Long noncoding RNA signatures induced by TLR7 and type I IFN signaling in activated human plasmacytoid dendritic cells

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Abstract

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Long noncoding RNAs (lncRNAs) exhibit highly lineage-specific expression and act through diverse mechanisms to exert control over a wide range of cellular processes. LncRNAs can function as potent modulators of innate immune responses through control of transcriptional and post-transcriptional regulation of mRNA expression and processing. Recent studies have demonstrated that lncRNAs participate in the regulation of antiviral responses and autoimmune disease. Despite their emerging role as immune mediators, the mechanisms that govern lncRNA expression and function have only begun to be characterized. In the present study, we explore the role of lncRNAs in human plasmacytoid dendritic cells (pDC), which are critical sentinel sensors of viral infection

and contribute to the development of autoimmune disease. Using genome-wide sequencing approaches we dissect the contributions of Toll-like receptor 7 (TLR7) and type I interferon (IFN-I) in the regulation of coding and noncoding RNA expression in CAL-1 pDC treated with R848 or IFNβ. Functional enrichment analysis reveals both the unique and synergistic roles of TLR7 and IFN-I signaling in the orchestration of pDC function. These observations were consistent with primary cell immune responses elicited by detection of viral infection. We identified and characterized the conditional TLR7- and IFN-I-dependent regulation of 588 lncRNAs. Dysregulation of these lncRNAs could significantly alter pDC maturation, IFN-I and inflammatory cytokine production, antigen presentation, costimulation or tolerance cues, turnover, or localization, all consequential events during viral infection or IFN-I-driven autoimmune diseases such as systemic lupus erythematosus (SLE). These findings demonstrate the differential responsiveness of lncRNAs to unique immune stimuli, uncover regulatory mechanisms of lncRNA expression, and reveal a novel and tractable platform for the study of lncRNA expression and function.

TABLE OF CONTENTS

List of Figures

List of Tables

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DEDICATION

For my father,

who framed my perception of all that exists in the living world

through a constant refrain: “survival advantage”.

February 5, 1946 – July 25, 2014

And for my mother,

who gave me a microscope when I asked for a telescope.

Chapter 1. Introduction

1.1 Innate immune antiviral defense

Detection of microbial infection by the innate immune system relies critically upon pattern recognition receptor (PRR) activation by conserved microbial motifs[1]. Pathogen-derived nucleic acids engage with a variety of PRRs that are specialized to sense the various unique nucleic acid species characteristic of different infectious organisms (**Fig. 1**). Toll-like receptors (TLRs) are an ancient family of PRRs, 10 of which are expressed in humans in cell-type-specific combinations. TLRs are transmembrane proteins distributed throughout various subcellular compartments, with TLRs 3, 7, 8, and 9 expressed in intracellular endosomal compartments and specialized in the detection of viral and bacterial nucleic acids[2]. Engagement of nucleic acids with these TLRs results in signaling through MyD88 or TRIF proteins to activate downstream signal cascades via mitogen associated protein kinase (MAPK), NF-kB and interferon regulatory factors (IRFs). These signals initiate production of inflammatory molecules which act locally and distally to restrain infectious processes, protect uninfected tissues, recruit cells of the immune system to the site of infection and promote their activation.

Type I interferons (IFN-I) are inflammatory proteins rapidly and abundantly produced upon detection of nucleic acids by many PRRs[3] (**Fig. 1**). IFN-I act on nearly all cell types through the heterodimeric IFN-I receptor (IFNAR), triggering intracellular signaling cascades primarily through the interferon stimulated gene factor 3 (ISGF3) complex, composed of signal transducer and activator of transcription (STAT) 1, STAT2 and IRF9[4]. Signaling by IFN-I results in the transcriptional induction of hundreds of IFN-

stimulated genes (ISGs) which confer protective antiviral activities and limit the spread of viral infections[5, 6, 7]. IFN-I also acts upon cells of the immune system to activate innate and adaptive immune responses to control infection[8]. Similarly, aberrant detection of host-derived endogenous nucleic acids by TLRs stimulates release of IFN-I, which drives inflammation and promotes self-antigen-specific autoreactivity in many systemic autoimmune diseases and interferonopathies[9]. Such aberrant IFN-I signaling is implicated in the pathogenesis of systemic lupus erythematosus (SLE)[10], where an elevated ISG signature in patient blood correlates with disease severity[11, 12]. Further evidence of the role of IFN-I in facilitating the initiation of autoreactivity is also provided by the observation that IFNAR-deficient mice are protected from the development of disease in adjuvant-induced and genetic SLE models[13, 14].

1.2 **Plasmacytoid dendritic cells**

Innate immune PRR detection of infectious motifs by dendritic cells (DC) results in subsequent release of inflammatory molecules such as IFN-I, as well as direct engagement and activation of cells of the adaptive immune system[15]. Through innate immune detection of infection and presentation of pathogen components for the generation of antigen-specific adaptive immune protection, DCs bridge the intersection of innate and adaptive immunity and are thus strategic targets for the manipulation of autoimmune responses[16]. Plasmacytoid dendritic cells (pDC) have been extensively implicated in the pathogenesis of SLE[17, 18] as a major source of IFN-I, generating up to 1,000 times more than any other cell type[19, 20]. Acting as phagocytic surveillance sentinels[21], pDC are specialized in the detection of nucleic acids and production of IFN-I through high constitutive expression of the endosomal TLR7 and TLR9, which engage

single stranded RNA (ssRNA) or unmethylated CpG DNA, respectively[22, 23] (**Fig. 1**). IFN-I produced by pDC is key in initiating protective resistance to infection in peripheral tissues and modulating the activity of other immune cells, as studies in murine models of pDC depletion have shown[24]. Additionally, IFN-I is essential to the function of pDC, as auto/paracrine IFN-I signaling in activated pDC results in amplification of subsequent IFN-I, inflammatory cytokine production, and modulation of pDC effector functions such as expression of costimulatory molecules and chemokine receptors[25, 26, 27, 28, 29, 30, 31, 32, 33, 34].

While production of IFN-I upon TLR engagement is a major role of pDC, they also exhibit diverse functions in immune orchestration which position them as critical regulators of inflammation[16]. pDC produce inflammatory cytokines and chemokines that activate and recruit other cells of the innate and adaptive immune system, such as monocytes, NK, T and B cells[35, 36, 37, 38, 39, 40, 41]. They are also gatekeepers of autoimmunity and excessive inflammation through their roles in antigen presentation to T cells, promotion of T regulatory responses and restraint of cytotoxic T cells[42, 43, 44, 45, 46, 47, 48]. Given the critical role of pDC in antiviral responses and initiation of autoimmune disease, a thorough understanding of factors which regulate their diverse activities is important as dysregulation of such elements could lead to pivotal outcomes in disease. In particular, factors that control transcriptional, post-transcriptional and post-translational regulatory processes which govern the magnitude of cytokine and chemokine production in pDC may lay a foundation for insufficient antiviral responses or the development of autoimmune disease.

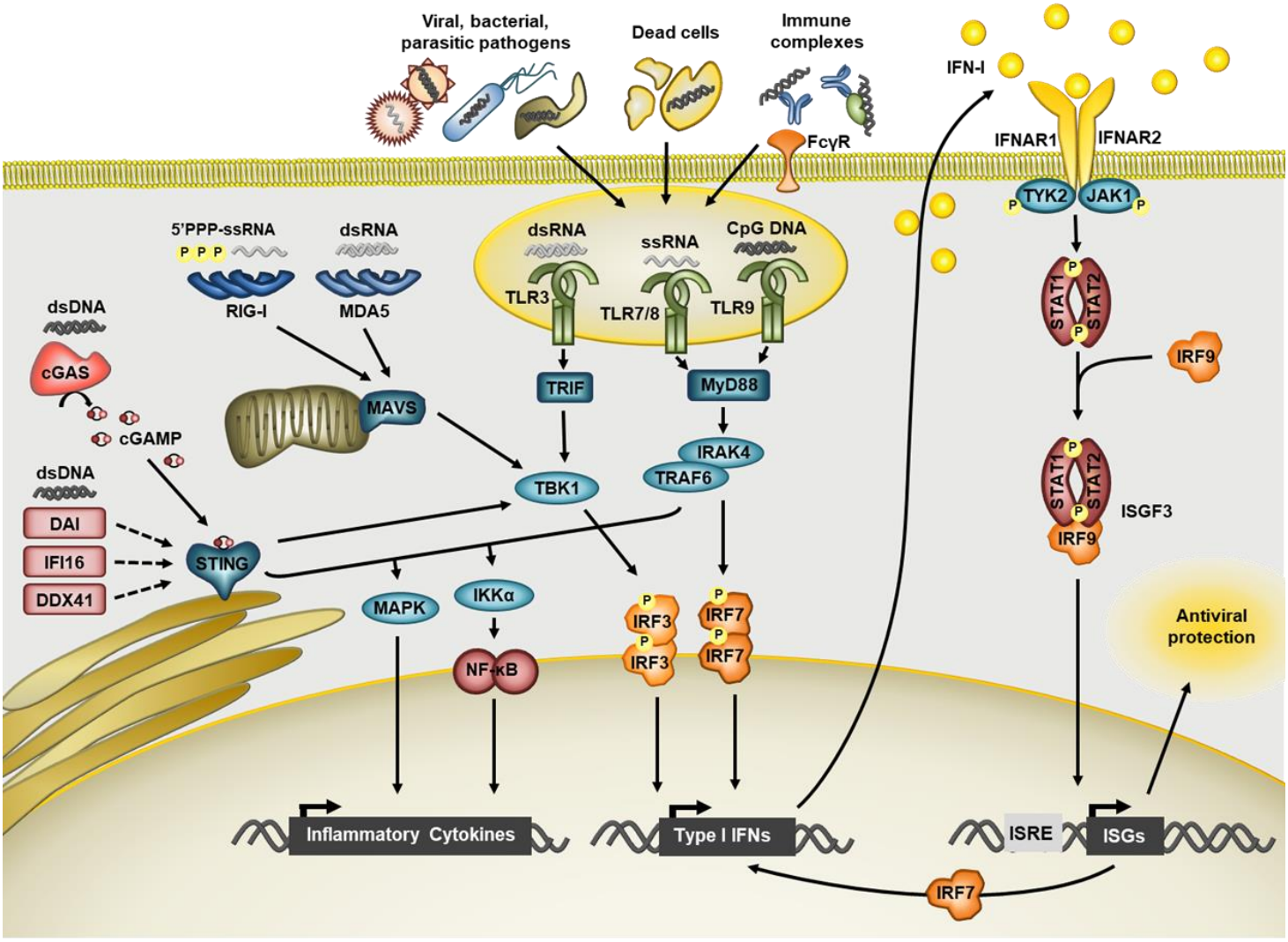

Figure 1. Intracellular nucleic acid sensing and type I interferon pathways.
In antigen presenting cells, phagocytic uptake of pathogen or cellular components imports foreign or self-derived nucleic acids into the endosome, where various nucleic acid species activate toll-like receptor (TLR) 3, which signals through TRIF, or TLRs 7/8 and 9 which signal through MyD88. Cytosolic detection of RNA occurs via RIG-I-like receptors (RLRs) which signal through MAVS at the mitochondrial membrane, and sensing of cytosolic interferon-stimulatory DNA (ISD) occurs via cyclic GMP-AMP synthase (cGAS) or various other sensors (DAI, IFI16, DDX41) which signal through STING at the endoplasmic reticulum (ER) membrane. Activation of each of these sensors triggers kinase- or second messenger-mediated signaling cascades to culminate in the activation and nuclear translocation of transcription factors that drive expression of type I IFNs (IFN-I; IFNA1, IFNA2, IFNA4, IFNA5, IFNA6, IFNA7, IFNA8, IFNA10, IFNA13, IFNA14, IFNA16, IFNA17, IFNA21) and proinflammatory cytokines (i.e. TNFA, IL6, IL12). RLRs and the ISD pathway primarily mediate IFN-I transcription through IRF3 homodimers, whereas TLR7/8 and 9 drive IFN-I expression via IRF7. Plasmacytoid dendritic cells (pDC) predominantly express TLRs 7/8 and 9, as well as high levels of IRF7, enabling nucleic acid sensing through endosomal uptake and rapid transcription of IFN-I genes. IFN-Is signal through a heterodimeric cell surface receptor (IFNAR1/2) to drive kinase-mediated activation of STAT1/2 heterodimers which, in conjunction with IRF9, form the interferon stimulated gene factor 3 (ISGF3) complex targeting interferon stimulated response elements (ISRE) upstream of interferon stimulated genes (ISGs). Transcription of ISGs results in expression of multiple effectors conferring antiviral protection. IRF7 is an ISG, thus in pDC auto/paracrine IFN-I signaling results in feedback-mediated amplification of TLR-driven IFN-I production and expression of proinflammatory cytokines or other TLR-dependent pDC effectors.

1.3 Principles and functions of long noncoding RNAs

1.3.1 *Discovery of long noncoding RNA*

Since the initial discovery of RNA, our understanding of its role in biological events has continually evolved beyond that of encoding a blueprint for protein synthesis. Technical challenges in RNA measurement have limited our ability to decipher a complete picture of its function within living tissues, and many decades-old questions are only now beginning to be feasibly answered with the advent of novel technologies. Advances in high-throughput sequencing technology have enabled researchers to comprehensively and objectively profile the human transcriptome at greater depth, producing an ever-expanding understanding of the realm of processes that involve RNA. Such studies have revealed that while ~95% of the human genome is transcribed and conservation analysis indicates 80% of the genome possesses functional significance[49], only ~2% of transcripts encode protein[50, 51, 52, 53], implying that the vast majority of genomic output is noncoding and functions in the cell through other means (**Fig. 2**). The functional importance of noncoding loci is also evidenced by findings from cross-species genome comparisons that sites of accelerated evolution are enriched among noncoding regions[54, 55] and from genome wide association studies (GWAS) that disease-associated variants fall outside protein-coding regions[56]. From the obsolete view of a genome full of “junk DNA” with RNA simply a transient intermediate between DNA source code and functional protein, noncoding RNAs (ncRNA) have emerged as key regulators of varied cellular processes through diverse mechanisms[57].

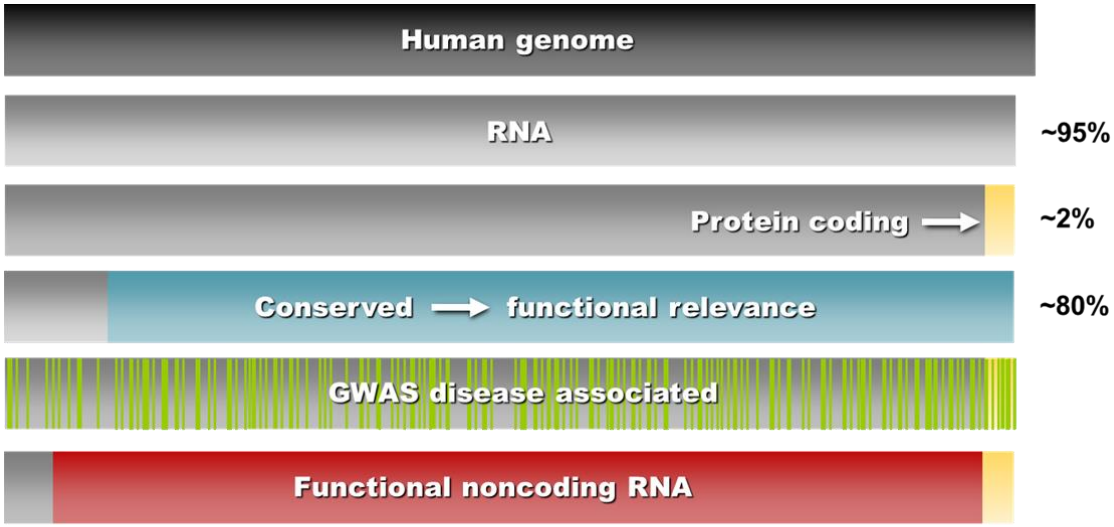

Figure 2. Genomic evidence for noncoding RNA functionality

Transcriptome sequencing across many organisms reveals abundant expression of long ncRNAs (lncRNAs) >200 nucleotides in length[58, 59] (**Fig. 3A**). Many lncRNAs share features of coding genes such as promoter-controlled expression, exon splicing, and polyadenylation[60, 61]. lncRNA biotypes are defined by their genomic origin relative to coding genes, and are derived from antisense transcription, coding gene introns, pseudogenes, standalone intergenic regions (long intergenic noncoding RNA, lincRNA) or from alternatively transcribed or spliced coding genes[50, 58, 61, 62] (**Fig. 3B**). In the absence of a requirement to maintain codon integrity, lncRNAs display a high degree of evolutionary permissiveness to mutation, resulting in highly species-specific signatures, with <10% of lncRNA loci conserved between human and mouse [63]. Additionally, sequencing within various human tissues has shown lncRNA expression to be extremely cell-type specific, even more so than coding or small noncoding RNA signatures[64, 65].

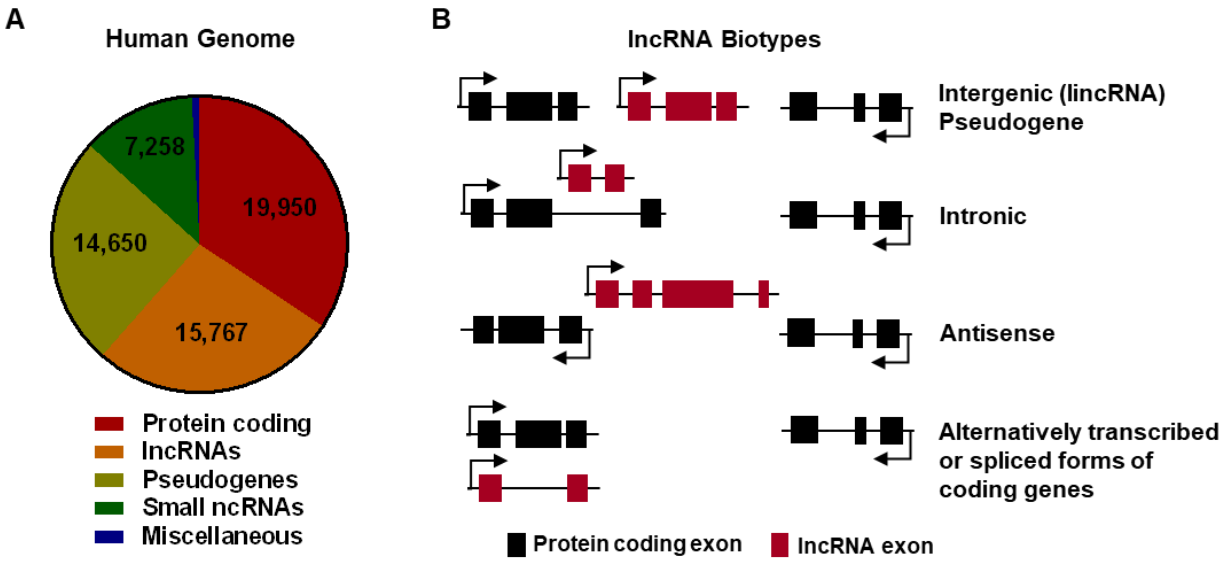

Figure 3. Genomic origins of lncRNAs
(A) Number of genes by type annotated in the human genome (GENCODE v25). (B) Origins relative to coding genes of transcripts which can function as lncRNAs. Adapted from Atianand 2017 AnnRevImmunol.

1.3.2 *Mechanistic diversity of lncRNAs*

While identification of novel lncRNAs is proceeding rapidly, functional mechanisms have been uncovered for very few transcripts. However, those investigated were found to play potent roles in a wide variety of cellular processes through diverse means, revealing a novel and complex layer of regulatory activity[66, 67]. Through sequence complementarity with other nucleic acids, lncRNAs have the capacity to capture and manipulate other transcripts. Regulation through sequence-directed means is illustrated by the roles of the small (21-31 nucleotide) ncRNAs [micro (miRNA), small nucleolar (snoRNA), small interfering (siRNA) and piwi-interacting (piwiRNA) RNAs], which are potent regulators of a wide range of cellular events through control of mRNA generation, abundance and translation[68, 69]. Sequence specificity also endows lncRNAs the ability to facilitate targeted modification of other nucleic acids through their association with

RNA/DNA-modifying proteins, however unlike small ncRNAs, lncRNAs possess intricate and labile secondary and tertiary structures which can influence their selective dynamic associations with other molecules[70]. Acting as structural scaffolds in facilitating or antagonizing protein colocalization, lncRNAs can modulate the functional interactions of key enzymes with other proteins and nucleic acids[71]. lncRNA primary and secondary structures influence their associations with RNA-binding proteins (RBPs), with conserved motifs enabling selective RBP binding at structurally accessible regions[72]. Mutations that disrupt sequence specificity, RBP motifs, or perturb secondary/tertiary structure may alter lncRNA function, and thereby dysregulate processes that define disease outcomes. It is a testament to the discretionary and versatile physical capacities of RNA that noncoding loci are sites rapid evolutionary change[73], implicating lncRNAs as a choice weapon utilized by both host and pathogen and indicating that lncRNA-mediated exchanges are vital players at the host-pathogen interface.

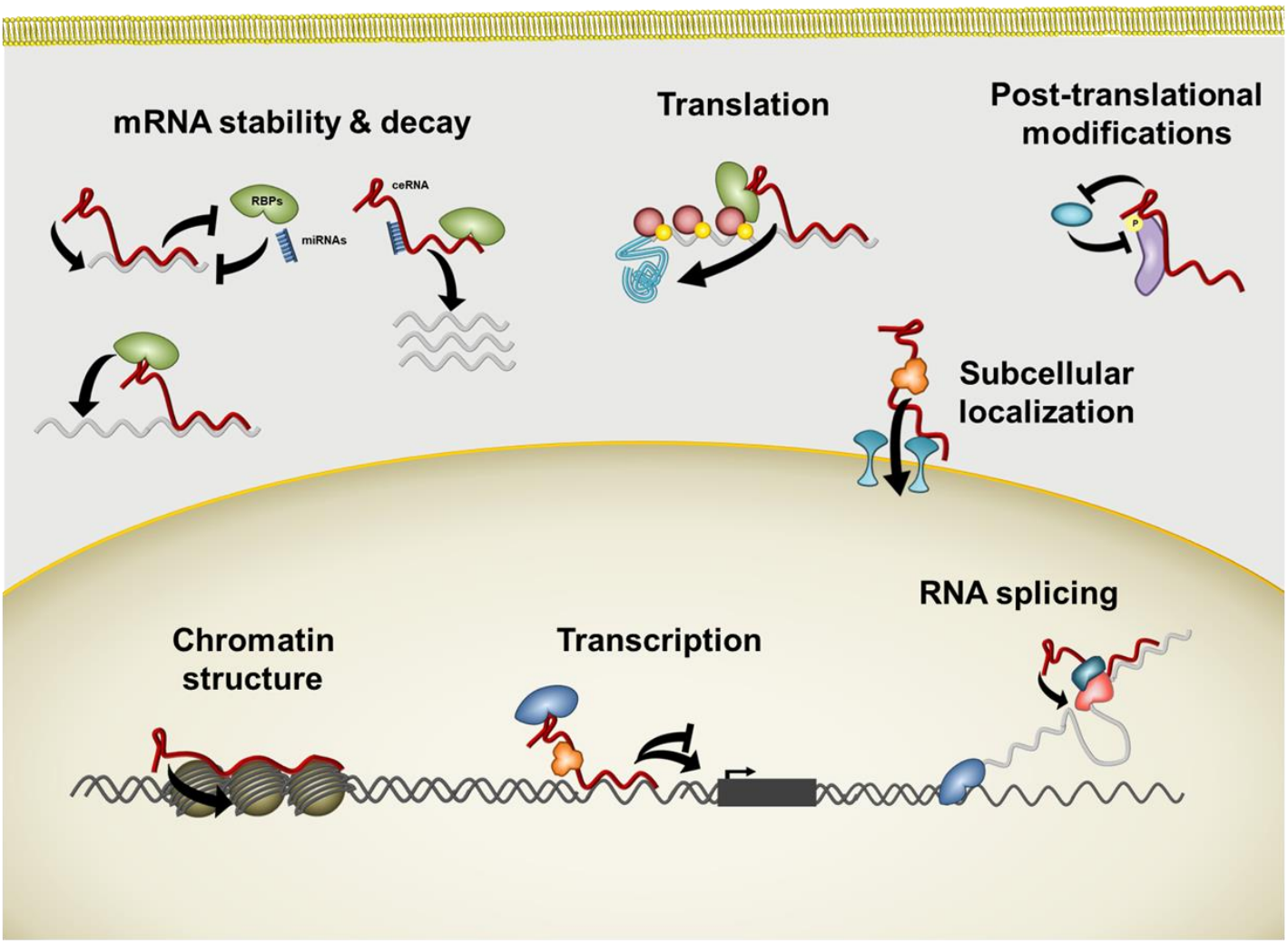

Figure 4. Mechanistic diversity of lncRNA functions
lncRNAs conduct diverse nuclear and cytoplasmic functions through their ability to bind proteins and nucleic acids. They act through a variety of modes, including scaffolding (chromatin, protein-protein, protein-RNA/DNA interactions), colocalization anchors, transcriptional or post-transcriptional nucleic acid modification site masking, decoy/competitive inhibition (ceRNA), post-translational protein modification site masking, or control of protein subcellular localization. lncRNAs facilitate or antagonize numerous biologic processes, including control of nuclear translocation, nuclear architecture, chromatin modification, transcription, splicing, mRNA stability/decay, translation, and post-translational modifications.

1.3.3 *Location-dependent lncRNA activities*

The functional activities of lncRNAs are largely linked to their subcellular localization[74]. When localized within the nucleus, lncRNAs function in epigenetic regulation[75] and transcriptional control[76]. Through recruitment, tethering or antagonism of

chromatin-modifying and transcriptional machinery, lncRNAs direct the concert of transcriptional activity[77]. By targeted guidance of these factors to select regions, lncRNAs control gene expression in *cis* to antisense or nearby genes[78] and in *trans* throughout the genome[79, 80], and can also play scaffolding roles in chromatin looping and nuclear architecture[81].

When localized within the cytosol, lncRNAs are key participants in the complex post-transcriptional layer of gene regulation[82]. lncRNAs termed competing endogenous RNAs (ceRNAs) function as decoys[83, 84], competing with messenger (m)RNAs for occupation of RBPs[85] and miRNAs[86, 87, 88, 89, 90], thereby relieving gene repression or stabilization by these factors. Cytoplasmic lncRNAs also modulate mRNA decay by masking RBP or miRNA binding sites, promote RNA stability through provision of structural support, impact protein levels through disruption of translational machinery, and control the activities of key signaling mediators through occupation of critical protein interaction or post-translational modification sites[91]. Through their ability to negatively regulate the repressive activities of miRNAs, RBPs or other enzymes, cytoplasmic lncRNAs are critical determinants of gene regulation. Cytoplasmic lncRNAs have also been shown to be key participants in intracellular signaling cascades[91] and to regulate nuclear translocation of transcription factors[92], thereby regulating gene expression upstream of transcription. However, the full breadth of these roles have yet to be elucidated in innate immunity, a context in which rapid gene induction and translation is crucial.

1.3.4 *Functional roles of lncRNAs in immune responses*

The myriad mechanistic means of lncRNAs to modulate cell-type specific gene expression profiles indicates broad potential for regulatory roles in immune responses[93]. Through control of lineage-defining transcription factors, lncRNAs have been shown to exert control over lineage development and differentiation within the hematopoietic system[91, 94, 95, 96, 97]. Host and pathogen-derived lncRNAs have been implicated in essential processes in immune responses[93, 98, 99] such as control over cytokine[80, 92, 100, 101, 102, 103, 104, 105, 106, 107] and antiviral gene[108, 109] expression, regulation of signal-mediating transcription factors such as NF-κB[110, 111] and STAT3[91], and negative regulation of PRR activation[112]. Studies implicating lncRNAs in innate antiviral immunity[113] have profiled marked changes in expression signatures during virus infection[108, 114, 115, 116, 117, 118, 119], much of which overlaps with IFN-I-inducibility. Several lncRNAs have been found to regulate IFN-I production[120, 121] and many lncRNAs have been defined through *in vitro* studies as IFN-I or PRR-inducible in various tissues[100, 107, 121, 122, 123, 124, 125, 126]. However, the mechanisms through which viral infection and inflammatory signals contribute to changes in lncRNA expression are poorly understood.

Profiling of lncRNA signatures and definition of their functional mechanisms among the various cell types and stimuli of the human immune system has only just begun. Neither IFN-I or TLR-modulated lncRNA expression changes nor lncRNA functions have been investigated in human pDC. While IFN-stimulated gene (ISG) profiling in pDC has revealed transcriptional profiles for protein coding genes[127, 128], until now noncoding loci were omitted from such studies. This study defines lncRNA expression profiles in human pDC following TLR ligation or IFN-I signaling, and we propose to a cytoplasmic role for

lncRNAs in modulating innate immune effectors in pDC (**Fig. 5**). We hypothesize that lncRNAs whose expression is modulated by these signals subsequently regulate various crucial elements of activated pDC function and demonstrate a platform upon which these roles may be further defined.

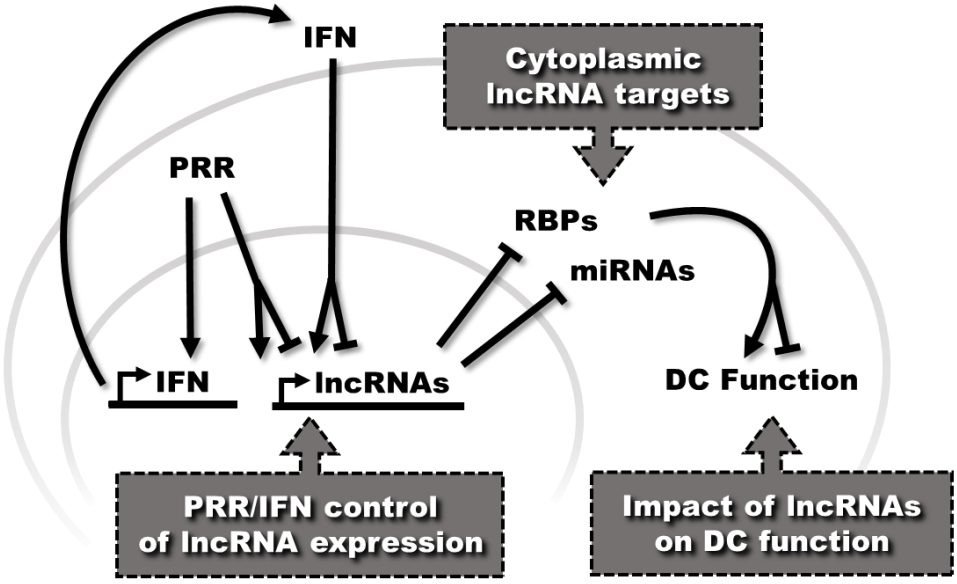

Figure 5. Research strategy for the study of lncRNAs in human pDC

Chapter 2. Defining long noncoding RNA signatures in human pDC

2.1 Introduction

High lineage specificity positions lncRNAs as critical regulators of cell subset differentiation and cell type-specific activities[95, 129]. However, the exact mechanisms through which lncRNAs modulate pDC development and function are unknown. While gene expression profiling in human pDC has defined cell type-specific coding genes [127], a comprehensive description of the long noncoding transcriptional landscape in pDCs has been challenging due to limited numbers of cells and low levels of lncRNA expression. To gain a better understanding of the noncoding transcriptional landscape in activated pDC, we undertook comprehensive transcriptome profiling in a tractable human pDC line following TLR7 engagement or IFN-I signaling. We identified novel subsets of lncRNA species that are differentially regulated by these innate immune stimuli, neighboring genes that are co-regulated with lncRNAs, and transcriptional regulators likely to coordinate lncRNA expression. We compared these transcriptome profiles with those of activated primary pDC, demonstrating that these data provide a solid platform for the study of lncRNA expression and their roles in pDC development and effector functions. We identified nuclear and cytoplasmic lncRNAs with known and unknown mechanisms which might impact pDC function. Further characterization of lncRNAs described in this study will identify new biomarkers and therapeutic targets for disease.

2.2 Results

2.2.1 *TLR7 and IFN-I signals drive broad transcriptional change in human pDC*

We utilized human CAL-1 cells, which phenotypically and functionally resemble primary peripheral pDC [130, 131], to understand the differences in the response to TLR7 and/or IFNAR ligation. CAL-1 cells were treated for 12 hours with the TLR7 agonist R848 in the presence or absence of the pan-IFN-I inhibitory protein B18R [132, 133], or treated with IFN-β, enabling dissection of the relative contributions of TLR and IFN-I signaling to gene expression (**Fig. 6A**). Treatment with R848 led to a significant induction of *IFNB1* expression, which was partially decreased upon blockade of the IFNAR-mediated TLR amplification loop (R848+B18R) (**Fig. 6B left, 6C**). Expression of the ISG, *MX1*, was induced by both R848 and IFN-β treatment and its expression was abrogated in the presence of B18R in R848-treated cells, demonstrating efficient IFN-I blockade (**Fig. 6B right, 6D-E**). Genome-wide transcriptional profiling was conducted on these samples for characterization of the coding and noncoding transcriptome changes in response to TLR7 and IFN-I stimulations (**Fig. 6F**).

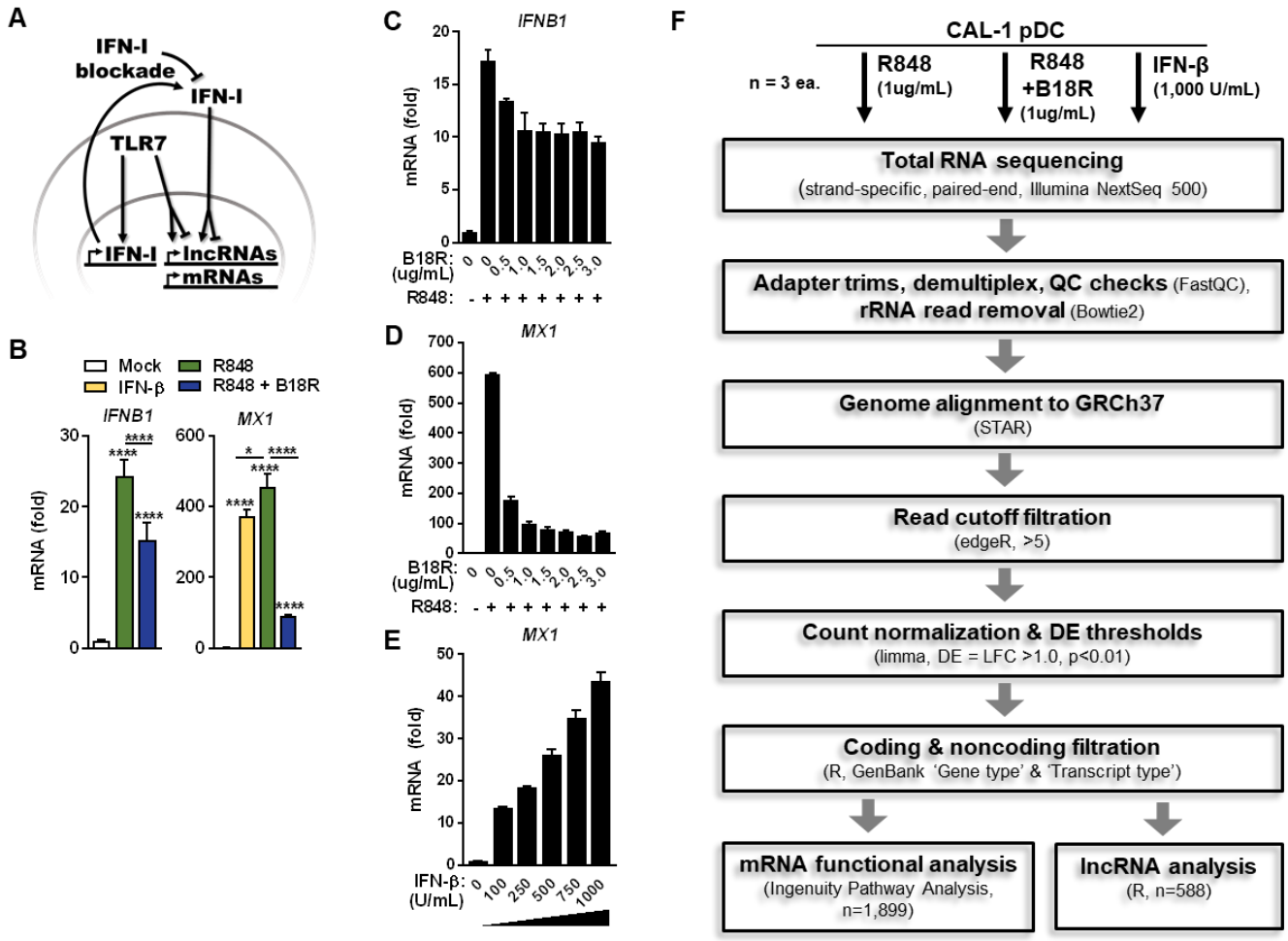

Figure 6. Experimental design for transcriptome sequencing of IFN-I and TLR7-stimulated human pDC
(A) Experimental schematic of TLR7 and IFN-I engagement in CAL-1 human pDC line used for transcriptome sequencing. (B) CAL-1 cells were stimulated with TLR7 agonist (R848) with or without blockade of type I IFN signaling by pan-IFN-I blocking protein (B18R) or treated with hIFN-β for 12 hours and *IFNB1, MX1* or mRNA quantified by RT-PCR normalized to GAPDH. (C) (C-E) CAL-1 cells were stimulated with TLR7 agonist (R848, 1ug/mL) with blockade of type I IFN signaling by titration of pan-IFN-I blocking protein (B18R) or treated with hIFN-β for 12 hours to determine optimal doses and IFNB1 and MX1 mRNA quantified by RT-PCR. (F) Schematic depicting RNA sequencing sample preparation and analysis pipeline.

Transcriptome profiling revealed substantial changes in gene expression in response to TLR ligation and IFN-I (**Fig. 7A**). Moreover, IFN-I blockade had a noticeable impact in the transcriptional signatures captured after R848 treatment (**Fig. 7A**). Statistical analysis of gene expression changes yielded 1,899 coding and 709 noncoding

significantly differentially expressed genes (DEGs) in response to R848, R848+B18R or IFN-β. Within the detected signatures we observed a high correlation between R848 and R848+B18R treatment (r^2= 0.736) and moderate correlations between R848 or IFN-β treatments (r^2= 0.430), consistent with the relative exposures to IFN-I and TLR engagement in each set (**Fig. 7B**). Consistent with this observation, the majority of the transcripts induced after IFN-β treatment were also found to respond to R848 stimulation. However, following IFN-I blockade, the induction of many of these transcripts was abrogated (**Fig. 7C**). Additionally, a contrast in fold change between R848 and R848+B18R conditions evidences the impact of IFN-I feedback in amplifying TLR-induced gene expression (**Fig. 7D**). These data demonstrate that although TLR7 and IFN-I activate distinct signaling pathways, IFN-I production amplifies TLR7 signaling in a feed forward loop, which enhances pDC responses to PRR ligands.

Functional analysis of coding DEGs across these stimulatory conditions revealed enrichment of gene sets pertaining to IFN-I signaling, PRR activation and dendritic cell (DC) functions (**Fig. 7E**). Accordingly, we observed enrichment of IFN signaling modules and strong induction of classical ISGs (**Table 1**) by TLR7 and IFN-I stimulations, which were markedly diminished by IFN-I blockade during TLR7 activation. In contrast, enrichment of DEGs related to DC functions such as antigen presentation and lymphocyte engagement were modulated primarily by TLR engagement versus IFN-I (**Fig. 7E**). However, control of DEG expression regulated by IRFs was similar downstream of both IFN-I and TLR stimulations, indicating redundancy in the pathways which control activity of these factors (**Fig. 7E**). To better understand the complexity of TLR7-mediated responses we implemented a regulatory network analysis which demonstrated robust

production of IFN-I (IFN-α and IFN-β), the activation of the signal transducer and activator of transcription (STAT; STAT1 and STAT3), IRFs, NF-κB (RELA, NFKB1, NFKBIA), and AP-1 (**Fig. 7F, left**). The strength of these activities was diminished in the absence of IFN-I signaling following TLR7 activation, but the diversity of mediators is preserved (**Fig. 7F, middle**). In contrast, responses to IFN-I were predicted to be primarily driven by STATs and IRFs, and there was a notable loss of NF-κB and AP1 activity (**Fig. 7F, right**). These data demonstrate that the stimulus-specific transcriptional response to TLR7 agonists and IFN-I is driven by unique transcription factors. Additionally, the induction of IFN-I further promotes cellular activation, cytokine production and maturation. Given the consistency of the observed gene expression patterns in CAL-1 cells with those observed in primary human and murine pDC[15, 134], this cell line provides a viable platform for the investigation of immune-responsive noncoding RNAs.

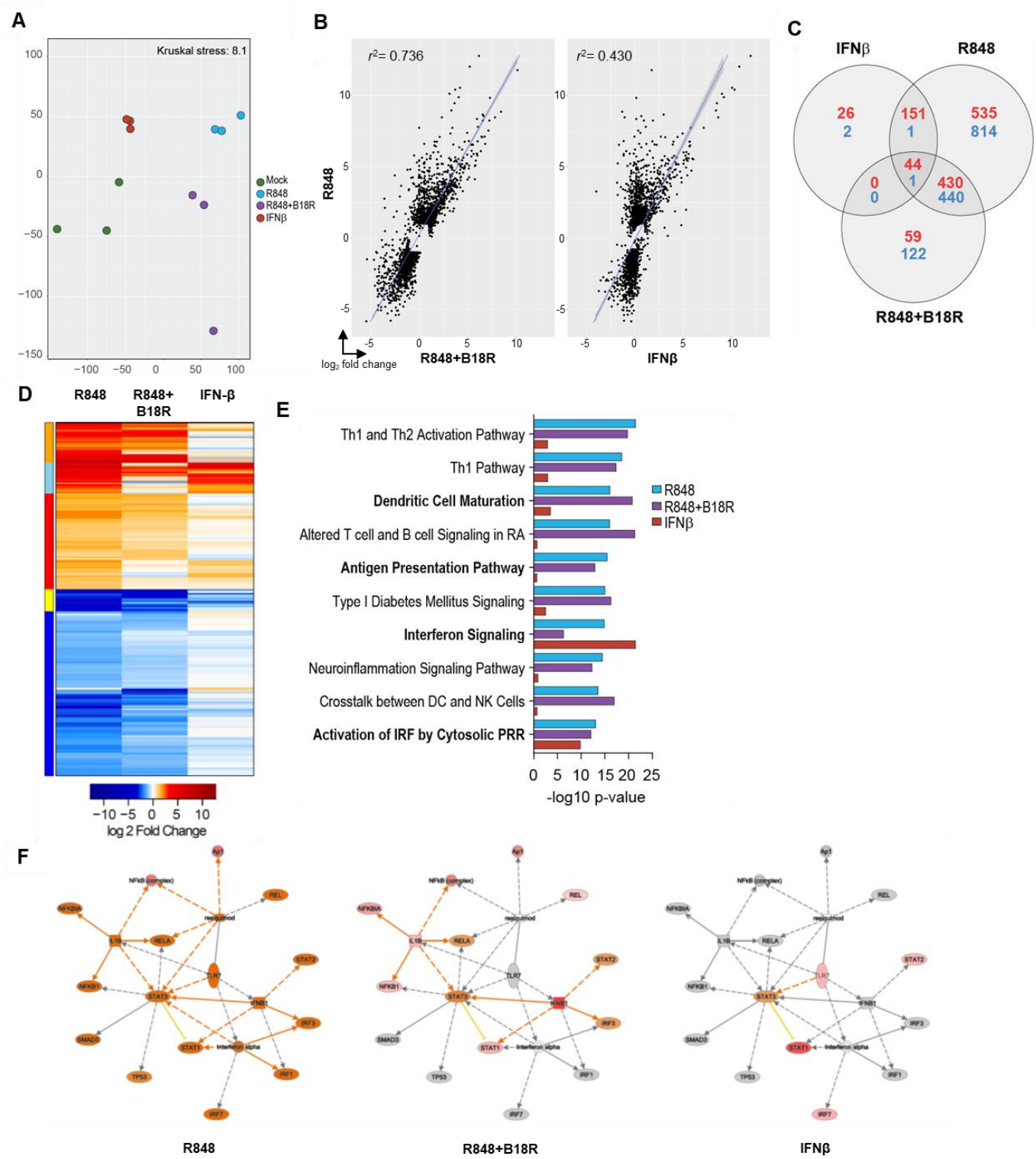

Figure 7. Transcriptome sequencing in IFN-I and TLR7-stimulated human pDC

(A) Multimensional scaling (MDS) of the whole genome transcriptome profiles from mock, IFN-β, R848 and R848+B18R-treated CAL-1 pDC. (B) Scatterplot depictions of correlation in the union of 2,610 differentially expressed (DE) genes in R848, R848+B18R and IFN-β stimulations. (C) Venn diagram depicting overlap across treatment conditions in the numbers of DE transcripts. Red indicates upregulated genes and blue indicates downregulated genes. (D) Heatmap showing unsupervised clustering of 2,610 genes DE over mock in any one condition. (E) Functional analysis of biologic pathways enriched within DE genes across each condition. (F) IPA molecular network mapping of DE genes within each condition.

Gene	log_2 fold change (over mock)		
	R848	R848+B18R	IFN-β
IFI44L	12.745	6.146	11.950
MX2	11.996	5.178	10.794
OAS2	10.526	4.200	10.046
IFI6	10.384	3.706	10.311
IFI44	10.109	3.100	8.985
EPSTI1	9.850	4.068	9.190
IFIT1	9.444	3.623	8.467
XAF1	8.941	3.295	8.372
CCL1	8.842	8.425	-1.317
IFNB1	8.534	6.618	0.660
OAS1	8.458	2.340	8.597
CCL2	8.437	7.801	-1.040
IL6	8.347	5.948	0.155
CD69	8.234	6.539	1.874
CD40	8.229	7.200	0.932
NR4A3	7.790	6.036	1.114
IFI27	7.729	-0.012	7.123
OASL	7.711	3.394	5.778
CDH1	7.530	7.108	0.337
CCR7	7.371	4.938	0.574
RIN2	7.312	3.223	4.936
EDN1	7.101	6.408	2.695
CSF2	7.092	6.738	-0.035
HELZ2	7.070	2.106	4.422
CTSH	7.046	6.453	3.147
IL18RAP	6.790	5.080	-1.142
SLAMF1	6.761	6.006	1.811
RASGRP3	6.758	4.689	3.989
ISG20	6.756	1.568	5.694
IFIT5	6.737	1.808	5.662
DYSF	6.585	6.790	0.435
C17orf99	6.556	5.498	2.052
IFIT2	6.546	3.514	3.909
BCL2A1	6.535	5.510	-1.646
SAMD9L	6.477	1.547	5.702
ISG15	6.410	1.713	5.658
ECE1	6.345	4.109	0.688
TNFSF18	6.186	4.421	0.013
SLIT2	6.104	5.378	1.547
IL32	6.091	6.322	-0.879
IFIT3	6.014	1.934	5.335
HLA-DOA	5.919	5.908	-0.124
FCGR1A	5.895	0.760	5.545
HIVEP3	5.879	5.571	-1.163
BOD1L2	5.840	4.948	1.282
MYH7	5.785	-0.533	5.241
SGK1	5.776	5.970	-1.143
DDX58	5.764	1.150	4.068
TNFSF10	5.721	2.228	3.086
TMCC3	5.641	3.705	4.312

Table 1. Top 50 TLR7-induced coding genes from transcriptome sequencing in CAL-1 pDC.

2.2.2 *TLR7 and IFN-I stimulations induce distinct lncRNA signatures*

Further characterization of the DEGs modulated in these conditions revealed that roughly one fourth are noncoding transcripts (**Fig. 8A**). Notably, in contrast to nearly equivalent numbers of up- and downregulated DEGs among coding loci, noncoding loci display a marked trend toward more genes downregulated than upregulated following TLR7 activation, and IFN-I blockade enhances this phenomenon (**Fig. 8A**). Amongst the noncoding transcripts, the majority (n=588) were identified as lncRNAs, with their distribution across lncRNA biotypes biased toward antisense transcripts (33%), standalone intergenic lincRNAs (30%), and pseudogenes (29%) (**Fig. 8B**). We did not capture a bias among lncRNA biotypes in the directionality of expression (**Fig. 8C**).

Unsupervised clustering of lncRNA relative expression yielded unique response modules, identifying lncRNAs under the regulation of either TLR7 or IFN-I **(Fig. 8D, Table 2)**. Consistent with the induction of coding ISGs by IFN-I, we identified 64 lncRNAs induced by exogenous IFN-β and whose induction is specifically dependent upon auto/paracrine IFN-I signaling in the context of TLR7 activation, as evidenced by the impact of IFN-I blockade (**Fig. 3E**). Conversely, we identified 89 lncRNAs which are generally indifferent to IFN-I signaling but are induced strictly by TLR engagement through mechanisms independent of IFN-I feedback (**Fig. 8F**). We identified a large set of lncRNAs (n=369) whose expression is inhibited following TLR engagement (**Fig. 8D).** Of these downregulated lncRNAs, 84 were markedly downregulated by R848 treatment despite IFN-I exposure **(Fig. 8G**). In sum, these 588 DE lncRNAs comprise a novel definition of lncRNA sensitivity to TLR and IFN-I stimuli.

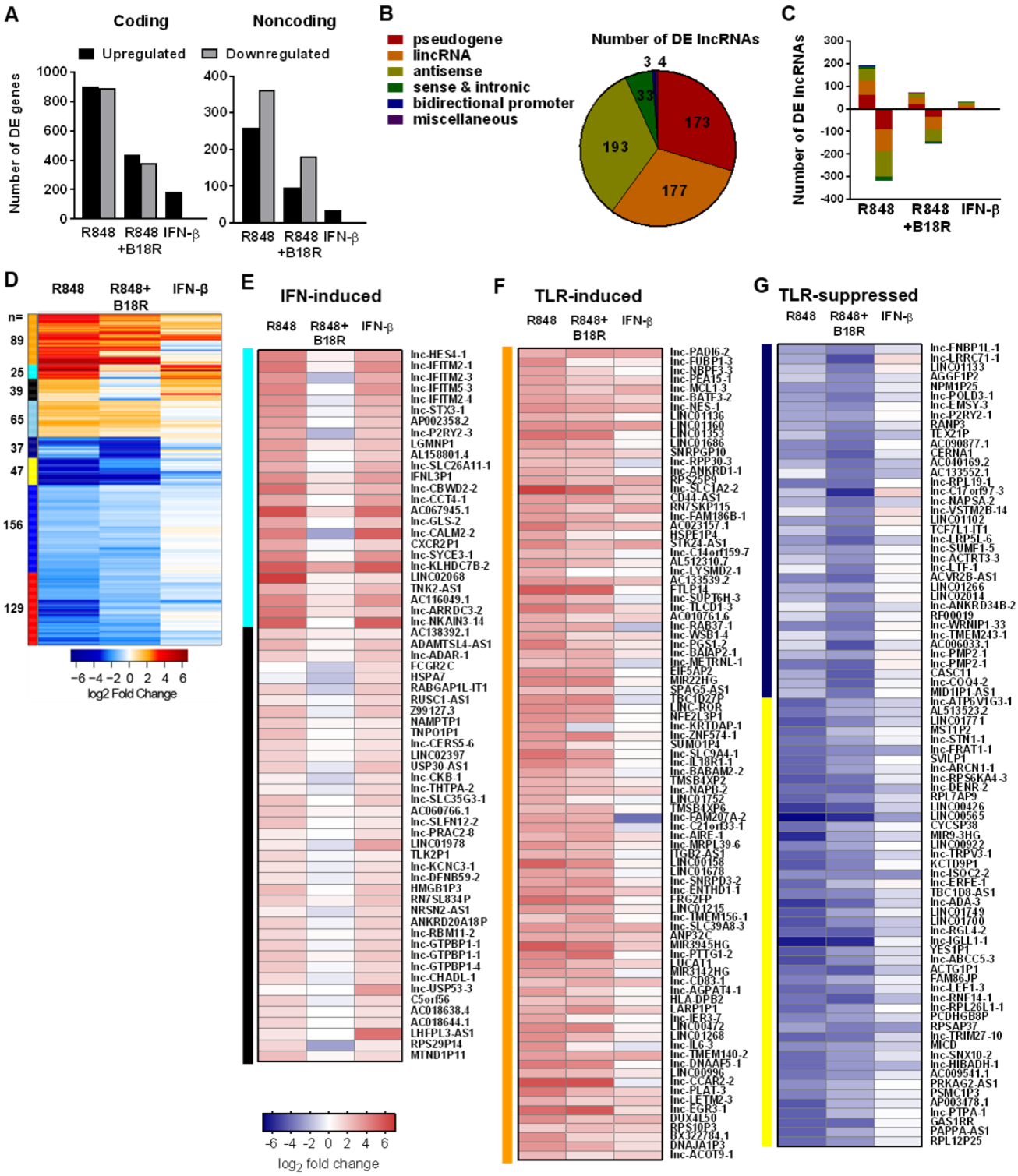

Figure 8. TLR- and IFN-I-modulated lncRNAs in human pDC

(A) Distribution of DE coding and noncoding transcripts after stimulation of CAL-1 cells. Distribution of lncRNA biotypes among (B) all lncRNAs DE within any one condition and (C) within each stimulation condition, plotted by directionality of fold change. (D) Heatmap of relative expression of 588 lncRNAs and unsupervised Euclidean distance clustering of transcripts. Annotated heatmaps were generated from modules representing unique immune responsiveness. These were defined as (E) IFN-I-induced lncRNAs (cyan and black clusters, n=64), (F) TLR7-induced lncRNAs (orange cluster, n=89), and (G) highly TLR-suppressed lncRNAs (dark blue and yellow clusters, n=84).

Transcript Type	Number of genes	
	Total detected	Significantly DE (log_2FC > 1.0, p < 0.01)
Coding	13,239	1,899
Noncoding	11,705	709
lncRNAs	9,072	588

Table 2. Number of coding and noncoding genes identified in transcriptome sequencing of CAL-1 pDC.

2.2.3 *TLR7-mediated gene expression changes reflect those observed in primary pDCs*

We have identified of TLR7-responsive coding and noncoding RNAs in *in vitro* pDC cultures. To evaluate whether these observations faithfully recapitulate the transcriptional changes captured in primary human pDCs we compared CAL-1 gene signatures with those of *ex vivo* virus-infected pDCs [135]. *In vitro* infection of pDC with influenza virus is reported to activate TLR7 to induce IFN-I and proinflammatory cytokines [42], and was shown induce differentiation of *ex vivo* primary human pDC into subsets which primarily produce IFN-I ("pDC1" which are PD-L1^{hi} CD80^{lo}), those with reduced IFN-I production but elevated antigen presentation capacity ("pDC3" which are PD-L1^{lo} CD80^{hi}), or an intermediate phenotype ("pDC2" which are which are PD-L1^{hi} CD80^{hi}), as measured by flow cytometry [135]. We compared the transcriptome of R848-stimulated CAL-1 with those of *in vitro* influenza-infected sort-purified primary pDC1, pDC2, and pDC3 cells. We observed a moderate correlation in coding DEG expression between TLR7-stimulated CAL-1 and virus infected primary pDC subsets (**Fig. 9A**). More importantly, we found a high to moderate degree of correlation in the expression of DE lncRNAs between CAL-1 and pDC1, pDC2 and pDC3 subsets (**Fig. 9B**). Correlation of these DEG patterns between mRNAs and lncRNAs reflects concordance between TLR7 ligands and culture

systems (**Fig. 9C**). Notably, we observe higher correlations between differentially expressed lncRNAs than mRNAs common to each dataset (**Fig. 9C**), consistent with the phenomenon of lncRNA signatures more tightly reflecting cellular lineage and activation states than coding gene signatures. Functional enrichment analysis of coding DEGs common to TLR7-stimulated CAL-1 and virus infected primary pDC revealed genes involved in inflammatory cytokine and IFN-I signaling and antiviral responses (**Fig. 9D**). This analysis demonstrates that the CAL-1 cell line displays similarities with primary human pDCs in their responses to TLR7 agonists.

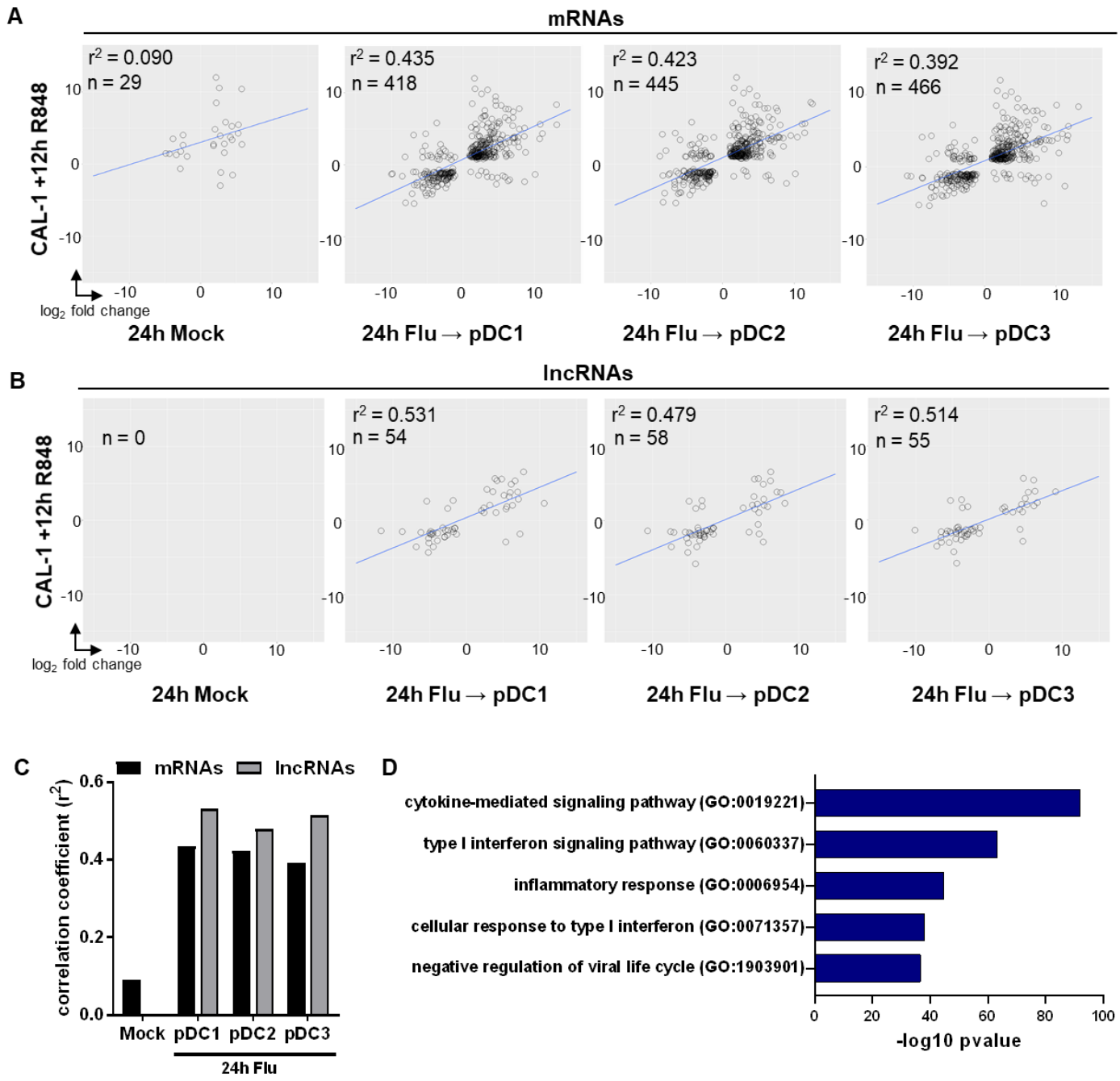

Figure 9. Transcriptomes of TLR-activated CAL-1 pDC correlate with *in vitro virus*-infected primary pDC
(A) Scatter plot and Pearson correlations of coding DEGs identified by RNA in CAL-1 pDC treated with R848 versus primary human pDC cultured *ex vivo* for 24 hours (mock) or infected with influenza virus for 24 hours and sort-purified by flow cytometry according to expression of PD-L1 and CD80 into subsets which produce IFN-I (pDC1), present antigen (pDC3) or have an intermediate phenotype (pDC2). DEG values were calculated as R848 fold over mock in CAL-1, and in primary pDCs mock as 24h media over *ex vivo*, and pDC1/2/3 as 24h infection over mock per pDC subset. (B) Scatter plot and Pearson correlations of lncRNA DEGs identified in R848-treated CAL-1 across mock and each influenza-infected pDC subset. (C) Bar plot of correlation coefficients listed in (A) and (B). (D) Combined scores of gene ontology (GO) term enrichment within coding DEGs identified in (A).

2.3 Discussion

Given the vast potential for discovery of novel mechanisms of immune response control by lncRNAs, we sought to describe the noncoding transcriptome of human pDC and develop a model system whereby functional analyses of lncRNA regulatory processes can be achieved. Collectively, these *in vitro* studies define the coding and noncoding transcriptome signatures of TLR7- and IFN-I-stimulated human pDC. We isolated the impact of IFN-I feedback following TLR activation and identify coding and noncoding gene sets sensitive to this influence. This investigation also delineates the concordance in DEG patterns of the CAL-1 line with primary human pDC. Our characterization of the lncRNA landscape in human pDC adds a critical element to the growing body of annotation of tissue- and stimulation-specific lncRNA expression profiles and lays a foundation for further study of their functions in immune responses.

Poor conservation of lncRNAs across species and accelerated evolution at these loci warrants comprehensive documentation of expression profiles in human cells. High lineage specificity and stimulation-specific expression changes add a further layer of analysis required to fully catalogue human noncoding genomic output. lncRNA expression profiles and individual functional roles have yet to be fully elucidated for the breadth of innate immune stimuli and human cellular subsets. Though coding ISGs have been extensively annotated, noncoding loci were omitted from such studies and very few descriptions of IFN-stimulated lncRNAs exist. Functional examples of lncRNAs induced by TLR activation or IFN-I illustrate the capacity of these molecular players to potently regulate this critical pathway in innate antiviral immunity[93]. Here, we present the transcriptome signatures of TLR7 and IFN-I stimulated human pDCs and identify lncRNAs

whose expression is modulated by these stimuli. In this analysis, we have defined patterns of lncRNA regulation by innate immune TLR activation and IFN-I signaling, subsetting cohorts of transcripts differentially induced or suppressed by TLR or IFN-I signaling. We also characterized the biotype distribution of these transcripts.

Restraint of inflammatory responses following TLR engagement is regulated by diverse means, including signaling by anti-inflammatory cytokines as well as transcriptional and post-transcriptional repression, and is critical in limiting tissue damage [136]. Several lncRNAs have been shown to function as negative regulators of inflammation[92, 103, 106, 107, 108, 110, 111, 112, 137], including IFN-I production[120, 121]. In our data set we observe a trend of substantially more noncoding genes being downregulated than upregulated following TLR stimulation compared to an equivalent distribution of coding DEG directionality. This phenomenon appears in our study to be a broad feature of lncRNAs and is unrestricted to certain lncRNA biotypes. We hypothesize that many of these downregulated transcripts act as negative regulators of cellular processes which are augmented following pDC TLR activation. Relief of the repression enforced by these lncRNAs via their downregulation after TLR activation would facilitate a burst of the cellular activities they restrain. Notably, downregulation of many lncRNAs has been observed in many cancers[138, 139, 140, 141], a phenomenon which is often a product of chronic inflammation, as well as following administration of probiotic dietary supplements[142]. While our findings indicate IRF and NF-κB-driven control of IFN-I and TLR-inducible lncRNAs, many factors such as miRNA-mediated decay or transcriptional repression may influence the downregulation of lncRNAs following TLR activation. Further study of the factors controlling lncRNA downregulation is warranted and may yield insight into

potential transcript functional mechanisms. Many of the downregulated lncRNAs identified in this study may function as a critical brake on inflammatory processes, thus dysfunction in their upstream regulators might have deleterious consequences due to uncontrolled inflammation. Additionally, the TLR7- and IFN-I-induced lncRNAs we have identified could also act as negative regulators of other repressors, as in the case of ceRNAs sponging miRNAs away from their target mRNAs, thereby facilitating increased mRNA stability and translation of inflammatory proteins. We hypothesize that many of the IFN/TLR-induced and TLR-suppressed lncRNAs identified in this study thus act as critical regulators of the cytokine production, antigen presentation and migratory activity that ensues once pDCs become activated.

Such regulatory activities have been described for lncRNAs in other pathways such as pluripotency, but in the context of innate immune PRR activation, these roles may contribute to the rapid and robust cytokine production that ensues following ligand engagement. This is particularly relevant to the function of pDC, whose primary role is production of IFNs and inflammatory cytokines following PRR activation. It has been reported that individual dendritic cells vary greatly in their cytokine production in response to a common stimulus[135, 143], and that in pDC such individual variability in IFN-I production is independent of the amplifying IFN-I feedback loop[144]. Differences in the propensity of individual cells to produce inflammatory cytokines could be dictated by the status of regulatory lncRNAs such as those identified in this study, which may be acting as potent repressors of cytokines in otherwise precocious producers. If the activity of such lncRNAs is dysregulated due to polymorphism or other anomalous influences, exaggerated cytokine production in response to infectious or innocuous stimuli may yield the

inflammatory foundation upon which the development of autoimmune pathogenesis depends. Further genetic and functional investigations of the lncRNAs identified in this study are thus warranted to identify their regulatory roles in IFN-I and inflammatory cytokine production or other cellular activities that may contribute to antiviral responses or aberrant pDC activity in systemic autoinflammatory disease.

Our dissection of the contribution of IFN-I to gene expression changes in the context of TLR7 activation uniquely identifies coding and noncoding genes whose upregulation in activated human pDC critically depends on IFN-I feedback. This phenomenon is reported to be due to the amplification of interferon response factor 7 (IRF7) levels by IFN-I feedback[30] which subsequently drives elevated TLR7-dependent gene induction, and our data support this hypothesis (data not shown). Disparate activity of these IFN-I-dependent genes may underly the mechanisms responsible for the effects of IFN-I antagonism therapy in SLE patients[145] and the protection from disease pathogenesis provided by IFNAR1$^{-/-}$ or blockade in murine SLE models. Thus, explorations of functional relevance and polymorphic risk factors within these coding and noncoding genes may reveal potential causal mechanisms in SLE and other IFN-I driven autoreactivities and interferonopathies.

Chapter 3. Immune-regulatory pathways govern TLR- and IFN-I-modulated lncRNAs and co-expressed neighboring coding genes

3.1 Introduction

Global analyses of lncRNA and neighboring coding gene expression patterns have revealed modest associations in expression levels[73]. Patterns of co-expression in IFN-I-induced lncRNAs and nearby coding genes have been identified in murine[115] and human [121, 122, 123, 124] epithelia and demonstrated for several lncRNA-neighboring ISG pairs[122]. Co-expression patterns between lncRNAs and coding neighbors are a naturally intuitive phenomenon for lncRNAs derived from alternative splice forms, antisense or intronic transcription of coding genes, as well as those derived from pseudogenes with preserved promoters which parallel their neighboring ancestral ortholog. However, for many of these transcripts, co-expression with a neighboring coding gene may be not only coincident but causal, as in the case of antisense lncRNA *cis* transcriptional control. Additionally, co-expressed pseudogenes may contain sequence similarity to their coding orthologues, thereby conferring ceRNA functions which enable relief of repressive elements targeting the coding gene. Thus, the roles of these lncRNA-coding gene neighbor pairs are intriguing points of study and are of particular relevance within contexts of robust transcriptional induction and protein production, such as those of TLR and IFN-I engagement.

3.2 **Results**

3.2.1 *Co-expression of lncRNAs with neighboring coding genes*

To better understand the distinct co-regulation of lncRNAs and neighboring genes by TLR7 and IFN-I signaling cascades we undertook a global co-expression analysis. First, we identified broad genomic distribution across the DE coding genes and lncRNAs (**Fig. 10A**). Then, we identified the nearest coding neighbor across the previously identified TLR7 and IFN-I-regulated modules. Noticeable concordance in the directionality of gene expression was noted between the IFN-I-induced lncRNAs and their neighboring genes (**Fig. 10B**). Similar co-expression was observed amongst the TLR-induced (**Fig. 10C**) and TLR-suppressed (**Fig. 10D**) lncRNAs and their neighboring genes, with some examples of inverse directionality, suggesting it is likely that lncRNA expression is transcriptionally regulated by similar factors regulating coding gene expression.

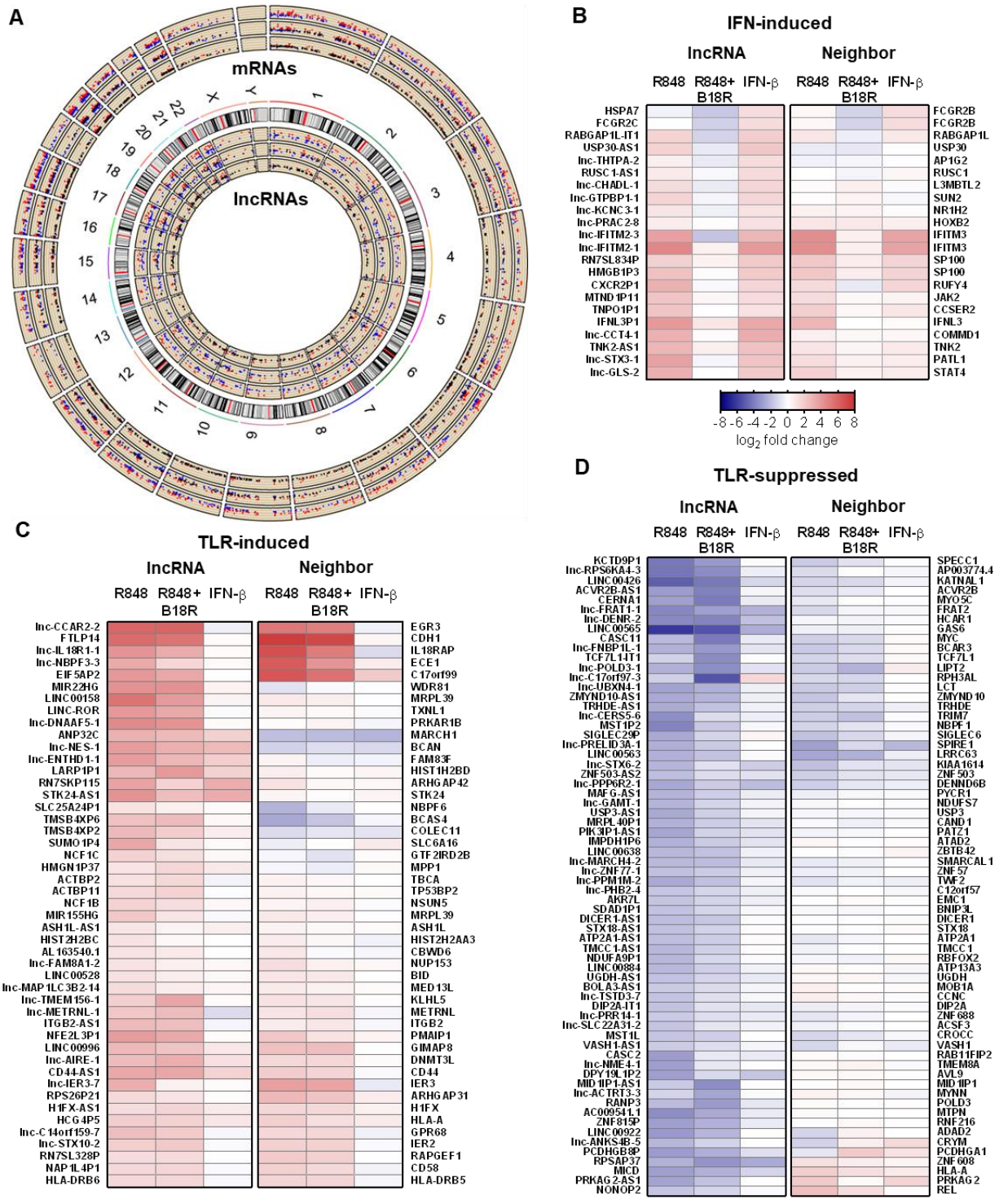

Figure 10. TLR/IFN-modulated lncRNAs are co-expressed with neighboring coding genes

(A) Circos plot of genomic distribution of all DE mRNAs (outer tracks) and lncRNAs (inner tracks) in CAL-1 pDC treated with R848, R848 +B18R, and IFNβ, respectively. Red indicates upregulated genes and blue indicates downregulates genes. Black denotes expressions values <|1|. Each condition is represented in an individual track, where the outermost track corresponds to R848 treatment and the innermost track corresponds to IFN-β. Heatmaps of DE lncRNAs (left) mapped against $\log_2$ fold change in expression of their nearest neighbor genes (right) across each condition for (B) IFN-induced lncRNAs, (C) TLR-induced lncRNAs, and (D) TLR-suppressed lncRNAs. Scatter plots and Pearson correlations relative expression over mock for each condition between lncRNAs and their nearest neighbor genes (as depicted in B-D) for (E) IFN-induced lncRNAs, (F) TLR-induced lncRNAs, and (G) TLR-suppressed lncRNAs.

3.2.2 *Canonical immune modulatory transcriptional control of lncRNA expression*

To explore the signaling and transcriptional influences responsible for lncRNA-neighbor gene co-expression, we performed functional analysis of the nearest neighbor coding genes. Functional enrichment analysis demonstrated that the coding genes neighboring IFN-induced lncRNAs are involved the response to IFN-I and STAT activation (IL-35, -23, -21 signaling pathways) (**Fig. 11A; top**). TLR-inducible lncRNAs were in close proximity with not only ISGs, but also genes involved in antigen presentation (**Fig. 11A; middle**). Interestingly, TLR-suppressed lncRNAs were found near genes involved in hormonal regulation and membrane integrity pathways (**Fig. 11A; bottom**). Given the parallels in functional enrichment between these nearest-neighbor coding genes (**Fig. 11A**) and those observed from the global signatures (**Fig. 7E**), these IFN-responsive and TLR-responsive lncRNAs are likely under the regulation of the previously identified master regulators of TLR7 and IFNAR-mediated signaling (**Fig. 7F**).

We leveraged the ENCODE ChIP-seq database to identify transcription factor binding sites upstream of the transcriptional start site of the IFN- and TLR-responsive lncRNA loci. We found an appreciable enrichment of STAT, IRF, NF-κB, and activator protein 1 (AP-1) binding at sites proximal to a subset of lncRNAs (**Fig. 11B**). Overall, IFN-induced lncRNAs display enhanced representation of IRF binding sites and AP-1 binding sites (**Fig. 11C**), while TLR-induced lncRNAs display enhanced representation of NF-κB and AP-1 binding sites (**Fig. 11D**). In contrast, TLR-suppressed lncRNAs displayed less enrichment of binding sites for these TFs (**Fig. 11E**), suggesting that TLR activation likely

induces additional negative regulators of gene expression. Collectively, these analyses demonstrate the role of TLR- and IFN-I-dependent transcriptional mediators in control of lncRNAs and their neighboring coding gene expression patterns in pDC, and specifically implicate and NF-κB and IRF proteins in their respective transcriptional control. These data suggest that many lncRNAs and their neighboring genes are coordinately regulated by similar canonical TLR- and IFN-I-signaling pathway mediators.

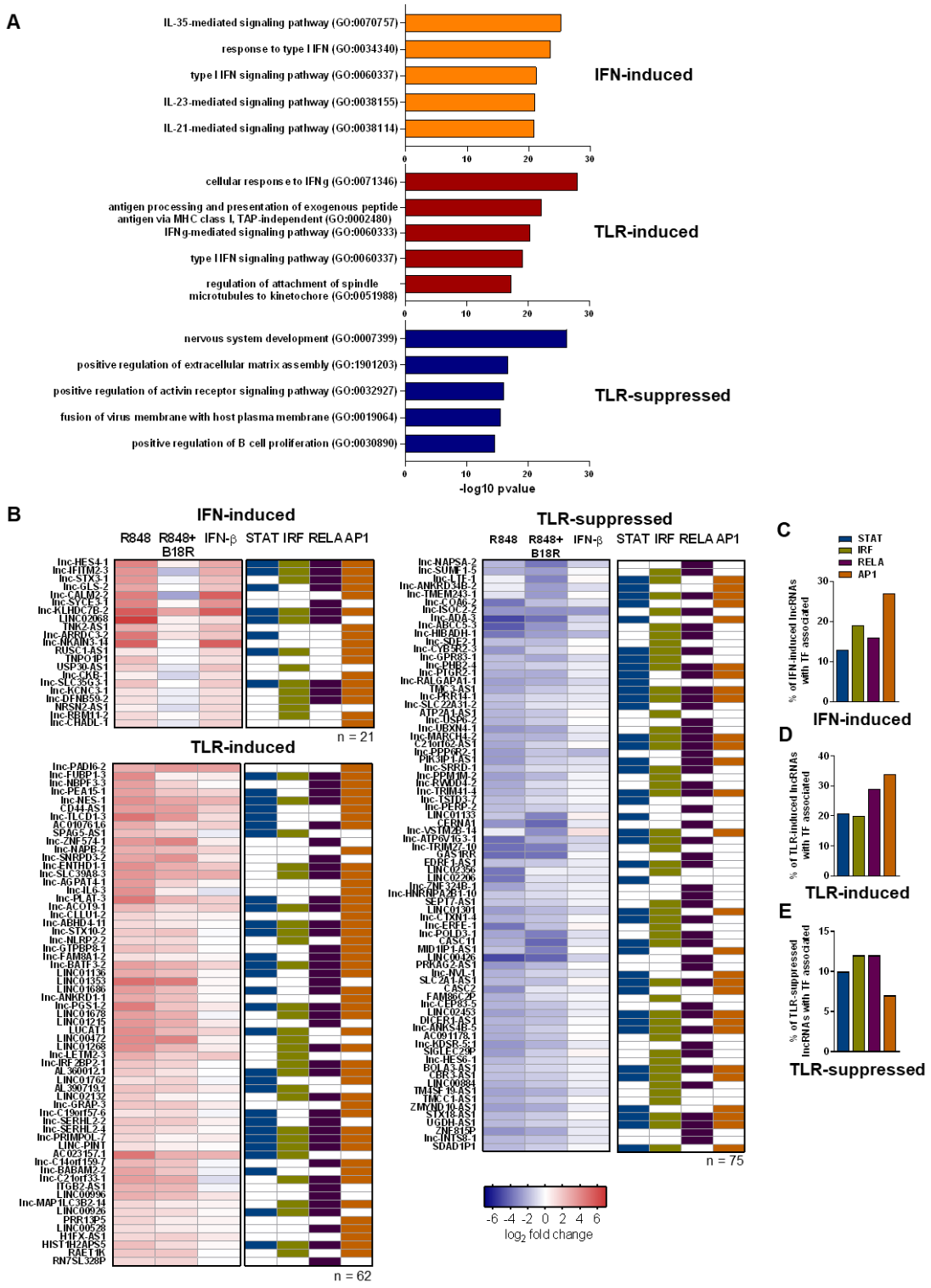

Figure 11. Immune modulatory control of lncRNA expression changes

(A) Combined scores of gene ontology (GO) term enrichment of nearest neighbor coding gene sets identified in Figs. 5B-D for IFN- and TLR-induced and TLR-suppressed lncRNAs whose nearest neighbor mapped to a coding gene . Heatmaps of expression change within each stimulatory condition of these (B) IFN- and TLR-induced and TLR-suppressed lncRNAs plotted against matrices of select immune-regulatory transcription factors (TF) associated within 500bp upstream of these lncRNA loci as determined by ChIP-seq (via ENCODE). Color-filled cells indicate association by each TF. Quantification of the proportion of all IFN- or TLR-induced/suppressed lncRNAs that show enrichment by each TF as outlined in (B) for (C) IFN-induced lncRNAs, (D) TLR-induced lncRNAs, and (E) TLR-suppressed lncRNAs.

3.3 Discussion

We defined genomic distribution of TLR- and IFN-I-modulated lncRNAs and performed an analysis of parallel and inverse expression patterns in their neighboring genes. This analysis revealed co-expression patterns between DE lncRNAs and neighboring TLR- or IFN-inducible coding genes. This phenomenon may be a product of regulation of these neighboring loci by similar transcription factors, and our analysis of enrichment of several factors known to control coding gene expression following these stimuli supports this hypothesis. Specifically, we found that IFN- and TLR-induced lncRNA groups are notably enriched for IRF and NF-κB associations, respectively. These analyses suggest that canonical pathways dictating IFN-I and TLR-driven coding transcriptional changes extend to noncoding loci as well. Additionally, lncRNAs identified in this analysis whose expression positively or inversely correlates with that of their coding neighbor may act as *cis* transcriptional or post-transcriptional regulators of the neighbor gene, thus further investigation of these co-expressed lncRNA-mRNA neighbor pairs is warranted.

Chapter 4. IFN-I- and TLR-modulated lncRNAs have diverse cytoplasmic functions

4.1 Introduction

lncRNAs termed competing endogenous RNA (ceRNA) function as decoys, competing with mRNAs for occupation of RBPs and microRNAs (miRNAs), thereby relieving gene repression or stabilization by these factors[83]. These activities occur within the cytosol, where lncRNAs can also modulate mRNA decay by masking RBP or miRNA binding sites, structurally support mRNA stability, disrupt translational machinery, and control the activities of signaling mediators through occupation of protein interaction or post-translational modification sites[87, 146]. Through their ability to regulate the repressive activities of miRNAs, RBPs or other enzymes, cytoplasmic lncRNAs are critical determinants of post-transcriptional gene regulation and cellular signaling. The demonstrated roles of lncRNAs in regulating critical intracellular signaling events, nuclear translocation, and post-transcriptional control over mRNA decay and translation position them as potentially potent regulators of the dynamic cytoplasmic events that occur in activated pDC, however these roles have not been investigated. We posit that cytoplasmic TLR and IFN-I induced lncRNAs regulate the abundance of pDC cytokine mRNAs and sensitivity to extracellular signals, and we establish an *in vitro* system to probe such functions.

4.2 **Results**

4.2.1 *Selection of lncRNAs for further study*

The limited abundance of primary pDC in peripheral blood (~1% of PBMC) is prohibitive for investigations of lncRNA function. To test the validity of CAL-1 as a model for exploration of functionally relevant lncRNAs, we screened these transcriptome profiles in conjunction with reports of known lncRNA functions to identify 6 lncRNAs representative of each TLR/IFN modulation group for further study: lnc-DC (*WFDC21P, LOC645638*), lnc-SIPA1L1-2 *(LOC145474),* lnc-ROR (*LINC-ROR*), lnc-515 (*LINC00515*), lnc-578 (*LINC00578*), and lnc-1133 (*LINC01133*) (**Table 3**).

lncRNA	Symbol	Gene ID	Ensembl ID	Transcript ID	Location	Exons	Splice Variants	bp	Type
Lnc-DC	WFDC21P	645638	ENSG00000261040	NR_030732.1	Chr 14: 71,487,861-71,489,714 forward strand	3	8	447-630	Pseudogene
Lnc-SIPA1L1-2	LOC145474	145474	N/A	NR_027046.1	Chr 14: 71,487,861-71,489,714 forward strand	1	1	1854	Misc ncRNA
Lnc-ROR	LINC-ROR	100885779	ENSG00000258609	NR_048536.1	Chr 18: 57,054,559-57,072,119 reverse strand	4	1	2630	Intergenic (lincRNA)
Lnc-515	LINC00515	282566	ENSG00000260583	NR_024092.1	Chr 21: 25,582,770-25,583,326 reverse strand	1	1	557	Antisense
Lnc-578	LINC00578	100505566	ENSG00000228221	NR_047568.1	Chr 3: 177,441,921-177,752,704 forward strand	4	1	1222	Intergenic (lincRNA)
Lnc-1133	LINC01133	100505633	ENSG00000224259	NR_038849.1	Chr 1: 159,961,218-159,984,750 forward strand	3	3	1113, 1266, 1996	Intergenic (lincRNA)

Table 3. Attributes of 6 TLR/IFN-modulated lncRNAs selected for further study in CAL-1 pDC.

4.2.2 *Validation of lncRNA expression in myeloid lineages*

Validation of expression changes by RT-PCR in IFN-β and R848 +/- B18R treated CAL-1 cells revealed lnc-DC and lnc-SIPA1L1-2 displaying IFN-I and TLR inducibility, lnc-ROR and lnc-515 induced only by TLR stimulation, and lnc-1133 and lnc-578 showing significant downregulation following TLR engagement (**Fig. 12A**). Analysis of transcript

modulation kinetics across 18 hours following stimulation revealed variable patterns in the timing of inducibility/suppressibility in response to these stimuli, with evident effects of waves of auto/paracrine cytokine signaling as well as the influence of translated ISGs in boosting lncRNA levels (**Fig. 12B**). We also tested the response of these lncRNAs to TLR9 agonism with CpG DNA, resulting in expression changes comparable to those observed in TLR7 stimulation (**Fig. 12C**). Modulation in response to TLR2 and TLR4 agonists in myeloid conventional dendritic cell (cDC) lineages showed similar patterns of change in abundance of these lncRNAs to that of TLR7 stimulation in pDC, with the exceptions in lnc-ROR and lnc-578 (**Figs. 12D-F**). We found similar patterns of expression in primary human myeloid lineages (monocytes and monocyte-derived DC) following TLR or IFN-I stimulations (**Figs. 12G-H**).

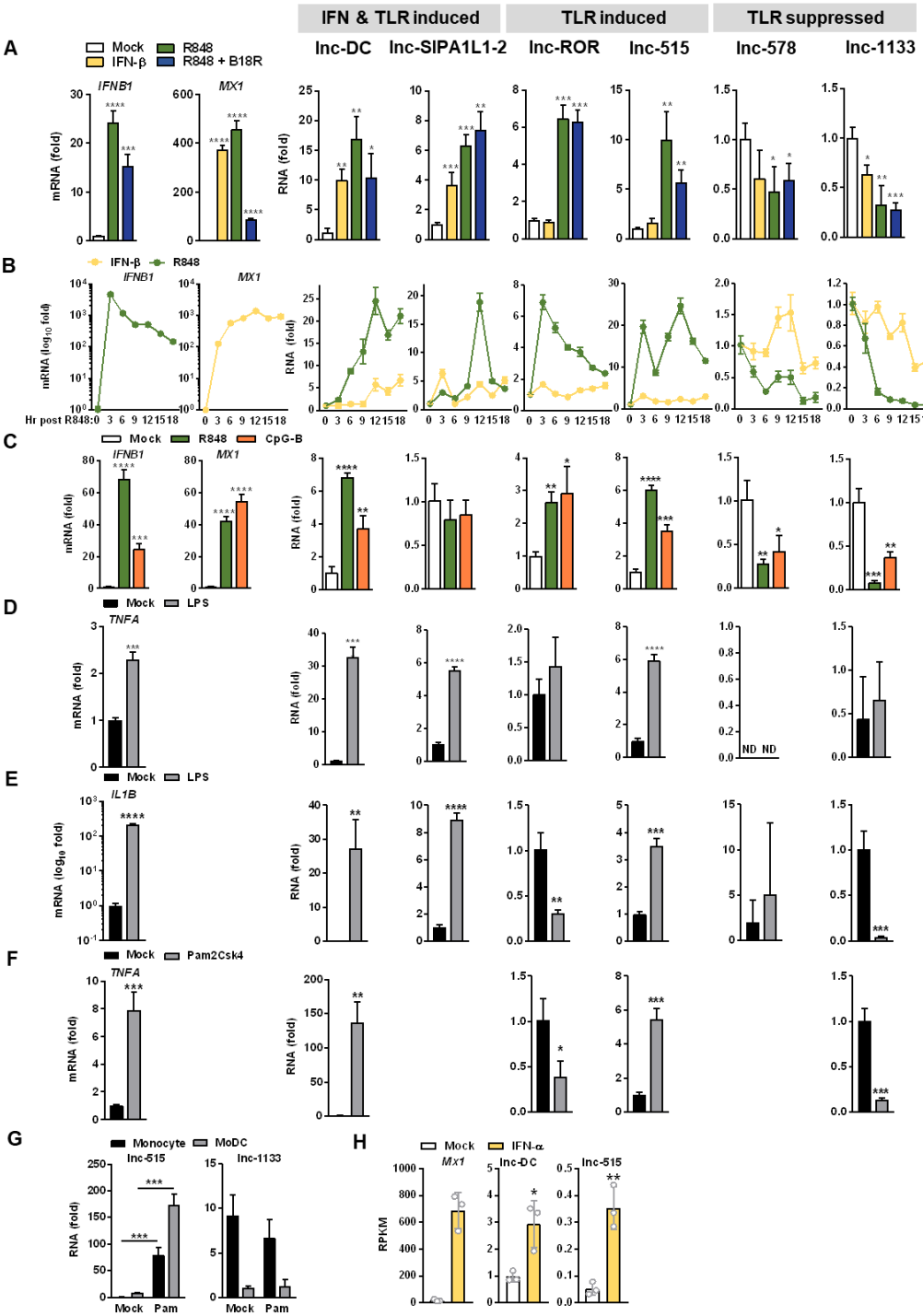

Figure 12. Expression patterns of select lncRNAs in pDC and cDC lineages

RT-PCR quantification of positive controls (labeled above plot) and lncRNA (labeled at top of figure panel A) expression changes normalized to GAPDH in CAL-1 pDC treated with (A) IFN-β (1,000U/mL), or R848 (1ug/mL) +/- B18R (1ug/mL) for 12 hours, (B) IFN-β (1,000U/mL) or R848 (1ug/mL) for 18 hours, or (C) R848 (1ug/mL) or CpG-B (1uM) for 6 hours. (D) MUTZ3 human mDC progenitor cells were differentiated to immature mDC with GM-CSF (100ng/mL), IL-4 (10ng/mL) and TNFα (2.5ng/mL) for 7 days and stimulated with LPS (100ng/mL). lncRNA transcript abundance was quantified by RT-PCR normalized to GAPDH or indicated as not detected (ND). Control and lncRNA transcripts measured by RT-PCR normalized to HPRT in (E) THP-1 monocyte-derived DC (moDC) generated by differentiation in GM-CSF (100 ng/ml) and IL-4 (100 ng/ml) for 6 days, with half of the medium replaced on day 3, and stimulated with 100ng/mL LPS for 12 hours or (F) Pam2CSK4 (100ng/mL) for 12 hours. lncRNA abundance was measured by RT-PCR normalized to GAPDH in (G) primary human moDC generated via culture in GM-CSF (100 ng/ml) and IL-4 (100 ng/ml) for 6 days, with half of the medium replaced on day 3, and stimulated with 100ng/mL Pam2CSK4 (Pam) for 12 hours. (H) RPKM values plotted from primary human monocytes treated with IFN-α for 6 hours (obtained via GSE72502).

4.2.3 *Identification of cytoplasmic lncRNAs*

Studies of unknown lncRNA mechanisms of action are particularly biochemically challenging to approach. However, lncRNAs which localize within the cytoplasm are subject to the same RNA interference (RNAi) decay modalities as mRNAs and are thus readily susceptible to siRNA-mediated knockdown, making interrogations of their function tractable. We focused our analysis on lncRNAs within this cytoplasmic niche. We determined the subcellular localization of the 6 validated lncRNAs in CAL-1 pDC via fractionation of nuclear and cytoplasmic compartments, identifying 3 of 6 lncRNAs enriched within each compartment (**Fig. 13A**). Consistent with prior reports, lnc-DC[91], lnc-ROR[89] and lnc-1133[147] localize within the cytosol, a finding also evidenced by their sensitivity to RNAi (**Fig. 13B**). Our demonstration of the amenability of this culture system to RNAi manipulations as well as the consistency of lncRNA localization and modulation patterns in CAL-1 with reports of their behavior in other tissues and primary cells supports the validity of this model for subsequent lncRNA characterizations.

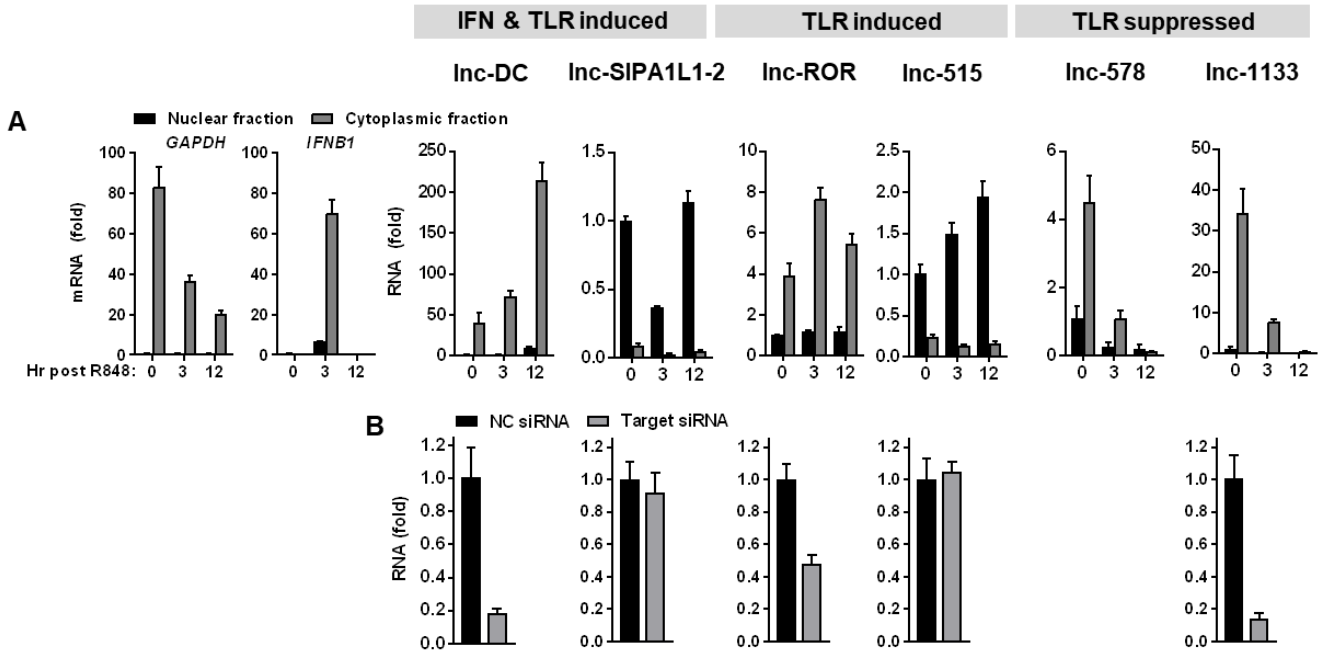

Figure 13. Identification of cytoplasmic TLR/IFN-modulated lncRNAs
(A) RT-PCR measurement of *GAPDH, IFNB1,* and TLR/IFN-modulated lncRNAs as indicated in the nuclear and cytoplasmic extracts of CAL-1 stimulated with R848 for 0, 3 or 12 hours. Transcript abundance is quantified relative to the lowest expressing sample in each set. (B) Transcript levels of lnc-DC, lnc-SIP1AL1-2, lnc-ROR, lnc-515 and lnc-1133 measured by RT-PCR normalized to GAPDH in CAL-1 cells nucleofected with 1uM siRNA targeting each lncRNA (Target) or negative control (NC) siRNA for 18 hours prior to harvest. Cells were stimulated with 1ug/mL R848 for 12 hours (lnc-DC, lnc-SIP1AL1-2, lnc-515) or 3 hours (lnc-ROR) prior to harvest or not stimulated (lnc-1133).

4.2.4 *Cytoplasmic lncRNA functions in pDC*

To characterize the functional impacts of the cytoplasmic lncRNAs we identified in human pDC, we performed siRNA knockdowns of lnc-DC, lnc-ROR and lnc-1133 in CAL-1 at time points following R848 stimulation when transcript levels were most abundant (**Fig. 12B**) and measured gene expression changes relative to stimulation-paired control siRNA by microarray. We found distinct gene expression changes in response to knockdown of each of these noncoding transcripts, indicating that each play functional roles in human pDC (**Fig.14A**). Notably, despite occurring in the same cell type, each of these knockdowns generated highly unique sets of differentially expressed genes (**Fig. 14B**), indicating their distinct and specific functionalities. Functional analysis of the gene

sets unique to knockdown of each lncRNA revealed impacts on unexpected pathways. While lnc-DC is reported to modulate STAT3 activity in cDC[91], its knockdown in pDC modulated genes involved in the production of IL-1β (**Fig. 14C, top**). Similarly, knockdown of lnc-ROR in CAL-1 failed to modulate the controllers of proliferation and differentiation it is reported to regulate in epithelial tissues[89] (**Fig. 14C, middle**). Additionally, knockdown of lnc-1133 implicated its role in metabolic respiratory control, a novel finding not entirely inconsistent with its reported control over tumor growth and metastasis[147] (**Fig. 14C, bottom**). Collectively, these data demonstrate the discrete impacts that modulation in the levels of just one lncRNA can have, indicate tissue-specific functions for these transcripts, and implicate these three cytoplasmic lncRNAs in playing functional roles in human pDC.

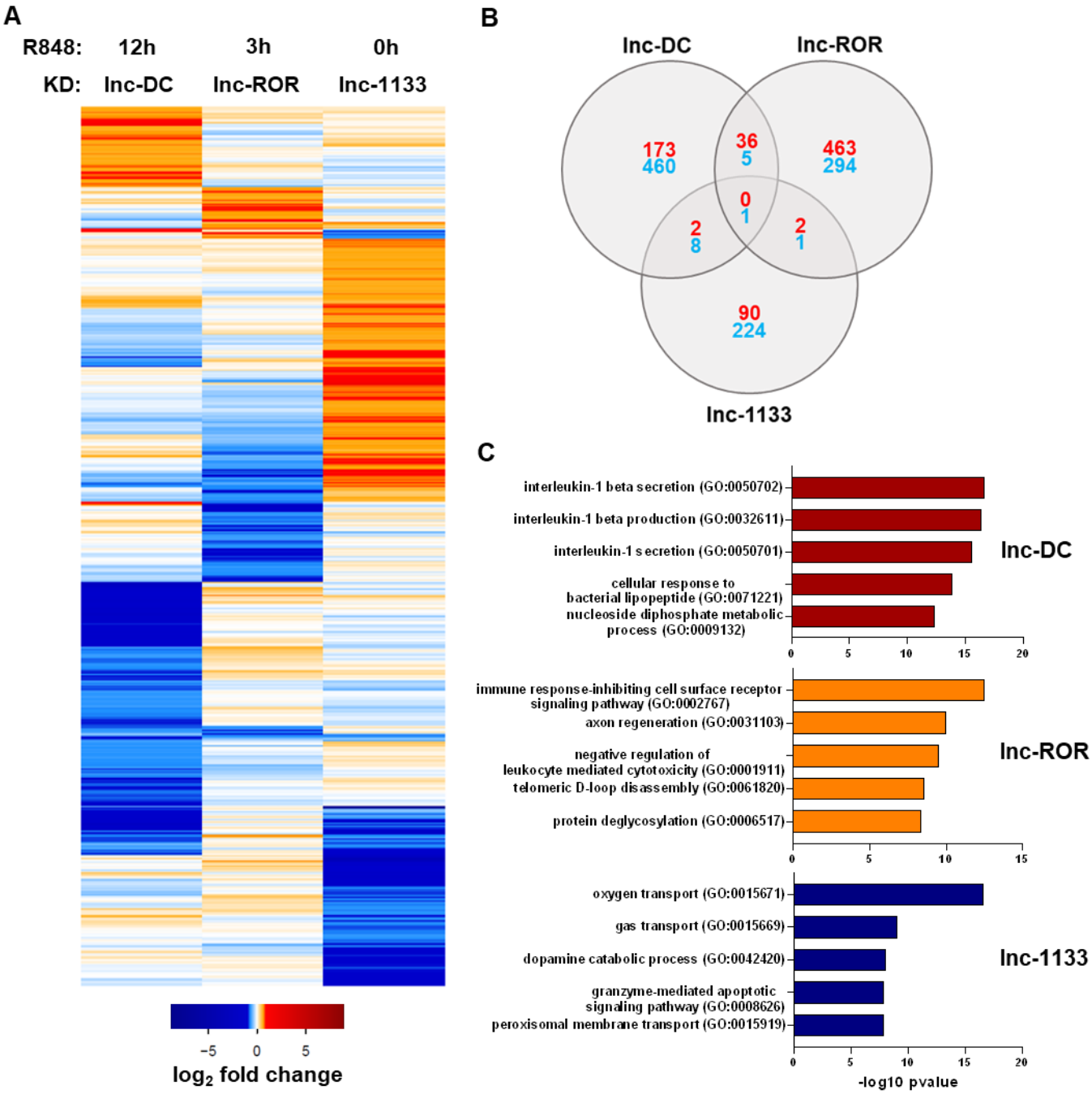

Figure 14. Gene expression changes after knockdown of cytoplasmic lncRNAs
Quantification by microarray of gene expression changes following knockdown (KD) of lnc-DC, lnc-ROR and lnc-1133 as depicted in Fig. 8B. CAL-1 cells were nucleofected with 1uM siRNA targeting each lncRNA or negative control (NC) siRNA for 18 hours prior to harvest. Cells were stimulated with 1ug/mL R848 for 12 hours (lnc-DC and NC) or 3 hours (lnc-ROR and NC) prior to harvest or not stimulated (lnc-1133 and NC) to capture peak levels for each lncRNA within the NC sample and maximum difference relative to the KD sample. Fold changes are represented relative to stimulation-paired NC. Data represent one biological and technical replicate.

4.3 Discussion

Nuclear lncRNAs within this gene set may act in *cis* to regulate transcription of their neighboring coding genes[78]. Cytoplasmic lncRNAs co-expressed with a neighboring coding gene may function as ceRNAs for the negative regulatory factors targeting the neighbor gene, thereby facilitating enhanced protein production[84]. This is especially relevant in the case of pseudogene lncRNAs which contain high sequence similarity to their coding orthologs[148]. We validated expression changes of six lncRNAs representative of TLR- and IFN-I-modulated signatures and determined their subcellular localization, identifying three cytoplasmic regulators whose activities may modulate pDC function.

We discovered that expression of lnc-DC, a cytoplasmic noncoding pseudogene specific to dendritic cells and reported to promote STAT3 activity via antagonism of SHP1-STAT3 interaction [91], is induced following TLR7 and IFN-I stimulation in pDC and cDC. Our findings suggest that DCs experience increased sensitivity to STAT3-mediated signals, such as those of IL-6 and IL-10, through elevated expression of lnc-DC in the hours subsequent to TLR activation, peaking at 12 hours post stimulation. However, whether the signal-amplifying role of lnc-DC might extend to other STAT proteins has not been explored, nor has the contribution of lnc-DC to pDC function been defined. Notably, lnc-DC polymorphisms associate with SLE incidence and levels are diminished in SLE patient blood relative to healthy controls [149], despite elevated IFN-I signaling and pDC activation in this context, indicating possible negative regulatory feedback in chronic exposure states *in vivo*.

We found that a well-described cytoplasmic ceRNA, lnc-ROR, is induced upon TLR activation in CAL-1. In epithelial tissues this transcript sponges miR-145 from targets critical to control of pluripotency [150]. However, we did not observe measurable levels of miR-145 in CAL-1 (data not shown), nor did our analysis of gene expression changes in the absence of lnc-ROR implicate its known pluripotency targets, suggesting that in pDC lnc-ROR may exert other ceRNA functions which relieve repression of mRNAs essential to pDC activation. Identification of other miRNAs predicted to bind lnc-ROR, as well as their mRNA targets, is thus warranted to examine the role of this ceRNA in regulating pDC gene expression.

Among the many lncRNAs downregulated following TLR7 stimulation, we identified lnc-1133, cytoplasmic ceRNA which is also reported to sponge miRNAs from targets critical in cell proliferation and implicated in several cancers [147, 151, 152, 153]. We show that lnc-1133 undergoes rapid and sustained downregulation in pDC and myeloid lineages following TLR stimulations. While we did not observe an impact on CAL-1 proliferation or viability with its knockdown (data not shown), lnc-1133 may act as a ceRNA for other lineage-specific targets or exert other cytoplasmic functions in this cell type. Further study of this transcript in hematopoietic lineages and TLR-activated epithelial tissues is thus warranted to determine the full range of its functions. These examples of functional cytoplasmic regulators illustrate the potential influence lncRNAs may have on pDC biology.

Chapter 5. Materials and Methods

Cell culture and reagents: CAL-1 pDC (a kind gift from Takahiro Maeda) were cultured as previously reported [131]. Cells were rested for 6 hours in 0.1% FBS CAL-1 media prior to stimulations with 1,000U/mL recombinant human IFN-β (PBL Interferon Source), 1ug/mL R848 (InvivoGen) with or without 1ug/mL recombinant vaccinia virus B18R protein (Thermo Fisher Scientific), or CpG-B (InvivoGen). Stimulated cells were incubated at 37°C for the indicated times. $20x10^6$ frozen MUTZ3 progenitor cells were resuspended in 40mL 20% MEM-alpha containing ribo/deoxyribonucleosides, 20% FBS and 10% conditioned medium from bladder carcinoma cell line 5638 and cultured at 37°C for 4 days. Differentiation to immature cDC was induced via culture at 0.25 $x10^6$/mL MEM-alpha 20% FBS without 10% bladder line media with the addition of 100 ng/mL GM-CSF (PeproTech), 10 ng/mL IL-4 (Shenandoah Biotechnology), 2.5 ng/mL TNF-α for 7 days, replacing half medium after 3 days. Cells were then stimulated with 100ng/mL LPS (Invivogen) for 12 hours. THP-1 moDC were derived and stimulated as previously described [154].

Primary cell analyses: Primary monocytes and monocyte-derived DC were obtained, cultured and stimulated with LPS or Pam2CSK4 as previous described [154].

Subcellular fractionation: CAL-1 cells were cultured and stimulated as described above for 0, 3 and 12 hours. RNA from nuclear and cytoplasmic cellular fractions was isolated from cell lysates using the Ambion PARIS™ system (Thermo Fisher Scientific) per the manufacturer's instructions.

siRNA knockdowns: CAL-1 pDC were nucleofected with 1uM siRNAs at $1.2x10^6$ cells/well in supplemented SF media from the SF cell line kit (Lonza) using an Amaxa nucleofector with 96-well plate strips (Lonza) on program DN-100 per the manufacturer's instructions. siRNAs were obtained from IDT targeting lncRNAs using the following sequences: lnc-DC 5′-GAGTTATCTTAAGGATCAT -3′, lnc-1133 5' CUUGCAGGAAGGAUGGAUUCUCC (CA) 3', lnc-ROR 5'-GGAGAGGAAGCCTGAGAGT-3'. Proprietary triplicate siRNA mixes were obtained from Dharmacon targeting lncRNAs: lnc-515 SMARTpool: Lincode LINC00515 siRNA R-189581-00-0005, lnc-SIPA1L1-2 SMARTpool: Lincode Loc145474 siRNA R189240-00-0005. Nucleofected cells were rested and stimulated with R848 as described above for the indicated times, with RNA collection at 18 hours post-nucleofection.

RNA isolation, reverse transcription, and quantification of gene expression: Total RNA was extracted via NucleoSpin RNA extraction kit (Macherey-Nagel) and cDNA was reverse transcribed with the QuantiTect RT kit (Qiagen) according to the manufacturer's instructions. TaqMan qPCR was conducted using the ViiA7 qPCR system (Life Technologies) with the following probe assays were obtained from IDT: lnc-DC Hs.PT.58.4925540, lnc-SIP1AL1-2 Hs.PT.58.24273269.g, lnc-ROR Hs.PT.58.40897944, lnc-515 Hs.PT.56a.24878664.g, lnc-578 Hs.PT.58.23294839, lnc-1133 Hs.PT.58.40059900. Gene expression levels were normalized to HPRT or GAPDH as indicated. Data were analyzed using the QuantStudio real-time PCR software (Applied Biosystems).

RNA sequencing and bioinformatic analysis: CAL-1 cells (1 million cells/mL) were cultured in 0.1% FBS CAL-1 media in triplicate in a 12-well tissue culture plate for 6 hours prior to stimulation with 1,000 U/mL recombinant human IFN-β (PBL Interferon Source) or 1ug/mL R848 (Invitrogen) or 1ug/mL R848 (Invitrogen) + 1ug/mL recombinant vaccinia virus B18R protein (Thermo Fisher Scientific) or mock stimulation. Stimulated cells were incubated for 12 hours at 37°C and RNA was isolated as described above. RNA integrity was determined using the RNA 6000 Nano Kit with a 2100 bioanalyzer (Agilent Genomics) and quantified using the fluorometric Qubit™ RNA BR assay kit (Invitrogen). cDNA libraries were prepared with the TruSeq Stranded mRNA Library Prep Kit and sequenced on an Ilumina NextSeq 500 sequencer. Library preparation, QC, and sequencing was carried out by Seattle Genomics (www.seattlegenomics.com). Both the genome sequence (fasta) and gene transfer files (gtf) for human were obtained using igenomes (https://support.illumina.com/sequencing/sequencing_software/igenome.html). Raw RNAseq data (Fastq files) were demultiplexed and checked for quality (FastQC, version 0.11.3). Ribosomal RNA was digitally removed using Bowtie2 (version 2.3.4). Host were mapped to the human genome (GRCh37) using STAR (version 2.5.3a) and converted into gene counts with HTSeq (version 0.6.1). Prior to statistical analysis, genes were filtered with a cutoff of a mean of five or greater read counts across all samples using R statistical programming language (version 3.4.3) and 'edgeR' (version 3.20.9) using RStudio. Gene counts were normalized using voom and statistical analysis for differential expression was conducted using 'limma' (version 3.34.8) in R. Fold changes were

calculated for R848 over mock, R848+B18R over mock, and IFN-β over mock with cutoffs for DE = >1.0 $\log_2$ fold, $p<0.01$. Functional analysis of coding gene transcriptional responses was performed using Ingenuity Pathway Analysis (IPA).

Transcript annotation: Annotation of lncRNAs was done by filtering transcripts through "transcript biotype" designations and universal nomenclatures were derived from LNCipedia database [155]. Chromosome location, start and end positions, and strand orientation of the lncRNAs were retrieved using 'biomaRt' (version 2.34.2) in R. Circos plots were generated using the RCircos (version 1.2.0) package in R.

Nearest neighbor analysis: Nearest neighbor genes for DE lncRNAs were identified with the seq2pathway package in R statistical programming language using RStudio. Functional analysis of nearest neighbor coding genes via gene ontology term enrichment was performed using Enrichr[156].

Transcription factor enrichment analysis: Transcription factor enrichment was performed using the ENCODE Chip-seq dataset (wgEncodeRegTfbsClusteredV3.bed) obtained from UCSC (http://hgdownload.soe.ucsc.edu/goldenPath/hg19/encodeDCC/wgEncodeRegTfbsClustered/). The analysis was restricted to the proximal 500bp upstream of lncRNAs. The transcription factor binding site and lncRNA promoter overlaps were identified using the Bioconductor 'GenomicRanges' (version 1.32.3) package in R statistical programming language (version 3.5.0) using RStudio.

Microarray: RNA was harvested at 18 hours post-nucleofection from CAL-1 cells nucleofected as described above with negative control (NC) siRNA or siRNAs targeting cytoplasmic lncRNAs lnc-DC, lnc-ROR and lnc-1133 (as described above) and treated with R848 (1ug/mL) for 12 hours (lnc-DC and NC), 3 hours (lnc-ROR and NC), or not stimulated (lnc-1133 and NC). Fluorescently labeled probes were generated from each sample using Agilent one-color LowInput Quick Amp Labeling Kit (Agilent Technologies). Individual cRNA samples were then hybridized to oligonucleotide microarrays for gene expression profiling using SurePrint G3 Human Gene Expression v2 Microarray Kit (G4851A; Agilent Technologies). The primary transcriptomic data was extracted and quantile normalized using the 'normalizeBetweenArrays' methods using the 'limma' package in R.

Statistical analysis: GraphPad Prism 7.0 (GraphPad Software, Inc.) was used for statistical analysis and plotting of RT-PCR data. Significance in gene expression differences is plotted as mean ± SEM was determined using two-tailed unpaired Student's *t*-test for stimulations versus mock or by one-way ANOVA across multiple stimulations ($p<0.05$, indicated by asterisk *). Pearson correlation analyses and plots were performed using the ggplot package in R statistical programming language using RStudio.

Data dissemination: The data generated in this study are available via the following accession identifiers on the NCBI GEO database (GSE117127). RNA sequencing data from primary monocytes treated with IFN-α for 6 hours was retrieved from the NCBI-GEO

database under accession number GSE72502[157]. RNA sequencing data from influenza-infected primary pDC1/2/3 subset was retrieved from NCBI-GEO database under accession number GSE84204[135].

Chapter 6. Concluding Remarks

Our description of TLR/IFN-modulated lncRNAs provides a foundation for identification of novel functional mechanisms and extensive interrogation of potential players in disease. The paucity of pDC in peripheral blood and the generally low expression levels of lncRNAs are major limitations to functional lncRNA analysis in primary pDC *ex vivo.* Through comparison with influenza-infected primary human pDC, we have established that lncRNA signatures in the CAL-1 pDC line resemble primary pDC and defined their core common elements, demonstrating that this a suitable model amenable to the biochemical manipulations required for subsequent interrogations of lncRNA function in pDC. As noncoding loci are sites of evolutionary pressure at host-pathogen interface, we posit that the lncRNA transcripts identified in this study may play vital roles in host defense to viral infection and, when aberrantly expressed or dysfunctional, in autoimmune disease. Further interrogations of the mechanisms utilized by these lncRNAs to regulate pDC function is thus warranted in the *in vitro* system we have established. Given demonstrated effectiveness of RNA-based interventions in disease[158], this characterization of the lncRNA landscape during the innate immune response reveals potential novel targets for treatment of viral infection and IFN-driven autoimmunity. We foresee the capacity of lncRNAs defined in this study to act through diverse mechanisms to modulate DC activities, wielding outcome-defining influence during infection and autoimmune disease.

REFERENCES

1. Takeuchi, O. & Akira, S. Pattern recognition receptors and inflammation. *Cell* **140**, 805-820 (2010).

2. Barbalat, R., Ewald, S.E., Mouchess, M.L. & Barton, G.M. Nucleic acid recognition by the innate immune system. *Annual review of immunology* **29**, 185-214 (2011).

3. Thompson, M.R., Kaminski, J.J., Kurt-Jones, E.A. & Fitzgerald, K.A. Pattern recognition receptors and the innate immune response to viral infection. *Viruses* **3**, 920-940 (2011).

4. Ivashkiv, L.B. & Donlin, L.T. Regulation of type I interferon responses. *Nature reviews. Immunology* **14**, 36-49 (2014).

5. Swiecki, M. & Colonna, M. Type I interferons: diversity of sources, production pathways and effects on immune responses. *Current opinion in virology* **1**, 463-475 (2011).

6. Sadler, A.J. & Williams, B.R. Interferon-inducible antiviral effectors. *Nature reviews. Immunology* **8**, 559-568 (2008).

7. Hoffmann, H.H., Schneider, W.M. & Rice, C.M. Interferons and viruses: an evolutionary arms race of molecular interactions. *Trends in immunology* **36**, 124-138 (2015).

8. Gonzalez-Navajas, J.M., Lee, J., David, M. & Raz, E. Immunomodulatory functions of type I interferons. *Nature reviews. Immunology* **12**, 125-135 (2012).

9. Crowl, J.T., Gray, E.E., Pestal, K., Volkman, H.E. & Stetson, D.B. Intracellular Nucleic Acid Detection in Autoimmunity. *Annual review of immunology* **35**, 313-336 (2017).

10. Elkon, K.B. & Wiedeman, A. Type I IFN system in the development and manifestations of SLE. *Curr Opin Rheumatol* **24**, 499-505 (2012).

11. Baechler, E.C. *et al*. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 2610-2615 (2003).

12. Kirou, K.A. *et al*. Activation of the interferon-alpha pathway identifies a subgroup of systemic lupus erythematosus patients with distinct serologic features and active disease. *Arthritis Rheum* **52**, 1491-1503 (2005).

13. Agrawal, H. *et al*. Deficiency of type I IFN receptor in lupus-prone New Zealand mixed 2328 mice decreases dendritic cell numbers and activation and protects from disease. *Journal of immunology* **183**, 6021-6029 (2009).

14. Nacionales, D.C. *et al*. Deficiency of the type I interferon receptor protects mice from experimental lupus. *Arthritis Rheum* **56**, 3770-3783 (2007).

15. Merad, M., Sathe, P., Helft, J., Miller, J. & Mortha, A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annual review of immunology* **31**, 563-604 (2013).

16. Swiecki, M. & Colonna, M. The multifaceted biology of plasmacytoid dendritic cells. *Nature reviews. Immunology* **15**, 471-485 (2015).

17. Rowland, S.L. *et al*. Early, transient depletion of plasmacytoid dendritic cells ameliorates autoimmunity in a lupus model. *The Journal of experimental medicine* **211**, 1977-1991 (2014).

18. Sisirak, V. *et al*. Genetic evidence for the role of plasmacytoid dendritic cells in systemic lupus erythematosus. *The Journal of experimental medicine* **211**, 1969-1976 (2014).

19. Cella, M. *et al*. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nature medicine* **5**, 919-923 (1999).

20. Siegal, F.P. *et al*. The nature of the principal type 1 interferon-producing cells in human blood. *Science* **284**, 1835-1837 (1999).

21. Reizis, B., Colonna, M., Trinchieri, G., Barrat, F. & Gilliet, M. Plasmacytoid dendritic cells: one-trick ponies or workhorses of the immune system? *Nature reviews. Immunology* **11**, 558-565 (2011).

22. Hemmi, H. *et al*. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. *Nature immunology* **3**, 196-200 (2002).

23. Jarrossay, D., Napolitani, G., Colonna, M., Sallusto, F. & Lanzavecchia, A. Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells. *European journal of immunology* **31**, 3388-3393 (2001).

24. Swiecki, M. & Colonna, M. Unraveling the functions of plasmacytoid dendritic cells during viral infections, autoimmunity, and tolerance. *Immunological reviews* **234**, 142-162 (2010).

25. Gautier, G. *et al.* A type I interferon autocrine-paracrine loop is involved in Toll-like receptor-induced interleukin-12p70 secretion by dendritic cells. *The Journal of experimental medicine* **201**, 1435-1446 (2005).

26. Asselin-Paturel, C. *et al.* Type I interferon dependence of plasmacytoid dendritic cell activation and migration. *The Journal of experimental medicine* **201**, 1157-1167 (2005).

27. Kim, S. *et al.* Self-priming determines high type I IFN production by plasmacytoid dendritic cells. *European journal of immunology* **44**, 807-818 (2014).

28. Bao, M. & Liu, Y.J. Regulation of TLR7/9 signaling in plasmacytoid dendritic cells. *Protein Cell* **4**, 40-52 (2013).

29. Liao, A.P. *et al.* Human plasmacytoid dendritic cell accumulation amplifies their type 1 interferon production. *Clinical immunology* **136**, 130-138 (2010).

30. Prakash, A., Smith, E., Lee, C.K. & Levy, D.E. Tissue-specific positive feedback requirements for production of type I interferon following virus infection. *The Journal of biological chemistry* **280**, 18651-18657 (2005).

31. Pantel, A. *et al.* Direct type I IFN but not MDA5/TLR3 activation of dendritic cells is required for maturation and metabolic shift to glycolysis after poly IC stimulation. *PLoS biology* **12**, e1001759 (2014).

32. Siren, J., Pirhonen, J., Julkunen, I. & Matikainen, S. IFN-alpha regulates TLR-dependent gene expression of IFN-alpha, IFN-beta, IL-28, and IL-29. *Journal of immunology* **174**, 1932-1937 (2005).

33. Oh, J.Z., Kurche, J.S., Burchill, M.A. & Kedl, R.M. TLR7 enables cross-presentation by multiple dendritic cell subsets through a type I IFN-dependent pathway. *Blood* **118**, 3028-3038 (2011).

34. Lorenzi, S. *et al.* Type I IFNs control antigen retention and survival of CD8alpha(+) dendritic cells after uptake of tumor apoptotic cells leading to cross-priming. *Journal of immunology* **186**, 5142-5150 (2011).

35. Dalod, M. *et al.* Interferon alpha/beta and interleukin 12 responses to viral infections: pathways regulating dendritic cell cytokine expression in vivo. *The Journal of experimental medicine* **195**, 517-528 (2002).

36. Gibson, S.J. *et al.* Plasmacytoid dendritic cells produce cytokines and mature in response to the TLR7 agonists, imiquimod and resiquimod. *Cellular immunology* **218**, 74-86 (2002).

37. Megjugorac, N.J., Young, H.A., Amrute, S.B., Olshalsky, S.L. & Fitzgerald-Bocarsly, P. Virally stimulated plasmacytoid dendritic cells produce chemokines and induce migration of T and NK cells. *Journal of leukocyte biology* **75**, 504-514 (2004).

38. Bendriss-Vermare, N. *et al.* Virus overrides the propensity of human CD40L-activated plasmacytoid dendritic cells to produce Th2 mediators through synergistic induction of IFN-{gamma} and Th1 chemokine production. *Journal of leukocyte biology* **78**, 954-966 (2005).

39. Cox, K. *et al.* Plasmacytoid dendritic cells (PDC) are the major DC subset innately producing cytokines in human lymph nodes. *Journal of leukocyte biology* **78**, 1142-1152 (2005).

40. Zucchini, N. *et al.* Individual plasmacytoid dendritic cells are major contributors to the production of multiple innate cytokines in an organ-specific manner during viral infection. *International immunology* **20**, 45-56 (2008).

41. Matta, B.M., Raimondi, G., Rosborough, B.R., Sumpter, T.L. & Thomson, A.W. IL-27 production and STAT3-dependent upregulation of B7-H1 mediate immune regulatory functions of liver plasmacytoid dendritic cells. *Journal of immunology* **188**, 5227-5237 (2012).

42. Cella, M., Facchetti, F., Lanzavecchia, A. & Colonna, M. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. *Nature immunology* **1**, 305-310 (2000).

43. Salio, M. *et al.* Plasmacytoid dendritic cells prime IFN-gamma-secreting melanoma-specific CD8 lymphocytes and are found in primary melanoma lesions. *European journal of immunology* **33**, 1052-1062 (2003).

44. Colonna, M. & Cella, M. Crosspresentation: plasmacytoid dendritic cells are in the business. *Immunity* **27**, 419-421 (2007).

45. Ito, T. *et al.* Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand. *The Journal of experimental medicine* **204**, 105-115 (2007).

46. Lui, G. *et al.* Plasmacytoid dendritic cells capture and cross-present viral antigens from influenza-virus exposed cells. *PloS one* **4**, e7111 (2009).

47. Mittelbrunn, M. *et al.* Imaging of plasmacytoid dendritic cell interactions with T cells. *Blood* **113**, 75-84 (2009).

48. Tel, J. *et al.* Human plasmacytoid dendritic cells phagocytose, process, and present exogenous particulate antigen. *Journal of immunology* **184**, 4276-4283 (2010).

49. Consortium, E.P. An integrated encyclopedia of DNA elements in the human genome. *Nature* **489**, 57-74 (2012).

50. Djebali, S. *et al.* Landscape of transcription in human cells. *Nature* **489**, 101-108 (2012).

51. Consortium, E.P. *et al.* Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* **447**, 799-816 (2007).

52. Gregory, T.R. Synergy between sequence and size in large-scale genomics. *Nature reviews. Genetics* **6**, 699-708 (2005).

53. Guttman, M., Russell, P., Ingolia, N.T., Weissman, J.S. & Lander, E.S. Ribosome profiling provides evidence that large noncoding RNAs do not encode proteins. *Cell* **154**, 240-251 (2013).

54. Kapusta, A. & Feschotte, C. Volatile evolution of long noncoding RNA repertoires: mechanisms and biological implications. *Trends in genetics : TIG* **30**, 439-452 (2014).

55. Necsulea, A. *et al.* The evolution of lncRNA repertoires and expression patterns in tetrapods. *Nature* **505**, 635-640 (2014).

56. Ricano-Ponce, I. & Wijmenga, C. Mapping of immune-mediated disease genes. *Annual review of genomics and human genetics* **14**, 325-353 (2013).

57. Cech, T.R. & Steitz, J.A. The noncoding RNA revolution-trashing old rules to forge new ones. *Cell* **157**, 77-94 (2014).

58. Derrien, T. *et al.* The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. *Genome research* **22**, 1775-1789 (2012).

59. Atkinson, S.R., Marguerat, S. & Bahler, J. Exploring long non-coding RNAs through sequencing. *Seminars in cell & developmental biology* **23**, 200-205 (2012).

60. Hung, T. *et al.* Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters. *Nature genetics* **43**, 621-629 (2011).

61. Cabili, M.N. *et al.* Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. *Genes & development* **25**, 1915-1927 (2011).

62. Griffiths-Jones, S. Annotating noncoding RNA genes. *Annual review of genomics and human genetics* **8**, 279-298 (2007).

63. Johnsson, P., Lipovich, L., Grander, D. & Morris, K.V. Evolutionary conservation of long non-coding RNAs; sequence, structure, function. *Biochimica et biophysica acta* **1840**, 1063-1071 (2014).

64. Kim, D.H. *et al.* Single-cell transcriptome analysis reveals dynamic changes in lncRNA expression during reprogramming. *Cell stem cell* **16**, 88-101 (2015).

65. Gloss, B.S. & Dinger, M.E. The specificity of long noncoding RNA expression. *Biochimica et biophysica acta* **1859**, 16-22 (2016).

66. Kung, J.T., Colognori, D. & Lee, J.T. Long noncoding RNAs: past, present, and future. *Genetics* **193**, 651-669 (2013).

67. Ulitsky, I. & Bartel, D.P. lincRNAs: genomics, evolution, and mechanisms. *Cell* **154**, 26-46 (2013).

68. Castel, S.E. & Martienssen, R.A. RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. *Nature reviews. Genetics* **14**, 100-112 (2013).

69. Djuranovic, S., Nahvi, A. & Green, R. A parsimonious model for gene regulation by miRNAs. *Science* **331**, 550-553 (2011).

70. Cook, K.B., Hughes, T.R. & Morris, Q.D. High-throughput characterization of protein-RNA interactions. *Briefings in functional genomics* **14**, 74-89 (2015).

71. Hogg, J.R. & Collins, K. Structured non-coding RNAs and the RNP Renaissance. *Current opinion in chemical biology* **12**, 684-689 (2008).

72. Li, X., Kazan, H., Lipshitz, H.D. & Morris, Q.D. Finding the target sites of RNA-binding proteins. *Wiley interdisciplinary reviews. RNA* **5**, 111-130 (2014).

73. Kutter, C. *et al.* Rapid turnover of long noncoding RNAs and the evolution of gene expression. *PLoS genetics* **8**, e1002841 (2012).

74. Cabili, M.N. *et al.* Localization and abundance analysis of human lncRNAs at single-cell and single-molecule resolution. *Genome biology* **16**, 20 (2015).

75. Mercer, T.R. & Mattick, J.S. Structure and function of long noncoding RNAs in epigenetic regulation. *Nature structural & molecular biology* **20**, 300-307 (2013).

76. Bonasio, R. & Shiekhattar, R. Regulation of transcription by long noncoding RNAs. *Annual review of genetics* **48**, 433-455 (2014).

77. Khalil, A.M. *et al.* Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 11667-11672 (2009).

78. Villegas, V.E. & Zaphiropoulos, P.G. Neighboring Gene Regulation by Antisense Long Non-Coding RNAs. *International journal of molecular sciences* **16**, 3251-3266 (2015).

79. Rinn, J.L. *et al.* Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. *Cell* **129**, 1311-1323 (2007).

80. Gomez, J.A. *et al.* The NeST long ncRNA controls microbial susceptibility and epigenetic activation of the interferon-gamma locus. *Cell* **152**, 743-754 (2013).

81. Bergmann, J.H. & Spector, D.L. Long non-coding RNAs: modulators of nuclear structure and function. *Current opinion in cell biology* **26**, 10-18 (2014).

82. Yoon, J.H., Abdelmohsen, K. & Gorospe, M. Posttranscriptional gene regulation by long noncoding RNA. *Journal of molecular biology* **425**, 3723-3730 (2013).

83. Tay, Y., Rinn, J. & Pandolfi, P.P. The multilayered complexity of ceRNA crosstalk and competition. *Nature* **505**, 344-352 (2014).

84. Salmena, L., Poliseno, L., Tay, Y., Kats, L. & Pandolfi, P.P. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell* **146**, 353-358 (2011).

85. Hammerle, M. *et al.* Posttranscriptional destabilization of the liver-specific long noncoding RNA HULC by the IGF2 mRNA-binding protein 1 (IGF2BP1). *Hepatology* **58**, 1703-1712 (2013).

86. Tay, Y. *et al.* Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. *Cell* **147**, 344-357 (2011).

87. Jalali, S., Bhartiya, D., Lalwani, M.K., Sivasubbu, S. & Scaria, V. Systematic transcriptome wide analysis of lncRNA-miRNA interactions. *PloS one* **8**, e53823 (2013).

88. Wang, J. *et al.* CREB up-regulates long non-coding RNA, HULC expression through interaction with microRNA-372 in liver cancer. *Nucleic acids research* **38**, 5366-5383 (2010).

89. Wang, Y. *et al.* Endogenous miRNA sponge lincRNA-RoR regulates Oct4, Nanog, and Sox2 in human embryonic stem cell self-renewal. *Developmental cell* **25**, 69-80 (2013).

90. Cesana, M. *et al.* A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. *Cell* **147**, 358-369 (2011).

91. Wang, P. *et al.* The STAT3-binding long noncoding RNA lnc-DC controls human dendritic cell differentiation. *Science* **344**, 310-313 (2014).

92. Willingham, A.T. *et al.* A strategy for probing the function of noncoding RNAs finds a repressor of NFAT. *Science* **309**, 1570-1573 (2005).

93. Atianand, M.K., Caffrey, D.R. & Fitzgerald, K.A. Immunobiology of Long Noncoding RNAs. *Annual review of immunology* **35**, 177-198 (2017).

94. Zhang, X. *et al.* A myelopoiesis-associated regulatory intergenic noncoding RNA transcript within the human HOXA cluster. *Blood* **113**, 2526-2534 (2009).

95. Alvarez-Dominguez, J.R. & Lodish, H.F. Emerging mechanisms of long noncoding RNA function during normal and malignant hematopoiesis. *Blood* **130**, 1965-1975 (2017).

96. Kotzin, J.J. *et al.* The long non-coding RNA Morrbid regulates Bim and short-lived myeloid cell lifespan. *Nature* **537**, 239-243 (2016).

97. Ranzani, V. *et al.* The long intergenic noncoding RNA landscape of human lymphocytes highlights the regulation of T cell differentiation by linc-MAF-4. *Nature immunology* **16**, 318-325 (2015).

98. Heward, J.A. & Lindsay, M.A. Long non-coding RNAs in the regulation of the immune response. *Trends in immunology* **35**, 408-419 (2014).

99. Turner, M., Galloway, A. & Vigorito, E. Noncoding RNA and its associated proteins as regulatory elements of the immune system. *Nature immunology* **15**, 484-491 (2014).

100. Carpenter, S. *et al.* A long noncoding RNA mediates both activation and repression of immune response genes. *Science* **341**, 789-792 (2013).

101. Li, Z. *et al.* The long noncoding RNA THRIL regulates TNFalpha expression through its interaction with hnRNPL. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 1002-1007 (2014).

102. Chan, J. *et al.* Cutting Edge: A Natural Antisense Transcript, AS-IL1alpha, Controls Inducible Transcription of the Proinflammatory Cytokine IL-1alpha. *Journal of immunology* **195**, 1359-1363 (2015).

103. Atianand, M.K. *et al.* A Long Noncoding RNA lincRNA-EPS Acts as a Transcriptional Brake to Restrain Inflammation. *Cell* **165**, 1672-1685 (2016).

104. Tong, Q. *et al.* LincRNA-Cox2 modulates TNF-alpha-induced transcription of Il12b gene in intestinal epithelial cells through regulation of Mi-2/NuRD-mediated epigenetic histone modifications. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **30**, 1187-1197 (2016).

105. Hu, G. *et al.* LincRNA-Cox2 Promotes Late Inflammatory Gene Transcription in Macrophages through Modulating SWI/SNF-Mediated Chromatin Remodeling. *Journal of immunology* **196**, 2799-2808 (2016).

106. Castellanos-Rubio, A. *et al.* A long noncoding RNA associated with susceptibility to celiac disease. *Science* **352**, 91-95 (2016).

107. Cui, H. *et al.* The human long noncoding RNA lnc-IL7R regulates the inflammatory response. *European journal of immunology* **44**, 2085-2095 (2014).

108. Ouyang, J. *et al.* NRAV, a long noncoding RNA, modulates antiviral responses through suppression of interferon-stimulated gene transcription. *Cell host & microbe* **16**, 616-626 (2014).

109. Bidet, K., Dadlani, D. & Garcia-Blanco, M.A. G3BP1, G3BP2 and CAPRIN1 are required for translation of interferon stimulated mRNAs and are targeted by a dengue virus non-coding RNA. *PLoS pathogens* **10**, e1004242 (2014).

110. Rapicavoli, N.A. *et al.* A mammalian pseudogene lncRNA at the interface of inflammation and anti-inflammatory therapeutics. *eLife* **2**, e00762 (2013).

111. Liu, B. *et al.* A cytoplasmic NF-kappaB interacting long noncoding RNA blocks IkappaB phosphorylation and suppresses breast cancer metastasis. *Cancer cell* **27**, 370-381 (2015).

112. Jiang, M. *et al.* Self-Recognition of an Inducible Host lncRNA by RIG-I Feedback Restricts Innate Immune Response. *Cell* **173**, 906-919 e913 (2018).

113. Li, Z. & Rana, T.M. Decoding the noncoding: Prospective of lncRNA-mediated innate immune regulation. *RNA biology* **11** (2014).

114. Peng, X. *et al.* Unique signatures of long noncoding RNA expression in response to virus infection and altered innate immune signaling. *mBio* **1** (2010).

115. Josset, L. *et al.* Annotation of long non-coding RNAs expressed in collaborative cross founder mice in response to respiratory virus infection reveals a new class of interferon-stimulated transcripts. *RNA biology* **11**, 875-890 (2014).

116. Yin, Z. *et al.* lncRNA expression signatures in response to enterovirus 71 infection. *Biochemical and biophysical research communications* **430**, 629-633 (2013).

117. Zhang, Q., Chen, C.Y., Yedavalli, V.S. & Jeang, K.T. NEAT1 long noncoding RNA and paraspeckle bodies modulate HIV-1 posttranscriptional expression. *mBio* **4**, e00596-00512 (2013).

118. Winterling, C. *et al.* Evidence for a crucial role of a host non-coding RNA in influenza A virus replication. *RNA biology* **11**, 66-75 (2014).

119. Ouyang, J., Hu, J. & Chen, J.L. lncRNAs regulate the innate immune response to viral infection. *Wiley interdisciplinary reviews. RNA* **7**, 129-143 (2016).

120. Valadkhan, S. & Gunawardane, L.S. lncRNA-mediated regulation of the interferon response. *Virus research* **212**, 127-136 (2016).

121. Kambara, H. *et al.* Negative regulation of the interferon response by an interferon-induced long non-coding RNA. *Nucleic acids research* **42**, 10668-10680 (2014).

122. Carnero, E. *et al.* Type I Interferon Regulates the Expression of Long Non-Coding RNAs. *Frontiers in immunology* **5**, 548 (2014).

123. Barriocanal, M., Carnero, E., Segura, V. & Fortes, P. Long Non-Coding RNA BST2/BISPR is Induced by IFN and Regulates the Expression of the Antiviral Factor Tetherin. *Frontiers in immunology* **5**, 655 (2014).

124. Kambara, H. *et al.* Regulation of Interferon-Stimulated Gene BST2 by a lncRNA Transcribed from a Shared Bidirectional Promoter. *Frontiers in immunology* **5**, 676 (2014).

125. Dave, R.K. *et al.* Regulated expression of PTPRJ/CD148 and an antisense long noncoding RNA in macrophages by proinflammatory stimuli. *PloS one* **8**, e68306 (2013).

126. NE, I.I. *et al.* Long non-coding RNAs and enhancer RNAs regulate the lipopolysaccharide-induced inflammatory response in human monocytes. *Nature communications* **5**, 3979 (2014).

127. Waddell, S.J. *et al.* Dissecting interferon-induced transcriptional programs in human peripheral blood cells. *PloS one* **5**, e9753 (2010).

128. Miller, J.C. *et al.* Deciphering the transcriptional network of the dendritic cell lineage. *Nature immunology* **13**, 888-899 (2012).

129. Fatica, A. & Bozzoni, I. Long non-coding RNAs: new players in cell differentiation and development. *Nature reviews. Genetics* **15**, 7-21 (2014).

130. Maeda, T. *et al.* A novel plasmacytoid dendritic cell line, CAL-1, established from a patient with blastic natural killer cell lymphoma. *International journal of hematology* **81**, 148-154 (2005).

131. Steinhagen, F. *et al.* IRF-5 and NF-kappaB p50 co-regulate IFN-beta and IL-6 expression in TLR9-stimulated human plasmacytoid dendritic cells. *European journal of immunology* **43**, 1896-1906 (2013).

132. Symons, J.A., Alcami, A. & Smith, G.L. Vaccinia virus encodes a soluble type I interferon receptor of novel structure and broad species specificity. *Cell* **81**, 551-560 (1995).

133. Colamonici, O.R., Domanski, P., Sweitzer, S.M., Larner, A. & Buller, R.M. Vaccinia virus B18R gene encodes a type I interferon-binding protein that blocks interferon alpha transmembrane signaling. *The Journal of biological chemistry* **270**, 15974-15978 (1995).

134. Heidkamp, G.F. *et al.* Human lymphoid organ dendritic cell identity is predominantly dictated by ontogeny, not tissue microenvironment. *Sci Immunol* **1** (2016).

135. Alculumbre, S.G. *et al.* Diversification of human plasmacytoid predendritic cells in response to a single stimulus. *Nature immunology* **19**, 63-75 (2018).

136. Murray, P.J. & Smale, S.T. Restraint of inflammatory signaling by interdependent strata of negative regulatory pathways. *Nature immunology* **13**, 916-924 (2012).

137. Yoon, J.H. *et al.* LincRNA-p21 suppresses target mRNA translation. *Molecular cell* **47**, 648-655 (2012).

138. Cancer Genome Atlas Research, N. *et al.* The Cancer Genome Atlas Pan-Cancer analysis project. *Nature genetics* **45**, 1113-1120 (2013).

139. Terashima, M., Tange, S., Ishimura, A. & Suzuki, T. MEG3 Long Noncoding RNA Contributes to the Epigenetic Regulation of Epithelial-Mesenchymal Transition in Lung Cancer Cell Lines. *The Journal of biological chemistry* **292**, 82-99 (2017).

140. Wu, Y. *et al.* Downregulation of the long noncoding RNA GAS5-AS1 contributes to tumor metastasis in non-small cell lung cancer. *Scientific reports* **6**, 31093 (2016).

141. Yang, Y. *et al.* Recurrently deregulated lncRNAs in hepatocellular carcinoma. *Nature communications* **8**, 14421 (2017).

142. Nunez-Acuna, G., Detree, C., Gallardo-Escarate, C. & Goncalves, A.T. Functional Diets Modulate lncRNA-Coding RNAs and Gene Interactions in the Intestine of Rainbow Trout Oncorhynchus mykiss. *Marine biotechnology* **19**, 287-300 (2017).

143. Shalek, A.K. *et al.* Single-cell RNA-seq reveals dynamic paracrine control of cellular variation. *Nature* **510**, 363-369 (2014).

144. Bauer, J. *et al.* Cutting Edge: IFN-beta Expression in the Spleen Is Restricted to a Subpopulation of Plasmacytoid Dendritic Cells Exhibiting a Specific Immune Modulatory Transcriptome Signature. *Journal of immunology* **196**, 4447-4451 (2016).

145. Kirou, K.A. & Gkrouzman, E. Anti-interferon alpha treatment in SLE. *Clinical immunology* **148**, 303-312 (2013).

146. Forero, A., So, L. & Savan, R. Re-evaluating Strategies to Define the Immunoregulatory Roles of miRNAs. *Trends in immunology* **38**, 558-566 (2017).

147. Wu, X.S. *et al.* LncRNA-PAGBC acts as a microRNA sponge and promotes gallbladder tumorigenesis. *EMBO reports* **18**, 1837-1853 (2017).

148. An, Y., Furber, K.L. & Ji, S. Pseudogenes regulate parental gene expression via ceRNA network. *Journal of cellular and molecular medicine* **21**, 185-192 (2017).

149. Li, J. *et al.* Association of long noncoding RNAs expression levels and their gene polymorphisms with systemic lupus erythematosus. *Scientific reports* **7**, 15119 (2017).

150. Pan, Y. *et al.* The Emerging Roles of Long Noncoding RNA ROR (lincRNA-ROR) and its Possible Mechanisms in Human Cancers. *Cell Physiol Biochem* **40**, 219-229 (2016).

151. Zhang, J.H., Li, A.Y. & Wei, N. Downregulation of long non-coding RNA LINC01133 is predictive of poor prognosis in colorectal cancer patients. *European review for medical and pharmacological sciences* **21**, 2103-2107 (2017).

152. Kong, J. *et al.* Long non-coding RNA LINC01133 inhibits epithelial-mesenchymal transition and metastasis in colorectal cancer by interacting with SRSF6. *Cancer letters* **380**, 476-484 (2016).

153. Zhang, J., Zhu, N. & Chen, X. A novel long noncoding RNA LINC01133 is upregulated in lung squamous cell cancer and predicts survival. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* **36**, 7465-7471 (2015).

154. Lim, C., Hong, M. & Savan, R. Human IL-22 binding protein isoforms act as a rheostat for IL-22 signaling. *Science signaling* **9**, ra95 (2016).

155. Volders, P.J. *et al.* An update on LNCipedia: a database for annotated human lncRNA sequences. *Nucleic acids research* **43**, D174-180 (2015).

156. Chen, E.Y. *et al.* Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC bioinformatics* **14**, 128 (2013).

157. Hung, T. *et al.* The Ro60 autoantigen binds endogenous retroelements and regulates inflammatory gene expression. *Science* **350**, 455-459 (2015).

158. Lieberman, J. Tapping the RNA world for therapeutics. *Nature structural & molecular biology* **25**, 357-364 (2018).

VITA

Rochelle is a native of Idaho, where she completed high school one year early, graduating as valedictorian in 2001. She began undergraduate studies at Seattle Pacific University that year and, after a four-year absence due to chronic illness, completed her Bachelor of Arts degree in Biology in 2009. Rochelle's personal experience with an illness driven by immunologic dysfunction ignited her interest in immunology, which she pursued from 2009-2011 as a technician in clinical immunology research. She worked in the labs of Drs. Philip Greenberg and Stanley Riddell at the Fred Hutchinson Cancer Research Center, where she aided in development of T-cell receptor libraries and characterization of T-cell subsets for adoptive immunotherapy clinical trials. Rochelle began graduate studies in the Department of Immunology at the University of Washington in 2011, joining the lab of Dr. Ram Savan in 2012 and, after a two-year absence due to recurrent illness, completed her doctoral work in 2018. Rochelle was fortunate to receive support from several trainee fellowships during her tenure as a predoctoral student: 2011-2012 Cancer Research Institute Emphasis Pathway in Tumor Immunology & UW Department of Immunology Training Grant; and from 2013-2014 and 2014-2015 as a TL1 Scholar in the Multidisciplinary Predoctoral Clinical Research Training Program from the University of Washington Institute of Translational Health Sciences.