64**	5.37E-03
mad1l1	MAD1 mitotic arrest deficient like 1	**3.63**	3.03E-02
pygb	glycogen phosphorylase B	**3.63**	4.47E-02
naaa	N-acylethanolamine acid amidase	**3.63**	4.48E-02
gorasp2	golgi reassembly stacking protein 2	**3.62**	3.91E-02
prph2	peripherin 2	**3.60**	1.86E-02
slc5a3	solute carrier family 5 member 3	**3.60**	2.29E-02
nadsyn1	NAD synthetase 1	**3.58**	4.69E-02
nras	neuroblastoma RAS viral oncogene homolog	**3.58**	4.69E-02
gabrb3	gamma-aminobutyric acid type A receptor beta3 subunit	**3.55**	4.78E-03
smg9	SMG9, nonsense mediated mRNA decay factor	**3.55**	1.65E-02
rdh13	retinol dehydrogenase 13	**3.53**	3.67E-02
coq8b	coenzyme Q8B	**3.47**	5.21E-02
pmp22	peripheral myelin protein 22	**3.47**	2.32E-03
krt18	keratin 18	**3.46**	4.83E-02
st6galnac6	ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 6	**3.46**	1.18E-02
plgrkt	plasminogen receptor with a C-terminal lysine	**3.45**	3.48E-02
nudt8	nudix hydrolase 8	**3.44**	3.17E-02
cldn11	claudin 11	**3.44**	2.96E-04
grwd1	glutamate rich WD repeat containing 1	**3.44**	2.21E-02
chst7	carbohydrate sulfotransferase 7	**3.43**	2.66E-03
myadm	myeloid associated differentiation marker	**3.41**	5.39E-02
tm9sf2	transmembrane 9 superfamily member 2	**3.40**	2.28E-02
psmd13	proteasome 26S subunit, non-ATPase 13	**3.39**	9.86E-05
anxa13	annexin A13	**3.39**	2.66E-02
pi16	peptidase inhibitor 16	**3.38**	7.54E-05
lgalsl	galectin like	**3.36**	8.23E-03
gzmh	granzyme H	**3.35**	8.88E-03
sesn3	sestrin 3	**3.35**	1.50E-02
clk2	CDC like kinase 2	**3.35**	1.49E-02
pcdh8	protocadherin 8	**3.34**	5.60E-02
btaf1	B-TFIID TATA-box binding protein associated factor 1	**3.34**	3.08E-02
ccnjl	cyclin J-like	**3.34**	5.71E-02
fam102a	family with sequence similarity 102 member A	**3.33**	1.68E-02
cav1	caveolin 1	**3.32**	2.16E-03
fam83g	family with sequence similarity 83 member G	**3.32**	4.07E-02
cpne3	copine 3	**3.30**	1.73E-02
mafg	MAF bZIP transcription factor G	**3.30**	5.83E-03
synpr	synaptoporin	**3.29**	1.71E-02
faah2	fatty acid amide hydrolase 2	**3.29**	6.93E-03
acot1	acyl-CoA thioesterase 1	**3.28**	1.96E-02
lyz	lysozyme	**3.26**	1.02E-02
entpd4	ectonucleoside triphosphate diphosphohydrolase 4	**3.25**	2.86E-02
pitpnc1	phosphatidylinositol transfer protein, cytoplasmic 1	**3.22**	1.97E-03
rabep1	rabaptin, RAB GTPase binding effector protein 1	**3.22**	1.57E-02
arhgap4	Rho GTPase activating protein 4	**3.22**	1.79E-03
erc2	ELKS/RAB6-interacting/CAST family member 2	**3.21**	9.19E-03
nemf	nuclear export mediator factor	**3.21**	6.62E-02
rnaseh2a	ribonuclease H2 subunit A	**3.20**	1.05E-02
lrif1	ligand dependent nuclear receptor interacting factor 1	**3.20**	1.96E-02
ripply1	ripply transcriptional repressor 1	**3.20**	3.39E-02
arhgap45	Rho GTPase activating protein 45	**3.20**	1.16E-02
tsku	tsukushi, small leucine rich proteoglycan	**3.20**	6.30E-02
chaf1b	chromatin assembly factor 1 subunit B	**3.19**	5.61E-02
sorcs1	sortilin related VPS10 domain containing receptor 1	**3.19**	3.98E-03
c1qc	complement C1q C chain	**3.18**	1.72E-03
ppib	peptidylprolyl isomerase B	**3.18**	4.25E-02
plekhf2	pleckstrin homology and FYVE domain containing 2	**3.17**	1.33E-02
hbz	hemoglobin subunit zeta	**3.17**	1.38E-02
crispld1	cysteine rich secretory protein LCCL domain containing 1	**3.17**	2.41E-03
nup107	nucleoporin 107	**3.16**	1.23E-02
msrb1	methionine sulfoxide reductase B1	**3.16**	2.79E-02
prss57	protease, serine 57	**3.16**	6.54E-02
dlst	dihydrolipoamide S-succinyltransferase	**3.15**	4.21E-02
tmem53	transmembrane protein 53	**3.15**	1.95E-02
noa1	nitric oxide associated 1	**3.14**	3.83E-02
spon1	spondin 1	**3.14**	9.58E-03
hint3	histidine triad nucleotide binding protein 3	**3.13**	2.21E-03
galnt5	polypeptide N-acetylgalactosaminyltransferase 5	**3.13**	1.00E-02
rnd1	Rho family GTPase 1	**3.13**	1.93E-02
arhgap40	Rho GTPase activating protein 40	**3.12**	2.54E-04
lrrfip1	LRR binding FLII interacting protein 1	**3.12**	1.74E-02
znf592	zinc finger protein 592	**3.12**	2.02E-02
slc2a9	solute carrier family 2 member 9	**3.12**	4.81E-02
bcr	BCR, RhoGEF and GTPase activating protein	**3.12**	1.54E-02
bscl2	BSCL2, seipin lipid droplet biogenesis associated	**3.11**	2.11E-02
raf1	Raf-1 proto-oncogene, serine/threonine kinase	**3.11**	8.19E-03
chuk	conserved helix-loop-helix ubiquitous kinase	**3.10**	1.32E-03
sptbn2	spectrin beta, non-erythrocytic 2	**3.10**	5.53E-03
pc	pyruvate carboxylase	**3.09**	6.70E-04
lmo1	LIM domain only 1	**3.09**	3.72E-02
pla2g3	phospholipase A2 group III	**3.09**	3.21E-02
exoc2	exocyst complex component 2	**3.09**	7.74E-04
usf1	upstream transcription factor 1	**3.09**	3.42E-02
dph5	diphthamide biosynthesis 5	**3.08**	7.71E-02
cab39l	calcium binding protein 39 like	**3.08**	4.77E-03
tbc1d7	TBC1 domain family member 7	**3.08**	1.98E-02
mfsd2a	major facilitator superfamily domain containing 2A	**3.08**	4.10E-02
hbe1	hemoglobin subunit epsilon 1	**3.08**	1.80E-04
nptx2	neuronal pentraxin 2	**3.07**	5.42E-02

oraov1	oral cancer overexpressed 1	**3.07**	7.28E-02
uba3	ubiquitin like modifier activating enzyme 3	**3.06**	3.93E-02
kctd13	potassium channel tetramerization domain containing 13	**3.05**	1.66E-04
phf3	PHD finger protein 3	**3.05**	5.11E-04
lgals8	galectin 8	**3.05**	2.99E-02
adam15	ADAM metallopeptidase domain 15	**3.04**	7.09E-02
plin1	perilipin 1	**3.04**	8.11E-02
tbc1d10a	TBC1 domain family member 10A	**3.04**	2.50E-02
zc2hc1a	zinc finger C2HC-type containing 1A	**3.04**	2.57E-02
st8sia5	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 5	**3.04**	8.10E-02
cept1	choline/ethanolamine phosphotransferase 1	**3.03**	7.42E-02
il6st	interleukin 6 signal transducer	**3.03**	2.40E-02
nid1	nidogen 1	**3.03**	2.82E-02
nptx1	neuronal pentraxin 1	**3.02**	4.77E-02
trim62	tripartite motif containing 62	**3.01**	3.44E-02
fxn	frataxin	**3.01**	4.70E-03
nup210	nucleoporin 210	**3.01**	5.26E-03
kazn	kazrin, periplakin interacting protein	**3.00**	5.66E-04
c3orf14	chromosome 3 open reading frame 14	**3.00**	4.90E-02
phf10	PHD finger protein 10	**2.98**	8.60E-02
aldh16a1	aldehyde dehydrogenase 16 family member A1	**2.98**	5.99E-02
fut9	fucosyltransferase 9	**2.97**	1.56E-02
mrps2	mitochondrial ribosomal protein S2	**2.97**	2.20E-02
epb41	erythrocyte membrane protein band 4.1	**2.96**	4.21E-02
lclat1	lysocardiolipin acyltransferase 1	**2.96**	2.21E-02
gtf2e2	general transcription factor IIE subunit 2	**2.95**	1.48E-02
ncr2	natural cytotoxicity triggering receptor 2	**2.95**	4.19E-02
dlat	dihydrolipoamide S-acetyltransferase	**2.95**	1.70E-03
kxd1	KxDL motif containing 1	**2.95**	2.04E-02
adgrg3	adhesion G protein-coupled receptor G3	**2.94**	8.80E-02
papd5	poly(A) RNA polymerase D5, non-canonical	**2.94**	3.51E-02
mal2	mal, T-cell differentiation protein 2 (gene/pseudogene)	**2.93**	8.12E-03
cntrob	centrobin, centriole duplication and spindle assembly protein	**2.93**	1.04E-02
vars2	valyl-tRNA synthetase 2, mitochondrial	**2.93**	3.95E-02
fastk	Fas activated serine/threonine kinase	**2.93**	1.51E-03
c9orf142	chromosome 9 open reading frame 142	**2.93**	1.82E-02
acadsb	acyl-CoA dehydrogenase, short/branched chain	**2.92**	6.70E-03
hmmr	hyaluronan mediated motility receptor	**2.92**	8.52E-02
gpatch4	G-patch domain containing 4	**2.92**	9.79E-10
src	SRC proto-oncogene, non-receptor tyrosine kinase	**2.92**	6.48E-02
rnpep	arginyl aminopeptidase	**2.92**	9.20E-02
mnx1	motor neuron and pancreas homeobox 1	**2.91**	1.62E-02
snx12	sorting nexin 12	**2.91**	4.29E-02
rrbp1	ribosome binding protein 1	**2.90**	5.02E-02
bcam	basal cell adhesion molecule (Lutheran blood group)	**2.90**	1.01E-02
pla2g1b	phospholipase A2 group IB	**2.90**	4.80E-02
pla2g6	phospholipase A2 group VI	**2.90**	3.50E-02
cdk5	cyclin dependent kinase 5	**2.90**	7.07E-02
chmp6	charged multivesicular body protein 6	**2.90**	8.02E-02
cltcl1	clathrin heavy chain like 1	**2.89**	1.04E-02
fbp1	fructose-bisphosphatase 1	**2.89**	9.31E-02
vcl	vinculin	**2.89**	2.38E-02
neu1	neuraminidase 1	**2.89**	5.82E-02
itgal	integrin subunit alpha L	**2.88**	1.16E-02
scpep1	serine carboxypeptidase 1	**2.88**	5.80E-02
lmf2	lipase maturation factor 2	**2.88**	9.37E-02
slc45a1	solute carrier family 45 member 1	**2.88**	3.58E-02
amdhd2	amidohydrolase domain containing 2	**2.87**	5.73E-02
hmgb3	high mobility group box 3	**2.87**	7.32E-03
flot1	flotillin 1	**2.87**	4.47E-02
mdh1	malate dehydrogenase 1	**2.87**	9.60E-02
scarb2	scavenger receptor class B member 2	**2.87**	2.33E-02
josd2	Josephin domain containing 2	**2.87**	3.45E-02
scamp3	secretory carrier membrane protein 3	**2.86**	6.18E-02
ppih	peptidylprolyl isomerase H	**2.86**	9.00E-02
snx5	sorting nexin 5	**2.85**	2.10E-03
malrd1	MAM and LDL receptor class A domain containing 1	**2.85**	7.32E-03
synj2bp	synaptojanin 2 binding protein	**2.85**	2.35E-02
hip1r	huntingtin interacting protein 1 related	**2.85**	6.47E-05
tp63	tumor protein p63	**2.85**	5.53E-02
ppm1h	protein phosphatase, Mg2+/Mn2+ dependent 1H	**2.85**	3.27E-02
gabra2	gamma-aminobutyric acid type A receptor alpha2 subunit	**2.84**	3.26E-03
man1b1	mannosidase alpha class 1B member 1	**2.84**	3.50E-03
dnaja2	DnaJ heat shock protein family (Hsp40) member A2	**2.84**	4.06E-03
tbl1x	transducin beta like 1X-linked	**2.84**	5.98E-02
stag2	stromal antigen 2	**2.83**	2.19E-02
alas1	5'-aminolevulinate synthase 1	**2.83**	4.07E-02
kif20b	kinesin family member 20B	**2.83**	2.35E-02
rbbp6	RB binding protein 6, ubiquitin ligase	**2.83**	4.04E-02
prkar2b	protein kinase cAMP-dependent type II regulatory subunit beta	**2.83**	7.89E-02
cdc20	cell division cycle 20	**2.82**	6.65E-03
dyrk3	dual specificity tyrosine phosphorylation regulated kinase 3	**2.82**	4.28E-02
trip10	thyroid hormone receptor interactor 10	**2.82**	5.04E-02
unc45b	unc-45 myosin chaperone B	**2.82**	9.48E-02
mia2	melanoma inhibitory activity 2	**2.82**	1.58E-02
smarcd3	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3	**2.82**	3.26E-02
sptan1	spectrin alpha, non-erythrocytic 1	**2.81**	1.72E-03
ecm1	extracellular matrix protein 1	**2.81**	4.89E-02
cldnd1	claudin domain containing 1	**2.81**	7.62E-02
cryzl1	crystallin zeta like 1	**2.81**	1.25E-02
arid1b	AT-rich interaction domain 1B	**2.80**	3.93E-03
slc12a6	solute carrier family 12 member 6	**2.80**	1.31E-02
endod1	endonuclease domain containing 1	**2.80**	8.52E-02
rad51c	RAD51 paralog C	**2.80**	9.64E-02
tmem14a	transmembrane protein 14A	**2.80**	9.69E-02
timm44	translocase of inner mitochondrial membrane 44	**2.80**	1.42E-02
calcoco2	calcium binding and coiled-coil domain 2	**2.79**	3.02E-02
btg1	BTG anti-proliferation factor 1	**2.79**	5.43E-02
wt1	Wilms tumor 1	**2.79**	5.89E-02
cfh	complement factor H	**2.78**	3.39E-02
ppp1r13b	protein phosphatase 1 regulatory subunit 13B	**2.78**	4.94E-02
epn1	epsin 1	**2.77**	7.91E-03
ece2	endothelin converting enzyme 2	**2.77**	9.58E-03
igf1r	insulin like growth factor 1 receptor	**2.77**	1.02E-02
elmo3	engulfment and cell motility 3	**2.77**	9.54E-02
prrc1	proline rich coiled-coil 1	**2.76**	1.41E-02
fmo5	flavin containing monooxygenase 5	**2.76**	6.80E-03
trpc4ap	transient receptor potential cation channel subfamily C member 4 associated protein	**2.76**	2.64E-02
synm	synemin	**2.76**	1.39E-02
osbpl11	oxysterol binding protein like 11	**2.76**	4.85E-02
fancb	Fanconi anemia complementation group B	**2.76**	9.10E-02
olfm4	olfactomedin 4	**2.75**	2.21E-02
coasy	Coenzyme A synthase	**2.75**	4.40E-02
larp7	La ribonucleoprotein domain family member 7	**2.75**	6.79E-02
ttc14	tetratricopeptide repeat domain 14	**2.75**	8.71E-02
peg10	paternally expressed 10	**2.75**	4.09E-02
lpin3	lipin 3	**2.74**	2.09E-02
klhl21	kelch like family member 21	**2.74**	9.51E-02
rbms1	RNA binding motif single stranded interacting protein 1	**2.74**	1.49E-02
dus1l	dihydrouridine synthase 1 like	**2.74**	2.11E-02
npat	nuclear protein, coactivator of histone transcription	**2.74**	8.23E-02
slc26a5	solute carrier family 26 member 5	**2.73**	9.45E-02
zfhx2	zinc finger homeobox 2	**2.73**	3.25E-02
osbpl3	oxysterol binding protein like 3	**2.73**	2.30E-02
smc1a	structural maintenance of chromosomes 1A	**2.73**	4.36E-03
mchr1	melanin concentrating hormone receptor 1	**2.73**	3.02E-02
slc25a14	solute carrier family 25 member 14	**2.72**	4.62E-02
foxp1	forkhead box P1	**2.72**	3.28E-03
gpx3	glutathione peroxidase 3	**2.71**	2.76E-02
plcxd3	phosphatidylinositol specific phospholipase C X domain containing 3	**2.71**	6.59E-02
s100a14	S100 calcium binding protein A14	**2.71**	3.85E-02
brpf1	bromodomain and PHD finger containing 1	**2.71**	6.43E-02
tmem50b	transmembrane protein 50B	**2.70**	3.45E-03
camk2d	calcium/calmodulin dependent protein kinase II delta	**2.70**	2.12E-03
ndel1	nudE neurodevelopment protein 1 like 1	**2.69**	2.01E-03
smyd4	SET and MYND domain containing 4	**2.69**	4.96E-02
med1	mediator complex subunit 1	**2.69**	9.87E-02
ncoa4	nuclear receptor coactivator 4	**2.69**	4.95E-02
cldn19	claudin 19	**2.69**	5.29E-02
usp46	ubiquitin specific peptidase 46	**2.69**	2.60E-02
polg2	DNA polymerase gamma 2, accessory subunit	**2.69**	4.26E-02
mat2b	methionine adenosyltransferase 2B	**2.69**	8.92E-02

sema4b	semaphorin 4B	**2.68**	8.50E-03
osbpl2	oxysterol binding protein like 2	**2.68**	2.56E-02
homer3	homer scaffolding protein 3	**2.68**	4.43E-02
smyd5	SMYD family member 5	**2.68**	9.45E-02
usp24	ubiquitin specific peptidase 24	**2.67**	5.50E-03
ilk	integrin linked kinase	**2.67**	5.70E-03
nthl1	nth like DNA glycosylase 1	**2.67**	2.84E-02
ctss	cathepsin S	**2.67**	4.53E-02
tmem214	transmembrane protein 214	**2.66**	1.48E-02
itpkb	inositol-trisphosphate 3-kinase B	**2.66**	9.41E-03
clec4e	C-type lectin domain family 4 member E	**2.66**	4.49E-02
cmtr2	cap methyltransferase 2	**2.66**	4.02E-04
kcnj5	potassium voltage-gated channel subfamily J member 5	**2.66**	7.03E-02
slc16a1	solute carrier family 16 member 1	**2.66**	6.57E-03
b4galt7	beta-1,4-galactosyltransferase 7	**2.66**	1.67E-02
klhl42	kelch like family member 42	**2.65**	4.55E-02
fam69b	family with sequence similarity 69 member B	**2.65**	3.15E-02
esyt3	extended synaptotagmin 3	**2.65**	2.41E-02
irx3	iroquois homeobox 3	**2.65**	9.92E-02
tuft1	tuftelin 1	**2.65**	7.53E-03
hyal2	hyaluronoglucosaminidase 2	**2.64**	4.82E-02
sil1	SIL1 nucleotide exchange factor	**2.64**	6.51E-02
ttc17	tetratricopeptide repeat domain 17	**2.64**	3.99E-02
depdc1	DEP domain containing 1	**2.63**	4.62E-02
gys1	glycogen synthase 1	**2.63**	3.48E-02
eprs	glutamyl-prolyl-tRNA synthetase	**2.63**	1.12E-02
atg5	autophagy related 5	**2.63**	1.93E-02
fbrsl1	fibrosin like 1	**2.63**	3.19E-02
lrrc41	leucine rich repeat containing 41	**2.63**	1.72E-03
neurl1	neuralized E3 ubiquitin protein ligase 1	**2.63**	8.36E-02
fam150a	family with sequence similarity 150 member A	**2.63**	9.01E-02
ppp1r2	protein phosphatase 1 regulatory inhibitor subunit 2	**2.62**	4.14E-02
b4galnt3	beta-1,4-N-acetyl-galactosaminyltransferase 3	**2.62**	8.99E-02
baz1b	bromodomain adjacent to zinc finger domain 1B	**2.62**	1.60E-02
exoc5	exocyst complex component 5	**2.62**	3.19E-02
piwil2	piwi like RNA-mediated gene silencing 2	**2.62**	2.94E-02
ccdc50	coiled-coil domain containing 50	**2.62**	2.24E-02
arhgap35	Rho GTPase activating protein 35	**2.62**	1.74E-02
rpap3	RNA polymerase II associated protein 3	**2.61**	2.08E-03
znf106	zinc finger protein 106	**2.61**	1.04E-02
sgk3	serum/glucocorticoid regulated kinase family member 3	**2.61**	1.60E-02
osbp	oxysterol binding protein	**2.61**	4.28E-02
mettl10	methyltransferase like 10	**2.61**	8.86E-02
rsbn1	round spermatid basic protein 1	**2.61**	1.19E-02
slc17a9	solute carrier family 17 member 9	**2.61**	2.13E-02
klf5	Kruppel like factor 5	**2.61**	5.89E-02
lhx9	LIM homeobox 9	**2.60**	6.64E-02
tnpo2	transportin 2	**2.60**	1.23E-02
bmpr1a	bone morphogenetic protein receptor type 1A	**2.60**	9.76E-02
zc3h7b	zinc finger CCCH-type containing 7B	**2.60**	3.16E-02
prkaca	protein kinase cAMP-activated catalytic subunit alpha	**2.59**	2.46E-02
chp1	calcineurin like EF-hand protein 1	**2.59**	3.02E-02
chkb	choline kinase beta	**2.59**	1.44E-02
lrp10	LDL receptor related protein 10	**2.59**	2.57E-02
ormdl1	ORMDL sphingolipid biosynthesis regulator 1	**2.59**	3.49E-02
irs2	insulin receptor substrate 2	**2.59**	1.99E-02
wbp1	WW domain binding protein 1	**2.59**	2.61E-02
clcn5	chloride voltage-gated channel 5	**2.59**	3.21E-02
ganab	glucosidase II alpha subunit	**2.59**	3.92E-02
ccdc129	coiled-coil domain containing 129	**2.59**	8.20E-02
cacul1	CDK2 associated cullin domain 1	**2.58**	2.41E-02
myh9l1	myosin, heavy chain 9, non-muscle-like 1	**2.58**	3.20E-02
ccna1	cyclin A1	**2.58**	7.06E-02
pcsk5	proprotein convertase subtilisin/kexin type 5	**2.58**	2.14E-02
szrd1	SUZ RNA binding domain containing 1	**2.58**	5.29E-02
rbm41	RNA binding motif protein 41	**2.58**	6.23E-02
znf318	zinc finger protein 318	**2.57**	1.63E-02
kiaa1468	KIAA1468	**2.57**	4.96E-02
zbtb1	zinc finger and BTB domain containing 1	**2.57**	9.06E-02
zfyve9	zinc finger FYVE-type containing 9	**2.57**	2.26E-02
ankrd17	ankyrin repeat domain 17	**2.57**	4.60E-02
pank1	pantothenate kinase 1	**2.57**	2.71E-02
mxra8	matrix remodeling associated 8	**2.56**	2.01E-02
myo1b	myosin IB	**2.56**	2.69E-02
dus3l	dihydrouridine synthase 3 like	**2.56**	3.56E-02
kit	KIT proto-oncogene receptor tyrosine kinase	**2.56**	5.79E-02
tmpo	thymopoietin	**2.55**	3.50E-02
nlrc3	NLR family CARD domain containing 3	**2.55**	5.75E-02
tubgcp2	tubulin gamma complex associated protein 2	**2.55**	5.38E-02
ankrd13a	ankyrin repeat domain 13A	**2.55**	1.29E-03
pomgnt1	protein O-linked mannose N-acetylglucosaminyltransferase 1 (beta 1,2-)	**2.55**	1.01E-02
pdcd6ip	programmed cell death 6 interacting protein	**2.55**	1.70E-02
prkca	protein kinase C alpha	**2.55**	2.51E-02
lipa	lipase A, lysosomal acid type	**2.55**	7.19E-02
gpcpd1	glycerophosphocholine phosphodiesterase 1	**2.55**	7.10E-03
gulp1	GULP, engulfment adaptor PTB domain containing 1	**2.55**	6.18E-02
nudt14	nudix hydrolase 14	**2.54**	3.58E-03
ptrf	polymerase I and transcript release factor	**2.54**	3.37E-02
reep2	receptor accessory protein 2	**2.54**	1.20E-02
sgpl1	sphingosine-1-phosphate lyase 1	**2.54**	1.83E-02
gclc	glutamate-cysteine ligase catalytic subunit	**2.54**	2.08E-02
fam129b	family with sequence similarity 129 member B	**2.54**	3.44E-02
ipo13	importin 13	**2.54**	3.59E-02
dennd3	DENN domain containing 3	**2.54**	1.65E-02
cacnb1	calcium voltage-gated channel auxiliary subunit beta 1	**2.54**	5.52E-02
ubtf	upstream binding transcription factor, RNA polymerase I	**2.54**	6.54E-02
rab24	RAB24, member RAS oncogene family	**2.53**	2.05E-02
zfyve28	zinc finger FYVE-type containing 28	**2.53**	2.47E-02
hvcn1	hydrogen voltage gated channel 1	**2.53**	5.18E-02
cgn	cingulin	**2.53**	4.19E-02
tex10	testis expressed 10	**2.53**	2.20E-02
kntc1	kinetochore associated 1	**2.53**	6.11E-02
mcfd2	multiple coagulation factor deficiency 2	**2.53**	1.45E-02
supt6h	SPT6 homolog, histone chaperone	**2.53**	3.13E-02
acap2	ArfGAP with coiled-coil, ankyrin repeat and PH domains 2	**2.53**	8.63E-02
tbp	TATA-box binding protein	**2.52**	2.55E-04
abhd5	abhydrolase domain containing 5	**2.52**	3.01E-02
hp1bp3	heterochromatin protein 1 binding protein 3	**2.52**	3.57E-02
htr5a	5-hydroxytryptamine receptor 5A	**2.52**	8.75E-02
pgrmc1	progesterone receptor membrane component 1	**2.52**	1.42E-02
arhgef7	Rho guanine nucleotide exchange factor 7	**2.52**	9.76E-02
crebzf	CREB/ATF bZIP transcription factor	**2.52**	7.77E-03
l3mbtl3	l(3)mbt-like 3 (Drosophila)	**2.52**	4.36E-02
rad51b	RAD51 paralog B	**2.51**	3.92E-02
znf570	zinc finger protein 570	**2.51**	5.06E-03
march6	membrane associated ring-CH-type finger 6	**2.51**	4.94E-02
tcf20	transcription factor 20	**2.51**	1.11E-02
med13	mediator complex subunit 13	**2.51**	3.35E-02
znf148	zinc finger protein 148	**2.51**	4.04E-02
stk3	serine/threonine kinase 3	**2.51**	2.66E-03
axin1	axin 1	**2.51**	1.45E-02
nsmaf	neutral sphingomyelinase activation associated factor	**2.51**	3.81E-02
ubac2	UBA domain containing 2	**2.51**	7.38E-02
secisbp2l	SECIS binding protein 2 like	**2.50**	1.26E-02
znf641	zinc finger protein 641	**2.50**	2.08E-02
hook1	hook microtubule tethering protein 1	**2.50**	1.93E-02
plod2	procollagen-lysine,2-oxoglutarate 5-dioxygenase 2	**2.50**	2.69E-02
atg3	autophagy related 3	**2.50**	4.80E-02
gapvd1	GTPase activating protein and VPS9 domains 1	**2.50**	1.59E-02
timeless	timeless circadian clock	**2.49**	6.87E-02
hgsnat	heparan-alpha-glucosaminide N-acetyltransferase	**2.49**	1.57E-02
hla-drb5	major histocompatibility complex, class II, DR beta 5	**2.49**	2.91E-02
cystm1	cysteine rich transmembrane module containing 1	**2.49**	1.50E-02
pde4b	phosphodiesterase 4B	**2.49**	5.74E-03
psmb10	proteasome subunit beta 10	**2.48**	1.58E-04
exosc1	exosome component 1	**2.48**	7.05E-02
lzts2	leucine zipper tumor suppressor 2	**2.48**	1.52E-02
ppip5k1	diphosphoinositol pentakisphosphate kinase 1	**2.48**	3.80E-02
rho	rhodopsin	**2.48**	5.63E-02
hhla2	HERV-H LTR-associating 2	**2.48**	8.15E-02
ano1	anoctamin 1	**2.48**	2.67E-02
kidins220	kinase D-interacting substrate 220kDa	**2.48**	7.22E-02
nqo1	NAD(P)H quinone dehydrogenase 1	**2.48**	8.39E-02
edem3	ER degradation enhancing alpha-mannosidase like protein 3	**2.48**	2.34E-02
ostm1	osteopetrosis associated transmembrane protein 1	**2.48**	7.14E-02
erlec1	endoplasmic reticulum lectin 1	**2.48**	7.60E-02

coa4	cytochrome c oxidase assembly factor 4 homolog	**2.48**	8.36E-04
bcas2	breast carcinoma amplified sequence 2	**2.47**	6.93E-06
anln	anillin actin binding protein	**2.47**	2.91E-02
eif4enif1	eukaryotic translation initiation factor 4E nuclear import factor 1	**2.47**	4.33E-02
ptdss1	phosphatidylserine synthase 1	**2.47**	1.54E-02
lurap1l	leucine rich adaptor protein 1 like	**2.47**	2.09E-02
senp5	SUMO1/sentrin specific peptidase 5	**2.47**	7.32E-02
ttc39c	tetratricopeptide repeat domain 39C	**2.47**	3.28E-03
fam213a	family with sequence similarity 213 member A	**2.47**	6.54E-02
tnrc6a	trinucleotide repeat containing 6A	**2.47**	7.13E-02
tatdn2	TatD DNase domain containing 2	**2.47**	8.15E-02
kmt5c	lysine methyltransferase 5C	**2.47**	5.15E-04
jam2	junctional adhesion molecule 2	**2.47**	2.27E-02
stard5	StAR related lipid transfer domain containing 5	**2.47**	2.65E-02
arhgap17	Rho GTPase activating protein 17	**2.47**	7.17E-02
mmab	methylmalonic aciduria (cobalamin deficiency) cblB type	**2.46**	2.72E-02
atg14	autophagy related 14	**2.46**	3.14E-02
zmiz2	zinc finger MIZ-type containing 2	**2.46**	4.77E-02
sh2d4a	SH2 domain containing 4A	**2.46**	3.57E-05
ube2h	ubiquitin conjugating enzyme E2 H	**2.46**	4.38E-02
mrpl13	mitochondrial ribosomal protein L13	**2.46**	1.93E-02
cks1b	CDC28 protein kinase regulatory subunit 1B	**2.46**	4.46E-02
brms1l	breast cancer metastasis-suppressor 1-like	**2.46**	5.64E-02
lamc3	laminin subunit gamma 3	**2.45**	3.07E-03
zfx	zinc finger protein, X-linked	**2.45**	5.18E-02
c3	complement C3	**2.45**	3.67E-02
ppig	peptidylprolyl isomerase G	**2.45**	1.91E-02
tfap4	transcription factor AP-4	**2.45**	2.85E-02
akr1b1	aldo-keto reductase family 1 member B	**2.44**	2.08E-03
ap3m2	adaptor related protein complex 3 mu 2 subunit	**2.44**	9.97E-02
zkscan8	zinc finger with KRAB and SCAN domains 8	**2.44**	5.89E-06
arcn1	archain 1	**2.44**	1.60E-02
rfc2	replication factor C subunit 2	**2.44**	1.63E-02
nacc1	nucleus accumbens associated 1	**2.44**	5.14E-02
mgat1	mannosyl (alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase	**2.44**	7.17E-02
gapdh	glyceraldehyde-3-phosphate dehydrogenase	**2.44**	3.60E-03
pkp3	plakophilin 3	**2.44**	5.10E-02
tspan16	tetraspanin 16	**2.43**	1.71E-02
ldb1	LIM domain binding 1	**2.43**	2.32E-02
atp9b	ATPase phospholipid transporting 9B (putative)	**2.43**	2.63E-02
arfgef2	ADP ribosylation factor guanine nucleotide exchange factor 2	**2.43**	3.35E-02
tmx1	thioredoxin related transmembrane protein 1	**2.43**	4.06E-02
znf462	zinc finger protein 462	**2.43**	4.16E-02
inhbe	inhibin beta E subunit	**2.43**	4.89E-02
hadhb	hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), beta subunit	**2.43**	6.32E-03
bud31	BUD31 homolog	**2.43**	5.47E-02
rtca	RNA 3'-terminal phosphate cyclase	**2.43**	7.97E-02
hipk1	homeodomain interacting protein kinase 1	**2.43**	3.47E-02
trak2	trafficking kinesin protein 2	**2.43**	4.77E-02
bbs5	Bardet-Biedl syndrome 5	**2.43**	5.48E-02
znrf2	zinc and ring finger 2	**2.43**	7.64E-02
wdr81	WD repeat domain 81	**2.42**	2.72E-02
ntpcr	nucleoside-triphosphatase, cancer-related	**2.42**	2.90E-02
iws1	IWS1, SUPT6H interacting protein	**2.42**	3.42E-02
alg13	ALG13, UDP-N-acetylglucosaminyltransferase subunit	**2.42**	6.86E-02
ice1	interactor of little elongation complex ELL subunit 1	**2.42**	8.79E-02
dgcr8	DGCR8, microprocessor complex subunit	**2.41**	6.14E-02
trrap	transformation/transcription domain associated protein	**2.41**	6.80E-02
rnf2	ring finger protein 2	**2.41**	5.92E-02
slc25a29	solute carrier family 25 member 29	**2.41**	7.20E-02
acsl6	acyl-CoA synthetase long-chain family member 6	**2.41**	5.17E-02
srms	src-related kinase lacking C-terminal regulatory tyrosine and N-terminal myristylation sites	**2.41**	7.59E-02
pip5k1a	phosphatidylinositol-4-phosphate 5-kinase type 1 alpha	**2.41**	8.92E-02
hebp2	heme binding protein 2	**2.41**	3.48E-02
kiaa1147	KIAA1147	**2.41**	8.21E-02
smad1	SMAD family member 1	**2.41**	1.38E-02
fcgr1a	Fc fragment of IgG receptor Ia	**2.41**	2.03E-02
pnpla6	patatin like phospholipase domain containing 6	**2.40**	7.04E-02
rybp	RING1 and YY1 binding protein	**2.40**	2.73E-03
glyr1	glyoxylate reductase 1 homolog	**2.40**	3.30E-03
tmem63b	transmembrane protein 63B	**2.40**	4.84E-02
aldh18a1	aldehyde dehydrogenase 18 family member A1	**2.40**	2.44E-03
zyg11b	zyg-11 family member B, cell cycle regulator	**2.40**	4.78E-02
mtss1	MTSS1, I-BAR domain containing	**2.40**	8.51E-02
jup	junction plakoglobin	**2.40**	9.69E-02
e2f8	E2F transcription factor 8	**2.39**	7.57E-02
pfn2	profilin 2	**2.39**	4.56E-02
chrna10	cholinergic receptor nicotinic alpha 10 subunit	**2.39**	6.25E-02
c16orf70	chromosome 16 open reading frame 70	**2.39**	1.38E-02
lsm7	LSM7 homolog, U6 small nuclear RNA and mRNA degradation associated	**2.39**	4.02E-02
bcat2	branched chain amino acid transaminase 2	**2.39**	2.83E-02
snx17	sorting nexin 17	**2.38**	2.81E-02
plekhh1	pleckstrin homology, MyTH4 and FERM domain containing H1	**2.38**	3.38E-02
ccdc88c	coiled-coil domain containing 88C	**2.38**	9.04E-03
atp6v0e1	ATPase H+ transporting V0 subunit e1	**2.38**	8.53E-02
tnrc18	trinucleotide repeat containing 18	**2.38**	3.10E-02
lamtor1	late endosomal/lysosomal adaptor, MAPK and MTOR activator 1	**2.38**	4.33E-02
slc36a1	solute carrier family 36 member 1	**2.38**	5.99E-02
tbc1d1	TBC1 domain family member 1	**2.37**	3.66E-02
tshr	thyroid stimulating hormone receptor	**2.37**	6.41E-02
porcn	porcupine homolog (Drosophila)	**2.37**	5.65E-02
ap2b1	adaptor related protein complex 2 beta 1 subunit	**2.37**	3.80E-02
sgpp1	sphingosine-1-phosphate phosphatase 1	**2.36**	5.34E-03
mtfr1	mitochondrial fission regulator 1	**2.36**	2.84E-02
ccl19	C-C motif chemokine ligand 19	**2.36**	5.52E-02
edem1	ER degradation enhancing alpha-mannosidase like protein 1	**2.36**	3.63E-02
rad23a	RAD23 homolog A, nucleotide excision repair protein	**2.36**	5.70E-02
nucb2	nucleobindin 2	**2.36**	4.04E-02
pgbd4	piggyBac transposable element derived 4	**2.36**	5.51E-02
rfng	RFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase	**2.36**	6.59E-02
slc25a19	solute carrier family 25 member 19	**2.36**	7.17E-02
lin54	lin-54 DREAM MuvB core complex component	**2.36**	8.33E-02
ccnyl1	cyclin Y like 1	**2.36**	7.53E-03
phf20l1	PHD finger protein 20-like 1	**2.36**	1.08E-02
rab11fip4	RAB11 family interacting protein 4	**2.36**	1.02E-02
harbi1	harbinger transposase derived 1	**2.36**	6.37E-02
scly	selenocysteine lyase	**2.36**	9.91E-02
rragc	Ras related GTP binding C	**2.36**	2.41E-02
tgfbr2	transforming growth factor beta receptor 2	**2.35**	5.21E-02
acot9	acyl-CoA thioesterase 9	**2.35**	3.31E-10
trim36	tripartite motif containing 36	**2.35**	2.28E-02
rftn2	raftlin family member 2	**2.35**	8.63E-02
slc38a3	solute carrier family 38 member 3	**2.35**	3.53E-02
lmo7	LIM domain 7	**2.35**	2.00E-02
tia1	TIA1 cytotoxic granule associated RNA binding protein	**2.35**	4.84E-02
naf1	nuclear assembly factor 1 ribonucleoprotein	**2.35**	3.63E-02
llgl1	LLGL1, scribble cell polarity complex component	**2.34**	3.67E-02
adamts9	ADAM metallopeptidase with thrombospondin type 1 motif 9	**2.34**	3.69E-02
eaf2	ELL associated factor 2	**2.34**	6.86E-02
slc39a7	solute carrier family 39 member 7	**2.34**	1.90E-02
rab40c	RAB40C, member RAS oncogene family	**2.34**	6.53E-02
kdsr	3-ketodihydrosphingosine reductase	**2.34**	1.47E-02
rab4b	RAB4B, member RAS oncogene family	**2.34**	5.74E-02
nt5c2	5'-nucleotidase, cytosolic II	**2.34**	6.25E-02
rab3ip	RAB3A interacting protein	**2.34**	6.68E-02
prodh	proline dehydrogenase 1	**2.33**	7.84E-02
mtor	mechanistic target of rapamycin	**2.33**	9.37E-02
zer1	zyg-11 related cell cycle regulator	**2.33**	8.18E-02
pip4k2a	phosphatidylinositol-5-phosphate 4-kinase type 2 alpha	**2.33**	3.09E-02
sh3gl1	SH3 domain containing GRB2 like 1, endophilin A2	**2.33**	4.03E-02
arrdc3	arrestin domain containing 3	**2.33**	4.88E-02
homez	homeobox and leucine zipper encoding	**2.33**	6.73E-02
inpp4a	inositol polyphosphate-4-phosphatase type I A	**2.33**	7.08E-02
rnaset2	ribonuclease T2	**2.33**	6.11E-02
psmb5	proteasome subunit beta 5	**2.33**	5.12E-03

dcun1d4	defective in cullin neddylation 1 domain containing 4	**2.33**	4.73E-02
smurf1	SMAD specific E3 ubiquitin protein ligase 1	**2.32**	1.83E-02
adgra3	adhesion G protein-coupled receptor A3	**2.32**	4.13E-02
got1	glutamic-oxaloacetic transaminase 1	**2.32**	6.64E-02
wnk2	WNK lysine deficient protein kinase 2	**2.32**	3.45E-02
trim16	tripartite motif containing 16	**2.32**	3.34E-02
ciz1	CDKN1A interacting zinc finger protein 1	**2.32**	4.40E-02
rabgap1	RAB GTPase activating protein 1	**2.32**	9.50E-02
clint1	clathrin interactor 1	**2.32**	1.51E-02
iffo2	intermediate filament family orphan 2	**2.31**	2.44E-02
c10orf54	chromosome 10 open reading frame 54	**2.31**	6.29E-02
sostdc1	sclerostin domain containing 1	**2.31**	3.36E-02
pacsin2	protein kinase C and casein kinase substrate in neurons 2	**2.31**	3.39E-02
zmym1	zinc finger MYM-type containing 1	**2.31**	3.85E-02
ptprj	protein tyrosine phosphatase, receptor type J	**2.31**	3.19E-02
psip1	PC4 and SFRS1 interacting protein 1	**2.31**	4.08E-02
stx18	syntaxin 18	**2.31**	5.78E-02
stk38	serine/threonine kinase 38	**2.31**	7.69E-03
washc2c	WASH complex subunit 2C	**2.31**	6.69E-02
ccpg1	cell cycle progression 1	**2.30**	1.51E-02
rpa3	replication protein A3	**2.30**	9.41E-02
dek	DEK proto-oncogene	**2.30**	1.46E-05
atp8b1	ATPase phospholipid transporting 8B1	**2.30**	4.31E-04
lamc1	laminin subunit gamma 1	**2.30**	9.44E-03
fermt1	fermitin family member 1	**2.30**	2.40E-02
zc3h4	zinc finger CCCH-type containing 4	**2.30**	5.02E-02
mon1b	MON1 homolog B, secretory trafficking associated	**2.30**	7.06E-02
slc27a1	solute carrier family 27 member 1	**2.30**	5.94E-02
app	amyloid beta precursor protein	**2.30**	5.72E-02
mastl	microtubule associated serine/threonine kinase like	**2.30**	3.15E-02
rfx1	regulatory factor X1	**2.30**	4.18E-02
snrk	SNF related kinase	**2.29**	3.64E-02
dcn	decorin	**2.29**	4.46E-02
uhrf1	ubiquitin like with PHD and ring finger domains 1	**2.29**	5.30E-02
c17orf49	chromosome 17 open reading frame 49	**2.29**	8.63E-02
lxn	latexin	**2.29**	1.18E-02
ncam2	neural cell adhesion molecule 2	**2.29**	1.74E-02
aco2	aconitase 2	**2.29**	2.41E-02
m6pr	mannose-6-phosphate receptor, cation dependent	**2.29**	5.82E-02
asb13	ankyrin repeat and SOCS box containing 13	**2.29**	3.54E-02
rictor	RPTOR independent companion of MTOR complex 2	**2.29**	4.61E-02
wdfy2	WD repeat and FYVE domain containing 2	**2.29**	2.88E-02
trim46	tripartite motif containing 46	**2.29**	3.57E-02
actn3	actinin alpha 3	**2.29**	5.75E-02
slc25a10	solute carrier family 25 member 10	**2.29**	8.04E-02
kdelr3	KDEL endoplasmic reticulum protein retention receptor 3	**2.29**	8.92E-02
rlf	rearranged L-myc fusion	**2.29**	1.54E-02
cpd	carboxypeptidase D	**2.29**	4.44E-02
gmds	GDP-mannose 4,6-dehydratase	**2.29**	5.16E-02
ccdc22	coiled-coil domain containing 22	**2.28**	2.58E-02
podxl2	podocalyxin like 2	**2.28**	3.69E-03
slc31a2	solute carrier family 31 member 2	**2.28**	2.01E-02
tfcp2	transcription factor CP2	**2.28**	2.39E-02
pou3f1	POU class 3 homeobox 1	**2.28**	2.92E-02
tmem87a	transmembrane protein 87A	**2.28**	4.90E-02
polr2e	RNA polymerase II subunit E	**2.28**	4.98E-02
rab10	RAB10, member RAS oncogene family	**2.28**	8.16E-02
srebf2	sterol regulatory element binding transcription factor 2	**2.28**	8.40E-02
zscan20	zinc finger and SCAN domain containing 20	**2.28**	3.57E-02
fbxo22	F-box protein 22	**2.28**	2.99E-02
pgd	phosphogluconate dehydrogenase	**2.28**	9.47E-02
acvr2a	activin A receptor type 2A	**2.28**	2.32E-02
cmtm6	CKLF like MARVEL transmembrane domain containing 6	**2.28**	2.47E-02
znf512b	zinc finger protein 512B	**2.28**	3.60E-02
atp2a1	ATPase sarcoplasmic/endoplasmic reticulum Ca2+ transporting 1	**2.28**	4.03E-02
atad1	ATPase family, AAA domain containing 1	**2.28**	4.27E-02
cic	capicua transcriptional repressor	**2.28**	6.15E-02
extl3	exostosin like glycosyltransferase 3	**2.27**	6.33E-02
aldoa	aldolase, fructose-bisphosphate A	**2.27**	2.01E-03
mpeg1	macrophage expressed 1	**2.27**	5.10E-02
hgs	hepatocyte growth factor-regulated tyrosine kinase substrate	**2.27**	3.83E-02
chrnb1	cholinergic receptor nicotinic beta 1 subunit	**2.27**	4.50E-02
prpf38a	pre-mRNA processing factor 38A	**2.27**	8.09E-02
dctn1	dynactin subunit 1	**2.27**	1.89E-02
dnajc4	DnaJ heat shock protein family (Hsp40) member C4	**2.27**	8.64E-02
plp2	proteolipid protein 2	**2.27**	9.20E-02
impdh2	inosine monophosphate dehydrogenase 2	**2.26**	5.53E-03
prss23	protease, serine 23	**2.26**	6.03E-03
snx2	sorting nexin 2	**2.26**	3.05E-02
kif21a	kinesin family member 21A	**2.26**	8.33E-02
kif3a	kinesin family member 3A	**2.26**	3.19E-02
oser1	oxidative stress responsive serine rich 1	**2.26**	3.78E-02
kiaa0368	KIAA0368	**2.26**	6.85E-02
snap91	synaptosome associated protein 91	**2.26**	1.71E-02
uggt1	UDP-glucose glycoprotein glucosyltransferase 1	**2.26**	3.22E-02
asph	aspartate beta-hydroxylase	**2.26**	4.89E-02
gramd1a	GRAM domain containing 1A	**2.25**	1.93E-02
gabra6	gamma-aminobutyric acid type A receptor alpha6 subunit	**2.25**	5.10E-02
sgcb	sarcoglycan beta	**2.25**	3.73E-02
cbx3	chromobox 3	**2.25**	4.70E-02
tinf2	TERF1 interacting nuclear factor 2	**2.25**	5.27E-02
zbed5	zinc finger BED-type containing 5	**2.25**	8.41E-02
tbc1d14	TBC1 domain family member 14	**2.25**	1.58E-02
mapre2	microtubule-associated protein, RP/EB family, member 2	**2.25**	2.42E-02
cdc27	cell division cycle 27	**2.25**	6.89E-02
plpp1	phospholipid phosphatase 1	**2.25**	4.80E-02
exosc10	exosome component 10	**2.24**	2.39E-03
chn1	chimerin 1	**2.24**	4.61E-03
mdfic	MyoD family inhibitor domain containing	**2.24**	7.10E-03
pank2	pantothenate kinase 2	**2.24**	1.37E-02
ppp2r5d	protein phosphatase 2 regulatory subunit B'delta	**2.24**	2.87E-02
far1	fatty acyl-CoA reductase 1	**2.24**	7.33E-02
pak1	p21 (RAC1) activated kinase 1	**2.24**	4.19E-02
znf438	zinc finger protein 438	**2.24**	9.66E-02
tgfbrap1	transforming growth factor beta receptor associated protein 1	**2.24**	5.54E-02
gosr2	golgi SNAP receptor complex member 2	**2.24**	8.28E-03
cat	catalase	**2.24**	6.18E-02
myo9b	myosin IXB	**2.24**	6.64E-02
guk1	guanylate kinase 1	**2.24**	2.35E-02
mtr	5-methyltetrahydrofolate-homocysteine methyltransferase	**2.24**	4.95E-02
rgl1	ral guanine nucleotide dissociation stimulator like 1	**2.24**	7.72E-02
znf853	zinc finger protein 853	**2.24**	8.22E-02
mfn2	mitofusin 2	**2.23**	1.87E-02
asap3	ArfGAP with SH3 domain, ankyrin repeat and PH domain 3	**2.23**	4.15E-02
wdr1	WD repeat domain 1	**2.23**	8.88E-02
zdhhc9	zinc finger DHHC-type containing 9	**2.23**	2.46E-02
prrc2c	proline rich coiled-coil 2C	**2.23**	7.54E-02
tes	testin LIM domain protein	**2.23**	4.97E-02
cnnm2	cyclin and CBS domain divalent metal cation transport mediator 2	**2.23**	4.16E-02
ankrd27	ankyrin repeat domain 27	**2.23**	7.17E-02
trip13	thyroid hormone receptor interactor 13	**2.23**	2.15E-02
pikfyve	phosphoinositide kinase, FYVE-type zinc finger containing	**2.23**	2.83E-02
atg9a	autophagy related 9A	**2.23**	1.03E-02
uba1	ubiquitin like modifier activating enzyme 1	**2.23**	4.47E-02
crhr2	corticotropin releasing hormone receptor 2	**2.23**	8.39E-02
smyd3	SET and MYND domain containing 3	**2.23**	9.93E-02
stk10	serine/threonine kinase 10	**2.22**	8.33E-02
gbf1	golgi brefeldin A resistant guanine nucleotide exchange factor 1	**2.22**	4.16E-02
myot	myotilin	**2.22**	6.13E-02
lgals9	galectin 9	**2.22**	9.30E-02
slc39a13	solute carrier family 39 member 13	**2.22**	5.52E-02
alg10	ALG10, alpha-1,2-glucosyltransferase	**2.22**	4.00E-02
gucy2f	guanylate cyclase 2F, retinal	**2.22**	8.70E-02
sema4c	semaphorin 4C	**2.21**	2.70E-02
prelid1	PRELI domain containing 1	**2.21**	4.37E-02
mmgt1	membrane magnesium transporter 1	**2.21**	6.97E-02
vps39	VPS39, HOPS complex subunit	**2.21**	7.20E-02
phf20	PHD finger protein 20	**2.21**	4.04E-02

rfxap	regulatory factor X associated protein	**2.21**	5.09E-02
pnpt1	polyribonucleotide nucleotidyltransferase 1	**2.21**	1.96E-02
alg1	ALG1, chitobiosyldiphosphodolichol beta-mannosyltransferase	**2.21**	2.09E-02
tm9sf4	transmembrane 9 superfamily member 4	**2.21**	7.43E-02
prickle2	prickle planar cell polarity protein 2	**2.21**	8.25E-02
nfkbil1	NFKB inhibitor like 1	**2.21**	9.54E-02
otud4	OTU deubiquitinase 4	**2.21**	6.42E-02
fam126a	family with sequence similarity 126 member A	**2.21**	1.82E-02
wbp1l	WW domain binding protein 1-like	**2.21**	3.66E-02
slc27a2	solute carrier family 27 member 2	**2.21**	8.45E-02
lrp1b	LDL receptor related protein 1B	**2.21**	5.87E-02
rab21	RAB21, member RAS oncogene family	**2.20**	1.52E-02
amhr2	anti-Mullerian hormone receptor type 2	**2.20**	2.83E-02
rxrg	retinoid X receptor gamma	**2.20**	9.87E-02
atp2a3	ATPase sarcoplasmic/endoplasmic reticulum Ca2+ transporting 3	**2.20**	3.43E-02
nfkbiz	NFKB inhibitor zeta	**2.20**	1.17E-02
gjb6	gap junction protein beta 6	**2.20**	7.32E-02
cerk	ceramide kinase	**2.20**	8.28E-02
zdhhc3	zinc finger DHHC-type containing 3	**2.20**	9.01E-02
emp2	epithelial membrane protein 2	**2.20**	2.26E-06
ptpn9	protein tyrosine phosphatase, non-receptor type 9	**2.20**	3.66E-02
slc35f6	solute carrier family 35 member F6	**2.20**	8.76E-02
bok	BOK, BCL2 family apoptosis regulator	**2.20**	6.16E-02
nceh1	neutral cholesterol ester hydrolase 1	**2.19**	7.63E-02
lims1	LIM zinc finger domain containing 1	**2.19**	7.93E-02
n4bp3	NEDD4 binding protein 3	**2.19**	6.83E-02
dtx3	deltex E3 ubiquitin ligase 3	**2.19**	5.54E-02
znf180	zinc finger protein 180	**2.19**	3.27E-02
h2afv	H2A histone family member V	**2.19**	3.38E-02
slc30a4	solute carrier family 30 member 4	**2.19**	4.45E-02
grk3	G protein-coupled receptor kinase 3	**2.19**	4.69E-02
gjc2	gap junction protein gamma 2	**2.19**	5.26E-02
ube2q2	ubiquitin conjugating enzyme E2 Q2	**2.19**	6.00E-02
adora1	adenosine A1 receptor	**2.19**	7.44E-02
ralgps2	Ral GEF with PH domain and SH3 binding motif 2	**2.19**	8.24E-02
triobp	TRIO and F-actin binding protein	**2.19**	8.69E-02
rnf157	ring finger protein 157	**2.19**	3.71E-02
gid8	GID complex subunit 8 homolog	**2.18**	1.27E-03
stk17a	serine/threonine kinase 17a	**2.18**	4.68E-02
cnot4	CCR4-NOT transcription complex subunit 4	**2.18**	7.15E-02
dhx33	DEAH-box helicase 33	**2.18**	7.82E-03
ugdh	UDP-glucose 6-dehydrogenase	**2.18**	1.39E-02
ubr5	ubiquitin protein ligase E3 component n-recognin 5	**2.18**	1.68E-02
gnal	G protein subunit alpha L	**2.18**	4.20E-02
ifnar1	interferon alpha and beta receptor subunit 1	**2.18**	4.44E-02
git2	GIT ArfGAP 2	**2.18**	4.82E-02
mybl2	MYB proto-oncogene like 2	**2.18**	9.67E-02
klc4	kinesin light chain 4	**2.18**	7.89E-02
limch1	LIM and calponin homology domains 1	**2.18**	3.09E-02
tmem106b	transmembrane protein 106B	**2.18**	4.57E-02
ankrd40	ankyrin repeat domain 40	**2.18**	4.72E-02
rhog	ras homolog family member G	**2.18**	5.56E-02
impdh1	inosine monophosphate dehydrogenase 1	**2.17**	6.62E-02
hdac2	histone deacetylase 2	**2.17**	3.74E-02
adam28	ADAM metallopeptidase domain 28	**2.17**	8.66E-02
slc35a4	solute carrier family 35 member A4	**2.17**	1.05E-02
map2k4	mitogen-activated protein kinase kinase 4	**2.17**	9.28E-02
cdca4	cell division cycle associated 4	**2.17**	3.04E-02
serinc2	serine incorporator 2	**2.17**	5.62E-04
emc8	ER membrane protein complex subunit 8	**2.17**	9.14E-02
nr1h3	nuclear receptor subfamily 1 group H member 3	**2.17**	9.50E-02
rps6ka3	ribosomal protein S6 kinase A3	**2.16**	2.55E-02
ddx31	DEAD-box helicase 31	**2.16**	3.05E-02
baz1a	bromodomain adjacent to zinc finger domain 1A	**2.16**	4.98E-02
cab39	calcium binding protein 39	**2.16**	5.71E-02
znf233	zinc finger protein 233	**2.16**	3.07E-02
elmod3	ELMO domain containing 3	**2.16**	3.22E-02
tnfaip1	TNF alpha induced protein 1	**2.16**	7.59E-02
ppp4c	protein phosphatase 4 catalytic subunit	**2.16**	5.54E-02
cluh	clustered mitochondria homolog	**2.16**	1.43E-05
ngly1	N-glycanase 1	**2.16**	3.41E-02
hmgn1	high mobility group nucleosome binding domain 1	**2.16**	2.42E-02
tmc6	transmembrane channel like 6	**2.16**	5.53E-02
sdhaf2	succinate dehydrogenase complex assembly factor 2	**2.16**	8.35E-02
npc1	NPC intracellular cholesterol transporter 1	**2.16**	4.67E-02
cul5	cullin 5	**2.16**	4.95E-02
hebp1	heme binding protein 1	**2.15**	1.64E-02
znf850	zinc finger protein 850	**2.15**	7.68E-02
homer1	homer scaffolding protein 1	**2.15**	7.41E-03
mtfr1l	mitochondrial fission regulator 1 like	**2.15**	3.61E-02
tpra1	transmembrane protein adipocyte associated 1	**2.15**	8.37E-02
vdac3	voltage dependent anion channel 3	**2.15**	5.22E-02
syndig1	synapse differentiation inducing 1	**2.15**	8.17E-02
farp2	FERM, ARH/RhoGEF and pleckstrin domain protein 2	**2.15**	1.78E-02
vkorc1	vitamin K epoxide reductase complex subunit 1	**2.15**	5.66E-02
tdrd1	tudor domain containing 1	**2.15**	7.46E-02
fgl1	fibrinogen like 1	**2.15**	2.47E-02
dclk2	doublecortin like kinase 2	**2.15**	4.48E-02
col6a1	collagen type VI alpha 1 chain	**2.15**	8.48E-02
psmd11	proteasome 26S subunit, non-ATPase 11	**2.14**	5.97E-02
jmjd6	arginine demethylase and lysine hydroxylase	**2.14**	7.15E-02
znf513	zinc finger protein 513	**2.14**	7.93E-02
trmt12	tRNA methyltransferase 12 homolog	**2.14**	2.01E-02
dcaf6	DDB1 and CUL4 associated factor 6	**2.14**	5.60E-02
cd9	CD9 molecule	**2.14**	7.79E-02
sept7	septin 7	**2.14**	3.96E-02
h3f3c	H3 histone family member 3C	**2.14**	6.54E-02
tef	TEF, PAR bZIP transcription factor	**2.14**	7.65E-02
chic1	cysteine rich hydrophobic domain 1	**2.14**	4.45E-02
gatad2b	GATA zinc finger domain containing 2B	**2.14**	1.49E-02
kif5b	kinesin family member 5B	**2.14**	3.21E-02
ufm1	ubiquitin fold modifier 1	**2.14**	7.10E-02
asah1	N-acylsphingosine amidohydrolase 1	**2.14**	8.60E-02
pgrmc2	progesterone receptor membrane component 2	**2.13**	6.54E-02
ssh3	slingshot protein phosphatase 3	**2.13**	3.26E-02
vps26b	VPS26, retromer complex component B	**2.13**	2.41E-02
arfip1	ADP ribosylation factor interacting protein 1	**2.13**	7.03E-02
setd5	SET domain containing 5	**2.13**	6.44E-02
zcchc8	zinc finger CCHC-type containing 8	**2.13**	8.27E-02
scoc	short coiled-coil protein	**2.13**	5.54E-02
setd1a	SET domain containing 1A	**2.13**	6.41E-02
zmynd8	zinc finger MYND-type containing 8	**2.13**	5.13E-03
pafah1b3	platelet activating factor acetylhydrolase 1b catalytic subunit 3	**2.13**	4.22E-02
arhgef18	Rho/Rac guanine nucleotide exchange factor 18	**2.13**	7.79E-02
f2rl1	F2R like trypsin receptor 1	**2.12**	1.85E-02
ift74	intraflagellar transport 74	**2.12**	3.78E-02
cables1	Cdk5 and Abl enzyme substrate 1	**2.12**	4.94E-02
bzw2	basic leucine zipper and W2 domains 2	**2.12**	7.11E-02
g6pc	glucose-6-phosphatase catalytic subunit	**2.12**	3.18E-02
kiaa0355	KIAA0355	**2.12**	8.49E-02
pip5k1b	phosphatidylinositol-4-phosphate 5-kinase type 1 beta	**2.12**	4.65E-02
rxrb	retinoid X receptor beta	**2.12**	7.73E-02
mcoln1	mucolipin 1	**2.12**	9.49E-02
ugt2a1	UDP glucuronosyltransferase family 2 member A1 complex locus	**2.12**	2.86E-02
dlg5	discs large MAGUK scaffold protein 5	**2.12**	3.57E-02
arhgef6	Rac/Cdc42 guanine nucleotide exchange factor 6	**2.12**	3.89E-02
asap2	ArfGAP with SH3 domain, ankyrin repeat and PH domain 2	**2.12**	4.37E-02
exoc7	exocyst complex component 7	**2.12**	6.75E-02
steap3	STEAP3 metalloreductase	**2.12**	6.96E-02
unc5d	unc-5 netrin receptor D	**2.12**	9.09E-02
duox1	dual oxidase 1	**2.12**	4.66E-02
abcd3	ATP binding cassette subfamily D member 3	**2.12**	6.14E-02
ccdc66	coiled-coil domain containing 66	**2.11**	5.72E-02
fem1a	fem-1 homolog A	**2.11**	4.84E-02
lrp2	LDL receptor related protein 2	**2.11**	6.55E-02
kif1b	kinesin family member 1B	**2.11**	1.98E-02
cers2	ceramide synthase 2	**2.11**	5.88E-02
sltm	SAFB like transcription modulator	**2.11**	6.62E-02
zfand6	zinc finger AN1-type containing 6	**2.11**	7.61E-02
slc7a3	solute carrier family 7 member 3	**2.11**	1.56E-02
slc6a12	solute carrier family 6 member 12	**2.11**	9.31E-02
pdcd4	programmed cell death 4 (neoplastic transformation inhibitor)	**2.11**	4.84E-03
gpm6a	glycoprotein M6A	**2.11**	1.19E-02
tmem57	transmembrane protein 57	**2.11**	4.80E-02
st13	suppression of tumorigenicity 13 (colon carcinoma) (Hsp70 interacting protein)	**2.11**	7.87E-02
mbp	myelin basic protein	**2.10**	7.52E-02

kif1c	kinesin family member 1C	**2.10**	8.59E-02
mocos	molybdenum cofactor sulfurase	**2.10**	2.35E-02
tmem63c	transmembrane protein 63C	**2.10**	2.97E-02
atf6b	activating transcription factor 6 beta	**2.10**	3.25E-02
cdkn2aip	CDKN2A interacting protein	**2.10**	4.96E-02
dmgdh	dimethylglycine dehydrogenase	**2.10**	6.08E-02
fgd4	FYVE, RhoGEF and PH domain containing 4	**2.10**	2.15E-02
canx	calnexin	**2.10**	5.63E-02
cd82	CD82 molecule	**2.10**	2.23E-02
gsn	gelsolin	**2.10**	2.72E-02
racgap1	Rac GTPase activating protein 1	**2.10**	5.33E-02
snap47	synaptosome associated protein 47	**2.10**	1.93E-03
pitrm1	pitrilysin metallopeptidase 1	**2.10**	4.36E-02
map7d2	MAP7 domain containing 2	**2.10**	4.69E-02
chmp7	charged multivesicular body protein 7	**2.10**	5.18E-02
slc25a16	solute carrier family 25 member 16	**2.10**	8.97E-02
mrm3	mitochondrial rRNA methyltransferase 3	**2.09**	9.41E-03
dnajc5b	DnaJ heat shock protein family (Hsp40) member C5 beta	**2.09**	7.52E-02
ssbp1	single stranded DNA binding protein 1	**2.09**	9.69E-02
lbp	lipopolysaccharide binding protein	**2.09**	9.90E-02
sept10	septin 10	**2.09**	4.73E-03
hif1an	hypoxia inducible factor 1 alpha subunit inhibitor	**2.09**	6.08E-02
gemin2	gem nuclear organelle associated protein 2	**2.09**	1.20E-02
dnm3	dynamin 3	**2.09**	5.58E-02
mob1b	MOB kinase activator 1B	**2.09**	3.34E-02
eif3j	eukaryotic translation initiation factor 3 subunit J	**2.08**	4.58E-02
psmc6	proteasome 26S subunit, ATPase 6	**2.08**	1.56E-02
fzd4	frizzled class receptor 4	**2.08**	1.99E-02
hyou1	hypoxia up-regulated 1	**2.08**	3.75E-02
atp6v0d1	ATPase H+ transporting V0 subunit d1	**2.08**	5.18E-02
tmem120b	transmembrane protein 120B	**2.08**	3.01E-02
fam222a	family with sequence similarity 222 member A	**2.08**	8.34E-02
lbx1	ladybird homeobox 1	**2.08**	9.68E-02
fstl1	follistatin like 1	**2.08**	3.52E-02
tdrd7	tudor domain containing 7	**2.08**	5.40E-02
rab6a	RAB6A, member RAS oncogene family	**2.08**	9.31E-02
ezr	ezrin	**2.08**	2.41E-02
yod1	YOD1 deubiquitinase	**2.08**	6.35E-02
zranb3	zinc finger RANBP2-type containing 3	**2.08**	6.32E-02
c1qtnf4	C1q and tumor necrosis factor related protein 4	**2.07**	6.44E-03
snrpc	U1 small nuclear ribonucleoprotein C	**2.07**	4.69E-02
slc15a4	solute carrier family 15 member 4	**2.07**	7.63E-02
arl15	ADP ribosylation factor like GTPase 15	**2.07**	9.63E-02
mrpl19	mitochondrial ribosomal protein L19	**2.07**	9.80E-02
c2cd2	C2 calcium dependent domain containing 2	**2.07**	6.03E-02
eif5b	eukaryotic translation initiation factor 5B	**2.07**	8.94E-03
tmprss9	transmembrane protease, serine 9	**2.07**	4.44E-02
rab37	RAB37, member RAS oncogene family	**2.07**	4.66E-02
mgst3	microsomal glutathione S-transferase 3	**2.07**	4.84E-02
rmi2	RecQ mediated genome instability 2	**2.07**	3.39E-02
gramd4	GRAM domain containing 4	**2.06**	2.05E-02
ndfip1	Nedd4 family interacting protein 1	**2.06**	6.17E-02
c20orf27	chromosome 20 open reading frame 27	**2.06**	4.83E-02
osgin1	oxidative stress induced growth inhibitor 1	**2.06**	9.10E-02
pcyt1a	phosphate cytidylyltransferase 1, choline, alpha	**2.06**	3.41E-02
gbe1	1,4-alpha-glucan branching enzyme 1	**2.06**	8.99E-02
mgmt	O-6-methylguanine-DNA methyltransferase	**2.06**	9.55E-02
ddc	dopa decarboxylase	**2.06**	1.86E-02
micall1	MICAL like 1	**2.06**	6.64E-02
trip12	thyroid hormone receptor interactor 12	**2.06**	7.10E-02
sec23a	Sec23 homolog A, coat complex II component	**2.06**	5.09E-02
znf652	zinc finger protein 652	**2.06**	9.06E-02
rnf11	ring finger protein 11	**2.05**	9.68E-02
wwp2	WW domain containing E3 ubiquitin protein ligase 2	**2.05**	2.81E-02
rspry1	ring finger and SPRY domain containing 1	**2.05**	4.03E-02
atp6v0a1	ATPase H+ transporting V0 subunit a1	**2.05**	6.03E-02
sgo2	shugoshin 2	**2.05**	7.65E-05
znf346	zinc finger protein 346	**2.05**	4.75E-02
ttc16	tetratricopeptide repeat domain 16	**2.05**	2.42E-02
rps6kb2	ribosomal protein S6 kinase B2	**2.05**	5.77E-02
fam3a	family with sequence similarity 3 member A	**2.05**	3.40E-02
trim39	tripartite motif containing 39	**2.05**	5.16E-02
git1	GIT ArfGAP 1	**2.05**	6.08E-02
efna4	ephrin A4	**2.05**	6.80E-02
zfr2	zinc finger RNA binding protein 2	**2.05**	7.03E-02
sowaha	sosondowah ankyrin repeat domain family member A	**2.05**	8.28E-02
ell2	elongation factor for RNA polymerase II 2	**2.05**	6.25E-02
tbc1d9	TBC1 domain family member 9	**2.05**	9.02E-02
pik3ca	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	**2.04**	5.41E-02
cyp2r1	cytochrome P450 family 2 subfamily R member 1	**2.04**	6.73E-02
ykt6	YKT6 v-SNARE homolog (S. cerevisiae)	**2.04**	4.77E-03
srp54	signal recognition particle 54	**2.04**	2.29E-02
spag9	sperm associated antigen 9	**2.04**	6.09E-02
spin3	spindlin family member 3	**2.04**	7.90E-02
tspo	translocator protein	**2.04**	8.60E-02
kif2c	kinesin family member 2C	**2.04**	5.68E-03
tab1	TGF-beta activated kinase 1 (MAP3K7) binding protein 1	**2.04**	4.41E-02
znf280d	zinc finger protein 280D	**2.04**	1.09E-02
tram1	translocation associated membrane protein 1	**2.04**	5.54E-02
mpp5	membrane palmitoylated protein 5	**2.04**	3.68E-02
cyb5b	cytochrome b5 type B	**2.04**	5.20E-02
cr1l	complement C3b/C4b receptor 1 like	**2.04**	5.94E-02
dhx38	DEAH-box helicase 38	**2.04**	9.49E-02
galnt18	polypeptide N-acetylgalactosaminyltransferase 18	**2.04**	1.17E-02
fnbp1	formin binding protein 1	**2.04**	5.24E-02
piezo1	piezo type mechanosensitive ion channel component 1	**2.04**	5.45E-02
cc2d1a	coiled-coil and C2 domain containing 1A	**2.04**	5.97E-02
shc1	SHC adaptor protein 1	**2.04**	7.61E-02
actr3	ARP3 actin related protein 3 homolog	**2.04**	7.97E-02
atp2a2	ATPase sarcoplasmic/endoplasmic reticulum Ca2+ transporting 2	**2.04**	8.28E-02
dctn4	dynactin subunit 4	**2.03**	3.80E-02
mfge8	milk fat globule-EGF factor 8 protein	**2.03**	6.72E-02
blnk	B-cell linker	**2.03**	4.19E-02
pxmp4	peroxisomal membrane protein 4	**2.03**	5.35E-02
abcc4	ATP binding cassette subfamily C member 4	**2.03**	9.53E-02
mre11	MRE11 homolog, double strand break repair nuclease	**2.03**	2.79E-02
plcg1	phospholipase C gamma 1	**2.03**	3.19E-02
lrrc8c	leucine rich repeat containing 8 family member C	**2.03**	4.03E-02
aplp1	amyloid beta precursor like protein 1	**2.03**	6.13E-02
efcab11	EF-hand calcium binding domain 11	**2.03**	9.01E-02
gclm	glutamate-cysteine ligase modifier subunit	**2.03**	9.69E-02
rasgrp2	RAS guanyl releasing protein 2	**2.03**	1.92E-02
calu	calumenin	**2.03**	3.34E-02
hspa4	heat shock protein family A (Hsp70) member 4	**2.03**	3.95E-02
anapc1	anaphase promoting complex subunit 1	**2.03**	9.70E-02
mapk14	mitogen-activated protein kinase 14	**2.03**	5.87E-02
tpp2	tripeptidyl peptidase 2	**2.03**	6.72E-02
ccnk	cyclin K	**2.03**	9.15E-02
kif2a	kinesin family member 2A	**2.03**	9.32E-02
mal	mal, T-cell differentiation protein	**2.03**	7.36E-03
eno2	enolase 2	**2.03**	4.01E-03
cbs/cbsl	cystathionine-beta-synthase	**2.02**	9.37E-02
wee2	WEE1 homolog 2	**2.02**	1.32E-03
acbd3	acyl-CoA binding domain containing 3	**2.02**	6.85E-02
xbp1	X-box binding protein 1	**2.02**	3.53E-02
cd151	CD151 molecule (Raph blood group)	**2.02**	7.17E-02
glce	glucuronic acid epimerase	**2.02**	7.42E-02
zhx1	zinc fingers and homeoboxes 1	**2.02**	7.76E-02
ubr1	ubiquitin protein ligase E3 component n-recognin 1	**2.02**	4.12E-02
rcc2	regulator of chromosome condensation 2	**2.02**	5.53E-02
nol9	nucleolar protein 9	**2.02**	5.99E-02
angpt1	angiopoietin 1	**2.02**	9.53E-02
znf763	zinc finger protein 763	**2.02**	1.36E-02
fbxl12	F-box and leucine rich repeat protein 12	**2.02**	8.41E-02
fam199x	family with sequence similarity 199, X-linked	**2.02**	3.14E-02
clta	clathrin light chain A	**2.02**	6.78E-02
flcn	folliculin	**2.02**	8.16E-02
tecpr1	tectonin beta-propeller repeat containing 1	**2.02**	8.41E-02
arl5b	ADP ribosylation factor like GTPase 5B	**2.02**	4.23E-02
rab4a	RAB4A, member RAS oncogene family	**2.02**	4.92E-02
cdr2l	cerebellar degeneration related protein 2 like	**2.02**	9.01E-02
dync1i2	dynein cytoplasmic 1 intermediate chain 2	**2.01**	6.53E-02
cttn	cortactin	**2.01**	6.56E-02
dcaf13	DDB1 and CUL4 associated factor 13	**2.01**	8.79E-02
tacr3	tachykinin receptor 3	**2.01**	9.72E-02
mapk9	mitogen-activated protein kinase 9	**2.01**	6.46E-02
ano5	anoctamin 5	**2.01**	8.55E-02
arfip2	ADP ribosylation factor interacting protein 2	**2.01**	4.96E-02
rab11fip1	RAB11 family interacting protein 1	**2.01**	5.57E-02

prkcd	protein kinase C delta	**2.01**	6.65E-02
apaf1	apoptotic peptidase activating factor 1	**2.01**	9.81E-02
ing5	inhibitor of growth family member 5	**2.01**	3.88E-02
grn	granulin precursor	**2.01**	4.96E-02
me3	malic enzyme 3	**2.01**	5.71E-02
lysmd3	LysM domain containing 3	**2.01**	6.81E-02
usp5	ubiquitin specific peptidase 5	**2.01**	8.93E-02
naa40	N(alpha)-acetyltransferase 40, NatD catalytic subunit	**2.01**	1.54E-02
ap1s3	adaptor related protein complex 1 sigma 3 subunit	**2.01**	6.32E-02
ago4	argonaute 4, RISC catalytic component	**2.01**	8.45E-02
ccnt1	cyclin T1	**2.01**	9.30E-02
exoc8	exocyst complex component 8	**2.01**	3.57E-02
stx5	syntaxin 5	**2.01**	5.86E-02
b2m	beta-2-microglobulin	**2.01**	9.00E-03
usf2	upstream transcription factor 2, c-fos interacting	**2.01**	2.78E-02
mlxip	MLX interacting protein	**2.01**	3.82E-02
gns	glucosamine (N-acetyl)-6-sulfatase	**2.01**	4.23E-02
c1qb	complement C1q B chain	**2.01**	7.60E-02
pgls	6-phosphogluconolactonase	**2.00**	2.55E-03
oaf	out at first homolog	**2.00**	3.01E-02
cep76	centrosomal protein 76	**2.00**	6.91E-02
letm1	leucine zipper and EF-hand containing transmembrane protein 1	**2.00**	4.74E-02
nsf	N-ethylmaleimide sensitive factor, vesicle fusing ATPase	**2.00**	5.79E-02
trak1	trafficking kinesin protein 1	**2.00**	5.99E-02
celf1	CUGBP, Elav-like family member 1	**2.00**	6.42E-02
trip6	thyroid hormone receptor interactor 6	**2.00**	6.42E-02
rps6ka1	ribosomal protein S6 kinase A1	**2.00**	6.53E-02
znf800	zinc finger protein 800	**2.00**	4.64E-02
snx10	sorting nexin 10	**2.00**	4.82E-02
pgam1	phosphoglycerate mutase 1	**2.00**	5.01E-02
ubp1	upstream binding protein 1 (LBP-1a)	**2.00**	7.71E-02
bax	BCL2 associated X, apoptosis regulator	**2.00**	5.18E-02
ncln	nicalin	**2.00**	9.00E-02
parva	parvin alpha	**1.99**	3.96E-02
lamp1	lysosomal associated membrane protein 1	**1.99**	6.26E-02
rab3il1	RAB3A interacting protein like 1	**1.99**	8.21E-02
glrx5	glutaredoxin 5	**1.99**	2.38E-02
bzw1	basic leucine zipper and W2 domains 1	**1.99**	1.24E-02
fermt2	fermitin family member 2	**1.99**	3.47E-02
ddit4l	DNA damage inducible transcript 4 like	**1.99**	5.15E-02
map2k7	mitogen-activated protein kinase kinase 7	**1.99**	7.81E-02
rbpjl2	recombination signal binding protein for immunoglobulin kappa J region-like 2	**1.99**	9.01E-02
smc4	structural maintenance of chromosomes 4	**1.99**	4.82E-02
nip7	NIP7, nucleolar pre-rRNA processing protein	**1.99**	6.42E-02
prps1l3	phosphoribosyl pyrophosphate synthetase 1-like 3	**1.99**	8.91E-02
sacm1l	SAC1 suppressor of actin mutations 1-like (yeast)	**1.98**	2.41E-02
cuedc1	CUE domain containing 1	**1.98**	8.60E-02
rab40b	RAB40B, member RAS oncogene family	**1.98**	1.66E-02
prkar1a	protein kinase cAMP-dependent type I regulatory subunit alpha	**1.98**	2.97E-02
txnrd3	thioredoxin reductase 3	**1.98**	5.54E-02
ern1	endoplasmic reticulum to nucleus signaling 1	**1.98**	5.93E-02
tapt1	transmembrane anterior posterior transformation 1	**1.98**	8.18E-02
capn2	calpain 2	**1.98**	9.73E-02
acp2	acid phosphatase 2, lysosomal	**1.98**	2.34E-05
inppl1	inositol polyphosphate phosphatase like 1	**1.98**	3.26E-02
ypel4	yippee like 4	**1.98**	6.02E-02
ptpn1	protein tyrosine phosphatase, non-receptor type 1	**1.98**	7.69E-02
tmem192	transmembrane protein 192	**1.98**	9.82E-02
gigyf1	GRB10 interacting GYF protein 1	**1.98**	8.10E-02
tbc1d10b	TBC1 domain family member 10B	**1.98**	9.88E-02
dbp	D-box binding PAR bZIP transcription factor	**1.98**	5.56E-02
ppfibp1	PPFIA binding protein 1	**1.98**	4.87E-02
ubap2l	ubiquitin associated protein 2 like	**1.98**	9.37E-02
stk24	serine/threonine kinase 24	**1.97**	9.31E-03
pla2g4a	phospholipase A2 group IVA	**1.97**	6.14E-02
sirt5	sirtuin 5	**1.97**	6.93E-02
pex5	peroxisomal biogenesis factor 5	**1.97**	9.27E-02
mxd1	MAX dimerization protein 1	**1.97**	7.19E-02
als2	ALS2, alsin Rho guanine nucleotide exchange factor	**1.97**	7.28E-02
add1	adducin 1	**1.97**	8.46E-02
c1orf21	chromosome 1 open reading frame 21	**1.97**	5.38E-02
nfkbie	NFKB inhibitor epsilon	**1.97**	8.12E-03
lztfl1	leucine zipper transcription factor like 1	**1.96**	4.39E-02
mtg2	mitochondrial ribosome associated GTPase 2	**1.96**	5.91E-02
itfg2	integrin alpha FG-GAP repeat containing 2	**1.96**	6.86E-02
serp1	stress associated endoplasmic reticulum protein 1	**1.96**	8.27E-02
plod3	procollagen-lysine,2-oxoglutarate 5-dioxygenase 3	**1.96**	2.81E-02
strn	striatin	**1.96**	7.03E-02
s100a4	S100 calcium binding protein A4	**1.96**	9.53E-02
pcmtd2	protein-L-isoaspartate (D-aspartate) O-methyltransferase domain containing 2	**1.96**	5.07E-02
krt8	keratin 8	**1.96**	6.13E-02
map3k6	mitogen-activated protein kinase kinase kinase 6	**1.96**	7.09E-02
mcam	melanoma cell adhesion molecule	**1.96**	7.12E-02
toe1	target of EGR1, member 1 (nuclear)	**1.96**	1.09E-02
lmbr1	limb development membrane protein 1	**1.96**	4.36E-02
cul4b	cullin 4B	**1.96**	4.65E-02
etnk1	ethanolamine kinase 1	**1.96**	6.60E-02
sestd1	SEC14 and spectrin domain containing 1	**1.96**	4.03E-02
ccng2	cyclin G2	**1.95**	4.26E-02
ap1m1	adaptor related protein complex 1 mu 1 subunit	**1.95**	4.28E-02
tm2d2	TM2 domain containing 2	**1.95**	7.11E-02
suz12	SUZ12 polycomb repressive complex 2 subunit	**1.95**	5.02E-02
sept6	septin 6	**1.95**	6.41E-02
mpp6	membrane palmitoylated protein 6	**1.95**	6.53E-02
ccne2	cyclin E2	**1.95**	4.89E-02
ralb	RAS like proto-oncogene B	**1.95**	6.49E-02
pkn2	protein kinase N2	**1.95**	6.78E-02
rsad1	radical S-adenosyl methionine domain containing 1	**1.95**	2.10E-02
aldh3b1	aldehyde dehydrogenase 3 family member B1	**1.95**	6.17E-02
ppp3cc	protein phosphatase 3 catalytic subunit gamma	**1.95**	8.07E-02
cdyl	chromodomain Y-like	**1.95**	8.23E-02
dclre1b	DNA cross-link repair 1B	**1.95**	8.50E-02
mzf1	myeloid zinc finger 1	**1.95**	8.75E-02
camk2g	calcium/calmodulin dependent protein kinase II gamma	**1.95**	5.37E-02
rara	retinoic acid receptor alpha	**1.95**	6.26E-02
tead1	TEA domain transcription factor 1	**1.95**	7.25E-02
sept5	septin 5	**1.95**	2.73E-02
ublcp1	ubiquitin like domain containing CTD phosphatase 1	**1.95**	3.54E-02
shmt2	serine hydroxymethyltransferase 2	**1.95**	3.81E-02
zcchc17	zinc finger CCHC-type containing 17	**1.95**	4.33E-02
pi4k2b	phosphatidylinositol 4-kinase type 2 beta	**1.95**	7.87E-02
mapt	microtubule associated protein tau	**1.94**	3.30E-02
cdc42	cell division cycle 42	**1.94**	4.04E-02
sptlc2	serine palmitoyltransferase long chain base subunit 2	**1.94**	5.50E-02
sall4	spalt like transcription factor 4	**1.94**	7.64E-02
vps53	VPS53, GARP complex subunit	**1.94**	7.70E-02
ufl1	UFM1 specific ligase 1	**1.94**	7.71E-02
znf681	zinc finger protein 681	**1.94**	7.76E-02
prom2	prominin 2	**1.94**	2.17E-02
tbc1d12	TBC1 domain family member 12	**1.94**	3.65E-02
znf430	zinc finger protein 430	**1.94**	6.46E-02
tmem248	transmembrane protein 248	**1.94**	7.93E-02
acbd5	acyl-CoA binding domain containing 5	**1.94**	8.03E-02
mapkapk3	mitogen-activated protein kinase-activated protein kinase 3	**1.94**	1.35E-02
plppr5	phospholipid phosphatase related 5	**1.94**	1.69E-02
rassf2	Ras association domain family member 2	**1.94**	9.89E-02
enah	enabled homolog (Drosophila)	**1.94**	5.70E-02
ctns	cystinosin, lysosomal cystine transporter	**1.94**	3.80E-02
thra	thyroid hormone receptor, alpha	**1.94**	6.02E-02
crip2	cysteine rich protein 2	**1.94**	6.05E-02
mau2	MAU2 sister chromatid cohesion factor	**1.94**	7.18E-02
minos1	mitochondrial inner membrane organizing system 1	**1.94**	4.97E-02
kyat1	kynurenine aminotransferase 1	**1.93**	3.48E-02
ttc3	tetratricopeptide repeat domain 3	**1.93**	2.54E-02
nfyc	nuclear transcription factor Y subunit gamma	**1.93**	7.73E-02
tprg1	tumor protein p63 regulated 1	**1.93**	7.76E-02
cask	calcium/calmodulin dependent serine protein kinase	**1.93**	8.14E-02
tmem184c	transmembrane protein 184C	**1.93**	3.27E-02
gpr146	G protein-coupled receptor 146	**1.93**	9.51E-02
rab8a	RAB8A, member RAS oncogene family	**1.93**	4.22E-02
fez2	fasciculation and elongation protein zeta 2	**1.93**	6.27E-02
trove2	TROVE domain family member 2	**1.93**	5.61E-02
pitpnb	phosphatidylinositol transfer protein beta	**1.93**	9.57E-02
ifi35	interferon induced protein 35	**1.93**	1.60E-02
st3gal1	ST3 beta-galactoside alpha-2,3-sialyltransferase 1	**1.93**	5.30E-02

lnpep	leucyl and cystinyl aminopeptidase	**1.93**	8.33E-02
tmem164	transmembrane protein 164	**1.92**	6.21E-02
narfl	nuclear prelamin A recognition factor like	**1.92**	6.64E-02
sord	sorbitol dehydrogenase	**1.92**	4.88E-02
fsd1l	fibronectin type III and SPRY domain containing 1 like	**1.92**	7.11E-02
ptpn4	protein tyrosine phosphatase, non-receptor type 4	**1.92**	8.84E-02
mob4	MOB family member 4, phocein	**1.92**	3.03E-02
ccar2	cell cycle and apoptosis regulator 2	**1.92**	6.36E-02
emc3	ER membrane protein complex subunit 3	**1.92**	6.48E-02
brd9	bromodomain containing 9	**1.92**	7.65E-02
insig1	insulin induced gene 1	**1.92**	9.50E-02
amer1	APC membrane recruitment protein 1	**1.92**	1.90E-02
xpo5	exportin 5	**1.92**	8.36E-02
fbxo28	F-box protein 28	**1.92**	1.92E-02
cnot9	CCR4-NOT transcription complex subunit 9	**1.92**	3.27E-02
r3hcc1l	R3H domain and coiled-coil containing 1 like	**1.92**	6.14E-02
slc9a6	solute carrier family 9 member A6	**1.92**	4.49E-02
ostc	oligosaccharyltransferase complex non-catalytic subunit	**1.92**	7.03E-02
tp53	tumor protein p53	**1.92**	7.17E-02
mrpl41	mitochondrial ribosomal protein L41	**1.92**	8.24E-02
hsp90aa1	heat shock protein 90 alpha family class A member 1	**1.91**	3.29E-02
apex2	apurinic/apyrimidinic endodeoxyribonuclease 2	**1.91**	7.80E-02
ctdnep1	CTD nuclear envelope phosphatase 1	**1.91**	5.60E-03
plppr3	phospholipid phosphatase related 3	**1.91**	8.90E-02
aacs	acetoacetyl-CoA synthetase	**1.91**	3.96E-02
ulk2	unc-51 like autophagy activating kinase 2	**1.91**	9.09E-02
agpat4	1-acylglycerol-3-phosphate O-acyltransferase 4	**1.91**	6.74E-02
anxa11	annexin A11	**1.91**	5.83E-02
enpp1	ectonucleotide pyrophosphatase/phosphodiesterase 1	**1.91**	7.56E-02
ppil2	peptidylprolyl isomerase like 2	**1.91**	7.88E-02
alad	aminolevulinate dehydratase	**1.91**	1.13E-02
mmadhc	methylmalonic aciduria and homocystinuria, cblD type	**1.91**	7.13E-02
usp4	ubiquitin specific peptidase 4	**1.91**	3.10E-02
ccne1	cyclin E1	**1.91**	4.08E-02
leng8	leukocyte receptor cluster member 8	**1.91**	6.71E-02
hecw2	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 2	**1.91**	9.21E-02
fam83d	family with sequence similarity 83 member D	**1.91**	9.90E-02
znf384	zinc finger protein 384	**1.91**	5.34E-02
znf878	zinc finger protein 878	**1.91**	6.68E-02
crkl	CRK like proto-oncogene, adaptor protein	**1.91**	8.22E-02
ppp1r14a	protein phosphatase 1 regulatory inhibitor subunit 14A	**1.90**	7.43E-02
spns1	sphingolipid transporter 1 (putative)	**1.90**	9.60E-02
fam49a	family with sequence similarity 49 member A	**1.90**	2.44E-03
vmp1	vacuole membrane protein 1	**1.90**	4.85E-02
urgcp	upregulator of cell proliferation	**1.90**	5.55E-02
plxnc1	plexin C1	**1.90**	6.01E-02
perp	PERP, TP53 apoptosis effector	**1.90**	7.85E-02
klhl25	kelch like family member 25	**1.90**	7.86E-02
aebp2	AE binding protein 2	**1.90**	2.80E-02
slc3a2	solute carrier family 3 member 2	**1.90**	8.79E-02
fignl1	fidgetin like 1	**1.90**	9.76E-02
snx30	sorting nexin family member 30	**1.90**	6.19E-02
tesk2	testis-specific kinase 2	**1.90**	6.86E-02
pawr	pro-apoptotic WT1 regulator	**1.90**	1.40E-02
ddx46	DEAD-box helicase 46	**1.90**	3.48E-02
dnajc14	DnaJ heat shock protein family (Hsp40) member C14	**1.90**	5.33E-02
mtdh	metadherin	**1.90**	6.81E-02
upf3b	UPF3 regulator of nonsense transcripts homolog B (yeast)	**1.89**	5.13E-02
nde1	nudE neurodevelopment protein 1	**1.89**	5.87E-03
scml2	sex comb on midleg-like 2 (Drosophila)	**1.89**	6.99E-02
khdrbs1	KH RNA binding domain containing, signal transduction associated 1	**1.89**	7.50E-02
slc37a1	solute carrier family 37 member 1	**1.89**	7.51E-02
crcp	CGRP receptor component	**1.89**	7.61E-02
smarca2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2	**1.89**	7.90E-02
lmbrd1	LMBR1 domain containing 1	**1.89**	9.73E-02
plekhg5	pleckstrin homology and RhoGEF domain containing G5	**1.89**	9.88E-02
tom1l2	target of myb1 like 2 membrane trafficking protein	**1.89**	9.52E-02
srsf5	serine and arginine rich splicing factor 5	**1.89**	6.36E-02
gimap7	GTPase, IMAP family member 7	**1.89**	7.05E-02
hcfc2	host cell factor C2	**1.89**	7.33E-02
dap	death associated protein	**1.89**	8.01E-02
rab5c	RAB5C, member RAS oncogene family	**1.89**	9.09E-02
zyx	zyxin	**1.89**	2.21E-02
tmem165	transmembrane protein 165	**1.89**	8.70E-02
fkbp5	FK506 binding protein 5	**1.89**	1.47E-03
smarcd2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 2	**1.89**	7.21E-02
c9orf69	chromosome 9 open reading frame 69	**1.89**	3.83E-02
csdc2	cold shock domain containing C2	**1.89**	7.84E-02
tmem79	transmembrane protein 79	**1.88**	1.93E-02
tubg1	tubulin gamma 1	**1.88**	6.47E-02
cnnm4	cyclin and CBS domain divalent metal cation transport mediator 4	**1.88**	8.46E-02
zbtb48	zinc finger and BTB domain containing 48	**1.88**	9.45E-02
cdk20	cyclin dependent kinase 20	**1.88**	9.83E-02
fbxo21	F-box protein 21	**1.88**	7.01E-02
gne	glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase	**1.88**	4.70E-02
oaz1	ornithine decarboxylase antizyme 1	**1.88**	6.98E-02
gfod1	glucose-fructose oxidoreductase domain containing 1	**1.88**	9.18E-02
pex14	peroxisomal biogenesis factor 14	**1.88**	7.43E-02
ergic1	endoplasmic reticulum-golgi intermediate compartment 1	**1.88**	3.19E-02
st3gal2	ST3 beta-galactoside alpha-2,3-sialyltransferase 2	**1.88**	5.72E-02
dennd6a	DENN domain containing 6A	**1.88**	5.76E-02
thap12	THAP domain containing 12	**1.88**	3.97E-02
trim11	tripartite motif containing 11	**1.88**	5.78E-02
cnpy4	canopy FGF signaling regulator 4	**1.88**	6.85E-02
stra6l	STRA6-like	**1.88**	7.26E-02
tbc1d17	TBC1 domain family member 17	**1.88**	7.50E-02
srpk1	SRSF protein kinase 1	**1.87**	7.29E-02
cnn3	calponin 3	**1.87**	8.99E-02
mark3	microtubule affinity regulating kinase 3	**1.87**	7.02E-02
nt5e	5'-nucleotidase ecto	**1.87**	3.49E-02
spdl1	spindle apparatus coiled-coil protein 1	**1.87**	4.69E-02
tk2	thymidine kinase 2, mitochondrial	**1.87**	9.12E-02
srsf11	serine and arginine rich splicing factor 11	**1.87**	2.54E-02
naa30	N(alpha)-acetyltransferase 30, NatC catalytic subunit	**1.87**	8.64E-02
pja2	praja ring finger ubiquitin ligase 2	**1.87**	9.14E-02
flvcr2	feline leukemia virus subgroup C cellular receptor family member 2	**1.87**	9.53E-02
cbx1	chromobox 1	**1.87**	3.20E-02
pudp	pseudouridine 5'-phosphatase	**1.87**	9.60E-02
dnaja3	DnaJ heat shock protein family (Hsp40) member A3	**1.86**	5.13E-02
aes	amino-terminal enhancer of split	**1.86**	7.28E-02
bcl10	B-cell CLL/lymphoma 10	**1.86**	3.38E-02
nfe2l1	nuclear factor, erythroid 2 like 1	**1.86**	5.17E-02
wrb	tryptophan rich basic protein	**1.86**	8.28E-02
ube2g1	ubiquitin conjugating enzyme E2 G1	**1.86**	4.37E-02
pou2f3	POU class 2 homeobox 3	**1.86**	7.72E-02
yme1l1	YME1 like 1 ATPase	**1.86**	8.82E-02
med6	mediator complex subunit 6	**1.86**	6.72E-02
slc30a9	solute carrier family 30 member 9	**1.86**	6.73E-02
cps1	carbamoyl-phosphate synthase 1	**1.86**	9.27E-02
chrnb3	cholinergic receptor nicotinic beta 3 subunit	**1.86**	6.21E-02
fam120b	family with sequence similarity 120B	**1.86**	6.91E-02
elmo1	engulfment and cell motility 1	**1.86**	5.31E-02
dnajb1	DnaJ heat shock protein family (Hsp40) member B1	**1.86**	9.13E-02
aen	apoptosis enhancing nuclease	**1.86**	2.83E-02
tcaim	T-cell activation inhibitor, mitochondrial	**1.86**	4.82E-02
atxn7l3	ataxin 7 like 3	**1.85**	4.36E-02
paip2b	poly(A) binding protein interacting protein 2B	**1.85**	4.48E-02
znf585a	zinc finger protein 585A	**1.85**	9.26E-02
pdia3	protein disulfide isomerase family A member 3	**1.85**	6.40E-03
bmp2k	BMP2 inducible kinase	**1.85**	8.34E-02
cul1	cullin 1	**1.85**	8.94E-02
arntl2	aryl hydrocarbon receptor nuclear translocator like 2	**1.85**	8.61E-02
slc25a42	solute carrier family 25 member 42	**1.85**	8.81E-02
pmpca	peptidase, mitochondrial processing alpha subunit	**1.85**	5.61E-02

map2k5	mitogen-activated protein kinase kinase 5	**1.85**	8.66E-03
akt1	AKT serine/threonine kinase 1	**1.85**	3.13E-02
znf568	zinc finger protein 568	**1.85**	1.87E-02
uqcr10	ubiquinol-cytochrome c reductase, complex III subunit X	**1.85**	4.58E-02
c21orf2	chromosome 21 open reading frame 2	**1.85**	5.73E-02
tlk2	tousled like kinase 2	**1.85**	7.57E-02
washc3	WASH complex subunit 3	**1.85**	9.55E-02
hacd3	3-hydroxyacyl-CoA dehydratase 3	**1.85**	8.56E-02
taok2	TAO kinase 2	**1.85**	9.63E-02
ppp1r3c	protein phosphatase 1 regulatory subunit 3C	**1.84**	1.54E-02
dym	dymeclin	**1.84**	5.15E-02
ssbp3	single stranded DNA binding protein 3	**1.84**	4.31E-02
otud3	OTU deubiquitinase 3	**1.84**	7.16E-02
gtpbp4	GTP binding protein 4	**1.84**	6.15E-03
ets1	ETS proto-oncogene 1, transcription factor	**1.83**	1.86E-02
gosr1	golgi SNAP receptor complex member 1	**1.83**	3.76E-02
gsk3b	glycogen synthase kinase 3 beta	**1.83**	6.63E-02
syne2	spectrin repeat containing nuclear envelope protein 2	**1.83**	5.36E-02
fam134a	family with sequence similarity 134 member A	**1.83**	5.74E-02
rhot1	ras homolog family member T1	**1.83**	7.54E-02
taf3	TATA-box binding protein associated factor 3	**1.83**	8.52E-02
csnk1a1l	casein kinase 1 alpha 1 like	**1.83**	2.17E-02
bbs4	Bardet-Biedl syndrome 4	**1.83**	5.41E-02
rtf1	RTF1 homolog, Paf1/RNA polymerase II complex component	**1.83**	5.83E-02
adprhl2	ADP-ribosylhydrolase like 2	**1.83**	5.00E-02
krt15	keratin 15	**1.83**	9.82E-02
myl6	myosin light chain 6	**1.83**	6.95E-02
hn1	hematological and neurological expressed 1	**1.83**	7.71E-02
akt3	AKT serine/threonine kinase 3	**1.83**	9.04E-02
arpc5	actin related protein 2/3 complex subunit 5	**1.83**	6.46E-02
ptpn11	protein tyrosine phosphatase, non-receptor type 11	**1.83**	2.76E-02
rabgap1l	RAB GTPase activating protein 1 like	**1.83**	7.32E-02
luc7l	LUC7 like	**1.83**	9.63E-02
ahcyl1	adenosylhomocysteinase like 1	**1.83**	9.65E-02
lrpap1	LDL receptor related protein associated protein 1	**1.83**	3.21E-03
hsd17b14	hydroxysteroid 17-beta dehydrogenase 14	**1.83**	3.29E-02
tgfbr3	transforming growth factor beta receptor 3	**1.82**	7.72E-02
parn	poly(A)-specific ribonuclease	**1.82**	3.63E-02
gorasp1	golgi reassembly stacking protein 1	**1.82**	4.33E-02
rrm1	ribonucleotide reductase catalytic subunit M1	**1.82**	4.77E-02
lrrc58	leucine rich repeat containing 58	**1.82**	4.33E-02
u2af2	U2 small nuclear RNA auxiliary factor 2	**1.82**	6.19E-02
plk4	polo like kinase 4	**1.82**	6.96E-02
tfe3	transcription factor binding to IGHM enhancer 3	**1.82**	9.18E-02
adam17	ADAM metallopeptidase domain 17	**1.82**	3.44E-02
rnft2	ring finger protein, transmembrane 2	**1.82**	3.47E-02
orai1	ORAI calcium release-activated calcium modulator 1	**1.82**	7.95E-02
znf217	zinc finger protein 217	**1.82**	8.63E-02
ctnnd1	catenin delta 1	**1.82**	2.17E-02
mapk6	mitogen-activated protein kinase 6	**1.82**	4.82E-02
sept2	septin 2	**1.82**	3.28E-02
abcb7	ATP binding cassette subfamily B member 7	**1.82**	6.85E-02
mat1a	methionine adenosyltransferase 1A	**1.82**	7.86E-02
znf117	zinc finger protein 117	**1.82**	8.81E-02
kdf1	keratinocyte differentiation factor 1	**1.82**	2.07E-02
zmpste24	zinc metallopeptidase STE24	**1.82**	3.75E-02
igf2r	insulin like growth factor 2 receptor	**1.82**	8.45E-02
c6orf89	chromosome 6 open reading frame 89	**1.82**	8.79E-02
ppp1cb	protein phosphatase 1 catalytic subunit beta	**1.81**	2.75E-02
pafah1b1	platelet activating factor acetylhydrolase 1b regulatory subunit 1	**1.81**	4.03E-02
usp19	ubiquitin specific peptidase 19	**1.81**	9.51E-02
mrpl12	mitochondrial ribosomal protein L12	**1.81**	9.63E-02
sap30l	SAP30 like	**1.81**	4.25E-02
hmbs	hydroxymethylbilane synthase	**1.81**	1.82E-02
aak1	AP2 associated kinase 1	**1.81**	6.00E-02
bsg	basigin (Ok blood group)	**1.81**	4.51E-02
banf1	barrier to autointegration factor 1	**1.81**	2.48E-02
ciao1	cytosolic iron-sulfur assembly component 1	**1.81**	6.68E-02
bet1	Bet1 golgi vesicular membrane trafficking protein	**1.81**	8.55E-02
ube3a	ubiquitin protein ligase E3A	**1.81**	4.37E-02
kpna5	karyopherin subunit alpha 5	**1.81**	7.55E-02
arf5	ADP ribosylation factor 5	**1.81**	1.25E-02
supt5h	SPT5 homolog, DSIF elongation factor subunit	**1.81**	9.28E-02
rab7a	RAB7A, member RAS oncogene family	**1.80**	3.66E-02
ppp2r2d	protein phosphatase 2 regulatory subunit Bdelta	**1.80**	4.52E-02
mycn	v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog	**1.80**	7.52E-02
gpr180	G protein-coupled receptor 180	**1.80**	9.34E-02
brd8	bromodomain containing 8	**1.80**	7.59E-02
manea	mannosidase endo-alpha	**1.80**	9.79E-02
cops8	COP9 signalosome subunit 8	**1.80**	1.37E-02
c2orf69	chromosome 2 open reading frame 69	**1.80**	8.60E-02
armt1	acidic residue methyltransferase 1	**1.80**	5.40E-02
ezh1	enhancer of zeste 1 polycomb repressive complex 2 subunit	**1.80**	5.61E-02
acss2	acyl-CoA synthetase short-chain family member 2	**1.80**	9.27E-02
esyt2	extended synaptotagmin 2	**1.80**	8.16E-02
znf230	zinc finger protein 230	**1.80**	2.20E-02
cdc45	cell division cycle 45	**1.80**	6.85E-02
zscan29	zinc finger and SCAN domain containing 29	**1.80**	7.00E-02
tomm20	translocase of outer mitochondrial membrane 20	**1.80**	9.94E-02
hsd17b12	hydroxysteroid 17-beta dehydrogenase 12	**1.80**	3.42E-02
shoc2	SHOC2, leucine rich repeat scaffold protein	**1.80**	3.56E-02
daglb	diacylglycerol lipase beta	**1.79**	5.60E-02
setd4	SET domain containing 4	**1.79**	1.28E-02
cachd1	cache domain containing 1	**1.79**	2.41E-02
st14	suppression of tumorigenicity 14	**1.79**	9.11E-02
brap	BRCA1 associated protein	**1.79**	9.61E-02
ube2l3	ubiquitin conjugating enzyme E2 L3	**1.79**	8.06E-02
rps6kb1	ribosomal protein S6 kinase B1	**1.79**	8.24E-02
rpn2	ribophorin II	**1.79**	8.39E-02
baz2a	bromodomain adjacent to zinc finger domain 2A	**1.79**	7.95E-02
hsp90b1	heat shock protein 90 beta family member 1	**1.79**	2.41E-02
map1lc3b	microtubule associated protein 1 light chain 3 beta	**1.79**	4.22E-02
arap1	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 1	**1.79**	6.42E-02
pdlim4	PDZ and LIM domain 4	**1.79**	2.75E-02
rabif	RAB interacting factor	**1.79**	2.83E-02
srf	serum response factor	**1.79**	7.55E-02
ctsz	cathepsin Z	**1.79**	6.51E-03
slc25a21	solute carrier family 25 member 21	**1.79**	5.68E-02
tmem55a	transmembrane protein 55A	**1.79**	7.27E-02
agap1	ArfGAP with GTPase domain, ankyrin repeat and PH domain 1	**1.78**	5.13E-02
tmem50a	transmembrane protein 50A	**1.78**	9.66E-02
stom	stomatin	**1.78**	3.86E-02
hras	HRas proto-oncogene, GTPase	**1.78**	7.70E-02
lepr	leptin receptor	**1.78**	8.26E-02
fundc1	FUN14 domain containing 1	**1.78**	6.29E-02
ptar1	protein prenyltransferase alpha subunit repeat containing 1	**1.78**	8.75E-02
tceanc2	transcription elongation factor A N-terminal and central domain containing 2	**1.78**	8.34E-02
trim25	tripartite motif containing 25	**1.78**	5.33E-02
hla-b	major histocompatibility complex, class I, B	**1.78**	7.34E-02
fblim1	filamin binding LIM protein 1	**1.78**	2.61E-02
cltb	clathrin light chain B	**1.78**	8.44E-02
c1orf52	chromosome 1 open reading frame 52	**1.78**	5.49E-02
vapb	VAMP associated protein B and C	**1.78**	6.43E-02
lin9	lin-9 DREAM MuvB core complex component	**1.78**	6.85E-02
arl8a	ADP ribosylation factor like GTPase 8A	**1.77**	6.13E-02
abr	active BCR-related	**1.77**	6.27E-02
cyth1	cytohesin 1	**1.77**	9.51E-02
renbp	renin binding protein	**1.77**	6.28E-02
kcnk6	potassium two pore domain channel subfamily K member 6	**1.77**	7.19E-02
ap4e1	adaptor related protein complex 4 epsilon 1 subunit	**1.77**	6.30E-02
ccna2	cyclin A2	**1.77**	2.73E-02
smarcad1	SWI/SNF-related, matrix-associated actin-dependent regulator of chromatin, subfamily a, containing DEAD/H box 1	**1.77**	3.62E-02
pi4kb	phosphatidylinositol 4-kinase beta	**1.76**	4.63E-02
pde3b	phosphodiesterase 3B	**1.76**	7.00E-02
ahcyl2	adenosylhomocysteinase like 2	**1.76**	9.66E-02
mitd1	microtubule interacting and trafficking domain containing 1	**1.76**	5.13E-02
slc25a36	solute carrier family 25 member 36	**1.76**	6.17E-02
iqgap1	IQ motif containing GTPase activating protein 1	**1.76**	9.66E-02
eif4g2	eukaryotic translation initiation factor 4 gamma 2	**1.76**	5.19E-02
fem1c	fem-1 homolog C	**1.76**	7.90E-02
inpp5k	inositol polyphosphate-5-phosphatase K	**1.76**	7.76E-02

dnajc3	DnaJ heat shock protein family (Hsp40) member C3	**1.76**	4.36E-02
spcs2	signal peptidase complex subunit 2	**1.76**	4.48E-02
padi4	peptidyl arginine deiminase 4	**1.76**	6.89E-02
creb1	cAMP responsive element binding protein 1	**1.76**	8.35E-02
tmem163	transmembrane protein 163	**1.76**	8.55E-02
zbtb11	zinc finger and BTB domain containing 11	**1.76**	8.41E-02
tgfa	transforming growth factor alpha	**1.75**	7.06E-02
morc2	MORC family CW-type zinc finger 2	**1.75**	9.24E-02
gtf2e1	general transcription factor IIE subunit 1	**1.75**	3.83E-02
agpat5	1-acylglycerol-3-phosphate O-acyltransferase 5	**1.75**	2.28E-02
lamtor5	late endosomal/lysosomal adaptor, MAPK and MTOR activator 5	**1.75**	2.88E-02
ipmk	inositol polyphosphate multikinase	**1.75**	4.47E-02
stx16	syntaxin 16	**1.75**	9.08E-02
mlec	malectin	**1.75**	9.31E-02
h2afz	H2A histone family member Z	**1.75**	5.43E-02
cdc123	cell division cycle 123	**1.75**	6.30E-02
bin2	bridging integrator 2	**1.75**	8.08E-02
syncrip	synaptotagmin binding cytoplasmic RNA interacting protein	**1.75**	8.45E-02
txlna	taxilin alpha	**1.75**	7.94E-02
dgcr2	DiGeorge syndrome critical region gene 2	**1.74**	3.84E-02
cpsf2	cleavage and polyadenylation specific factor 2	**1.74**	9.61E-02
mdm4	MDM4, p53 regulator	**1.74**	1.56E-02
c19orf47	chromosome 19 open reading frame 47	**1.74**	7.52E-02
cfl1	cofilin 1	**1.74**	7.60E-02
hn1l	hematological and neurological expressed 1 like	**1.74**	9.87E-02
bcl7b	BCL tumor suppressor 7B	**1.74**	4.63E-02
kat7	lysine acetyltransferase 7	**1.74**	5.18E-02
f11r	F11 receptor	**1.74**	6.17E-02
washc1	WASH complex subunit 1	**1.74**	8.25E-02
mybpc3	myosin binding protein C, cardiac	**1.74**	7.52E-02
rac1	ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)	**1.74**	9.03E-02
zmat2	zinc finger matrin-type 2	**1.74**	6.58E-02
mcph1	microcephalin 1	**1.74**	1.89E-02
carf	calcium responsive transcription factor	**1.74**	8.05E-02
pdha1	pyruvate dehydrogenase (lipoamide) alpha 1	**1.73**	4.46E-02
nae1	NEDD8 activating enzyme E1 subunit 1	**1.73**	2.28E-02
copa	coatomer protein complex subunit alpha	**1.73**	6.17E-02
ccdc92	coiled-coil domain containing 92	**1.73**	1.87E-02
atp6v1c1	ATPase H+ transporting V1 subunit C1	**1.73**	4.40E-02
lrrc73	leucine rich repeat containing 73	**1.73**	6.70E-02
rbbp4	RB binding protein 4, chromatin remodeling factor	**1.73**	7.43E-02
ammecr1	Alport syndrome, mental retardation, midface hypoplasia and elliptocytosis chromosomal region gene 1	**1.73**	7.76E-02
tmed9	transmembrane p24 trafficking protein 9	**1.73**	9.33E-02
ifih1	interferon induced with helicase C domain 1	**1.73**	9.44E-03
tm2d1	TM2 domain containing 1	**1.73**	6.83E-02
tmub1	transmembrane and ubiquitin like domain containing 1	**1.73**	2.97E-02
efna1	ephrin A1	**1.73**	9.28E-02
p4hb	prolyl 4-hydroxylase subunit beta	**1.73**	9.36E-02
ahcy	adenosylhomocysteinase	**1.73**	9.93E-02
mlx	MLX, MAX dimerization protein	**1.72**	2.46E-02
umad1	UBAP1-MVB12-associated (UMA) domain containing 1	**1.72**	8.05E-02
rap2b	RAP2B, member of RAS oncogene family	**1.72**	9.66E-02
cyba	cytochrome b-245 alpha chain	**1.72**	3.04E-02
yy1	YY1 transcription factor	**1.72**	4.95E-02
rnf114	ring finger protein 114	**1.72**	5.42E-02
znf441	zinc finger protein 441	**1.72**	1.36E-02
magi3	membrane associated guanylate kinase, WW and PDZ domain containing 3	**1.72**	5.08E-02
atf7ip	activating transcription factor 7 interacting protein	**1.72**	7.23E-02
znf226	zinc finger protein 226	**1.72**	9.68E-02
sntb2	syntrophin beta 2	**1.72**	9.89E-02
tmed2	transmembrane p24 trafficking protein 2	**1.72**	6.05E-02
znf658	zinc finger protein 658	**1.72**	6.48E-02
capza1	capping actin protein of muscle Z-line alpha subunit 1	**1.71**	5.11E-02
rdh10	retinol dehydrogenase 10 (all-trans)	**1.71**	6.61E-02
mtm1	myotubularin 1	**1.71**	7.35E-02
rhpn1	rhophilin Rho GTPase binding protein 1	**1.71**	7.24E-02
ska2	spindle and kinetochore associated complex subunit 2	**1.71**	7.17E-03
rtn3	reticulon 3	**1.71**	2.21E-02
socs2	suppressor of cytokine signaling 2	**1.71**	6.46E-02
asxl1	additional sex combs like 1, transcriptional regulator	**1.71**	4.10E-02
ube2m	ubiquitin conjugating enzyme E2 M	**1.71**	6.20E-02
trim21	tripartite motif containing 21	**1.71**	6.83E-02
elovl6	ELOVL fatty acid elongase 6	**1.71**	7.63E-02
slc25a22	solute carrier family 25 member 22	**1.71**	7.87E-02
rab11b	RAB11B, member RAS oncogene family	**1.71**	9.23E-02
nusap1	nucleolar and spindle associated protein 1	**1.71**	4.57E-02
myo6	myosin VI	**1.70**	4.24E-02
fam45a	family with sequence similarity 45 member A	**1.70**	7.82E-02
esrp2	epithelial splicing regulatory protein 2	**1.70**	4.47E-02
sgk1	serum/glucocorticoid regulated kinase 1	**1.70**	7.66E-02
mtap	methylthioadenosine phosphorylase	**1.70**	9.40E-02
gpt2	glutamic--pyruvic transaminase 2	**1.70**	4.16E-02
abcb10	ATP binding cassette subfamily B member 10	**1.70**	7.82E-02
gmfb	glia maturation factor beta	**1.70**	8.84E-02
rala	RAS like proto-oncogene A	**1.70**	6.45E-02
lamtor4	late endosomal/lysosomal adaptor, MAPK and MTOR activator 4	**1.70**	9.23E-02
znf576	zinc finger protein 576	**1.70**	7.33E-02
rbm42	RNA binding motif protein 42	**1.70**	8.72E-02
ift46	intraflagellar transport 46	**1.70**	2.73E-02
dcaf8	DDB1 and CUL4 associated factor 8	**1.70**	6.36E-02
hbs1l	HBS1 like translational GTPase	**1.70**	6.57E-02
cenpk	centromere protein K	**1.69**	3.88E-03
rarres3	retinoic acid receptor responder 3	**1.69**	2.42E-02
nudcd2	NudC domain containing 2	**1.69**	4.74E-02
bcap29	B-cell receptor associated protein 29	**1.69**	6.97E-02
gna13	G protein subunit alpha 13	**1.69**	7.00E-02
sh3bgrl3	SH3 domain binding glutamate rich protein like 3	**1.69**	7.26E-02
tnfrsf14	TNF receptor superfamily member 14	**1.69**	9.20E-02
grb7	growth factor receptor bound protein 7	**1.69**	6.62E-02
nme3	NME/NM23 nucleoside diphosphate kinase 3	**1.68**	7.27E-02
dpf2	double PHD fingers 2	**1.68**	7.55E-02
rnf41	ring finger protein 41	**1.68**	8.19E-02
ssb	Sjogren syndrome antigen B	**1.68**	2.71E-02
frzb	frizzled-related protein	**1.68**	3.96E-02
ccdc6	coiled-coil domain containing 6	**1.68**	5.26E-02
klhl22	kelch like family member 22	**1.68**	7.98E-02
hspa8	heat shock protein family A (Hsp70) member 8	**1.68**	2.90E-02
prune1	prune exopolyphosphatase	**1.68**	9.59E-02
zdhhc7	zinc finger DHHC-type containing 7	**1.68**	5.11E-02
rpl9	ribosomal protein L9	**1.68**	7.64E-02
slmap	sarcolemma associated protein	**1.68**	9.27E-02
stx7	syntaxin 7	**1.67**	8.80E-03
naif1	nuclear apoptosis inducing factor 1	**1.67**	7.49E-02
prelid3b	PRELI domain containing 3B	**1.67**	8.71E-02
elovl1	ELOVL fatty acid elongase 1	**1.67**	7.48E-02
prkra	protein activator of interferon induced protein kinase EIF2AK2	**1.67**	8.53E-02
npepps	aminopeptidase puromycin sensitive	**1.67**	8.08E-02
arid4a	AT-rich interaction domain 4A	**1.67**	4.40E-02
rhbdl2	rhomboid like 2	**1.67**	8.70E-02
clic1	chloride intracellular channel 1	**1.67**	9.95E-02
nmt1	N-myristoyltransferase 1	**1.67**	3.43E-02
id2	inhibitor of DNA binding 2, HLH protein	**1.66**	1.07E-02
tmem64	transmembrane protein 64	**1.66**	3.85E-02
aatf	apoptosis antagonizing transcription factor	**1.66**	5.78E-02
dbnl	drebrin like	**1.66**	9.09E-02
kctd5	potassium channel tetramerization domain containing 5	**1.66**	4.61E-02
slc12a9	solute carrier family 12 member 9	**1.66**	8.77E-02
rnaseh1	ribonuclease H1	**1.66**	9.60E-02
ssfa2	sperm specific antigen 2	**1.66**	2.73E-02
tbc1d15	TBC1 domain family member 15	**1.66**	6.58E-02
galnt2	polypeptide N-acetylgalactosaminyltransferase 2	**1.66**	6.67E-02
gcsh	glycine cleavage system protein H	**1.66**	6.97E-02
tmem107	transmembrane protein 107	**1.66**	7.90E-02
cenpo	centromere protein O	**1.66**	8.97E-02
etaa1	Ewing tumor associated antigen 1	**1.65**	4.33E-02
arpc4	actin related protein 2/3 complex subunit 4	**1.65**	4.69E-02
c12orf29	chromosome 12 open reading frame 29	**1.65**	9.24E-02
thbs4	thrombospondin 4	**1.65**	3.33E-02
vps4b	vacuolar protein sorting 4 homolog B	**1.65**	6.84E-02
ttc13	tetratricopeptide repeat domain 13	**1.65**	9.65E-02
fn1	fibronectin 1	**1.65**	5.64E-02

mthfd2	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase	**1.65**	9.24E-03
taf1a	TATA-box binding protein associated factor, RNA polymerase I subunit A	**1.64**	1.04E-02
pacsin1	protein kinase C and casein kinase substrate in neurons 1	**1.64**	8.54E-02
tdrd6	tudor domain containing 6	**1.64**	8.30E-02
fam46c	family with sequence similarity 46 member C	**1.64**	9.21E-02
selenoh	selenoprotein H	**1.64**	2.34E-02
abi1	abl interactor 1	**1.64**	4.64E-02
picalm	phosphatidylinositol binding clathrin assembly protein	**1.64**	5.75E-02
aktip	AKT interacting protein	**1.64**	6.13E-02
tbc1d9b	TBC1 domain family member 9B	**1.63**	5.09E-02
rps3	ribosomal protein S3	**1.63**	6.06E-02
zcchc10	zinc finger CCHC-type containing 10	**1.63**	5.25E-03
dnajb6	DnaJ heat shock protein family (Hsp40) member B6	**1.63**	6.80E-02
spryd7	SPRY domain containing 7	**1.63**	9.47E-02
tspan1	tetraspanin 1	**1.63**	6.31E-02
cdc34	cell division cycle 34	**1.63**	6.53E-02
znf845	zinc finger protein 845	**1.63**	7.39E-02
glud1	glutamate dehydrogenase 1	**1.63**	4.27E-02
xpo4	exportin 4	**1.63**	9.12E-02
ptbp1	polypyrimidine tract binding protein 1	**1.63**	6.05E-02
mettl6	methyltransferase like 6	**1.62**	2.37E-02
appbp2	amyloid beta precursor protein binding protein 2	**1.62**	4.27E-02
cul9	cullin 9	**1.62**	3.26E-02
ankrd13c	ankyrin repeat domain 13C	**1.62**	5.88E-02
rabl3	RAB, member of RAS oncogene family like 3	**1.62**	5.14E-02
sec61a1	Sec61 translocon alpha 1 subunit	**1.62**	7.74E-02
xrn1	5'-3' exoribonuclease 1	**1.62**	9.67E-02
sfxn3	sideroflexin 3	**1.62**	9.40E-02
cep97	centrosomal protein 97	**1.62**	2.49E-02
calm1	calmodulin 1	**1.62**	4.09E-02
nrm	nurim (nuclear envelope membrane protein)	**1.62**	8.90E-02
gpa33	glycoprotein A33	**1.62**	6.35E-03
pid1	phosphotyrosine interaction domain containing 1	**1.62**	1.01E-02
icmt	isoprenylcysteine carboxyl methyltransferase	**1.62**	7.70E-02
osbpl1a	oxysterol binding protein like 1A	**1.62**	9.10E-02
cactin	cactin, spliceosome C complex subunit	**1.62**	9.53E-02
arfgef1	ADP ribosylation factor guanine nucleotide exchange factor 1	**1.62**	3.92E-02
ccdc50	coiled-coil domain containing 50	**1.62**	5.38E-02
nmnat3	nicotinamide nucleotide adenylyltransferase 3	**1.61**	4.64E-02
tom1	target of myb1 membrane trafficking protein	**1.61**	5.08E-02
rac2	ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)	**1.61**	6.25E-02
taf13	TATA-box binding protein associated factor 13	**1.61**	1.99E-02
cdc42ep4	CDC42 effector protein 4	**1.61**	5.20E-02
ube2v1	ubiquitin conjugating enzyme E2 V1	**1.61**	5.60E-02
dffb	DNA fragmentation factor subunit beta	**1.61**	5.18E-02
uqcrc1	ubiquinol-cytochrome c reductase core protein I	**1.61**	8.35E-02
cables2	Cdk5 and Abl enzyme substrate 2	**1.61**	9.93E-02
cep120	centrosomal protein 120	**1.61**	3.78E-02
eif2a	eukaryotic translation initiation factor 2A	**1.61**	9.79E-02
pbdc1	polysaccharide biosynthesis domain containing 1	**1.61**	9.83E-02
csnk1g2	casein kinase 1 gamma 2	**1.60**	9.12E-03
sdf4	stromal cell derived factor 4	**1.60**	8.51E-02
idh3g	isocitrate dehydrogenase 3 (NAD(+)) gamma	**1.60**	9.07E-02
larp6	La ribonucleoprotein domain family member 6	**1.60**	2.99E-02
scml4	sex comb on midleg-like 4 (Drosophila)	**1.60**	9.70E-02
ptpn21	protein tyrosine phosphatase, non-receptor type 21	**1.59**	8.08E-02
eif2s3	eukaryotic translation initiation factor 2 subunit gamma	**1.59**	2.43E-02
tpcn1	two pore segment channel 1	**1.59**	7.95E-02
mllt3	MLLT3, super elongation complex subunit	**1.58**	7.61E-02
gnai1	G protein subunit alpha i1	**1.58**	4.99E-02
rock2	Rho associated coiled-coil containing protein kinase 2	**1.58**	5.02E-02
rergl	RERG like	**1.58**	3.30E-02
rest	RE1 silencing transcription factor	**1.58**	1.60E-02
nuak2	NUAK family kinase 2	**1.58**	8.90E-02
ap1s2	adaptor related protein complex 1 sigma 2 subunit	**1.57**	9.98E-02
fkbp3	FK506 binding protein 3	**1.57**	7.13E-02
ist1	IST1, ESCRT-III associated factor	**1.57**	8.51E-02
nhp2	NHP2 ribonucleoprotein	**1.57**	7.75E-02
mcm10	minichromosome maintenance 10 replication initiation factor	**1.57**	4.48E-02
myrip	myosin VIIA and Rab interacting protein	**1.57**	8.62E-02
wwtr1	WW domain containing transcription regulator 1	**1.56**	3.20E-02
sesn1	sestrin 1	**1.56**	9.37E-02
slc29a1	solute carrier family 29 member 1 (Augustine blood group)	**1.56**	7.13E-02
etnk2	ethanolamine kinase 2	**1.56**	5.17E-02
lman2l	lectin, mannose binding 2 like	**1.56**	7.68E-02
col1a1	collagen type I alpha 1 chain	**1.56**	3.46E-02
ric8b	RIC8 guanine nucleotide exchange factor B	**1.56**	9.30E-02
gnl1	G protein nucleolar 1 (putative)	**1.56**	9.41E-02
senp7	SUMO1/sentrin specific peptidase 7	**1.56**	5.61E-02
znf143	zinc finger protein 143	**1.56**	8.26E-02
fam219a	family with sequence similarity 219 member A	**1.55**	1.15E-02
bcl7a	BCL tumor suppressor 7A	**1.54**	5.46E-02
fnbp4	formin binding protein 4	**1.54**	5.60E-02
rrm2	ribonucleotide reductase regulatory subunit M2	**1.54**	5.72E-02
higd1a	HIG1 hypoxia inducible domain family member 1A	**1.54**	7.88E-02
elovl7	ELOVL fatty acid elongase 7	**1.54**	9.76E-02
rexo4	REX4 homolog, 3'-5' exonuclease	**1.54**	2.89E-02
clpx	caseinolytic mitochondrial matrix peptidase chaperone subunit	**1.54**	7.41E-02
hsf1	heat shock transcription factor 1	**1.54**	8.97E-02
dkc1	dyskerin pseudouridine synthase 1	**1.53**	4.93E-02
fdx1l	ferredoxin 1 like	**1.53**	6.81E-02
arhgap1	Rho GTPase activating protein 1	**1.53**	2.47E-02
psma6	proteasome subunit alpha 6	**1.53**	5.67E-02
pa2g4	proliferation-associated 2G4	**1.53**	7.03E-02
ehd4	EH domain containing 4	**1.53**	8.11E-02
srm	spermidine synthase	**1.52**	3.94E-02
miga2	mitoguardin 2	**1.52**	5.10E-02
csde1	cold shock domain containing E1	**1.52**	5.46E-02
lrrfip2	leucine rich repeat (in FLII) interacting protein 2	**1.52**	4.32E-02
pip4k2b	phosphatidylinositol-5-phosphate 4-kinase type 2 beta	**1.52**	5.19E-02
kctd15	potassium channel tetramerization domain containing 15	**1.52**	4.48E-02
nemp1	nuclear envelope integral membrane protein 1	**1.52**	3.73E-02
elf1	E74 like ETS transcription factor 1	**1.52**	5.99E-02
grb2	growth factor receptor bound protein 2	**1.52**	7.55E-02
cltc	clathrin heavy chain	**1.51**	4.65E-02
top1	topoisomerase (DNA) I	**1.51**	6.14E-02
znf169	zinc finger protein 169	**1.51**	7.96E-02
hlf	HLF, PAR bZIP transcription factor	**1.51**	2.43E-02
c5orf22	chromosome 5 open reading frame 22	**1.51**	8.65E-02
dolpp1	dolichyldiphosphatase 1	**1.51**	9.34E-02
atp6v1a	ATPase H+ transporting V1 subunit A	**1.51**	3.16E-02
znf350	zinc finger protein 350	**1.51**	9.09E-02
tpm4	tropomyosin 4	**1.50**	4.53E-02
pls3	plastin 3	**1.50**	6.74E-02
cs	citrate synthase	**1.50**	7.68E-02
capn5	calpain 5	**1.50**	6.85E-02

Gene symbol	Gene name (downregulated)	**Fold change**	P-adjusted value
fbxl5	F-box and leucine rich repeat protein 5	**-253.53**	6.59E-53
copz1	coatomer protein complex subunit zeta 1	**-149.40**	2.34E-34
st3gal3	ST3 beta-galactoside alpha-2,3-sialyltransferase 3	**-111.35**	1.85E-29
ccdc125	coiled-coil domain containing 125	**-106.67**	4.53E-34
chaf1a	chromatin assembly factor 1 subunit A	**-53.37**	8.02E-20
rngtt	RNA guanylyltransferase and 5'-phosphatase	**-41.44**	3.16E-16
nsmce2	NSE2/MMS21 homolog, SMC5-SMC6 complex SUMO ligase	**-34.11**	2.78E-15
ing1	inhibitor of growth family member 1	**-28.86**	1.26E-12
sart3	squamous cell carcinoma antigen recognized by T-cells 3	**-26.80**	3.09E-12
npepl1	aminopeptidase-like 1	**-23.93**	1.28E-21
znf205	zinc finger protein 205	**-17.59**	1.22E-08
uqcrfs1	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	**-16.23**	1.29E-08
msto1	misato 1, mitochondrial distribution and morphology regulator	**-15.23**	2.58E-10
nipbl	NIPBL, cohesin loading factor	**-15.20**	1.98E-02
spint2	serine peptidase inhibitor, Kunitz type 2	**-13.10**	4.06E-07
rgs9	regulator of G-protein signaling 9	**-12.82**	1.52E-06
esam	endothelial cell adhesion molecule	**-12.79**	6.29E-12
gtf3c5	general transcription factor IIIC subunit 5	**-10.89**	1.28E-05
iba57	IBA57 homolog, iron-sulfur cluster assembly	**-10.56**	4.30E-06

phb2	prohibitin 2	**-10.37**	1.66E-06
cdca7	cell division cycle associated 7	**-9.86**	4.19E-06
galc	galactosylceramidase	**-9.04**	1.87E-05
pros1	protein S (alpha)	**-8.94**	1.49E-05
hnrnph3	heterogeneous nuclear ribonucleoprotein H3	**-8.66**	1.51E-04
amh	anti-Mullerian hormone	**-7.91**	6.40E-05
inha	inhibin alpha subunit	**-7.89**	2.37E-03
gtpbp10	GTP binding protein 10	**-7.77**	6.63E-05
znf300	zinc finger protein 300	**-7.61**	5.69E-06
ivd	isovaleryl-CoA dehydrogenase	**-7.46**	5.53E-05
pggt1b	protein geranylgeranyltransferase type I subunit beta	**-7.33**	2.27E-04
top2b	topoisomerase (DNA) II beta	**-7.24**	7.17E-04
pdxk	pyridoxal (pyridoxine, vitamin B6) kinase	**-7.22**	2.75E-10
dll1	delta like canonical Notch ligand 1	**-7.22**	7.40E-08
tdg	thymine DNA glycosylase	**-7.15**	3.20E-04
col4a2	collagen type IV alpha 2 chain	**-7.10**	9.10E-07
c8orf76	chromosome 8 open reading frame 76	**-7.05**	9.81E-06
tysnd1	trypsin domain containing 1	**-7.02**	1.54E-05
pgk1	phosphoglycerate kinase 1	**-6.81**	8.02E-04
nek2	NIMA related kinase 2	**-6.71**	2.61E-06
mis18bp1	MIS18 binding protein 1	**-6.42**	5.00E-05
chka	choline kinase alpha	**-6.40**	1.21E-02
opn5	opsin 5	**-6.30**	6.16E-07
chmp4b	charged multivesicular body protein 4B	**-5.95**	3.03E-03
uxs1	UDP-glucuronate decarboxylase 1	**-5.93**	3.04E-03
slc7a11	solute carrier family 7 member 11	**-5.88**	4.62E-02
surf4	surfeit 4	**-5.78**	2.01E-06
rps23	ribosomal protein S23	**-5.76**	1.38E-03
lonp2	lon peptidase 2, peroxisomal	**-5.64**	1.72E-04
nmrk2	nicotinamide riboside kinase 2	**-5.58**	2.55E-06
ube2b	ubiquitin conjugating enzyme E2 B	**-5.57**	3.15E-03
mcoln3	mucolipin 3	**-5.54**	4.62E-03
sdhaf3	succinate dehydrogenase complex assembly factor 3	**-5.51**	5.80E-03
ints7	integrator complex subunit 7	**-5.45**	2.16E-03
helq	helicase, POLQ-like	**-5.41**	2.51E-03
rpl18a	ribosomal protein L18a	**-5.39**	4.91E-03
mrpl40	mitochondrial ribosomal protein L40	**-5.39**	1.09E-06
gpat4	glycerol-3-phosphate acyltransferase 4	**-5.36**	1.16E-03
atp23	ATP23 metallopeptidase and ATP synthase assembly factor homolog	**-5.30**	1.50E-03
plekhm1	pleckstrin homology and RUN domain containing M1	**-5.30**	2.23E-03
avil	advillin	**-5.26**	7.23E-03
aggf1	angiogenic factor with G-patch and FHA domains 1	**-5.24**	5.85E-03
dab2	DAB2, clathrin adaptor protein	**-5.14**	3.18E-02
slc9a1	solute carrier family 9 member A1	**-5.13**	8.99E-03
mettl13	methyltransferase like 13	**-5.11**	4.27E-03
sppl2b	signal peptide peptidase like 2B	**-5.10**	3.61E-08
ints9	integrator complex subunit 9	**-5.07**	1.82E-03
gemin6	gem nuclear organelle associated protein 6	**-5.05**	2.23E-03
hps3	HPS3, biogenesis of lysosomal organelles complex 2 subunit 1	**-5.03**	2.10E-03
akr7a2	aldo-keto reductase family 7 member A2	**-4.95**	1.34E-05
zdhhc11	zinc finger DHHC-type containing 11	**-4.86**	6.32E-03
tf	transferrin	**-4.85**	1.44E-04
nudc	nuclear distribution C, dynein complex regulator	**-4.82**	5.29E-04
fhl2	four and a half LIM domains 2	**-4.81**	6.03E-03
snf8	SNF8, ESCRT-II complex subunit	**-4.80**	1.59E-03
hsd3b2	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2	**-4.74**	3.35E-04
itgbl1	integrin subunit beta like 1	**-4.73**	6.28E-03
pik3c3	phosphatidylinositol 3-kinase catalytic subunit type 3	**-4.73**	4.06E-04
hist2h3a	histone cluster 2 H3 family member a	**-4.72**	6.38E-05
ift20	intraflagellar transport 20	**-4.69**	1.69E-03
txn2	thioredoxin 2	**-4.63**	2.38E-03
scfd1	sec1 family domain containing 1	**-4.63**	2.56E-03
mpped2	metallophosphoesterase domain containing 2	**-4.63**	4.73E-03
a3galt2	alpha 1,3-galactosyltransferase 2	**-4.63**	1.09E-02
ptges2	prostaglandin E synthase 2	**-4.63**	1.41E-02
abca1	ATP binding cassette subfamily A member 1	**-4.62**	1.50E-02
camkmt	calmodulin-lysine N-methyltransferase	**-4.59**	1.72E-03
atp1b1	ATPase Na+/K+ transporting subunit beta 1	**-4.53**	4.25E-03
rab1b	RAB1B, member RAS oncogene family	**-4.49**	7.78E-07
arpp19	cAMP regulated phosphoprotein 19	**-4.49**	1.18E-05
atg4b	autophagy related 4B cysteine peptidase	**-4.45**	6.20E-03
dnttip2	deoxynucleotidyltransferase terminal interacting protein 2	**-4.44**	9.81E-03
myc	v-myc avian myelocytomatosis viral oncogene homolog	**-4.43**	8.81E-04
med8	mediator complex subunit 8	**-4.43**	1.98E-02
sri	sorcin	**-4.41**	5.06E-03
chst10	carbohydrate sulfotransferase 10	**-4.40**	2.22E-03
washc5	WASH complex subunit 5	**-4.37**	1.51E-03
timm23	translocase of inner mitochondrial membrane 23	**-4.35**	2.41E-03
fcf1	FCF1 rRNA-processing protein	**-4.32**	3.16E-05
c3orf33	chromosome 3 open reading frame 33	**-4.31**	1.70E-02
pars2	prolyl-tRNA synthetase 2, mitochondrial (putative)	**-4.31**	8.35E-03
nop14	NOP14 nucleolar protein	**-4.30**	1.93E-02
psd2	pleckstrin and Sec7 domain containing 2	**-4.29**	3.12E-03
pdlim1	PDZ and LIM domain 1	**-4.29**	1.96E-02
slc24a2	solute carrier family 24 member 2	**-4.28**	1.32E-02
ccnd2	cyclin D2	**-4.28**	2.22E-02
ncapd2	non-SMC condensin I complex subunit D2	**-4.25**	6.57E-04
rnps1	RNA binding protein with serine rich domain 1	**-4.25**	9.83E-03
ap1m2	adaptor related protein complex 1 mu 2 subunit	**-4.20**	2.48E-02
dhrs3	dehydrogenase/reductase 3	**-4.20**	2.28E-02
ssx2ip	SSX family member 2 interacting protein	**-4.19**	3.08E-02
cdc14a	cell division cycle 14A	**-4.15**	5.02E-03
wasf3	WAS protein family member 3	**-4.15**	1.17E-02
angel1	angel homolog 1	**-4.10**	2.79E-02
ftsj3	FtsJ homolog 3	**-4.10**	8.55E-03
tmem198	transmembrane protein 198	**-4.07**	2.54E-02
dhrs7b	dehydrogenase/reductase 7B	**-4.06**	2.49E-02
acot11	acyl-CoA thioesterase 11	**-4.06**	1.40E-03
lrp1	LDL receptor related protein 1	**-4.03**	5.26E-03
ggt7	gamma-glutamyltransferase 7	**-3.99**	1.74E-02
ercc2	ERCC excision repair 2, TFIIH core complex helicase subunit	**-3.98**	7.60E-03
slc25a3	solute carrier family 25 member 3	**-3.98**	9.44E-03
ddx23	DEAD-box helicase 23	**-3.97**	1.13E-03
rev3l	REV3 like, DNA directed polymerase zeta catalytic subunit	**-3.97**	2.63E-02
wars	tryptophanyl-tRNA synthetase	**-3.96**	1.77E-02
dcun1d3	defective in cullin neddylation 1 domain containing 3	**-3.92**	1.29E-03
wdr24	WD repeat domain 24	**-3.90**	1.74E-02
slc38a8	solute carrier family 38 member 8	**-3.90**	3.00E-02
xpc	XPC complex subunit, DNA damage recognition and repair factor	**-3.88**	8.86E-03
taf6l	TATA-box binding protein associated factor 6 like	**-3.86**	6.35E-03
sf3b5	splicing factor 3b subunit 5	**-3.85**	8.88E-04
ttyh1	tweety family member 1	**-3.84**	1.21E-08
ube2z	ubiquitin conjugating enzyme E2 Z	**-3.82**	3.58E-02
dynll2	dynein light chain LC8-type 2	**-3.82**	2.75E-02
timm23b	translocase of inner mitochondrial membrane 23 homolog B	**-3.78**	2.92E-02
patz1	POZ/BTB and AT hook containing zinc finger 1	**-3.77**	5.13E-04
katnbl1	katanin regulatory subunit B1 like 1	**-3.74**	4.38E-03
smc5	structural maintenance of chromosomes 5	**-3.74**	1.27E-02
slbp	stem-loop binding protein	**-3.72**	4.04E-02
nxpe3	neurexophilin and PC-esterase domain family member 3	**-3.71**	2.59E-02
brf1	BRF1, RNA polymerase III transcription initiation factor 90 kDa subunit	**-3.70**	1.12E-03
asnsd1	asparagine synthetase domain containing 1	**-3.70**	1.43E-02
cd302	CD302 molecule	**-3.69**	6.71E-03
fam19a2	family with sequence similarity 19 member A2, C-C motif chemokine like	**-3.69**	1.89E-02
il1rl1	interleukin 1 receptor like 1	**-3.69**	4.21E-02
coq9	coenzyme Q9	**-3.68**	2.47E-02
allc	allantoicase	**-3.67**	2.08E-02
ogfod3	2-oxoglutarate and iron dependent oxygenase domain containing 3	**-3.66**	3.78E-02
sssca1	Sjogren syndrome/scleroderma autoantigen 1	**-3.66**	8.54E-03
wdr3	WD repeat domain 3	**-3.65**	4.47E-03
trim2	tripartite motif containing 2	**-3.65**	1.11E-02
bhlhe40	basic helix-loop-helix family member e40	**-3.65**	1.54E-02
mtpn	myotrophin	**-3.64**	2.70E-02
blcap	bladder cancer associated protein	**-3.62**	6.73E-03
galnt6	polypeptide N-acetylgalactosaminyltransferase 6	**-3.62**	2.22E-02
timm10b	translocase of inner mitochondrial membrane 10B	**-3.62**	4.46E-02
ccndbp1	cyclin D1 binding protein 1	**-3.62**	1.16E-02
mon1a	MON1 homolog A, secretory trafficking associated	**-3.61**	4.00E-03

tmem41a	transmembrane protein 41A	**-3.59**	2.33E-03
mettl3	methyltransferase like 3	**-3.59**	1.41E-02
cbln1	cerebellin 1 precursor	**-3.57**	4.49E-02
med23	mediator complex subunit 23	**-3.55**	2.97E-02
usmg5	up-regulated during skeletal muscle growth 5 homolog (mouse)	**-3.55**	4.76E-02
aadacl4	arylacetamide deacetylase like 4	**-3.55**	7.70E-03
anxa2	annexin A2	**-3.54**	4.21E-03
st3gal4	ST3 beta-galactoside alpha-2,3-sialyltransferase 4	**-3.53**	6.68E-05
atp1a1	ATPase Na+/K+ transporting subunit alpha 1	**-3.53**	2.82E-02
cyfip2	cytoplasmic FMR1 interacting protein 2	**-3.52**	5.55E-03
otx1	orthodenticle homeobox 1	**-3.52**	2.34E-02
aplnr	apelin receptor	**-3.52**	8.08E-04
stab2	stabilin 2	**-3.52**	5.97E-03
llph	LLP homolog, long-term synaptic facilitation	**-3.52**	2.36E-02
lamb1	laminin subunit beta 1	**-3.51**	2.13E-02
ccdc93	coiled-coil domain containing 93	**-3.51**	3.88E-02
crym	crystallin mu	**-3.50**	2.20E-02
tacc1	transforming acidic coiled-coil containing protein 1	**-3.49**	2.02E-02
nek1	NIMA related kinase 1	**-3.49**	3.29E-02
zbtb18	zinc finger and BTB domain containing 18	**-3.49**	8.66E-03
gsap	gamma-secretase activating protein	**-3.48**	4.74E-02
b4galt4	beta-1,4-galactosyltransferase 4	**-3.48**	4.78E-02
herc1	HECT and RLD domain containing E3 ubiquitin protein ligase family member 1	**-3.46**	1.11E-02
napa	NSF attachment protein alpha	**-3.46**	8.97E-03
knl1	kinetochore scaffold 1	**-3.46**	1.62E-04
nelfa	negative elongation factor complex member A	**-3.45**	4.81E-02
clgn	calmegin	**-3.44**	2.69E-02
rnf103	ring finger protein 103	**-3.44**	4.21E-03
rnf10	ring finger protein 10	**-3.44**	4.88E-02
slc39a3	solute carrier family 39 member 3	**-3.42**	3.79E-02
tmem43	transmembrane protein 43	**-3.41**	1.09E-02
myef2	myelin expression factor 2	**-3.41**	6.03E-03
cyp19a1	cytochrome P450 family 19 subfamily A member 1	**-3.40**	1.91E-02
st5	suppression of tumorigenicity 5	**-3.40**	3.60E-02
pomt2	protein O-mannosyltransferase 2	**-3.37**	2.04E-02
setdb1	SET domain bifurcated 1	**-3.37**	5.48E-02
cd2ap	CD2 associated protein	**-3.36**	2.63E-03
espl1	extra spindle pole bodies like 1, separase	**-3.36**	1.42E-02
slc6a2	solute carrier family 6 member 2	**-3.35**	3.60E-02
arl4d	ADP ribosylation factor like GTPase 4D	**-3.34**	7.41E-03
c1orf198	chromosome 1 open reading frame 198	**-3.34**	1.91E-02
slc9a7	solute carrier family 9 member A7	**-3.33**	2.89E-03
wdr83os	WD repeat domain 83 opposite strand	**-3.32**	2.90E-02
ddx41	DEAD-box helicase 41	**-3.32**	4.79E-03
epc2	enhancer of polycomb homolog 2	**-3.32**	3.24E-03
dpp3	dipeptidyl peptidase 3	**-3.31**	1.38E-02
misp	mitotic spindle positioning	**-3.31**	3.63E-02
bmi1	BMI1 proto-oncogene, polycomb ring finger	**-3.31**	2.21E-02
rps27l	ribosomal protein S27 like	**-3.31**	3.63E-02
htra2	HtrA serine peptidase 2	**-3.31**	4.43E-02
slc25a38	solute carrier family 25 member 38	**-3.30**	1.67E-02
fam198b	family with sequence similarity 198 member B	**-3.29**	5.63E-02
gdf3	growth differentiation factor 3	**-3.29**	5.73E-02
aqr	aquarius intron-binding spliceosomal factor	**-3.29**	3.19E-02
ndufs2	NADH:ubiquinone oxidoreductase core subunit S2	**-3.28**	6.35E-02
fam109a	family with sequence similarity 109 member A	**-3.28**	6.22E-02
sox3	SRY-box 3	**-3.27**	2.38E-03
slc20a1	solute carrier family 20 member 1	**-3.27**	5.26E-03
gart	phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase	**-3.27**	2.07E-02
arhgap23	Rho GTPase activating protein 23	**-3.27**	2.92E-02
atp13a2	ATPase 13A2	**-3.27**	2.35E-02
rexo1	RNA exonuclease 1 homolog	**-3.26**	1.38E-02
sephs1	selenophosphate synthetase 1	**-3.25**	5.26E-02
cited2	Cbp/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 2	**-3.25**	2.90E-02
snrpg	small nuclear ribonucleoprotein polypeptide G	**-3.24**	5.91E-02
thbs3	thrombospondin 3	**-3.23**	3.33E-03
zbtb12	zinc finger and BTB domain containing 12	**-3.22**	1.93E-02
chchd3	coiled-coil-helix-coiled-coil-helix domain containing 3	**-3.22**	2.96E-02
rps9	ribosomal protein S9	**-3.22**	3.18E-02
tmem204	transmembrane protein 204	**-3.21**	3.01E-02
tardbp	TAR DNA binding protein	**-3.21**	6.39E-02
ada	adenosine deaminase	**-3.20**	3.70E-02
birc5	baculoviral IAP repeat containing 5	**-3.20**	6.05E-02
tle1	transducin like enhancer of split 1	**-3.19**	1.49E-02
cadm4	cell adhesion molecule 4	**-3.19**	2.46E-02
eif4g3	eukaryotic translation initiation factor 4 gamma 3	**-3.19**	6.15E-03
mrpl44	mitochondrial ribosomal protein L44	**-3.19**	4.96E-03
uso1	USO1 vesicle transport factor	**-3.18**	2.33E-03
tp53bp1	tumor protein p53 binding protein 1	**-3.18**	1.96E-02
ggct	gamma-glutamylcyclotransferase	**-3.18**	2.23E-02
narf	nuclear prelamin A recognition factor	**-3.18**	5.13E-02
qtrt2	queuine tRNA-ribosyltransferase accessory subunit 2	**-3.18**	2.88E-02
atxn7	ataxin 7	**-3.18**	8.02E-03
stxbp1	syntaxin binding protein 1	**-3.18**	3.19E-02
ing2	inhibitor of growth family member 2	**-3.17**	9.80E-04
fam172a	family with sequence similarity 172 member A	**-3.17**	1.93E-02
mepce	methylphosphate capping enzyme	**-3.17**	1.69E-03
serpine1	serpin family E member 1	**-3.17**	3.52E-07
pknox1	PBX/knotted 1 homeobox 1	**-3.16**	2.58E-02
ercc4	ERCC excision repair 4, endonuclease catalytic subunit	**-3.16**	4.14E-03
zfhx3	zinc finger homeobox 3	**-3.16**	8.19E-03
plin2	perilipin 2	**-3.16**	5.94E-02
ppp2r3b	protein phosphatase 2 regulatory subunit B"beta	**-3.15**	3.22E-02
fam131a	family with sequence similarity 131 member A	**-3.15**	6.63E-02
tbx15	T-box 15	**-3.15**	7.82E-03
cox10	COX10, heme A:farnesyltransferase cytochrome c oxidase assembly factor	**-3.14**	6.93E-02
lig3	DNA ligase 3	**-3.13**	6.49E-03
eif3d	eukaryotic translation initiation factor 3 subunit D	**-3.12**	7.03E-02
cyb561	cytochrome b561	**-3.12**	2.05E-04
fcho1	FCH domain only 1	**-3.12**	4.12E-02
irf1	interferon regulatory factor 1	**-3.11**	5.53E-02
ptp4a2	protein tyrosine phosphatase type IVA, member 2	**-3.10**	7.63E-02
kcmf1	potassium channel modulatory factor 1	**-3.09**	5.23E-02
itgb1bp1	integrin subunit beta 1 binding protein 1	**-3.09**	2.38E-02
kctd10	potassium channel tetramerization domain containing 10	**-3.09**	5.78E-02
rad21	RAD21 cohesin complex component	**-3.07**	3.23E-03
prpf6	pre-mRNA processing factor 6	**-3.07**	1.35E-02
asb11	ankyrin repeat and SOCS box containing 11	**-3.07**	6.78E-02
ccdc12	coiled-coil domain containing 12	**-3.06**	3.77E-02
pemt	phosphatidylethanolamine N-methyltransferase	**-3.06**	7.11E-02
nup43	nucleoporin 43	**-3.06**	4.20E-02
atrx	ATRX, chromatin remodeler	**-3.06**	4.84E-02
gmeb1	glucocorticoid modulatory element binding protein 1	**-3.05**	3.50E-02
ccdc24	coiled-coil domain containing 24	**-3.05**	7.94E-02
sun2	Sad1 and UNC84 domain containing 2	**-3.05**	6.58E-02
txndc5	thioredoxin domain containing 5	**-3.04**	4.38E-02
fen1	flap structure-specific endonuclease 1	**-3.04**	6.03E-02
cep295	centrosomal protein 295	**-3.03**	1.21E-02
ankrd52	ankyrin repeat domain 52	**-3.03**	3.80E-02
dpp9	dipeptidyl peptidase 9	**-3.03**	2.43E-02
tpi1	triosephosphate isomerase 1	**-3.02**	5.46E-02
col4a5	collagen type IV alpha 5 chain	**-3.02**	2.62E-04
eif4a2	eukaryotic translation initiation factor 4A2	**-3.01**	3.42E-02
tmem200b	transmembrane protein 200B	**-3.01**	7.68E-02
vps50	VPS50, EARP/GARPII complex subunit	**-3.01**	4.22E-02
a2m	alpha-2-macroglobulin	**-3.00**	3.66E-02
plcl2	phospholipase C like 2	**-3.00**	3.85E-02
ppp2r5c	protein phosphatase 2 regulatory subunit B'gamma	**-2.99**	2.37E-02
samd4a	sterile alpha motif domain containing 4A	**-2.98**	2.38E-02
gnai3	G protein subunit alpha i3	**-2.98**	8.63E-02
ube2k	ubiquitin conjugating enzyme E2 K	**-2.97**	2.50E-03
rps13	ribosomal protein S13	**-2.97**	4.58E-02
lmo4	LIM domain only 4	**-2.97**	6.39E-02
reep6	receptor accessory protein 6	**-2.97**	1.96E-02
znf574	zinc finger protein 574	**-2.97**	1.98E-02
foxo3	forkhead box O3	**-2.95**	1.84E-02
mlh3	mutL homolog 3	**-2.95**	1.62E-02
selenos	selenoprotein S	**-2.94**	1.70E-02
cndp2	CNDP dipeptidase 2 (metallopeptidase M20 family)	**-2.94**	3.18E-03
mthfd1	methylenetetrahydrofolate dehydrogenase, cyclohydrolase and formyltetrahydrofolate synthetase 1	**-2.94**	1.03E-02
ulk4	unc-51 like kinase 4	**-2.93**	2.61E-02
ttll12	tubulin tyrosine ligase like 12	**-2.93**	2.64E-02
cnksr1	connector enhancer of kinase suppressor of Ras 1	**-2.93**	4.63E-02

rida	reactive intermediate imine deaminase A homolog	**-2.93**	8.91E-02
ttc25	tetratricopeptide repeat domain 25	**-2.93**	1.82E-02
fat1	FAT atypical cadherin 1	**-2.93**	2.30E-02
grpel1	GrpE like 1, mitochondrial	**-2.93**	6.05E-02
gtf2a1l	general transcription factor IIA subunit 1 like	**-2.93**	8.95E-02
ogt	O-linked N-acetylglucosamine (GlcNAc) transferase	**-2.93**	4.27E-03
sbds	SBDS ribosome assembly guanine nucleotide exchange factor	**-2.92**	1.09E-02
alkbh8	alkB homolog 8, tRNA methyltransferase	**-2.92**	3.21E-02
zc4h2	zinc finger C4H2-type containing	**-2.91**	9.32E-02
rfx7	regulatory factor X7	**-2.91**	5.87E-02
ppic	peptidylprolyl isomerase C	**-2.91**	9.23E-02
pkig	protein kinase (cAMP-dependent, catalytic) inhibitor gamma	**-2.91**	2.53E-02
zzef1	zinc finger ZZ-type and EF-hand domain containing 1	**-2.91**	6.22E-02
pon2	paraoxonase 2	**-2.91**	7.95E-02
afdn	afadin, adherens junction formation factor	**-2.90**	1.72E-02
fam129a	family with sequence similarity 129 member A	**-2.90**	8.12E-02
tial1	TIA1 cytotoxic granule associated RNA binding protein like 1	**-2.89**	7.66E-02
sec22b	SEC22 homolog B, vesicle trafficking protein (gene/pseudogene)	**-2.89**	3.26E-02
p3h2	prolyl 3-hydroxylase 2	**-2.89**	9.51E-02
b4galt1	beta-1,4-galactosyltransferase 1	**-2.88**	3.50E-02
mnat1	MNAT1, CDK activating kinase assembly factor	**-2.88**	7.20E-02
jmjd1c	jumonji domain containing 1C	**-2.88**	2.28E-02
med16	mediator complex subunit 16	**-2.88**	8.91E-02
cenpj	centromere protein J	**-2.88**	3.56E-02
znf131	zinc finger protein 131	**-2.88**	4.88E-02
sat1	spermidine/spermine N1-acetyltransferase 1	**-2.87**	2.12E-02
herc3	HECT and RLD domain containing E3 ubiquitin protein ligase 3	**-2.87**	7.62E-02
acad11	acyl-CoA dehydrogenase family member 11	**-2.87**	5.84E-02
olfml2b	olfactomedin like 2B	**-2.86**	7.11E-02
phgdh	phosphoglycerate dehydrogenase	**-2.85**	2.83E-02
ankar	ankyrin and armadillo repeat containing	**-2.85**	2.47E-02
smpd1	sphingomyelin phosphodiesterase 1	**-2.85**	5.33E-02
zzz3	zinc finger ZZ-type containing 3	**-2.84**	1.71E-02
gsg1l	GSG1 like	**-2.83**	5.18E-02
prmt5	protein arginine methyltransferase 5	**-2.83**	9.37E-02
brwd1	bromodomain and WD repeat domain containing 1	**-2.82**	3.86E-02
tceb2	transcription elongation factor B (SIII), polypeptide 2	**-2.82**	2.02E-02
epcam	epithelial cell adhesion molecule	**-2.82**	9.28E-02
dennd2d	DENN domain containing 2D	**-2.81**	9.07E-03
pdcd11	programmed cell death 11	**-2.81**	9.10E-03
aadat	aminoadipate aminotransferase	**-2.81**	4.34E-02
g0s2	G0/G1 switch 2	**-2.81**	5.05E-02
dact3	dishevelled binding antagonist of beta catenin 3	**-2.81**	2.81E-03
dennd6b	DENN domain containing 6B	**-2.81**	7.92E-02
cdk8	cyclin dependent kinase 8	**-2.81**	4.12E-02
tnr	tenascin R	**-2.80**	1.20E-02
scrn3	secernin 3	**-2.80**	4.69E-02
osbpl10	oxysterol binding protein like 10	**-2.80**	6.08E-02
fam193a	family with sequence similarity 193 member A	**-2.80**	6.99E-02
fau	FAU, ubiquitin like and ribosomal protein S30 fusion	**-2.80**	1.07E-03
c4a/c4b	complement C4B (Chido blood group)	**-2.80**	7.64E-02
clspn	claspin	**-2.80**	3.70E-02
dgat2	diacylglycerol O-acyltransferase 2	**-2.79**	2.61E-02
fnta	farnesyltransferase, CAAX box, alpha	**-2.79**	2.16E-02
plod1	procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	**-2.79**	5.15E-02
klf11	Kruppel like factor 11	**-2.79**	5.54E-02
atr	ATR serine/threonine kinase	**-2.79**	3.61E-02
sorl1	sortilin related receptor 1	**-2.78**	1.57E-02
lgals9b	galectin 9B	**-2.78**	9.90E-02
farp1	FERM, ARH/RhoGEF and pleckstrin domain protein 1	**-2.78**	7.45E-02
rps7	ribosomal protein S7	**-2.78**	7.62E-02
rnf38	ring finger protein 38	**-2.78**	2.21E-02
pdk2	pyruvate dehydrogenase kinase 2	**-2.77**	3.65E-02
pdia2	protein disulfide isomerase family A member 2	**-2.76**	2.02E-02
fam110b	family with sequence similarity 110 member B	**-2.76**	3.47E-02
prpf31	pre-mRNA processing factor 31	**-2.75**	3.67E-02
parp12	poly(ADP-ribose) polymerase family member 12	**-2.75**	8.34E-02
tmcc1	transmembrane and coiled-coil domain family 1	**-2.75**	4.70E-02
vezf1	vascular endothelial zinc finger 1	**-2.75**	5.12E-02
ttc4	tetratricopeptide repeat domain 4	**-2.75**	7.90E-02
ehmt1	euchromatic histone lysine methyltransferase 1	**-2.74**	1.52E-03
rbm26	RNA binding motif protein 26	**-2.74**	1.86E-02
wdr83	WD repeat domain 83	**-2.74**	2.01E-04
spg11	spastic paraplegia 11 (autosomal recessive)	**-2.74**	3.63E-02
scap	SREBF chaperone	**-2.74**	3.98E-04
ilvbl	ilvB acetolactate synthase like	**-2.73**	3.73E-02
paqr8	progestin and adipoQ receptor family member 8	**-2.73**	3.83E-02
acin1	apoptotic chromatin condensation inducer 1	**-2.73**	5.01E-02
ormdl2	ORMDL sphingolipid biosynthesis regulator 2	**-2.73**	7.27E-02
dcun1d5	defective in cullin neddylation 1 domain containing 5	**-2.72**	3.44E-02
arhgap25	Rho GTPase activating protein 25	**-2.72**	2.90E-02
alx4	ALX homeobox 4	**-2.72**	8.07E-02
dnajc13	DnaJ heat shock protein family (Hsp40) member C13	**-2.71**	5.13E-02
ttc33	tetratricopeptide repeat domain 33	**-2.71**	6.64E-02
tti1	TELO2 interacting protein 1	**-2.71**	7.33E-02
sgk494	uncharacterized serine/threonine-protein kinase SgK494	**-2.71**	2.63E-02
akap1	A-kinase anchoring protein 1	**-2.71**	4.39E-02
ttc28	tetratricopeptide repeat domain 28	**-2.71**	1.30E-02
timp2	TIMP metallopeptidase inhibitor 2	**-2.70**	7.13E-02
med26	mediator complex subunit 26	**-2.70**	4.16E-02
atp1a3	ATPase Na+/K+ transporting subunit alpha 3	**-2.70**	8.04E-02
mycbp2	MYC binding protein 2, E3 ubiquitin protein ligase	**-2.70**	5.51E-02
dopey2	dopey family member 2	**-2.70**	6.14E-02
rpl35	ribosomal protein L35	**-2.69**	1.53E-02
mrm1	mitochondrial rRNA methyltransferase 1	**-2.69**	5.53E-02
pxk	PX domain containing serine/threonine kinase like	**-2.69**	5.18E-02
exosc2	exosome component 2	**-2.68**	3.06E-02
dnmt1	DNA methyltransferase 1	**-2.68**	3.51E-02
ifrd2	interferon related developmental regulator 2	**-2.68**	5.93E-02
hectd4	HECT domain E3 ubiquitin protein ligase 4	**-2.68**	3.83E-02
fam53b	family with sequence similarity 53 member B	**-2.68**	5.14E-02
fam13a	family with sequence similarity 13 member A	**-2.68**	4.78E-03
sh3pxd2b	SH3 and PX domains 2B	**-2.68**	6.26E-02
pdhx	pyruvate dehydrogenase complex component X	**-2.67**	5.13E-02
ccdc158	coiled-coil domain containing 158	**-2.67**	7.80E-02
iah1	isoamyl acetate-hydrolyzing esterase 1 homolog	**-2.67**	9.81E-02
zbtb20	zinc finger and BTB domain containing 20	**-2.67**	2.54E-02
pigm	phosphatidylinositol glycan anchor biosynthesis class M	**-2.67**	1.16E-04
spice1	spindle and centriole associated protein 1	**-2.66**	2.09E-02
rps20	ribosomal protein S20	**-2.66**	8.25E-02
tram2	translocation associated membrane protein 2	**-2.66**	2.09E-02
rbmx	RNA binding motif protein, X-linked	**-2.66**	7.88E-02
wnt3	Wnt family member 3	**-2.66**	6.08E-04
znf835	zinc finger protein 835	**-2.66**	1.01E-03
serpinb6	serpin family B member 6	**-2.66**	5.99E-02
zpr1	ZPR1 zinc finger	**-2.66**	7.44E-03
med21	mediator complex subunit 21	**-2.65**	2.47E-02
wipi1	WD repeat domain, phosphoinositide interacting 1	**-2.65**	6.52E-02
safb	scaffold attachment factor B	**-2.65**	3.75E-02
apob	apolipoprotein B	**-2.65**	3.99E-02
znf470	zinc finger protein 470	**-2.65**	9.65E-02
vwf	von Willebrand factor	**-2.65**	6.24E-03
hnmt	histamine N-methyltransferase	**-2.64**	2.83E-03
agtrap	angiotensin II receptor associated protein	**-2.64**	7.30E-02
igsf3	immunoglobulin superfamily member 3	**-2.64**	2.17E-02
rrp9	ribosomal RNA processing 9, small subunit (SSU) processome component, homolog (yeast)	**-2.64**	5.08E-02
nckap1	NCK associated protein 1	**-2.64**	2.17E-02
nup37	nucleoporin 37	**-2.64**	5.93E-02
il13ra2	interleukin 13 receptor subunit alpha 2	**-2.64**	8.45E-02
sash1	SAM and SH3 domain containing 1	**-2.64**	7.32E-02
scrib	scribbled planar cell polarity protein	**-2.63**	4.95E-02
tpst1	tyrosylprotein sulfotransferase 1	**-2.63**	4.39E-02
rnf44	ring finger protein 44	**-2.63**	4.41E-02
cep68	centrosomal protein 68	**-2.62**	4.69E-02
herc4	HECT and RLD domain containing E3 ubiquitin protein ligase 4	**-2.62**	5.39E-03
atf3	activating transcription factor 3	**-2.62**	3.18E-02
cisd2	CDGSH iron sulfur domain 2	**-2.62**	8.71E-02
fam57b	family with sequence similarity 57 member B	**-2.62**	1.57E-02
kbtbd2	kelch repeat and BTB domain containing 2	**-2.62**	2.22E-02
cenpl	centromere protein L	**-2.61**	1.56E-02
cers5	ceramide synthase 5	**-2.61**	3.85E-02

dynlrb2	dynein light chain roadblock-type 2	**-2.61**	6.77E-02
apool	apolipoprotein O like	**-2.61**	7.00E-02
nr4a1	nuclear receptor subfamily 4 group A member 1	**-2.61**	2.93E-02
ptprk	protein tyrosine phosphatase, receptor type K	**-2.61**	3.07E-02
ccdc82	coiled-coil domain containing 82	**-2.60**	7.96E-02
ptpn22	protein tyrosine phosphatase, non-receptor type 22	**-2.60**	6.49E-02
zfp36l1	ZFP36 ring finger protein like 1	**-2.60**	6.21E-02
acbd4	acyl-CoA binding domain containing 4	**-2.60**	5.22E-02
cherp	calcium homeostasis endoplasmic reticulum protein	**-2.60**	3.06E-02
ube2f	ubiquitin conjugating enzyme E2 F (putative)	**-2.60**	8.49E-02
scamp4	secretory carrier membrane protein 4	**-2.59**	5.75E-02
celf2	CUGBP, Elav-like family member 2	**-2.59**	5.07E-02
tgfb3	transforming growth factor beta 3	**-2.58**	1.45E-02
dut	deoxyuridine triphosphatase	**-2.58**	4.69E-02
hspa13	heat shock protein family A (Hsp70) member 13	**-2.58**	6.58E-02
ubash3b	ubiquitin associated and SH3 domain containing B	**-2.58**	6.56E-02
rasa1	RAS p21 protein activator 1	**-2.58**	2.72E-03
agrn	agrin	**-2.57**	9.99E-03
rassf5	Ras association domain family member 5	**-2.57**	7.28E-02
lima1	LIM domain and actin binding 1	**-2.57**	8.10E-02
sowahc	sosondowah ankyrin repeat domain family member C	**-2.57**	9.44E-03
ralgapb	Ral GTPase activating protein non-catalytic beta subunit	**-2.57**	5.35E-02
crh	corticotropin releasing hormone	**-2.57**	9.06E-02
eif4b	eukaryotic translation initiation factor 4B	**-2.57**	5.07E-02
ncor1	nuclear receptor corepressor 1	**-2.56**	3.05E-02
dhdh	dihydrodiol dehydrogenase	**-2.56**	4.00E-02
csnk1a1	casein kinase 1 alpha 1	**-2.56**	3.26E-02
hesx1	HESX homeobox 1	**-2.56**	2.17E-02
guca1a	guanylate cyclase activator 1A	**-2.56**	2.25E-02
gss	glutathione synthetase	**-2.56**	8.90E-02
zdhhc23	zinc finger DHHC-type containing 23	**-2.55**	4.99E-02
dnmt3b	DNA methyltransferase 3 beta	**-2.55**	6.57E-04
robo1	roundabout guidance receptor 1	**-2.55**	2.08E-03
nxf1	nuclear RNA export factor 1	**-2.55**	3.89E-02
poldip2	DNA polymerase delta interacting protein 2	**-2.55**	5.28E-02
dnah17	dynein axonemal heavy chain 17	**-2.55**	6.37E-02
ssuh2	ssu-2 homolog (C. elegans)	**-2.55**	4.46E-02
yap1	Yes associated protein 1	**-2.55**	4.78E-02
slc22a6	solute carrier family 22 member 6	**-2.54**	9.26E-02
adamts2	ADAM metallopeptidase with thrombospondin type 1 motif 2	**-2.54**	1.30E-02
arl9	ADP ribosylation factor like GTPase 9	**-2.54**	2.20E-02
pnpla7	patatin like phospholipase domain containing 7	**-2.54**	3.96E-02
zfyve21	zinc finger FYVE-type containing 21	**-2.54**	3.48E-02
gtf2f2	general transcription factor IIF subunit 2	**-2.54**	5.00E-02
ptpru	protein tyrosine phosphatase, receptor type U	**-2.53**	5.41E-02
cit	citron rho-interacting serine/threonine kinase	**-2.53**	6.03E-02
arhgap32	Rho GTPase activating protein 32	**-2.53**	1.59E-02
mkrn1	makorin ring finger protein 1	**-2.53**	2.45E-02
fes	FES proto-oncogene, tyrosine kinase	**-2.53**	4.77E-02
bub1	BUB1 mitotic checkpoint serine/threonine kinase	**-2.53**	5.71E-02
elf2	E74 like ETS transcription factor 2	**-2.52**	1.01E-02
taok3	TAO kinase 3	**-2.52**	7.00E-02
nrarp	NOTCH-regulated ankyrin repeat protein	**-2.52**	7.25E-02
fkbp4	FK506 binding protein 4	**-2.52**	5.79E-02
cux2	cut like homeobox 2	**-2.52**	5.86E-02
mrs2	MRS2, magnesium transporter	**-2.52**	7.51E-04
rasl11b	RAS like family 11 member B	**-2.52**	3.51E-02
eno1	enolase 1	**-2.52**	3.41E-02
colec11	collectin subfamily member 11	**-2.52**	5.35E-02
chrdl1	chordin like 1	**-2.52**	2.49E-02
lfng	LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase	**-2.52**	3.48E-02
zfc3h1	zinc finger C3H1-type containing	**-2.51**	4.10E-04
sipa1l2	signal induced proliferation associated 1 like 2	**-2.51**	5.45E-02
axin2	axin 2	**-2.51**	2.24E-03
mgme1	mitochondrial genome maintenance exonuclease 1	**-2.51**	6.70E-02
mbnl2	muscleblind like splicing regulator 2	**-2.51**	1.61E-02
alms1	ALMS1, centrosome and basal body associated protein	**-2.51**	3.11E-02
cttnbp2	cortactin binding protein 2	**-2.50**	5.42E-02
lgr4	leucine rich repeat containing G protein-coupled receptor 4	**-2.50**	1.33E-02
aasdh	aminoadipate-semialdehyde dehydrogenase	**-2.50**	3.54E-02
usp8	ubiquitin specific peptidase 8	**-2.50**	8.62E-02
fmnl2	formin like 2	**-2.50**	5.78E-02
tuba3e	tubulin alpha 3e	**-2.50**	6.89E-02
erich6b	glutamate rich 6B	**-2.50**	4.83E-02
tuba3c	tubulin alpha 3c	**-2.49**	6.52E-02
kdm5a	lysine demethylase 5A	**-2.49**	4.25E-02
ins	insulin	**-2.49**	6.44E-02
commd10	COMM domain containing 10	**-2.49**	4.66E-02
hfe2	hemochromatosis type 2 (juvenile)	**-2.49**	2.50E-04
flna	filamin A	**-2.49**	7.38E-03
lama2	laminin subunit alpha 2	**-2.48**	2.07E-02
hectd1	HECT domain E3 ubiquitin protein ligase 1	**-2.48**	4.45E-02
crtc2	CREB regulated transcription coactivator 2	**-2.48**	3.42E-02
cox6b1	cytochrome c oxidase subunit 6B1	**-2.48**	7.88E-02
marveld2	MARVEL domain containing 2	**-2.48**	4.45E-02
rab36	RAB36, member RAS oncogene family	**-2.48**	9.80E-02
exd2	exonuclease 3'-5' domain containing 2	**-2.48**	4.34E-02
scaf1	SR-related CTD associated factor 1	**-2.47**	6.57E-02
ewsr1	EWS RNA binding protein 1	**-2.47**	8.44E-02
cdc23	cell division cycle 23	**-2.47**	2.28E-02
vps41	VPS41, HOPS complex subunit	**-2.47**	4.45E-02
fam173b	family with sequence similarity 173 member B	**-2.47**	6.37E-02
btbd2	BTB domain containing 2	**-2.46**	1.68E-03
mrpl34	mitochondrial ribosomal protein L34	**-2.46**	4.53E-02
copz2	coatomer protein complex subunit zeta 2	**-2.46**	1.53E-02
fahd2a	fumarylacetoacetate hydrolase domain containing 2A	**-2.46**	4.37E-02
rpl39	ribosomal protein L39	**-2.46**	4.80E-02
numa1	nuclear mitotic apparatus protein 1	**-2.46**	6.36E-02
lrmp	lymphoid restricted membrane protein	**-2.46**	5.41E-02
hnrnpa0	heterogeneous nuclear ribonucleoprotein A0	**-2.46**	5.67E-02
vps16	VPS16, CORVET/HOPS core subunit	**-2.45**	8.27E-02
med12	mediator complex subunit 12	**-2.45**	2.94E-02
vangl1	VANGL planar cell polarity protein 1	**-2.45**	2.10E-02
mcm4	minichromosome maintenance complex component 4	**-2.45**	4.66E-02
serpind1	serpin family D member 1	**-2.45**	6.52E-02
fbxw7	F-box and WD repeat domain containing 7	**-2.44**	4.99E-02
znf408	zinc finger protein 408	**-2.44**	8.60E-02
kmt2e	lysine methyltransferase 2E	**-2.44**	5.38E-02
ptn	pleiotrophin	**-2.44**	5.62E-02
zc3h12c	zinc finger CCCH-type containing 12C	**-2.44**	6.42E-02
pex19	peroxisomal biogenesis factor 19	**-2.43**	5.03E-02
tfap2e	transcription factor AP-2 epsilon	**-2.43**	6.57E-02
rev1	REV1, DNA directed polymerase	**-2.43**	9.06E-02
ppa1	pyrophosphatase (inorganic) 1	**-2.43**	7.75E-02
rpl8	ribosomal protein L8	**-2.43**	6.89E-02
rab31	RAB31, member RAS oncogene family	**-2.43**	5.84E-02
ilf3	interleukin enhancer binding factor 3	**-2.42**	4.50E-02
srprb	SRP receptor beta subunit	**-2.42**	6.88E-02
eps15l1	epidermal growth factor receptor pathway substrate 15 like 1	**-2.42**	4.28E-02
apc	APC, WNT signaling pathway regulator	**-2.42**	4.30E-02
tac1	tachykinin precursor 1	**-2.42**	6.05E-02
ccdc189	coiled-coil domain containing 189	**-2.42**	9.01E-02
map3k2	mitogen-activated protein kinase kinase kinase 2	**-2.42**	9.78E-02
serpinh1	serpin family H member 1	**-2.42**	9.90E-02
gspt1	G1 to S phase transition 1	**-2.42**	5.17E-02
pcdha8	protocadherin alpha 8	**-2.42**	7.70E-02
pum1	pumilio RNA binding family member 1	**-2.41**	5.25E-02
iqce	IQ motif containing E	**-2.41**	9.57E-02
kdm6a	lysine demethylase 6A	**-2.41**	6.73E-02
ckm	creatine kinase, M-type	**-2.41**	5.69E-02
ckb	creatine kinase B	**-2.41**	3.42E-02
tbc1d8	TBC1 domain family member 8	**-2.41**	8.07E-02
sec61b	Sec61 translocon beta subunit	**-2.41**	2.05E-02
ube2e2	ubiquitin conjugating enzyme E2 E2	**-2.41**	8.47E-02
trim71	tripartite motif containing 71	**-2.40**	6.00E-02
mrpl38	mitochondrial ribosomal protein L38	**-2.40**	9.45E-02
adra2b	adrenoceptor alpha 2B	**-2.40**	4.64E-02
map4k5	mitogen-activated protein kinase kinase kinase kinase 5	**-2.40**	5.62E-02
ggcx	gamma-glutamyl carboxylase	**-2.40**	8.36E-02
spc24	SPC24, NDC80 kinetochore complex component	**-2.40**	9.09E-02
slc7a7	solute carrier family 7 member 7	**-2.40**	3.41E-02
hibadh	3-hydroxyisobutyrate dehydrogenase	**-2.40**	5.52E-02
apmap	adipocyte plasma membrane associated protein	**-2.40**	1.09E-03
usp10	ubiquitin specific peptidase 10	**-2.40**	3.68E-02
ppip5k2	diphosphoinositol pentakisphosphate kinase 2	**-2.39**	1.69E-02
mfsd6	major facilitator superfamily domain containing 6	**-2.39**	2.94E-02

padi2	peptidyl arginine deiminase 2	**-2.39**	6.72E-03
ccnd1	cyclin D1	**-2.39**	8.93E-02
slc6a17	solute carrier family 6 member 17	**-2.39**	9.96E-02
fbxl21	F-box and leucine rich repeat protein 21 (gene/pseudogene)	**-2.39**	3.94E-02
kif13a	kinesin family member 13A	**-2.38**	1.91E-02
ndufs7	NADH:ubiquinone oxidoreductase core subunit S7	**-2.38**	4.52E-02
irf2bp2	interferon regulatory factor 2 binding protein 2	**-2.38**	5.79E-02
sbf2	SET binding factor 2	**-2.38**	6.90E-02
cct3	chaperonin containing TCP1 subunit 3	**-2.38**	1.95E-02
nfyb	nuclear transcription factor Y subunit beta	**-2.38**	2.35E-02
atxn2l	ataxin 2 like	**-2.37**	5.40E-02
epb42	erythrocyte membrane protein band 4.2	**-2.37**	7.42E-02
pebp1	phosphatidylethanolamine binding protein 1	**-2.37**	6.26E-02
bhlhe41	basic helix-loop-helix family member e41	**-2.37**	1.47E-02
pacs1	phosphofurin acidic cluster sorting protein 1	**-2.37**	6.59E-02
golga6l2	golgin A6 family-like 2	**-2.36**	5.90E-02
siah1	siah E3 ubiquitin protein ligase 1	**-2.36**	9.83E-02
sbf1	SET binding factor 1	**-2.36**	6.41E-02
gon4l	gon-4 like	**-2.36**	3.01E-02
mum1l1	MUM1 like 1	**-2.36**	4.98E-02
nmur1	neuromedin U receptor 1	**-2.36**	5.18E-02
map3k11	mitogen-activated protein kinase kinase kinase 11	**-2.36**	7.58E-02
znf703	zinc finger protein 703	**-2.36**	2.63E-02
sp4	Sp4 transcription factor	**-2.36**	7.35E-02
adam10	ADAM metallopeptidase domain 10	**-2.35**	3.37E-02
slc35b3	solute carrier family 35 member B3	**-2.35**	5.54E-02
usf3	upstream transcription factor family member 3	**-2.35**	1.96E-02
serpinc1	serpin family C member 1	**-2.35**	4.88E-02
trpm7	transient receptor potential cation channel subfamily M member 7	**-2.35**	5.54E-02
kat6a	lysine acetyltransferase 6A	**-2.35**	7.64E-02
socs3	suppressor of cytokine signaling 3	**-2.35**	9.40E-05
heatr4	HEAT repeat containing 4	**-2.35**	6.62E-02
fut10	fucosyltransferase 10	**-2.34**	9.25E-06
msmb	microseminoprotein beta	**-2.34**	7.31E-02
ctnnbl1	catenin beta like 1	**-2.34**	8.12E-02
nfkb1	nuclear factor kappa B subunit 1	**-2.34**	5.52E-02
ahctf1	AT-hook containing transcription factor 1	**-2.34**	7.21E-02
sp3	Sp3 transcription factor	**-2.34**	8.63E-02
pak3	p21 (RAC1) activated kinase 3	**-2.34**	8.14E-03
xpo1	exportin 1	**-2.34**	4.09E-02
parp10	poly(ADP-ribose) polymerase family member 10	**-2.34**	5.73E-02
opa1	OPA1, mitochondrial dynamin like GTPase	**-2.34**	6.76E-02
neil1	nei like DNA glycosylase 1	**-2.34**	8.14E-02
plk2	polo like kinase 2	**-2.34**	1.54E-02
itprip	inositol 1,4,5-trisphosphate receptor interacting protein	**-2.34**	8.36E-02
tsta3	tissue specific transplantation antigen P35B	**-2.33**	5.93E-02
fam120c	family with sequence similarity 120C	**-2.33**	5.10E-02
ago3	argonaute 3, RISC catalytic component	**-2.33**	3.98E-02
zranb1	zinc finger RANBP2-type containing 1	**-2.33**	3.09E-02
tmem132b	transmembrane protein 132B	**-2.33**	7.66E-02
med14	mediator complex subunit 14	**-2.33**	2.03E-02
pear1	platelet endothelial aggregation receptor 1	**-2.32**	5.33E-03
gimap4	GTPase, IMAP family member 4	**-2.32**	3.64E-02
sumf2	sulfatase modifying factor 2	**-2.32**	4.42E-02
hcfc1	host cell factor C1	**-2.32**	6.55E-02
btbd6	BTB domain containing 6	**-2.32**	2.24E-02
plekha5	pleckstrin homology domain containing A5	**-2.32**	9.44E-02
yipf6	Yip1 domain family member 6	**-2.32**	1.06E-03
net1	neuroepithelial cell transforming 1	**-2.32**	1.68E-02
tnrc6c	trinucleotide repeat containing 6C	**-2.32**	3.78E-02
max	MYC associated factor X	**-2.32**	5.77E-02
eif2s2	eukaryotic translation initiation factor 2 subunit beta	**-2.32**	7.32E-02
ubxn10	UBX domain protein 10	**-2.32**	5.90E-02
kiaa1217	KIAA1217	**-2.32**	8.83E-02
uvrag	UV radiation resistance associated	**-2.31**	9.08E-02
gtf2h4	general transcription factor IIH subunit 4	**-2.31**	6.44E-03
hsd17b8	hydroxysteroid 17-beta dehydrogenase 8	**-2.30**	2.02E-02
thyn1	thymocyte nuclear protein 1	**-2.30**	4.19E-02
zswim6	zinc finger SWIM-type containing 6	**-2.30**	2.63E-02
dock9	dedicator of cytokinesis 9	**-2.30**	3.98E-02
trim45	tripartite motif containing 45	**-2.30**	4.58E-02
col4a1	collagen type IV alpha 1 chain	**-2.30**	5.67E-03
lrwd1	leucine rich repeats and WD repeat domain containing 1	**-2.30**	4.05E-02
cd200	CD200 molecule	**-2.30**	6.41E-02
fam83a	family with sequence similarity 83 member A	**-2.30**	4.80E-02
anp32e	acidic (leucine-rich) nuclear phosphoprotein 32 family, member E	**-2.29**	8.80E-03
epb41l2	erythrocyte membrane protein band 4.1 like 2	**-2.29**	5.53E-02
plekhg1	pleckstrin homology and RhoGEF domain containing G1	**-2.29**	1.18E-02
mtss1l	MTSS1L, I-BAR domain containing	**-2.29**	4.08E-02
rprd2	regulation of nuclear pre-mRNA domain containing 2	**-2.29**	5.84E-02
bptf	bromodomain PHD finger transcription factor	**-2.29**	6.90E-02
arid1a	AT-rich interaction domain 1A	**-2.29**	5.86E-02
rapgef1	Rap guanine nucleotide exchange factor 1	**-2.29**	6.10E-02
ilf2	interleukin enhancer binding factor 2	**-2.28**	6.28E-02
zp3	zona pellucida glycoprotein 3	**-2.28**	4.31E-02
pnrc2	proline rich nuclear receptor coactivator 2	**-2.28**	5.72E-02
capn13	calpain 13	**-2.28**	1.15E-02
lonrf3	LON peptidase N-terminal domain and ring finger 3	**-2.28**	5.29E-02
kmt2d	lysine methyltransferase 2D	**-2.28**	9.30E-02
stk19	serine/threonine kinase 19	**-2.27**	5.83E-02
tsc22d3	TSC22 domain family member 3	**-2.27**	3.11E-02
llgl2	LLGL2, scribble cell polarity complex component	**-2.27**	3.09E-02
hspd1	heat shock protein family D (Hsp60) member 1	**-2.27**	3.81E-02
snrpd1	small nuclear ribonucleoprotein D1 polypeptide	**-2.27**	4.62E-02
tpd52l2	tumor protein D52 like 2	**-2.27**	8.12E-02
vwa5a	von Willebrand factor A domain containing 5A	**-2.26**	7.79E-02
mettl12	methyltransferase like 12	**-2.26**	8.84E-02
ncald	neurocalcin delta	**-2.26**	9.01E-02
taf4b	TATA-box binding protein associated factor 4b	**-2.26**	1.03E-02
nsun5	NOP2/Sun RNA methyltransferase family member 5	**-2.25**	4.35E-02
tnfrsf9	TNF receptor superfamily member 9	**-2.25**	1.57E-02
large1	LARGE xylosyl- and glucuronyltransferase 1	**-2.25**	3.94E-02
neil3	nei like DNA glycosylase 3	**-2.25**	6.96E-02
hey2	hes related family bHLH transcription factor with YRPW motif 2	**-2.25**	7.06E-02
anxa4	annexin A4	**-2.25**	6.92E-02
lcmt1	leucine carboxyl methyltransferase 1	**-2.25**	5.58E-02
eef1b2	eukaryotic translation elongation factor 1 beta 2	**-2.25**	7.11E-02
piga	phosphatidylinositol glycan anchor biosynthesis class A	**-2.25**	8.12E-02
echdc2	enoyl-CoA hydratase domain containing 2	**-2.25**	1.07E-02
cdc26	cell division cycle 26	**-2.25**	3.05E-02
lpcat3	lysophosphatidylcholine acyltransferase 3	**-2.25**	2.97E-02
polr2b	RNA polymerase II subunit B	**-2.24**	8.17E-02
tmem238	transmembrane protein 238	**-2.24**	1.15E-02
mxi1	MAX interactor 1, dimerization protein	**-2.24**	2.07E-02
ncoa6	nuclear receptor coactivator 6	**-2.24**	3.76E-02
dhx8	DEAH-box helicase 8	**-2.24**	4.12E-02
ctgf	connective tissue growth factor	**-2.24**	6.04E-02
prdm2	PR/SET domain 2	**-2.24**	5.62E-03
casq1	calsequestrin 1	**-2.24**	8.48E-02
sorbs2	sorbin and SH3 domain containing 2	**-2.23**	7.63E-02
ror2	receptor tyrosine kinase like orphan receptor 2	**-2.23**	8.17E-02
rnf34	ring finger protein 34	**-2.23**	4.25E-03
heyl	hes related family bHLH transcription factor with YRPW motif-like	**-2.23**	2.25E-02
mrps22	mitochondrial ribosomal protein S22	**-2.23**	7.22E-02
habp4	hyaluronan binding protein 4	**-2.23**	1.35E-02
fhl1	four and a half LIM domains 1	**-2.23**	6.82E-02
slc22a13	solute carrier family 22 member 13	**-2.22**	1.43E-03
pdgfa	platelet derived growth factor subunit A	**-2.22**	2.89E-02
ttc9c	tetratricopeptide repeat domain 9C	**-2.22**	4.12E-02
znf281	zinc finger protein 281	**-2.22**	5.18E-02
ace	angiotensin I converting enzyme	**-2.22**	5.45E-02
rpl5	ribosomal protein L5	**-2.22**	6.51E-02
fxr2	FMR1 autosomal homolog 2	**-2.22**	4.60E-02
syt13	synaptotagmin 13	**-2.22**	4.99E-02
ip6k1	inositol hexakisphosphate kinase 1	**-2.22**	7.20E-02
stt3a	STT3A, catalytic subunit of the oligosaccharyltransferase complex	**-2.21**	1.27E-02
lrrc2	leucine rich repeat containing 2	**-2.21**	4.59E-02
gdi2	GDP dissociation inhibitor 2	**-2.21**	1.70E-02
col1a2	collagen type I alpha 2 chain	**-2.21**	4.22E-02
eif4h	eukaryotic translation initiation factor 4H	**-2.21**	5.25E-02
znf41	zinc finger protein 41	**-2.21**	1.51E-02
nudcd3	NudC domain containing 3	**-2.21**	1.15E-02
kiaa0556	KIAA0556	**-2.21**	4.38E-02

dyrk1a	dual specificity tyrosine phosphorylation regulated kinase 1A	**-2.21**	5.89E-02
dcaf12	DDB1 and CUL4 associated factor 12	**-2.21**	8.24E-02
ids	iduronate 2-sulfatase	**-2.21**	4.04E-02
tubgcp4	tubulin gamma complex associated protein 4	**-2.21**	4.22E-02
puf60	poly(U) binding splicing factor 60	**-2.21**	6.29E-02
ago1	argonaute 1, RISC catalytic component	**-2.20**	4.94E-05
cetn3	centrin 3	**-2.20**	5.92E-02
papolg	poly(A) polymerase gamma	**-2.20**	2.07E-02
zc3h13	zinc finger CCCH-type containing 13	**-2.20**	8.40E-02
rps18	ribosomal protein S18	**-2.20**	9.36E-02
spata13	spermatogenesis associated 13	**-2.19**	5.72E-02
fam208a	family with sequence similarity 208 member A	**-2.19**	9.22E-04
ncoa3	nuclear receptor coactivator 3	**-2.19**	1.28E-02
atp5j	ATP synthase, H+ transporting, mitochondrial Fo complex subunit F6	**-2.19**	9.55E-02
lrig2	leucine rich repeats and immunoglobulin like domains 2	**-2.19**	9.61E-02
daz2	deleted in azoospermia 2	**-2.19**	7.03E-02
pnisr	PNN interacting serine and arginine rich protein	**-2.19**	9.15E-02
ppp1r14c	protein phosphatase 1 regulatory inhibitor subunit 14C	**-2.19**	9.34E-02
taok1	TAO kinase 1	**-2.18**	9.84E-02
bahd1	bromo adjacent homology domain containing 1	**-2.18**	5.12E-03
tspan13	tetraspanin 13	**-2.18**	3.42E-02
hsp90ab1	heat shock protein 90 alpha family class B member 1	**-2.18**	4.61E-02
mvp	major vault protein	**-2.18**	8.40E-02
pepd	peptidase D	**-2.18**	3.65E-02
wasl	Wiskott-Aldrich syndrome like	**-2.17**	4.63E-02
plxna1	plexin A1	**-2.17**	5.32E-02
tab3	TGF-beta activated kinase 1/MAP3K7 binding protein 3	**-2.17**	6.27E-02
cfap65	cilia and flagella associated protein 65	**-2.17**	3.34E-02
hps4	HPS4, biogenesis of lysosomal organelles complex 3 subunit 2	**-2.17**	4.12E-02
sytl4	synaptotagmin like 4	**-2.16**	3.59E-02
pex11b	peroxisomal biogenesis factor 11 beta	**-2.16**	6.36E-02
nf1	neurofibromin 1	**-2.16**	3.85E-02
thoc2	THO complex 2	**-2.16**	4.79E-02
psme4	proteasome activator subunit 4	**-2.15**	7.93E-02
psmd1	proteasome 26S subunit, non-ATPase 1	**-2.15**	8.10E-02
stx6	syntaxin 6	**-2.15**	5.67E-02
pnp	purine nucleoside phosphorylase	**-2.15**	1.72E-02
tbc1d22a	TBC1 domain family member 22A	**-2.15**	7.87E-02
macf1	microtubule-actin crosslinking factor 1	**-2.15**	5.49E-02
rhoa	ras homolog family member A	**-2.15**	5.97E-02
glrb	glycine receptor beta	**-2.15**	9.89E-02
mta1	metastasis associated 1	**-2.15**	4.39E-02
chd2	chromodomain helicase DNA binding protein 2	**-2.14**	3.72E-02
glg1	golgi glycoprotein 1	**-2.14**	9.79E-03
bmt2	base methyltransferase of 25S rRNA 2 homolog	**-2.14**	2.69E-02
cspp1	centrosome and spindle pole associated protein 1	**-2.14**	2.41E-02
emsy	EMSY, BRCA2 interacting transcriptional repressor	**-2.14**	5.95E-02
carmil2	capping protein regulator and myosin 1 linker 2	**-2.14**	6.77E-02
slc35d1	solute carrier family 35 member D1	**-2.14**	4.95E-02
cstf2	cleavage stimulation factor subunit 2	**-2.14**	4.76E-02
faf1	Fas associated factor 1	**-2.13**	3.03E-02
lmnb1	lamin B1	**-2.13**	6.27E-04
mtrr	5-methyltetrahydrofolate-homocysteine methyltransferase reductase	**-2.13**	6.42E-02
orc3	origin recognition complex subunit 3	**-2.13**	1.79E-02
scaf8	SR-related CTD associated factor 8	**-2.13**	9.25E-02
dnajc25	DnaJ heat shock protein family (Hsp40) member C25	**-2.13**	8.74E-02
slc25a43	solute carrier family 25 member 43	**-2.13**	6.68E-02
spen	spen family transcriptional repressor	**-2.13**	8.68E-02
kmt2a	lysine methyltransferase 2A	**-2.12**	3.79E-02
gpatch2	G-patch domain containing 2	**-2.12**	6.47E-02
ptpro	protein tyrosine phosphatase, receptor type O	**-2.12**	9.36E-02
dyrk1b	dual specificity tyrosine phosphorylation regulated kinase 1B	**-2.12**	9.40E-02
mkl1	megakaryoblastic leukemia (translocation) 1	**-2.12**	4.68E-02
degs2	delta 4-desaturase, sphingolipid 2	**-2.12**	8.21E-02
ppp2cb	protein phosphatase 2 catalytic subunit beta	**-2.12**	9.56E-02
polr3d	RNA polymerase III subunit D	**-2.11**	3.57E-05
dars	aspartyl-tRNA synthetase	**-2.11**	5.18E-02
nars	asparaginyl-tRNA synthetase	**-2.11**	6.14E-03
chd7	chromodomain helicase DNA binding protein 7	**-2.11**	7.26E-02
sft2d2	SFT2 domain containing 2	**-2.11**	9.06E-02
phka1	phosphorylase kinase regulatory subunit alpha 1	**-2.11**	9.44E-03
nup214	nucleoporin 214	**-2.11**	4.10E-02
arpc2	actin related protein 2/3 complex subunit 2	**-2.11**	7.13E-02
csgalnact1	chondroitin sulfate N-acetylgalactosaminyltransferase 1	**-2.11**	1.36E-02
cldn5	claudin 5	**-2.11**	3.01E-02
ndufv3	NADH:ubiquinone oxidoreductase subunit V3	**-2.11**	8.94E-02
kiaa0100	KIAA0100	**-2.11**	1.45E-02
clcn3	chloride voltage-gated channel 3	**-2.11**	4.93E-02
abhd16a	abhydrolase domain containing 16A	**-2.11**	6.25E-02
cdk7	cyclin dependent kinase 7	**-2.11**	7.45E-02
zswim8	zinc finger SWIM-type containing 8	**-2.10**	1.65E-02
vangl2	VANGL planar cell polarity protein 2	**-2.10**	5.38E-02
cdca8	cell division cycle associated 8	**-2.10**	3.59E-02
ubl3	ubiquitin like 3	**-2.10**	9.62E-12
eif4e2	eukaryotic translation initiation factor 4E family member 2	**-2.09**	6.03E-02
uba52	ubiquitin A-52 residue ribosomal protein fusion product 1	**-2.09**	6.87E-02
spata2	spermatogenesis associated 2	**-2.09**	3.35E-02
sh3rf1	SH3 domain containing ring finger 1	**-2.09**	4.04E-02
slc44a1	solute carrier family 44 member 1	**-2.09**	5.10E-02
nelfe	negative elongation factor complex member E	**-2.09**	5.44E-02
pogz	pogo transposable element with ZNF domain	**-2.09**	6.17E-02
lonp1	lon peptidase 1, mitochondrial	**-2.09**	6.29E-02
plxdc2	plexin domain containing 2	**-2.09**	4.01E-02
cux1	cut like homeobox 1	**-2.09**	5.33E-02
akap9	A-kinase anchoring protein 9	**-2.09**	4.07E-02
sart1	squamous cell carcinoma antigen recognized by T-cells 1	**-2.09**	6.58E-02
xylt2	xylosyltransferase 2	**-2.09**	9.16E-02
insr	insulin receptor	**-2.08**	6.71E-02
jarid2	jumonji and AT-rich interaction domain containing 2	**-2.08**	3.89E-02
trim24	tripartite motif containing 24	**-2.08**	5.60E-02
magi1	membrane associated guanylate kinase, WW and PDZ domain containing 1	**-2.08**	6.50E-02
skiv2l2	Ski2 like RNA helicase 2	**-2.08**	7.45E-02
panx2	pannexin 2	**-2.08**	3.98E-02
cdk18	cyclin dependent kinase 18	**-2.08**	7.71E-02
vps37b	VPS37B, ESCRT-I subunit	**-2.08**	1.52E-02
ep400	E1A binding protein p400	**-2.08**	3.94E-02
pmpcb	peptidase, mitochondrial processing beta subunit	**-2.08**	5.31E-02
ankrd11	ankyrin repeat domain 11	**-2.08**	7.63E-02
lama1	laminin subunit alpha 1	**-2.08**	1.33E-02
psmd6	proteasome 26S subunit, non-ATPase 6	**-2.08**	5.62E-02
gaa	glucosidase alpha, acid	**-2.08**	7.11E-02
nudt5	nudix hydrolase 5	**-2.08**	8.12E-02
vgll4	vestigial like family member 4	**-2.08**	8.49E-02
eef2kmt	eukaryotic elongation factor 2 lysine methyltransferase	**-2.07**	8.63E-02
ube2c	ubiquitin conjugating enzyme E2 C	**-2.07**	9.09E-02
ucp2	uncoupling protein 2	**-2.07**	6.54E-02
fars2	phenylalanyl-tRNA synthetase 2, mitochondrial	**-2.07**	7.48E-02
kat8	lysine acetyltransferase 8	**-2.07**	3.49E-02
rbm25	RNA binding motif protein 25	**-2.07**	7.80E-02
clp1	cleavage and polyadenylation factor I subunit 1	**-2.07**	7.92E-02
atp13a1	ATPase 13A1	**-2.07**	4.37E-02
rbm5	RNA binding motif protein 5	**-2.07**	3.46E-02
adck5	aarF domain containing kinase 5	**-2.07**	5.85E-02
ndufa13	NADH:ubiquinone oxidoreductase subunit A13	**-2.07**	6.41E-02
prkd3	protein kinase D3	**-2.07**	3.72E-02
sf3b1	splicing factor 3b subunit 1	**-2.07**	3.38E-02
ergic3	ERGIC and golgi 3	**-2.07**	3.67E-02
swt1	SWT1, RNA endoribonuclease homolog	**-2.06**	6.64E-02
kri1	KRI1 homolog	**-2.06**	7.10E-02
mars	methionyl-tRNA synthetase	**-2.06**	3.76E-02
terf2	telomeric repeat binding factor 2	**-2.06**	7.43E-02
gga3	golgi associated, gamma adaptin ear containing, ARF binding protein 3	**-2.06**	8.99E-03
ankrd22	ankyrin repeat domain 22	**-2.06**	9.58E-03
uroc1	urocanate hydratase 1	**-2.06**	2.68E-02
notch1	notch 1	**-2.06**	8.11E-02
idh2	isocitrate dehydrogenase (NADP(+)) 2, mitochondrial	**-2.06**	9.52E-02

rnf113a1	ring finger protein 113A1	**-2.06**	1.89E-02
adgrl3	adhesion G protein-coupled receptor L3	**-2.06**	3.90E-02
gucy2c	guanylate cyclase 2C	**-2.06**	8.02E-03
fam135a	family with sequence similarity 135 member A	**-2.06**	1.25E-02
ttll11	tubulin tyrosine ligase like 11	**-2.06**	2.99E-02
sema4g	semaphorin 4G	**-2.06**	4.74E-02
itgb2	integrin subunit beta 2	**-2.06**	8.52E-02
hivep1	human immunodeficiency virus type I enhancer binding protein 1	**-2.05**	7.47E-02
ccar1	cell division cycle and apoptosis regulator 1	**-2.05**	4.92E-02
tuba1c	tubulin alpha 1c	**-2.05**	6.08E-02
wdr43	WD repeat domain 43	**-2.05**	6.51E-02
tpm3	tropomyosin 3	**-2.05**	2.02E-02
stard4	StAR related lipid transfer domain containing 4	**-2.05**	2.27E-02
dcaf10	DDB1 and CUL4 associated factor 10	**-2.05**	5.59E-02
kif1a	kinesin family member 1A	**-2.05**	7.95E-02
glyctk	glycerate kinase	**-2.05**	6.64E-02
syn1	synapsin I	**-2.05**	3.01E-02
inhbb	inhibin beta B subunit	**-2.04**	1.98E-02
hnrnpu	heterogeneous nuclear ribonucleoprotein U	**-2.04**	5.00E-02
tlk1	tousled like kinase 1	**-2.04**	6.04E-02
mef2d	myocyte enhancer factor 2D	**-2.04**	5.51E-02
arnt2	aryl hydrocarbon receptor nuclear translocator 2	**-2.04**	6.64E-02
jade2	jade family PHD finger 2	**-2.04**	2.57E-02
huwe1	HECT, UBA and WWE domain containing 1, E3 ubiquitin protein ligase	**-2.04**	2.86E-02
znf770	zinc finger protein 770	**-2.04**	9.45E-02
ppp6r1	protein phosphatase 6 regulatory subunit 1	**-2.04**	9.76E-02
lair1	leukocyte associated immunoglobulin like receptor 1	**-2.04**	5.60E-02
arpc5l	actin related protein 2/3 complex subunit 5 like	**-2.04**	6.65E-02
acsf2	acyl-CoA synthetase family member 2	**-2.04**	6.72E-02
gstt3	glutathione S-transferase, theta 3	**-2.04**	2.63E-05
dhrs4	dehydrogenase/reductase 4	**-2.04**	2.77E-02
ythdc1	YTH domain containing 1	**-2.04**	5.18E-02
ccdc61	coiled-coil domain containing 61	**-2.03**	6.04E-02
mpg	N-methylpurine DNA glycosylase	**-2.03**	5.25E-02
trim65	tripartite motif containing 65	**-2.03**	4.46E-02
hivep3	human immunodeficiency virus type I enhancer binding protein 3	**-2.03**	5.18E-02
tnni1	troponin I1, slow skeletal type	**-2.03**	5.33E-02
slc25a12	solute carrier family 25 member 12	**-2.03**	6.71E-02
plpp7	phospholipid phosphatase 7 (inactive)	**-2.03**	8.46E-02
slc7a6	solute carrier family 7 member 6	**-2.03**	9.71E-02
agtpbp1	ATP/GTP binding protein 1	**-2.03**	4.87E-02
cd209	CD209 molecule	**-2.03**	5.95E-02
selenon	selenoprotein N	**-2.03**	1.70E-02
ptges3	prostaglandin E synthase 3	**-2.03**	8.28E-02
top3a	topoisomerase (DNA) III alpha	**-2.02**	4.27E-02
kng1	kininogen 1	**-2.02**	9.94E-03
ndufs1	NADH:ubiquinone oxidoreductase core subunit S1	**-2.02**	6.51E-02
ncbp3	nuclear cap binding subunit 3	**-2.02**	2.63E-02
tyk2	tyrosine kinase 2	**-2.02**	4.50E-02
cgrrf1	cell growth regulator with ring finger domain 1	**-2.02**	6.83E-02
fibp	FGF1 intracellular binding protein	**-2.02**	3.31E-02
tfam	transcription factor A, mitochondrial	**-2.02**	5.14E-02
numbl	NUMB like, endocytic adaptor protein	**-2.02**	6.29E-02
dnajc17	DnaJ heat shock protein family (Hsp40) member C17	**-2.01**	4.83E-02
tmc7	transmembrane channel like 7	**-2.01**	5.22E-02
ino80d	INO80 complex subunit D	**-2.01**	6.42E-02
paxbp1	PAX3 and PAX7 binding protein 1	**-2.01**	4.04E-02
kti12	KTI12 chromatin associated homolog	**-2.01**	8.18E-02
znf510	zinc finger protein 510	**-2.01**	4.69E-02
mob2	MOB kinase activator 2	**-2.01**	6.57E-02
cadm1	cell adhesion molecule 1	**-2.01**	7.99E-02
klhl17	kelch like family member 17	**-2.00**	4.77E-03
atp6v0a2	ATPase H+ transporting V0 subunit a2	**-2.00**	3.19E-02
rb1	RB transcriptional corepressor 1	**-2.00**	4.07E-02
snx1	sorting nexin 1	**-2.00**	4.33E-02
zfyve1	zinc finger FYVE-type containing 1	**-2.00**	4.25E-02
cse1l	chromosome segregation 1 like	**-2.00**	6.52E-02
prdm6	PR/SET domain 6	**-2.00**	8.61E-02
nfkb2	nuclear factor kappa B subunit 2	**-2.00**	9.70E-02
cnot1	CCR4-NOT transcription complex subunit 1	**-2.00**	3.70E-02
uevld	UEV and lactate/malate dehyrogenase domains	**-2.00**	4.22E-02
ticrr	TOPBP1 interacting checkpoint and replication regulator	**-1.99**	4.22E-03
msl1	male specific lethal 1 homolog	**-1.99**	7.19E-02
grk5	G protein-coupled receptor kinase 5	**-1.99**	9.53E-02
dnajc9	DnaJ heat shock protein family (Hsp40) member C9	**-1.99**	6.97E-02
nxt2	nuclear transport factor 2 like export factor 2	**-1.99**	3.17E-02
plxna2	plexin A2	**-1.99**	5.60E-02
prr12	proline rich 12	**-1.99**	5.58E-02
oplah	5-oxoprolinase (ATP-hydrolysing)	**-1.99**	6.49E-02
ndufb2	NADH:ubiquinone oxidoreductase subunit B2	**-1.99**	6.59E-02
kalrn	kalirin, RhoGEF kinase	**-1.99**	4.88E-02
foxk1	forkhead box K1	**-1.99**	7.88E-02
gphn	gephyrin	**-1.98**	2.18E-02
lrrc1	leucine rich repeat containing 1	**-1.98**	5.99E-02
asrgl1	asparaginase like 1	**-1.98**	8.13E-02
tcirg1	T-cell immune regulator 1, ATPase H+ transporting V0 subunit a3	**-1.98**	8.14E-02
hadh	hydroxyacyl-CoA dehydrogenase	**-1.98**	8.00E-03
rsf1	remodeling and spacing factor 1	**-1.98**	5.11E-02
zdhhc5	zinc finger DHHC-type containing 5	**-1.98**	6.69E-02
efcab1	EF-hand calcium binding domain 1	**-1.98**	9.89E-02
itpka	inositol-trisphosphate 3-kinase A	**-1.97**	5.54E-02
rraga	Ras related GTP binding A	**-1.97**	5.58E-02
gal3st3	galactose-3-O-sulfotransferase 3	**-1.97**	4.65E-02
glrx	glutaredoxin	**-1.97**	7.89E-02
depdc7	DEP domain containing 7	**-1.97**	9.40E-02
gas8	growth arrest specific 8	**-1.97**	5.27E-02
twsg1	twisted gastrulation BMP signaling modulator 1	**-1.97**	6.12E-02
mtif3	mitochondrial translational initiation factor 3	**-1.97**	8.70E-02
znf319	zinc finger protein 319	**-1.97**	6.68E-02
ttyh3	tweety family member 3	**-1.97**	9.67E-02
tulp4	tubby like protein 4	**-1.96**	2.30E-02
iqgap3	IQ motif containing GTPase activating protein 3	**-1.96**	6.00E-02
gnas	GNAS complex locus	**-1.96**	6.23E-02
ncf2	neutrophil cytosolic factor 2	**-1.96**	7.27E-02
znf286a	zinc finger protein 286A	**-1.96**	5.90E-02
vrk3	vaccinia related kinase 3	**-1.96**	2.08E-02
sbno1	strawberry notch homolog 1	**-1.96**	4.44E-02
klc1	kinesin light chain 1	**-1.96**	6.50E-02
pik3c2a	phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 alpha	**-1.95**	6.70E-02
serac1	serine active site containing 1	**-1.95**	1.99E-02
znf175	zinc finger protein 175	**-1.95**	5.73E-02
mast4	microtubule associated serine/threonine kinase family member 4	**-1.95**	8.61E-02
id1	inhibitor of DNA binding 1, HLH protein	**-1.95**	2.41E-03
ints5	integrator complex subunit 5	**-1.95**	2.54E-02
tmem56	transmembrane protein 56	**-1.95**	1.56E-02
fndc3b	fibronectin type III domain containing 3B	**-1.95**	3.79E-02
slc26a6	solute carrier family 26 member 6	**-1.95**	3.96E-02
cdh4	cadherin 4	**-1.95**	6.58E-02
parpbp	PARP1 binding protein	**-1.95**	3.93E-02
rhoj	ras homolog family member J	**-1.95**	4.22E-02
atp1a2	ATPase Na+/K+ transporting subunit alpha 2	**-1.94**	6.98E-02
lsr	lipolysis stimulated lipoprotein receptor	**-1.94**	6.59E-02
atp6v1c2	ATPase H+ transporting V1 subunit C2	**-1.94**	4.82E-02
atp5j2	ATP synthase, H+ transporting, mitochondrial Fo complex subunit F2	**-1.94**	6.35E-02
sec23ip	SEC23 interacting protein	**-1.94**	8.34E-02
atxn7l2	ataxin 7 like 2	**-1.94**	5.15E-02
slc35f2	solute carrier family 35 member F2	**-1.94**	6.69E-02
pabpc1	poly(A) binding protein cytoplasmic 1	**-1.94**	8.59E-02
znf791	zinc finger protein 791	**-1.93**	9.51E-02
tnni2	troponin I2, fast skeletal type	**-1.93**	2.75E-02
pitpnm3	PITPNM family member 3	**-1.93**	6.78E-02
dhx40	DEAH-box helicase 40	**-1.93**	5.69E-02
psmc3ip	PSMC3 interacting protein	**-1.93**	2.24E-02
parp4	poly(ADP-ribose) polymerase family member 4	**-1.93**	8.61E-02
usp44	ubiquitin specific peptidase 44	**-1.93**	4.10E-02
ppil6	peptidylprolyl isomerase like 6	**-1.93**	5.08E-02
pitpna	phosphatidylinositol transfer protein alpha	**-1.93**	5.27E-02
bcl3	B-cell CLL/lymphoma 3	**-1.92**	2.52E-02
raver2	ribonucleoprotein, PTB binding 2	**-1.92**	4.95E-02
fosl2	FOS like 2, AP-1 transcription factor subunit	**-1.92**	2.95E-02
ppp5c	protein phosphatase 5 catalytic subunit	**-1.92**	7.21E-02
prickle1	prickle planar cell polarity protein 1	**-1.92**	9.83E-02
anks1a	ankyrin repeat and sterile alpha motif domain containing 1A	**-1.92**	3.98E-03
fh	fumarate hydratase	**-1.92**	9.81E-02
hmox2	heme oxygenase 2	**-1.92**	1.57E-02

aph1a	aph-1 homolog A, gamma-secretase subunit	**-1.92**	2.24E-02
hist2h2ab	histone cluster 2 H2A family member b	**-1.92**	9.07E-05
hsh2d	hematopoietic SH2 domain containing	**-1.92**	4.89E-02
pcolce2	procollagen C-endopeptidase enhancer 2	**-1.91**	1.97E-02
dusp8	dual specificity phosphatase 8	**-1.91**	6.12E-02
bach2	BTB domain and CNC homolog 2	**-1.91**	7.06E-02
cxorf23	chromosome X open reading frame 23	**-1.91**	7.96E-02
nsrp1	nuclear speckle splicing regulatory protein 1	**-1.91**	5.71E-02
sh2d4b	SH2 domain containing 4B	**-1.91**	8.06E-02
sec24c	SEC24 homolog C, COPII coat complex component	**-1.91**	8.84E-02
pcgf1	polycomb group ring finger 1	**-1.91**	5.93E-02
prpf39	pre-mRNA processing factor 39	**-1.91**	9.69E-02
ppm1k	protein phosphatase, Mg2+/Mn2+ dependent 1K	**-1.91**	1.96E-03
mcu	mitochondrial calcium uniporter	**-1.91**	5.68E-03
gas2l3	growth arrest specific 2 like 3	**-1.91**	3.96E-02
tfeb	transcription factor EB	**-1.91**	7.02E-02
ddb1	damage specific DNA binding protein 1	**-1.91**	8.44E-02
sumo2	small ubiquitin-like modifier 2	**-1.91**	1.91E-02
foxk2	forkhead box K2	**-1.91**	4.83E-02
ankle2	ankyrin repeat and LEM domain containing 2	**-1.90**	5.11E-02
tmsb10	thymosin beta 10	**-1.90**	8.11E-02
dgat1	diacylglycerol O-acyltransferase 1	**-1.90**	2.75E-02
ncor2	nuclear receptor corepressor 2	**-1.90**	3.21E-02
phyhd1	phytanoyl-CoA dioxygenase domain containing 1	**-1.89**	5.58E-02
cdk11a	cyclin dependent kinase 11A	**-1.89**	4.11E-02
tesk1	testis-specific kinase 1	**-1.89**	4.27E-02
slc16a9	solute carrier family 16 member 9	**-1.89**	8.73E-02
etf1	eukaryotic translation termination factor 1	**-1.89**	4.94E-02
ctdspl2	CTD small phosphatase like 2	**-1.89**	5.82E-02
arl2bp	ADP ribosylation factor like GTPase 2 binding protein	**-1.89**	9.94E-02
map3k1	mitogen-activated protein kinase kinase kinase 1	**-1.89**	7.47E-02
kiaa1109	KIAA1109	**-1.89**	9.27E-02
ptprz1	protein tyrosine phosphatase, receptor type Z1	**-1.89**	7.78E-02
med15	mediator complex subunit 15	**-1.89**	3.92E-02
cnot3	CCR4-NOT transcription complex subunit 3	**-1.89**	3.98E-02
znf687	zinc finger protein 687	**-1.89**	4.74E-02
basp1	brain abundant membrane attached signal protein 1	**-1.89**	5.31E-02
sf1	splicing factor 1	**-1.88**	4.84E-02
btg3	BTG anti-proliferation factor 3	**-1.88**	6.48E-02
mtmr14	myotubularin related protein 14	**-1.88**	7.13E-02
nampt	nicotinamide phosphoribosyltransferase	**-1.88**	6.14E-02
rpp25l	ribonuclease P/MRP subunit p25 like	**-1.88**	9.20E-02
klhl7	kelch like family member 7	**-1.88**	5.19E-02
ibtk	inhibitor of Bruton tyrosine kinase	**-1.88**	2.18E-02
fcho2	FCH domain only 2	**-1.88**	6.74E-02
ddi2	DNA damage inducible 1 homolog 2	**-1.87**	3.45E-02
slc27a6	solute carrier family 27 member 6	**-1.87**	4.45E-02
egr1	early growth response 1	**-1.87**	2.79E-02
rdh16	retinol dehydrogenase 16 (all-trans)	**-1.87**	5.18E-02
znf778	zinc finger protein 778	**-1.87**	6.15E-02
plekhg3	pleckstrin homology and RhoGEF domain containing G3	**-1.87**	6.97E-03
znf879	zinc finger protein 879	**-1.87**	5.48E-02
cbl	Cbl proto-oncogene	**-1.87**	7.81E-02
arf6	ADP ribosylation factor 6	**-1.87**	2.98E-02
sppl3	signal peptide peptidase like 3	**-1.87**	3.19E-02
kpnb1	karyopherin subunit beta 1	**-1.87**	3.60E-02
il1r1	interleukin 1 receptor type 1	**-1.87**	7.10E-02
gas6	growth arrest specific 6	**-1.87**	3.80E-02
tmem147	transmembrane protein 147	**-1.87**	8.63E-02
zmym4	zinc finger MYM-type containing 4	**-1.86**	7.28E-02
sall3	spalt like transcription factor 3	**-1.86**	7.25E-02
ccnh	cyclin H	**-1.86**	4.29E-02
cd93	CD93 molecule	**-1.86**	1.41E-02
zufsp	zinc finger with UFM1 specific peptidase domain	**-1.86**	4.99E-02
polr1c	RNA polymerase I subunit C	**-1.86**	7.35E-02
dcaf7	DDB1 and CUL4 associated factor 7	**-1.86**	4.25E-03
riok3	RIO kinase 3	**-1.86**	7.01E-02
azi2	5-azacytidine induced 2	**-1.86**	8.88E-02
galnt8	polypeptide N-acetylgalactosaminyltransferase 8	**-1.86**	2.17E-02
pfkfb4	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4	**-1.85**	8.07E-02
rttn	rotatin	**-1.85**	4.24E-02
bcl9	B-cell CLL/lymphoma 9	**-1.85**	4.12E-02
mgat5	mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase	**-1.85**	7.06E-02
rpl36a	ribosomal protein L36a	**-1.85**	6.81E-02
tmem55b	transmembrane protein 55B	**-1.85**	8.17E-02
polr3h	RNA polymerase III subunit H	**-1.85**	9.76E-02
mtmr6	myotubularin related protein 6	**-1.85**	9.56E-02
tubgcp6	tubulin gamma complex associated protein 6	**-1.85**	1.61E-02
nr0b2	nuclear receptor subfamily 0 group B member 2	**-1.85**	2.56E-02
cdk9	cyclin dependent kinase 9	**-1.85**	2.67E-02
srsf4	serine and arginine rich splicing factor 4	**-1.85**	8.91E-02
klf6	Kruppel like factor 6	**-1.85**	5.01E-02
rab19	RAB19, member RAS oncogene family	**-1.84**	2.04E-02
fbxo11	F-box protein 11	**-1.84**	6.68E-02
casc4	cancer susceptibility candidate 4	**-1.84**	4.06E-02
tle3	transducin like enhancer of split 3	**-1.84**	5.96E-02
ubr4	ubiquitin protein ligase E3 component n-recognin 4	**-1.84**	9.62E-02
znf341	zinc finger protein 341	**-1.84**	4.94E-02
sgip1	SH3 domain GRB2 like endophilin interacting protein 1	**-1.83**	4.49E-02
phf23	PHD finger protein 23	**-1.83**	7.92E-02
ube3d	ubiquitin protein ligase E3D	**-1.83**	9.01E-02
dld	dihydrolipoamide dehydrogenase	**-1.83**	9.59E-02
mrpl51	mitochondrial ribosomal protein L51	**-1.83**	9.73E-02
jakmip1	janus kinase and microtubule interacting protein 1	**-1.83**	7.85E-02
nicn1	nicolin 1	**-1.83**	2.92E-02
ino80b	INO80 complex subunit B	**-1.83**	9.73E-02
nt5c3a	5'-nucleotidase, cytosolic IIIA	**-1.83**	5.00E-02
brd2	bromodomain containing 2	**-1.83**	5.02E-02
znf618	zinc finger protein 618	**-1.83**	6.13E-02
csad	cysteine sulfinic acid decarboxylase	**-1.83**	7.02E-02
maz	MYC associated zinc finger protein	**-1.82**	7.85E-02
ints3	integrator complex subunit 3	**-1.82**	4.37E-02
chd4	chromodomain helicase DNA binding protein 4	**-1.82**	7.19E-02
atp2b1	ATPase plasma membrane Ca2+ transporting 1	**-1.82**	3.83E-02
mphosph8	M-phase phosphoprotein 8	**-1.82**	6.49E-02
hddc2	HD domain containing 2	**-1.82**	2.75E-02
acp5	acid phosphatase 5, tartrate resistant	**-1.82**	4.84E-02
ubr2	ubiquitin protein ligase E3 component n-recognin 2	**-1.82**	5.62E-02
brpf3	bromodomain and PHD finger containing 3	**-1.82**	6.18E-02
kank1	KN motif and ankyrin repeat domains 1	**-1.82**	1.24E-02
bicd2	BICD cargo adaptor 2	**-1.81**	3.86E-02
tmbim6	transmembrane BAX inhibitor motif containing 6	**-1.81**	2.22E-02
mbd2	methyl-CpG binding domain protein 2	**-1.81**	4.63E-02
ccdc130	coiled-coil domain containing 130	**-1.81**	4.99E-02
setd2	SET domain containing 2	**-1.81**	6.85E-02
kansl1l	KAT8 regulatory NSL complex subunit 1 like	**-1.81**	6.17E-02
nhej1	non-homologous end joining factor 1	**-1.81**	4.94E-02
bdh1	3-hydroxybutyrate dehydrogenase, type 1	**-1.81**	8.48E-02
rreb1	ras responsive element binding protein 1	**-1.81**	8.51E-02
camk4	calcium/calmodulin dependent protein kinase IV	**-1.81**	4.99E-03
themis2	thymocyte selection associated family member 2	**-1.81**	4.30E-02
wdr55	WD repeat domain 55	**-1.81**	9.92E-02
kiaa1456	KIAA1456	**-1.81**	2.64E-02
vps37a	VPS37A, ESCRT-I subunit	**-1.81**	6.15E-02
vars	valyl-tRNA synthetase	**-1.81**	7.41E-02
abi2	abl interactor 2	**-1.81**	3.75E-02
atrip	ATR interacting protein	**-1.80**	1.24E-02
zfpl1	zinc finger protein like 1	**-1.80**	3.39E-02
rars	arginyl-tRNA synthetase	**-1.80**	9.20E-02
grb10	growth factor receptor bound protein 10	**-1.80**	1.73E-02
tmem129	transmembrane protein 129	**-1.80**	6.28E-02
fbxw11	F-box and WD repeat domain containing 11	**-1.80**	6.53E-02
ccdc112	coiled-coil domain containing 112	**-1.80**	7.86E-02
lrrc61	leucine rich repeat containing 61	**-1.80**	8.37E-02
ei24	EI24, autophagy associated transmembrane protein	**-1.80**	9.33E-02
znf782	zinc finger protein 782	**-1.80**	8.29E-02
pgm1	phosphoglucomutase 1	**-1.79**	4.89E-02
mast3	microtubule associated serine/threonine kinase 3	**-1.79**	7.46E-02
paics	phosphoribosylaminoimidazole carboxylase and phosphoribosylaminoimidazolesuccinocarboxamide synthase	**-1.79**	2.81E-02
fsd1	fibronectin type III and SPRY domain containing 1	**-1.79**	7.51E-02
ltv1	LTV1 ribosome biogenesis factor	**-1.79**	3.60E-02
zbtb40	zinc finger and BTB domain containing 40	**-1.79**	9.32E-02
cdk5r1	cyclin dependent kinase 5 regulatory subunit 1	**-1.79**	6.17E-02
sumf1	sulfatase modifying factor 1	**-1.79**	6.62E-02
hnrnpc	heterogeneous nuclear ribonucleoprotein C (C1/C2)	**-1.78**	4.82E-02
bmp7	bone morphogenetic protein 7	**-1.78**	5.61E-02
ap3d1	adaptor related protein complex 3 delta 1 subunit	**-1.78**	4.21E-02
sh3glb2	SH3 domain containing GRB2 like endophilin B2	**-1.78**	6.03E-02

gabpa	GA binding protein transcription factor alpha subunit	**-1.77**	7.58E-02
zxdc	ZXD family zinc finger C	**-1.77**	6.17E-02
kpna2	karyopherin subunit alpha 2	**-1.77**	9.21E-02
mical3	microtubule associated monooxygenase, calponin and LIM domain containing 3	**-1.77**	7.72E-02
c11orf74	chromosome 11 open reading frame 74	**-1.77**	5.84E-02
ski	SKI proto-oncogene	**-1.76**	6.06E-02
zbtb34	zinc finger and BTB domain containing 34	**-1.76**	4.06E-02
rbm17	RNA binding motif protein 17	**-1.76**	7.63E-02
rad54b	RAD54 homolog B (S. cerevisiae)	**-1.76**	9.64E-02
pmp2	peripheral myelin protein 2	**-1.76**	9.75E-02
trim32	tripartite motif containing 32	**-1.76**	1.58E-02
dstyk	dual serine/threonine and tyrosine protein kinase	**-1.76**	4.02E-02
cep44	centrosomal protein 44	**-1.76**	7.49E-02
rnf146	ring finger protein 146	**-1.76**	8.71E-02
srsf10	serine and arginine rich splicing factor 10	**-1.76**	9.72E-02
utp20	UTP20, small subunit processome component	**-1.76**	4.14E-02
kif13b	kinesin family member 13B	**-1.76**	6.24E-02
ndst2	N-deacetylase and N-sulfotransferase 2	**-1.76**	5.39E-02
helb	DNA helicase B	**-1.76**	8.08E-02
marcks	myristoylated alanine rich protein kinase C substrate	**-1.76**	1.44E-02
madd	MAP kinase activating death domain	**-1.76**	5.28E-02
acp6	acid phosphatase 6, lysophosphatidic	**-1.76**	6.91E-02
thrap3	thyroid hormone receptor associated protein 3	**-1.76**	4.98E-03
trnau1ap	tRNA selenocysteine 1 associated protein 1	**-1.76**	8.39E-02
ngrn	neugrin, neurite outgrowth associated	**-1.76**	8.99E-02
wasf2	WAS protein family member 2	**-1.75**	4.96E-02
nbeal1	neurobeachin like 1	**-1.75**	7.71E-02
enkd1	enkurin domain containing 1	**-1.75**	6.42E-02
gatad2a	GATA zinc finger domain containing 2A	**-1.75**	9.21E-02
brd4	bromodomain containing 4	**-1.75**	7.89E-02
crebrf	CREB3 regulatory factor	**-1.75**	8.36E-02
gigyf2	GRB10 interacting GYF protein 2	**-1.75**	4.13E-02
msl2	male-specific lethal 2 homolog (Drosophila)	**-1.75**	4.45E-03
glcci1	glucocorticoid induced 1	**-1.75**	7.25E-02
snrnp25	small nuclear ribonucleoprotein U11/U12 subunit 25	**-1.74**	7.55E-02
pom121	POM121 transmembrane nucleoporin	**-1.74**	2.85E-02
mios	meiosis regulator for oocyte development	**-1.74**	8.18E-02
ogfr	opioid growth factor receptor	**-1.74**	3.18E-05
bend3	BEN domain containing 3	**-1.74**	7.33E-02
suco	SUN domain containing ossification factor	**-1.74**	3.77E-02
rap1gds1	Rap1 GTPase-GDP dissociation stimulator 1	**-1.74**	8.40E-02
hexb	hexosaminidase subunit beta	**-1.73**	3.29E-02
tgif1	TGFB induced factor homeobox 1	**-1.73**	5.53E-02
stim1	stromal interaction molecule 1	**-1.73**	7.64E-02
ddx17	DEAD-box helicase 17	**-1.73**	5.42E-02
slain1	SLAIN motif family member 1	**-1.73**	5.85E-02
myl7	myosin light chain 7	**-1.73**	7.66E-02
lrit1	leucine rich repeat, Ig-like and transmembrane domains 1	**-1.73**	9.27E-02
lgals3	galectin 3	**-1.73**	2.00E-03
specc1	sperm antigen with calponin homology and coiled-coil domains 1	**-1.73**	3.86E-03
samd4b	sterile alpha motif domain containing 4B	**-1.73**	5.03E-02
tut1	terminal uridylyl transferase 1, U6 snRNA-specific	**-1.73**	7.48E-02
ildr1	immunoglobulin like domain containing receptor 1	**-1.73**	4.33E-02
col18a1	collagen type XVIII alpha 1 chain	**-1.73**	5.72E-02
znhit6	zinc finger HIT-type containing 6	**-1.73**	3.34E-03
gopc	golgi associated PDZ and coiled-coil motif containing	**-1.73**	1.21E-02
cep85	centrosomal protein 85	**-1.73**	8.94E-02
hspa9	heat shock protein family A (Hsp70) member 9	**-1.73**	3.24E-02
naa38	N(alpha)-acetyltransferase 38, NatC auxiliary subunit	**-1.73**	8.57E-02
elk3	ELK3, ETS transcription factor	**-1.72**	2.24E-02
prpf4b	pre-mRNA processing factor 4B	**-1.72**	7.87E-02
pole	DNA polymerase epsilon, catalytic subunit	**-1.72**	3.02E-02
dmwd	dystrophia myotonica, WD repeat containing	**-1.72**	5.49E-02
etv5	ETS variant 5	**-1.72**	6.41E-02
hira	histone cell cycle regulator	**-1.72**	7.16E-02
eef1g	eukaryotic translation elongation factor 1 gamma	**-1.72**	9.84E-02
dnajc8	DnaJ heat shock protein family (Hsp40) member C8	**-1.72**	2.00E-02
ivns1abp	influenza virus NS1A binding protein	**-1.72**	6.33E-02
tcp11l2	t-complex 11 like 2	**-1.72**	1.37E-02
gadd45b	growth arrest and DNA damage inducible beta	**-1.72**	6.70E-02
eif2b1	eukaryotic translation initiation factor 2B subunit alpha	**-1.72**	7.82E-02
nasp	nuclear autoantigenic sperm protein	**-1.72**	3.71E-02
smdt1	single-pass membrane protein with aspartate rich tail 1	**-1.72**	5.19E-02
cep350	centrosomal protein 350	**-1.72**	6.96E-02
kdm1a	lysine demethylase 1A	**-1.72**	7.55E-02
ilkap	ILK associated serine/threonine phosphatase	**-1.72**	9.63E-02
slc36a4	solute carrier family 36 member 4	**-1.71**	6.89E-03
psmd2	proteasome 26S subunit, non-ATPase 2	**-1.71**	8.56E-02
mrpl39	mitochondrial ribosomal protein L39	**-1.71**	1.53E-03
acsbg2	acyl-CoA synthetase bubblegum family member 2	**-1.71**	3.92E-02
slc10a3	solute carrier family 10 member 3	**-1.71**	4.47E-02
znf648	zinc finger protein 648	**-1.71**	8.34E-02
znf225	zinc finger protein 225	**-1.71**	8.15E-02
cbll1	Cbl proto-oncogene like 1	**-1.71**	5.72E-02
fastkd2	FAST kinase domains 2	**-1.71**	3.81E-02
tubb4b	tubulin beta 4B class IVb	**-1.71**	5.49E-02
kiaa1324	KIAA1324	**-1.71**	6.25E-02
nfatc2	nuclear factor of activated T-cells 2	**-1.71**	6.63E-02
nono	non-POU domain containing, octamer-binding	**-1.71**	5.51E-03
cfap206	cilia and flagella associated protein 206	**-1.71**	4.59E-02
klf9	Kruppel like factor 9	**-1.71**	7.95E-02
znf629	zinc finger protein 629	**-1.71**	5.15E-02
pdf	peptide deformylase (mitochondrial)	**-1.71**	9.47E-02
pygo1	pygopus family PHD finger 1	**-1.71**	1.37E-02
dicer1	dicer 1, ribonuclease III	**-1.71**	7.21E-02
nup205	nucleoporin 205	**-1.71**	7.39E-02
tbcel	tubulin folding cofactor E like	**-1.70**	6.64E-02
igbp1	immunoglobulin (CD79A) binding protein 1	**-1.70**	9.18E-02
zfyve16	zinc finger FYVE-type containing 16	**-1.70**	7.69E-02
tfg	TRK-fused gene	**-1.70**	8.10E-02
atp6ap1	ATPase H+ transporting accessory protein 1	**-1.70**	9.33E-02
sec14l1	SEC14 like lipid binding 1	**-1.70**	5.54E-02
prkd2	protein kinase D2	**-1.70**	5.90E-02
phc1	polyhomeotic homolog 1	**-1.70**	7.00E-02
znf691	zinc finger protein 691	**-1.70**	4.90E-02
catsperg	cation channel sperm associated auxiliary subunit gamma	**-1.70**	7.48E-02
smarca1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1	**-1.69**	6.57E-02
cerkl	ceramide kinase like	**-1.69**	6.81E-02
kif4a	kinesin family member 4A	**-1.69**	6.64E-02
nnt	nicotinamide nucleotide transhydrogenase	**-1.69**	1.29E-02
zdhhc6	zinc finger DHHC-type containing 6	**-1.69**	6.17E-02
xpo7	exportin 7	**-1.69**	4.29E-02
armc1	armadillo repeat containing 1	**-1.69**	5.84E-02
nfu1	NFU1 iron-sulfur cluster scaffold	**-1.69**	8.39E-02
tmem209	transmembrane protein 209	**-1.69**	6.69E-02
ybey	ybeY metallopeptidase (putative)	**-1.69**	9.49E-02
rab35	RAB35, member RAS oncogene family	**-1.69**	5.31E-02
arpc3	actin related protein 2/3 complex subunit 3	**-1.69**	5.44E-02
cnr1	cannabinoid receptor 1	**-1.69**	7.51E-02
nckipsd	NCK interacting protein with SH3 domain	**-1.68**	1.62E-02
ankrd13d	ankyrin repeat domain 13D	**-1.68**	1.82E-02
cacna1d	calcium voltage-gated channel subunit alpha1 D	**-1.68**	2.28E-02
acly	ATP citrate lyase	**-1.68**	8.84E-02
rabep2	rabaptin, RAB GTPase binding effector protein 2	**-1.68**	7.06E-02
cxxc1	CXXC finger protein 1	**-1.68**	5.76E-02
wdr91	WD repeat domain 91	**-1.68**	9.82E-02
smpd3	sphingomyelin phosphodiesterase 3	**-1.68**	4.84E-02
ylpm1	YLP motif containing 1	**-1.68**	7.56E-02
psen2	presenilin 2	**-1.68**	4.22E-02
jade1	jade family PHD finger 1	**-1.68**	5.57E-02
apbb1	amyloid beta precursor protein binding family B member 1	**-1.67**	5.15E-02
fah	fumarylacetoacetate hydrolase	**-1.67**	6.37E-02
kif20a	kinesin family member 20A	**-1.67**	6.58E-02
dpy19l1	dpy-19 like 1	**-1.67**	7.34E-02
rpl4	ribosomal protein L4	**-1.67**	6.99E-02
crip1	cysteine rich protein 1	**-1.67**	7.28E-02
pzp	PZP, alpha-2-macroglobulin like	**-1.66**	8.45E-02
mtmr2	myotubularin related protein 2	**-1.66**	8.79E-02
cald1	caldesmon 1	**-1.66**	3.59E-02
abhd10	abhydrolase domain containing 10	**-1.66**	4.30E-02

eftud2	elongation factor Tu GTP binding domain containing 2	**-1.66**	7.13E-02
ndufa4	NDUFA4, mitochondrial complex associated	**-1.66**	1.00E-01
c5orf42	chromosome 5 open reading frame 42	**-1.65**	6.59E-02
r3hdm1	R3H domain containing 1	**-1.65**	2.72E-02
timm17b	translocase of inner mitochondrial membrane 17 homolog B (yeast)	**-1.65**	9.03E-02
baz2b	bromodomain adjacent to zinc finger domain 2B	**-1.65**	9.83E-02
dnajc7	DnaJ heat shock protein family (Hsp40) member C7	**-1.65**	4.84E-02
rapgef2	Rap guanine nucleotide exchange factor 2	**-1.65**	6.71E-02
esrrb	estrogen related receptor beta	**-1.64**	5.60E-02
tmem177	transmembrane protein 177	**-1.64**	7.35E-02
rbm43	RNA binding motif protein 43	**-1.64**	3.71E-02
ipo9	importin 9	**-1.64**	2.54E-02
snx21	sorting nexin family member 21	**-1.64**	7.21E-02
srpra	SRP receptor alpha subunit	**-1.64**	9.69E-02
fmr1	fragile X mental retardation 1	**-1.64**	7.91E-02
rapgefl1	Rap guanine nucleotide exchange factor like 1	**-1.64**	2.13E-02
gtf2h1	general transcription factor IIH subunit 1	**-1.64**	2.24E-02
ppp3r1	protein phosphatase 3 regulatory subunit B, alpha	**-1.64**	5.29E-02
phospho1	phosphoethanolamine/phosphocholine phosphatase	**-1.64**	9.01E-02
rbks	ribokinase	**-1.64**	9.06E-02
vps72	vacuolar protein sorting 72 homolog	**-1.64**	6.54E-02
fdft1	farnesyl-diphosphate farnesyltransferase 1	**-1.64**	7.17E-02
grhl1	grainyhead like transcription factor 1	**-1.64**	4.55E-02
hck	HCK proto-oncogene, Src family tyrosine kinase	**-1.63**	5.40E-02
adam12	ADAM metallopeptidase domain 12	**-1.63**	4.65E-02
tomm22	translocase of outer mitochondrial membrane 22	**-1.63**	9.90E-02
fez1	fasciculation and elongation protein zeta 1	**-1.63**	8.93E-02
ranbp3	RAN binding protein 3	**-1.63**	6.40E-02
fgr	FGR proto-oncogene, Src family tyrosine kinase	**-1.63**	3.99E-02
slc39a10	solute carrier family 39 member 10	**-1.63**	6.76E-02
lrp5	LDL receptor related protein 5	**-1.62**	4.99E-02
orai2	ORAI calcium release-activated calcium modulator 2	**-1.62**	5.68E-03
ptgr1	prostaglandin reductase 1	**-1.62**	4.96E-02
slc4a2	solute carrier family 4 member 2	**-1.62**	7.59E-02
lrrc8d	leucine rich repeat containing 8 family member D	**-1.62**	9.51E-02
srsf3	serine and arginine rich splicing factor 3	**-1.62**	9.76E-02
tor4a	torsin family 4 member A	**-1.62**	5.99E-02
creld2	cysteine rich with EGF like domains 2	**-1.62**	9.60E-02
eif4a1	eukaryotic translation initiation factor 4A1	**-1.62**	9.15E-02
rnf170	ring finger protein 170	**-1.62**	9.27E-02
slc35b4	solute carrier family 35 member B4	**-1.62**	9.90E-02
senp6	SUMO1/sentrin specific peptidase 6	**-1.62**	8.23E-02
slc7a6os	solute carrier family 7 member 6 opposite strand	**-1.62**	2.10E-02
tars	threonyl-tRNA synthetase	**-1.62**	7.66E-02
larp4b	La ribonucleoprotein domain family member 4B	**-1.62**	4.79E-02
chic2	cysteine rich hydrophobic domain 2	**-1.62**	5.01E-02
inpp1	inositol polyphosphate-1-phosphatase	**-1.62**	9.18E-02
kat5	lysine acetyltransferase 5	**-1.61**	5.92E-02
psd3	pleckstrin and Sec7 domain containing 3	**-1.61**	8.25E-02
cd276	CD276 molecule	**-1.61**	1.87E-02
actl6a	actin like 6A	**-1.61**	2.97E-02
ubc	ubiquitin C	**-1.61**	8.70E-02
ikzf4	IKAROS family zinc finger 4	**-1.61**	3.40E-02
nbl1	neuroblastoma 1, DAN family BMP antagonist	**-1.61**	4.72E-02
kifc3	kinesin family member C3	**-1.61**	7.36E-02
gtf3c4	general transcription factor IIIC subunit 4	**-1.61**	2.86E-02
gyg1	glycogenin 1	**-1.61**	9.61E-02
anapc7	anaphase promoting complex subunit 7	**-1.61**	6.98E-02
jag1	jagged 1	**-1.61**	7.04E-02
ip6k2	inositol hexakisphosphate kinase 2	**-1.61**	3.18E-02
chchd6	coiled-coil-helix-coiled-coil-helix domain containing 6	**-1.61**	7.17E-02
mrpl17	mitochondrial ribosomal protein L17	**-1.61**	9.50E-02
c5orf15	chromosome 5 open reading frame 15	**-1.61**	4.45E-02
fkbp7	FK506 binding protein 7	**-1.61**	9.33E-02
phactr1	phosphatase and actin regulator 1	**-1.61**	9.69E-02
ccdc84	coiled-coil domain containing 84	**-1.60**	7.13E-02
pard6a	par-6 family cell polarity regulator alpha	**-1.60**	6.53E-02
atf5	activating transcription factor 5	**-1.60**	5.08E-02
myh9	myosin heavy chain 9	**-1.60**	7.21E-02
ctbp2	C-terminal binding protein 2	**-1.60**	3.42E-02
f3	coagulation factor III, tissue factor	**-1.60**	9.89E-02
ddx39a	DExD-box helicase 39A	**-1.60**	4.67E-02
matr3	matrin 3	**-1.60**	5.82E-02
dhrs13	dehydrogenase/reductase 13	**-1.59**	4.72E-02
recql	RecQ like helicase	**-1.59**	4.82E-02
pphln1	periphilin 1	**-1.59**	2.23E-02
rin2	Ras and Rab interactor 2	**-1.59**	7.58E-02
poldip3	DNA polymerase delta interacting protein 3	**-1.59**	9.98E-02
atl3	atlastin GTPase 3	**-1.59**	2.73E-02
arhgap11a	Rho GTPase activating protein 11A	**-1.59**	4.37E-02
zc3h3	zinc finger CCCH-type containing 3	**-1.59**	6.63E-02
ext1	exostosin glycosyltransferase 1	**-1.59**	9.99E-02
vac14	Vac14, PIKFYVE complex component	**-1.59**	4.36E-02
frk	fyn related Src family tyrosine kinase	**-1.59**	4.74E-02
stk26	serine/threonine protein kinase 26	**-1.59**	6.64E-02
adra1b	adrenoceptor alpha 1B	**-1.59**	4.78E-02
e4f1	E4F transcription factor 1	**-1.59**	7.68E-02
sec24b	SEC24 homolog B, COPII coat complex component	**-1.59**	4.09E-02
eml4	echinoderm microtubule associated protein like 4	**-1.59**	7.90E-02
sp2	Sp2 transcription factor	**-1.59**	3.19E-02
haus5	HAUS augmin like complex subunit 5	**-1.59**	4.01E-02
gnb1l	G protein subunit beta 1 like	**-1.58**	7.43E-02
aif1l	allograft inflammatory factor 1 like	**-1.58**	4.04E-02
ggnbp2	gametogenetin binding protein 2	**-1.58**	9.36E-02
sirt1	sirtuin 1	**-1.58**	4.19E-02
esco1	establishment of sister chromatid cohesion N-acetyltransferase 1	**-1.58**	4.17E-02
kiaa0753	KIAA0753	**-1.58**	2.72E-02
prrc2b	proline rich coiled-coil 2B	**-1.58**	5.18E-02
nectin4	nectin cell adhesion molecule 4	**-1.58**	3.22E-02
apoh	apolipoprotein H	**-1.58**	7.33E-02
birc6	baculoviral IAP repeat containing 6	**-1.58**	5.58E-02
igf2bp1	insulin like growth factor 2 mRNA binding protein 1	**-1.58**	7.51E-02
hacd2	3-hydroxyacyl-CoA dehydratase 2	**-1.58**	6.98E-02
setd3	SET domain containing 3	**-1.58**	9.51E-02
pard6g	par-6 family cell polarity regulator gamma	**-1.57**	6.72E-02
pkd2	polycystin 2, transient receptor potential cation channel	**-1.57**	7.59E-02
ahr	aryl hydrocarbon receptor	**-1.57**	8.58E-02
taf5	TATA-box binding protein associated factor 5	**-1.57**	5.64E-02
atp5d	ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit	**-1.57**	9.48E-02
fam134c	family with sequence similarity 134 member C	**-1.57**	9.88E-02
phf6	PHD finger protein 6	**-1.56**	4.54E-02
usp25	ubiquitin specific peptidase 25	**-1.56**	8.85E-02
eogt	EGF domain specific O-linked N-acetylglucosamine transferase	**-1.56**	4.74E-02
psmd4	proteasome 26S subunit, non-ATPase 4	**-1.56**	8.62E-02
smg8	SMG8, nonsense mediated mRNA decay factor	**-1.56**	8.23E-02
epc1	enhancer of polycomb homolog 1	**-1.56**	6.12E-02
zfand3	zinc finger AN1-type containing 3	**-1.56**	7.81E-02
tubb4a	tubulin beta 4A class IVa	**-1.56**	9.30E-02
ddx28	DEAD-box helicase 28	**-1.56**	6.76E-02
tshz3	teashirt zinc finger homeobox 3	**-1.55**	4.65E-02
tmem97	transmembrane protein 97	**-1.55**	5.14E-02
icam1	intercellular adhesion molecule 1	**-1.55**	6.95E-02
f10	coagulation factor X	**-1.55**	9.89E-02
fam124b	family with sequence similarity 124 member B	**-1.55**	1.48E-02
atp6v0c	ATPase H+ transporting V0 subunit c	**-1.55**	5.35E-02
prpf3	pre-mRNA processing factor 3	**-1.55**	6.03E-02
tjp2	tight junction protein 2	**-1.54**	2.94E-02
dscr3	DSCR3 arrestin fold containing	**-1.54**	6.17E-02
cdc25c	cell division cycle 25C	**-1.54**	1.30E-02
rnf185	ring finger protein 185	**-1.54**	4.32E-02
bcor	BCL6 corepressor	**-1.54**	8.91E-02
limk2	LIM domain kinase 2	**-1.54**	6.41E-02
pwwp2b	PWWP domain containing 2B	**-1.54**	8.47E-02
sec24a	SEC24 homolog A, COPII coat complex component	**-1.54**	7.06E-02
tbc1d2	TBC1 domain family member 2	**-1.54**	1.14E-02
adssl1	adenylosuccinate synthase like 1	**-1.53**	6.05E-02
rnpc3	RNA binding region (RNP1, RRM) containing 3	**-1.53**	7.35E-02
patl1	PAT1 homolog 1, processing body mRNA decay factor	**-1.53**	9.57E-02
arhgef2	Rho/Rac guanine nucleotide exchange factor 2	**-1.53**	3.23E-02
mex3b	mex-3 RNA binding family member B	**-1.53**	9.16E-02
coch	cochlin	**-1.52**	7.48E-02
sertad2	SERTA domain containing 2	**-1.52**	8.54E-02
fnbp1l	formin binding protein 1 like	**-1.52**	2.35E-02
nfs1	NFS1, cysteine desulfurase	**-1.52**	2.90E-02
cyr61	cysteine rich angiogenic inducer 61	**-1.52**	6.54E-02

dusp7	dual specificity phosphatase 7	**-1.51**	6.17E-02
gpatch8	G-patch domain containing 8	**-1.51**	7.34E-02
tespa1	thymocyte expressed, positive selection associated 1	**-1.51**	7.20E-02
drg1	developmentally regulated GTP binding protein 1	**-1.51**	3.48E-02
haus8	HAUS augmin like complex subunit 8	**-1.51**	4.20E-02
polr3a	RNA polymerase III subunit A	**-1.50**	2.57E-02
pkp4	plakophilin 4	**-1.50**	5.98E-02
d1pas1	DNA segment, Chr 1, Pasteur Institute 1	**-1.50**	5.70E-02
armc5	armadillo repeat containing 5	**-1.50**	9.44E-03
ndufs3	NADH:ubiquinone oxidoreductase core subunit S3	**-1.50**	3.91E-02
coq5	coenzyme Q5, methyltransferase	**-1.50**	5.98E-02
gldc	glycine decarboxylase	**-1.50**	3.18E-02
cc2d1b	coiled-coil and C2 domain containing 1B	**-1.50**	6.31E-02
cldn6	claudin 6	**-1.50**	2.83E-02

Table 2.3. Changes in expression of ovarian genes after treatment of females with E2 for three days. The table lists ovarian contigs regulated by E2 after three days, identified by DESeq2 (base mean >10, fold change ≥1.5 or -1.5≤, P-adj <0.1), showing fold change in expression relative to control values.

Most significantly altered ovary contigs 3 days after E2 treatment

Top contigs (Basemean >10, fold change ≥1.5 or ≤-1.5, P-adjusted ≤0.1)

Gene symbol	Gene name (upregulated)	**Fold change**	P-adjusted value
mitf	melanogenesis associated transcription factor	**41.16**	8.66E-59
rnf212	ring finger protein 212	**18.51**	1.18E-26
tmpo	thymopoietin	**14.87**	4.29E-04
fam227a	family with sequence similarity 227 member A	**12.81**	1.58E-19
bsdc1	BSD domain containing 1	**12.39**	1.30E-18
arrdc2	arrestin domain containing 2	**11.10**	9.04E-22
znf432	zinc finger protein 432	**10.27**	9.36E-16
hoxc13	homeobox C13	**9.24**	3.81E-19
gpbp1l1	GC-rich promoter binding protein 1 like 1	**9.05**	8.96E-14
katna1	katanin catalytic subunit A1	**8.78**	2.45E-13
slc30a7	solute carrier family 30 member 7	**6.60**	1.26E-09
pcbp4	poly(rC) binding protein 4	**6.39**	1.32E-15
rpl29	ribosomal protein L29	**5.10**	1.04E-07
lonrf2	LON peptidase N-terminal domain and ring finger 2	**4.91**	1.66E-07
wee2	WEE1 homolog 2	**4.41**	1.62E-05
chek2	checkpoint kinase 2	**4.23**	3.17E-05
ercc1	ERCC excision repair 1, endonuclease non-catalytic subunit	**4.21**	3.63E-05
nid1	nidogen 1	**3.96**	9.97E-04
anxa13	annexin A13	**3.67**	6.88E-04
tsku	tsukushi, small leucine rich proteoglycan	**3.43**	1.78E-03
ugt2a1	UDP glucuronosyltransferase family 2 member A1 complex locus	**3.25**	6.88E-04
ccdc92	coiled-coil domain containing 92	**3.09**	1.04E-02
sh3bgrl	SH3 domain binding glutamate rich protein like	**3.09**	1.12E-02
tmod4	tropomodulin 4	**3.07**	1.17E-02
myoz2	myozenin 2	**3.04**	9.30E-03
ssb	Sjogren syndrome antigen B	**3.00**	9.15E-09
paip2b	poly(A) binding protein interacting protein 2B	**3.00**	3.63E-05
c9orf142	chromosome 9 open reading frame 142	**2.93**	1.22E-02
adipor2	adiponectin receptor 2	**2.93**	1.22E-02
amd1	adenosylmethionine decarboxylase 1	**2.78**	4.07E-02
grin3b	glutamate ionotropic receptor NMDA type subunit 3B	**2.71**	1.63E-02
prph2	peripherin 2	**2.67**	7.52E-02
kif16b	kinesin family member 16B	**2.65**	7.94E-02
tmem39a	transmembrane protein 39A	**2.63**	8.11E-02
ptbp1	polypyrimidine tract binding protein 1	**2.63**	6.87E-04
sra1	steroid receptor RNA activator 1	**2.59**	6.16E-02
c14orf166	chromosome 14 open reading frame 166	**2.57**	3.15E-03
gabra2	gamma-aminobutyric acid type A receptor alpha2 subunit	**2.57**	8.69E-02
zmym1	zinc finger MYM-type containing 1	**2.42**	3.93E-02
sostdc1	sclerostin domain containing 1	**2.27**	2.72E-02
cr1	complement C3b/C4b receptor 1 (Knops blood group)	**2.25**	3.24E-02
pdcl3	phosducin like 3	**2.05**	7.29E-02
med6	mediator complex subunit 6	**2.04**	9.68E-02
mmgt1	membrane magnesium transporter 1	**1.98**	6.16E-02
hes5	hes family bHLH transcription factor 5	**1.95**	4.24E-02
crip2	cysteine rich protein 2	**1.93**	1.21E-03
rasgrp2	RAS guanyl releasing protein 2	**1.83**	1.57E-02
trim45	tripartite motif containing 45	**1.78**	1.02E-02
dusp6	dual specificity phosphatase 6	**1.78**	1.85E-02
st6galnac4	ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 4	**1.73**	6.53E-03
arhgap1	Rho GTPase activating protein 1	**1.63**	2.78E-02

Gene symbol	Gene name (downregulated)	**Fold change**	P-adjusted value
kcnab2	potassium voltage-gated channel subfamily A regulatory beta subunit 2	**-1.87**	9.42E-02
parp11	poly(ADP-ribose) polymerase family member 11	**-1.98**	6.53E-03
ogfr	opioid growth factor receptor	**-1.99**	1.70E-06
dcaf7	DDB1 and CUL4 associated factor 7	**-2.01**	2.43E-04
trim29	tripartite motif containing 29	**-2.04**	8.69E-02
rer1	retention in endoplasmic reticulum sorting receptor 1	**-2.09**	4.99E-05
tagln	transgelin	**-2.23**	7.11E-02
sec14l2	SEC14 like lipid binding 2	**-2.25**	1.07E-02
adam12	ADAM metallopeptidase domain 12	**-2.27**	6.06E-02
elovl1	ELOVL fatty acid elongase 1	**-2.49**	4.72E-05
eif4a2	eukaryotic translation initiation factor 4A2	**-2.54**	6.95E-02
sh3glb2	SH3 domain containing GRB2 like endophilin B2	**-2.61**	5.13E-02
azi2	5-azacytidine induced 2	**-2.62**	3.46E-02
mkrn1	makorin ring finger protein 1	**-2.67**	1.31E-04
fars2	phenylalanyl-tRNA synthetase 2, mitochondrial	**-2.69**	6.33E-02
gdf3	growth differentiation factor 3	**-2.70**	5.41E-02
pcgf5	polycomb group ring finger 5	**-2.71**	2.33E-02
med26	mediator complex subunit 26	**-2.72**	6.34E-02
muc5b	mucin 5B, oligomeric mucus/gel-forming	**-2.76**	5.38E-02
tmem132d	transmembrane protein 132D	**-2.85**	2.80E-02
chka	choline kinase alpha	**-2.86**	8.90E-03
cdc42	cell division cycle 42	**-2.88**	2.24E-02
odf2	outer dense fiber of sperm tails 2	**-2.89**	1.39E-02
fbxo36	F-box protein 36	**-2.92**	2.31E-02
maf1	MAF1 homolog, negative regulator of RNA polymerase III	**-2.98**	8.90E-03
csnk2a2	casein kinase 2 alpha 2	**-3.05**	9.86E-04
ccdc24	coiled-coil domain containing 24	**-3.14**	9.06E-03
tctex1d2	Tctex1 domain containing 2	**-3.15**	1.38E-03
vwf	von Willebrand factor	**-3.16**	4.48E-05
itgbl1	integrin subunit beta like 1	**-3.25**	6.88E-04
meis2	Meis homeobox 2	**-3.37**	1.74E-03
pcgf1	polycomb group ring finger 1	**-3.37**	2.62E-03
rbm38	RNA binding motif protein 38	**-3.51**	9.33E-04
slc19a3	solute carrier family 19 member 3	**-3.56**	6.88E-04
npl	N-acetylneuraminate pyruvate lyase	**-3.58**	6.77E-04
acp5	acid phosphatase 5, tartrate resistant	**-3.99**	4.82E-03
sf3b5	splicing factor 3b subunit 5	**-4.10**	9.88E-06
urm1	ubiquitin related modifier 1	**-4.23**	3.63E-05
glra4	glycine receptor alpha 4	**-4.38**	1.06E-06
bcl2l1	BCL2 like 1	**-4.66**	4.85E-06
slc9a3r1	SLC9A3 regulator 1	**-8.22**	3.65E-13
fbxo6	F-box protein 6	**-8.93**	1.49E-13
ddt	D-dopachrome tautomerase	**-10.53**	1.52E-25
syt10	synaptotagmin 10	**-21.59**	1.39E-07
gk	glycerol kinase	**-24.22**	2.00E-32
ppp1r15b	protein phosphatase 1 regulatory subunit 15B	**-111.43**	1.36E-91
hsp90b1	heat shock protein 90 beta family member 1	**-456.98**	4.59E-200

Table 2.4. Canonical pathways significantly altered in ovaries of females treated with 11-KT for three days, identified by Ingenuity® Pathway Analysis software. Pathway, -Log_{10} P-value, z-score, ratio of genes represented in data set to total genes reported in pathway. Top 45 pathways displayed. Gray shaded rows indicate pathways relevant to topics discussed in the text.

Ingenuity Canonical Pathways altered by 11-KT at day 3	-Log (P-value)	z-score	ratio of genes
Germ Cell-Sertoli Cell Junction Signaling	13.00		73/173
Integrin Signaling	13.00	3.53	86/219
Remodeling of Epithelial Adherens Junctions	10.80	0.89	37/68
Rac Signaling	10.40	0.57	52/117
Epithelial Adherens Junction Signaling	10.20		60/146
Sertoli Cell-Sertoli Cell Junction Signaling	10.20		69/178
Molecular Mechanisms of Cancer	9.54		117/374
Insulin Receptor Signaling	9.40	0.94	57/141
Signaling by Rho Family GTPases	9.32	1.73	85/247
Actin Cytoskeleton Signaling	8.87	2.26	79/228
Pyridoxal 5'-phosphate Salvage Pathway	8.68		33/65
Breast Cancer Regulation by Stathmin1	8.64		74/208
Neuregulin Signaling	8.54	3.18	40/88
Phagosome Maturation	8.51		56/144
Tight Junction Signaling	8.41		62/167
PI3K/AKT Signaling	8.29	1.18	50/124
RhoA Signaling	8.07	2.24	49/122
Huntington's Disease Signaling	8.00	0.91	80/241
mTOR Signaling	7.85	1.66	69/199
NGF Signaling	7.78	1.94	47/117
Estrogen Receptor Signaling	7.75		50/128
RhoGDI Signaling	7.73	-2.45	62/173
AMPK Signaling	7.68	0.58	66/189
Glucocorticoid Receptor Signaling	7.56		90/287
14-3-3-mediated Signaling	7.49	0.71	50/130
ILK Signaling	7.37	1.78	67/196
Ephrin Receptor Signaling	7.22	1.70	61/174
Mitotic Roles of Polo-Like Kinase	7.18	0.26	31/66
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes	7.16	2.40	39/93
Protein Ubiquitination Pathway	7.14		81/255
Androgen Signaling	7.12	0.66	46/116
Prostate Cancer Signaling	7.01		39/94
Clathrin-mediated Endocytosis Signaling	6.89		66/197
ERK/MAPK Signaling	6.71	2.39	66/199
HIPPO signaling	6.64	0.00	36/86
Regulation of eIF4 and p70S6K Signaling	6.57	2.12	55/157
NRF2-mediated Oxidative Stress Response	6.52	2.96	64/193
TGF-β Signaling	6.49	0.93	36/87
Superpathway of Inositol Phosphate Compounds	6.49		73/230
Regulation of Actin-based Motility by Rho	6.42	1.86	37/91
IGF-1 Signaling	6.35	2.54	41/106
Reelin Signaling in Neurons	6.28		37/92
Gap Junction Signaling	6.25		57/168
Telomerase Signaling	6.18	1.18	42/111
RAR Activation	6.07		62/190

Table 2.5. Canonical pathways significantly altered in ovaries of females treated with E2 for three days, identified by Ingenuity® Pathway Analysis software. Pathway, -Log_{10} P-value, number of genes represented in data set from total genes reported in pathway, gene IDs. No pathways were assigned a z-score.

Ingenuity Canonical Pathways altered by E2 at day 3	-Log (P-value)	ratio of genes	gene IDs
NAD Salvage Pathway II	2.20	2/26	*acp5,nmnat3*
Spermine Biosynthesis	2.04	1/2	*amd1*
Spermidine Biosynthesis I	2.04	1/2	*amd1*
p53 Signaling	1.84	3/111	*bcl2l1,trim29,chek2*
Eumelanin Biosynthesis	1.64	1/5	*ddt*
Glycerol Degradation I	1.56	1/6	*gk*
NAD Biosynthesis III	1.56	1/6	*nmnat3*
Phosphatidylcholine Biosynthesis I	1.50	1/7	*chka*
NAD Salvage Pathway III	1.50	1/7	*nmnat3*
Mitotic Roles of Polo-Like Kinase	1.43	2/66	*hsp90b1,chek2*
Phosphatidylethanolamine Biosynthesis II	1.39	1/9	*chka*
PPARα/RXRα Activation	1.31	3/178	*hsp90b1,gk,adipor2*

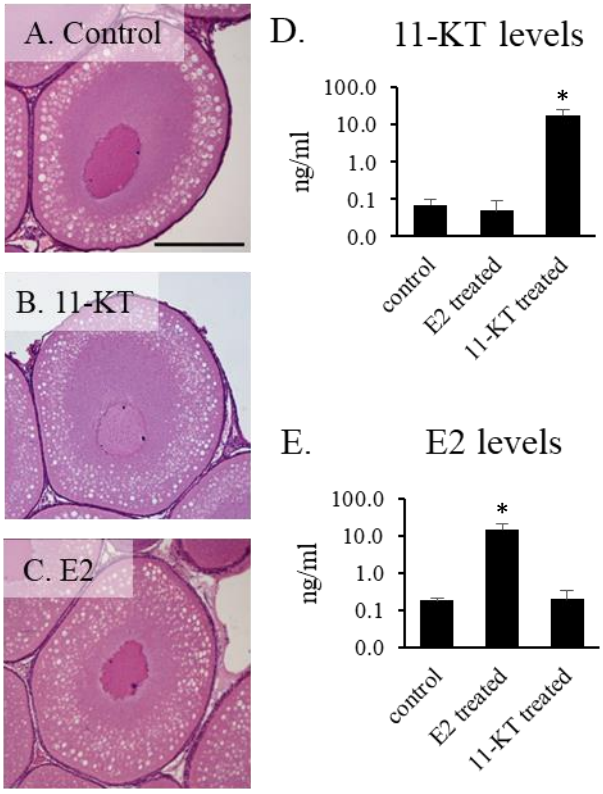

Figure 2.1. Ovarian stage and plasma sex steroid levels. Ovarian tissue from fish displaying early secondary growth ovarian follicles with similar cortical alveoli abundance were chosen from each treatment, (A) control, (B) 11-KT, and (C) E2, for RNA-seq analysis. Plasma 11-KT (D) and E2 (E) levels were measured three days after implant. Asterisks indicate significant elevation in plasma steroid levels ($P < 0.05$).

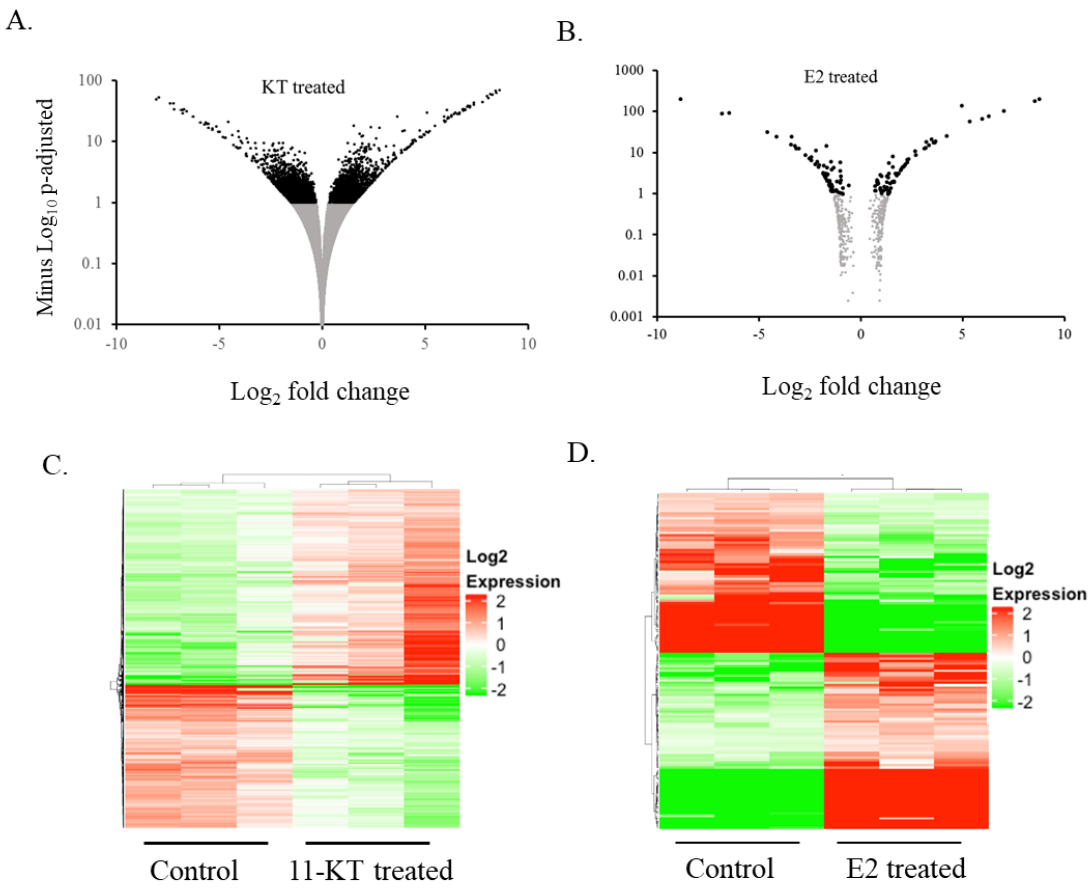

Figure 2.2. The expression of contigs in ovaries from females following short term treatment with 11-KT or E2. All contigs with a calculated p-adjusted value ($-Log_{10}$), plotted by fold change (Log_2). Black dots represent contigs significantly altered by 11-KT (A) or E2 (B). Cluster analysis (DESeq2) of differentially expressed contigs (DESeq2, basemean>10, P-adj<0.1) after three days of 11-KT (C) or E2 (D) treatment. Expression of contigs (rows) is displayed for three independent samples (columns), with red representing up-regulation and green representing down-regulation from the mean expression value (white) of each contig. Each column represents data from ovaries of a single individual.

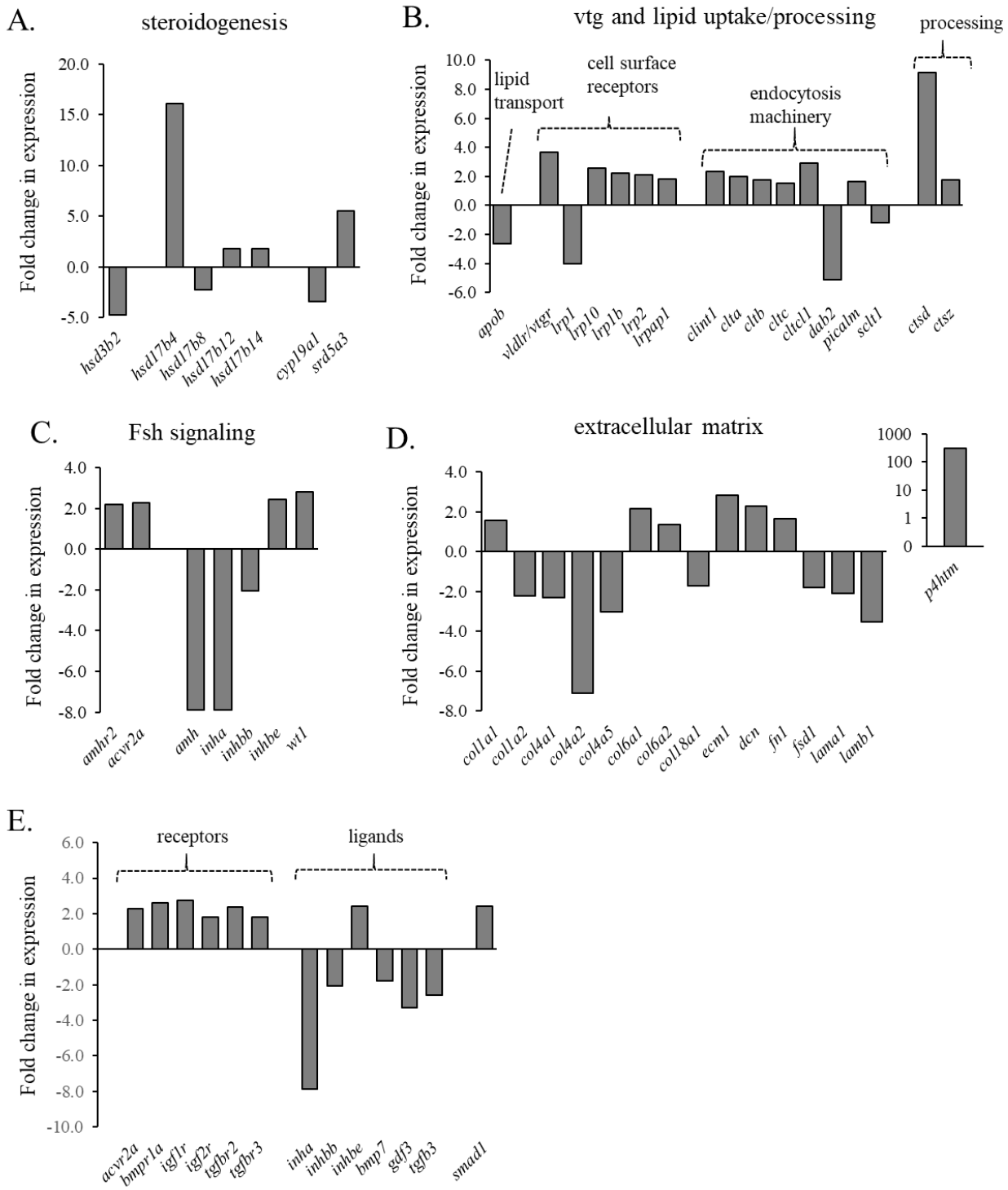

Figure 2.3. The expression of contigs altered by 11-KT treatment related to the morphology and function of the ovarian follicle. The fold expression compared to controls is displayed for contigs mapped to genes involved in (A) steroidogenesis, (B) vitellogenesis and lipid uptake and processing, (C) Fsh signaling, (D) the extracellular matrix, and (E) *tgfb* superfamily members.

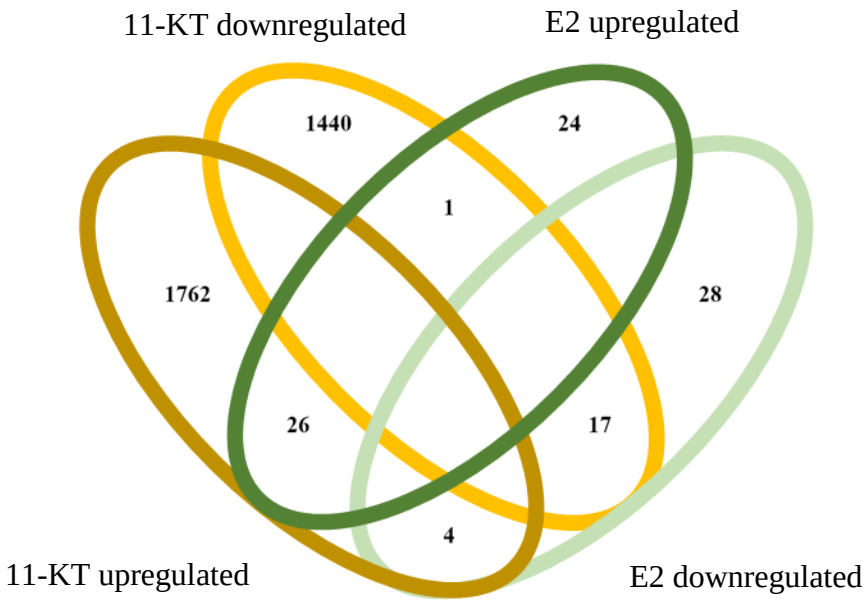

Figure 2.4. Comparison of genes that were significantly altered (DESeq2 p<0.1) by 11-KT and E2 treatment in the coho salmon ovary. Venn diagram illustrating the number of differentially expressed genes (annotated to human or zebrafish orthologs) in common between 11-KT (dark and light yellow) and E2 treatment (dark and light green). Nearly half of the genes altered by E2 were also altered by 11-KT, and the expression of the majority of these was altered in the same direction between treatments.

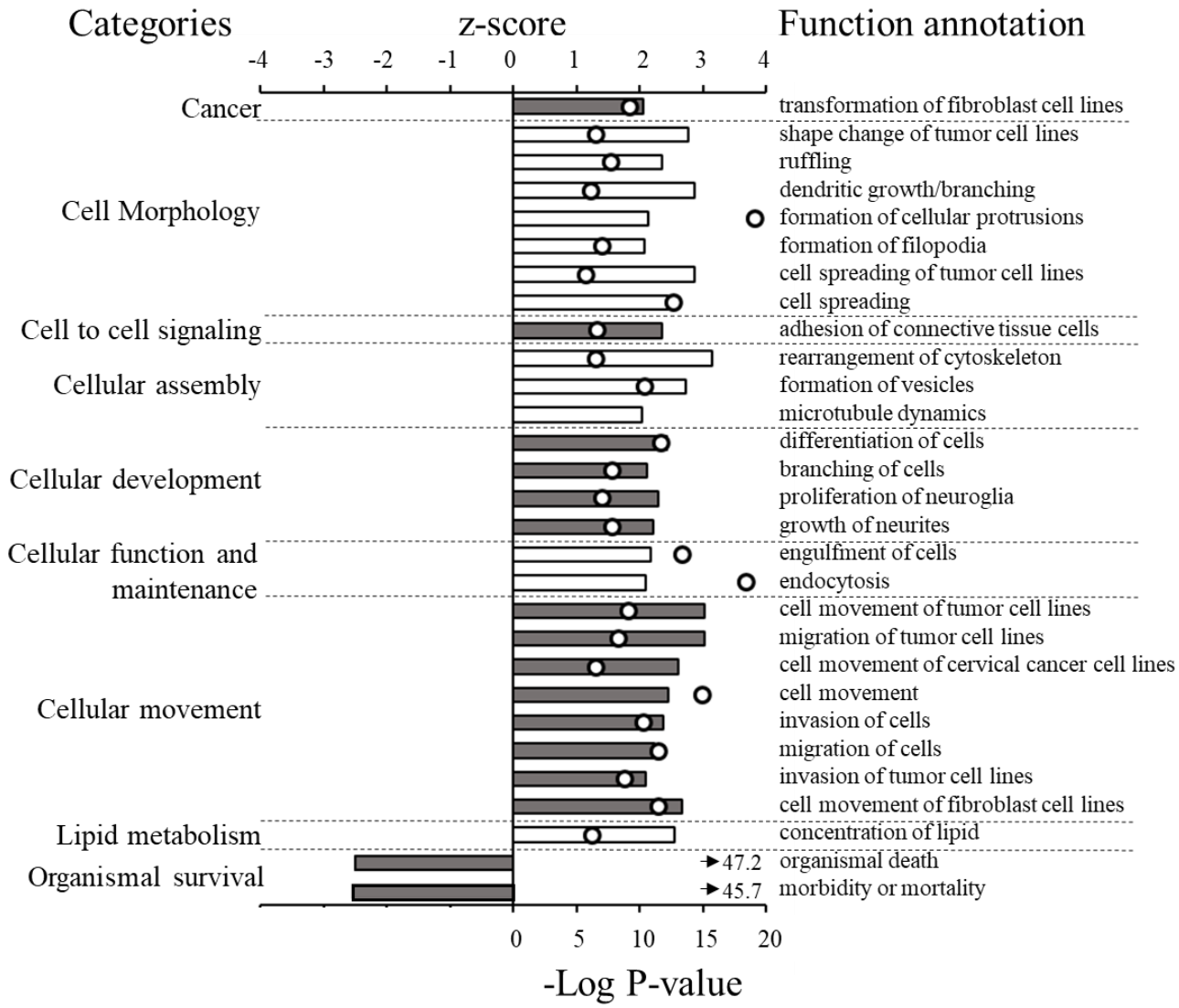

Figure 2.5. Biological functions in the ovary altered by 11-KT treatment. Many biological functions were predicted to be altered in response to 11-KT treatment by IPA pathway analysis, including cellular functions, movement, and morphology, as well as lipid metabolism. Functions are predicted to be activated or inactivated based on a positive or negative z-score (top axis, ≥2 or -2≤ is considered significantly predictive). The P-value (white dots, lower axis, $-\text{Log}_{10}$ P ≥1.3 [P ≤0.05]) indicates the likelihood that a function is accurately associated with the genes in our data set. Arrows indicate $-\text{Log}_{10}$ P-value is greater than the bounds of the axis. Bar color indicates biological functions within the same category.

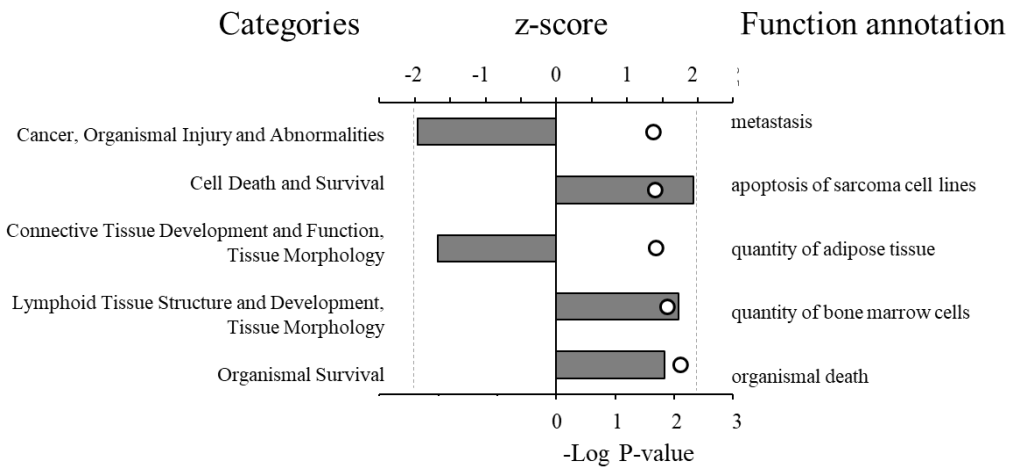

Figure 2.6. Biological functions in the ovary altered by E2 treatment. Relatively few biological functions were closely associated with E2 treatment and none were considered significantly altered. Functions are predicted to be activated or inactivated based on a positive or negative z-score (top axis, ≥2 or -2≤ is considered significantly predictive). The P-value (white dots, lower axis-Log_{10} P ≥1.3 [P ≤0.05]) indicates the likelihood that a function is accurately associated with the genes in our data set.

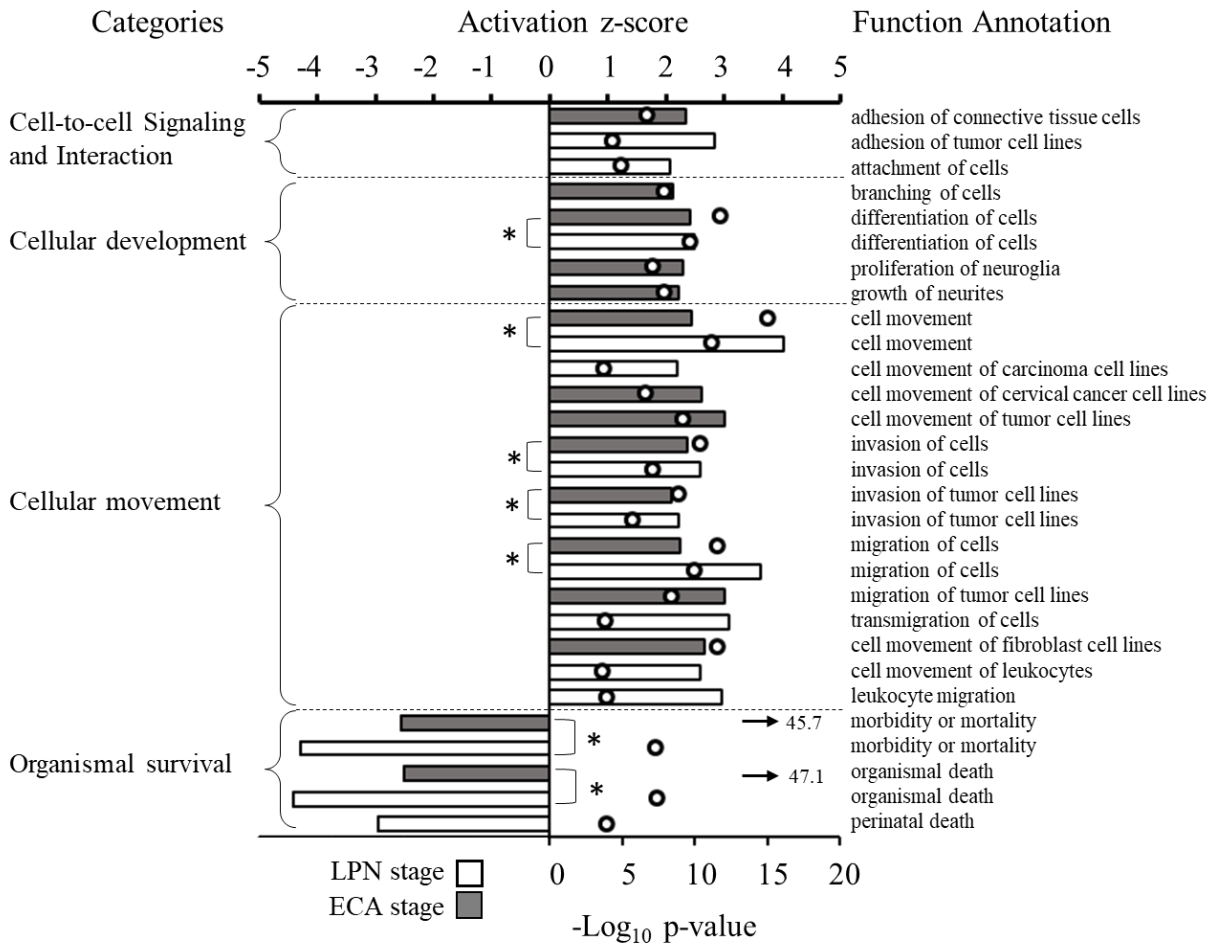

Figure 2.7. Altered biological functions in the ovary from categories in common between 11-KT treated primary and early secondary growth coho salmon. Many of the same processes were predicted to be altered by 11-KT after 3-days at both late primary growth (white bars) and early secondary growth (gray bars) stages. A previously published DEG list [9] was interrogated using the IPA biological function analysis and compared with the biological functions predicted to be altered in the current study. A positive or negative z-score (top axis, ≥2 or -2≤ is considered significantly predictive). The P-value (white dots, lower axis-Log_{10} P ≥1.3 [P ≤0.05]) indicates the likelihood that a function is accurately associated with the genes in our data set. Arrows indicate –Log_{10} P-value is greater than the bounds of the axis. Starred functions were significantly altered at both stages.

CHAPTER 3: In vivo treatment with 17α-ethinylestradiol alters the ovarian transcriptome in previtellogenic coho salmon.

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Introduction

Estrogens are ancient signaling molecules. The genes encoding the aromatase enzyme (cytochrome P450, family 19, subfamily a; Cyp19a) and estrogen receptors (Esr), that mediate the synthesis and biological action of endogenous steroidal estrogens, respectively, are conserved in vertebrate species, and the vertebrate ancestral steroid receptor likely functioned as an estrogen receptor [1,2]. It is no surprise then, that concomitant with the increasing complexity of vertebrates through evolution, estrogen signaling has attained a remarkable diversity of functions. In fish, endogenous steroidal estrogens, predominantly estradiol-17β (E2), have taken on a central role in many reproductive functions in female development. E2 has been implicated in gonadal sex determination in many species (reviewed in [3]) since E2 synthesis is necessary for ovarian differentiation and blocking E2 synthesis results in testis formation. Oogonial proliferation is induced by E2, an action that is blocked by inhibiting E2 synthesis [4]. During primary and previtellogenic secondary development, E2 stimulates the formation of cortical alveoli and is particularly effective in promoting growth of the secondary ovarian follicle [5,6]. The maintenance of the ovarian phenotype is also under the control of E2 since disruption of E2 synthesis causes post-pubertal medaka females to undergo sex reversal [7]. The most well defined role for E2 in ovarian growth and function is in the stimulation of hepatic vitellogenin (Vtg) production (reviewed in Lubzens et al. [8]), the yolk protein precursor that is cleaved and incorporated into developing oocytes. Additional control of reproductive development by E2 occurs through feedback on the brain, increasing gonadotropin releasing hormone (Gnrh) content

by modulating the expression of kisspeptins (*kiss1* and *kiss2*), and both kisspeptin and neurokinin B receptors (*kiss1ra*, *kiss2r*, *tac3ra* and *tac3rb*) [9,10], and on pituitary function by decreasing follicle stimulating hormone (Fsh) and increasing luteinizing hormone (Lh) synthesis or release [11]. Given the abundance of effects of endogenous estrogens, exogenous estrogenic chemicals could cause disruption along many points of the hypothalmus-pituitary-ovary-liver (HPOL) axis.

Many contaminants in the environment can severely affect internal homeostasis and endocrine processes in aquatic species. The term endocrine disrupting chemical (EDC) refers to any exogenous chemical contaminant that alters normal endocrine function by mimicking or blocking endogenous hormonal synthesis, secretion, transport, action, or elimination [12,13]. Many EDCs have been shown to be either estrogenic or to antagonize estrogen receptor (Esr) signaling [14]. The large number of studies on xenoestrogens in fish is probably due to the characterization of an easily measured biomarker of estrogen exposure, the induction of Vtg synthesis. Early evidence of environmental endocrine disruption was measured by the aberrant expression of hepatic *vtg* [15–17], and the feminization [18] of male fish downstream from wastewater treatment plants.

A synthetic steroidal estrogen, 17α-ethinylestradiol (EE2), is an ingredient in oral contraceptives and commonly found in the aquatic environment due to effluent from sewage treatment plants or septic systems. EE2 was designed to have a long half-life and high estrogen receptor affinity. It is both not easily metabolized and is lipophilic, and thus can accumulate in body lipids [19]. Worldwide, EE2 is commonly found in surface waters at levels around 10 ng/L, but has been observed in concentrations as high as 273 ng/L [20–23]. EE2 is a potent agonist of estrogen

receptors (Esr), with equal or higher (10x) binding affinity than E2 in fish [24], and may be up to 30-fold higher than endogenous estrogens [25].

In laboratory experiments on various fish species, acute EE2 exposure induced the expression of *vtg* or synthesis of Vtg in vivo [20,26–31] and in vitro [32–34], and chronic exposures have caused reproductive failure [21] and complete feminization followed by population collapse [35]. These results are in line with the effects from exposure to wastewater effluent, in which EE2 was present [36].

The laboratory exposure approach identified only a small suite of biological responses, most notably the increase in hepatic expression of *vtg*, which have been used in further lab and field-based studies to demonstrate exposure to xenoestrogens. However, these studies largely ignored the overall biological responses. Rather, they aimed to develop and evaluate estrogen sensitive biomarkers.

Several studies have identified the effects of EE2 on the brain and pituitary, including alterations in the expression of genes involved in many cell processes: cell signaling, cell metabolism, cell growth [37], and cholesterol biosynthesis [38]. In fish, EE2 appears to affect the expression of genes involved in the regulation of circadian rhythm [38,39] and dramatically increases luteinizing hormone beta subunit (*lhb*), and decreases follicle stimulating hormone beta subunit (*fshb*) expression in the pituitary [39]. EE2 also decreases *lhb* expression in the pituitary in mature zebrafish [40] and in pituitary cells from mature medaka, mRNA levels for *lhb* and *fshb* were altered by all estrogenic EDC treatments tested [41]. These effects may lead to downstream

impacts on ovarian development and function. Fsh, in particular, plays an important role in ovarian follicle development. Fsh stimulates ovarian E2 production via the regulation of expression of steroidogenic enzymes [42,43] and E2 subsequently feeds back on the brain and pituitary, and can affect gonadotropins that are important for ovarian growth [11]. The effects of EE2 on aspects of these processes suggests that it could have consequences downstream of the pituitary in the HPOL axis.

In the ovary, several transcriptomic studies found that EE2 disrupted the expression of genes involved in cell cycle progression and mitochondrial function in maturing female zebrafish [44], and altered steroidogenesis in rare minnow [45] and largemouth bass [46]. However, the ovaries of these species contain ovarian follicles that develop asynchronously, potentially confounding interpretation of results from this highly dynamic tissue. An in vitro exposure using previtellogenic ovaries of Atlantic salmon [47], a species that undergoes synchronous ovarian development, also identified alterations in steroidogenic potential using a targeted gene expression approach. Overall, previous studies have shown that EE2 disrupts normal reproductive development, affects gonadal differentiation, alters steroidogenesis and steroid receptor signaling, and can potentially reduce reproductive output. However, there is still a need for a comprehensive analysis of the impacts of EE2 on ovarian gene expression, especially at specific developmental stages, to determine the mechanisms underlying these documented effects.

The follicle cell layers surrounding the oocyte, comprised of granulosa cell and theca cells, and the extracellular matrix, provide biochemical and mechanical support for the developing oocyte

throughout previtellogenic ovarian development. Endogenous sex steroids that are synthesized locally in this follicle layer participate in regulating ovarian gene expression, and provide hormonal feedback on the brain and pituitary, modulating the release of pituitary gonadotropins Fsh and Lh, which in turn modulate sex steroid synthesis in the ovary [8,48]. Exposure to low physiological levels of estrogens in vivo have been shown to decrease the expression of transcripts encoding steroidogenic proteins in rainbow trout ovaries [49], reducing steroidogenic capacity of the ovary. Thus, EE2 might impact both endogenous estrogen, androgen, and progestin signaling. Additionally, the expression of a number of ovarian follicle genes is regulated by E2 ([5]; unpublished RNA-Seq data), and a number of genes that encode structural proteins and contain androgen or estrogen response elements are expressed in the ovary throughout previtellogenic development [50]. Although differential effects between EE2 and E2 on gene expression have been reported [51], there is considerable overlap in biological actions and EE2 is up to 10 times more estrogenic [25]. Since E2 is essential to the development of secondary previtellogenic and vitellogenic follicles, and also exerts negative feedback to suppress Fsh secretion, EE2 could potentially disrupt reproductive processes dependent on Fsh or E2 signaling, including steroidogenesis in the previtellogenic ovary.

The aim of this study was to identify the effects of waterborne exposure to environmentally relevant concentrations of EE2 on ovarian gene expression in post-smolt coho salmon, a stage when they migrate to the ocean through urban waterways. This is also a stage when coho salmon ovarian follicles are in late primary growth and transitioning to early secondary growth. The coho salmon model provides a platform to identify the effects of EE2 on ovarian morphology and function. Coho salmon are a semelparous species, spawning a single time prior to expiry,

thus ovaries contain a single cohort of ovarian follicles that follow synchronous development. This allows for the analysis of effects at specific developmental stages. Disregulation of ovarian gene expression in response to EE2 could lead to reductions in fecundity, egg quality, or interrupt the seasonal checkpoints that determine future spawning success. Perturbation during previtellogenic growth in the expression of genes that are necessary for later processes such as vitellogenesis could impact egg quality or subsequent embryonic development. This study was done in conjunction with Harding et al. [39], in which the expression of hundreds of genes in the pituitary of coho salmon was altered by EE2 exposure, including significant induction of pituitary *lhb* expression and other genes involved in gonadotropin releasing hormone (Gnrh) signaling. Thus, EE2 could alter function at multiple parts of the gonadotropin signaling pathway.

Methods

Animal maintenance

Juvenile coho salmon from the Issaquah Hatchery stock were reared as previously described in Harding et al. [39], at the Northwest Fisheries Science Center hatchery facilities (Seattle, WA) in recirculated 10–10.5° C fresh water under a simulated natural photoperiod. Fish were fed a standard commercial diet according to manufacturer's guidelines. At approximately 16 months of age, fish were transferred to Battelle Pacific Northwest National Laboratory, Marine Science Laboratory (MSL, Sequim, WA) and over a 14-day period, were acclimated to seawater. Morbidity and mortality occurring over the course of the study were assumed to be a result of failure to acclimate to seawater. Water quality parameters were monitored throughout the study.

All fish were maintained according to the guidelines established by the Institutional Animal Care and Use Committee of Battelle.

In order to identify sex, fish were anesthetized in buffered tricaine methanesulfonate (0.05% MS-222; Argent Laboratories, Redmond, WA) and tagged with passive-integrated transponder (PIT) tags. Fin tissue was collected and analyzed for genetic sex using a molecular marker for the Y chromosome [52].

Waterborne Exposure

The waterborne exposure was previously detailed in Harding et al. [39]. Briefly, concentrated 17α-ethinylestradiol (EE2, >99% purity, Sigma-Aldrich Chemical Co., St Louis, MO) aqueous stock solution at 240 µg/L was added to exposure tanks with a peristaltic pump at a flow rate of 7–9 ml/h to achieve the nominal concentration of 12–15 ng/L. Fish in the previtellogenic stage (cortical alveolar stage) of secondary ovarian follicle growth were randomly assigned to 370-L circular fiberglass tanks (2 tanks/treatment, 10 females and 10 males/tank) and exposed continuously to control or EE2-treated seawater for up to 46 days. Control and EE2 exposure water samples were analyzed at four different times during the six-week exposure experiment. There was no EE2 detected in control tanks and the time-weighted age concentrations for EE2 exposure tanks were 17.7 and 16.4 ng EE2/L.

Sample collection

After 1 week and approximately 6 weeks (46 days) after the start of EE2 treatment, 4 females per treatment were euthanized in buffered MS-222. Fork length, body weight, and gonad weight

were recorded and gonadosomatic index (GSI) was calculated. Blood was collected with heparinized 1-ml syringes and plasma separated by centrifugation at 1000 x g for 15 minutes. One ovary was flash frozen in liquid N2 for later RNA isolation, and the other was fixed in 0.5 mL of HistoChoice™ tissue fixative (AMRESCO, Solon, OH) for 72 hours followed by Bouin's fixative for 24 hours for histology analysis.

Estradiol-17β radioimmunoassay

Plasma E2 levels were measured by radioimmunoassay (RIA) using the double antibody Estradiol-17β ^{125}I RIA kit (MP Biomedicals LLC, Solon, OH) according to the manufacturer's instructions. This kit has previously been validated and used to measure circulating E2 in coho salmon plasma [52].

Histological analysis

Fixed ovaries were dehydrated in increasing concentrations of ethanol, followed by two washes in xylene, and embedded in paraffin wax. Sections with a thickness of 5 µm were cut and mounted on microscope slides and stained with hematoxylin and eosin. Ovarian stage was scored based on previously published criteria [6,52]. Average ovarian follicle volume was calculated from 15 follicles per samples that were sectioned through the nucleus, using image analysis software (NIS-elements, Nikon, USA).

RNA extraction

Total RNA was extracted from frozen ovaries using Tri-Reagent (Molecular Research Center, Cincinnati, OH) according to manufacturer's instruction. RNA yield was assessed using a

Nanodrop ND-1000 (ThermoFisher Scientific, Waltham, MA). Equal quantities of total RNA from each sample (four control and four EE2 treated individuals each collected at 1 and 6 weeks) were submitted to the University of Washington Hi-Throughput Genomics Unit for quality checking, library preparation, and 36 bp single end sequencing.

RNASeq and pathway analysis

Bioinformatics were performed using the DRAP pipeline as described in Cabau et al.[53] and Monson et al. [50]. Briefly, sequences were quality trimmed using Trim Galore v0.4.0 [54] and assembled into a de novo backbone with Drap v1.8 [53] and Oases v0.2.09 [55]. Contiguous sequences (contigs) that had FPKM (fragments per kilobase of transcript per million mapped reads) greater than 1 and had sequence lengths greater than 200 bp were retained. These contigs were annotated using BlastX against the NCBI non-redundant protein database (nr) and partially non-redundant nucleotide database (nt). Gene level count estimates were made using RSEM v.1.2.31 [56] and bowtie2 v2.2.6 [57] and differential expression was determined using DESeq2 [58]. Contigs with a P-adjusted (P-adj) value ≤ 0.1 were considered significantly altered between control and EE2 treatment. Gene clustering at 1 and 6 weeks was performed using cluster::agnes package in R with the Spearman method [59] following log2 transformation. Data was centered on a mean expression value to improve visualization of expression differences.

Ingenuity Pathway Analysis® (IPA) software was used to conduct pathway and network analyses and predict the effects of steroid treatment on biological functions, as described in Monson et al. [50]. Contigs were initially mapped to zebrafish orthologs using BLASTN against the Ensembl *Danio rerio* gene database (v.Zv9.72). However, some zebrafish genes have not

been mapped to mammalian orthologs, so the remaining contigs were mapped to the *Homo sapiens* transcript database (v.GRCH37.72) for inclusion in IPA. If more than one contig ($P \leq 0.05$) mapped to the same gene, the average expression value of those contigs was used as the gene expression value in further analyses. The expression patterns of the zebrafish and human gene orthologs were compared to the IPA database to estimate altered canonical pathways and biological functions (Fisher exact test $P \leq 0.05$ [-log10 P-value ≥ 1.3]). Zebrafish nomenclature is used throughout, although due to the use of this software, human gene names are used in places where annotation to the zebrafish database was not possible.

Results

Morphometrics and ovarian stage

There were no significant differences between control and EE2 treated fish at either 1 or 6 weeks in body weight, fork length, or GSI. However, a significant increase was observed in the GSI of control and treated fish between 1 and 6 weeks, corresponding to a slight, but not statistically significant advancement in ovarian stage at 6 weeks. Ovarian follicle stage was variable across all individuals (ranging from late perinucleolar to late cortical alveolus stages). In the fish collected for sequencing, no difference was observed in ovarian follicle volume (Fig. 1A) or in ovarian stage (Fig. 1B); fish displayed an early cortical alveolus phenotype (Fig. 1B) (data previously published in Harding et al. [39]).

Illumina sequencing and RNA-Seq analysis

Sequencing of the ovarian transcriptome resulted in 1,205,972,329 single end reads with an average length of 36 bases. All reads were retained after quality trimming. De novo backbone

assembly generated 58,270 annotate contigs ranging from 201 to 18,572 bases with a mean of 991 bases. RNA-Seq analysis identified 279 and 30 contigs that were differentially expressed between control and EE2 treatment groups (DESeq2, P-adj <0.1, |fold change| >1.5) after 1 (Fig. 2A) and 6 weeks (Fig. 2B), respectively. Cluster analysis of significantly altered contigs indicated clear distinctions between control and EE2 treatment groups at week 1 (Fig. 3A) and week 6 (Fig 3B).

Gene level analysis

In order to identify presumptive biological functions and pathways in the ovary that are affected by EE2, zebrafish and human orthologous genes were used to interrogate the IPA Knowledgebase. Following annotation, 135 differentially expressed genes (DEGs, DESeq2, P-adjusted <0.1) at 1 week (Table 1) and 17 DEGs at 6 weeks (Table 2) were identified. After 1 week of EE2 treatment, the expression of 70 genes was increased and 65 showed a decrease, and after 6 weeks of EE2 treatment, the expression of 8 genes was increased and 9 genes exhibited decreased expression (Fig. 4). At 1 week, the expression of three follicular genes involved in ovarian steroidogenesis was decreased in the ovary: *cytochrome p450 family 17 subfamily a member 1* (*cyp17a1*, -1.48 fold, P-adj <0.08), *hydroxysteroid (11-beta) dehydrogenase 2* (*hsd11b2*, -1.52 fold, P-adj <0.05), and *cytochrome p450 family 19 subfamily a member 1* (*cyp19a1*, -1.60 fold, P-adj <0.02).

After 1 week (Table 3), 59 canonical pathways that were significantly associated with EE2 treatment (P <0.05; $-\text{Log}_{10}$ P >1.30) were identified. The most highly associated canonical pathways were hepatic fibrosis/hepatic stellate cell activation ($-\text{Log}_{10}$ P =4.83), endoplasmic

reticulum stress pathway ($-Log_{10}$ P =3.62), and axonal guidance signaling ($-Log_{10}$ P =3.46). Two canonical pathways were putatively activated by EE2 treatment, according to the calculated IPA z-score: both sphingosine-1-phosphate signaling, and endothelin-I signaling had z-scores =2. After 6 weeks of treatment, 9 canonical pathways in the ovary were significantly associated with E2 treatment (Table 4), although none were assigned a z-score.

Putative biological functions in the ovary were altered after exposure to EE2 for 1 week and 6 weeks. After 1 week, 500 biological functions were significantly associated with EE2 treatment ($-Log_{10}$ P >1.30), 8 of which were considered significantly altered ($|z| \geq 2$) in categories of behavior, cancer and organismal injury, cellular movement, endocrine system development and function, protein synthesis, and tissue development (Figure 5). At week 6, 199 biological functions were significantly associated with the EE2 treatment, although none were significantly altered.

Comparison of the ovarian transcriptome of control fish after 1-week and 6-weeks

After 6 weeks of culture in treatment tanks, the expression of 320 contigs (Fig. 6) that could be mapped to 236 zebrafish or human orthologous genes were identified in the ovary that were differentially expressed compared to the 1-week control samples. The most highly altered transcripts encode *translocator protein* (3.35 fold proteins, P-adj <0.01), *homeobox c13* (2.44 fold, P-adj <0.001), *hepatocyte growth factor* (-2.34 fold, P-adj <0.001) and *potassium calcium-activated channel mb2* (-2.30 fold, P-adj <0.001). The expression of the gene encoding ovarian *cyp19a1* was also decreased by 2.01 fold (P-adj <0.05) in the 6-week samples.

Four canonical pathways out of 219 significantly associated with the 6-week controls were predicted to be significantly inhibited: cardiac hypertrophy signaling, Rac (Gtpase) signaling, hepatocyte growth factor (Hgf) signaling, and p53 signaling (-Log10 P >1.30) had activation z-scores <-2. Significant inhibition (z-score ≤-2) of 27 biological functions (Fig. 7) in categories of cancer and organismal injury, cardiovascular system development and function, cell death and survival, cell morphology, cell to cell signaling, cellular assembly and organization, cellular movement, gene expression, and lipid metabolism and small molecule biochemistry was identified at 6-weeks. A single biological function, in the category of organismal survival, was predicted to be activated, organismal death ($-Log_{10}$ P =2.40, Z-score =2.36).

Discussion

The effects of EE2 on the brain, liver, and testis of teleosts are fairly well characterized, but a comprehensive analysis of the potential effects on ovarian gene expression is lacking. In the current study we used an RNA-Seq approach to identify alterations in the ovarian transcriptome after a short-term in vivo exposure of previtellogenic coho salmon to environmentally relevant levels of EE2. EE2 treatment resulted in the altered expression of 135 genes after 1 week, but far fewer after 6 weeks. The treatment was sufficient to induce hepatic *vtg* expression, verifying the effectiveness of the treatment in inducing classic estrogenic effects on the liver. But, there was no impact on endogenous E2 levels and no morphological signs of impact on ovarian follicles. Nonetheless, alterations to the ovarian transcriptome suggest potential functional consequences in in the ovary. Comparison of controls between 1 and 6 weeks revealed greater differences in expression than between control and treated samples at either time point. This suggests developmental changes over time were more profound than the effects of the EE2 exposure.

Effects on morphology and hepatic vitellogenin expression from 1 and 6-week EE2 treatment

In the present study, no significant differences were observed in ovarian follicle stage or GSI, and E2 levels were not altered at 1 or 6 weeks by EE2 treatment compared to controls (previously reported in Harding et al. [39]). This is in contrast to observations in other species in which ovarian morphology of zebrafish [60,61], the GSI of fathead minnow [28,62], zebrafish [60,61], and rare minnow [45], and plasma steroid concentrations in common roach [63] and zebrafish [64] were altered by treatment with environmentally relevant concentrations of EE2.

In iteroparous and semelparous salmonids that undergo seasonal breeding, estrogenic contaminants did not produce similar effects on the ovary. In female rainbow trout, in vivo treatment with E2 did not alter GSI [65], and treatment with nonylphenol did not affect ovarian stage [66]. In vitro EE2 treatment of previtellogenic Atlantic salmon ovarian fragments did not alter ovarian morphology, but did affect sex steroid production [67]. In contrast to these findings, both in vivo and in vitro exposures of previtellogenic secondary growth coho salmon ovarian follicles to the endogenous estrogen, E2, caused a marked increase in ovarian follicle volume [6,5], suggesting differences in the mode of action of E2 and exogenous estrogenic contaminants [51] in the ovary. Although, there were differences in study design between the current study and those described for Atlantic salmon or E2 treated coho salmon that may underly the disparity in results. The ovary of semelparous species such as coho salmon may also be less "stress sensitive" than other species, due to only having a single clutch of eggs and thus only a single chance to ovulate. In support of this idea, a study using previtellogenic coho salmon, delayed

ovarian follicle progression and widespread atresia were not observed until after 14-weeks of nutritional stress [68,69].

Despite the lack of a direct impact on ovarian morphology, or sex steroid levels, the exposure levels of EE2 in our study were sufficient to induce hepatic *vtg* expression, at both 1 and 6 weeks [39], to similar levels reported in rainbow trout exposed to EE2 at a similar concentration [31]. This suggests that current treatment regime was sufficient to induce estrogen receptor signaling and alter gene expression patterns in the liver. In the pituitary glands of the same fish used in the current study, Harding et al., [39] identified 218 and 670 contigs that were significantly altered at 1 and 6-weeks, indicating major impacts of this treatment on the pituitary.

Steroidal androgens and estrogens have been shown to directly affect primary and previtellogenic secondary ovarian follicle growth in coho salmon (Chapters 1 and 2), suggesting that this developmental point could potentially be impacted by contaminants that alter steroid signaling. In vitro treatment of ovarian fragments from these stages with 11-KT or E2 caused rapid increases in ovarian follicle volume [6], and in vivo treatment of juvenile female coho salmon with E2 led to significant growth of ovarian follicles after 10 days [5,50] and major alterations in ovarian gene expression. Many of the genes altered by 11-KT or E2 encode proteins involved in reproductive development, hormone synthesis, and extracellular matrix components. The expression of numerous other genes involved in cell signaling, and other cellular processes was also altered. Thus, we expected EE2 would have similar effects to E2 on the ovarian transcriptome. Surprisingly, EE2 altered the ovarian transcriptome but the suite of genes affected was considerably different than those altered by E2.

The effects of EE2 on the ovarian transcriptome

The expression of 269 contigs that could be mapped to 135 genes at 1 week and 36 contigs that could be be mapped to 17 genes at week 6 was altered by in vivo EE2 treatment. This is similar in magnitude to the effects of EE2 on the transcriptome of the ovary [44–46], or other tissues [30,39,44,70–72] in various species of fish.

Effects of EE2 on steroidogenic enzymes. Among the most highly altered genes after 1 week were those encoding steroidogenic enzymes. The expression of cytochrome P450 family 17 subfamily A member 1 (*cyp17a1*) and cytochrome P450 family 19 subfamily A member 1 (*cyp19a1*) was significantly decreased. These genes are integral to the production of sex steroids. Cyp17a1 has a dual function: it has both 17α-hydroxylase activity and 17,20-lysase activity, and catalyzes the conversion of progesterone to 17α-hydroxyprogesterone (17α-OHP) and then into androstenedione, respectively. 17α-OHP is an immediate precursor of C19 androgens (e.g., testosterone [T]), in the sex steroid biosynthesis pathway. Cyp19a1 is the enzyme responsible for the conversion of T to E2. Thus, EE2 may impact the steroidogenic potential in previtellogenic coho ovary, specifically reducing the capacity to produce E2. These results are similar what was observed in an acute and short-term in vitro exposure of juvenile Atlantic salmon ovarian fragments to EE2; the expression genes involved in the initial rate-limiting steps of steroidogenesis, *steroidogenic acute regulatory protein* (*star*) and *cytochrome P450, subfamily XIA, polypeptide 1* (*cyp11a1*, also known as *p450scc*) as well as *cyp19a1* was decreased [67], although *star* was not altered in the current study. In contrast, *p450scc* expression was increased

in zebrafish by EE2 in vivo [44] and in Atlantic cod ovaries by the weak estrogen 4-nonylphenol in vitro [67,73].

The ovarian expression of *hsd11b2* was also decreased by EE2 treatment after 1 week, further indicating disruption in steroidogenic capacity. The protein encoded by *hsd11b2* is required for the production of 11-oxygenated androgens, such as the teleost androgen 11-KT, and also the catabolism of cortisol to cortisone. At primary and previtellogenic secondary growth stages, 11-KT has been shown to dramatically promote ovarian follicle development in shortfinned eel [74], cod [75], and coho salmon [6,50]. Additionally, 11-KT treatment in vivo ([50]; unpublished RNA-Seq data) alters the expression of hundreds of genes in the ovary involved in various cellular and metabolic processes, including vitellogenin and lipid uptake. Thus, a reduction in ovarian 11-KT levels could affect ovarian follicle growth and although EE2 is known to induce *vtg* expression in the liver, effects on the ovary could disrupt subsequent uptake into the developing oocyte.

Ovarian canonical pathways and biological functions affected by EE2. In order to identify possible perturbation of cellular processes and functions, the IPA Knowledgebase was interrogated using the genes and expression values identified by RNA-Seq. Signaling pathways were the most prevalent (34/59) canonical pathways associated with EE2 treatment at 1 week. Indeed, the majority of the most highly associated pathways (by P-value) with EE2 treatment contained one or both of the genes phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*pik3ca,* 1.23-fold increase) and G protein subunit alpha q (*gnaq,* 1.63-fold increase). These genes encode orthologs to mammalian proteins that are involved in well-known

signaling mechanisms. Pik3ca (also known as P110α) is a phosphoinositide 3-kinase (Pi3k) subunit that can activate conserved signal transduction pathways (Pi3k/Akt/mTor). Gnaq is a subunit of a class of G proteins (Gq) that activate Phospohlipase C (Plc), which is a class of intracellular enzymes involved in signal transduction. Two canonical pathways were predicted to be significantly activated by EE2 treatment using the IPA z-score algorithm. Sphingosine-1-phosphate signaling and endothelin signaling had z-scores =2. Signaling through these pathways regulates many cellular processes including cell migration, differentiation, and survival (reviewed in Davenport et al. [76] Patmanathan et al. [77]). Fewer pathways were associated with EE2 treatment after 6 weeks, and were identified by only one (8/9) or two (1/9) genes.

The biological function application of IPA was used to interrogate known interactions between gene products and known cause-effect relationships between genes to help identify potential biological responses to EE2 in the ovary. IPA identified eight biological functions at 1 week that were predicted to be significantly altered by EE2. Notably, functions of *synthesis of hormone*, *metabolism of protein*, *synthesis of protein*, and *formation of gland* were all predicted to be inactivated (z-score <-2.0), supporting the hypothesis that EE2 has a direct impact on normal ovarian processes. Additional functions in categories of behavior and cancer were predicted to be inactivated and functions in categories of embryonic development, organismal development, organismal survival, and post-translational modification were assigned z-scores that indicate near-significant inactivation. A single biological function was predicted to be activated, *cell movement of leukocytes* (z-score >2), although positive z-scores of other functions in the categories of cell movement, cellular development, cell-to-cell signaling, and cell morphology were near significant. Interestingly, biological functions in these categories were also predicted

to be activated by short-term 11-KT treatment in previtellogenic secondary ovarian follicles (unpublished RNA-Seq data).

Effects of EE2 on the reproductive axis. The reduced capacity for the ovary to produce sex steroids has implications on normal hormonal signaling processes across the hypothalamus-pituitary-ovary-liver (HPOL) axis. In female fish and other female oviparous vertebrates, environmental signals are transduced by the hypothalamus, triggering pituitary synthesis and release of gonadotropins (Fsh or Lh) by Gnrh. Circulating gonadotropins signal through receptors on somatic cells surrounding the ovarian follicle to produce E2, which in turn stimulates hepatic *vtg* expression. The hormones synthesized and released throughout the HPOL axis participate in both positive and negative feedback loops, creating an intricate network of pathways that leads to temporal synchronization in the production of functional fertilizable eggs (see reviews by Lubzens et al. [48,78]). Alterations along any point of the HPOL axis can affect the development of a functional oocyte and may impact fecundity.

In addition to the transcriptome sequencing performed on the ovary in the current study, RNA-Seq was performed on the pituitary from the fish in the same study (reported in Harding et al. [39]). In the pituitary, EE2 dramatically altered the expression of genes and pathways related to reproduction. The expression of transcripts encoding the gonadotropin subunit *lhb* (dramatic increase) and *fshb* (decrease) was altered, as well as transcripts encoding genes involved in Gnrh signaling pathway, possibly contributing to the dramatic *lhb* induction. These results, and the reduced expression of steroidogenic enzymes in the ovary indicate that EE2 interferes with

multiple levels of the HPOL axis and could potentially disrupt the fine-tuning necessary to control reproductive development.

The impact of EE2 on the ovary could be direct or indirect through impacts on gonadotropin secretion [39], which regulate gonadal steroidogenesis. The expression of pituitary *lhb* is generally low in sub-adult salmonids and plasma levels remain low or undetectable until late vitellogenesis, peaking during final oocyte maturation and ovulation [79–83]. In contrast, Fsh levels rise during late primary and early secondary growth, peaking during vitellogenesis and declining prior to ovulation [52,84,85]. Plasma levels of E2 follow the trend of Fsh [85] and indeed while Lh has been shown to be equipotent in stimulating E2 production in vitro [84,86], Fsh is primarily responsible for the increase in plasma E2 in vivo [52,82,85]. Fsh has also been implicated in Vtg uptake [87], and regulates a number of genes in the ovarian follicle related to growth and development [42,43,88]. There is no evidence so far that the experimental induction of Lh beta has biological consequences. Sex steroids also may indirectly regulate Gnrh synthesis and thus indirectly regulate gonadotropins, through Kisspeptin/Nkb [9,10].

Estrogen signaling in the ovary

Direct effects of EE2 on the ovarian transcriptome would be expected to occur via classical nuclear and/or membrane estrogen receptors. In the current study, the only *esr* isoform expressed in the ovary at detectable levels was *esr2b*/*erβ2*, identified by the mapping of three contigs in our de novo backbone (annotated to the zebrafish ortholog *esr2*). An additional contig was also identified as the membrane receptor *g-protein coupled estrogen receptor 1* (*gper1*). Thus, it is a reasonable assumption that any direct transcriptional effects of EE2 on the ovary were mediated

by either or both Esr2b or Gper1. However, it is likely that at least some of the transcriptional changes in the ovary were a result of systemic effects due to use of an in vivo model, especially as other factors in the reproductive axis were significantly altered after 1 week [39].

Estrogen signaling occurs through the classical (genomic) binding of Esr isoforms or through binding of membrane associated Esr or Gper. In genomic signaling, the ligand activates nuclear Esr to bind estrogen response elements, thereby regulating transcription of target genes. Non-genomic estrogen signaling can occur when ligands bind membrane-associated receptors, subsequently activating rapid downstream signaling through cAMP and protein kinases (Mapk, Pi3k, Pkc). There is additional evidence in mammals for estrogen signaling mechanisms through a likely receptor for estradiol-17α (estrogen receptor X), or through a splice variant of Esr1 (ERα36) that binds a GPER-specific agonist and is activated by antiestrogens (see review by Soltysik and Czekaj [89]). Thus, the view of estrogen signaling through estrogen receptors as a simple transcriptional activator is a likely a gross oversimplification, and there is a great deal yet to be understood in the pharmacological properties and transcriptional consequences of estrogen signaling.

In the rainbow trout ovary, a salmonid in the same genus as coho salmon, there are four isoforms of the classical nuclear *esr* [90] that display different expression patterns throughout the reproductive cycle [91]. This is in contrast to most other teleosts, in which only three isoforms are present [92]. Early in ovarian follicle growth, *esr2* (*esr2a*/*erβ1* and *esr2b*/*erβ2*) isoforms were expressed at higher levels, corresponding to low plasma E2 and GSI, and to declining plasma Vtg levels from the previously ovulated clutch of eggs [91]. In the same study, the

expression of *esr1* isoforms (*esr1a*/*erα1* and *esr1b*/*erα2*), were generally low, only increasing (*erα1*) in the later stages of the reproductive cycle. This is in agreement with the results of the current study in which *esr2* was the only identified nuclear *esr*. In fish, two *gper* isoforms have been described. Although ovarian expression across development has not been characterized, major roles for Gper in final oocyte maturation [93], and the maintenance of meiotic arrest by estrogens [94] have been described (reviewed by Thomas [95]).

There have been several studies in fish that described differences in receptor activation by native and exogenous estrogens [92] or consequences of exposure on gene expression [51]. In general, activation of Esr isoforms differs only slightly between endogenous and exogenous estrogenic steroids, and transcriptional consequences have been attributed to differences in the bioactivity of endogenous steroidal estrogens or xenoestrogen contaminants. In fish, the only direct link between a biological function and an Esr isoform is the activation of Esr1a in the liver driving Vtg synthesis [96]. While other major biological effects of estrogens are well known, the receptor isoforms that mediate them is unclear.

Ovarian condition after 6 weeks

There was no histological indication of differences in the ovary after 6 weeks of culture. Ovarian follicle volume was unchanged and only a slight non-significant increase in the ratio of later stage follicles was observed [39], although GSI was increased in both control and EE2 treated fish at 6 weeks compared to 1 week. The ovarian transcriptome, however, was significantly altered in the 6-week control fish compared to the 1-week control fish and more contigs were significantly altered in control fish between week 1 and week 6 than by EE2 treatment at either 1

or 6 weeks. Thus, developmental differences over time were greater than EE2 effects at either time point.

Among the most downregulated transcripts at 6-weeks were *aromatase* and *insulin-like growth factor 1 receptor* (*igf1r*), the former encoding the enzyme responsible for E2 production and the latter mediating the signal of insulin like growth factor-1 (Igf1). Both Igf1 signaling and the expression of ovarian *cyp19a1* are known to increase during previtellogenic secondary growth [78]. Although ovarian *igf1r* expression was decreased by short-term Fsh treatment in vitro [42], an increase in circulating Fsh and the subsequent expression of *cyp19a1*, and increase in plasma E2 levels are hallmarks of secondary growth. Igf1 signaling has also been implicated in the regulation of the capacity for E2 biosynthesis [97,98]. Thus, a decrease in *cyp19a1* and *igf1r* expression could indicate impairment of normal ovarian endocrine functions. The expression of *cyp19a1* was reduced in fasted juvenile coho salmon, but only after 14-weeks [69], prior to any morphological indications of fasting effects on the ovary. In the same fish, transcripts for kruppel-like factor 6 (*klf6*) were increased in fasted fish. In the present study, the expression of a related zinc finger transcription factor gene, kruppel-like factor 5 (*klf5*) was increased after 6-weeks in control fish. The precise function of Klf5 in teleosts is unknown, although in mammals, the KLF5 protein interacts with tumor protein 53 (TP53) to regulate the expression of *survivin*, an anti-apoptotic gene [99]. The increased *klf5* expression in the present study may be an indication of the activation of anti-apoptotic processes in the ovary. Mammalian TP53 also suppresses *Igf1r* expression [100].

Pathway analysis identified 28 biological functions in the ovary that were significantly altered at 6 weeks compared to 1 week in control fish. The majority of these functions were predicted to be inactivated, including functions in categories of cell signaling, cell assembly, cell movement, and cell morphology. Additionally, functions related to gene expression were predicted to be inactivated, which may explain the lower quantity of DEGs after six weeks of EE2 treatment compared to one week. The lone biological function predicted to be activated was organismal death. Overall, these predictions suggest that the ovarian function may be diminished after 6 weeks of culture. In contrast, our previous studies ([50]; unpublished RNA-Seq data) identified pathways in cell assembly, cell movement, and cell morphology categories that were predicted to be activated by 11-KT in short term in vivo treatment of late primary and early secondary coho salmon. At these developmental stages, 11-KT promotes growth of ovarian follicles [5,6]. Thus, the predicted inactivation of these pathways in the present study may indicate reduced ovarian growth during the six-week study. Given that the experimental fish did not grow in length or weight during the six-week study period [39] and other studies have shown that progression of previtellogenic growth is affected by body growth in coho salmon [52], this result is not altogether surprising. Since ovarian growth may have been impaired due to the poor body growth, the ovary may also have been less responsive to the exogenous EE2 by six weeks. The diminished effects of EE2 on the ovarian transcriptome as six-weeks versus one-week support this idea.

Conclusions

Short-term in vivo exposure to EE2 caused widespread changes in the ovarian transcriptome, reducing the expression of steroidogenic enzymes. Although ovarian morphology was

unaffected, pathway analysis indicated that EE2 affected cellular function and hormone synthesis in the ovary. Since EE2 is a potent Esr agonist, these results provide insight into estrogen actions on the ovary, and possible xenoestrogenic disruption of normal function.

This experiment was conducted as an in vivo exposure, therefore alterations in ovarian function could be due to systemic effects of EE2, in particular the effects on the pituitary. Circadian rhythm signaling was a major function altered in the pituitary [39], suggesting that EE2 may affect timing along the reproductive axis. The timing of reproductive development is especially important in a semelparous species, as maturation must correspond to a single seasonal spawning event. This experiment was carried out during a single ovarian follicle stage, cortical alveolus stage (previtellogenic secondary growth) and it remains to be seen what the long-term effects EE2 would be on ovarian morphology and fertility. The results were also confounded with poor growth of fish during the six week period, which appeared to inhibit progression of ovarian growth in control fish.

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Table 3.1. Changes in expression of ovarian genes after 1 week of EE2 treatment, identified by DESeq2 (base mean >10, fold change ≥1.5 or -1.5≤, P-adj <0.1). Gray shaded rows indicate genes that are discussed in the text.

Top contigs (base mean >10, adjusted *p*-value <0.1

Gene symbol	Gene title upregulated	Fold change	Adjusted *p*-value
pdgfra	platelet derived growth factor receptor alpha	1.96	0.000
nlgn3	neuroligin 3	1.74	0.002
sema4c	semaphorin 4C	1.71	0.002
evl	Enah/Vasp-like	1.71	0.001
gnaq	G protein subunit alpha q	1.63	0.006
tgm1	transglutaminase 1	1.61	0.013
gtf3c3	general transcription factor IIIC subunit 3	1.60	0.014
liph	lipase H	1.60	0.006
kcnk2	potassium two pore domain channel subfamily K member 2	1.60	0.005
wdr19	WD repeat domain 19	1.60	0.001
lrrc75b	leucine rich repeat containing 75B	1.59	0.015
myo9b	myosin IXB	1.57	0.015
plec	plectin	1.53	0.041
ibtk	inhibitor of Bruton tyrosine kinase	1.52	0.003
angpt1	angiopoietin 1	1.52	0.040
sfmbt1	Scm-like with four mbt domains 1	1.52	0.005
st8sia6	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 6	1.51	0.053
rassf5	Ras association domain family member 5	1.51	0.055
eno1	enolase 1	1.51	0.028
mfsd2b	major facilitator superfamily domain containing 2B	1.51	0.059
ednrb	endothelin receptor type B	1.50	0.056
fam83c	family with sequence similarity 83 member C	1.49	0.072
bcl6	B-cell CLL/lymphoma 6	1.49	0.065
nppc	natriuretic peptide C	1.47	0.099
gan	gigaxonin	1.47	0.099
gpr20	G protein-coupled receptor 20	1.47	0.099
parp8	poly(ADP-ribose) polymerase family member 8	1.46	0.005
calcrl	calcitonin receptor like receptor	1.46	0.089
antxr1	anthrax toxin receptor 1	1.46	0.083
celsr1	cadherin EGF LAG seven-pass G-type receptor 1	1.45	0.056
itga10	integrin subunit alpha 10	1.44	0.084

mgat4b	mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isozyme B	1.43	0.089
tenm4	teneurin transmembrane protein 4	1.43	0.056
npffr1	neuropeptide FF receptor 1	1.42	0.044
theg	theg spermatid protein	1.42	0.027
ccdc103	coiled-coil domain containing 103	1.42	0.088
tspo	translocator protein	1.41	0.018
glra2	glycine receptor alpha 2	1.40	0.088
kpna6	karyopherin subunit alpha 6	1.40	0.026
endod1	endonuclease domain containing 1	1.40	0.085
enpp6	ectonucleotide pyrophosphatase/phosphodiesterase 6	1.39	0.037
kif23	kinesin family member 23	1.38	0.038
calr	calreticulin	1.38	0.015
col4a3	collagen type IV alpha 3	1.38	0.055
ncoa3	nuclear receptor coactivator 3	1.38	0.012
hs3st1	heparan sulfate-glucosamine 3-sulfotransferase 1	1.38	0.044
crebrf	CREB3 regulatory factor	1.37	0.017
taok3	TAO kinase 3	1.37	0.004
itga4	integrin subunit alpha 4	1.36	0.099
gramd4	GRAM domain containing 4	1.36	0.024
fam60a	family with sequence similarity 60 member A	1.35	0.016
glod4	glyoxalase domain containing 4	1.35	0.083
hps1	HPS1, biogenesis of lysosomal organelles complex 3 subunit 1	1.34	0.055
kctd2	potassium channel tetramerization domain containing 2	1.34	0.089
col5a2	collagen type V alpha 2	1.34	0.056
adamts8	ADAM metallopeptidase with thrombospondin type 1 motif 8	1.34	0.099
atxn7	ataxin 7	1.33	0.004
pigh	phosphatidylinositol glycan anchor biosynthesis class H	1.33	0.001
spats1	spermatogenesis associated serine rich 1	1.33	0.042
abtb1	ankyrin repeat and BTB domain containing 1	1.31	0.031
col26a1	collagen type XXVI alpha 1	1.29	0.099
msi1	musashi RNA binding protein 1	1.28	0.049
errfi1	ERBB receptor feedback inhibitor 1	1.27	0.098
rabl2b	RAB, member of RAS oncogene family-like 2B	1.27	0.000
fzd9	frizzled class receptor 9	1.27	0.090
polb	polymerase (DNA) beta	1.25	0.002
pik3ca	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	1.23	0.019
lysmd4	LysM domain containing 4	1.21	0.043

fbxo10	F-box protein 10	1.20	0.086
bzw1	basic leucine zipper and W2 domains 1	1.17	0.089

Gene symbol	Gene title downregulated	Fold change	Adjusted *p*-value
grm4	glutamate receptor, metabotropic 4	-2.02	0.000
clec4f	C-type lectin domain family 4 member F	-1.82	0.000
cdx2	caudal type homeobox 2	-1.78	0.000
sp140l	SP140 nuclear body protein like	-1.69	0.002
negr1	neuronal growth regulator 1	-1.69	0.003
vmn2r1	vomeronasal 2, receptor 1	-1.68	0.002
adcy3	adenylate cyclase 3	-1.62	0.000
saxo2	stabilizer of axonemal microtubules 2	-1.61	0.012
cyp19a1	cytochrome P450 family 19 subfamily A member 1	-1.60	0.012
fbxl18	F-box and leucine-rich repeat protein 18	-1.55	0.017
ephb4	EPH receptor B4	-1.55	0.033
carmil3	capping protein regulator and myosin 1 linker 3	-1.54	0.000
gpr137b	G protein-coupled receptor 137B	-1.54	0.017
slc5a7	solute carrier family 5 member 7	-1.54	0.024
hsd11b2	hydroxysteroid (11-beta) dehydrogenase 2	-1.52	0.040
abcc2	ATP binding cassette subfamily C member 2	-1.52	0.051
fn1	fibronectin 1	-1.51	0.054
cyr61	cysteine rich angiogenic inducer 61	-1.51	0.024
mnx1	motor neuron and pancreas homeobox 1	-1.51	0.051
st6galnac6	ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 6	-1.51	0.057
neurl1	neuralized E3 ubiquitin protein ligase 1	-1.51	0.057
dbi	diazepam binding inhibitor (GABA receptor modulator, acyl-CoA binding protein)	-1.50	0.003
kcnd3	potassium voltage-gated channel subfamily D member 3	-1.49	0.070
cyp17a1	cytochrome P450 family 17 subfamily A member 1	-1.48	0.078
gsta4	glutathione S-transferase, alpha 4	-1.47	0.000
fam174b	family with sequence similarity 174 member B	-1.47	0.025
pax6	paired box 6	-1.47	0.089
trim28	tripartite motif containing 28	-1.47	0.006
gmeb1	glucocorticoid modulatory element binding protein 1	-1.44	0.039
dnpep	aspartyl aminopeptidase	-1.44	0.094
glra3	glycine receptor alpha 3	-1.43	0.018
rhbdd1	rhomboid domain containing 1	-1.42	0.065
c2cd4c	C2 calcium-dependent domain containing 4C	-1.40	0.027
fuk	fucokinase	-1.40	0.032
arid1b	AT-rich interaction domain 1B	-1.40	0.065
manba	mannosidase beta	-1.39	0.051

tgfb1	transforming growth factor beta 1	-1.38	0.031
pak1	p21 protein (Cdc42/Rac)-activated kinase 1	-1.38	0.065
ngrn	neugrin, neurite outgrowth associated	-1.38	0.077
slc22a6	solute carrier family 22 member 6	-1.37	0.017
ddx55	DEAD-box helicase 55	-1.36	0.003
mrps22	mitochondrial ribosomal protein S22	-1.36	0.007
ribc2	RIB43A domain with coiled-coils 2	-1.36	0.059
pde4dip	phosphodiesterase 4D interacting protein	-1.35	0.080
nr4a1	nuclear receptor subfamily 4 group A member 1	-1.34	0.050
allc	allantoicase	-1.33	0.020
kdm8	lysine demethylase 8	-1.33	0.000
alg13	ALG13, UDP-N-acetylglucosaminyltransferase subunit	-1.33	0.032
phldb1	pleckstrin homology like domain family B member 1	-1.32	0.042
hdx	highly divergent homeobox	-1.32	0.078
zp2	zona pellucida glycoprotein 2	-1.32	0.041
dnaaf5	dynein (axonemal) assembly factor 5	-1.29	0.037
timp2	TIMP metallopeptidase inhibitor 2	-1.29	0.031
tram2	translocation associated membrane protein 2	-1.25	0.065
strbp	spermatid perinuclear RNA binding protein	-1.24	0.087
krtcap2	keratinocyte associated protein 2	-1.24	0.007
ccdc125	coiled-coil domain containing 125	-1.23	0.050
atf4	activating transcription factor 4	-1.21	0.029
myl6	myosin light chain 6	-1.20	0.049
u2surp	U2 snRNP associated SURP domain containing	-1.20	0.089
prrc2c	proline rich coiled-coil 2C	-1.19	0.099
prickle2	prickle planar cell polarity protein 2	-1.18	0.051
cyb561d1	cytochrome b561 family member D1	-1.18	0.087
aldh6a1	aldehyde dehydrogenase 6 family member A1	-1.17	0.055
eif2s3	eukaryotic translation initiation factor 2 subunit gamma	-1.16	0.021

Table 3.2. Changes in expression of ovarian genes after 6 weeks of EE2 treatment, identified by DESeq2 (base mean >10, fold change ≥1.5 or -1.5≤, P-adj <0.1).

Top contigs (base mean >10, adjusted *P*-value <0.1

Gene symbol	Gene title upregulated	Fold change	Adjusted *P*-value
bcan	brevican	2.18	0.0E+00
fdxacb1	ferredoxin-fold anticodon binding domain containing 1	1.76	5.0E-03
antxr2	anthrax toxin receptor 2	1.75	4.0E-03
tcf12	transcription factor 12	1.68	1.7E-02
gabra6	gamma-aminobutyric acid type A receptor alpha6 subunit	1.65	2.6E-02
nlrp3	NLR family, pyrin domain containing 3	1.63	2.6E-02
kcnk4	potassium two pore domain channel subfamily K member 4	1.60	6.3E-02
polk	polymerase (DNA) kappa	1.35	6.3E-02
Gene symbol	**Gene title downregulated**	**Fold change**	**Adjusted *P*-value**
tex11	testis expressed 11	-1.88	0.0E+00
camkk2	calcium/calmodulin-dependent protein kinase kinase 2	-1.62	3.5E-02
kdelr3	KDEL endoplasmic reticulum protein retention receptor 3	-1.62	4.4E-02
cdo1	cysteine dioxygenase type 1	-1.62	3.5E-02
zbed1	zinc finger BED-type containing 1	-1.57	0.0E+00
dmbt1	deleted in malignant brain tumors 1	-1.57	5.1E-02
rpa3	replication protein A3	-1.53	6.6E-02
mat2a	methionine adenosyltransferase 2A	-1.38	9.0E-03
rce1	Ras converting CAAX endopeptidase 1	-1.19	9.8E-02

Table 3.3. Top canonical pathways, by z-score and –Log P value, significantly altered in the ovarian follicles of females treated with EE2 for 1 week, identified by Ingenuity® Pathway Analysis software. Pathway, -Log_{10} P-value, ratio of genes in dataset compared to pathway, z-score, genes in pathway represented in the dataset.

Ingenuity Canonical Pathways	-Log_{10} (p-value)	Ratio	z-score	Molecules
Sphingosine-1-phosphate Signaling	2.19	4/126	2	*pik3ca, adcy3, pdgfra, gnaq*
Endothelin-1 Signaling	1.51	4/200	2	*pik3ca, ednrb, adcy3, gnaq*
Hepatic Fibrosis / Hepatic Stellate Cell Activation	4.83	8/187		*col5a2, fn1, ednrb, myl6, tgfb1, col4a3, pdgfra, timp2*
Endoplasmic Reticulum Stress Pathway	3.62	3/21		*calr, atf4, taok3*
Axonal Guidance Signaling	3.46	10/455		*adamts8, pik3ca, ephb4, pak1, myl6, gnaq, fzd9, rassf5, sema4c, itga4*
PAK Signaling	3.45	5/103		*pik3ca, pak1, myl6, pdgfra, itga4*
Ephrin Receptor Signaling	3.21	6/177		*ephb4, pak1, angpt1, gnaq, atf4, itga4*
TR/RXR Activation	2.47	4/105		*eno1, pik3ca, strbp, ncoa3*
CXCR4 Signaling	2.45	5/173		*pik3ca, pak1, myl6, adcy3, gnaq*
Paxillin Signaling	2.35	4/113		*pik3ca, pak1, itga10, itga4*
CREB Signaling in Neurons	2.26	5/192		*pik3ca, adcy3, gnaq, atf4, grm4*
Corticotropin Releasing Hormone Signaling	2.25	4/121		*adcy3, nr4a1, gnaq, atf4*
G-Protein Coupled Receptor Signaling	2.22	6/278		*enpp6, pik3ca, adcy3, gnaq, atf4, grm4*
Role of Tissue Factor in Cancer	2.21	4/124		*pik3ca, pak1, gnaq, cyr61*
Renin-Angiotensin Signaling	2.15	4/129		*pik3ca, pak1, adcy3, gnaq*
Molecular Mechanisms of Cancer	2.13	7/383		*pik3ca, pak1, tgfb1, adcy3, gnaq, fzd9, itga4*
GNRH Signaling	2.08	4/135		*pak1,adcy3,gnaq,atf4*
Leukocyte Extravasation Signaling	2.05	5/216		*pik3ca,myl6,rassf5,timp2,itga4*
Ephrin B Signaling	2.01	3/75		*ephb4,pak1,gnaq*
P2Y Purigenic Receptor Signaling Pathway	2.01	4/142		*pik3ca,adcy3,gnaq,atf4*
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	1.97	6/316		*pik3ca,fn1,tgfb1,gnaq,atf4,fzd9*
Angiopoietin Signaling	1.95	3/79		*pik3ca, pak1, angpt1*
Human Embryonic Stem Cell Pluripotency	1.95	4/148		*pik3ca, tgfb1, pdgfra, fzd9*
Actin Cytoskeleton Signaling	1.92	5/231		*pik3ca, pak1, fn1, myl6, itga4*
Estrogen-Dependent Breast Cancer Signaling	1.92	3/81		*pik3ca, cyp19a1, atf4*

Table 3.4. Canonical pathways significantly altered in ovaries of females treated with EE2 for 6 weeks, identified by Ingenuity® Pathway Analysis software. Pathway, -Log_{10} P-value.

Ingenuity Canonical Pathways	-log(p-value)	Ratio	Molecules
Cell Cycle Control of Chromosomal Replication	1.69	1/27	*rpa3*
Cysteine Biosynthesis III (mammalia)	1.63	1/31	*mat2a*
Inflammasome pathway	1.76	1/23	*nlrp3*
L-cysteine Degradation I	2.08	1/11	*cdo1*
Methionine Degradation I (to Homocysteine)	1.74	1/24	*mat2a*
Nucleotide Excision Repair Pathway	1.58	1/35	*rpa3*
S-adenosyl-L-methionine Biosynthesis	2.22	1/8	*mat2a*
Superpathway of Methionine Degradation	2.96	2/65	*mat2a, cdo1*
Taurine Biosynthesis	2.22	2/8	*cdo1*

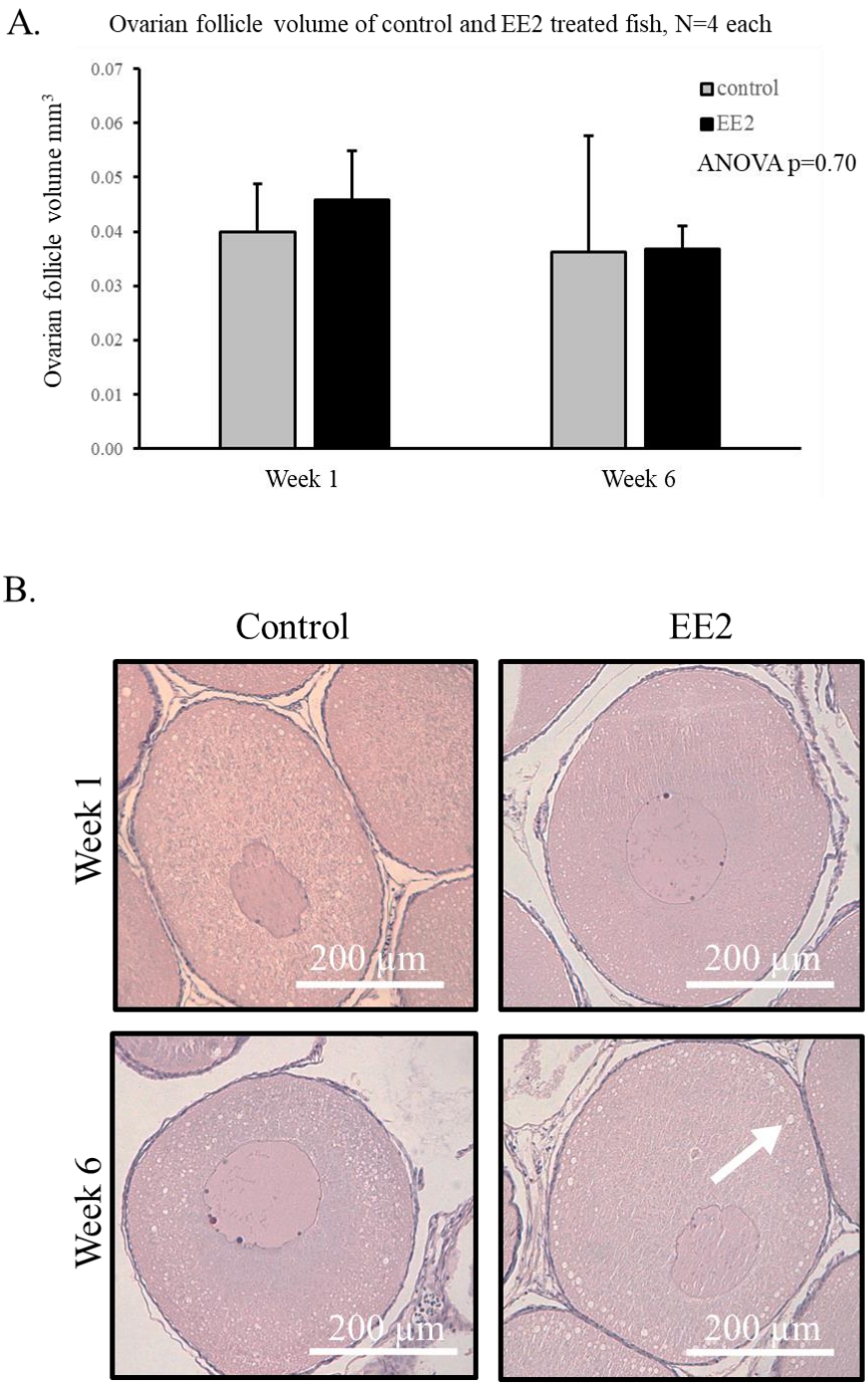

Figure 3.1. Morphology of ovarian follicles. (A) Ovarian follicles from control and EE2 treatment grous did not differ in volume (ANOVA, P=0.7) at 1 or 6 weeks. (B) Representative samples of ovarian follicles from each treatment group were at the cortical alveoli stage of ovarian development. Cortical alveoli are indicated by white arrow. Scale bar = 200 µm.

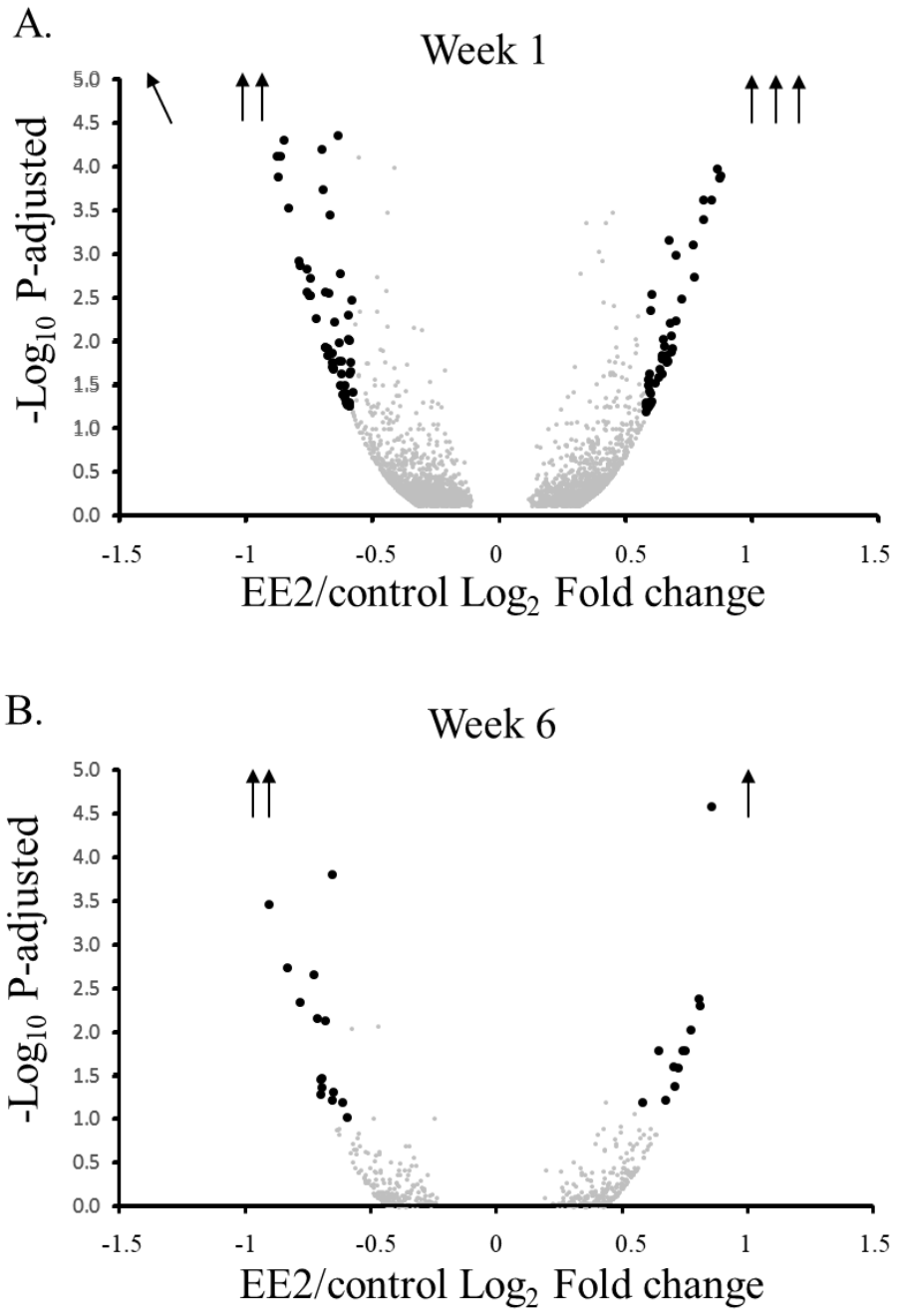

Figure 3.2. Contigs with altered expression in the ovary following EE2 treatment. At week 1(A) and week 6 (B) RNA-Seq analysis identified 279 and 30 contigs, respectively that were differentially expressed (black dots) between control and EE2 treatment groups (DESeq2, $-Log_{10}$ P-adj >1.30, Log_2 fold change ≥|0.58|). Arrows indicate significant contigs outside of axes range.

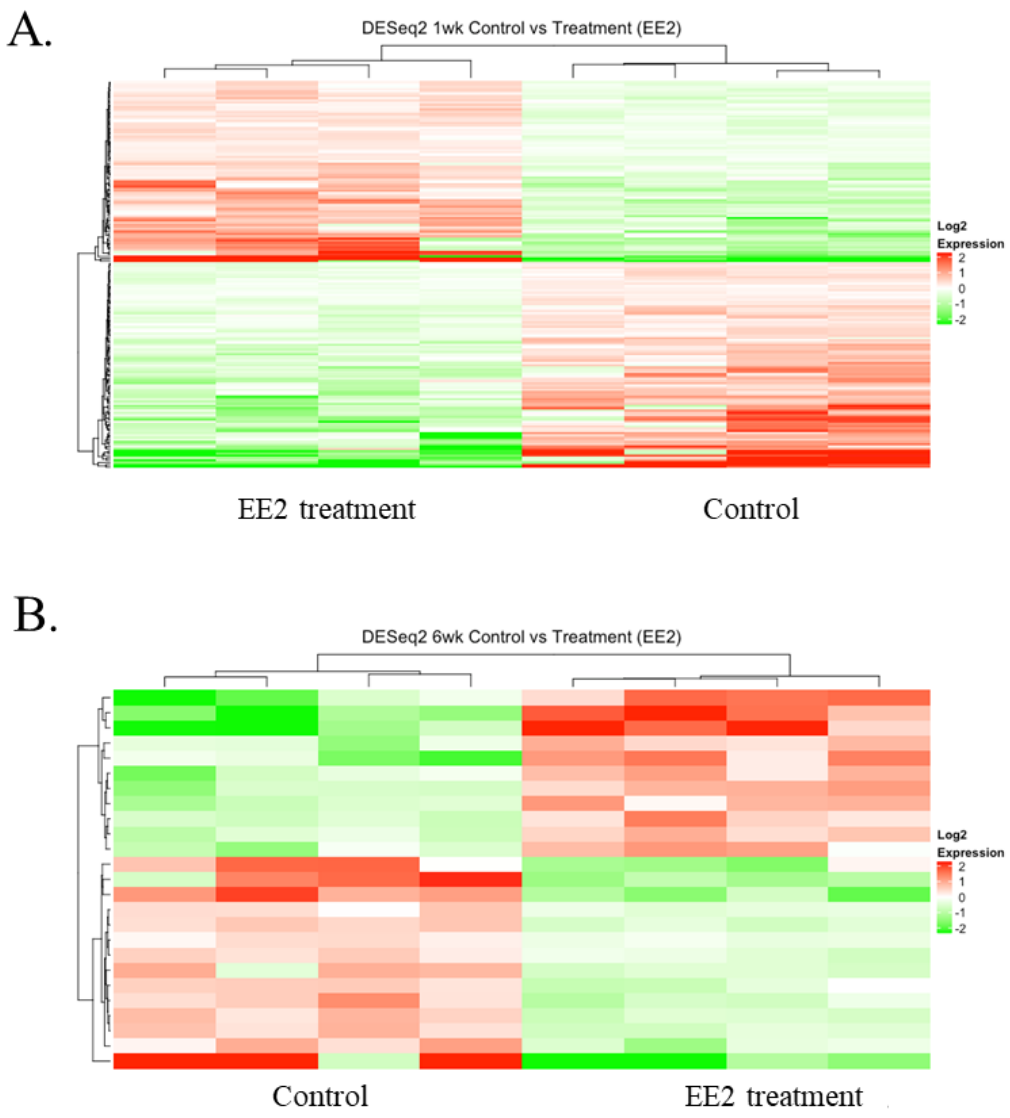

Figure 3.3. Cluster analysis of DEGs. Cluster analysis of genes that were significantly differentially expressed between control and E2 treatment at 1 (A) and 6 (B) weeks demonstrates a clear difference in expression pattern between treatment groups. Expression of contigs (rows) is displayed for three independent samples (columns), with red indicating up-regulation and green representing down regulation from the mean expression value (white). DESeq2, Log_{10} P-adj >1.30.

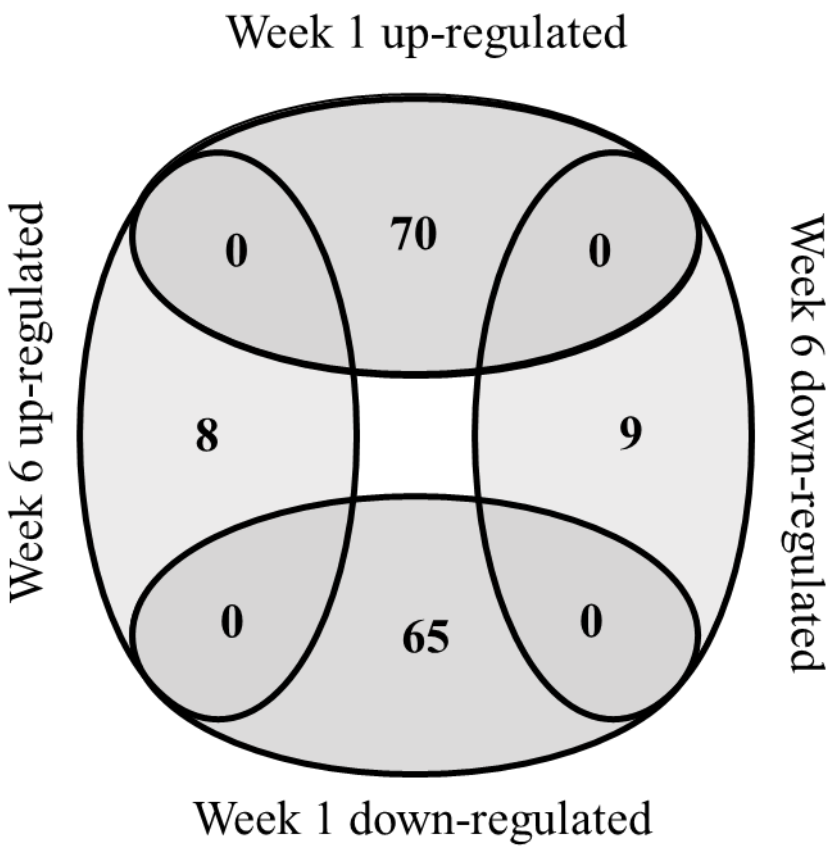

Figure 3.4. Comparison of genes that were significantly altered (DESeq2, Log_{10} P-adj >1.30) by EE2 in the juvenile coho ovary. Venn diagram of DEGs at 1 and 6 weeks, annotated to zebrafish or human orthologs, illustrates the number of genes that were altered by each treatment. There is no overlap in DEGs identified at either time point. 135 DEGs were identified at 1 week and 17 DEGs at 6 weeks.

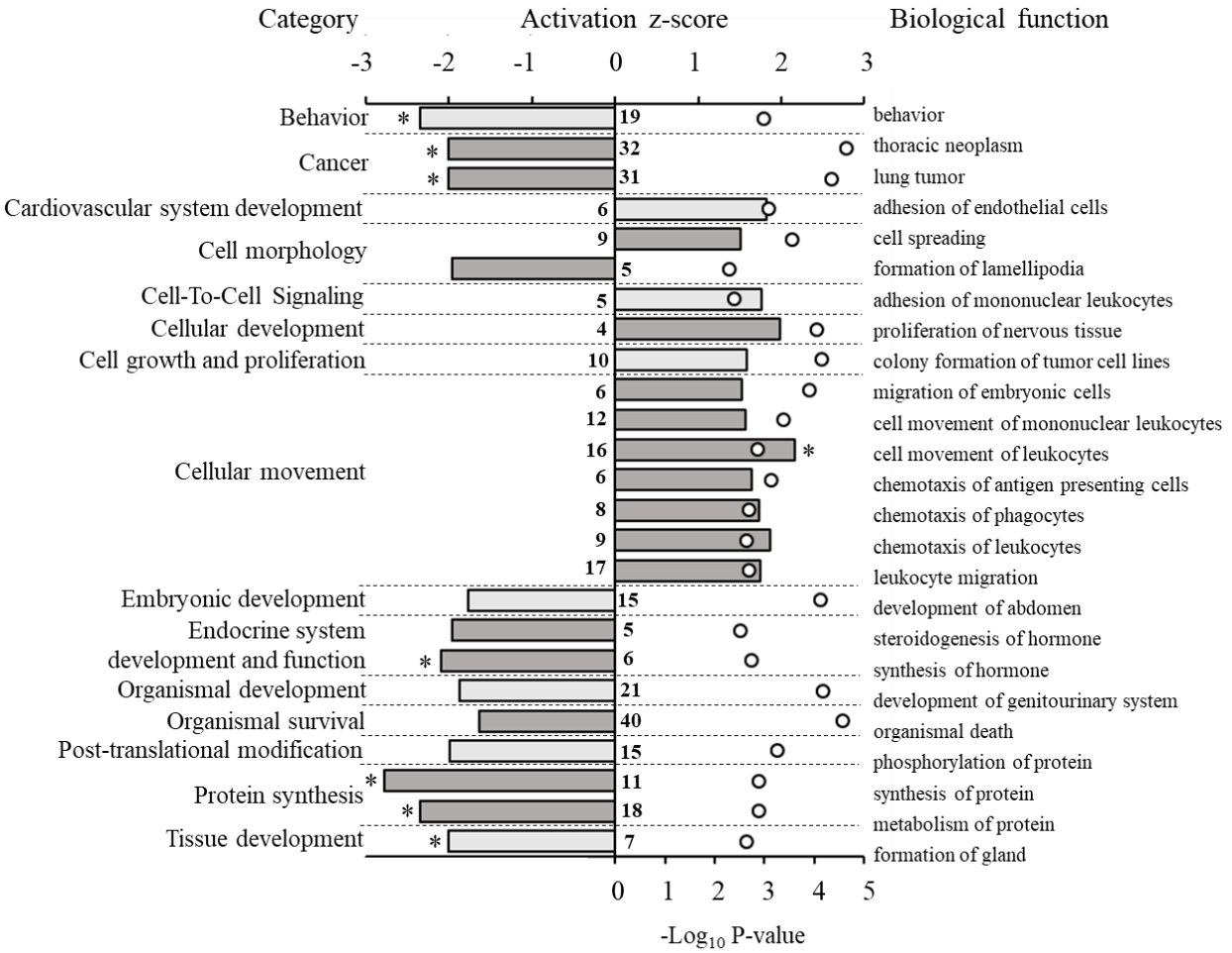

Figure 3.5. Biological functions in the ovary altered by 1 week of EE2 treatment. 8 biological functions were significantly altered by EE2 treatment as identified by IPA pathway analysis. All biological functions with a z-score |>1.5|, and $-Log_{10}$ P-value >1.3 (P<0.05, which indicates a biological function is significantly associated with the dataset) are displayed; biological functions with a z-score >|2| were considered to be significantly predictive of activation or inactivation, indicated by an asterisk, based on positive or negative z-score respectively. 7/8 biological functions that were significant altered were predicted to be inactivated, including functions in categories of *endocrine system development and function*, *protein synthesis*, and *tissue development*. Bar color indicates biological functions share the same function category.

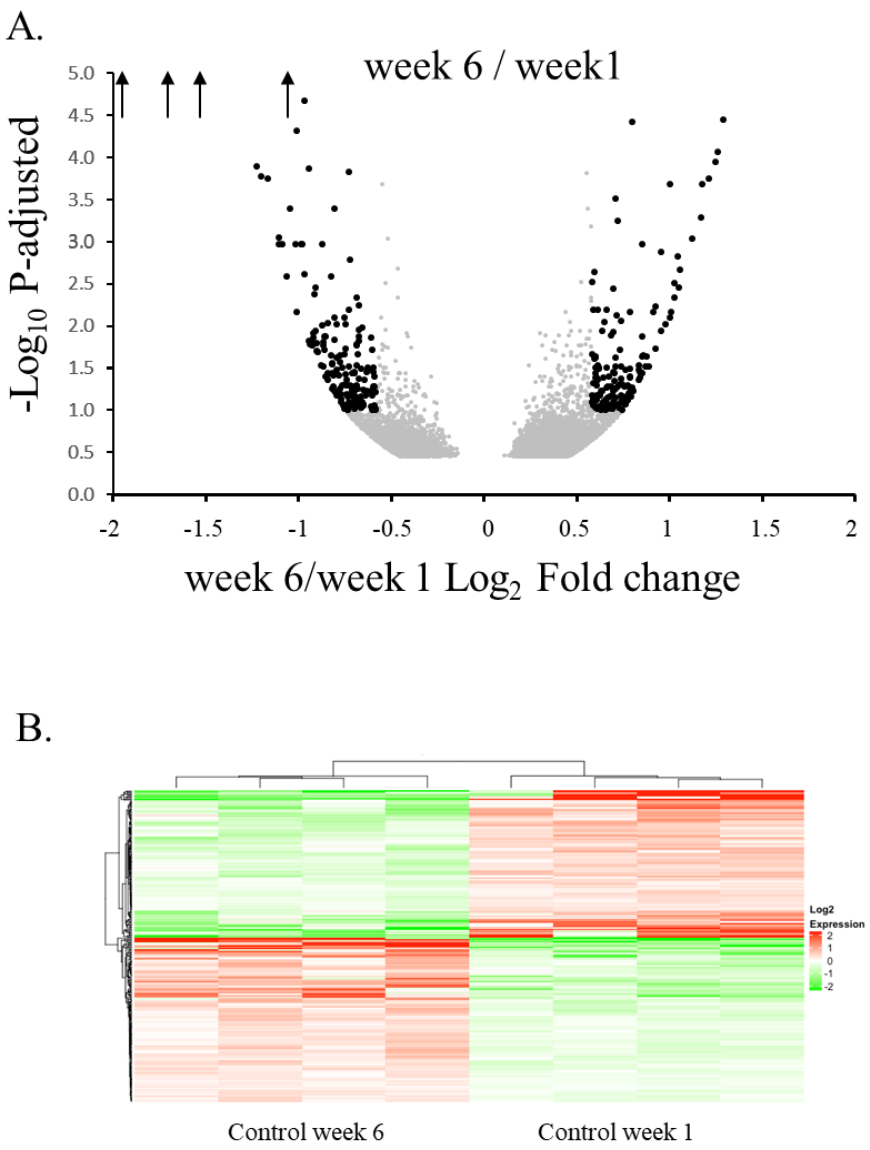

Figure 3.6. Gene expression differences in ovaries of untreated females at week 6 compared to week 1. (A) After 6 weeks RNA-Seq analysis identified 320 contigs that were differentially expressed (black dots) from week 1 controls (DESeq2, $-Log_{10}$ P-adj >1.30, Log_2 fold change ≥|0.58|). Arrows indicate contigs outside if axes range. (B) Contigs mapped to zebrafish or human orthologs (rows) are displayed for three independent samples (columns), with red indicating higher expression and green representing lower expression from the mean expression value (white). DESeq2, Log_{10} P-adj >1.30.

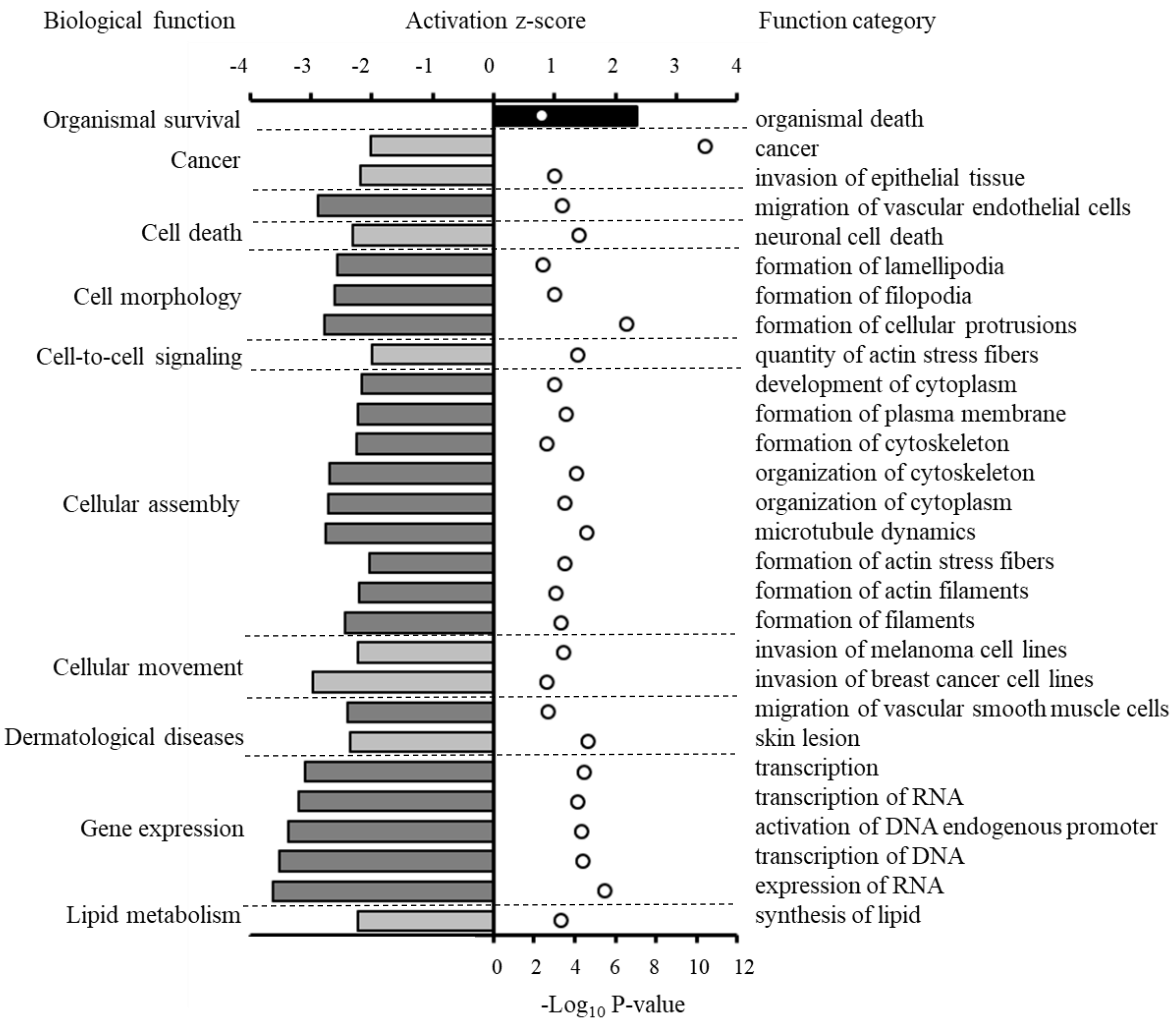

Figure 3.7. Biological functions in control fish predicted to be altered at week 6 compared to week 1. Biological functions in several cellular and developmental categories, as well as gene expression and lipid metabolism were predicted to be inactivated at week 6, compared to week 1 in ovaries of control fish. Organismal death is the only biological function predicted to be significantly activated. All biological functions with a z-score |>2| were considered significantly activated or inactivated, and $-Log_{10}$ P-value >1.3 (P<0.05), which indicates a biological function is significantly predictive given gene expression in the dataset, are displayed. Bar color indicates biological functions within the same function category.

Synthesis and Conclusion

The work presented here creates a comprehensive picture of the transcriptional response to sex steroids in the developing ovary. By utilizing a deep sequencing of the ovarian transcriptome in a non-model organism, this work also describes a pathway for identifying altered expression patterns of even relatively rare transcripts. The data discussed in Chapters 1 and 2 highlight transcriptional responses that precede morphological endpoints, uncovering possible mechanisms for androgen-induced ovarian growth in teleosts. The steroidal androgen steroid used in these studies is non-aromatizable, meaning it cannot be converted into an estrogen, as can other androgens such as testosterone. Thus, the transcriptional responses are likely responses to the androgen signal or downstream consequences of androgen signaling. Since both studies were conducted in vivo, the effects of the androgen could be direct at the level of the ovary, or indirect via effects on other organs that modulate ovarian function (e.g. pituitary, brain). Additionally, the data discussed in Chapter 3 presents valuable information on both the role of estrogens in regulating ovarian gene expression, but also provides a basis on which to interpret results from research into the effects of steroidal xenoestrogens.

A significant finding from this research is that the late primary growth and early secondary growth ovary is extremely sensitive to androgens. In salmon, these stages encompass the onset of the developmental phenomenon analogous to mammalian puberty, and thus the present study provides insight into the regulation of puberty onset. Androgens have been shown in several fish species to induce ovarian growth at this point in development [1–3], as is described in detail in Chapters 1 and 2. Of particular interest from these studies is the increase in expression of follicle stimulating hormone receptor (*fshr*) observed after androgen exposure in primary growth (pre-

pubertal) oocytes. This finding indicates a direct ovarian response to androgens that increases sensitivity to the initial signals in pubertal development. Similarly, in early secondary growth, the ovary was also extremely sensitive to the androgen treatment, which altered the expression of thousands of genes in the ovary, including genes involved in follicle stimulating hormone (Fsh) signaling, and steroidogenesis, as well as other hallmarks of secondary growth, and thus may mediate general pubertal development. Some of the major effects of androgen (magnitude of gene expression and number of genes in a given pathway) are in process associated with tissue remodeling and growth. Interestingly the transcriptomic response to short term (three-day) treatment with estradiol-17β (E2) was not as robust, even though E2 is known to be a potent ovarian growth promoter of secondary growth.

Much of what is known about puberty comes from studies on mammals. The onset of mammalian (hormonal) puberty is generally defined as the (re)initiation of the pulsatile gonadotropin surge that accompanies the estrous cycle, and thus the study of the underlying mechanisms controlling puberty has focused on mechanisms controlling GnRH secretion [4]. Signaling mechanisms associated with GnRH secretion, such as kisspeptin, insulin-like growth factor signaling, and leptin, coordinate body condition with the reproductive endocrine signal. Puberty is completed when secondary sexual characteristics develop and the gonads mature to reproductive capability, indicated by the first reproductive cycle in females and the ability to undertake reproduction in males.

As in mammals, the main function of puberty in fish is the activation of the reproductive axis to produce reproductive hormones leading to the maturation of gonadal function. While the first

production of fertile gametes [5] denotes the end product of puberty, the point of onset is not as clear, given the diversity in reproductive strategies in teleost lineages. Similar to mammals though, extra-gonadal regulatory input via the activation of the hypothalamus-pituitary-gonad (HPG) axis [6], resulting in increases in gonadotropin secretion, is generally understood to be a hallmark of puberty onset. This is accompanied by the accumulation of cortical alveoli in the oocyte which denotes the transition from the primary to secondary oocyte growth stage [6] and is followed by vitellogenesis in the ovary. In males the rapid proliferation of spermatogonia in testes occurs at this time [7].

The coho salmon model presented a unique opportunity to study mechanisms related to puberty onset. Coho salmon exhibit semelparity, reaching maturity and spawning only once just prior to expiry, and thus puberty (completion of the one and only reproductive cycle) in this species is a protracted process, typically taking three years. This made it feasible to identify and target the narrow developmental window just prior and just following morphological indications of puberty onset. In this context, these studies provide a blueprint for further exploration of factors controlling puberty onset, and the identified processes that are profoundly affected by androgens at this stage of development.

In addition to the work presented characterizing the effects of natural sex steroids, the response of the early secondary growth ovary to the potent synthetic xenoestrogen 17α-ethinylestradiol (EE2) was explored. While the morphological and transcriptomic responses were mild compared to the androgen exposure studies, the alterations in gene expression suggest disruption of steroidogenic capacity, possibly in compensation for the increase in estrogen signal from EE2.

This study was done in conjunction with Harding et al. [8], which identified massive alterations in gonadotropin expression in the pituitary of the same fish, and the potential effects of estrogens on circadian rhythm. When examined together, these studies indicate that EE2 may interfere with normal processes at multiple levels of the reproductive endocrine axis, and could potentially disrupt reproductive timing. However, this study was confounded by poor body growth in experimental fish which could have affected the responsiveness of the ovary to EE2 after six weeks.

In summary, this body of work has improved our understanding of sex steroid regulation of ovarian processes during a critical stage of development and further cemented coho salmon as a viable model for research into reproductive development and endocrinology. It is interesting to speculate, based on the androgen treatment results, that androgens may play an as important, if not a more important, role than estrogens at this point in ovarian development. While it is tempting to draw conclusions about functional and phenotypic changes due to the profound alterations in the ovarian transcriptome from these treatments, transcript abundance does not directly equate with protein abundance or function, and an examination of the ovarian (follicle cell) proteome would be necessary to test that hypothesis. Additionally, the source and identity of the full complement of endogenous androgens is not well understood at this point in development. Nor is there a satisfactory answer as to why androgen signaling may have such robust effects, given female reproductive development is known to be largely mediated by estrogen signaling.

Future considerations

While many novel transcripts associated with androgen treatment were identified in these studies, the differential effects of 11-ketotestosterone on anti mullerian hormone (*amh*) expression, between primary and secondary growth were some of the most interesting, and warrants further exploration. In mammals, the AMH protein is critical to sex determination and in reproductive females, AMH is produced by ovarian granulosa cells of preantral and antral follicles (primary and secondary follicles), where it may inhibit recruitment of these follicles by repressing FSH signaling, forming a selection mechanism for a dominant follicle in a particular reproductive cycle. It's unclear if Amh signaling forms a similar mechanism in teleosts, and very little experimental information exists regarding its function. However, the evidence that does exist supports the hypothesis that Amh signaling is necessary for normal ovarian development and function and may play a role in Fsh signaling. Different patterns of expression and regulation of *amh* have been observed in different species, which may reflect varying reproductive strategies across teleosts. It would be interesting to further characterize Amh signaling in several teleosts. A time course of *amh* and *amh-receptor* transcript and protein expression in a semelparous species such as coho salmon would be invaluable to identifying the ovarian stages where Amh may be important and the utilization of gene editing technologies, such as transgenesis incorporating *Cre/Lox* recombination, or knockout via Crispr/Cas9 or TALEN, in combination with characterized null-mutants for Amh or Amhr could allow for precise functional studies of Amh and Amh-receptor.

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Appendix. In vitro treatment of vitellogenic rainbow trout ovarian follicles with endocrine disrupting chemicals can alter basal production of estradiol-17β and the responsiveness to gonadotropins

Coauthors: Graham Young, Irvin Schultz

Introduction

Endogenous estrogenic steroid hormones, predominantly estradiol-17β (E2), play a central role in many reproductive functions in female development in fish, including gonadal sex determination (reviewed in [1], oogonial proliferation [2], promoting the secondary ovarian follicle phenotype [3,4] and maintenance of the ovary [5]. Additional control of reproductive development by E2 occurs through its regulation on hypothalamic gonadotropin releasing hormone (Gnrh) content via modulation of the expression of kisspeptins (*kiss1* and *kiss2*), both kisspeptin and neurokinin B receptors (*kiss1ra, kiss2r, tac3ra* and *tac3rb*) [6,7]. E2 also regulates pituitary gonadotropins by decreasing follicle stimulating hormone (Fsh) and increasing luteinizing hormone (Lh) synthesis or release [8].

The most well defined role for E2 in ovarian function is in the stimulation of Vtg production in the liver (reviewed in [9]. E2 is the primary steroid responsible for stimulating synthesis of the hepatic yolk protein precursor, vitellogenin (Vtg). Following the aromatization of testosterone (T) in ovarian follicle cells, primarily in response to Fsh signaling (Tyler, 1991), E2 is released into the blood stream where it binds hepatic estrogen receptors (Esrs) and triggers the hepatic synthesis of Vtg proteins. These are then selectively incorporated into the oocyte and cleaved into egg-yolk proteins, contributing to the massive growth of the secondary oocyte.

Many environmental contaminants are known to affect endocrine processes, and are thus classified as endocrine disrupting chemicals (EDCs). These contaminants mimic or block endogenous hormonal signals by affecting hormone synthesis, secretion, transport, action, or elimination [10,11]. In particular, many EDCs display estrogenic or anti-estrogenic properties. This may reflect the use of a well-characterized and easily measured biomarker of aberrant Vtg induction, an indicator of estrogen exposure. However, the direct effects of EDCs on the ovarian production of estrogens have not been well-explored.

Studies using in vivo exposures to measure the biological effects of EDCs have yielded valuable information, but suffer from several constraints including long exposure durations and the necessity of a large number of test animals, which limits the affordability of such tests [12]. Results from in vivo testing are also complicated by the interactions of endogenous endocrine processes which can obscure the direct target of an EDC. Therefore, the use of in vitro tissue culture has been proposed as an alternative method to better screen for EDCs (Lee et al., 2008; Schirmer, 2006).

Ovarian follicles cultured in vitro continue to respond to an exogenous Gth signal, and will continue to produce E2, a capacity that has used by researcher to investigate mechanisms of ovarian steroidogenesis (reviewed by Kagawa [13]). More recently the capacity of the ovarian follicle to remain sensitive to Gths during in vitro culture has been used to characterize the regulation of secondary growth follicles by Fsh [14], identifying numerous genes encoding steroidogenic proteins whose expression is under the control of Gths, presumably acting via the Fsh receptor. The expression of steroidogenic enzymes has been used as endpoints in studies

detecting various EDCs [15] and characterizing gonad specific responses [16]. The capacity of cultured ovarian follicles to maintain function in vitro makes it possible to screen for direct effects of EDCs on the ovary.

As part of a broader study to create a predictive model of EDC effects on reproduction, this study was conducted in order to identify direct impacts of EDCs on ovarian function. Ovarian follicles were assessed for the ability to produce E2 following exposure to a suite of EDCs, including estrogen receptor agonists, aromatase inhibitors, androgens, and SSRIs.

Methods

Chemicals

Hydroxyflutaminde, norsetraline, and 17β-trenbolone were purchased from Sigma Aldrich (St. Louis, MO), 17α-ethinylestradiol was purchased from steraloids (Newport, RI), prochloraz was purchased from Toronto Research Chemicals (North York, Ontario, CA), and fluoxetine and tamoxifen citrate were purchased from TCI America (Portland, OR). Concentrated stock solutions of each chemical were prepared by dissolving chemicals in methanol, and adding 10-100 µl to 50 or 100 ml bottles with aluminum caps and pierceable septums to bottles using a Hamilton syringe. The solvent was evaporated using N2 via sterile 25G needles, which were used to pierce the septum (one for venting and one for N2 introduction). Sterile trout Ringers was added in a similar manner, except a 21 G needle was used for introduction. Preparation of all test chemicals and media introduction was performed under sterile conditions. The nominal concentration of each chemical was determined from the volumes of chemical stock solutions and media added to the bottles. The bottles were placed on an orbital shaker for 24 hours at room

temperature. Following this, the solutions were stored in the dark at 4°C. For in vitro testing, serial dilutions of the stock solutions were made using sterile trout Ringer's media. The concentrations of the test chemicals were chosen to span the limits of solubility or cytotoxicity to environmentally relevant levels.

Animals and experimental procedure

Rainbow trout (Troutlodge, Sumner, WA) in the vitellogenic stage of ovarian growth were anesthetized in buffered 0.05% tricaine methanesulfonate until movement of the gill operculum ceased, followed by decapitation. Fork length and body weight were recorded. Ovaries were removed and weighed, then placed in 4°C trout Ringer's media. Gonadosomatic index was calculated (gonad weight / body weight x 100). Ovarian follicles were photographed using a Nikon SMZ1000 and diameter and circumference were measured using Nikon Elements BR 3.2 software.

Six technical replicates of ovarian fragments from each of three fish with the most similar GSI were used for each treatment concentration in each exposure experiment. Ovarian follicles were separated with forceps and small scissors and 10 follicles were placed in each well of a 24-well tissue culture plate with chilled sterile trout Ringer's media. After all of the wells were filled, the media was removed and replaced with 1 ml trout Ringer's containing test chemicals at the concentrations described for each chemical or with control Ringer's. Follicles were incubated in the test chemicals for 18 hours, all media were then removed and replaced with the same test chemical treatments, with or without 500 ng/ml salmon partial purified pituitary extract (referred to as sGTH challenge throughout; sGTH donated by Dr. Penny Swanson, NOAA) to stimulate

estradiol-17β (E2) production into the medium (Fig. A.1). After 6, 12, and 24 hours following the commencement of the sGTH challenge, 0.1 ml of the culture media was collected for assessment of E2 levels by radioimmunoassay. An additional trenbolone treatment was conducted with an initial 34-hour incubation followed by 6 hours with or without sGTH challenge. During all treatments plates with follicles were gently swirled on an orbital shaker at 14°C.

E2 Assays

Media samples were heat treated at 80°C for 1 hour and E2 was measured by radioimmunoassay, as described by Sower and Schreck [17], and modified by Fitzpatrick et al. [18]. Validation and characteristics of this assay has been reported previously.

Data are presented in three ways: (A) the levels of E2 in the media after 6 hours of culture in test chemical treatment following the media change after the initial 18 hour exposure (24 hours total treatment time); (B) the levels of E2 after a 6-hour challenge with sGTH and test chemical treatment (24 hours total treatment time); and (C) the responsiveness of the follicles to the sGTH challenge after test chemical treatment (fold change between basal E2 levels and sGTH-induced E2 levels for each test chemical treatment concentration) after a 6-hour challenge with sGTH (with the exception of EE2 treatment, in which results are shown for a 24-hour challenge with sGTH). The concentration of E2 in the media, and fold change in responsiveness to sGTH challenge was compared across treatments by ANOVA followed by Tukey HSD with significance accepted at $P < 0.05$.

Quantative PCR analysis

Total RNA from follicles used in the EE2 exposures was extracted using Qiagen RNEasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA pellets were re-suspended in DNAse/RNAse free water (Sigma-Aldrich). Total RNA concentrations in extracts were determined using a NanoDrop ND-100 (NanoDrop Technologies, Wilmington, DE). Genes encoding enzymes involved in the synthesis of E2 or C19 androgens were selected for quantitative PCR (qPCR). Total RNA (1 µg) was reverse transcribed using Superscript IV reverse transcriptase (Thermo Fisher Scientific), random primers, and RNasin RNase inhibitor (Promega). Gene specific primers from previously published primer sequences [19], were purchased from Integrated DNA Technologies (Coralville, IA).

Total volume for qPCR was 25 µl, and consisted of 12.5 µl Power SYBR Green Master Mix (ABI/Invitrogen, Foster City, CA), 150 nM of gene specific forward and reverse primers, and 0.2 ng cDNA template based on total RNA loaded into the RT reaction. Assays were run on an ABI 7500 Sequence Detector in 96-well plates using standard cycling conditions: 50°C for 2 mins, 95°C for 10 mins, followed by 40 cycles consisting of 95°C for 15 secs, and 60°C for 1 min. A standard curve constructed from five concentrations of serially diluted cDNA synthesized from pooled ovary RNA (ranging from 0.004 to 4 ng) was included in each assay. No cDNA template controls (NTC) and no amplification controls (NAC, reverse transcriptase excluded from RT reaction) were included in each assay and showed no amplification over 40 cycles of PCR. Melt curve analyses showed single signals for all genes and no peaks were observed in NTC or NAC wells. Data were expressed relative to the expression of eukaryotic elongation factor 1 alpha (*eef1a*), which we have validated previously for use as a normalizer for salmonid ovarian tissue

[20]. The mean expression level of each gene transcript was compared between control and treatment samples at each time point using ANOVA and Tukey HSD with significance accepted at $P < 0.05$.

Results

Morphometrics, ovarian follicle size, and basal E2 production

Ovaries from early-mid vitellogenic rainbow trout contained follicles that ranged in diameter from 1 mm to 3 mm. Body weight ranged from 1.63 ± 0.12 kg to 2.21 ± 0.16 kg and GSI ranged from 0.64 ± 0.05 to 5.71 ± 0.31. There was a positive correlation between ovarian follicle size and basal E2 production (Fig. A.2A) after 24 hours of culture in control media, although smaller follicles were significantly more responsive to a 6-hour sGTH challenge, after 18 hours of culture in control medium (Fig. A.2B).

The effects of test chemical treatments on E2 production.

Basal E2 levels were not significantly reduced by EE2 treatment (Fig. A.3A). The E2 levels induced by sGTH challenge were significantly less at concentrations greater than 100 nM (Fig. 3B). After 24 hours of sGTH challenge, the fold change in E2 levels between EE2 treatment with sGTH challenge and EE2 treatment alone was significantly reduced at concentrations ≥0.1 nM EE2 (Fig. A.3C).

Basal E2 levels were significantly reduced by concentrations of prochloraz ≥2.8 µM (Fig. A.4A). The E2 levels induced by sGTH challenge were less at the highest prochloraz concentration

tested (14 µM; Fig. A.4B). There was a clear dose response in E2 levels from prochloraz treatment, but the effect on basal E2 or sGTH induced E2 levels, from treatments less than 2.8 µM or 14 µM, respectively, were not statistically significant. There was no difference in the fold change between prochloraz treatment with sGTH challenge and prochloraz treatment alone (Fig. A.4C).

Twenty-four hours of trenbolone treatment significantly elevated basal E2 levels (Fig. A.5A), but did not alter the E2 levels following sGTH (Fig. A.5B-C). A 40-hour trenbolone treatment (Fig. A.5B-D) similarly elevated basal E2 production, although at lower concentrations (1.6 µM and 9.4 µM, Fig. A.5D). There was a shallow U-shaped dose response to trenbolone with sGTH challenge and E2 levels were significantly increased with 2.1 µM trenbolone (Fig. A.5E). There was also a decrease in the fold change associated with sGTH challenge at the highest trenbolone concentration (Fig. A.5F).

Basal E2 levels were reduced after 24 hours of treatment with 500 nM tamoxifen (Fig. A.6A), but tamoxifen did not affect sGTH-induced E2 production (Fig. A.6B) and there was no change in in the responsiveness to sGTH (Fig. A.6C). Fluoxetine did not affect basal E2 levels (Fig. A.7A) or sGTH-induced E2 levels (Fig. A.7B), although the response to sGTH challenge was reduced by 50 µM fluoxetine (Fig. A.7C). There were no effects on basal E2 levels, sGTH-stimulated E2 levels, or the ovarian follicle response to sGTH from flutamide (Fig. A.8), 4-hydroxytamoxifen (Fig. A.9), norfluoxetine (Fig. A.10), hydroxyflutamide (Fig. A.11) treatment.

The expression of steroidogenic enzymes following EE2 treatment

Follicle stimulating hormone receptor (*fshr*) (Fig. A.12A) and luteinizing hormone receptor (*lhr*) (Fig. A.12B) transcripts were reduced slightly, but not significantly following sGTH challenge. The expression of steroidogenic acute regulatory protein (*star*) was consistently increased 6-8 fold after sGTH (Fig. A.12C) although the only statistically significant treatment was the control (no EE2 exposure). Transcripts of cytochrome p450 family 11 subfamily a polypeptide 1 (*cyp11a1*, also known as *p450 side chain cleavage*) were not significantly altered by sGTH challenge (Fig. A.12D). Transcript levels of hydroxy-delta-5-steroid dehydrogenase, 3 beta (*hsd3b*) were consistently increased by sGTH treatment (Fig. A.12E), although only the 10 nM and 100 nM EE2 concentrations were near significant ($p=0.06$). Transcript levels of cytochrome P450 family 17 subfamily a member 1 (*cyp17a1*) (Fig. A.12F) were not significantly altered by sGTH. Transcript levels of cytochrome P450 family 11 subfamily b (*cyp11b*) were consistently increased by sGTH treatment (Fig. A.12G), and were statistically significant with the 10 nM EE2 treatment ($P < 0.05$). Transcript levels of hydroxysteroid 11-beta dehydrogenase 1 (*hsd11b*) (Fig. A.12H) and cytochrome p450 family 19 subfamily a member 1 (*cyp19a1*, also known as *aromatase*) (Fig. A.12I) were unchanged by sGTH challenge.

Discussion

The adverse outcome pathway (AOP) concept was established to identify key events and causal linkages between contaminant exposure and biological responses in order to generate predictive power in a decision-making context [21]. Within the AOP framework, a molecular initiating event causes perturbation across levels of biological organization, creating a linkage between direct sub-cellular responses, through a series of key events, to population dynamics. Existing

knowledge is integrated with novel molecular endpoints or measured responses, not from a specific contaminant, but on targets potentially modulated by those contaminants on consequently multi-level effects in vivo [22].

It is generally accepted that estrogen signaling is required for female reproductive development across vertebrate species, and perturbation of ovarian estrogen synthesis could have dramatic effects at several points in the hypothalamus-pituitary-ovary-liver (HPOL) axis, and thus on reproduction. In the current study, several EDCs were identified that directly affected E2 production in rainbow ovarian follicles trout vitro. These three chemicals have distinct modes of action in affecting E2 synthesis or production, and in the context of the broader modeling project, represent distinct paths to a key event in an AOP.

EE2 treatment at environmentally relevant concentrations significantly reduced the ability of ovarian follicles to respond to sGTH stimulation. There was also a slight, albeit not statistically significant decrease in basal E2 production at the highest EE2 test concentration. Treatment with the aromatase inhibitor, prochloraz, caused a significant decrease in basal E2 production, but did not reduce the responsiveness to sGTH. Treatment with the synthetic, non-aromatizable androgen, 17β-trenbolone, at relatively high concentrations caused an increase in in E2 production, and during a longer-term incubation, reduced the responsiveness to sGTH.

EE2 is a potent estrogen with up to 10-fold higher affinity for Esr than native E2. Numerous studies have shown that EE2 impacts the reproductive endocrine axis and several studies have shown impacts on ovarian steroidogenesis [23–25]. Much of the effects thus far have been

attributed to impacts higher in the HPOL-axis, and a recent transcriptome analysis on the pituitary of EE2 treated coho salmon [26] showed massive dysregulation of GTH expression.

The ability of prochloraz to inhibit E2 production is well-characterized. Prochloraz acts as a direct inhibitor of aromatase activity, preventing the conversion of testosterone to E2, but is also a weak androgen receptor (Ar) and Esr antagonist. Similar results were observed in fathead minnow [27]: plasma or media E2 levels were reduced after only 6 hours of treatment in vitro, and 12 hours in vivo, respectively. This was correlated with an increase in *cyp19a1a* expression, which has also been observed in zebrafish [28], medaka [29], and in other fathead minnow studies [30], and explained as compensation for reduced aromatase activity.

Trenbolone treatment led to a significant increase in basal E2 production, although following a longer-term incubation (40 hours vs. 18 hours), the response to sGTH was reduced. The pattern of E2 production observed in the longer incubation was similar to plasma concentrations of both T and E2 observed following a 21-day exposure in fathead minnow [31]. At low concentrations, trenbolone caused a slight decrease (not statistically significant in the current study) in E2, but E2 levels were significantly elevated at concentrations above 0.35 µg/ml. This U-shaped dose response has been reported for androgenic and antiandrogenic chemicals in mammalian studies (reviewed by Gray et al [32]), and has been suggested to be a result of feedback on higher levels of the HPOL axis [31], which does not explain the results observed in the current study. A short loop feedback on ovarian androgen production may exist, in which low levels directly inhibit testosterone production whereas high levels downregulate androgen receptor expression resulting in loss of feedback inhibition. Other studies have shown little impact of trenbolone on E2 levels,

but have reported it to be slightly estrogenic [33]. Regardless, ovarian follicle sensitivity to trenbolone likely reflects a critical role that androgens play in the normal female reproductive endocrine system.

Of the other potential EDCs tested, only tamoxifen, which is weak Esr antagonist, and fluoxetine, a selective serotonin reuptake inhibitor had an impact on E2 production, and only at concentrations near the limits of solubility. Interestingly, the tamoxifen metabolite 4-hydroxytamoxifen, which has a significantly higher affinity for the Esr, and thus is a much more potent Esr antagonist, had no effects.

Effects of EE2 and sGTH on the expression of steroidogenesis related genes

One of the most compelling results from these test chemical exposures was the effect of EE2 on the ovarian follicle response to sGTH. In order to identify potential impacts on gene transcription underlying this effect, the expression of genes encoding gonadotropin receptors (*fshr* and *lhr*), and steroidogenic enzymes was measured. There were no statistically significant changes in expression from EE2 treatment, but unsurprisingly several transcripts were significantly increased following sGTH challenge (*star* and *cyp11b*). Other transcripts showed trends towards increased (*hsd3b*) or decreased (*fshr*, *lhr*) expression following sGTH challenge. Fsh stimulates ovarian E2 production at least in part via regulation of the expression of steroidogenic genes [14,34]. In previtellogenic coho salmon, in vitro treatment with Fsh increased the ovarian expression of *star*, *hsd3b*, and *lhr*, temporarily decreased the expression of *fshr*, and altered other steroidogenesis related genes [34]. The major rate limiting steps in the biosynthesis of E2 is the

transport of cholesterol from the cytoplasm to the mitochondria by the Star protein, and the final aromatization of testosterone to E2 by Cyp19a1 (aromatase). The expression of *cyp19a1* was not altered by sGTH in the present study, but previous studies have observed significant declines in basal *cyp19a1* expression during in vitro culture [34]. It is possible that the observed increase in E2 production by sGTH in the current study was due to an increase in substrate for aromatization via an increase in expression of genes upstream of aromatase in steroid biosynthesis, similar to the mechanism proposed by Luckenbach et al. [34]. Aromatase activity was not measured, but basal levels of Cyp19a1 may have been sufficient to produce E2 in the absence of a transcriptional response of *cyp19a1* to sGTH. There is not a consistent correlation between *cyp19a1* expression and aromatase activity [35]. Studies in other teleosts have shown variable transcriptional responses of steroidogenic genes to gonadotropins, but in general *star*, *hsd3b*, *cyp11a1*, and *cyp19a1*, transcript were common targets for Fsh or Lh acting through the Fsh receptor (reviewed in Lubzens et al., [9,36]).

Although a significant transcriptional response to EE2 was not observed in this study, several transcripts encoding genes in the steroidogenic pathway showed trends that may be biologically significant. Basal levels of *fshr* transcript were slightly increased by EE2 at 1 nM and 10 nM. Transcript levels of *cyp11a1* were increased 2-fold by EE2 at 10 nM and 100 nM, and was doubled in response to sGTH in the 1nM EE2 treatment. Basal transcript levels of *hsd3b* were slightly decreased by EE2 treatment at 100 nM. Transcript levels of *cyp17a1* showed an increasing trend in the response to sGTH with increasing EE2 concentrations. Although the trends in transcript levels do not correlate well with the reduced response in E2 production from sGTH, and the relatively low sample size and short duration of exposure may have led to the lack

of statistical significance in the gene expression experiments, these results do suggest a compensatory increase in steroidogenic capacity and Fsh responsiveness.

The expression of several of these genes was altered in response to estrogens in other studies. In several species, increased ovarian expression of *cyp11a1* has been reported in response to exposure to EE2 [25] or 4-nonylphenol [37] in vitro, and EE2 in vivo [38], although after longer exposures than the current study. In previtellogenic coho salmon, the expression of *cyp17a1* was significantly decreased by in vivo exposure to environmentally relevant levels of EE2 for 1-week (unpublished RNA-Seq data) and in vivo treatment of rainbow trout with E2 also reduced *star* and *hsd3b* transcript levels [39]. In several studies, *cyp19a1* transcript levels were reduced by in vivo exposure to EE2 [24,40] or E2 [41], or in vitro exposure to EE2 [25] suggesting short-loop negative feedback on the potential for E2 production. Together, these results demonstrate that EE2 may have direct impacts on ovarian steroid biosynthesis through the alteration of steroidogenic transcript levels.

In vitro model

The broader context of this study was to quantify a set of effects from common EDCs across different tissues in the HOPL to provide data for the creation of an AOP for reproductive endocrine system in fish. In addition to ovarian follicle culture, the effects on the ability of pituitary cells ability to produce GTHs and on hepatocytes to produce vitellogenin following EDC exposure were tested separately by collaborators. For this reason, vitellogenic stage ovarian

follicles were used. During vitellogenesis, the ovarian follicle is sensitive to Gth signals and in response produces E2 which stimulates hepatic vitellogenesis [36,42].

Ovarian follicle size was strongly correlated with endogenous E2 production in vitro. Smaller follicles showed less capacity for E2 production, but were far more sensitive to the Gth signal than larger follicles. It is not surprising then, that exposure of larger follicles to contaminants (prochloraz, tamoxifen, and 4-hydroxytamoxifen) did not significantly alter the response to sGTH. Although exposure of the smallest follicles to either EE2 or fluoxetine did not cause significant alterations in basal E2 production, there was a significant reduction in the response to sGTH. However the effects of fluoxetine were only apparent at a very high concentration. Test chemicals were not tested multiple times with follicles of different sizes, but any continuation of this project should use both large and small follicles with each test chemical in order to identify effects on the responsiveness to GTHs (small follicles) and the effects during maximal E2 production (larger follicles).

Conclusions

In the terms of AOP, the results of this study describe multiple potential paths to the key event of altered E2 production resulting from EDC exposure. Any change in E2 levels could impact multiple points on the HOPL axis, and impact reproductive potential. While the impacts of the majority of test chemicals were only apparent at high concentrations, the reduction in responsiveness to sGTH from EE2 treatment was striking, and at concentrations well within those observed in the environment. The method developed to quantify E2 production, and the

ovarian follicle response to sGTH in vitro was most effective when follicles were smaller (~2 mm^3, early vitellogenesis). Additionally, the high variance observed in this study could be reduced by increasing the number of replicates in any future work.

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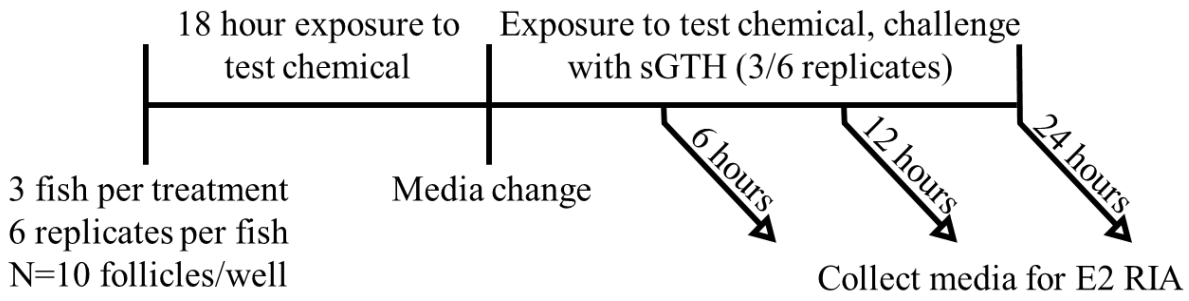

Figure A.1. Treatment design. Six replicates of 10 follicles from each of three rainbow trout were incubated in the test chemicals. After 18 hours, all media was removed and replaced with identical test chemical treatments, with or without sGTH (500 ng/ml, three of six replicates) to elicit estradiol-17β (E2) production. After 6, 12, and 24 hours following the commencement of the sGTH challenge, 0.1 ml of the culture media was collected for assessment of E2 levels by radioimmunoassay. After 24 hours of sGTH challenge, follicles from the EE2 treatment were collected for target gene qPCR.

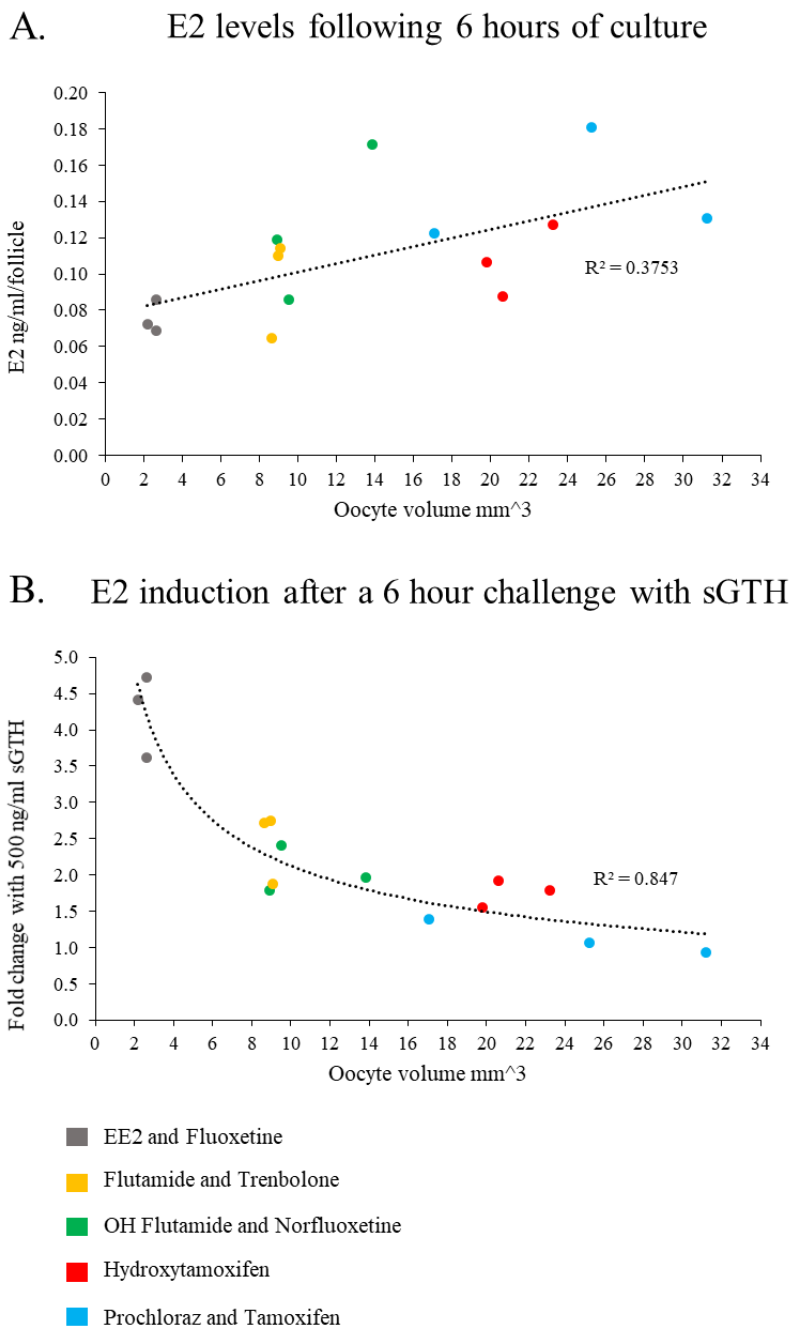

Figure A.2. Ovarian follicle size is correlated to basal E2 production and responsiveness to sGTH. (A) Basal E2 production increases with ovarian follicle volume, although (B) smaller ovarian follicles are more responsive to sGTH challenge. Follicles were initially cultured for 18 hours, then following a complete media change, were cultured for an additional 6 hours, or challenged with sGTH for 6 hours prior to media collection for E2 RIA. E2 levels were calculated from the mean of three technical replicates per fish, N=3 fish. Follicles used for each treatment are labeled by color. EE2 and fluoxetine (gray), flutamide and trenbolone (yellow), OH flutamide and nor-fluoxetine (green), hydroxytamoxifen (red), and prochloraz and tamoxifen (blue).

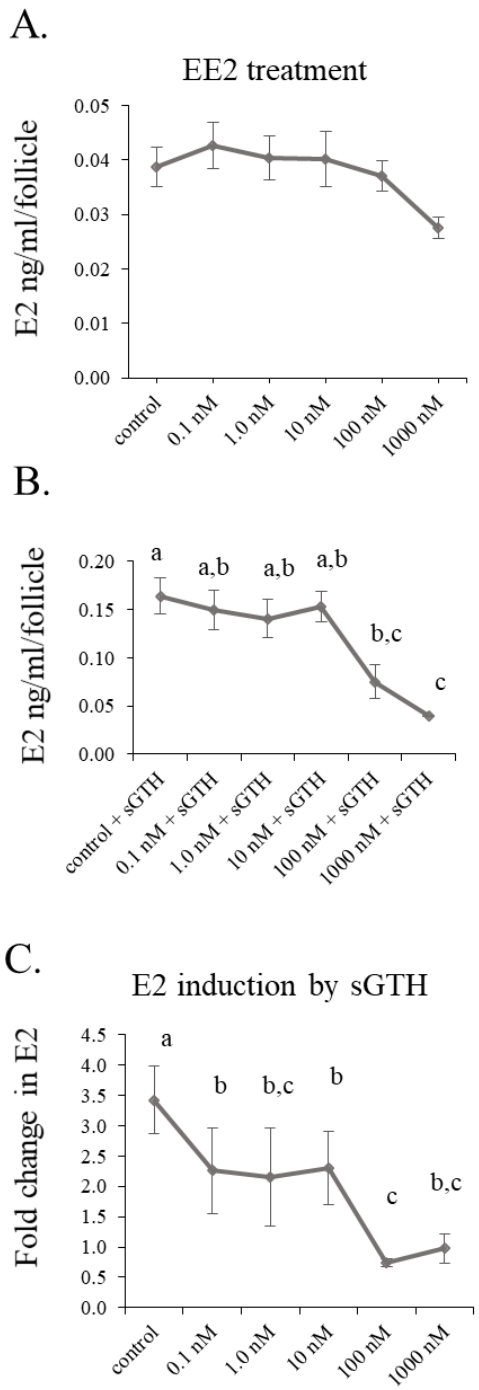

Figure A.3. EE2 treatment decreases the ovarian follicle response to sGTH. (A) Basal E2 production was not altered by EE2 after 24 hours of treatment, but the sGTH induced E2 production was significantly reduced by concentrations of EE2 ≥100 nM after 6 hours (B). After 24 hour with sGTH (42 hours of total culture time), the responsiveness of the follicles to sGTH was significantly reduced by concentrations of EE2 ≥0.1 nM (C). E2 levels were calculated from the means of 3 replicates from 3 fish. Treatments not sharing the same letter were statistically different (ANOVA, $P < 0.05$).

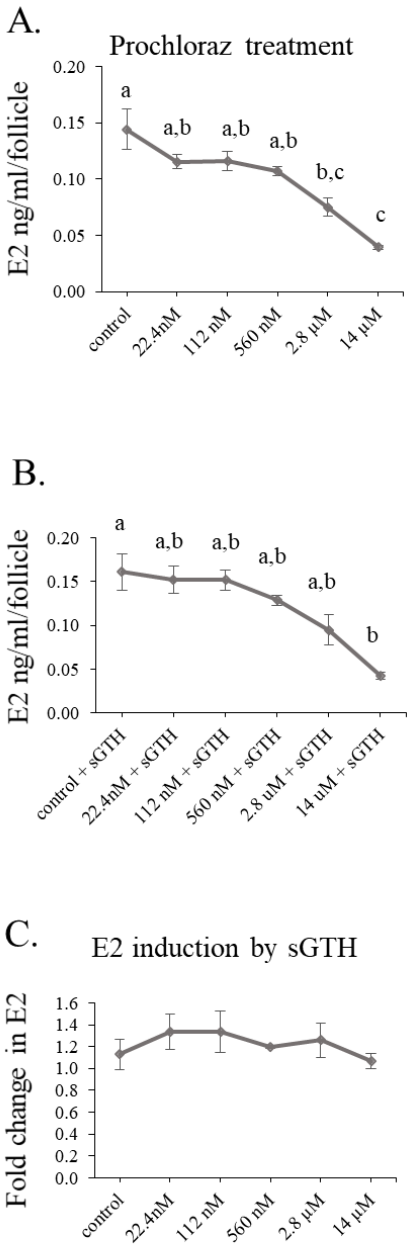

Figure A.4. Prochloraz decreases basal E2 production in the ovarian follicle. Prochloraz treatment significantly reduced basal E2 production at 2.8 µM, and further at 14 µM concentrations (A) after 24 hours of treatment. Prochloraz treatment for 24 hours with a 6-hour sGTH challenge produced a similar pattern as prochloraz alone (B), and the fold change in E2 production induced by sGTH was unchanged by prochloraz treatment (C). E2 levels were calculated from the means of 3 replicates from 3 fish. Treatments not sharing the same letter were statistically different (ANOVA, $P < 0.05$).

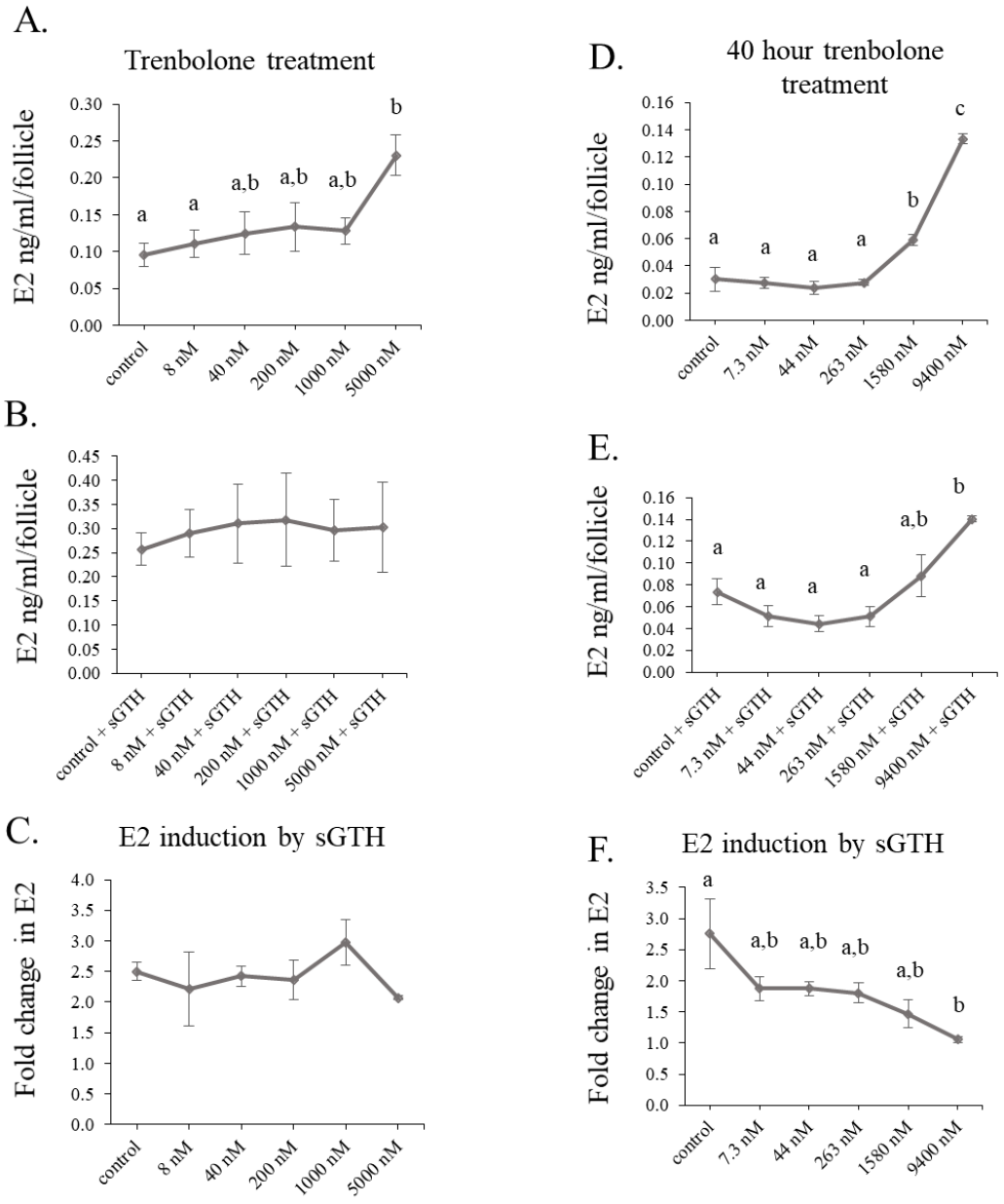

Figure A.5. Trenbolone increases basal E2 production, but decreases the Oovarian follicle response to sGTH. (A) Exposure to 5000 nM trenbolone for 24 hours significantly increased ovarian follicle E2 production, but did not impact E2 induction by sGTH challenge (B-C). Following a 40 hour exposure, (D) trenbolone treatments ≥1580 nM increased basal E2 production (E), and trenbolone at a concentration of 9400 nM also increased the responsiveness of the follicles to sGTH, although caused an overall reduction in the responsiveness of the follicles to sGTH (F). E2 levels were calculated from the means of 3 replicates from 3 fish. Treatments not sharing the same letter were statistically different (ANOVA, $P < 0.05$).

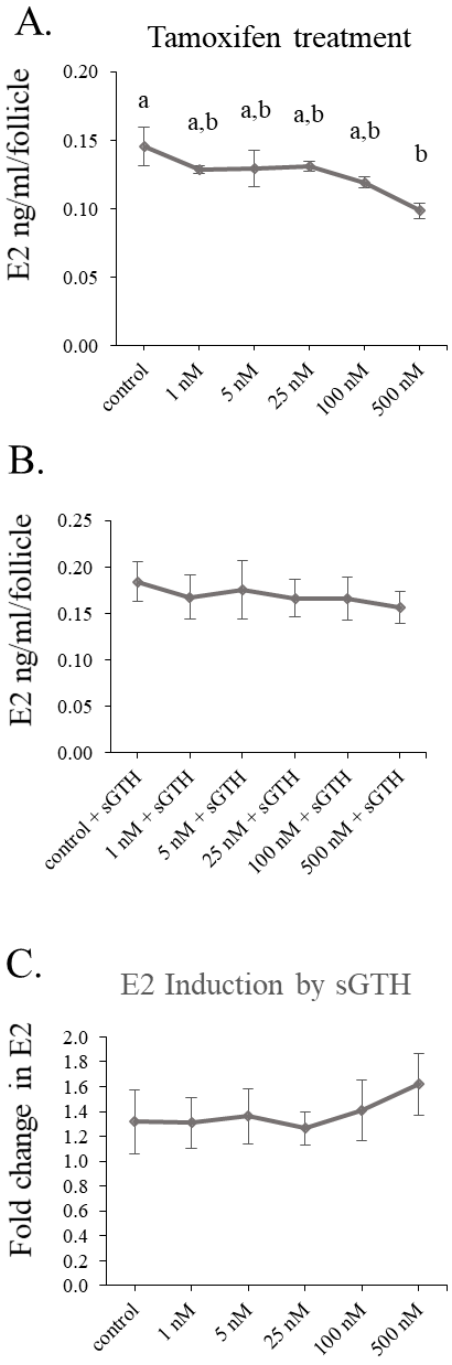

Figure A.6. Tamoxifen decreases basal ovarian follicle E2 production, but only at high concentrations. Tamoxifen at a concentration of 500 nM reduced basal E2 production (A), but did not alter sGTH induced E2 production (B), and there was no change in the responsiveness of the follicles to sGTH (C) after 24 hours of treatment. E2 levels were calculated from the means of 3 replicates from 3 fish. Treatments not sharing the same letter were statistically different (ANOVA, $P < 0.05$).

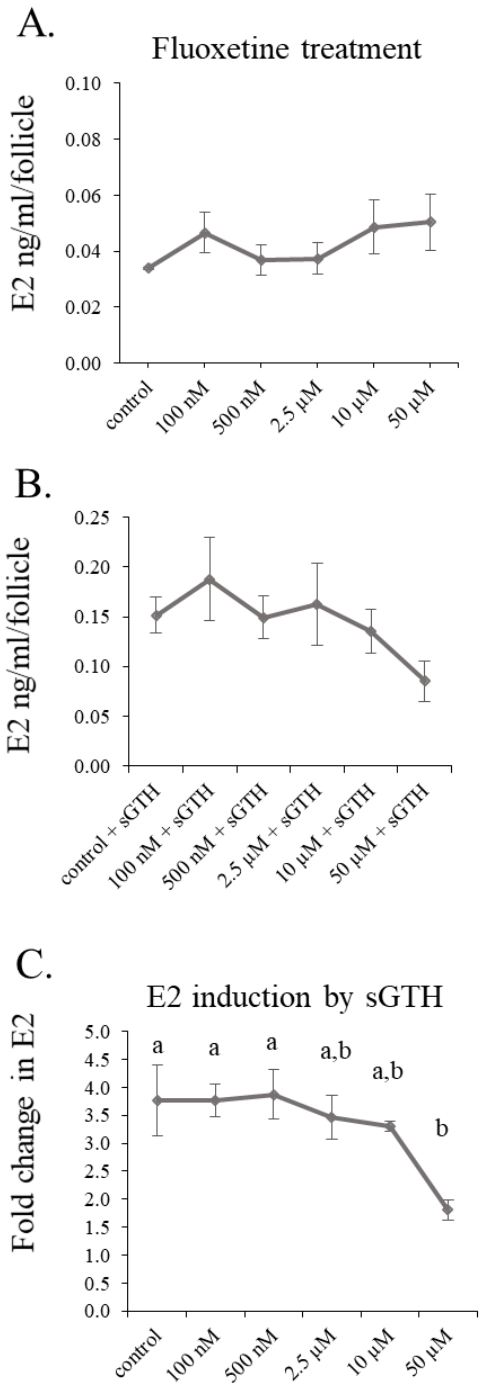

Figure A.7. Fluoxetine decreases the ovarian follicle response to sGTH, but only at very high concentrations. After 24 hours, there was no change in basal E2 production from fluoxetine exposure (A), nor and change in the induction of E2 by ovarian follicles from sGTH challenge (B). There was a significant reduction in the responsiveness to sGTH at very high concentrations of fluoxetine (C). E2 levels were calculated from the means of 3 replicates from 3 fish. Treatments not sharing the same letter were statistically different (ANOVA, $P < 0.05$).

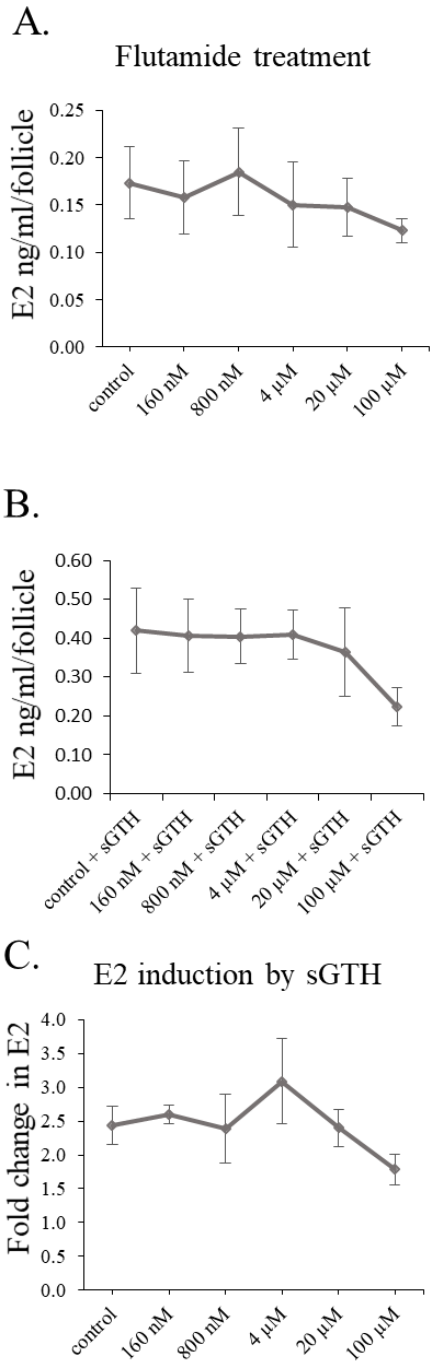

Figure A.8. Flutamide does not alter ovarian E2 production. Flutamide did not alter basal (A) or sGTH induced (B) E2 production after 24 hours of treatment, and there was no change in the responsiveness of the follicles to sGTH (C). E2 levels were calculated from the means of 3 replicates from 3 fish.

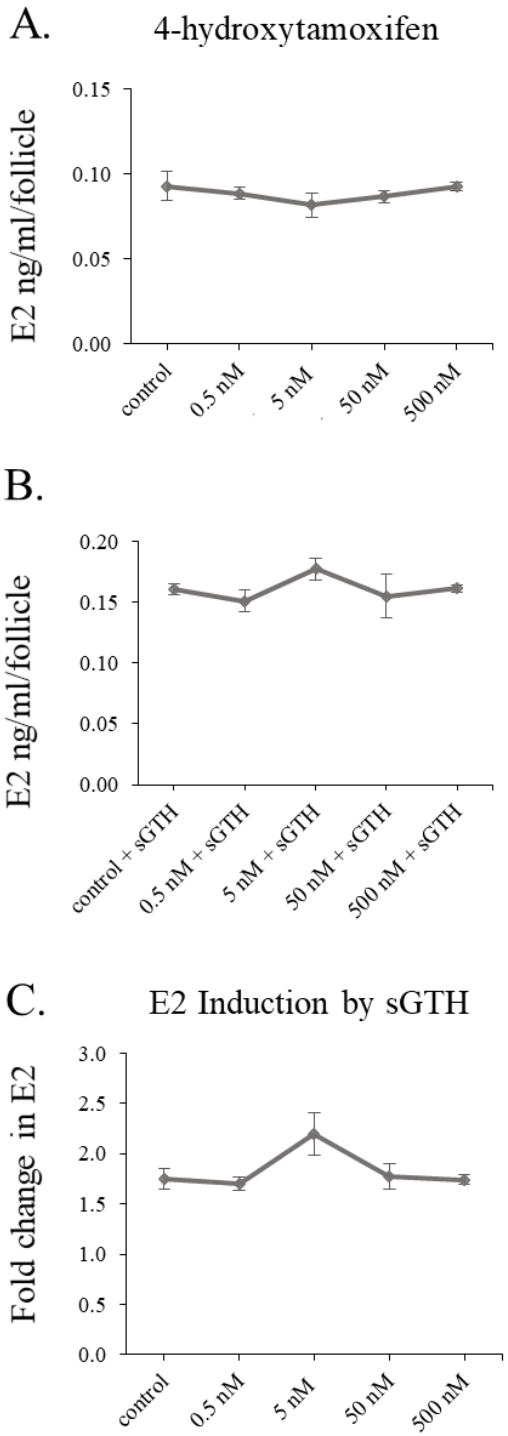

Figure A.9. 4-Hydroxytamoxifen does not alter ovarian follicle E2 production. 4-hydroxytamoxifen did not alter basal (A) or sGTH induced (B) E2 production after 24 hours of treatment, and there was no change in the responsiveness of the follicles to sGTH (C). E2 levels were calculated from the means of 3 replicates from 3 fish.

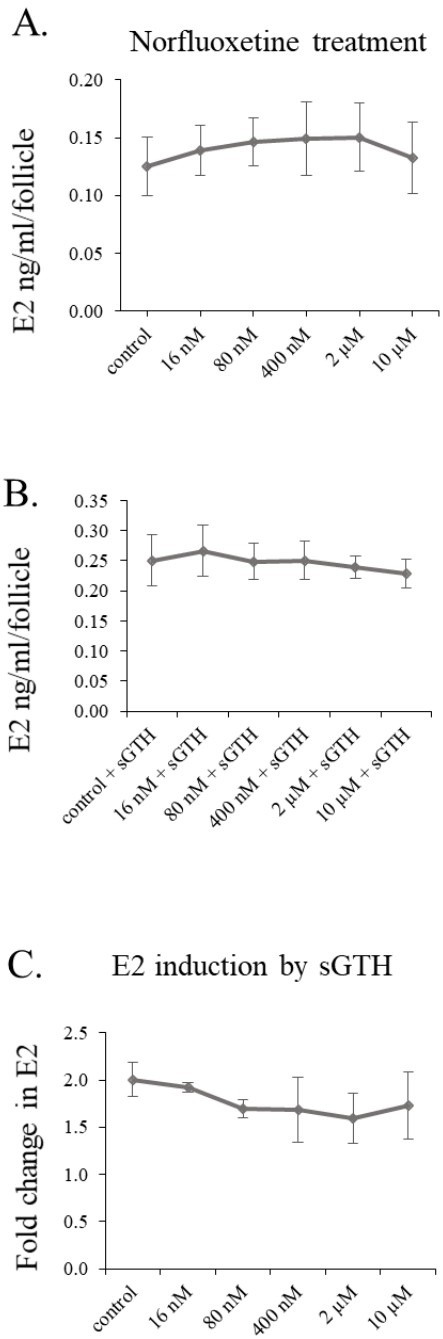

Figure A.10. Nor-Fluoxetine does not alter ovarian E2 production. Nor-fluoxetine did not alter basal (A) or sGTH induced (B) E2 production after 24 hours of treatment, and there was no change in the responsiveness of the follicles to sGTH (C). E2 levels were calculated from the means of 3 replicates from 3 fish.

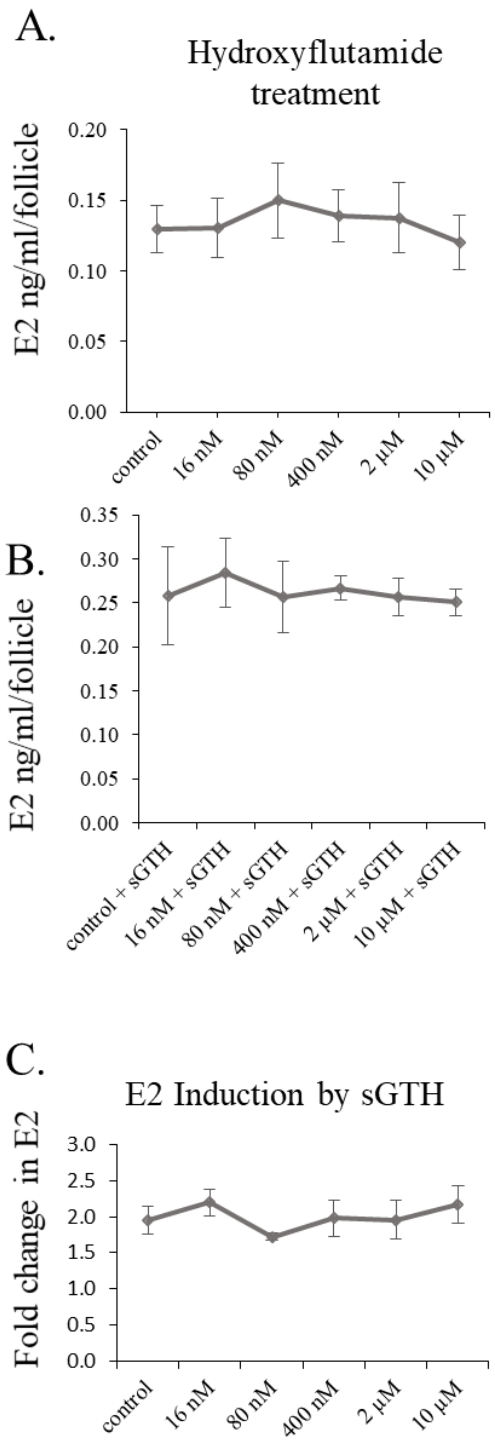

Figure A.11. Hydroxyflutamide does not alter ovarian E2 production. Hydroxyflutamide did not alter basal (A) or sGTH induced (B) E2 production, and there was no change in the responsiveness of the follicles to sGTH (C). E2 levels were calculated from the means of 3 replicates from 3 fish.

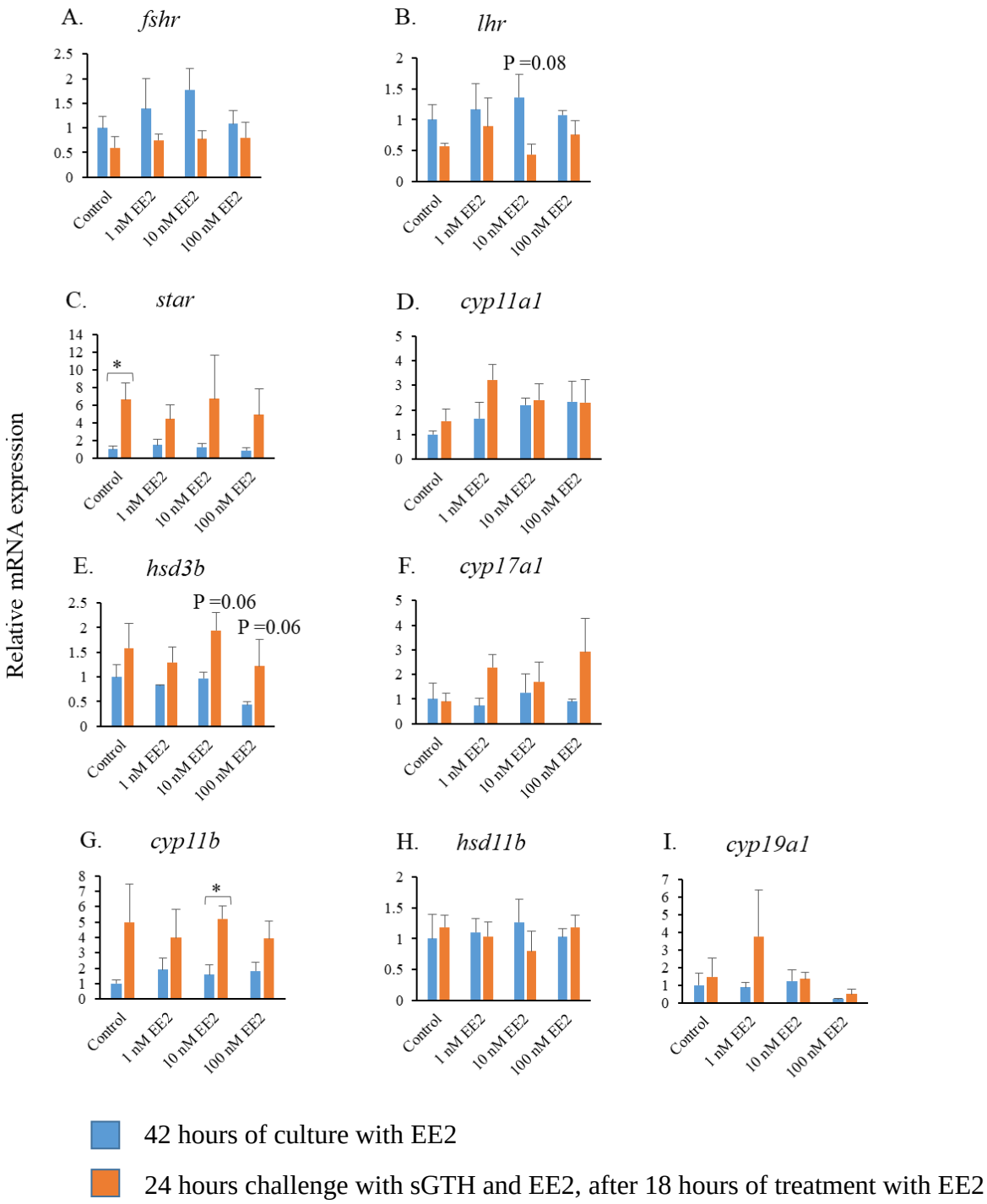

Figure A.12. EE2 does not alter steroidogenic gene expression in the ovarian follicle. EE2 did not alter the expression of genes encoding steroidogenic enzymes (A-I) after 42 hours of EE2 treatment, but after 24-hour challenge with sGTH, *star* (C) and *cyp11b* (E) expression were increased. Expression levels were calculated from the mean expression levels of 3 fish. Asterisks indicate statistical significance (ANOVA P <0.05).