Interleukin-1 beta signaling induces cell-intrinsic defense programs

Lauren D Aarreberg

A dissertation
submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

University of Washington
2019

Reading Committee:
Michael Gale, Jr., Chair
Ram Savan
Daniel Stetson

Program Authorized to Offer Degree:
Immunology

University of Washington

Abstract

Interleukin-1 beta signaling induces cell-intrinsic defense programs

Lauren D Aarreberg

Chair of the Supervisory Committee:
Michael Gale, Jr.
Department of Immunology

Interleukin-1 beta (IL-1β) is a pleiotropic mediator of inflammation and is produced in response to a wide range of stimuli. During infection, IL-1β production occurs in parallel with the onset of innate antimicrobial defenses, but the contribution of IL-1β signaling to cell-intrinsic immunity is not defined. Induction of interferon (IFN), IFN-stimulated genes (ISG) and inflammatory responses are critical for control of viral infection. We recently identified an essential linkage between stimulation of the inflammatory cytokine IL-1β and induction of ISGs that function as host restriction pathways against the emerging flavivirus, West Nile virus (WNV), *in vivo*. We utilized *ex vivo* global transcriptome analysis of primary dendritic cells, known targets of WNV replication, to define gene signatures required for this IL-1β-driven antiviral response. Dendritic cells that were deficient in IL-1 receptor signaling showed dysregulation of cell-intrinsic defense genes and loss of viral control during WNV infection. We found that IL-1β treatment, in the absence of infection, drove transcription of IFN and ISGs at late times following treatment. In delineating the mechanism of IL-1β-to-IFN signaling crosstalk, we found that exogenous IL-1β induces interferon regulatory factor 3 (IRF3) activation in human myeloid, fibroblast and epithelial cells. IRF3 activation by IL-1β is dependent upon the DNA sensing pathway adaptor, stimulator of interferon genes (STING), through the recognition of cytosolic mitochondrial DNA by cyclic GMP-AMP synthase (cGAS). IL-1β treatment results in IFN production and activation of IFN signaling to direct a potent innate immune response that also restricts Dengue virus infection. This study identifies a new function for IL-1β in the

onset or enhancement of cell-intrinsic immunity, with important implications for cGAS-STING in integrating inflammatory and microbial cues for host defense.

Ever tried. Ever failed. No matter. Try again. Fail again. Fail better.

- Samuel Beckett

Acknowledgements

Thank you to the many people who have been my mentors and mentees. I have learned so much from you over the years and your lessons will be carried well into the future. Thank you to Elizabeth Gray, Stephanie Cambier, Hannah Volkman, Abby Jarret and Snehal Ozarkar for guidance throughout my rotations and beyond. I would like to acknowledge Ty Crowl, Justin Roby, Lucy Young, Adriana Forero, Johannes Schwerk, Sunil Thomas, Kate Wuertz, Michael Davis, Andrey Shuvarikov, Alison Kell, Emily Hemann, Connor Driscoll, Rich Green, Courtney Wilkins, Kwan Chow, Amina Negash and Yueh-Ming Loo for critical discussion and training that were vital to the success of the projects herein. A special thanks to Katharina Esser-Nobis for her expertise and enthusiasm as we performed the Best Experiments Ever. Thank you to my 2013 cohort, Nicole Arroyo and Katie Wagner, for encouragement and friendship. Our achievements would not be possible without the administrative and structural support of Nanette Crochet, Elyse Verstelle, Jackie Berhorst, Melissa Peterson, Sarah Bland, Sandy Turner, Peggy McCune and countless others who promote our work.

I owe so much gratitude to my family and friends outside of academia who remind me every day why I am here. Thank you, Billy Hatfield, my husband and friend. I am grateful for your unwavering love and support. We have grown so much through these challenges and successes. I could dream of no better partner with whom to travel this path of lifelong learning.

Lastly, an enormous thank you to my doctoral supervisory committee: Michael Gale, Jr., Ph.D., Ram Savan, Ph.D., Daniel B. Stetson, Ph.D., Shiu-Lok Hu, Ph.D., John D. Scott, M.D., M.Sc., FIDSA.

Table of Contents

List of Figures

List of Tables

1. Introduction

1.1 Infection and immunity

An important role of the immune system is to limit infectious disease caused by microorganisms. These microbes may live on or inside the host to drive a broad spectrum of disease outcomes. Obligate intracellular parasites rely on host cells to replicate and include all viruses and select bacteria, protozoa, and fungi. Extracellular microbes utilize host cells or tissues at various stages of their life cycle. Microbe entry, trafficking, replication and release present several opportunities for recognition by the host immune system. For example, viral infection can involve fusion with the host cell plasma membrane or receptor-mediated endocytosis, followed by uncoating and replication of the viral genome. Both the cellular changes associated with the manipulation of host membranes and the direct recognition of viral components alert the host cell to infection and initiate an immune response. The early response to infection, termed innate immunity, is generally not pathogen-specific and can result in the expression of antimicrobial molecules and cytokines for the recruitment and activation of immune cells. Inflammation is a common feature of innate immunity and is characterized by dilation and permeability of blood vessels for increased blood flow and edema at the site of infection. Inflammatory responses potentiate immune cell influx and facilitate the uptake and presentation of pathogen-derived motifs, termed antigens, to cells of the adaptive immune system. Adaptive immunity, in contrast to innate immunity, develops during infection, is pathogen-specific, and ideally provides lifelong immunity against reinfection. B and T lymphocytes of the adaptive immune system can recognize nearly any antigen due to complex rearrangements of their receptor genes, yet they still rely on antigen-presenting cells, co-stimulation and a guiding cytokine milieu for their activation and differentiation. All these processes begin with the recognition of a microbe or infection-associated damage, highlighting the importance of innate immune sensing in protection against infectious disease.

1.2 Initiation of innate immune responses

1.2.1 Recognition of pathogen-associated molecular patterns (PAMPs)

Innate immunity serves to stop the spread of infection, direct adaptive immune responses and mediate tissue repair. Innate immune responses can be initiated by several classes of germline-encoded pattern recognition receptors (PRRs), which include Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), nucleotide-binding oligomerization domain and leucine-rich-repeat-

containing receptors (NLRs), C-type lectin receptors (CLRs) and cytosolic DNA sensors such as cyclic GMP-AMP synthase (cGAS) and absent in melanoma 2 (AIM2)-like receptors (ALRs). Different classes of pathogens express distinct PAMPs, such as nucleic acids, viral envelope proteins or bacterial flagellin, which can activate specific PRRs. Microbial detection initiates intracellular signaling cascades that culminate in cellular defense programs such as production of pro- and anti-inflammatory cytokines, phagocytosis, autophagy and cell death. Coincident detection by multiple PRRs, in combination with local cytokines, can lead to the integration of signaling pathways to tailor an appropriate immune response.

Critical to innate immune signaling are the adaptor proteins that link PRRs and cytokine receptors to effector molecules. These adaptors are comprised of domains that allow them to interact with both the receptor and downstream signaling components. In addition, intracellular signaling cascades are frequently modulated by protein kinases, which phosphorylate specific residues on target substrates, and ubiquitin ligases, which conjugate ubiquitin to lysine residues on target substrates. Signaling can be transmitted through several layers of sequentially activated kinases and ubiquitin ligases, some of which may require multiple residue modifications. Likewise, phosphatases and deubiquitinating enzymes provide regulatory support. Multiple steps of modification can provide more control and higher specificity in signaling events. Indeed, most pathways of innate immune activation converge on a handful of transcription factors [nuclear factor kappa B (NF-κB), activator protein 1 (AP-1), interferon regulatory factor (IRF) and signal transducer and activator of transcription (STAT) family members] but drive surprisingly diverse cellular outcomes with subtle differences in signal transduction (Häcker, 2006; Martin, 2002).

1.2.2 Toll-like receptors (TLRs)

TLRs are transmembrane proteins that exist as loosely associated dimers at the cell surface or within membrane-enclosed intracellular compartments, termed endosomes. Surface-localized TLR1, TLR2, TLR4, TLR5 and TLR6 recognize bacterial products, amyloid beta and oxidized low-density lipoproteins; endosomal TLR3, TLR7, and TLR8 recognize viral and host RNA; endosomal TLR9 recognizes unmethylated CpG-containing bacterial and viral DNA, as well as host DNA (De Nardo, 2015). As occurs with many receptor-mediated signalosomes, TLR ligand binding induces conformational changes to create a signaling platform for adaptor recruitment via homotypic interaction domains (O'Neill, 2007). All but TLR3 require the signaling adaptor myeloid differentiation factor 88 (MyD88) for activation of NF-κB and AP-1

transcription factors (see **1.3 IL-1R signaling**). TLR3 directly engages the signaling adaptor Toll/interleukin-1 receptor domain-containing adaptor inducing interferon beta (TRIF) for activation of IRF3 and downstream interferon (IFN) expression (Oshiumi, 2003a). TLR4 is the only TLR to engage both MyD88 and TRIF and does so in sequential order from two different cellular locations (Fitzgerald, 2003a; Kagan, 2008; Oshiumi, 2003b; Yamamoto, 2003). Endosomal TLRs can also induce IFN expression in a cell type-restricted manner via MyD88 and IRF7 (Honda, 2005; Kawai, 2004). TLR expression is tissue-, cell-, and temporally-restricted *in vivo*, with important implications for their access to activating ligands and roles in disease (Price, 2018).

1.2.3 Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs)

RIG-I and melanoma differentiation-associated gene 5 (MDA5) are unbound, intracellular PRRs that detect cytoplasmic viral RNA or modified host RNA through their central helicase domains (Yoneyama, 2005). RIG-I preferentially binds RNA with a 5'triphosphate group, which is not present in host RNA, and polyuridine-rich RNA, while MDA5 binds long, double stranded RNA with blunt ends (Hornung, 2006; Saito, 2008). Although RIG-I and MDA5 display unique ligand specificity and activation requirements (Loo, 2011), both RLRs signal through the adaptor molecule mitochondria antiviral signaling protein (MAVS), which is anchored to the outer membranes of mitochondria, peroxisomes and the endoplasmic reticulum (ER) (Kawai, 2005; Seth, 2005). MAVS acts as a signaling platform to facilitate the assembly of a number of ubiquitin ligases and serine kinases for expression of NF-κB- and IRF3-responsive antiviral genes (Liu, 2013; Paz, 2006). RIG-I signaling is autoregulated by interactions between its tandem caspase recruitment domains (CARDs) and repressor domain (RD) (Saito, 2007). Upon binding stimulatory RNA, RIG-I undergoes a conformational change coincident with ubiquitin- and CARD-dependent oligomerization and migration to MAVS (Kowalinski, 2011; Liu, 2012; Luo, 2011). MDA5 is not autoregulated by its RD-like motif but requires ubiquitination, phosphorylation and association with cofactors for activation of IFN responses (Loo, 2011). The third RLR, laboratory of genetics and physiology 2 (LGP2), lacks CARDs and has demonstrable positive and negative regulatory roles in RLR antiviral responses that may be expression-dependent (Saito, 2007; Satoh, 2010; Yoneyama, 2005). Though widely expressed, the RLRs can be induced during infection in IFN-dependent and -independent manners (Kang, 2004; Yount, 2007). This is a common feature of innate immune PRRs and underlies the spatiotemporal requirement for each PRR during innate immune activation.

1.2.4 Cytosolic DNA sensors

Several candidate cytosolic DNA sensors have been described in the literature and most require the adaptor protein stimulator of IFN genes (STING) for downstream signaling to NF-κB and IRF3 (Abe, 2014; Ishikawa, 2009). The first reported DNA sensor was DNA-dependent activator of IRFs (DAI), with conflicting evidence for its requirement in innate responses to transfected DNA and viral infection (Ishii, 2008; Lippmann, 2008; Takaoka, 2007; Wang, 2008). Since its discovery in 2013, cGAS has been shown to be essential for IFN programs stimulated by cytosolic delivery of DNA, aberrant accumulation of undigested cytosolic DNA, liberated mitochondrial DNA, DNA virus infection, RNA virus infection, retrovirus infection and bacterial infection (Costa Franco, 2018; Gao, 2013; Gray, 2015; Li, 2013; Rongvaux, 2014; Schoggins, 2014; Sun, 2013; Watson, 2012; White, 2014). The preponderance of evidence on cGAS has guided the field to accepting it as *the* cytosolic DNA sensor involved in interferon responses (Vance, 2016). Two members of the ALR family, AIM2 and IFNγ-inducible 16 (IFI16), have distinct immunostimulatory roles upon activation. Upon binding double stranded DNA, AIM2 forms a large signaling complex, termed inflammasome, that mediates caspase-1-dependent proteolytic activation of interleukin-1 (IL-1)β and IL-18 as well as the inflammatory form of cell death, pyroptosis (Bürckstümmer, 2009; Fernandes-Alnemri, 2009; Hornung, 2009). IFI16 is also shown to bind dsDNA, but instead induces STING-dependent IFN responses (Unterholzner, 2010). Later studies described a cooperative role of IFI16 and cGAS in STING signaling (Almine, 2017; Jønsson, 2017). The aspartate-glutamate-any amino acid-aspartate/histidine (DExD/H)-box helicases DDX3, DHX9, DHX36, and DDX41 have likewise been associated with innate DNA sensing, but their specific roles independent of, upstream of, or in tandem with cGAS-STING are understudied (DeFilippis, 2010; Kim, 2010; Zhang, 2011). As interferon-inducible genes and transcriptional regulators, these candidates may be vital downstream of STING activation (Fullam, 2013). Redundancies, spatiotemporal expression, and ligand specificity dictate the role of each of these sensors in the responses to cytosolic DNA (Unterholzner, 2013).

STING is perhaps unique in that it functions as both an adaptor (described above) and as a direct innate immune sensor of cyclic dinucleotides (CDN) produced by bacteria (Burdette, 2011; Yin, 2012). Upon binding CDNs synthesized by cGAS or bacteria, ER-localized STING traffics via the Golgi to autophagosome-like puncta where it can activate IRF3 for expression of IFN (Ishikawa, 2009; Konno, 2013;

Saitoh, 2009). Interestingly, the cGAS-CDN-STING pathway predates the evolution of IFN-based immunity (Margolis, 2017) and has been implicated in cell-autonomous defense mechanisms involving autophagy (Costa Franco, 2018; Liu, 2018; McFarlane, 2011; Moretti J., 2017; Rasmussen, 2011; Watson, 2012).

1.2.5 Nucleotide-binding oligomerization domain- and leucine-rich-repeat-containing receptors (NLRs)

NLRs respond to a broad range of stimuli that emerge during infection or tissue damage, including microbial products, peptide aggregates, ATP, uric acid crystals, reactive oxygen species and oxidized mitochondrial DNA (Latz, 2013; Malik, 2017). These unbound, intracellular PRRs are linked to multiple signaling pathways via structurally variable effector domains. NLR members contain a C-terminal leucine rich repeat (LRR) domain and a central nucleotide-binding oligomerization domain (NOD). Based on their N-terminal domains, NLRs are categorized as NOD- LRR- and pyrin domain (PYD)-containing (NLRP) receptors or NOD-, LRR- and CARD-containing (NLRC) receptors. As described above for AIM2, NLRP activation can result in the formation of an inflammasome complex consisting of apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and caspase-1 to initiate autocatalytic activation of caspase-1, cleavage of pro-IL-1β and pro-IL-18, and pyroptotic cell death (Malik, 2017). The NLRC subgroup act as important intracellular bacteria sensors for activation of inflammatory responses.

NLRP1 was the first sensor identified to form an inflammasome and is unusual in that it contains a function-to-find domain (FIIND) and a C-terminal CARD (Martinon, 2002). NLRP1 is activated by bacterial peptidoglycan and the murine ortholog NLRP1B can be activated by anthrax lethal toxin (Boyden, 2006; Faustin, 2007). NLRP3 inflammasome activation usually requires two distinct steps: priming and inflammasome assembly. Priming can occur downstream of TLR stimulation and other NF-κB and AP-1 activating pathways for increased expression of inflammasome components and pro-IL-1β. Subsequent cellular distress signals trigger inflammasome assembly via mechanisms that are not fully defined but frequently involve mitochondrial stress (Zhou, 2011). Other NLRs identified as NLRP6 and NLRP12 have inflammasome-independent functions that may negatively regulate inflammatory responses (Allen, 2012; Anand, 2012). NOD1 and NOD2 of the NLRC family recognize bacterial peptidoglycan and signal via homotypic CARD-CARD interactions with the inflammatory kinase, receptor interacting serine/threonine protein kinase 2 (RIPK2), for activation of NF-κB and AP-1 (Moreira, 2012). NLRC4 is the only NLRC

member shown to form an inflammasome. Its activation depends on NLR family apoptosis inhibitory protein (NAIP)-mediated sensing of bacterial flagellin and components of the type III secretion system (Kofoed, 2011; Malik, 2017; Zhao, 2011). In addition to the maturation and release of IL-1β and IL-18, NLRC4 promotes the production of inflammatory eicosanoids and pyroptosis-mediated shedding of infected epithelial cells (Sellin, 2014; von Moltke, 2012).

Interestingly, IFN signaling is required for the activation of some inflammasomes in bacterial infection. IFN signaling induces the expression of guanylate binding proteins (GBPs) and immunity-related GTPase family member B10 (IRGB10), which mediate bacterial lysis and release of inflammasome-stimulating PAMPs into the cytosol (Man, 2016, 2015; Meunier, 2014, 2015). In other contexts, IFN is shown to inhibit inflammasome activation and IL-1 production, highlighting the complicated crosstalk between IFN and inflammation in aseptic and sterile disease (Guarda et al., 2011; Lopez de Padilla and Niewold, 2016; Novikov et al., 2011).

1.2.6. C-type lectin receptors (CLRs)

Myeloid cells and subsets of lymphocytes express members of the CLR family on their cell surface or as soluble opsonins for the recognition of mannose, fucose and glucan carbohydrate structures (Geijtenbeek, 2009; Patin, 2018). While CLRs are largely studied in antifungal immunity, their ability to bind diverse carbohydrate structures also allows for their recognition of viruses, bacteria and helminths (Geijtenbeek, 2009). CLRs signal via association with the immunoreceptor tyrosine-based activation motif (ITAM)-containing Fc receptor gamma-chain (FcRγ) or through direct activation of protein kinases or phosphatases that complex with their cytoplasmic tails (Geijtenbeek, 2009; Patin, 2018). Some CLRs initiate NF-κB and AP-1 activation, while other CLRs have not been shown to induce gene expression in the absence of additional PRR signaling (Geijtenbeek, 2009). A proposed ‘phagocytic synapse’ formed by CLR clustering may allow cells to differentiate between direct binding of a microbe versus detection of shed surface components, thereby determining the appropriate immune response (Goodridge, 2011). CLR signaling can therefore impact TLR signaling and modulate antigen uptake and presentation by dendritic cells, with important consequences for adaptive immunity.

1.2.7. Recognition of pathogen-associated activities and damage-associated molecular patterns (DAMPs)

PRRs can also detect a broad array of host-derived structural and biochemical motifs that are produced in response to infection or stress-induced damage, termed DAMPs. Under homeostatic conditions, DAMPs are present below an activation threshold, are sequestered from PRRs, or are otherwise structurally unrecognizable, and do not stimulate immune responses. Liberation or modification of the DAMP can result in its recognition by PRRs (Schaefer, 2014). DAMPs can originate from the extracellular or intracellular space and include a diverse range of molecules, from ATP to nucleic acids, oxidized lipoproteins to whole mitochondria. Extracellular DAMPs are typically proteolytically liberated from the extracellular matrix by host or microbial enzymes. Cell death is a major driver of the release of intracellular DAMPs, which can originate from mitochondria (mitochondrial DNA, N-formyl peptides, molecules modified by reactive oxygen species) (Krysko, 2011), the nucleus [redox-modified high mobility group protein B1 (HMGB1), complexed DNA, histones] (Pisetsky, 2014), and the cytosol (uric acid, S100 calcium-binding proteins, heat shock proteins) (Schaefer, 2014). Some DAMPs are recognized without release from the cell. Detection of self-nucleic acids is implicated in several autoinflammatory and autoimmune diseases but is largely avoided by compartmentalization of nucleic acids from nucleic acid-sensing receptors as well as the presence of cytosolic nucleases (Crowl, 2017). Stimulatory endogenous nucleic acids can arise from transcriptionally active retroelements (Volkman, 2014), damaged genomic DNA (Chatzinikolaou, 2014) and liberated mitochondrial DNA (Rongvaux, 2014; White, 2014). The major PRRs that recognize DAMPs are TLRs, NLRs, cGAS and receptor for advanced glycation endproducts (RAGE). DAMP-dependent crosstalk between PRRs and accessory molecules has been shown to differentiate signaling outcomes from those of PAMP-dependent PRR signaling, though the details of these effects are far from defined (Chun, 2010; Frey, 2013; Schaefer, 2014).

Beyond the recognition of structural motifs in PAMPs and DAMPs, cellular stress is an important indicator of infection that will modulate immune signaling. Such stress can include nutrient starvation, redox imbalance, cytoskeleton disruption, ER stress and the unfolded protein response. These “patterns of pathogenesis” indicate the presence of a pathogenic microbe and thereby control the quality of the response to PAMPs and DAMPs (Vance, 2009).

1.3 Interleukin-1 receptor (IL-1R) signaling

IL-1 cytokines are produced upon PAMP and DAMP recognition to initiate or amplify inflammatory responses. All forms of innate immune recognition can contribute to IL-1R signaling; transcriptional responses downstream of TLRs, RLRs, cytosolic DNA sensors, or CLRs are required for the transcription of inflammasome components and pro-IL-1β; post-translational responses downstream of NLRs are typically required for the maturation and release of bioactive IL-1β. The diversity of IL-1 inducers and the potency of IL-1 responses accounts for their central role in chronic inflammatory diseases (Dinarello, 2011). Primary sources of IL-1β are blood monocytes, macrophages, dendritic cells, and neutrophils while pre-formed IL-1α is released from damaged keratinocytes and endothelial cells (Dinarello, 2009a; Sims, 2010).

Signal transduction downstream of IL-1R results in the activation of NF-κB and AP-1 transcription factors to cooperatively induce the expression of canonical inflammatory genes (i.e. *IL6*, *IL8*, *CCL2*, and *IL1B* itself). The Toll/interleukin-1 receptor (TIR) homology domain of IL-1R1 is shared with IL-1 receptor accessory protein (IL-1RAcP), TLRs and cytoplasmic adaptor molecules that mediate TLR and IL-1R signaling (Martin, 2002). TIR-TIR interactions upon ligand-induced association of IL-1R1 and IL-1RAcP allows for the recruitment of MyD88, which then facilitates the phosphorylation and activation of the IL-1 receptor-associated kinases (IRAK)-4, -1 and -2 (Greenfeder S.A., 1995; Huang, 1997; Muzio, 1997; Suzuki, 2002; Wesche, 1997). Tumor necrosis factor (TNF) receptor-associated factor (TRAF)6 is recruited and phosphorylated TRAF6 migrates with IRAK1 to associate with transforming growth factor (TGF)β-activated kinase 1 (TAK1) and TAK1-binding proteins (TAB)-1 and -2/3 (Cao, 1996; Ishitani, 2003; Muzio, 1997; Ninomiya-Tsuji, 1999; Takaesu, 2000). This complex formation allows for the ubiquitination of TRAF6 and phosphorylation of TAK1 (Wang, 2001). Phosphorylated TAK1 activates inhibitor of NF-κB kinases (IKK)α and β, which can then phosphorylate IκB, leading to its ubiquitin-mediated degradation (Beg, 1993; Mercurio, 1997; Ninomiya-Tsuji, 1999; Wang, 2001; Zandi, 1997). Loss of IκB releases NF-κB/Rel family members (NF-κB1/p50, NF-κB2/p52, c-Rel, RelA/p65 and RelB) to form an assortment of heterodimers of active, DNA-binding transcription factors (Karin, 2000). TAK1 also activates mitogen-activated protein kinase (MAPK) signaling for control of AP-1 transcription factors.

TRAF family member-associated NF-κB activator (TANK) binding kinase 1 (TBK1) and IKKε were identified as novel IKK-related kinases and were originally studied in the context of NF-κB activation

(Pomerantz, 1999; Shimada, 1999). Conflicting evidence for roles of TBK1 and IKKε in the regulation of NF-κB gave way to studies that firmly established these kinases in the activation of IFN responses through phosphorylation and activation of IRF3 and IRF7 (Fitzgerald, 2003b; Sharma, 2003). As TBK1 and IKKε are now believed to be activated by ligands that lead to IRF activation and the production of IFN, their roles in IL-1R signaling remain undefined.

1.4 Unanswered questions

IL-1 cytokines are widely studied for their cell-extrinsic roles in propagating inflammation in infection and autoinflammatory disease. While a few early studies of IL-1α and IL-1β described their antiviral capacity *in vitro* (Fujita, 1989; Van Damme, 1983), the mechanisms and consequences of IL-1-induced cell-intrinsic immunity were not defined. Moreover, IL-1β induction and IL-1R signaling are shown to be integral to host immunity against pathogens such as Influenza A virus, West Nile virus, Japanese encephalitis virus, Legionella pneumophila and mycobacterium tuberculosis *in vivo*, but the intracellular mechanisms by which IL-1β regulates antimicrobial control are lacking (Copenhaver, 2015; Das et al., 2008; Durrant, 2013; Fremond, 2007; Ichinohe, 2009; King et al., 2007; Ramos, 2012).

PRRs and cytokine receptors are selectively expressed at sites that intersect with processes of microbial entry and replication (Sato, 2000). Additionally, localization within the cell dictates the functional purpose of a PRR (e.g. cell surface PRRs can direct phagocytosis while cytoplasmic PRRs can direct cell death) (Brubaker, 2015). During infection, PRRs do not act in isolation to induce antimicrobial defenses and cues that accompany PRR ligands (e.g. cytokines and cellular stress) indicate the severity of a microbial threat. As inflammasome activation and the synthesis of IL-1β are common features of the immunological response to infection we sought to study how IL-1R signaling influences pathogen recognition and resultant innate immune activation (**Figure 1.1**).

In the studies presented here, we provide evidence for IL-1β-mediated cell-intrinsic immune protection through the upregulation of antimicrobial genes. In Chapter 2 we address the role of IL-1R signaling in infection of primary macrophages and dendritic cells, known target cells of West Nile virus infection. We demonstrate that *ex vivo* cultures of *Il-1r*$^{-/-}$ macrophages and dendritic cells are unable to fully control WNV at late times post-infection, and that this lack of antiviral control is associated with a loss of effective IFN responses in these cells. Significantly, we show that IL-1β treatment, in the absence of

infection, results in the induction of IFNβ and ISGs. In Chapter 3 we present the molecular mechanisms by which IL-1β induces cell-intrinsic immunity and antiviral response genes in human myeloid and epithelial cells. IL-1R signaling results in the release and cytosolic sensing of mitochondrial DNA in these cells. We demonstrate that IL-1β activates a cGAS-STNG-IRF3 axis for potentiated responses to PAMPs and Dengue virus infection. This newly defined pathway of innate immune cytokine crosstalk describes a means for indirect sensing of microbial threats and illustrates an important function for IL-1β in host defense.

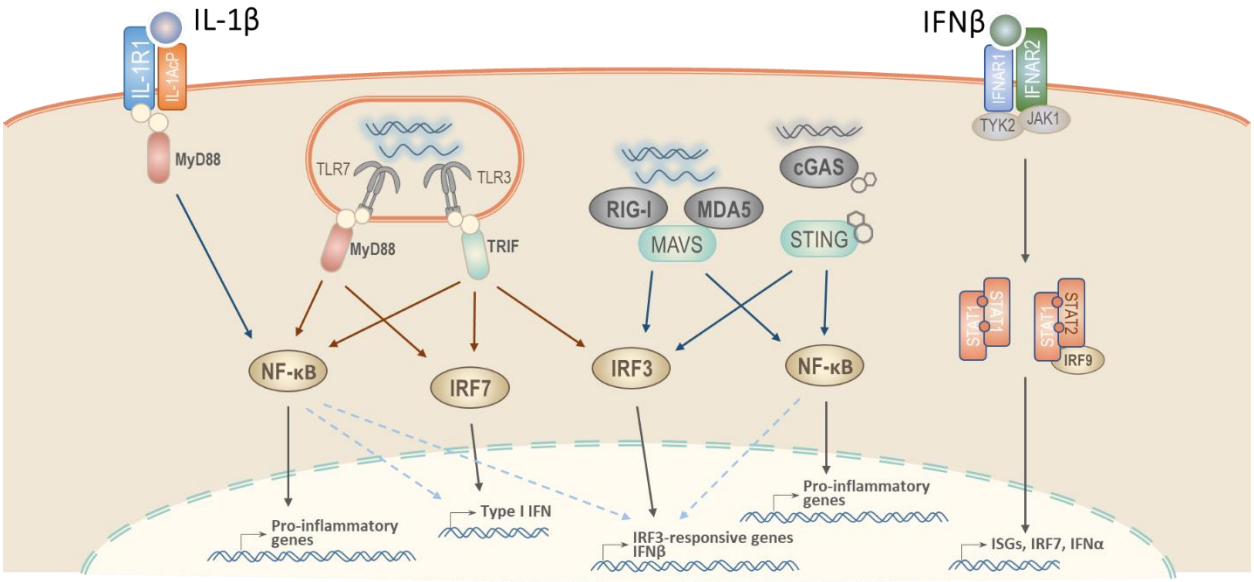


Figure 1.1. Pattern recognition receptors drive expression of antimicrobial response genes. TLRs, RLRs and cytosolic DNA sensors, among other PRRs not shown, utilize adaptor proteins and complex intracellular signaling pathways for the activation of NF-κB and IRF transcription factor families. These transcription factors coordinately drive expression of proinflammatory cytokines, IFN, antimicrobial response genes, and intermediary signaling molecules for potentiated microbial recognition and immune responses. Autocrine cytokine signaling by IL-1β and IFN modulate these processes.

2. Interleukin-1β signaling in dendritic cells induces antiviral interferon responses

Lauren D. Aarreberg, Courtney Wilkins, Hilario J. Ramos, Richard Green, Michael A. Davis, Kwan Chow, Michael Gale, Jr. (Aarreberg, 2018)

2.1 Introduction

Virus infection initiates innate immune and inflammatory responses that function to restrict viral replication and spread while serving to modulate the adaptive immune response for effective viral clearance. Type I interferon (IFN) and interleukin (IL)-1β are central mediators driving innate antiviral immunity and inflammation, respectively (Dinarello, 2009a; Sen, 2001; Sims, 2010; Stetson, 2006). Though both cytokines are typically induced during acute virus infection, the temporal nature of their induction over the course of specific virus infection, and how each cytokine influences the actions of the other to drive downstream gene expression are not well-understood. Evidence for positive and negative co-regulation of each can be found in pathogen- and cell-specific contexts (Mayer-Barber, 2017). Several studies have demonstrated that both IFN and IL-1β are critical cytokines for defense against West Nile virus (WNV), with distinct and concerted roles in directing host immunity (Durrant, 2013; Lazear et al., 2011; Ramos, 2012; Suthar et al., 2010).

WNV is a member of the single-stranded RNA virus family *flaviviridae*. Over the past 18 years, WNV has emerged into North America and continues to cause infection and disease (Gubler, 2007; Krow-Lucal, 2017). While the virus is normally maintained between mosquito and avian reservoirs, incidental infection of humans occurs through the bite of infected mosquitoes (Hayes et al., 2005; Suthar et al., 2013b). WNV initially replicates at the site of infection before spreading to the draining lymph nodes and spleen, where it replicates in subsets of macrophages and dendritic cells (DCs) (Samuel and Diamond, 2006). WNV is neurotropic, and although the virus is usually controlled in the periphery, it can spread to the central nervous system (CNS) where infection of neurons and induction of inflammation can lead to encephalitis and death (Davis et al., 2006; Samuel and Diamond, 2006; Sejvar et al., 2003). While inflammatory cell recruitment and function is necessary for limiting WNV pathogenesis, inflammation must be tightly controlled to prevent inflammatory-mediated destruction of CNS tissue and disease (Basu, 2004; Dinarello, 2009a, b; Shrestha et al., 2008).

The type I IFN response is a major component of antiviral innate immunity. Induction of IFNβ is triggered downstream of pattern recognition receptors (PRRs), including the RIG-I like receptors (RLRs) and toll-like receptors (TLRs) (Akira, 2001; Wilkins and Gale, 2010). PRRs recognize components of the virus and signal through conserved pathways to activate transcription factors belonging to the NF-κB and interferon regulatory factor (IRF) families to induce expression of IFNβ (Lazear et al., 2013; Mamane, 1999; Suthar et al., 2013b). IFNβ is secreted from the cell and acts in an autocrine and paracrine manner through the ubiquitous IFNα/β receptor (IFNAR) to activate its receptor-associated kinases. These kinases can in turn phosphorylate and activate signal transducer and activator of transcription (STAT)1 and STAT2 for the assembly of the ISGF3 complex, which acts to induce transcription of hundreds of ISGs that include known antiviral effector molecules (Wilkins and Gale, 2010). Components of the RLR-signaling pathway are absolutely required for host clearance of WNV, as mice deficient in RIG-I, MDA5, MAVS or IFNβ are unable to control WNV infection and are highly susceptible to WNV-induced mortality (Errett et al., 2013; Fredericksen et al., 2008; Lazear et al., 2011; Suthar et al., 2010).

IL-1β is one of a family of cytokines that include IL-1α, IL-18 and IL-33 (Dinarello, 2009a; Sims, 2010). Its primary receptor, IL-1 receptor (IL-1R), is homologous to the TLRs in its downstream signaling components and is constitutively expressed in most cell types (Dinarello, 1996). IL-1β signals through IL-1R to activate MyD88 and NF-κB and drive the expression of genes required for immune-mediated inflammation, effective adaptive immunity and antiviral control (Ben-Sasson et al., 2011; Dinarello, 1996; Kanneganti, 2010). IL-1β induction and secretion is stimulated by a number of viruses, including influenza A virus, herpes simplex virus, Sendai virus, vesicular stomatitis, hepatitis C virus, Dengue virus and St. Louis encephalitis virus (Chang, 1994; Kanneganti, 2010; Negash et al., 2013). Additionally, IL-1β-regulated inflammation in the brain is required for clearance of neurotropic viruses including WNV and Japanese encephalitis virus (Das et al., 2008; Durrant, 2013; King et al., 2007).

Inflammatory molecules such as IL-1β and type I IFN are generally considered to be mutually antagonistic (Mayer-Barber, 2017). IFNβ regulates inflammatory homeostasis by decreasing IL-1β production and inflammasome-mediated IL-1β processing, thereby preventing uncontrolled tissue destruction by inflammatory cytokines (Guarda et al., 2011; Hu et al., 2005; Mayer-Barber et al., 2011).

IRF3 was shown to suppress the expression of pro-inflammatory genes such as IL-1 and TNF in microglia (Tarassishin et al., 2011), while IL-1β was conversely found to decrease the ability of IRF3 to accumulate in the nucleus and bind to the interferon-sensitive response element (ISRE) in liver cells (Hisaeda et al., 2004). Additionally, IL-1β-induced eicosanoids were found to limit type I IFN production in an *in vivo* model of mycobacterium tuberculosis (Mayer-Barber, 2014). However, the cross-regulation of inflammatory and IFN responses is not entirely antagonistic, as mice defective in IL-1R or IFNAR show defects in both responses (Goritzka et al., 2014; Ichikawa et al., 2002; Ramos, 2012).

IL-1β induction through the NLRP3 inflammasome was recently identified as a key component in host immunity against WNV infection (Durrant, 2013; Ramos, 2012). WNV infection induced the acute production of IL-1β both *in vivo* and in *ex vivo* cortical neuron isolates. Loss of IL-1β signaling in IL-1R-deficient (*Il-1r*$^{-/-}$*)* mice led to enhanced accumulation of WNV in the CNS but not the periphery of infected mice, resulting in increased pathogenesis and mortality (Ramos, 2012). Importantly, we found that type I IFN levels were reduced in the draining lymph nodes and delayed in the CNS of WNV-infected mice in the absence of IL-1R signaling. Additionally, IL-1β and IFNβ acted synergistically to control WNV in *ex vivo* cultures of cortical neurons, suggesting cross-regulation between these cytokines that is required for effective antiviral control. As it has been suggested that myeloid cells promote WNV entry into the CNS via a “Trojan horse” mechanism (Samuel and Diamond, 2006), it is likely that the defect in viral control in *Il-1r*$^{-/-}$ mice may be partially due to the reduced IFN levels in the draining lymph nodes, allowing for decreased control of virus in macrophages and dendritic cells that go on to infiltrate the CNS and enhance encephalitic disease.

In this study we address the role of IL-1R signaling in infection of primary macrophages and dendritic cells, known target cells of WNV infection. We demonstrate that *ex vivo* cultures of *Il-1r*$^{-/-}$ macrophages and dendritic cells are unable to fully control WNV at late times post-infection, and that this lack of antiviral control is associated with a loss of effective type I IFN responses in these cells. Significantly, we show that IL-1β treatment of bone marrow derived dendritic cells (BMDCs) results in induction of IFNβ and ISGs at late time points post-treatment and in the absence of infection. Our data suggest that the cross-regulation between IL-1β and IFNβ is required to effectively clear WNV infection.

2.2 Results

2.2.1 IL-1β signaling is required for control of WNV infection in myeloid cells

Induction of type I IFN and the programming of an antiviral ISG response are critical for control of WNV replication (Errett et al., 2013; Fredericksen et al., 2008; Lazear et al., 2011; Suthar et al., 2010). Recently, we identified NLRP3 inflammasome activation and IL-1β signaling as key host restriction pathways important in maintaining optimal IFN and ISG responses to control WNV replication in neurons and the infected CNS (Ramos, 2012). In contrast to neurons, which are highly permissive to WNV replication, myeloid cells can control WNV replication in a type I IFN-dependent manner (Lazear et al., 2011). Therefore, in order to understand the mechanism by which IL-1β regulates antiviral control of WNV, we examined a requirement for this pathway in the control of WNV in primary myeloid cells. Bone marrow derived dendritic cells (BMDCs) and macrophages (BMM) were prepared from wildtype (WT) and IL-1R-deficient (*Il-1r*$^{-/-}$) mice and challenged with WNV. WNV replicated to similar titer in WT and *Il-1r*$^{-/-}$ at 24 hours post-infection in both BMDCs (**Figure 2.1A**) and BMMs (**Figure 2.1B**). However, while WT cells controlled WNV by 48 hours post-infection, *Il-1r*$^{-/-}$ cells showed increased viral replication and lack of viral control at this time (**Figures 2.1A, 2.1B**).

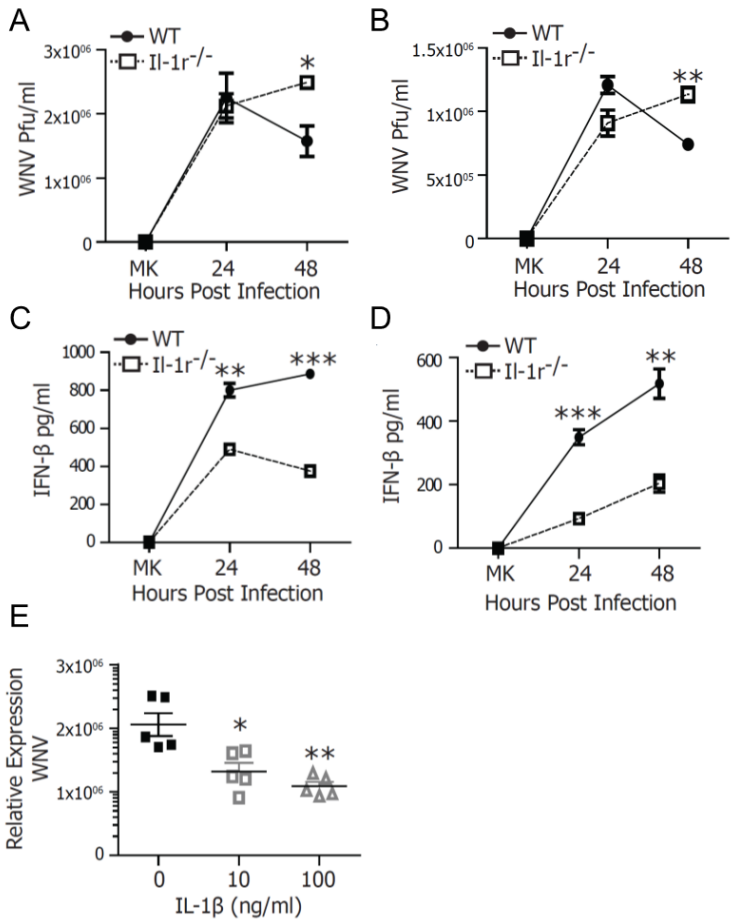


Figure 2.1. IL-1 signaling is required for WNV control. BMDCs (A, C) or BMMs (B, D) from WT or *Il-1r*$^{-/-}$ mice were infected with WNV at an MOI of 2.5 and compared with mock-infected cells. At 24 and 48hrs, WNV titers were determined by plaque assay (A, B) and IFNβ levels were measured by ELISA (C, D). (E) IL-1β (0, 10, or 100 ng/ml) was titrated onto WT BMDCs 24hrs prior to infection with WNV at an MOI of 2.5. WNV RNA was measured by qRT-PCR at 48hrs p.i. The data are averages of three (A to D) or five (E) independent experiments. Asterisks indicate differences that are statistically significant by Mann-Whitney U test (A, B) or by unpaired *t* test (C to E). MK, mock treatment. **p* <0.05, ***p* <0.01, ****p* <0.001

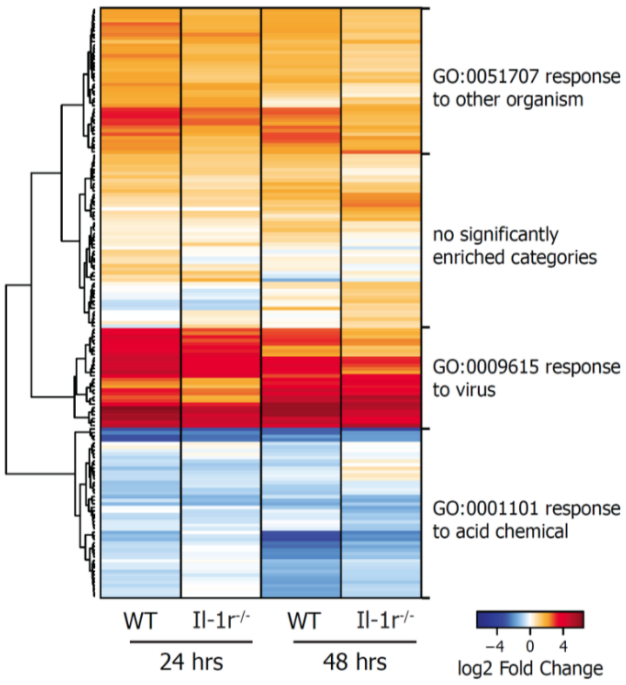


Figure 2.2. Genome-wide expression analysis of IL-1R-regulated genes. WT or *Il-1r*$^{-/-}$ BMDCs were mock infected or infected with WNV at an MOI of 2.5. Total RNA was extracted at 24 and 48hrs p.i. and subjected to Agilent Whole Mouse Genome Microarray analysis. Gene expression levels were determined as fold changes with respect to matched, mock-treated controls. A significant change is defined as a >1.5-fold increase or decrease with respect to mock treatment, with a BH-adjusted *p* value of <0.05. IL-1R-regulated genes were defined as those whose fold changes with respect to mock treatment in *Il-1r*$^{-/-}$ BMDCs were >1.5-fold decreases compared with WT cells, with a BH-adjusted *p* value of <0.05. WNV-induced expression of IL-1R-regulated genes was plotted on a heat map with hierarchical clustering by Euclidean distance. Gene clusters are labeled with the most significantly enriched biological process in that group.

The lack of viral control in IL-1R signaling-deficient cells suggested a similar defect in cell intrinsic immunity to the virus as we previously observed in neurons (Ramos, 2012). Therefore, we next examined type I IFN production in BMDCs and BMMs after WNV challenge. In accordance with the lack of viral control, both *Il-1r*$^{-/-}$ BMDCs and BMMs displayed a reduction in IFNβ secretion (**Figures 2.1C, 2.1D**). These data further identify IL-1β as a key host restriction factor involved in regulating antiviral immunity by modulating type I IFN responses.

We next examined whether IL-1β exposure was sufficient to mediate antiviral activity in myeloid cells. BMDCs were prepared from WT animals and pre-treated with 0, 10 or 100 ng/mL IL-1β. After 24hrs, cells were either challenged with WNV or left as uninfected controls. IL-1β treatment reduced WNV RNA levels by 2-5 fold compared to untreated cells (**Figure 2.1E**). Virus reduction was comparable to levels of inhibition observed in neurons, suggesting a global contribution for IL-1β in eliciting antiviral immunity against WNV (Ramos, 2012).

2.2.2 IL-1β drives antiviral gene signatures in dendritic cells

To examine the mechanism by which IL-1β participated in controlling WNV infection, we utilized global transcriptome analysis in BMDCs to define the gene signature associated with WNV infection and induction of host defense. BMDCs were prepared from WT or *Il-1r*$^{-/-}$ mice and were infected with WNV or left untreated as time-matched, mock controls. Total RNA was harvested at 24 and 48 hours post-infection

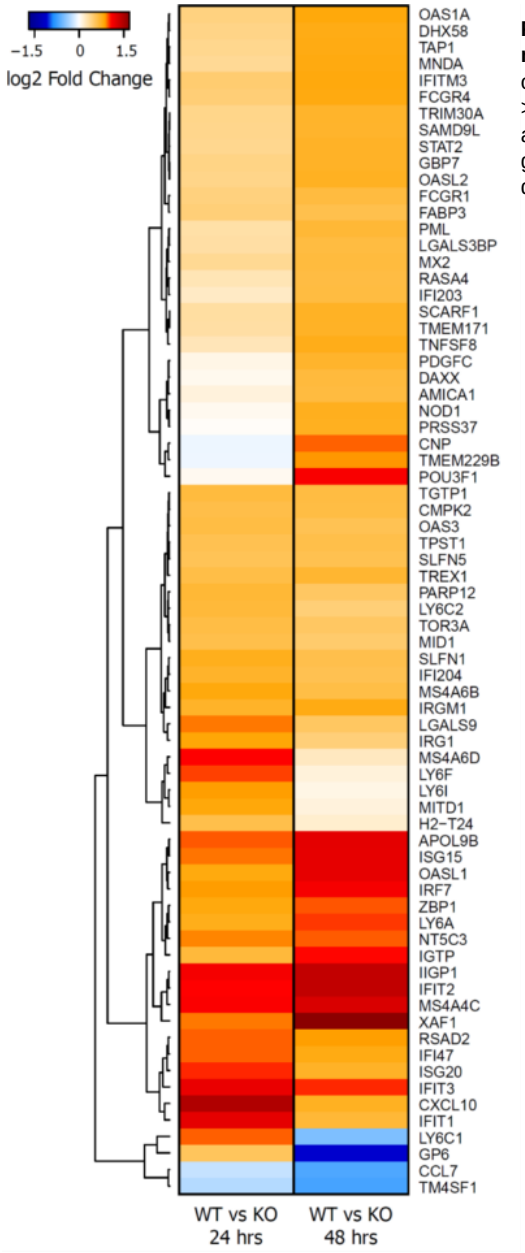


Figure 2.3. IL-1R signaling is necessary for induction of antiviral response genes. Antiviral response genes whose expression is more up- or downregulated during WNV infection in WT BMDCs as determined by a >1.5-fold increase or decrease with respect to IL-1R knockout BMDCs, with a BH-corrected *p* value of <0.05, in a microarray analysis. IL-1R-regulated genes were plotted on a heat map with hierarchical clustering by Euclidean distance.

and relative gene expression levels were determined by Agilent Whole-Genome mouse microarray (4x44K chip). Significant up- or down-regulation over mock controls was defined as a greater than 1.5-fold change in expression with a Benjamini-Hochberg corrected p-value < 0.05 (**Table S1**). Gene expression patterns driven by WNV infection of BMDCs were then compared between WT and *Il-1r*$^{-/-}$ cells to define genes whose expression is regulated by IL-1R signaling. Gene expression changes that differed significantly between the two genotypes (as defined by the same statistical criteria above) were visualized by heatmap for both 24 and 48 hour samples (**Figure 2.2**). Interestingly, genes dysregulated in *Il-1r*$^{-/-}$ BMDCs are involved in response to virus and response to other organisms (as determined by Enrichr analysis of Gene Ontology biological process (Chen, 2013)), indicating loss of antiviral control in

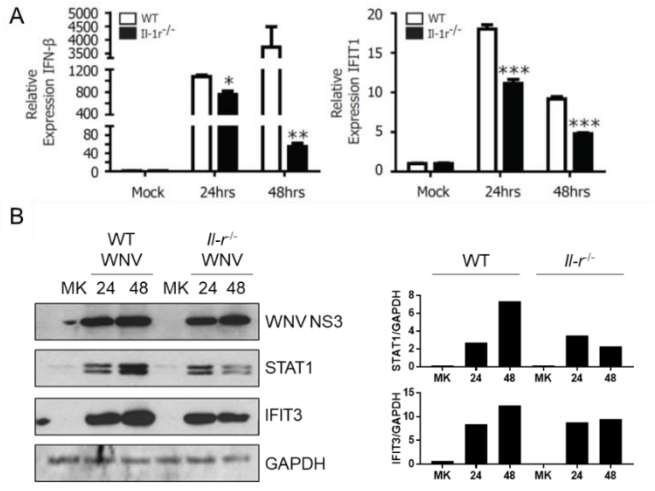


Figure 2.4. IL-1 signaling enhances antiviral responses. (A) WT or *Il-1r*$^{-/-}$ BMDCs were mock infected or infected with WNV at an MOI of 2.5. Expression of IFNβ and IFIT1 was measured by qRT-PCR at 24 and 48hrs p.i. relative to that in matched, mock-treated controls. (B) Total cell WNV NS3, STAT1, and IFIT3 protein levels were measured by immunoblotting with GAPDH as a loading control (left). Densitometry analyses of STAT1 and IFIT3 protein abundance were compared against GAPDH abundance for each condition (right). The data are the averages of three independent experiments. Asterisks indicate differences that are statistically significant between WT and *Il-1r*$^{-/-}$ cells by unpaired *t* test. MK, mock treatment. **p* <0.05, ***p* <0.01, ****p* <0.001

the absence of IL-1R signaling (**Figure 2.3, Table S2**). These data demonstrate that IL-1β signaling regulates innate immune response genes during WNV infection of DCs.

2.2.3 IL-1β signaling enhances ISG responses after WNV infection

To understand the effect of IL-1R signaling requirements on ISG induction following WNV infection, we examined ISG expression by qRT-PCR and immunoblot. The WNV-driven expression of *IFNβ*, an IRF3 and IRF7 target gene, is slightly reduced in *Il-1r*$^{-/-}$ BMDCs at 24 hours p.i. as compared to WT, but this difference in expression is exacerbated by 48 hours (**Figure 2.4A, left**). *IFIT1* is regulated by both IRF3 and IFN-responsive promoter sites, and it shows depressed expression in the absence of IL-1R at both times by qRT-PCR (**Figure 2.4A, right**) (Grandvaux N., 2002; Guo, 2000). Additionally, protein expression of the ISGs STAT1 and IFIT3 is not maintained during WNV infection in the absence of IL-1R signaling (**Figure 2.4B**). Together, these results confirm that ISG expression is negatively altered by the lack of IL-1β signaling in WNV-infected BMDCs. Moreover, curtailed expression of these genes appears to associate with the lack of control of WNV in *Il-1r*$^{-/-}$ BMDC at 48 hours post-infection (see **Figure 2.1A**).

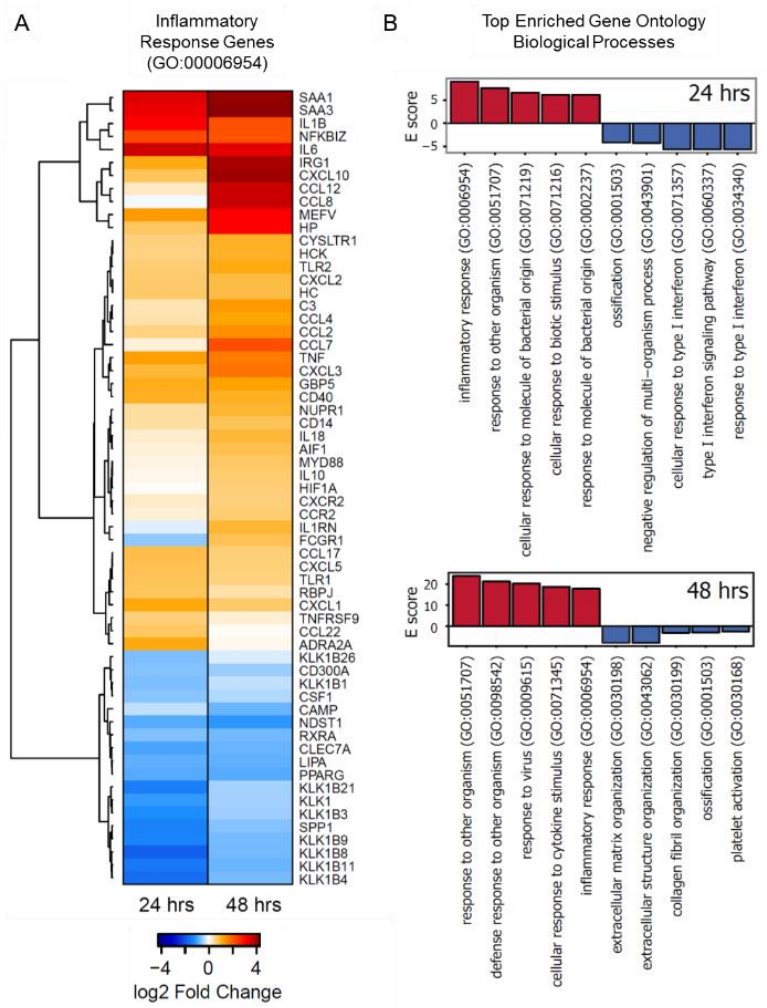


Figure 2.5. IL-1β treatment drives inflammatory response genes. (A) Genes mapping to the Gene Ontology biological process term inflammatory response (GO:0006954) whose expression is up- or downregulated by IL-1β treatment at 24 or 48hrs posttreatment as determined by a >1.5-fold increase or decrease with respect to matched, mock-treated cells, with a BH-corrected *p* value of <0.05, in a microarray analysis. (B) Genes upregulated at 24 and 48hrs after IL-1β treatment were assessed for enrichment of Gene Ontology biological processes. Significant enrichment is defined as a BH-adjusted *p* value of <0.05. Enrichment E scores refer to the negative log of the adjusted *p* value. The enrichment score top five significantly enriched categories in each direction are plotted for 24 (top) or 48 (bottom) hrs of IL-1β treatment.

2.2.4 IL-1β drives expression of IFNβ and ISGs in the absence of infection

The surprising dysregulation of ISGs in *Il-1r*$^{-/-}$ BMDCs during WNV infection led us to examine how IL-1R signaling affects gene expression in the absence of infection. As expected, WT BMDCs treated with IL-1β for 24 or 48 hours results in the increase (up-regulation) or decrease (down-regulation) in the expression of a number of genes mapping to inflammatory responses (**Figure 2.5A**) (Chen, 2013). An analysis of all genes whose expression is regulated by IL-1β treatment in WT BMDCs demonstrated that modules of genes enriched in inflammatory response genes and response to other organisms are upregulated at both 24 and 48 hours post-treatment, while genes involved in cytokine regulation and cellular response to IFNβ are induced at the later time point (**Figure 2.6A**, **Table S3**). Consistent with these results, Gene Ontology analysis of biological processes upregulated following IL-1β treatment revealed an increased enrichment of genes involved in response to virus, as well as a loss of enrichment of type I IFN signaling pathways from the downregulated gene sets (**Figure 2.5B**).

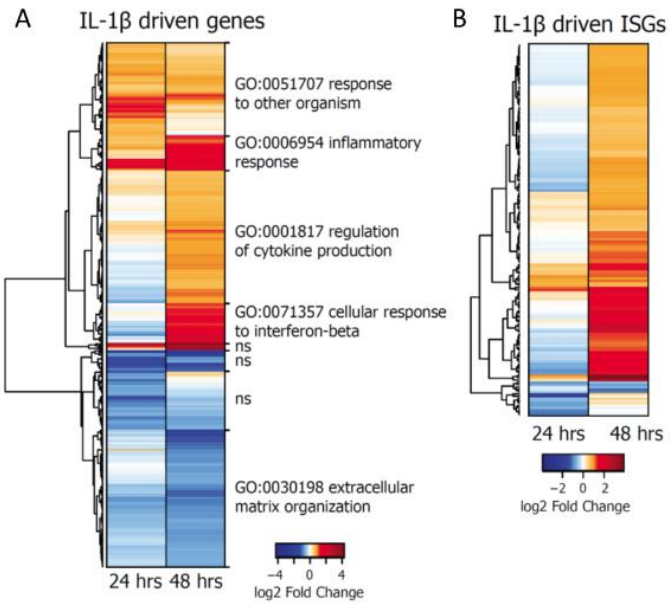


Figure 2.6. IL-1β treatment drives expression of IFNβ and ISGs. WT BMDCs were mock treated or treated with 100ng/ml IL-1β. Total RNA was extracted at 24 and 48hrs posttreatment and subjected to Agilent Whole Mouse Genome Microarray analysis. (A) Gene expression levels were determined as fold changes with respect to matched, mock-treated controls. A significant change is defined as a >1.5-fold increase or decrease with respect to mock treatment, with a BH-adjusted *p* value of <0.05. IL-1β-regulated genes were plotted on a heat map with hierarchical clustering by Euclidean distance. Gene clusters are labeled with the most significantly enriched biological process in that group. The abbreviation ns signifies no significantly enriched categories in that cluster. (B) IL-1β-driven genes were compared against genes found to be induced upon IFNβ treatment of WT BMDCs. ISGs regulated by IL-1β as defined for panel A were plotted on a heat map.

To determine whether any of these innate immune genes were ISGs, we compared the list of IL-1β driven genes to a published list of genes found to be induced following IFNβ treatment of WT BMDC (Lazear et al., 2015). We found that while a few ISGs are expressed at 24 hours post-treatment, many more were driven by IL-1β after 48 hours of treatment (**Figure 2.6B**, **Table S4**). Interestingly, a portion of the IL-1β-driven ISGs appear to be downregulated at 24 hours post-treatment but are then either back to baseline

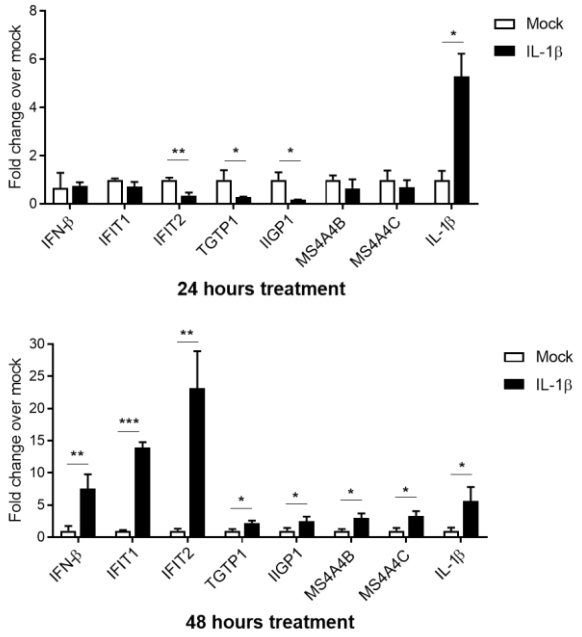


Figure 2.7. IL-1β treatment drives the expression of ISGs. WT BMDCs were mock treated or treated with 100ng/ml IL-1β for 24 or 48hrs. Total RNA was harvested and subjected to qRT-PCR to determine relative levels of gene expression. The data are the averages of three independent experiments. Asterisks indicate values that are statistically significantly different between mock-treated controls and IL-1β-treated cells by unpaired *t* test. **p* <0.05, ***p* <0.01, ****p* <0.001

or upregulated by 48 hours post-treatment. qRT-PCR analysis confirmed that *IFNβ* and several ISGs were transcriptionally silent or even downregulated at 24 hours after IL-1β treatment but were upregulated at 48 hours following IL-1β treatment alone (**Figure 2.7**). These results demonstrate that IL-1β signaling in BMDCs leads to expression of ISGs in BMDCs at late times post-treatment.

2.2.5 Signaling requirements of IL-1β driven responses

To identify the transcription factors linked with IL-1β signaling to drive ISGs and inflammatory molecules, we assessed the enrichment of promoter regions among lists of genes up- or down-regulated

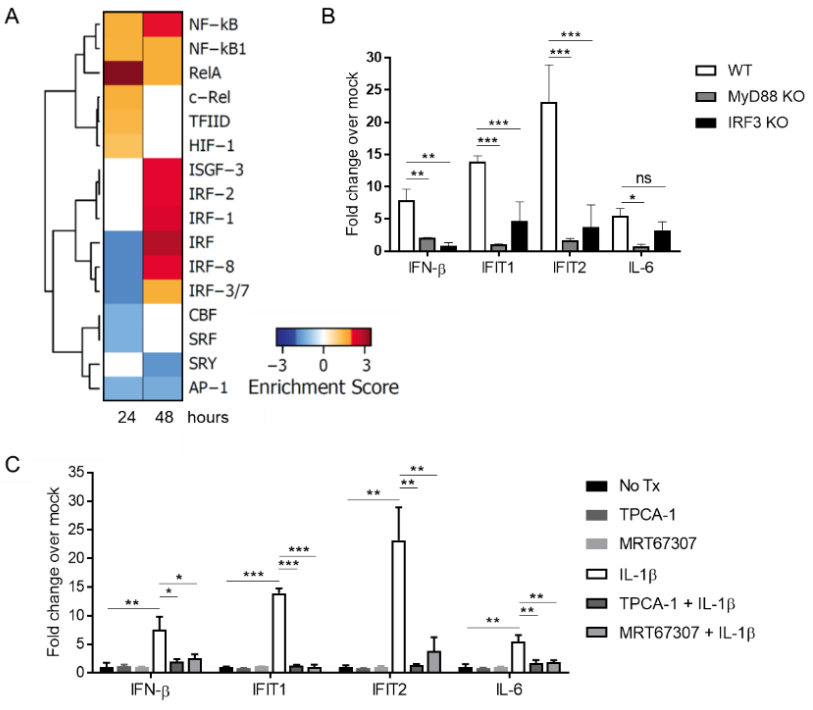


Figure 2.8. Signaling requirements of IL-1β-driven ISG responses. (A) Genes upregulated (red) or downregulated (blue) after 24 or 48hrs of IL-1β treatment were assessed for enriched transcription factor binding sites. Significantly enriched sites are considered those with an adjusted *p* value of <0.05. Enrichment scores are defined as the negative log of the adjusted *p* value. (B) WT, *Myd88*$^{-/-}$, and *Irf3*$^{-/-}$ BMDCs were mock treated or treated with 100ng/ml IL-1β for 48hrs. Gene expression levels were measured by qRT-PCR and are displayed relative to those of matched, mock-treated controls. (C) WT BMDCs were mock treated (No Tx) or pretreated with the IKKβ inhibitor TPCA-1 at 50nM or the TBK1/IKKε inhibitor MRT67307 at 2uM for 1hr and then mock treated or treated with 100ng/ml IL-1β for 48hrs. The data are averages of three independent experiments and represent fold changes with respect to respective mock-treated controls. Asterisks indicate differences that are statistically significant between the indicated groups by unpaired *t* test. **p* <0.05, ***p* <0.01, ****p* <0.001

following 24 or 48 hours of IL-1β treatment (**Figure 2.8A**). We found that the general IRF binding site and the IRF3/7 binding site are enriched within the list of downregulated genes at 24 hours post-IL-1β treatment but the IRF motifs are remarkably enriched in the list of 48 hour upregulated genes post-IL-1β treatment, consistent with IL-1β driving a distinct crosstalk toward an innate immune antiviral response at 48 hours post-treatment. The ISRE binding factor, ISGF3, is also enriched at 48 but not 24 hours post-treatment. Binding sites for the NF-κB family members cRel and RelA, as well as the general NF-κB binding site, are enriched at both 24 and 48 hours post-IL-1β treatment, although the enrichment pattern appears to be altered slightly at 48 hours. This overall pattern is consistent with a shift in IL-1β signaling from an NF-κB-driven inflammatory response to an IRF-driven antiviral response.

To confirm the role of IRF signaling in ISG induction after IL-1β treatment, we treated BMDCs from WT or *Irf3*$^{-/-}$ mice with IL-1β and assessed the expression of genes identified in our transcriptomics analysis (**Figure 2.8B**). While the induction of NF-κB-responsive *IL-6* was not affected by the loss of IRF3, *Irf3*$^{-/-}$ BMDCs were unable to express *IFNβ*. Similarly, ISGs *IFIT1* and *IFIT2* were largely reduced in expression upon IL-1β treatment of IRF3 KO BMDCs compared to WT cells. These data demonstrate that the induction of ISGs by IL-1β is indeed through an IRF-dependent mechanism. NF-κB- and IRF-mediated transcriptional activity depends on their regulation by the canonical and non-canonical IκB kinases (IKKs) (DiDonato, 1997; Fitzgerald, 2003b; Sharma, 2003). The canonical IKKs, IKKα and IKKβ, activate NF-κB via phosphorylation and subsequent degradation of the NF-κB inhibitory molecule, IκBα (DiDonato, 1997). The non-canonical IKKs include TBK1 and IKKε and are essential for the phosphorylation and activation of IRF3 (Fitzgerald, 2003b; Sharma, 2003). Additionally, IKKε can regulate innate immune effector genes via modulation of STAT1 (Perwitasari O., 2011; tenOever B.R., 2007). We examined the contribution of these kinases in IL-1β-induced gene expression through the use of pharmacological inhibitors against the canonical IKKs, (TPCA-1 (Podolin, 2005)) or the non-canonical IKKs (MRT67307 (Clark, 2011)). Interestingly, inhibition of either the canonical or the non-canonical IKKs completely prevented IL-1β-induced expression of *IFNβ*, *IFIT1* and *IFIT2* (**Figure 2.8C**). Additionally, both IKK families influence expression of the NF-κB- and ISGF3-responsive gene, *IL-6*. As NF-κB is necessary for the induction of IFNβ in this context, inhibition of the canonical IKKs could affect secondary response genes downstream of IFN (ie ISGF3-driven genes) (Thanos, 1992).

To define the signaling requirements of IL-1β-driven responses, we assessed whether the Toll-interleukin 1 receptor domain-containing adaptor protein MyD88 mediated this signature. WT and *Myd88*$^{-/-}$ BMDC were treated with IL-1β and gene expression was assessed by qRT-PCR. As expected, NF-κB- and IRF-mediated transcriptional changes induced by IL-1β are entirely dependent upon this essential signaling adaptor (**Figure 2.8B**). These results show that IL-1R/MyD88 signaling can activate both canonical and non-canonical IKKs to coordinately induce antiviral response genes through the actions of the NF-κB and IRF transcription factor families.

2.2.6. Model of IL-1β signaling

Finally, we used network analysis to examine the interplay between inflammatory and anti-inflammatory molecules following IL-1β treatment. As shown in **Figure 2.9**, we identified distinct regulatory nodes of IL-1β signaling according to our transcriptomics and kinase inhibitor data sets. At 24 hours post-treatment, mRNA expression of pro-inflammatory genes is high while expression of antiviral ISGs like interferon regulatory factor 7 (IRF7), a prominent biomarker of the antiviral/IFN response (Honda K., 2005), is notably repressed. However, by 48 hours post-treatment, expression of inflammatory genes is either reduced or not substantially increased as compared to 24 hours. This change is concomitant with the upregulation of genes with known inhibitory functions toward inflammatory cytokines (Couper K.N., 2008; Dinarello, 1996; Wong P.K., 2006). At 48 hours, IRF7 mRNA expression is induced, correlating with an increase in IRF3/7-responsive IFNβ and antiviral genes at later times post-IL-1β exposure. Together, these data sets demonstrate a dynamic regulation of IL-1β signaling outcome between inflammatory and antiviral in a cell-intrinsic manner.

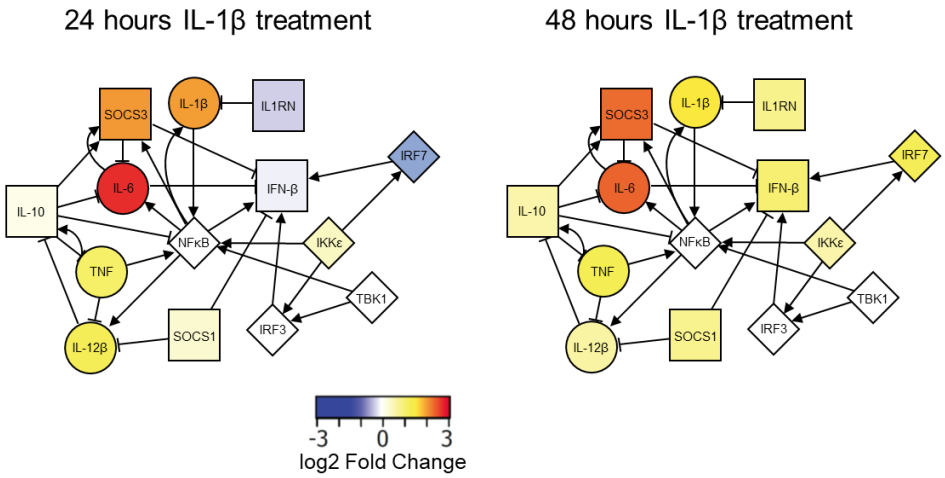


Figure 2.9. Model of IL-1β-driven ISG responses. (A) Network analysis of inflammatory and anti-inflammatory genes during IL-1β treatment. Nodes represent either genes induced by IL-1β treatment or signaling molecules and transcription factors regulating their expression. Circular nodes are considered inflammatory, whereas square nodes are considered anti-inflammatory. Diamond-shaped nodes represent signaling molecules and transcription factors involved in this network. Edges between nodes were curated from the InnateDB database and represent either activation (arrows) or inhibition (bars). Node fill colors represent log2-fold changes in expression following IL-1β treatment with respect to mock-treated cells at the times indicated.

2.3 Discussion

West Nile virus is an emerging mosquito-borne flavivirus that can result in serious illness, neuropathology, and death in a subset of infected individuals. Currently there are no vaccines or therapies for human use against West Nile virus. Immune control of West Nile infection requires inflammatory and antiviral responses, though the effect that each arm of this response has on the other is unclear. Our study reveals that loss of the IL-1R has a detrimental effect on antiviral responses to WNV in bone marrow derived dendritic cells and macrophages, leading to reduced type I IFN and increased viral replication. Additionally, multiple functional classes of ISGs are disrupted in *Il-1r*$^{-/-}$ cells in the induction and/or the maintenance of expression throughout infection. Bioinformatic modeling suggests that the pattern of response to ISGs in the presence or absence of IL-1 signaling may be determined by the temporal regulation of distinct transcription factors, and that the host transcription machinery is not optimally coordinated without some level of IL-1 signaling. Additionally, we found that IL-1β treatment of BMDCs led to early induction of pro-inflammatory genes but shifted at later times to inducing anti-inflammatory genes that serve to dampen the inflammatory response following IL-1β treatment. Our previous study demonstrated that cortical neurons lacking IL-1R actually produce more IFNβ in response to WNV (Ramos, 2012), suggesting that the specifics of cross-regulation between these pathways differs from that found in monocyte-derived cells.

Type I IFN and pro-inflammatory cytokines are known to each downregulate the production and function of the other (Mayer-Barber, 2017), suggesting that the induction of IFNβ at late times after IL-1β treatment may serve as a mechanism to balance antimicrobial inflammatory function with pathological inflammatory-mediated tissue damage. In our previous report, we found that subsets of microglia appear to become activated upon WNV entry to the CNS in infected mice (Ramos, 2012). However, these microglia did not return to basal states at late times of infection in *Il-1r*$^{-/-}$ mice as they did in WT mice. This outcome implies a role for the IL-1 signaling pathway in maintaining homeostatic balance of inflammation in the CNS, particularly in macrophage or DC-like cells. Consistent with this notion, IRF3 activation has been reported to act as a switch from pro-inflammatory "M1-like" to immunomodulatory "M2-like" phenotypes in microglia (Tarassishin et al., 2011), and IFN has been defined to have a role in homeostatic defense against IL-1-mediated inflammation and tissue damage (Hu et al., 2005).

Our data sets support a model of IL-1β to IRF3 crosstalk signaling in which at earlier times following IL-1β exposure of BMDCs, signaling through IL-1R and MyD88 to NF-κB leads to a canonical and well-described response of upregulation of inflammatory genes and cytokines to direct the classic inflammatory response to IL-1β. The opposing anti-inflammatory/antiviral response, including IRF3/7-mediated induction of IFNβ expression, is silent at these times after IL-1β to allow for efficient inflammatory responses. At later times post-treatment, IL-1β continues to drive gene expression of inflammatory cytokines through NF-κB, albeit at lower levels as compared to earlier times post-treatment. However, by this time following IL-1β exposure, signaling has begun a regulatory anti-inflammatory response, including the expression of type I IFN and ISGs. Coordinate activation of NF-κB and IRF transcription factors results in the expression of critical antiviral genes. This dynamic crosstalk of IL-1β and IFN pathways may serve to both control inflammatory responses as well as to sustain antiviral responses against WNV.

The crosstalk signaling by IL-1β to type I IFNs in cellular homeostasis is likely of particular importance beyond virus infection to impact autoimmune development and immune regulation. Depending on the particular autoimmune disease and stage of development, type I IFNs can promote disease through chemokine expression and antigen presentation or protect against damage through regulation of pro-inflammatory cytokines, including IL-1β and TNFα (Ivashkiv and Donlin, 2014). In clinical settings, inhibition

of IL-1β through specific agonists or through IFNβ therapy is useful in limiting development and progression of autoimmune and inflammatory-mediated diseases, including rheumatoid arthritis and multiple sclerosis (Dinarello, 2009b; Guarda et al., 2011; Lopez de Padilla and Niewold, 2016).

One of the best studied scenarios of IL-1β cross-regulation with type I IFN is in the context of Mycobacterium tuberculosis (*Mtb*) infection. IL-1β is absolutely required for effective host responses to *Mtb* infection (Mayer-Barber et al., 2011). However, virulent strains of *Mtb* selectively trigger induction of type I IFN that inhibits the expression of protective IL-1β expression (Novikov et al., 2011). Although this may also reflect an attempt by the host to limit inflammatory-mediated tissue damage, *Mtb* is able to utilize the response to enhance its own infection and pathogenesis. This response is also relevant during viral infections in *Mtb* patients, as type I IFN production during influenza infection exacerbates *Mtb* infection and disease progression (Redford et al., 2014). Conversely, IL-1β-induced eicosanoids were shown to inhibit the actions of type I IFN during influenza (Coulombe, 2014) or *Mtb* (Mayer-Barber, 2014) infection, with opposite outcomes for disease. These studies highlight the complicated interplay between inflammation and IFNs during microbial infection.

Other groups have also observed connections between IL-1β signaling and IFN induction. IL-1β was found to activate IRF3 in cultured human fetal astrocytes that then induced the expression of IRF7 and IFNβ (Rivieccio, 2005). The authors suggested that IL-1β produced by activated microglia may trigger IRF3 activity in astrocytes to amplify innate immune responses and provide a second line of defense against infection in the CNS. Additionally, TLR9-dependent activation of type I IFN and the anti-inflammatory cytokine IL-10 was found to be lacking in the absence of IL-1R, and BMDCs from *Il-1r*$^{-/-}$ mice failed to mount protective type I IFN responses following TLR9 or TLR3 stimulation (Gonzalez-Navajas et al., 2010). Another group found that Huh7 hepatoma cells co-treated with IFNα and IL-1β show potentiated ISG expression and phosphorylation of STAT1 but no ISG induction with IL-1β alone (Ichikawa et al., 2002), which may be a demonstration of enhanceosome activity in these cells (Wienerroither, 2015). IL-1R-mediated IFN production may not be limited to IL-1β, as IL-1α has also been shown to induce transcription of IFNβ mRNA in human foreskin fibroblasts (Fujita, 1989). These studies provide additional support to our

finding that IL-1β signaling can be intricately linked to the induction of IFNβ and ISGs in a cell-specific manner.

There are several potential mechanisms by which IL-1β signaling may shift to induction of IFNβ at late times post-treatment. One such mechanism may be shunting signaling between the adaptor molecule TNF receptor associated factor 3 (TRAF3). TRAF3 is essential for the induction of type I IFNs and IL-10 in BMMs, but is dispensable for the expression on pro-inflammatory cytokines (Hacker et al., 2006). TRAF3 must be ubiquitinated at lysine (K)48 residues and subsequently degraded for MyD88-dependent TLR signaling to produce pro-inflammatory cytokines, while non-degradative K63-linked self-ubiquitination of TRAF3 leads to IFNβ induction (Tseng et al., 2010). In other systems, IL-1 signaling has been shown to trigger the downregulation of Deubiquitinating Enzyme A (DUBA), which selectively cleaves K63-linked ubiquitin chains from TRAF3 to limit type I IFN responses (Gonzalez-Navajas et al., 2010). Although we have not detected this phenomenon in our system, the possibility remains that DUBA expression or function may be altered over the course of IL-1β treatment to manage the switch toward anti-inflammatory gene induction. Another possibility by which IL-1β treatment may lead to IRF-dependent IFNβ expression is by signaling through phosphoinositide-3-kinase (PI3K)-Akt. In microglia, overexpression of IRF3 via adenoviral vectors activated PI3K and Akt to induce the anti-inflammatory genes *IL-1RN*, *IL-10* and *IFNβ* (Tarassishin et al., 2011). The authors suggested that Akt signaling may suppress miR-155 to modulate cytokine production. IFN and inflammatory cytokine signaling have in other circumstances been found to induce cellular miRNAs that target components of IFN signaling (Ivashkiv and Donlin, 2014), so modulation of miRNA expression and function may be another mechanism by which IL-1β and IFNβ may cross-regulate each other. Certainly, there may be yet other mechanisms induced by IL-1β signaling that function to de-repress IRF-mediated signaling at late times after exposure as a means of inflammatory resolution.

In summary, our studies demonstrate a cell-intrinsic cross-regulation between IL-1β signaling and type I IFN responses in myeloid cells that is required for optimal control of WNV infection. Further defining the mechanisms by which pro-inflammatory signaling switches to activate anti-inflammatory cytokines and antiviral ISG responses may reveal novel targets for controlling dysregulated immune responses in

autoinflammatory disease as well as in response to pathogens with potential efficacy against other neuroinvasive viruses.

2.4 Methods

Materials. Recombinant murine IL-1β was purchased from Miltenyi Biotec Inc., reconstituted in sterile water, and stored as at a concentration of 100ug/ml at -20°C. TPCA-1 (Tocris) was reconstituted in ethanol, and stored at 10mM at -20°C. MRT67307 (Sigma) was reconstituted in sterile water and stored at a concentration of 15mg/ml at -20°C. Working concentrations are indicated in figure legends.

Viruses and cell lines. WNV isolate, TX 2002-HC (WNV-TX), was titered by a standard plaque assay on BHK-21 cells and working stocks of WNV-TX were generated as previously described (Suthar et al., 2010). BHK-21 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), HEPES, L-glutamine, sodium pyruvate, antibiotic-antimycotic solution, and nonessential amino acids.

Primary cell isolation and infection. C57BL/6 wild-type (WT), IL-1R deficient (*Il-1r*$^{-/-}$), and MyD88 deficient (*Myd88*$^{-/-}$) mice were described previously (Ramos, 2012). *Irf3*$^{-/-}$ mice were a kind gift from Dr. T. Taniguchi. All mice were genotyped for positive identification and were bred in specific pathogen-free conditions in the animal facility at the University of Washington. Experiments were performed in accordance with the University of Washington Institutional Animal Care and Use Committee guidelines. Bone marrow derived dendritic cells (BMDCs) were generated as follows: cells were isolated from the bone marrow of WT, *Il-1r*$^{-/-}$, *Myd88*$^{-/-}$ or *Irf3*$^{-/-}$ mice and cultured for 7 days in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% FBS, L-glutamine, sodium pyruvate, antibiotic-antimycotic solution and nonessential amino acids in the presence of 20ng/ml granulocyte-macrophage colony-stimulating factor and 20ng/ml interleukin-4 (PeproTech Inc.). Bone marrow derived macrophages (BMMs) were generated as follows: cells were isolated from the bone marrow of WT or *Il-1r*$^{-/-}$ mice and cultured for 7 days in DMEM supplemented with 10% FBS, L-glutamine, sodium pyruvate, antibiotic-antimycotic solution and nonessential amino acids in the presence of 40ng/ml macrophage colony-stimulating factor (PeproTech Inc.). $5x10^5$ BMDC or BMM were infected with WNV-TX at an MOI of 2.5 for 1 hour, washed and subsequently incubated for 24 or 48 hours in the appropriate medium before downstream analyses.

IFNβ ELISA. For detection of IFNβ in cell culture supernatants, 100ul of UV-inactivated supernatant was tested using mouse-specific ELISA kits from PBL Biomedical Laboratories according to the manufacturer's protocol.

Immunoblotting. Protein extracts (20ug) were analyzed by immunoblotting. The following primary antibodies were used to probe blots: goat anti-WNV NS3 (R&D systems), rabbit anti-ISG49 (IFIT3) kindly provided by Dr. G. Sen, rabbit anti-GAPDH (FL-335) (Santa Cruz) and rabbit anti-STAT1 (Cell Signaling). Secondary antibodies included peroxidase-conjugated goat anti-rabbit and donkey anti-goat (Jackson Immunoresearch). Densitometry analysis was performed using Image Studio Lite software (LI-COR).

RNA extraction and analysis. Total RNA was isolated from BMDCs using RNA extraction buffer (RLT, Qiagen), and the RNeasy kit according to the manufacturer's protocol (Qiagen). DNase treated RNA (Qiagen) was then reversed transcribed to cDNA using a 1:1 mixture of random hexamers and oligodT primers with the iScript select cDNA synthesis kit (Biorad). WNV-specific RNA copy number was measured by single-step Real Time-quantitative PCR (qRT-PCR) using Taqman technology via specific primer sets and probes as previously described (Suthar et al., 2010). Gene expression was assessed by one-step SYBR Green qRT-PCR using an ABI 7800 machine.

Specific primer sets for mouse GAPDH, IFNβ, IL-1β, IL-6, Ms4a4b, Ms4a4c, Iigp1, and Tgtp1, are as follows: mGAPDH forward: CAACTACATGGTCTACATGTTC, mGAPDH reverse: CTCGCTCCTGGAAGATG; mIFNb forward: GGAGATGACGGAGAAGATGC mIFNb reverse: CCCAGTGCTGGAGAAATTGT; mIL1b forward: ACGGACCCCAAAAGATGAAG; mIL1b reverse: CACGGGAAAGACACAGGTAG; mIL6 forward: GTTCTCTGGGAAATCGTGGA, mIL6 reverse: TGTACTCCAGGTAGCTATGG; mMs4a4b forward: TGCAGCAGGAGTGACACCTACAAA, mMs4a4b reverse: ACAGCCACACTGACTACACCCATT; mMs4a4c forward: CCTGTCAATTGCAGCAGGAGTGAA, mMs4a4c reverse: TGCAGCCAACACAGAGGTGATAGT; mIigp1 forward: AGTGTGCTCAATGTTGCTGTCACC, mIigp1 reverse: TTCATTCCCAATGCCTCTCAGGGT; mTgtp1 forward: TGCAAGTCTTACTGAGGCCACC, mTgtp1 reverse: ATGCTCCAGCCTTCATGGCTTCTA; mIFIT1 and mIFIT2 were purchased as pre-mixed SuperArray primer sets (Qiagen).

RNA preparation and oligonucleotide microarray processing. Total RNA was harvested for array analysis using Trisol LS. Samples were prepared and hybridized to Agilent mouse whole-genome oligonucleotide 4-by-44 microarrays as previously described (Suthar et al., 2013a).

Microarray analysis and bioinformatics. Microarray data were analyzed using the R statistical programming language and Bioconductor (Gentleman et al., 2004; R Development Core Team, 2012). Raw data were quantile normalized followed by linear modeling using the limma package (Smyth, 2004). Genes with significant changes following WNV infection or IL-1β treatment were defined by those with a >1.5-fold increase or decrease over genotype and time-matched controls with a Benjamini-Hochberg (BH) corrected p-value <0.05. WT and *Il-1r*$^{-/-}$ WNV responses over mock were quantitatively compared using the limma package with the same criteria as above. Microarray data have been deposited in the NCBI Gene Expression Omnibus under GEO Series accession number GSE109069 according to Minimum Information About a Microarray Experiment (MIAME) standards. Network analysis was run by manual curation using the InnateDB curated database and analysis tools (Lynn et al., 2010), and network images were created using Cytoscape (Cline et al., 2007; Shannon et al., 2003). Transcription factor binding site (based on Genome Browser PWMs) and Gene Ontology Biological Process enrichment was performed using Enrichr (Chen, 2013). Ranking of significant processes was determined by sorting on the Combined Score followed by sorting on the adjusted p-value.

Statistical analysis. Unpaired t-test was used to determine significant differences between the groups indicated in each figure for qRT-PCR and ELISA analyses. Virus titers were analyzed by Mann-Whitney U test to assess significance between genotypes at each time. All quantifications are displayed as mean ± standard deviation and were analyzed using Prism software (GraphPad Prism 7).

3. Interleukin-1β induces mitochondrial DNA release to activate innate immune signaling via cGAS-STING

Lauren D. Aarreberg, Katharina Esser-Nobis, Connor Driscoll, Andrey Shuvarikov, Justin A. Roby and Michael Gale, Jr.

3.1 Introduction

A timely and potent response to pathogens is critical for host defense against infection. Microbial and cellular cues of infection are detected by immune and non-immune cells via pattern recognition receptors (PRRs) to initiate innate and inflammatory cascades. PRRs include Toll-like receptors (TLRs), RIG-I-like receptors, nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and cytosolic DNA sensors such as cyclic GMP-AMP synthase (cGAS) (Paludan, 2013; Takeuchi, 2010). Differential expression of PRRs across cell types directs cell-specific innate immunity. PRRs recognize a broad array of structural and biochemical motifs that originate from the pathogen itself (pathogen-associated molecular patterns, PAMPs) or are products that are produced in response to infection or stress-induced damage (danger-associated molecular patterns, DAMPs). Under normal conditions, DAMPs are sequestered from PRRs, or are otherwise structurally unrecognizable, and do not stimulate immune responses. Liberation or modification of the DAMP can result in its recognition by PRRs (Schaefer, 2014). The spectrum of PPRs engaged during infection and responses to stress serve to direct the outcome of infection and immunity (Brubaker, 2015). For example, which PRRs are engaged can communicate the makeup (bacterial, fungal, viral), location (extracellular, endosomal) and pathogenicity (damage-inducing) of the offending microbe. PRR signaling converges on latent transcription factors such as nuclear factor kappa B (NF-κB), interferon regulatory factors (IRFs) and signal transducer and activator of transcription (STAT) proteins for the induction of gene expression involved in immune cell recruitment, signal transduction and direct antimicrobial activities (Paludan, 2013; Takeuchi, 2010).

Cytokine production and response comprise an important arm of host defense. Interferon beta (IFNβ), interferon lambda (IFNλ) and interleukin-1β (IL-1β) are pivotal cytokines of innate immunity and inflammation in the control of microbial infection. IFNs are produced as a result of PRR signaling that drives IRF3 activation. Upon binding its ubiquitously expressed, heterodimeric receptor comprised of IFNαβ receptor (IFNAR)1 and IFNAR2, IFNβ induces Janus kinase (JAK) activation and the assembly of IFN-

stimulated gene factor 3 (ISGF3; a heterotrimer of phosphorylated STAT1, STAT2 and IRF9) for the transcriptional upregulation of IFN-stimulated genes (ISGs). ISG products promote an antimicrobial state in infected and bystander cells (Brierley, 2002; Schneider, 2014). IFNλ initiates a largely analogous program, but its effects are limited to the few cell types expressing its unique receptor, namely epithelial, neuronal and myeloid cells (Hemann, 2017). IL-1β, a product of inflammasome activation, is a potent inducer of NF-κB-dependent gene transcription and can propagate inflammation, recruit immune cells and modulate adaptive immune responses (Dinarello, 2009a; Sims, 2010). The heterodimeric IL-1 receptor (IL-1R) consists of IL-1 receptor type I (IL-1R1) and IL-1 receptor accessory protein (IL-1RAcP) and is expressed at different levels across cell types.

IL-1β can initiate cell-intrinsic host restriction pathways against bacterial and viral infections but the intracellular mechanisms thereof are not fully defined (Copenhaver, 2015; Mayer-Barber, 2014; Ramos, 2012; Van Damme, 1983). We recently demonstrated that IL-1R signaling in primary murine myeloid cells regulates transcriptional activation to initiate or maintain ISG expression and limit West Nile virus infection (Aarreberg, 2018). Moreover, an intriguing recent study by Orzalli *et al*. defined the presence of an IL-1-induced, IRF1-dependent antiviral program in human fibroblasts and endothelial cells (Orzalli, 2018). Here we examined innate immune defense programs downstream of IL-1R in various cell types and reveal that exogenous IL-1β triggers TBK1-mediated IRF3 activation and autophagic flux through the DNA sensing pathway components cGAS and Stimulator of IFN genes (STING). This response depends upon the liberation and cytosolic sensing of mitochondrial DNA (mtDNA) and functions to potentiate pathogen-induced IFN production, IFN signaling and ISG expression. We also found that IL-1R1 is required for maximal IRF-directed immune responses to inflammasome-activating microbial products and Dengue virus infection. Our observations present a new mechanism in which IL-1β modulates STING activity for cell-intrinsic protection against microbial pathogens.

3.2 Results

3.2.1 Exogenous IL-1β activates IRF3

To determine the impact of IL-1β on the cell-intrinsic innate immune response, we analyzed IRF3 activation and immune gene expression upon IL-1β treatment of various cell types. Treatment of human

A549 epithelial cells with exogenous IL-1β resulted in phosphorylation of IRF3 at the essential activating phosphoacceptor reside Serine-386 (S386) (Mori, 2004), followed by increased abundance of IFIT1, a known IRF3 target gene (Grandvaux, 2002) (**Figure 3.1A**). Transcriptional induction of antiviral response genes *IFIT1* and *MX1* by IL-1β was lost in CRISPR-targeted A549 cells lacking IRF3 expression, but induction of the NF-κB-responsive gene, *CCL4*, was unaffected by the loss of IRF3 (**Figure 3.1B**). Additionally, we found that IRF3 is phosphorylated in response to IL-1β treatment in primary human foreskin fibroblasts (**Figure 3.1C**).

In PMA-differentiated, macrophage-like THP-1 cells, IL-1β treatment resulted in the delayed phosphorylation of IRF3 at S386 (**Figure 3.1D**). In THP-1 cells we found that IL-1β-induced *CCL4* expression occurs rapidly after IL-1β treatment and then subsides over 36 hours while antiviral response genes increase in expression over this period (**Figure 3.1E**). Of note, *CXCL10* is both IRF3- and NF-κB-responsive (Ohmori, 1995). Consistent with this dual induction program, *CXCL10* demonstrates a bimodal expression pattern in IL-1β-treated THP-1 macrophages (**Figure 3.1E**) and is only partially reduced in IRF3-deficient, IL-1β-treated A549 cells (**Figure 3.1B**). These responses were absent in IL-1R1-deficient cells, demonstrating that pathways of IRF3 and NF-κB activation are induced in response to IL-1β engagement of IL-1R (**Figure 3.2**).

We then evaluated the response to IL-1β in primary human monocyte-derived dendritic cells (moDC). MoDC treated with exogenous IL-1β express antiviral genes in a dose-response manner, with similar trends of immediate NF-κB activation that wanes over time and a gradual increase in IRF3 target genes and ISGs over 36 hours (**Figure 3.1F**). Notably, IL-1β induces concentration-dependent phosphorylation of IRF3 in primary moDC at 36 hours post-treatment (**Figure 3.1G**). The delayed kinetics of IRF3-responsive gene expression in human macrophages and dendritic cells match the kinetics of IL-1β-induced antiviral gene expression that occur in primary murine macrophages and dendritic cells (Aarreberg, 2018). Together, these observations demonstrate that exogenous IL-1β induces functional IRF3 activation in human cell lines of epithelial and myeloid origin as well as primary human fibroblasts and myeloid cells.

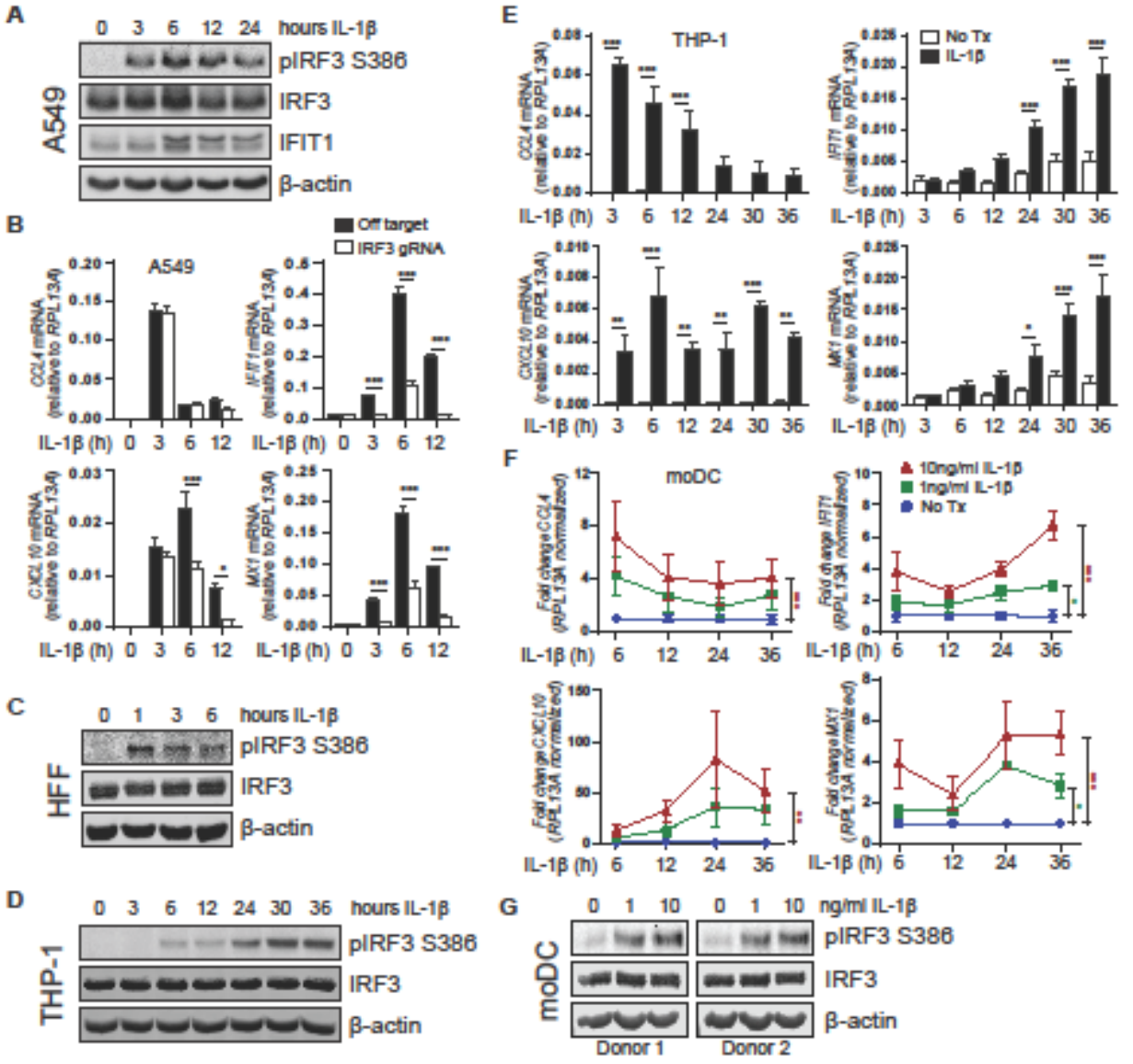


Figure 3.1 Exogenous IL-1β activates IRF3. (A) A549 were treated with media (0) or 10ng/ml IL-1β for 3-24hrs before protein analysis by immunoblot, *n*=4. (B) A549 transduced with lentiCRISPR/Cas9 and off target gRNA or IRF3-gRNA were treated with media (0) or 10ng/ml IL-1β for 3-12hrs before qRT-PCR analysis. Statistical analysis was performed using student's T test and Holm-Sidak to compare genotypes, *n*=6 with mean ± SEM. (C) Human foreskin fibroblasts were treated with media (0) or 10ng/ml IL-1β for 1-6hrs before protein analysis by immunoblot, *n*=3. (D) THP-1 were treated with media (0) or 10ng/ml IL-1β for 3-36hrs before protein analysis by immunoblot, *n*=4. (E) THP-1 were treated with media or 10ng/ml IL-1β for 3-36hrs before qRT-PCR analysis. Statistical analysis was performed using student's T test and Holm-Sidak to compare treatments, *n*=3 with mean ± SEM. (F) Primary human monocyte-derived dendritic cells (moDC) were treated with media or the indicated concentrations of IL-1β for 6-36hrs before qRT-PCR analysis. Statistical analysis was performed using two-way ANOVA and Dunnett's to compare mock to IL-1β treatments, *n*=3 with mean ± SEM. (G) moDC were treated with media or the indicated concentrations of IL-1β for 36hrs before protein analysis by immunoblot, *n*=2. *p<0.05, **p<0.01, ***p<0.001

3.2.2 Differential IRF requirements for IL-1β-induced antiviral gene programs

To characterize IL-1β-induced antiviral gene programs, we utilized droplet-based single-cell RNA-sequencing (scRNA-seq), wherein we identified heterogeneous responses in A549 cells treated with IL-1β over 3, 6 or 12 hours (**Figure 3.3A**). In total, we sequenced 7547 cells that passed knee filtering (1825 cells at 3 hours post-treatment, 3278 cells at 6 hours, and 2444 cells at 12 hours; see Methods). Of interest, scRNA-seq revealed that several ISGs, protein modifier genes involved in innate intracellular signaling, and apoptosis-regulating genes are induced or "upregulated" by IL-1β (**Figure 3.3B**, **Table S5**). Upregulated genes follow four general patterns of expression: 1) immediate and transient (e.g. *BCL3*, *IRF1*) 2) immediate and sustained (e.g. *CXCL1*, *CXCL2*) 3) delayed and transient (e.g. *GBP1, ISG20*) or 4) gradual (e.g. *B2M*, *CCL5*). We next examined predicted activation of upstream transcriptional regulators associated with significant change in measured gene expression at each time point. Transcriptional repressors such as Estrogen Receptor 1 (ESR1) and Nuclear Subfamily 3 Group C Member 1 (NR3C1) were predicted as inactivated upon IL-1β treatment (**Figure 3.3C**). Consistent with our signaling data (see **Figure 3.1**), NF-κB family members, IRF3 and other IFNβ enhanceosome members (Wathelet, 1998) are predicted to be active by three hours post-IL-1β treatment in A549 cells. While STAT1 is a predicted positive regulator at all three time points, STAT3 is predicted to be active late in the response to IL-1β.

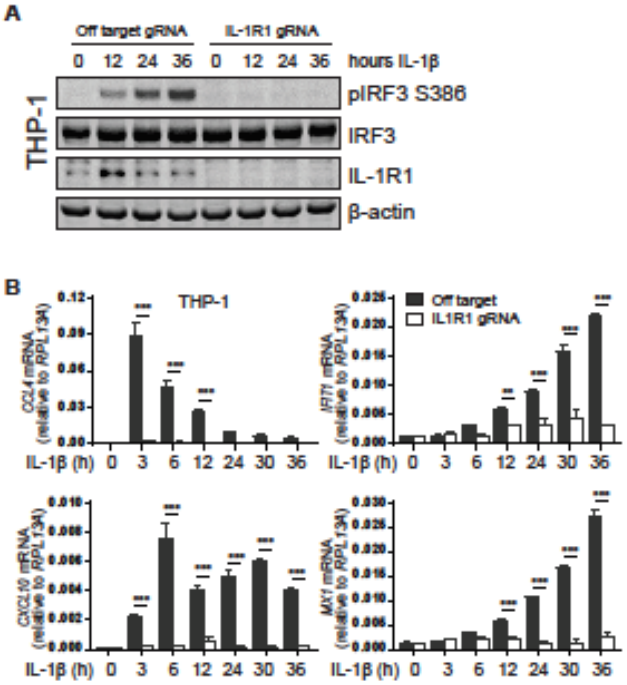


Figure 3.2. IL-1R1 is required for IL-1β signaling to IRF3. (A) THP-1 transduced with lentiCRISPR/Cas9 and off target gRNA or IL-1R1-gRNA were treated with media (0) or 10ng/ml IL-1β for 12-36hrs before protein analysis by immunoblot. (B) THP-1 transduced with lentiCRISPR/Cas9 and off target gRNA or IL-1R1-gRNA were treated with media (0) or 10ng/ml IL-1β for 3-36hrs before qRT-PCR analysis. Statistical analysis was performed using student's T test with Holm-Sidak to compare genotypes, *n*=4 with mean ± SEM. **p<0.01, ***p<0.001

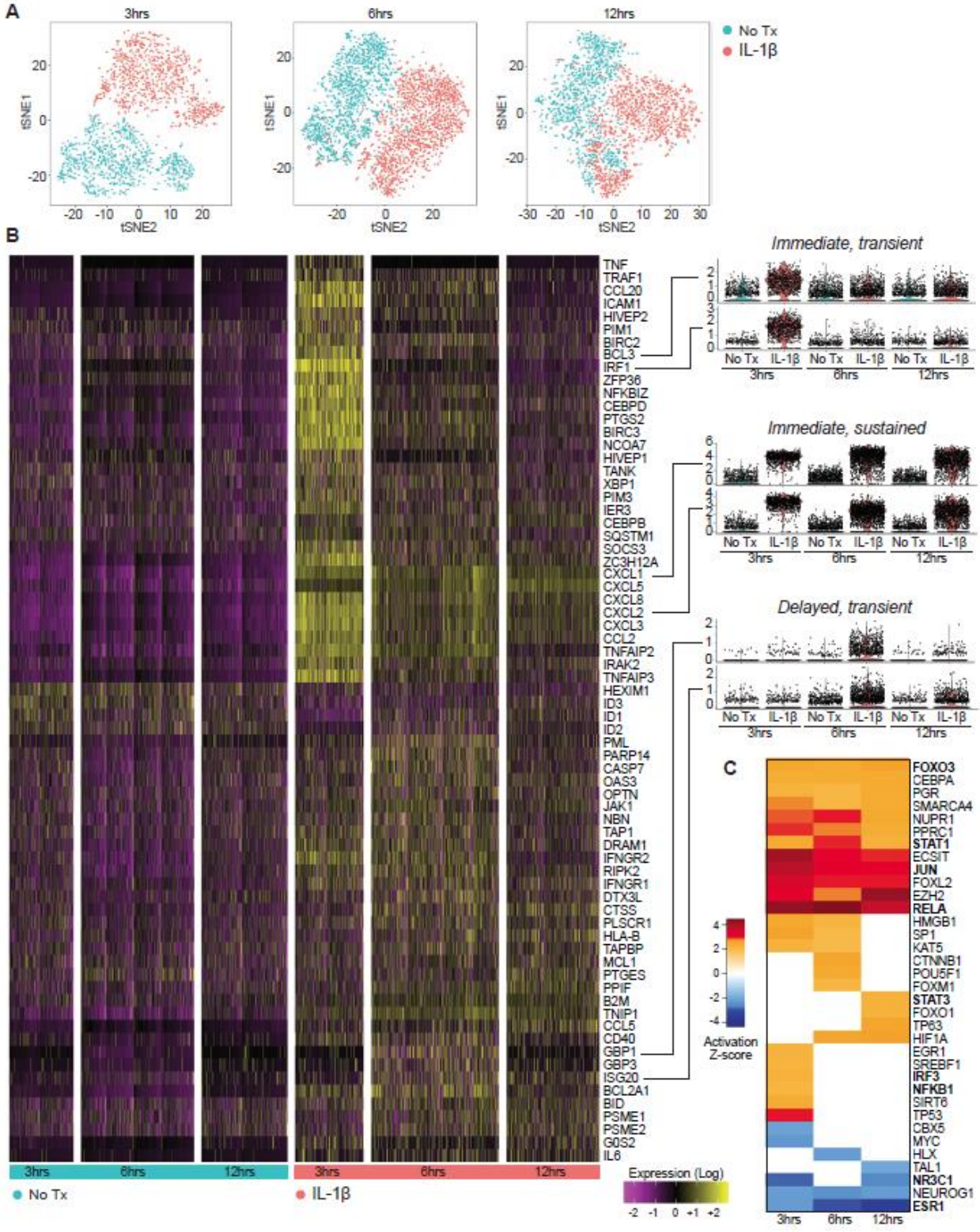


Figure 3.3. IL-1β initiates antiviral gene programs (A) A549 were treated with media or 10ng/ml IL-1β for 3, 6 or 12hrs before single cells were isolated and prepared for sequencing. tSNE analysis of scRNA-seq expression with each point representing a single cell treated with media (blue) or IL-1β (orange). (B) Left: Gene expression heatmap of selected differentially expressed genes comparing media and IL-1β treatment. Right: Violin plots representing normalized gene expression of select genes per cell. (C) Activation z-score heatmap of predicted transcription factor activity upon treatment with IL-1β.

The IRF family of transcription factors IRF1, IRF3, IRF5 and IRF7 have redundant promoter binding sites (Harada, 1989; Taniguchi, 2001) and we found that *IRF1* is highly upregulated by IL-1β treatment (see **Figure 3.3B**). We therefore ablated the expression of each IRF to determine their relative contributions to this IL-1β-induced antiviral gene signature. CRISPR-targeting of each IRF member in A549 cells revealed that both IRF1 and IRF3 contribute to IL-1β-mediated antiviral transcriptional responses, with also a partial reduction of *IFIT1* transcription observed in IRF7-deficient cells (**Figure 3.4B**). Of note, basal IRF1 expression is hardly detectable in A549 cells, and basal expression of IRF7 was dampened in IRF1 and IRF3 CRISPR-targeted A549 cells (**Figure 3.4A**). Additionally, CRISPR-targeting of IRF5 resulted in enhanced expression of IL-1β-induced genes, revealing a potential negative regulatory role for IRF5 in these cells. Immunoblot analysis revealed that exogenous IL-1β results in increased abundance of IRF1, IRF7 and IFIT1, but neither IRF7 or IFIT1 are produced in the absence of IRF3 **(Figure 3.4C**). Similarly,

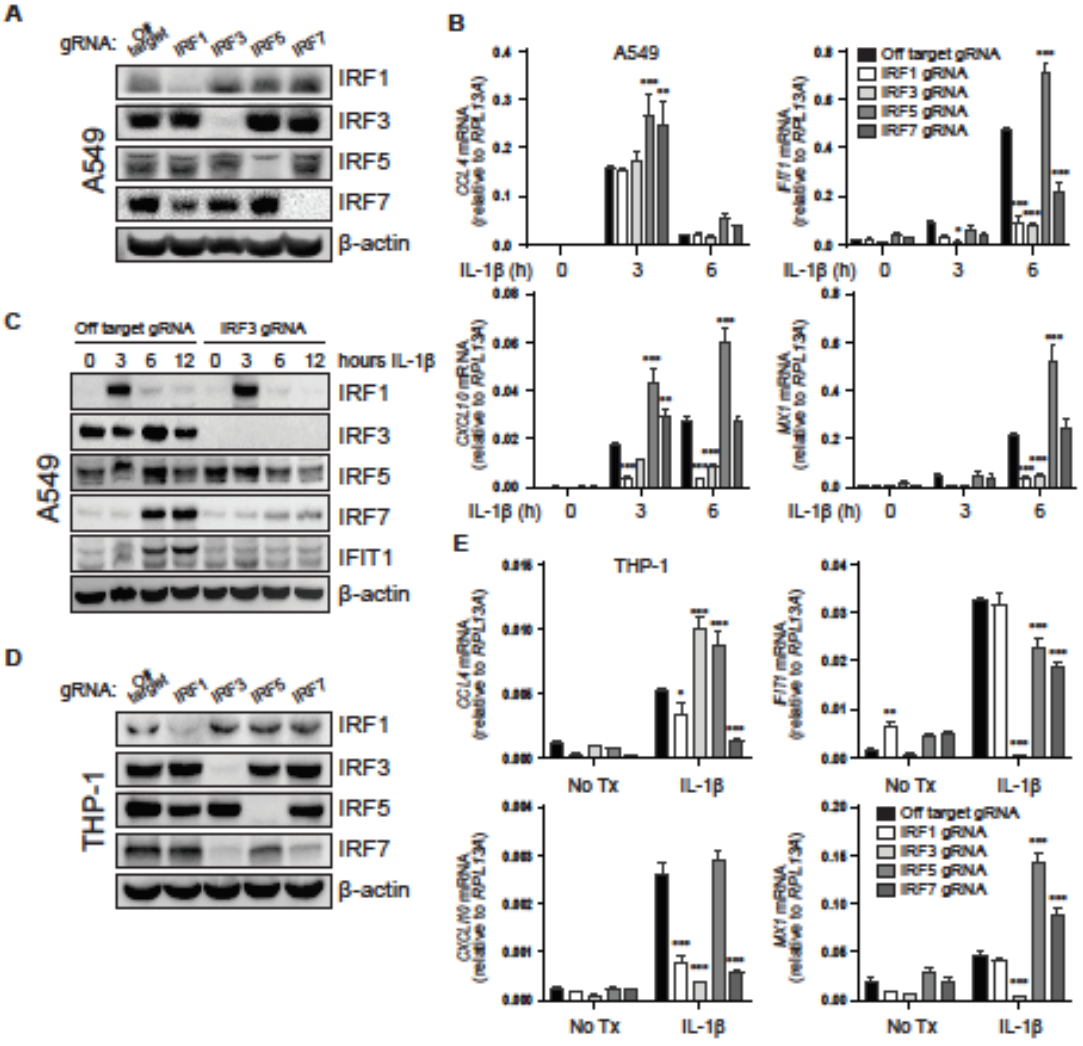


Figure 3.4. Differential IRF requirements for IL-1β-induced antiviral gene induction. (A) A549 were transduced with lentiCRISPR/Cas9 and off target gRNA or IRF1-, IRF3-, IRF5- or IRF7-gRNA before protein analysis by immunoblot. (B) A549 transduced with lentiCRISPR/Cas9 and off target gRNA or IRF1-, IRF3-, IRF5- or IRF7-gRNA were treated with media (0) or 10ng/ml IL-1β for 3-6hrs before qRT-PCR analysis. Statistical analysis was performed using two-way ANOVA with Dunnett's to compare knockouts to control cells, *n*=3 with mean ± SEM. (C) A549 transduced with lentiCRISPR/Cas9 and off target gRNA or IRF3-gRNA were treated with media (0) or 10ng/ml IL-1β for 3-12hrs before protein analysis by immunoblot. (D) THP-1 were transduced with lentiCRISPR/Cas9 and off target gRNA or IRF1-, IRF3-, IRF5- or IRF7-gRNA before protein analysis by immunoblot. (E) THP-1 transduced with lentiCRISPR/Cas9 and off target gRNA or IRF1-, IRF3-, IRF5- or IRF7-gRNA were treated with media (0) or 10ng/ml IL-1β for 36hrs before qRT-PCR analysis. Statistical analysis was performed using two-way ANOVA with Dunnett's to compare knockouts to control cells, *n*=4 with mean ± SEM. *p<0.05, **p<0.01, ***p<0.001

CRISPR-targeting of each IRF member in THP-1 macrophages showed differential requirements for each in the induction of antiviral response genes and a complete loss of signaling in the absence of IRF3 **(Figure 3.4E**). Of note, IRF3 CRISPR-targeted THP-1 cells express less IRF7 protein, which may impact antiviral responses gene expression in these cells **(Figure 3.4D**). Our data also indicate that IRF1 plays a role in the induction of *CXCL10* by IL-1β in either cell type, which is consistent with previous reports on the transcriptional regulation of this gene (Yarilina, 2008; Zaheer, 2010). These observations confirm that exogenous IL-1β can orchestrate complex transcriptional changes, including the activation of IRF1 (Orzalli, 2018) and IRF3, wherein IRF3 plays an essential role to direct the expression of target genes and ISGs in both human epithelial and myeloid cells.

3.2.3 IRF3 activation in response to IL-1β is cGAS- and STING-dependent

During PRR signaling IRF3 activation is conferred by the TBK1 and IKKε protein kinases, which coordinate with adaptor molecules MAVS, STING or TRIF to gain proximity to downstream substrates (Liu, 2015). These kinases are essential for phosphorylation of IRF3 (Fitzgerald, 2003b; Sharma, 2003), which then dimerizes and translocates to the nucleus to initiate transcription of *IFNB1, IFIT1,* and other IRF3-responsive genes. In our previous examination of signaling requirements during the murine myeloid response to IL-1β, we demonstrated a potent loss of antiviral response genes when cells were co-treated with a TBK1/IKKε inhibitor (Aarreberg, 2018).

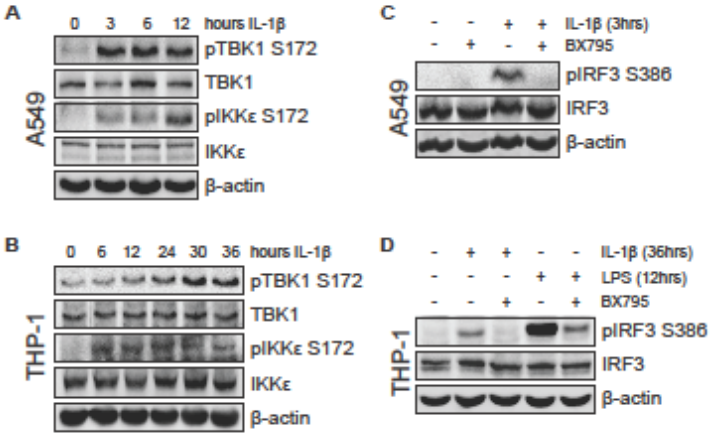


Figure 3.5. TBK1/IKKε mediate IL-1β-induced IRF3 phosphorylation. (A) A549 were treated with media (0) or 10ng/ml IL-1β for 3-12hrs before protein analysis by immunoblot. (B) THP-1 were treated with media (0) or 10ng/ml IL-1β for 6-36hrs before protein analysis by immunoblot (C) A549 were treated with media (0) or 10ng/ml IL-1β for 3hrs +/- 1hr pretreatment with DMSO or 1uM BX795 before protein analysis by immunoblot. (D) THP-1 were treated with media (0) or 10ng/ml IL-1β for 36hrs or 0.5ug/ml LPS for 12hrs +/- 1hr pretreatment with DMSO or 1uM BX795 before protein analysis by immunoblot.

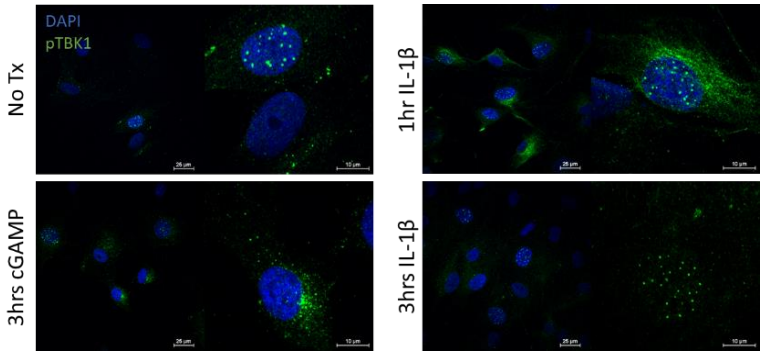
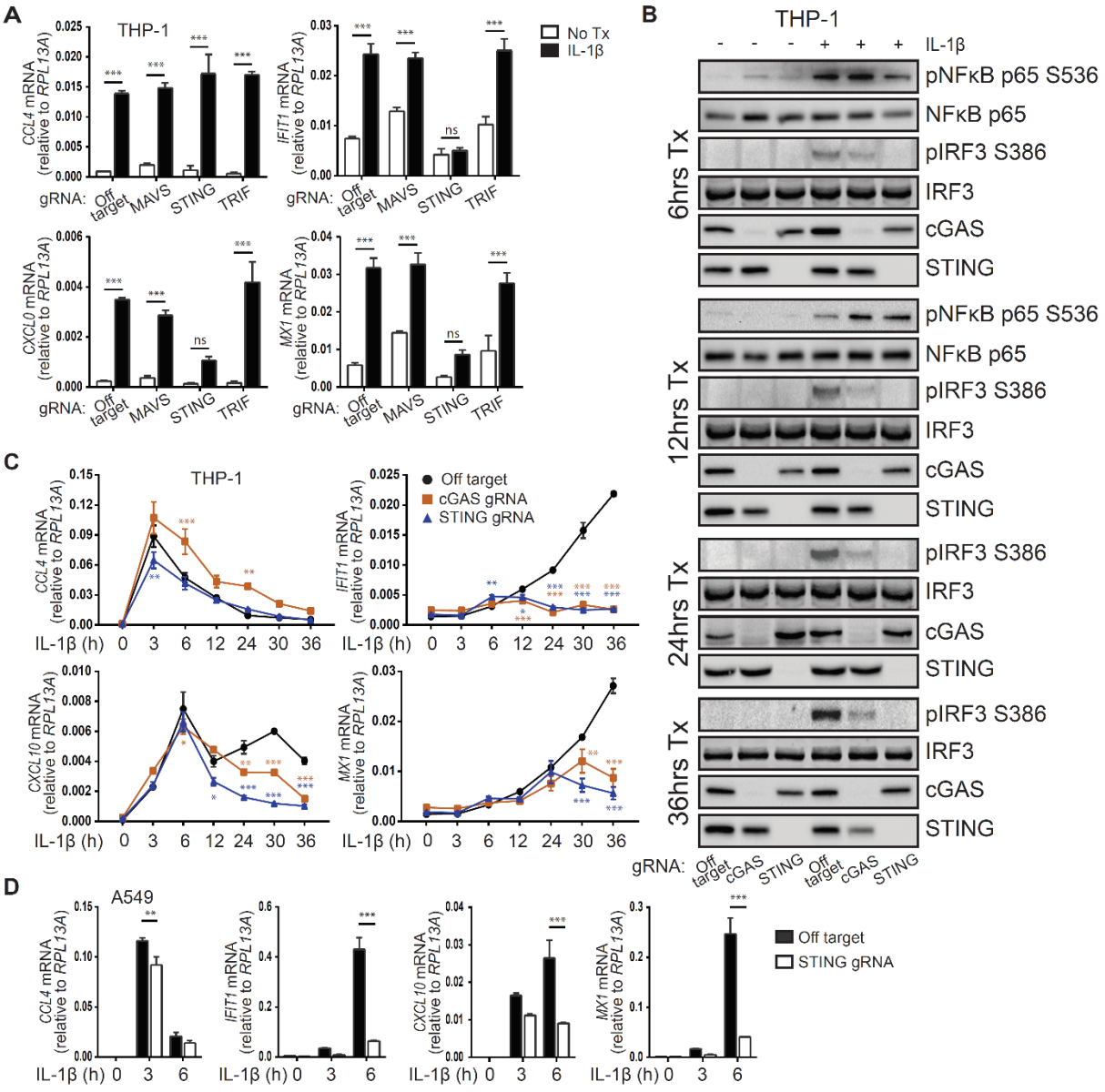


Figure 3.6. TBK1 activation in HFF. HFF were treated with media, transfected with 10ug/ml cGAMP, or treated with 10ng/ml IL-1β for the time indicated on coverslips. Cells were fixed and stained for confocal imaging of pTBK1 S172 (green). Nuclei were counterstained with DAPI (blue).

Therefore, we examined IL-1β-induced activation of TBK1 and IKKε in human epithelial cells and macrophages. Coincident with detectable phosphorylation of IRF3 (See **Figures 3.1A, 3.1D**), we observed sustained activation of TBK1 and IKKε in response to IL-1β treatment, as shown by phosphorylation at the activating phosphoacceptor residue Ser-172 on each protein (**Figures 3.5A, 3.5B**) (Kishore, 2002; Shimada, 1999). We also observed phosphorylation of TBK1 after 1 hour, but not 3 hours, of IL-1β treatment in HFF (**Figure 3.6**), which correlates with peak IRF3 activation in these cells (see **Figure 3.1C**). To confirm the role of these kinases in IL-1β-mediated IRF3 phosphorylation, we used a pharmacological inhibitor of TBK1/IKKε kinase activity, BX795 (Clark, 2009). Control cells treated with LPS exhibited robust activation of IRF3 that was suppressed by inhibitor treatment (**Figure 3.5D**). Pre-treatment with BX795 also prevented IL-1β-induced phosphorylation of IRF3 at S386, thereby expanding the critical role for these kinases to include IL-1β-induced IRF3 phosphorylation (**Figures 3.5C, 3.5D**).

Next, we tested whether the innate immune adaptor proteins MAVS, STING or TRIF were necessary to engage TBK1/IKKε-mediated activation of IRF3 in response to IL-1β. Remarkably, we found that macrophages lacking STING were unable to express IRF3-responsive genes upon IL-1β treatment, while MAVS- or TRIF-deficient cells were unaffected and fully respond to IL-1β (**Figure 3.7A**). STING is activated by cyclic dinucleotides synthesized by the cytosolic DNA sensor cGAS (Sun, 2013). We therefore treated cGAS and STING CRISPR-targeted THP-1 macrophages with IL-1β and examined phosphorylation

Figure 3.7. IRF3 activation in response to IL-1β is cGAS- and STING-dependent. (A) THP-1 transduced with lentiCRISPR/Cas9 and off target gRNA or MAVS-, STING- or TRIF-gRNA were treated with media or 10ng/ml IL-1β for 30hrs before qRT-PCR analysis. Statistical analysis was performed using two-way ANOVA and Bonferroni's to compare treatments, *n*=3 with mean ± SEM. (B) THP-1 transduced with lentiCRISPR/Cas9 and off target gRNA or cGAS- or STING-gRNA were treated with media or 10ng/ml IL-1β for 6-36hrs before protein analysis by immunoblot, *n*=2. (C) THP-1 transduced with lentiCRISPR/Cas9 and off target gRNA or cGAS- or STING-gRNA were treated with media (0) or 10ng/ml IL-1β for 3-36hrs before qRT-PCR analysis. Statistical analysis was performed using two-way ANOVA with Bonferroni's to compare knockouts to control cells, *n*=4 with mean ± SEM. (D) A549 transduced with lentiCRISPR/Cas9 and off target gRNA or STING-gRNA were treated with media (0) or 10ng/ml IL-1β for 3-6hrs before qRT-PCR analysis. Statistical analysis was performed using student's T test and Holm-Sidak to compare genotypes, *n*=3 with mean ± SEM. *p<0.05, **p<0.01, ***p<0.001



of IRF3 by immunoblot to assess the role of these DNA sensing pathway components in IL-1β-induced IRF3 activation. cGAS-deficiency resulted in greatly reduced phosphorylation of IRF3 compared to control cells, while STING-deficient cells showed a complete loss of IRF3 phosphorylation in response to IL-1β (**Figure 3.7B**). Importantly, cGAS- or STING-deficiency significantly reduced IL-1β-induced IRF3 target gene expression compared to control cells (**Figure 3.7C**). Activation of NF-κB was apparent in cGAS- and STING-deficient cells at 6 and 12 hours post treatment with IL-1β, with no detection of phosphorylated NF-κB by 24 hours (**Figure 3.7B**). Of note, cGAS-deficient cells demonstrated prolonged NF-κB phosphorylation and enhanced expression of *CCL4* compared to control cells, while STING-deficient cells

showed delayed phosphorylation of NF-κB and slightly reduced expression of *CCL4*. The difference in responses between cGAS and STING CRISPR-targeted cells may reflect a non-linear relationship between the two (Costa Franco, 2018; Holm, 2016). We also confirmed the requirement for STING in the non-myeloid response to IL-1β. STING CRISPR-targeted A549 cells showed a slight decrease in NF-κB-responsive gene, *CCL4*, a complete loss of ISGs *IFIT1* and *MX1*, and a partial loss of *CXCL10* induction compared to control cells (**Figure 3.7D**). In agreement with these data, IL-1β signaling results in the phosphorylation of STING at the critical phosphoacceptor residue Ser-366 (Liu, 2015) and a temporary loss of cGAS and STING protein levels in A549, indicative of their activation-induced degradation (Chen, 2016; Hu, 2016; Konno, 2013; Prabakaran, 2018) (**Figure 3.8**).

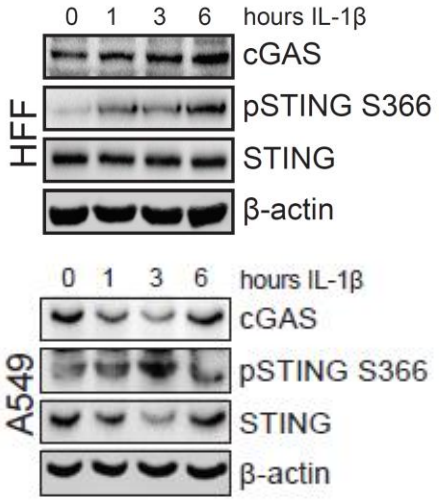


Figure 3.8. IL-1β induces STING activation. HFF or A549 were treated with media (0) or 10ng/ml IL-1β for 1-6hrs before protein analysis by immunoblot, *n*=2 per cell type.

Upon activation, STING migrates from the endoplasmic reticulum (ER) through the ER-Golgi intermediate compartments (ERGIC) to autophagosome-like vesicles (Dobbs, 2015; Ishikawa, 2009; Konno, 2013; Saitoh, 2009). To examine whether IL-1β treatment drives translocation of endogenous STING as

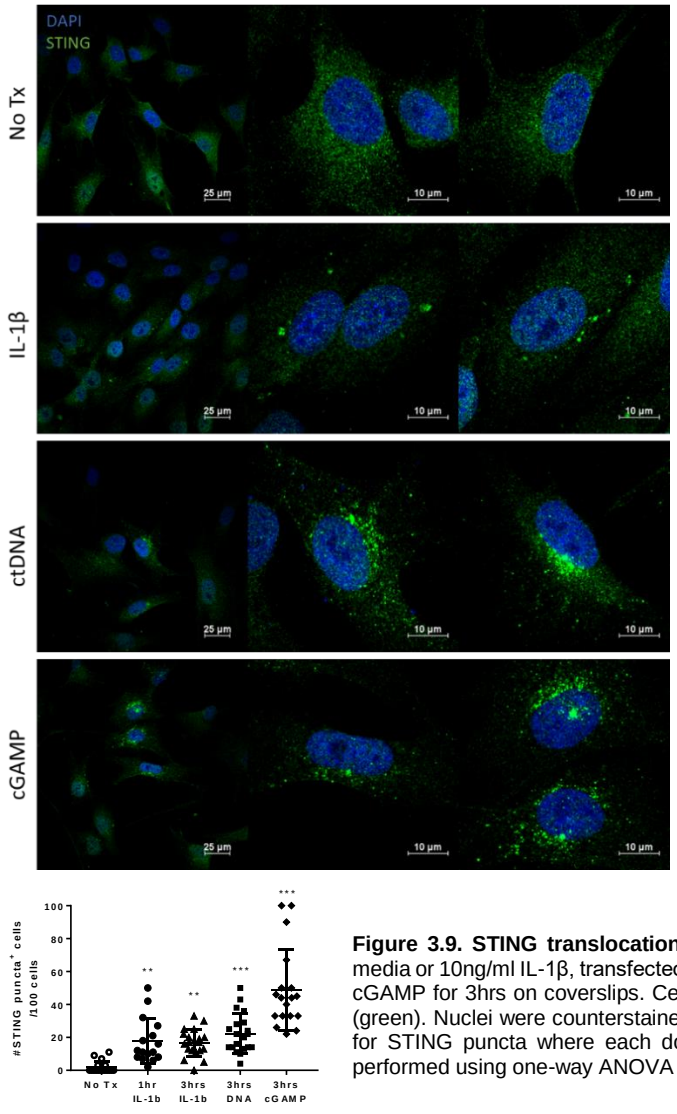


Figure 3.9. STING translocation upon IL-1R signaling. Above: HFF were treated with media or 10ng/ml IL-1β, transfected with 1ug/ml calf thymus DNA, or transfected with 10ug/ml cGAMP for 3hrs on coverslips. Cells were fixed and stained for confocal imaging of STING (green). Nuclei were counterstained with DAPI (blue). Below: Quantification of cells positive for STING puncta where each dot represents one field of view. Statistical analysis was performed using one-way ANOVA and Holm-Sidak, *n*=4 with mean ± SD.

another indicator of its activation, we utilized human foreskin fibroblasts (HFF), which express high levels of STING protein. As shown in previous reports, cytosolic DNA or exposure to the second messenger cyclic GMP-AMP (cGAMP) resulted in perinuclear relocalization of STING (**Figure 3.9**). Importantly, the aggregation of STING also occurred following IL-1β treatment (**Figure 3.9**), concurrent with phosphorylation of IRF3 at Ser-386 (See **Figure 3.1C**) and phosphorylation of STING at Ser-366 (see **Figure 3.8**). Together, these data show that IL-1β drives cGAS- and STING-dependent innate immune activation.

3.2.4 IL-1β initiates STING-dependent autophagic flux

We noticed that IL-1β-driven STING puncta are distinct in size and structure from canonical activators (see **Figure 3.9**), reminiscent of STING-containing autophagosome-like vesicles described previously (Dobbs, 2015; Ishikawa, 2009; Konno, 2013; Saitoh, 2009). STING-dependent autophagosomes are reported to function in antibacterial and antiviral defense (Liu, 2018; McFarlane, 2011; Moretti J., 2017; Rasmussen, 2011; Watson, 2012), as well as in the resolution of STING signaling (Prabakaran, 2018). Previously, Pilli *et al*. demonstrated that IL-1β induces autophagic killing of *M. tuberculosis* and that TBK1 is essential for autophagic maturation in this context (Pilli, 2012). We hypothesized that this process may be mediated by STING. The initiation of autophagy is indicated by the lipidation of microtubule-associated protein 1A/1B-light chain 3 (LC3), which can be detected as LC3-II by immunoblot (Kabeya, 2000; Klionsky, 2012). In agreement with Pilli *et al.*, we observed that exogenous IL-1β drives an increase in LC3-II levels (**Figure 3.10**). Importantly, IL-1β-induced LC3-II levels are further elevated by co-treatment with the autophagy maturation inhibitor bafilomycin A1. This demonstrates that the accumulation of LC3-II is due to increased autophagic flux and not a block in the completion of baseline autophagy (Klionsky, 2012). Notably, STING-deficient cells do not display increased levels of LC3-II upon treatment with IL-1β in the presence or absence of bafilomycin A1. Results from our scRNA-seq analysis also revealed that IL-1β induces the expression of guanylate binding

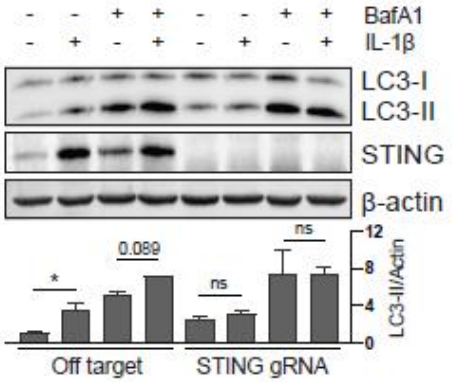


Figure 3.10. IL-1β initiates STING-dependent autophagic flux. A549 were treated with media or 10ng/ml IL-1β for 3hrs +/- 1hr pre-treatment with Bafilomycin A1 (BafA1). Above: Protein analysis by immunoblot. Below: Densitometry analysis of LC3-II protein abundance was compared against actin for each condition, *n=3*. **p* <0.05

protein (GBP) 1 and 3 (see **Figure 3.3B**, **Table S5**), which can act to couple autophagic machinery to pathogen-containing vacuoles (Costa Franco, 2018; Kim, 2011). Together, these data support a role for STING in IL-1β-mediated autophagic flux, which could function in innate antimicrobial defense as well as inflammatory resolution (Shi, 2012; Takahama, 2018; Zhong, 2016).

3.2.5 IL-1R signaling induces release of mitochondrial DNA to initiate innate immune activation

Previous studies have described a role for the DNA sensing pathway in the immunological response to dying cells (Ahn, 2012; Marichal, 2011). To determine if the IL-1β-cGAS-STING axis was a response to IL-1β-induced cell death, we assessed cell viability over a time course of IL-1β treatment in real-time using IncuCyte live cell imaging. While cell death was readily induced by pyroptotic or apoptotic stimuli, we observed no cell death in response to IL-1β treatment of either THP-1 macrophages or A549 epithelial cells (**Figure 3.11**).

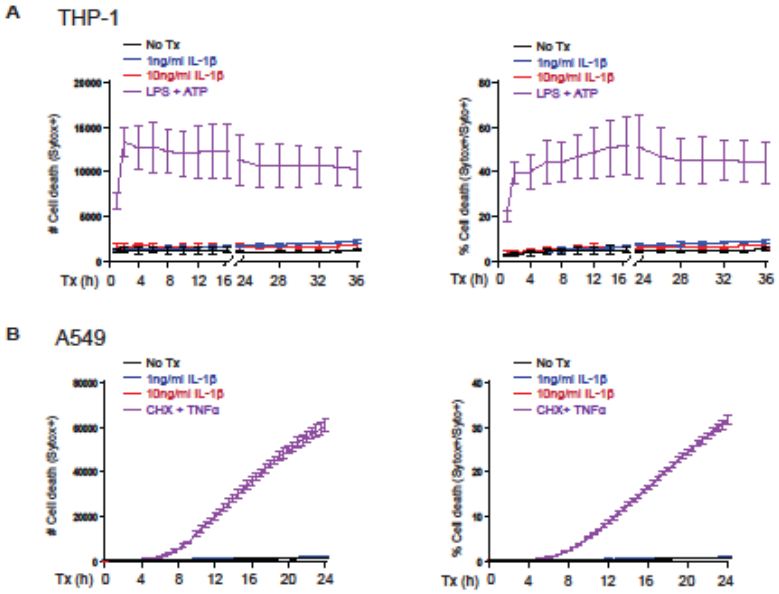


Figure 3.11. Exogenous IL-1β does not cause cell death. (A) THP-1 were treated with media, the indicated concentrations of IL-1β or 0.5ug/ml LPS + 5mM ATP and imaged by IncuCyte live cell imaging to quantify cell death over 36hrs. Left: Cell death number was quantified by counting Sytox-positive cells. Right: Percentage cell death was quantified by dividing Sytox-positive cells by Syto-positive cells. Data represent technical triplicates with mean ± SEM. (B) A549 were treated with media, the indicated concentrations of IL-1β or 10ng/ml TNFα + 10uM Cycloheximide (CHX) and imaged by IncuCyte live cell imaging to quantify cell death over 24hrs. Left: Cell death number was quantified by counting Sytox-positive cells. Right: Percentage cell death was quantified by dividing Sytox-positive cells by Syto-positive cells. Data represent technical triplicates with mean ± SEM.

Noting that liberated mitochondrial DNA (mtDNA) is shown to induce STING-dependent innate immune activation in response to microbial products and virus infection (Aguirre, 2017b; Rongvaux, 2014; Sun, 2017; West, 2015; White, 2014), we examined the possibility that IL-1β treatment induced mtDNA release to the cytoplasm as a DAMP sensed by cGAS. Cells were treated with IL-1β then subsequently fractionated to examine cytosolic mtDNA content (**Figure 3.12A**).

Immunoblot analysis confirmed the isolation of pure subcellular fractions of cytosolic, crude mitochondria and nuclear compartments, with no cross-contamination of mitochondria-associated proteins in the cytosolic or nuclear fractions (**Figures 3.12B, 3.13**). Cytosolic and nuclear fractions were then used to quantitate the level of DNA containing specific mitochondrial (*MT-ND1*, *D-loop*, *MT-CO2*, *MT-ATP6*) and nuclear (*RPL13A*) genes by qPCR. IL-1β-treated cells demonstrated a significant 3-6-fold enrichment of mtDNA in the cytosolic fraction compared to mock treated cells, indicating IL-1β induced the release of mtDNA (**Figure 3.12C**). A549 cells treated with IL-1β reached peak enrichment of cytosolic DNA

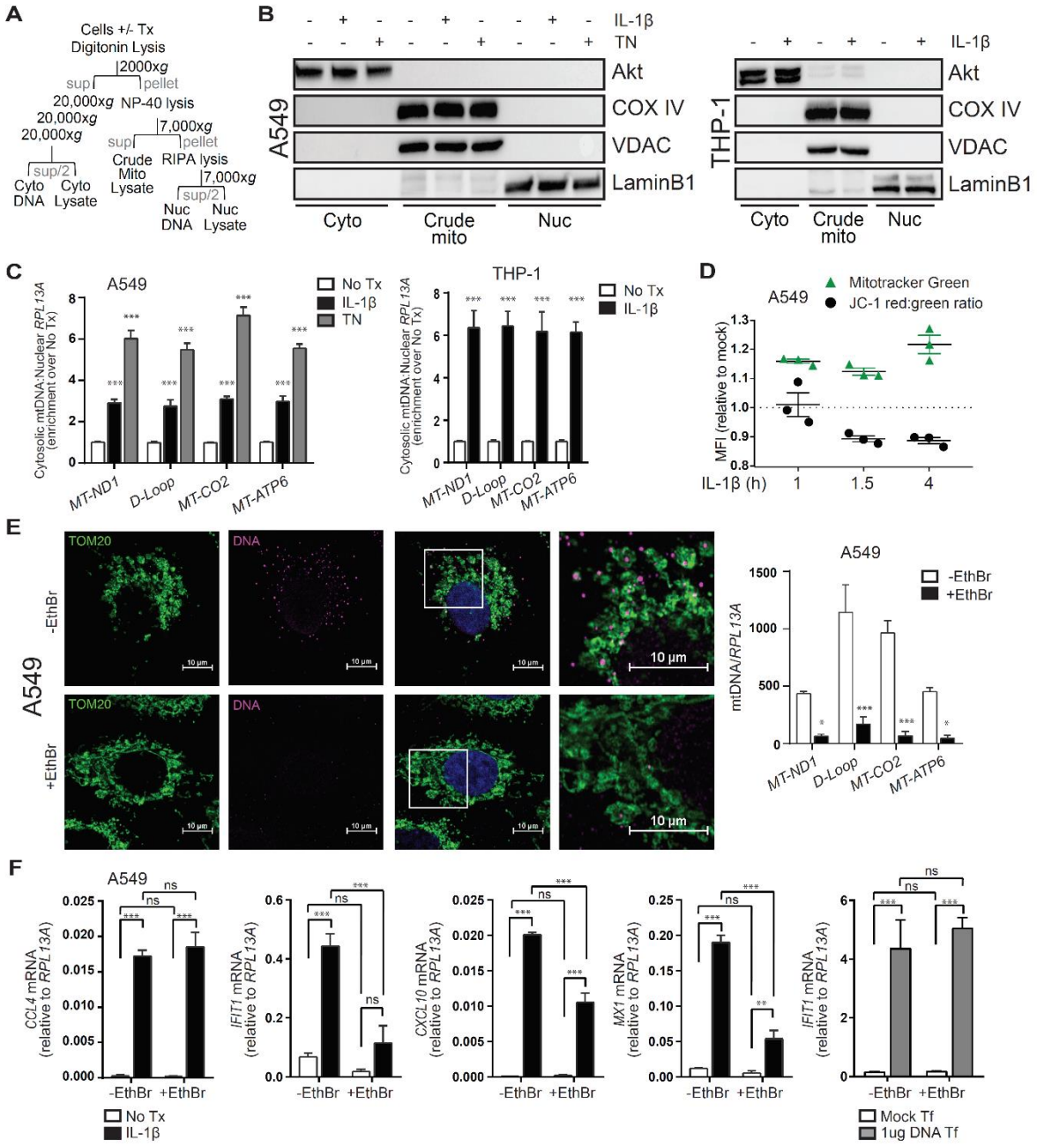
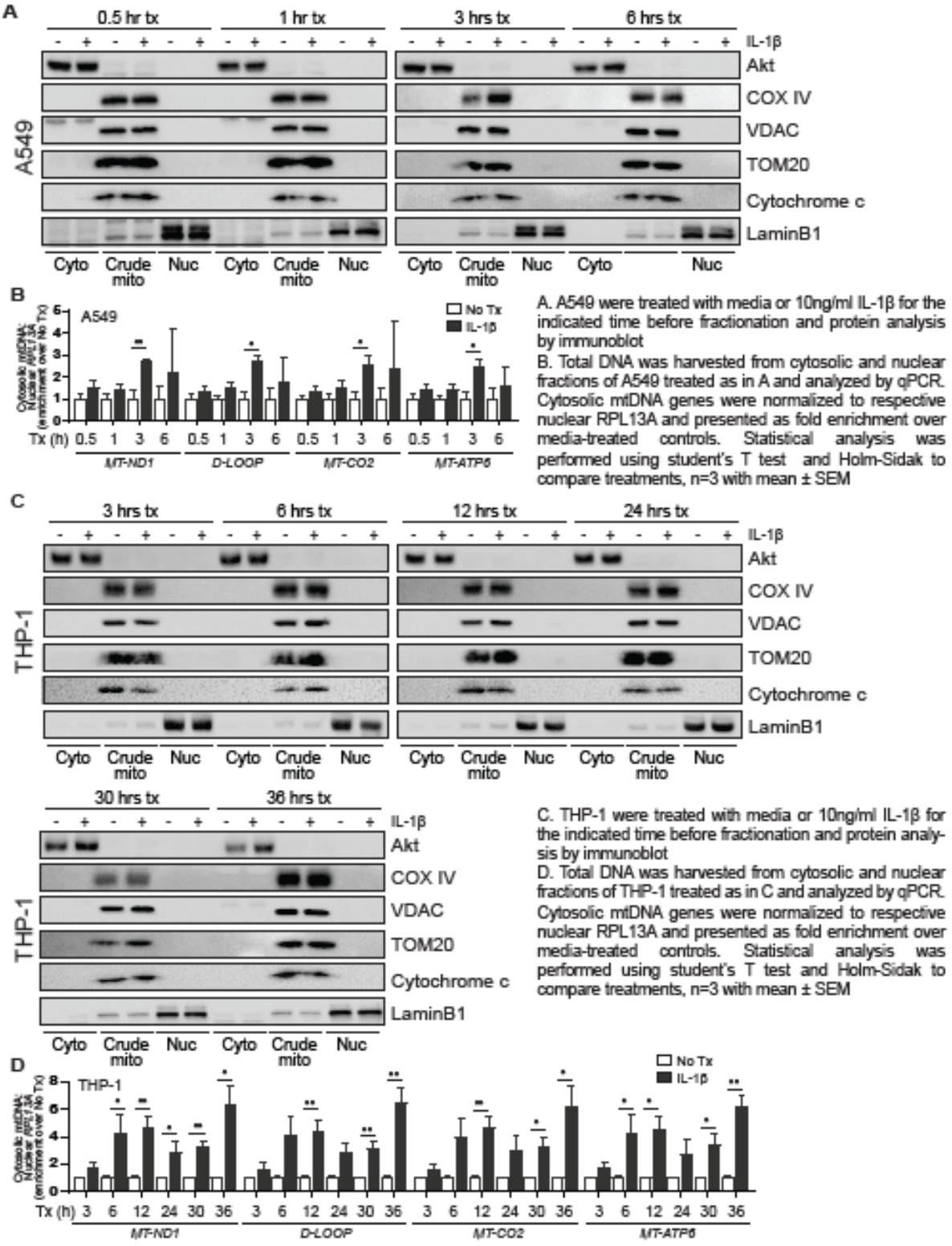


Figure 3.12. IL-1R signaling induces release of mitochondrial DNA to initiate innate immune activation. (A) Schematic of cell lysis and centrifugation for subcellular fractionation. (B) Left: A549 were treated with media, 10ng/ml IL-1β or 4ug/ml tunicamycin (TN) for 3hrs before fractionation and protein analysis by immunoblot. Right: THP-1 were treated with media or 10ng/ml IL-1β for 36hrs before fractionation and protein analysis by immunoblot. (C) Total DNA was harvested from cytosolic and nuclear fractions of A549 (left) or THP-1 (right) treated as in B and analyzed by qPCR. Cytosolic mtDNA genes were normalized to respective nuclear *RPL13A* and presented as fold enrichment over media-treated controls. Statistical analysis was performed using student's T test and Holm-Sidak to compare treatments, *n*=3 with mean ± SEM. (D) A549 were treated with media or 10ng/ml IL-1β for 0.5, 1 or 3.5hrs before incubation with Mitotracker Green or JC-1 dye for an additional 0.5hr. Cells were analyzed by flow cytometry to determine median fluorescence intensity (MFI) of the indicated dyes, *n*=3 with mean ± SEM. (E) A549 were cultured for 4 days +/- ethidium bromide (EthBr) to deplete mtDNA. Left: Untreated and treated cells were fixed on coverslips and stained for confocal imaging of mitochondria (TOM20, green) or DNA (magenta). Nuclei were counterstained with DAPI (blue). Right: Treated and untreated cells were subjected to total DNA extraction followed by qPCR analysis for quantification of mtDNA genes. Statistical analysis was performed using student's T test and Holm-Sidak to compare treatments, *n*=4 with mean ± SEM. (F) EthBr treated and untreated A549 were exposed to media or 10ng/ml IL-1β or transfected with 1ug/ml calf thymus DNA for 6hrs before qRT-PCR analysis. Statistical analysis was performed using two-way ANOVA and Bonferroni's to compare the indicated treatments, *n*=4 with mean ± SEM. Sup, supernatant. Cyto, cytoplasm. Mito, mitochondria. Nuc, nucleus. Tf, transfection *p<0.05, **p<0.01, ***p<0.001

accumulation by 3 hours post-treatment while detection at 6 hours was inconsistent between experiments (**Figure 3.13B**). THP-1 cells demonstrated a bimodal release of mtDNA into the cytoplasm; the earliest detection coincided with phosphorylation of IRF3 at 6 hours post-treatment (See **Figure 3.1D**) and peak enrichment was observed at 36 hours post-treatment (**Figure 3.13D**). Of note, the mitochondrial intermembrane space-resident protein, cytochrome c, was not detectable in the cytosol upon treatment with IL-1β, which is consistent with IL-1β-mediated release of mtDNA in the absence of cell death.

To determine if mtDNA served as a DAMP to stimulate STING activation in response to IL-1β, A549 cells were depleted of mtDNA using the DNA entercalating dye ethidium bromide to inhibit mtDNA replication (Hashiguchi, 2009; Rongvaux, 2014; White, 2014). After confirming by confocal microscopy and qPCR that ethidium bromide treated cells were depleted of mtDNA (**Figure 3.12.E**), we exposed both treated and untreated cells to IL-1β and examined the induction of NF-κB- and IRF3-responsive genes. mtDNA-deficient cells exposed to IL-1β show a significant reduction in IRF3-responsive genes while expression of the NF-κB-responsive gene, *CCL4*, was fully induced by IL-1β (**Figure 3.12F**). Importantly, mtDNA-deficient cells responded normally to cytosolic DNA treatment, indicating the DNA sensing pathways were intact. mtDNA encodes 13 polypeptide components of the mitochondrial respiratory chain and 2 rRNAs and 22 tRNAs necessary for their translation. Cells depleted of mtDNA retain mitochondrial structure as most mitochondrial genes are encoded in the nuclear genome (see **Figure 3.12E**). However, mtDNA depletion leads to dysfunctional oxidative phosphorylation and consequently may affect signaling processes linked to mitochondrial reduction-oxidation (redox) potential or superoxide generation (Chandel,

Figure 3.13. Temporal release of mitochondrial DNA upon IL-1β treatment.



1999; Hashiguchi, 2009). To test the importance of mitochondrial ROS in this pathway, which may also

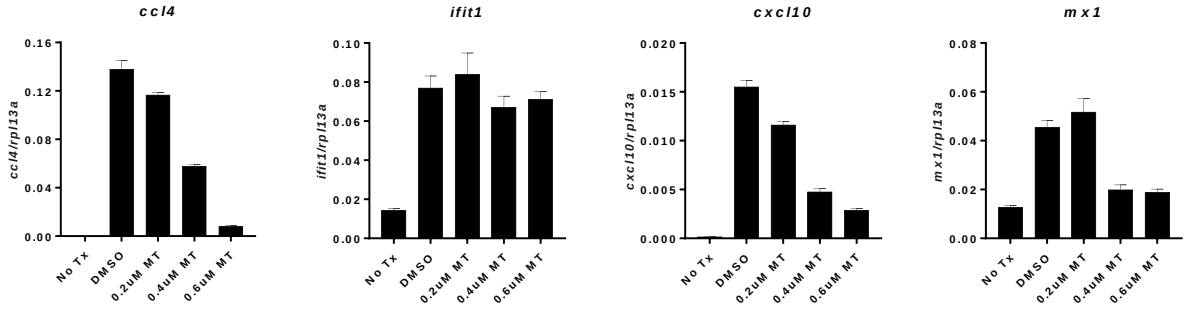


Figure 3.14. Mitochondrial ROS inhibition partially ablates IL-1R-induced antiviral gene expression. A549 were pre-treated with media, DMSO or increasing concentrations of MitoTEMPO (MT) for 1hr before treatment with media or 10ng/ml IL-1β for 3hrs and qRT-PCR analysis, *n=3*. No Tx = media alone; all other treatments include IL-1β.

have been reduced with mtDNA depletion, we utilized a specific scavenger of mitochondrial superoxide, MitoTEMPO. Pre-treatment with MitoTEMPO indeed ablated IL-1β-induced expression of *CXCL10* and *MX1*, but it also inhibited the expression of NF-κB-responsive gene, *CCL4* **(Figure 3.14**). Surprisingly, MitoTEMPO pre-treatment had no effect on IL-1β-induced expression of *IFIT1.* As mtDNA depletion coincided with the ablation of IL-1β-induced IRF3-responsive genes without perturbing *CCL4* expression, we concluded that mtDNA served as a DAMP to activate cGAS-STING-IRF3. Though we cannot conclusively rule out the contribution of mitochondrial ROS to this pathway.

To define the mechanism of mtDNA release, we examined mitochondrial morphology and association with mitophagy factors. Thorough immunofluorescence analyses if A549 cells and HFFs did not reveal stark differences in mitochondrial morphology or association with mitophagy factors between untreated and IL-1β-treated cells. However, we did note an association between mitochondria and STING aggregates in IL-1β-treated HFFs that could potentially represent a mechanism of STING-mediated quality control (**Figure 3.15**).

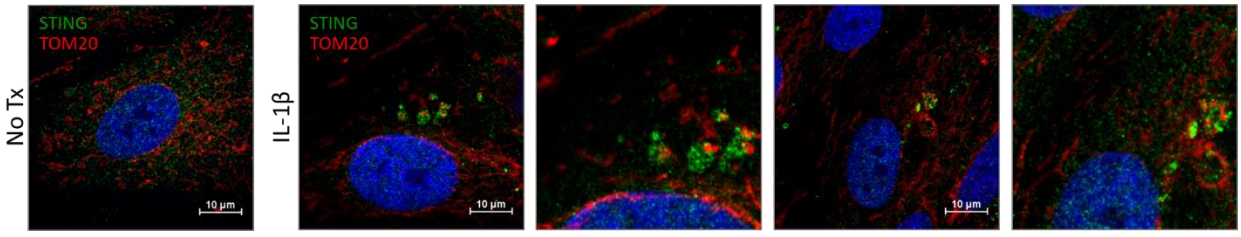


Figure 3.15. STING associates with mitochondria upon IL-1β treatment. HFF treated with media or 10ng/ml IL-1β for 3hrs were fixed on coverslips and stained for confocal imaging of mitochondria (TOM20, red) or STING (green). Nuclei were counterstained with DAPI (blue).

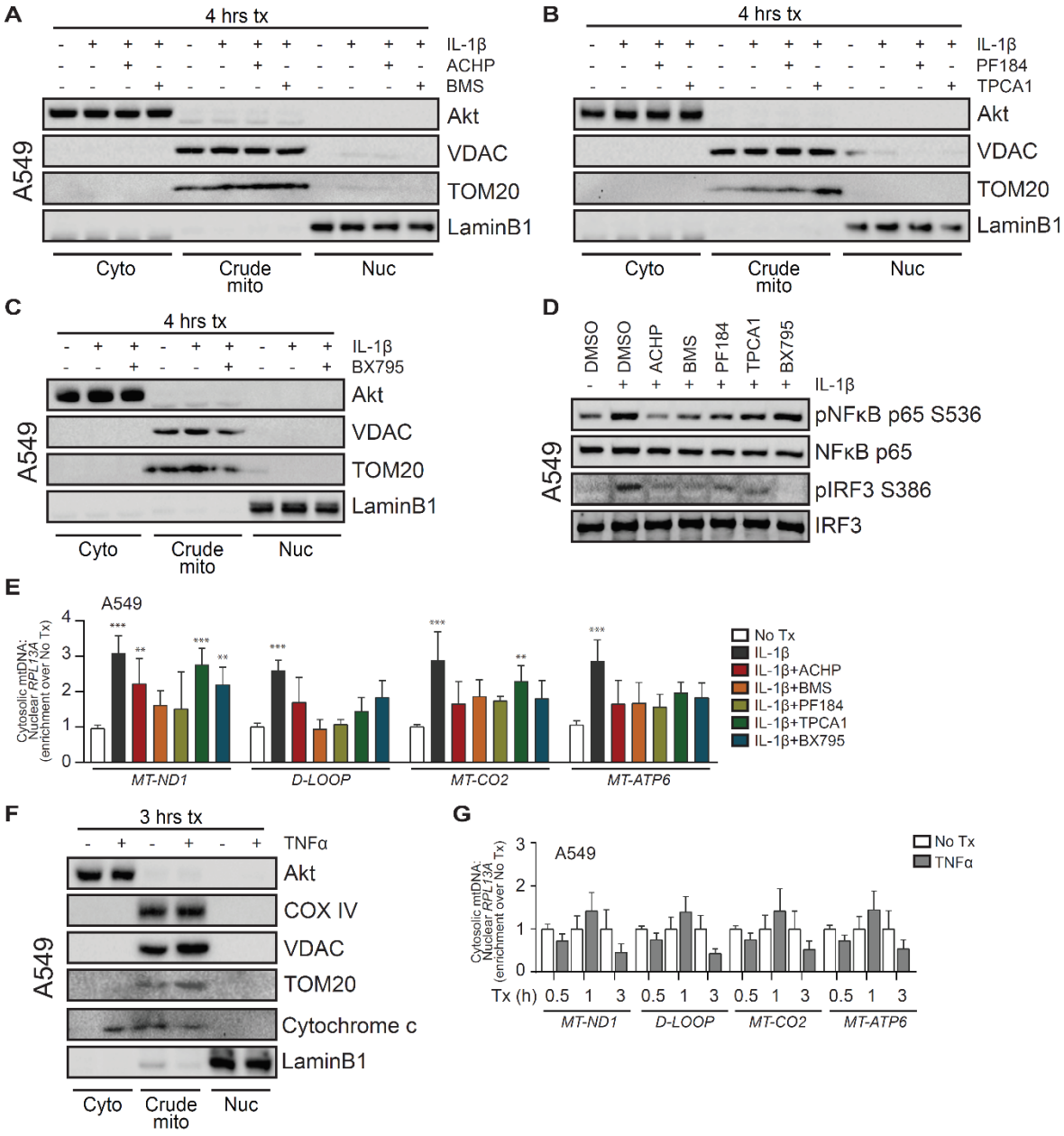


Figure 3.16. IL-1β-induced mtDNA release is NF-κB-dependent. (A) A549 were treated with media (0) or 10ng/ml IL-1β for 3hrs +/- 1hr pretreatment with DMSO or 10uM IKKα/β inhibitor (ACHP, BMS) before fractionation and protein analysis by immunoblot. (B) A549 were treated as in A with 500nM IKKβ inhibitor (PF184, TPCA1). (C) A549 were treated as in A with 1uM TBK1/IKKε inhibitor (BX795). (D) A549 were treated with media or 10ng/ml IL-1β for 3hrs +/- 1hr pretreatment with DMSO or the indicated inhibitors before protein analysis by immunoblot. (E) Total DNA was harvested from cytosolic and nuclear fractions of A549 treated as in A-C and analyzed by qPCR. Cytosolic mtDNA genes were normalized to respective nuclear *RPL13A* and presented as fold enrichment over media-treated controls. Statistical analysis was performed using two-way ANOVA with Bonferroni's to compare each treatment to mock, *n*=3 with mean ± SEM. (F) A549 were treated with media or 10ng/ml TNFα for 3hrs before fractionation and protein analysis by immunoblot. (G) Total DNA was harvested from cytosolic and nuclear fractions of A549 treated as in F and analyzed by qPCR. Statistical analysis was performed using student's T test and Holm-Sidak to compare treatments, *n*=3 with mean ± SEM. Cyto, cytoplasm. Mito, mitochondria. Nuc, nucleus. **p<0.01, ***p<0.001

Our observations of IL-1β-mediated autophagic flux (possibly mitophagy) led us to hypothesize that IL-1β treatment would drive a loss of mitochondrial mass. We therefore assessed mitochondrial mass by

flow cytometry analysis of Mitotracker Green (MTG) dye in A549 cells treated with IL-1β over time. To our surprise, we found that mtDNA detection in the cytosol at 3 hours post-treatment with IL-1β (see **Figures 3.12C, 3.13B**) followed an increase in mitochondrial mass, which was detectable as early as 1 hour post-treatment with IL-1β (**Figure 3.12D**). Additionally, we measured mitochondrial membrane potential (MMP) as a surrogate measure of mitochondrial membrane integrity. MMP was determined using JC-1 dye, which exhibits potential-dependent accumulation in mitochondria. At high MMP, JC-1 forms red fluorescent aggregates, whereas it becomes green fluorescent monomers at low MMP. Change in the ratio of red:green fluorescence intensity is used to determine a shift from high to low MMP. IL-1β treatment resulted in a decrease in mitochondrial membrane potential, which trailed an increase in mitochondrial mass but preceded detection of cytosolic mtDNA accumulation (**Figure 3.12D**).

To examine whether canonical IL-1R signaling via NF-κB contributes to mtDNA release, cells were pre-treated with inhibitors of IKKα/β (**Figure 3.16A**), IKKβ (**Figure 3.16B**) or TBK1/IKKε (**Figure 3.16C**) before exposure to IL-1β, subcellular fractionation and cytosolic mtDNA quantification. All IKKα/β and IKKβ inhibitors reduced NF-κB phosphorylation while TBK1/IKKε inhibition had no effect (**Figure 3.16D**). Loss of NF-κB activity resulted in a significant reduction in mtDNA release (**Figure 3.16E**), coincident with a reduction in phosphorylation of IRF3 (see **Figures 3.16D**). Use of the TBK1/IKKε inhibitor also reduced mtDNA release, suggesting a dual role for IKKα/β and TBK1/IKKε in this process. Of note, treatment with another NF-κB activator, TNFα, induced the release of cytochrome c to the cytosol without detectable enrichment of mtDNA (**Figures 3.16F, 3.16G**). Collectively, these results show that IL-1β induces the NF-κB-dependent release of mtDNA to the cytosol, which can then be detected by cGAS for activation of STING and IRF3.

3.2.6 IL-1β treatment drives IFN production and ISG expression

The interferon regulatory factor family of transcription factors play an integral role in the transcriptional induction of IFN (Honda, 2006; Osterlund, 2007). IRF3 is constitutively expressed in most cell types while IRF7 expression is induced by IFN (Marié, 1998; Sato, 1998). IRF3 therefore acts to induce small amounts of IFNβ and IFNλ, which then bind and activate their respective receptors and ISGF3 for

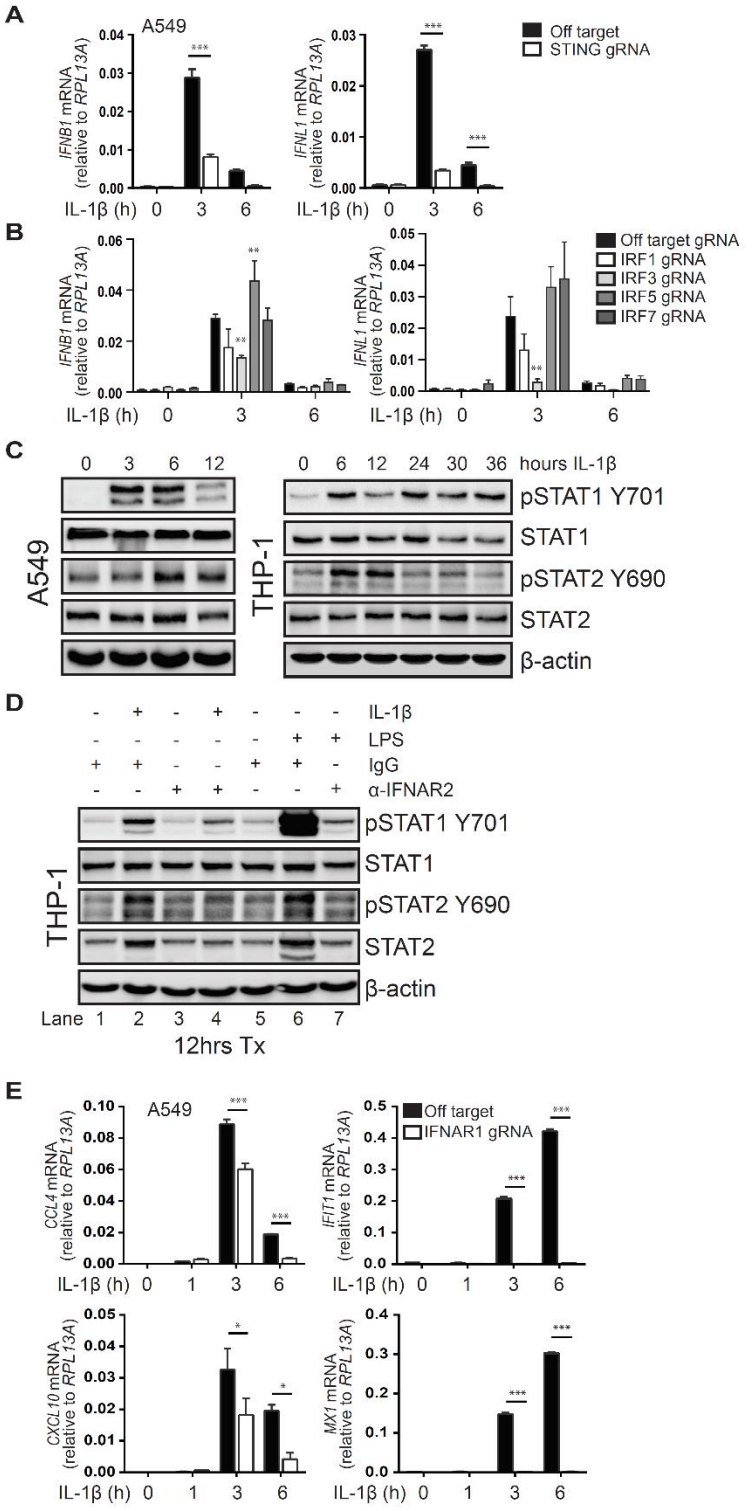


Figure 3.17. IL-1β treatment drives IFN production and ISG expression. (A) A549 transduced with lentiCRISPR/Cas9 and off target gRNA or STING-gRNA were treated with media (0) or 10ng/ml IL-1β for 3-6hrs before qRT-PCR analysis. Statistical analysis was performed using student's T test and Holm-Sidak to compare genotypes, *n*=3 with mean ± SEM. (B) A549 transduced with lentiCRISPR/Cas9 and off target gRNA or IRF1-, IRF3-, IRF5- or IRF7-gRNA were treated with media (0) or 10ng/ml IL-1β for 3-6hrs before qRT-PCR analysis. Statistical analysis was performed using two-way ANOVA with Dunnett's to compare knockouts to control cells, *n*=3 with mean ± SEM. (C) A549 (left) or THP-1 (right) were treated with media (0) or 10ng/ml IL-1β for the indicated time before protein analysis by immunoblot, *n*=2 (A549) or *n*=3 (THP-1). (D) THP-1 were treated with media, 10ng/ml IL-1β or 0.5ug/ml LPS in the presence of 0.5ug/ml IgG control or 0.5ug/ml anti-IFNAR2 for 12hrs before protein analysis by immunoblot, n=*2*. Lanes 1 and 5 are redundant. (E) A549 transfected with CRISPR/Cas9 and off target gRNA or IFNAR1-gRNA were treated with media (0) or 10ng/ml IL-1β for 1-6hrs before qRT-PCR analysis. Statistical analysis was performed using student's T test and Holm-Sidak to compare genotypes, *n*=3 with mean ± SEM. *p<0.05, **p<0.01, ***p<0.001

autocrine induction of IRF7 and other innate signaling components that facilitate secondary transcriptional responses (Brierley, 2002; Sato, 2000). In line with our observation of IRF3 activation, exogenous IL-1β treatment resulted in the transcriptional induction of IFNβ and IFNλ in a STING- and IRF3-dependent manner (**Figures 3.17A, 3.17B**). We also noted a partial role for IRF1 in the induction of these transcripts, consistent with recent observations by Orzalli *et al*. (Orzalli, 2018). Immunoblot analysis of STAT1 and STAT2 revealed IL-1β-induced phosphorylation at the activating phosphoacceptor residues Tyrosine-701 and Tyrosine-690, respectively. Phosphorylation of each STAT occurs with distinct kinetics in different cell types. In A549 cells,

phosphorylation of STAT1 occurs by 3 hours post-treatment and is reduced by 12 hours post-treatment, while phosphorylation of STAT2 is delayed and maintained at 12 hours in A549 cells (**Figure 3.17C**). In contrast, THP-1 macrophages treated with IL-1β demonstrate sustained STAT1 phosphorylation and transient STAT2 phosphorylation (**Figure 3.17D**). These observations may reflect a cell-specific response to IL-1β-induced cytokines that differentially regulate STAT1 and STAT2.

To affirm that STAT phosphorylation in IL-1β-treated cells was occurring in response to IFNβ production, cells were co-treated with IL-1β and neutralizing antibody against IFNAR2. Treatment with neutralizing antibody reduced, but did not fully prevent, IL-1β-induced phosphorylation of STAT1, supporting roles for IFN and other molecules (e.g. IL-6 (Hirahara, 2015)) in IL-1β- induced STAT modulation (**Figure 3.17E**). In contrast, STAT2 phosphorylation is completely inhibited by IFNAR2 neutralization. IFNAR2 neutralization completely ablated STAT phosphorylation in control cells co-treated with LPS. Although IL-1β-induced STAT1 phosphorylation may not be entirely IFNβ-dependent, IFNAR1-deficient

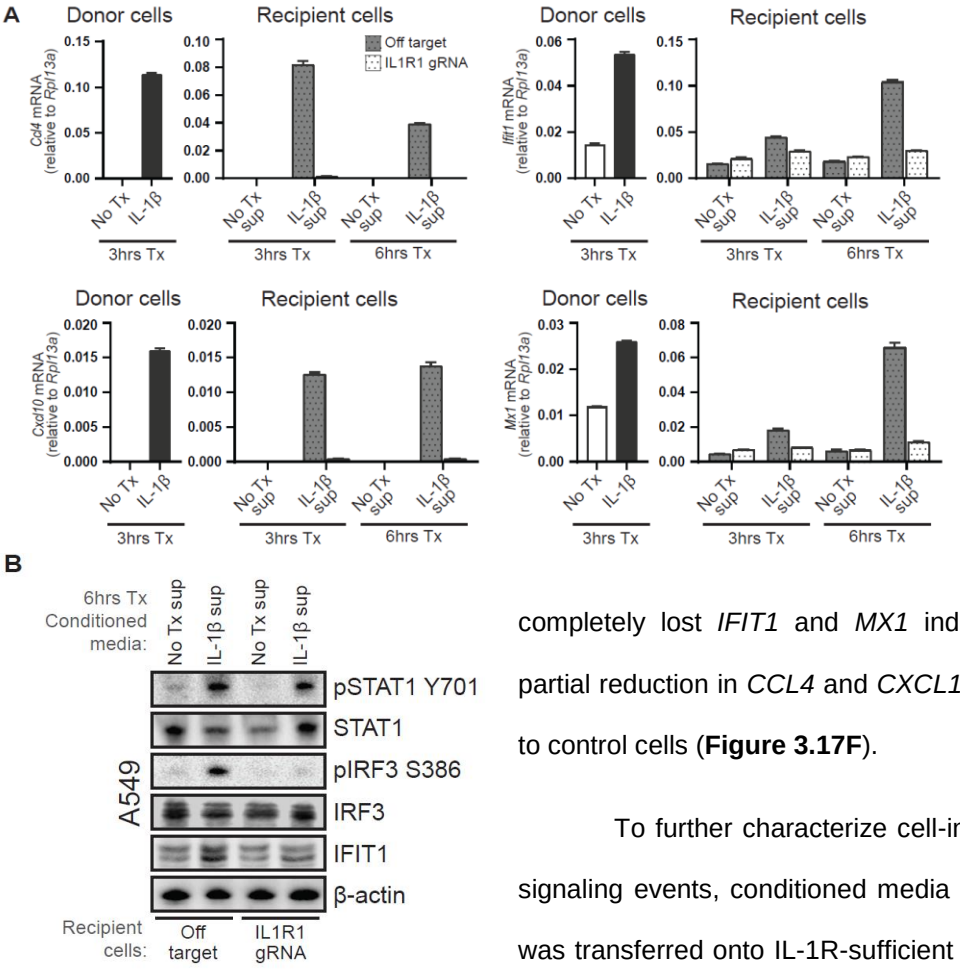


Figure 3.18. IL-1R and IFNAR signaling synergize for antiviral gene induction. (A) Left: qRT-PCR analysis of WT A549 "Donor" cells mock treated or treated with IL-1β for 3hrs with mean ± SEM; *n=3*. Right: qRT-PCR analysis of non-targeted or IL-1R1 CRISPR-targeted A549 "Recipient" cells cultured with supernatants (sup) from Donor cells for 3 or 6 hours with mean ± SEM; *n=3*. (B) Immunoblot analysis of Recipient cells after 6 hours with conditioned

cells treated with IL-1β completely lost *IFIT1* and *MX1* induction and exhibited a partial reduction in *CCL4* and *CXCL10* expression compared to control cells (**Figure 3.17F**).

To further characterize cell-intrinsic versus -extrinsic signaling events, conditioned media from IL-1β-treated cells was transferred onto IL-1R-sufficient or -deficient cells. qRT-

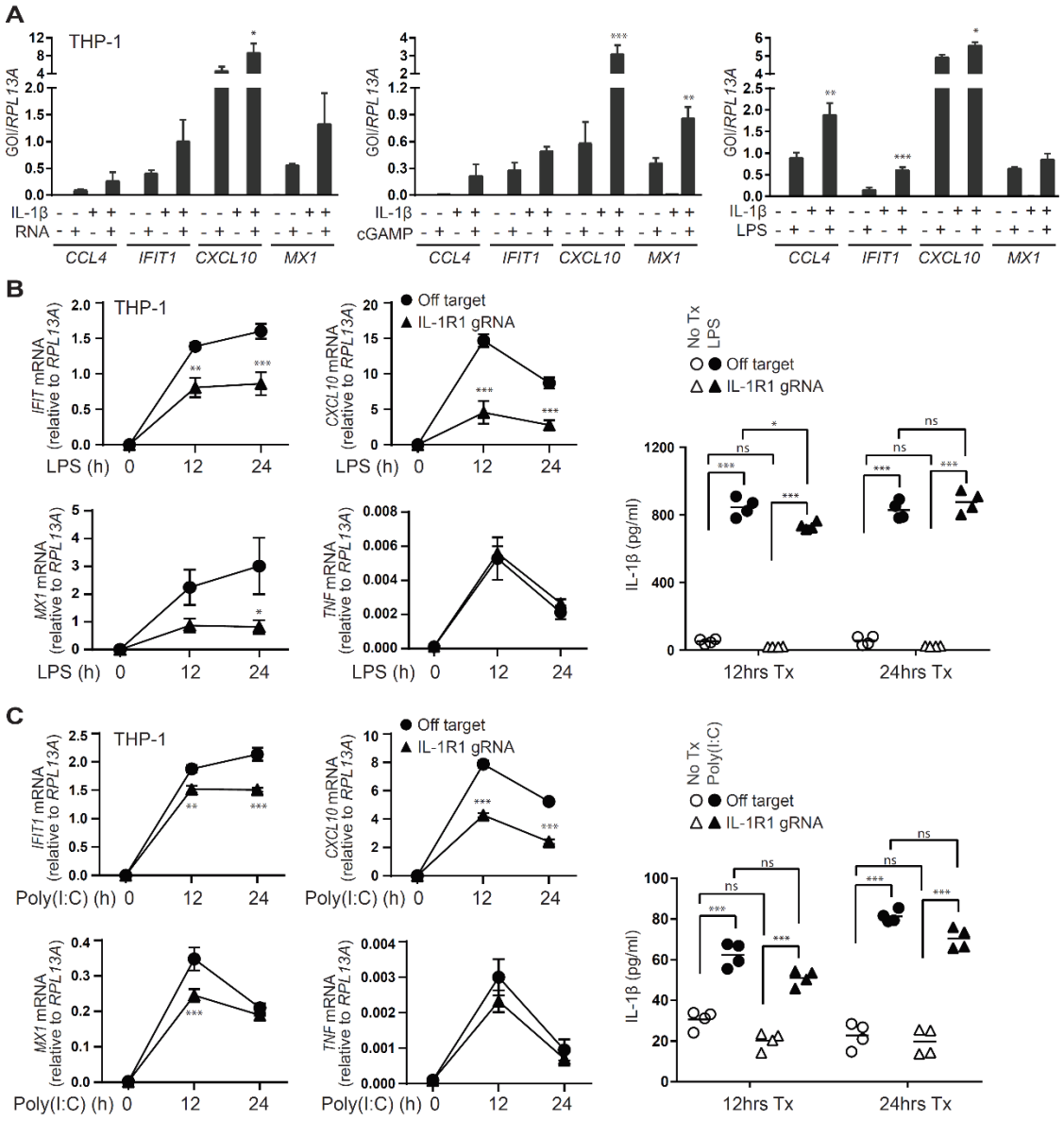


Figure 3.19. Synergistic response to IL-1β and PAMPs. (A) THP-1 were treated with media or 10ng/ml IL-1β for 30hrs before the addition of media or 0.1ug/ml PAMP RNA (left, transfected), 25ug/ml cGAMP (middle, exogenous) or 0.5ug/ml LPS (right) for 6hrs followed by qRT-PCR analysis. Statistical analysis was performed using one-way ANOVA and Holm-Sidak to compare the sum of monotreatments to cotreatment, *n*=4 (PAMP RNA) or *n*=3 (cGAMP, LPS) with mean ± SEM. THP-1 transduced with lentiCRISPR/Cas9 and off target gRNA or IL-1R1-gRNA were treated with media (0) or 0.5ug/ml LPS (B) or transfected with 1ug/ml Poly(I:C) (C) for 12-24hrs. (Left) The indicated transcripts were analyzed by qRT-PCR. Statistical analysis was performed using student's T test and Holm-Sidak to compare genotypes, *n*=4 with mean ± SEM. (Right) Cell-free supernatants were analyzed for IL-1β secretion by ELISA. Statistical analysis was performed using two-way ANOVA and Bonferroni's to compare the indicated treatments, *n*=4 with mean indicated. GOI, gene of interest *p<0.05, **p<0.01, ***p<0.001

PCR analysis revealed a marked requirement for IL-1R signaling in antiviral response gene induction (**Figure 3.18A**). This may indicate that even though IFN is present, there could be insufficient IFN to induce these genes without synergy between IFNAR and IL-1R signaling. Indeed, while conditioned media transferred onto IL-1R-sufficient and -deficient cells results in phosphorylation of STAT1, phosphorylation

of IRF3 and increased levels of IFIT1 are only observable in IL-1R-sufficient cells (**Figure 3.18B**). Thus IL-1β drives mtDNA DAMP accumulation to activate cGAS-STING for the production and autocrine response to IFN, modulation of STAT activity, and expression of ISGs.

3.2.7 Synergistic response to IL-1β and PAMPs

As IL-1β is produced in response to inflammatory stimuli, including microbial infection, (Jorgensen, 2015, 2017; Martinon, 2002), we hypothesized that the release of IL-1β may serve to prime or amplify IRF3-directed immune responses against pathogens. We therefore assessed the response to various PAMPs in combination with IL-1β to examine how IL-1β modulates pathogen recognition and response. We found that while IL-1β alone induces low levels of antiviral gene expression, IL-1β treatment in combination with PAMP activates NF-κB and IRF3 signaling at levels significantly above an additive response to IL-1β or PAMP. This innate activation synergy is observed when IL-1β treatment accompanies stimulation with cytosolic RNA, which signals through MAVS, cGAMP, which signals through STING, and LPS, which signals through TRIF **(Figure#**).

To evaluate how IL-1R imparts regulation of innate immune signaling when IL-1β and IFN are induced by microbial products, we examined the response to PAMPs that induce both cytokines in IL-1R1-sufficient or -deficient macrophages. Expression of IRF3- and IFN-responsive genes driven by LPS **(Figure** #) or cytosolic Poly(I:C) (polyinosinic-polycytidylic acid, used as an analog of viral double-stranded RNA, **Figure** #) were significantly reduced in the absence of IL-1R1, despite the production of comparable amounts of IL-1β. Notably, *TNFA* expression, which is not regulated by IRF3, was not affected by the absence of IL-1R1. These data demonstrate that IL-1R signaling is required for maximal IRF3-mediated immune activation against PAMPs that induce both IL-1β and IFN. These actions are consistent with observations that IL-1β enhances the innate response against West Nile virus infection *in vivo* and *in vitro* (Aarreberg, 2018; Ramos, 2012).

3.2.8 IL-1β-cGAS-STING-IRF3 axis restricts Dengue virus infection

RNA viruses produce no DNA intermediate but have been shown to be sensitive to STING signaling (Aguirre, 2017a; Ishikawa, 2009; Sun, 2017). To examine this newly defined IL-1β-cGAS-STING-IRF3

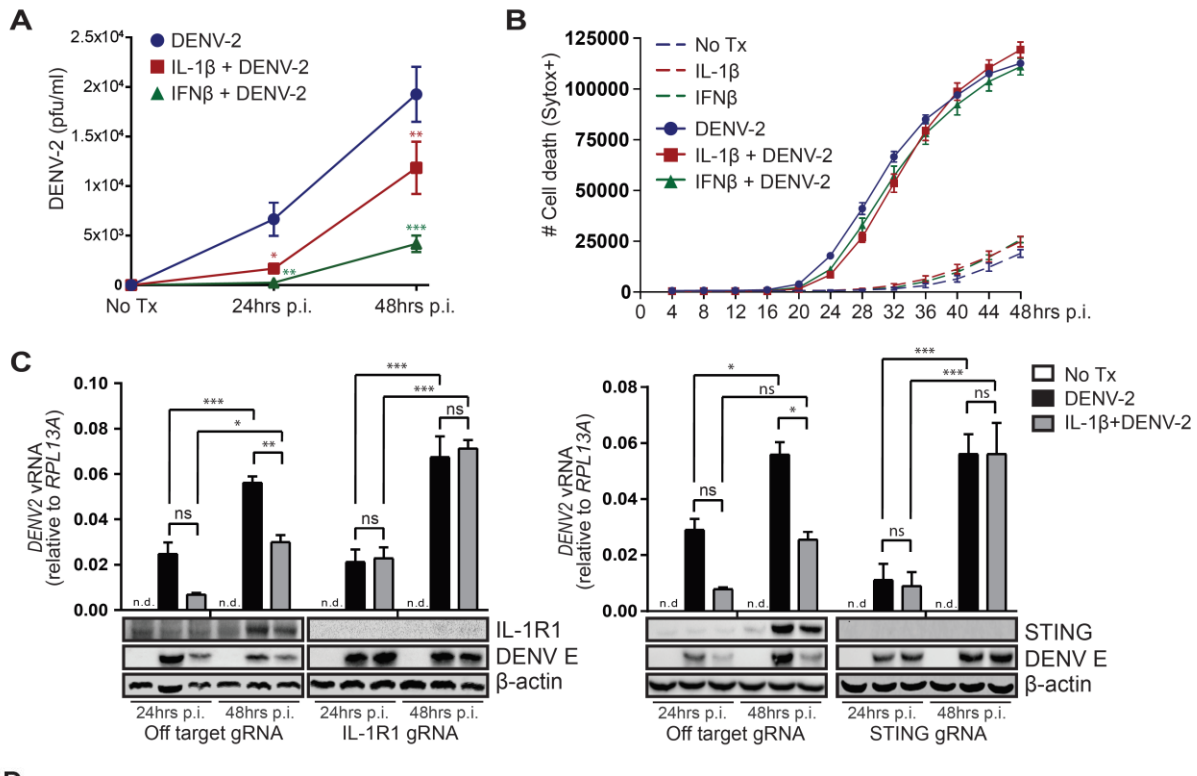


Figure 3.20. IL-1β-cGAS-STING-IRF3 axis restricts Dengue virus infection. (A) Analysis of DENV-2 infectious virus by plaque assay using cell-free supernatants from A549 pretreated with media, 10ng/ml IL-1β or 50IU/ml IFNβ for 6hrs before DENV-2 infection for 24- 48hrs (MOI=0.5). Statistical analysis was performed using two-way ANOVA and Bonferroni's to compare media to cytokine pretreatment, *n*=3 with mean ± SEM. (B) A549 pretreated with media, 10ng/ml IL-1β or 50IU/ml IFNβ for 6hrs were mock or DENV-2 infected (MOI=0.5) and imaged by IncuCyte live cell imaging to quantify cell death over 48hrs. Data represent technical triplicates with mean ± SEM. (C) A549 transduced with lentiCRISPR/Cas9 and off target gRNA or IL-1R1-gRNA (left) or STING-gRNA (right) were pretreated with media or 10ng/ml IL-1β for 6hrs before DENV-2 infection for 24-48hrs (MOI=0.5). Upper: viral RNA was analyzed by qRT-PCR. Statistical analysis was performed using two-way ANOVA and Bonferroni's to compare the indicated treatments, *n*=5 (IL-1R1) or *n*=3 (STING) with mean ± SEM. Lower: protein analysis by immunoblot, *n*=3 (IL-1R1) or *n*=2 (STING). (D) Primary human moDC were pretreated with media or 10ng/ml IL-1β for 6hrs before DENV-2 infection (MOI=1). 2hrs post-adsorption, media was replaced +/- 1ug/ml anti-IL-1R1. Protein analysis by immunoblot 24-48hrs p.i. DENV E was normalized to actin and quantified relative to 24hr DENV-2 infection, *n*=3. p.i., post-infection. pfu, plaque forming unit. vRNA, viral RNA *p<0.05, **p<0.01, ***p<0.001

innate immune program in the control of viral infection, we assessed this pathway in Dengue virus 2 (DENV-2) infection. In particular, DENV-2 induces both IL-1β production and STING signaling linked with the accumulation and sensing of mtDNA DAMPs (Aguirre, 2017a; Chang, 1994; Sun, 2017; Wu, 2013a, b). Exposure of A549 cells to IL-1β before infection was protective against DENV-2, as indicated by reduced infectious virus production at 24 and 48 hours post-infection (**Figure 3.20A**). IL-1β-mediated protection against DENV-2 infection was comparable to IFN pre-treatment at 24 hours but was less effective by 48 hours, confirming the importance of this signaling axis in early viral control. Of note,

pre-treatment with either cytokine was slightly cytoprotective at 24 hours post-infection, but cell death was comparable between all groups by 48 hours post-infection despite the reduction in infectious virus production with cytokine pre-treatment (**Figure 3.20B**). Cells that were responsive to IL-1β show a 45-70% reduction in viral RNA and a large reduction in viral E protein, while the protective effect of IL-1β is absent in IL-1R1-deficient cells (**Figure 3.20C**). Importantly, IL-1β-mediated protection against DENV-2 is entirely dependent upon STING, as STING-deficient cells show no reduction in viral RNA or viral E protein with IL-1β pre-treatment (**Figure 3.20C**). Primary human moDC pre-treated with IL-1β and infected with DENV-2 showed a reduction in viral E protein production at 24 and 48 hours post-infection compared to nontreated controls (**Figure 3.20D**). Treatment with a neutralizing antibody against IL-1R1 in the absence of exogenous IL-1β treatment had little effect on viral production in moDC. Contrary to our previous results with WNV infection of BMDC (Aarreberg, 2018), moDC may not produce high enough IL-1β themselves to mediate protection against DENV-2 infection through IL-1β autocrine signaling *in vitro* (**Figure 3.20D**) (Chang, 1994; Wu, 2013b). These data provide evidence for an IL-1β-driven antiviral program that is dependent on the activities of STING, thereby expanding the mechanisms in which the DNA sensing pathway imparts innate immune protection against RNA virus infection.

3.3 Discussion

Inflammasome activation and the synthesis of IL-1β are common features of the immunological response to bacterial and viral infections (Jorgensen, 2015, 2017; Mayer-Barber, 2017). Immune protection provided by IL-1β has commonly been attributed to cell-extrinsic mechanisms such as the recruitment and activation of neutrophils and lymphocytes or the production of acute phase proteins that mediate antimicrobial defense (Dinarello, 2009a; Sims, 2010). Few studies have focused on IL-1R signaling in the initiation of cell-intrinsic defense (Copenhaver, 2015; Mayer-Barber, 2014; Orzalli, 2018). Our observations support a model in which IL-1R signaling initiates innate defense programs through a cGAS-STING-IRF3 axis to drive the expression of IRF- and IFN-responsive genes as well as increased autophagic flux. Importantly, we have demonstrated a cell-intrinsic, antiviral effect of IL-1β against another flavivirus, Dengue virus. Dengue was of interest in this study due to its known induction of IL-1β, as well as its proven antagonism of cGAS, STING and the IFN pathway (Aguirre, 2017a, b; Chang, 1994; Wu, 2013a). Our study is consistent with the concept that indirect sensing of viral infection is a critical component of innate immune

defense. Moreover, synergy of IL-1β and IFN in bystander cells can be essential to overcome pathogen subversion of innate immune signaling and suppress viral spread.

Some evidence exists for infection-independent, inflammation-directed cytokine crosstalk. Yarilina *et al*. characterized a TNF-IRF1-IFN signaling loop in which the inflammatory cytokine TNF induces and synergizes with autocrine IFN to initiate expression of ISGs (Yarilina, 2008). In contrast to our data, which support a partial role for IRF1 and a primary role for IRF3 in IL-1β-induced ISG production in a range of cell types, Yarilina *et al*. emphasize that IRF1 is the chief driver of TNF-mediated ISG expression. However, their study also showed phosphorylation of IRF3 in human monocytes and a partial dependence on IRF3 for ISG expression in bone marrow derived macrophages treated with TNF. In the report by Orzalli *et al*., IL-1-induced ISG expression is IRF1-dependent yet occurs independent of TNF (Orzalli, 2018). These data warrant further investigation into whether and how IL-1 and TNF signaling may converge on IRF activation. These studies and our current study support a model of innate immune defense in which inflammatory cues prime cells for detection and response to invading pathogens and DAMPs by upregulating PRRs, their key signaling molecules, and antimicrobial effector genes.

IL-1β and stress-associated DAMPs can serve to contextualize stimuli perceived by the cell, and ultimately direct defensive or repair responses (Brubaker, 2015). We and others have shown that mitochondria are an important source of cellular DAMPs, and the detection of such initiates critical innate immune signaling programs (Krysko, 2011; Rongvaux, 2014; West, 2017, 2015; White, 2014). While our study demonstrates that IL-1β-driven IRF3 activation is dependent upon cytosolic detection of mtDNA, we note that the mechanism by which mtDNA is liberated is not yet known. Mitochondrial outer membrane permeabilization (MOMP) can result in the release of intermembrane space proteins and the initiation of intrinsic apoptosis (Tait, 2013). Three groups demonstrated that MOMP also allows for mtDNA release into the cytosol, which, in the absence of apoptotic caspase activation, is detected by cGAS for activation of STING (Riley, 2018; Rongvaux, 2014; White, 2014). Interestingly, our scRNA-seq analysis predicts that Forkhead box O3 (FOXO3) is activated upon IL-1β treatment; FOXO3 has been shown to induce transient MOMP, thus linking possible MOMP with mtDNA release (Hagenbuchner, 2013). Our study suggests that IL-1β can augment mitochondrial mass, decrease mitochondrial membrane potential and induce mtDNA

release without detectable cytochrome c release or cell death. Indeed, limited mitochondrial permeabilization can occur without triggering cell death (Ichim, 2015; Minamikawa, 1999; Tait, 2010). In our model, we might expect that IL-1R signaling results in NF-κB-dependent upregulation of inhibitor of apoptosis proteins (IAP) to promote cell survival (Stehlik, 1998; Wang, 1998). In fact, we found several anti-apoptotic genes to be upregulated by exogenous IL-1β.

Another mechanism by which IL-1β may mediate mitochondrial disruption could involve the production of mitochondrial reactive oxygen species (ROS). Mitochondria are a major source of ROS production and therefore particularly prone to oxidative damage. While high doses of ROS are generally considered cytotoxic, small doses can modulate early, intermediate and late events in cytokine signaling pathways by modifying key intermediary proteins (Brigelius-Flohé, 2004; Thannickal 2000). IL-1 stimulates the generation of ROS in a cell-specific manner via NAD(P)H oxidase and/or lipoxygenase (Bonizzi, 2000). West *et al.* demonstrated that a subset of TLRs (1, 2 and 4) activate a TNF receptor-associated factor (TRAF)6-mediated program to increase mitochondrial ROS production (West, 2011). Importantly, TRAF6 is an essential signal transducer in the IL-1R pathway, but is dispensable for TNF signaling (Cao, 1996). In agreement with our observation that TNF treatment does not drive release of mtDNA to the cytosol, West *et al.* found that endosomal TLRs and TNF did not drive TRAF6 relocalization to mitochondria for subsequent mitochondrial ROS production (West, 2011). Although the authors of this study did not investigate the effects of a respiratory burst on mitochondrial integrity or mtDNA release, mitochondrial ROS has been associated with reversible mitochondrial depolarization and mitochondrial permeability transition induction (Kowaltowski, 1999; Zorov, 2000). Whether and how IL-1β may initiate mitochondrial membrane permeabilization still needs to be resolved.

In conclusion, we have described a mechanism in which IL-1β signaling drives mitochondrial DNA release and detection by the cytosolic DNA sensor cGAS for activation of STING and IRF3 (**Figure 3.21**). Resultant signaling leads to the synthesis and autocrine response to IFN for the basal elevation of ISGs. In addition to the production of cell-autonomous defense proteins, the temporary upregulation of PRRs, IRFs and STATs may sensitize cells for subsequent pathogen detection and response. This report provides

mechanistic insight into a stress-induced pathway of STING activation that can restrict microbial infection, shape adaptive immunity and potentially exacerbate autoinflammatory and autoimmune diseases.

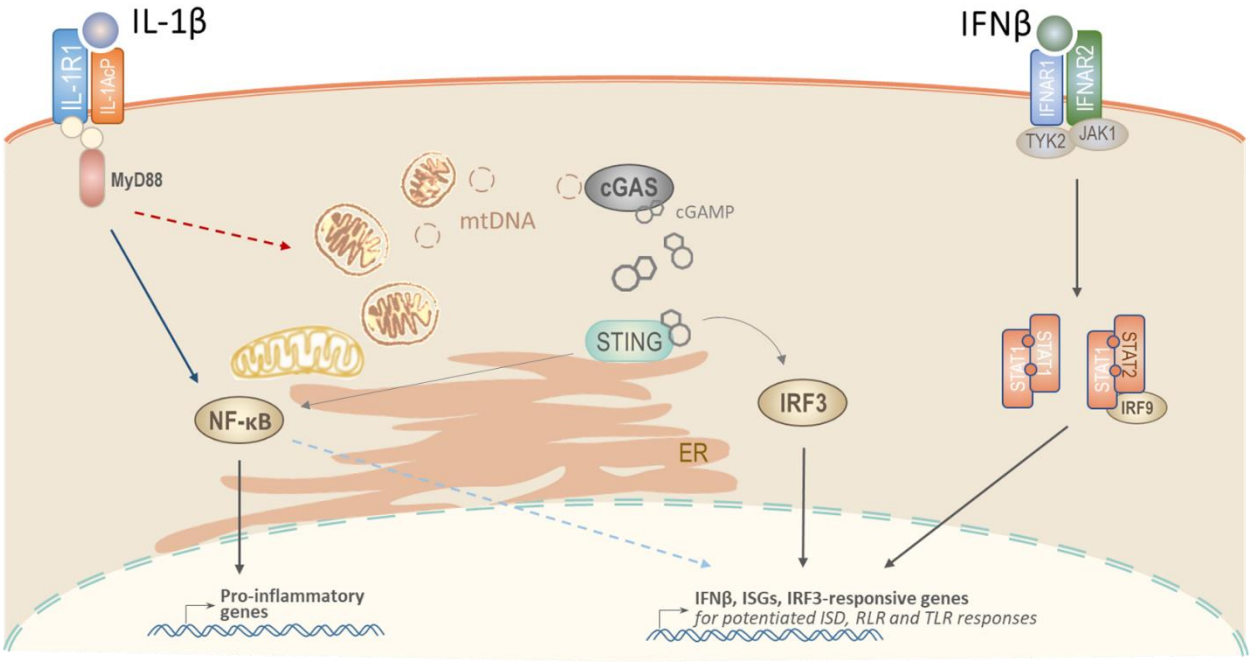


Figure 3.21. Model of IL-1β-cGAS-STING-IRF3 axis. IL-1R signaling via the canonical and non-canonical IKKs (not shown) leads to the disruption of mitochondrial homeostasis and cytosolic release of mitochondrial DNA (mtDNA) for detection by cGAS. Upon stimulation, cGAS produces the second messenger 2′3′-cGAMP, which binds to the ER-resident protein STING. STING then dimerizes and translocates from the ER to the Golgi, where it activates autophagic flux (not shown) and IRF3 for transcriptional induction of IFN and antimicrobial response genes. cGAS-STING signaling also induces activation of NF-κB, leading to proinflammatory cytokine expression and enhanceosome activity. Synergistic signaling by IL-1β and autocrine IFN potentiates microbial detection and resultant immune responses.

3.4 Methods

Cells and virus. A549, THP-1, HEK293T and Vero cells were obtained from the American Type Culture Collection (ATCC). TERT-immortalized human foreskin fibroblasts were provided by Dr. Daniel Stetson (University of Washington). THP-1 cells were cultured in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum (HI-FBS), 2mM L-glutamine, 1mM sodium pyruvate, 1x nonessential amino acids and antibiotic-antimycotic solution (Fisher), while all other cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented as above. THP-1 monocytes were differentiated to macrophages in 40nM PMA (Sigma) for 36 hours and rested for 36 hours without PMA before experiments were performed. Human whole blood was collected from six independent donors using an IRB-approved protocol. Whole blood was centrifuged using Ficoll-Paque PLUS (Fisher) to obtain peripheral blood mononuclear cells

(PBMCs). 6-10x10^6 PBMCs were rested in 15cm non-TC-treated plates (VWR) for 2 hours. After 2 hours, non- and semi-adherent cells were collected and cultured in complete RPMI medium supplemented with 50ng/ml GM-CSF and 100ng/ml IL-4 (Peprotech) for 6 days, replacing 2/3 media with complete RPMI+GM-CSF+IL-4 every other day to prepare monocyte-derive dendritic cells (MoDC). On day 6, MoDC were collected by gently scrapping. Cells were counted and seeded at 1x10^6/ml with the indicated treatments. Dengue virus type 2 New Guinea C (DENV-2) was propagated on Vero cells (MOI=0.05) for 5 days before cell-free supernatants were aliquoted and frozen at -80C. Thawed aliquots were used to determine viral titer by plaque assay on Vero cells.

Cell treatment and analysis. Recombinant human IL-1β (Enzo Life Sciences) was reconstituted in sterile water at 100ug/ml and used at 10ng/ml unless otherwise indicated. ACHP (Tocris) was reconstituted in DMSO at 20mM and used at 10uM. BMS (Tocris) was reconstituted in DMSO at 100mM and used at 10uM. BX795 (Invivogen) was reconstituted in DMSO at 10mM and used at 1uM. PF184 (Tocris) was reconstituted at 100mM and used at 0.5uM. TPCA1 (Tocris) was reconstituted at 100mM and used at 0.5uM. Tunicamycin (Sigma) was reconstituted in DMSO at 10mg/ml and used at 4ug/ml. HCV polyU/UC PAMP RNA was synthesized via *in vitro* transcription (Saito, 2008) and transfected at 0.1ug/ml using TransIT-LT1 Transfection Reagents (Mirus). cGAMP was a kind gift from Pingwei Li (Texas A&M University), reconstituted in sterile water at 1mg/ml and used exogenously at 25ug/ml (THP-1) or transfected at 10ug/ml using Lipofectamine 3000 reagents (A549, HFF). Calf thymus DNA solution (ctDNA, Life Technologies) was transfected at 1ug/ml using Lipofectamine 3000 reagents (ThermoFisher). LPS (Adipogen) was used on its own at 0.5ug/ml or in combination with 1mM ATP (Sigma). TNFα (Peprotech) was used on its own at 10ng/ml or in combination with 10uM Cyclohexamide (Sigma). Anti-IFNAR2 antibody (R&D Systems) was used to neutralize Type I IFN at 0.5ug/ml or mouse IgG (Sigma) was used as a control antibody at 0.5ug/ml. Recombinant human IFNβ (gift from Toray Industries, Japan) was used at 50, 100 or 1000IU/ml. Anti-IL-1R1 antibody (R&D Systems) was used to at 1ug/ml to neutralize endogenous IL-1. JC-1 (Invitrogen) was reconstituted at 5mM and used at 2.5uM. Mitotracker Green (Invitrogen) was reconstituted in DMSO at 1mM and used at 500nM.

LentiCRISPR. For CRISPR-targeting of the indicated genes, DNA oligos containing off target or targeting gRNAs were inserted into a Cas9-t2a-puro pRRL vector using the In-Fusion HD Cloning Kit (Clontech). Lentivirus pseudotyped with vesicular stomatitis virus envelope glycoprotein (VSV-G) was produced by transfection of $2x10^6$ HEK293T cells with the ProFection Mammalian Transfection System (Promega), 6ug gRNA-Cas9-t2a-puro pRRL lentivirus plasmid, 3ug psPAX-2 packaging plasmid and 1.5ug pVSV-G in 10cm plates for 48 hours before filtration of infectious supernatants with a 0.45uM filter. Target cells were transduced with the filtered viral supernatants for 24 hours, washed and cultured in fresh media for 24 hours before selection with 1.5ug/ml Puro (A549) or 5ug/ml Puro (THP-1) for 3-4 days. Targeting was evaluated by immunoblot and ablated signaling downstream of relevant stimuli (**Figure 3.**).

Viral infection and plaque assay. $1x10^5$ A549 cells were seeded in 12-well dishes for 36 hours before mock or cytokine treatment or $2x10^6$ moDC were seeded in 6-well dishes with mock or cytokine treatment. 6 hours post-treatment, media was removed, cells were washed twice with 1x PBS and incubated on a rocker with infectious media (complete DMEM with DENV-2 at MOI=0.5 for A549 or MOI=1 for moDC) for 2 hours. Infectious media was removed 2 hours post-adsorption and cells were incubated with fresh media for 24 or 48 hours before collection of infectious supernatants, total RNA or cellular lysates. $1.85x10^5$ Vero cells were seeded in 6-well dishes and mock- or virus-containing supernatants were serially diluted in complete DMEM. Vero cell monolayers were incubated in technical duplicate with dilutions, rocking for 1 hour. Cells were overlaid with 1% agarose and 6 days later plaques were visualized with a 1% agarose overlay containing 3.5% Neutral Red.

Immunoblot. Cells were lysed in RIPA buffer (Sigma) with freshly added protease inhibitor cocktail (Sigma), phosphatase inhibitor cocktail (Millipore) and Okadaic acid (Thermo). Lysates were separated on 8% Bis-Acrylamide SDS gels and transferred onto nitrocellulose membranes (Fisher Scientific).Membranes were blocked in Odyssey TBS Blocking Buffer (LI-COR) for 1 hour at room temperature (RT) before overnight 4C incubation with primary antibody diluted in blocking buffer. After washing, membranes were incubated for 1 hour at RT with HRP-conjugated secondary antibodies diluted in TBS-tween. Membranes were incubated in ECL (Fisher Scientific) and detected using a ChemiDoc XRS+ System (Bio-Rad).

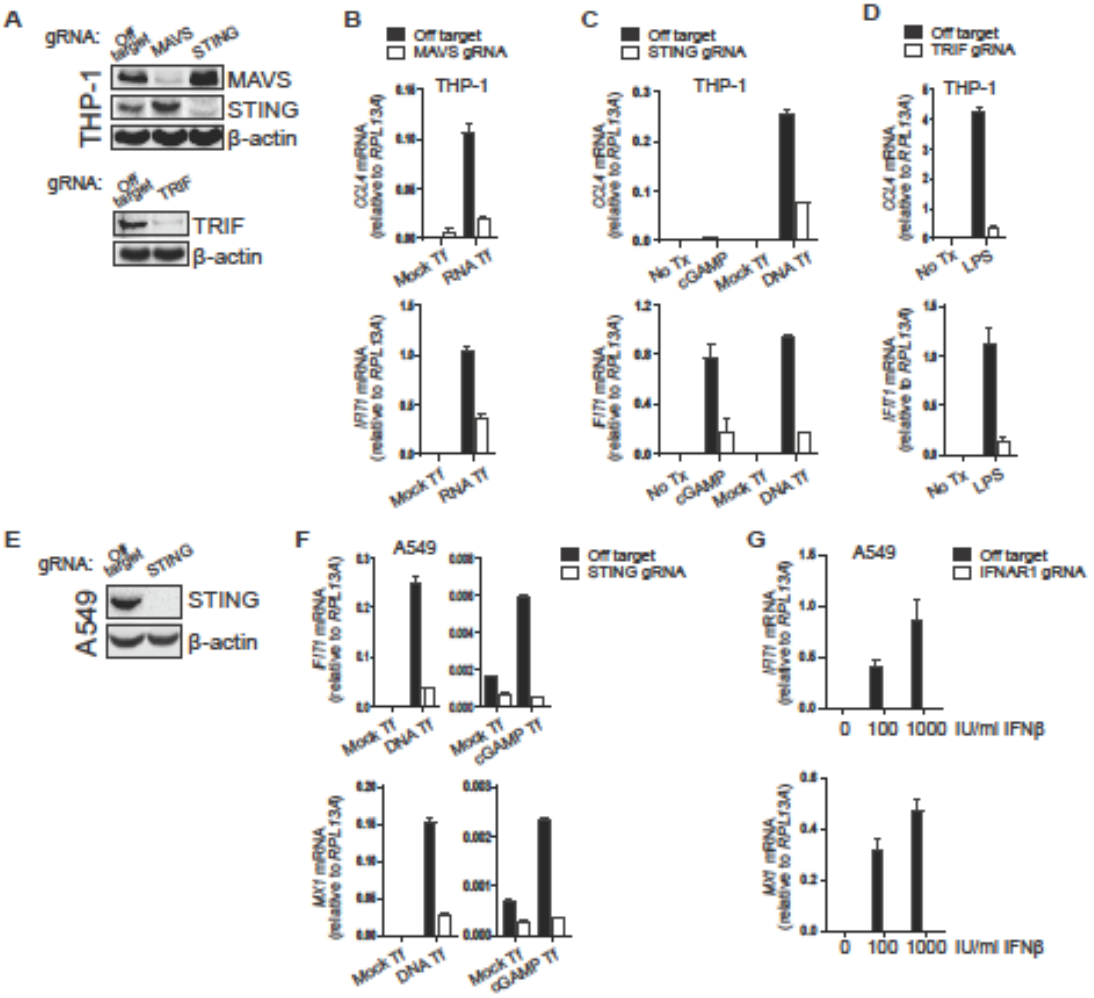


Figure 3.22 CRISPR-targeting analysis. (A) THP-1 were transduced with lentiCRISPR/Cas9 and off target gRNA or gRNA targeting MAVS, STING or TRIF before protein analysis by immunoblot. (B) THP-1 transduced with lentiCRISPR/Cas9 and off target gRNA or MAVS-gRNA were mock transfected or transfected with 0.1ug/ml PAMP RNA for 6hrs. (C) THP-1 transduced with lentiCRISPR/Cas9 and off target gRNA or STING-gRNA were treated with media or 25ug/ml exogenous cGAMP, mock transfected or transfected with 1ug/ml calf thymus DNA for 6hrs. (D) THP-1 transduced with lentiCRISPR/Cas9 and off target gRNA or TRIF-gRNA were treated with media or 0.5ug/ml LPS for 6hrs. (E) A549 were transduced with lentiCRISPR/Cas9 and off target gRNA or STING-gRNA before protein analysis by immunoblot. (F) A549 transduced with lentiCRISPR/Cas9 and off target gRNA or STING-gRNA were mock transfected, transfected with 1ug/ml calf thymus DNA, or transfected with 10ug/ml cGAMP for 6hrs. (G) A549 transfected with CRISPR/Cas9 and off target gRNA or IFNAR1-gRNA were treated with media or 100 or 1000IU/ml IFNβ for 12hrs. B-D, F-G. After treatment, the indicated transcripts were analyzed by qRT-PCR; *n*=3 per genotype with mean ± SEM. Tf, Transfection.

qRT-PCR. Total RNA was isolated from cell lysates using the RNeasy Kit (Qiagen) and digested with DNase I (Qiagen) on column. 200ng total RNA was subjected to cDNA synthesis using the iScript cDNA Synthesis Kit (BioRad). cDNA was diluted 1:4 in H_2O and qPCR was performed using SYBR Select Master Mix (Thermo) and gene specific primers on the ABI 7500 Real-Time PCR System.

Cell death assay. THP-1 macrophages and A549 cells were seeded at $1x10^5$ cells per well in 24-well dishes. 24 hours after seeding, cells were treated with 1 ng/ml or 10 ng/ml IL-1β, 0.5ug/ml LPS (Adipogen)

in combination with 1mM ATP (Sigma) to induce pyroptosis, or 10uM Cycloheximide (Sigma) in combination with 10ng/ml TNFα (Peprotech) to induce apoptosis. Alternatively, A549 cells were seeded as above and treated with 10ng/ml IL-1β or 50IU/ml IFNβ for 6 hours before infection with DENV-2 (MOI=0.5). Additionally, cells were treated with 100nM of the green fluorescent nucleic acid stains Syto or Sytox to quantify total number of cells or dead cells, respectively (ThermoFisher). Cells were then imaged with the IncuCyte imaging platform (Essen Bioscience) up to 48 hours post-treatment. At every 2 (THP-1) or 4 (A549) hours, four images were taken per well. Each treatment was run in triplicate. The ratio of Sytox-Green to Syto-Green counts was used to calculate percent cell death.

Subcellular fractionation. Subcellular fractionation and mitochondrial DNA quantification was adapted from West *et al*. (West, 2015) as follows: $4x10^6$ THP-1 or $2x10^6$ A549 cells were lysed in 100ul/$5x10^5$ cells Digitonin buffer (150mM NaCl, 50mM HEPES pH7.4, 25ug/ml Digitonin, Protease and phosphatase inhibitors) and incubated on a rotator at 4C for 10 min. Samples were centrifuged at 2,000x*g* for 10 min at 4C. Supernatants were transferred to fresh tubes and centrifuged three times at 20,000x*g* for 20 at 4C, transferring supernatants to fresh tubes between centrifugation steps to finally yield cytosolic fractions. The cytosolic fraction was split into two tubes (one for total DNA extraction and one for immunoblot analysis). The remaining pellet from the first spin was resuspended in ice-cold PBS to wash away Digitonin buffer. Samples were centrifuged at 2,000x*g* for 5 min at 4C. Wash was aspirated and samples were resuspended in 100ul/$5x10^5$ cells NP-40 buffer (150mM NaCl, 50mM HEPES pH7.4, 1% NP-40, protease and phosphatase inhibitors) and incubated on ice for 30 min. Samples were centrifuged at 7,000x*g* for 10 min at 4C to yield the crude mitochondria fraction for immunoblot analysis. The remaining pellet was resuspended in ice-cold PBS to wash away NP-40 buffer. Samples were split into two tubes (one for total DNA extraction and one for cellular lysates). One tube was centrifuged at 2,000x*g* for 5 min at 4C. Wash was aspirated and samples were resuspended in 50ul/$5x10^5$ cells RIPA buffer (Sigma) to yield nuclear fraction for immunoblot analysis. DNA was subsequently extracted from the appropriate cytosolic and nuclear fractions using the QIAmp DNA Mini Kit (Qiagen). 2ng cytosolic DNA was used for qPCR analysis of mitochondrial DNA using gene-specific primers (Aguirre, 2017b); nuclear gene *Rpl13a* was quantified from the respective nuclear fraction for normalization.

Flow cytometry. 1x10^5 A549 cells were seeded in 12-well dishes for 24 hours before treatment with media or cytokine. 30 minutes before collection, cells were treated with media containing JC-1 or Mitotracker Green dye and incubated at 37C. Cells were washed with 1x PBS and trypsinized, followed by another PBS wash before analysis by flow cytometry. Data were acquired using a FACSCanto (BD Biosciences) and analyzed with FlowJo 10.5.0 software.

Mitochondrial depletion. A549 cells were cultured with or without 150ng/ml Ethidium Bromide for 4 days (Hashiguchi, 2009). On Day 4, cells were washed with 1x PBS and trypsinized before counting and seeding for stimulation or microscopy. Total DNA was collected by alkaline extraction.

Immunofluorescence. 1x10^5 HFF or A549 cells were seeded on glass coverslips in a 24-well plate. For visualization of STING, 24 hours after seeding HFF, cells were treated with media, treated with 10ng/ml IL-1β, transfected with 1ug/ml calf thymus DNA or transfected with 10ug/ml cGAMP for 3 hours before fixation with 3% PFA for 15 min at RT. Cells were washed three times with 1x PBS before permeabilization with 0.5% Triton-X-100 for 15 min at RT. Cells were washed three times with 1x PBS before blocking with 3% BSA in PBS for 30 min at RT. Cells were stained overnight at 4C with primary rabbit antibody directed against STING (diluted 1:100 in blocking solution). The next day, cells were washed three times with 1x PBS before incubation with FITC-conjugated, anti-Rabbit IgG secondary antibody (Thermo Fisher) for 1 hour at RT. Nuclei were counterstain with DAPI (Thermo Fisher). Cells were washed four times with 1x PBS before coverslips were mounted onto glass slides using ProLong Gold (Thermo Fisher). STING slides were blinded before images were taken. Quantification of cells containing STING puncta was performed blinded using ImageJ software. For visualization of mitochondrial DNA, 24 hours after seeding A549, cells were fixed with 3% PFA for 15 min. Cells were permeabilized with 0.5% Triton-X-100 for 5 min (note the shorter permeabilization to prevent nuclear membrane permeabilization). Cells were washed three times with 1x PBS before blocking with 3% BSA in PBS for 30 min at RT. Cells were stained for 1 hour at RT temperature with primary mouse antibodies directed against DNA and TOM20. Cells were washed three times with 1x PBS before incubation with fluorophore-conjugated, isotype specific secondary antibodies (Thermo Fisher) for 1 hour at RT. Nuclei were counterstained with DAPI. Cells were washed and mounted as above. Images were acquired with a Nikon Eclipse Ti confocal microscope equipped with a 60x oil

immersion objective using the Nikon confocal software. Images were merged and processed using the Nikon confocal analysis software.

IL-1β ELISA. Cell-free supernatants were frozen and thawed before quantification of IL-1β was performed using a human IL-1β ELISA kit (BioLegend). Absorbance was read at 450nm and 570nm using the Epoch Microplate Spectrophotometer (BioTek). The absorbance at 570nm was subtracted from the absorbance at 450nm before calculating IL-1β concentration based upon a standard curve.

scRNA-seq. A549 cells were mock treated or treated with 10ng/ml IL-1β in technical duplicate for 3, 6 or 12 hours. Cells were isolated on the ddSEQ single-cell isolator, and samples prepared for sequencing using the SureCell WTA 3' Library Prep Kit (Illumina/Bio-Rad). All samples were sequenced on a NextSeq 500 to a depth of > 20,000 reads/cell, and raw reads were submitted to GEO under accession GSE120269. Single-cell UMI counts were generated within Illumina's SureCell RNA Single-Cell Basespace, where reads were mapped to the human genome version hg38 and only cells passing the knee-calling threshold were kept. All downstream filtering, normalization, and differential expression analyses were performed using the Seurat R package (Butler, 2018). Cells with fewer than 200 detected genes and greater than 5% of total reads assigned to ribosomal RNA were removed from the data, and expression was then log normalized with a scale factor of 10,000. Cellular counts were then batch-corrected for replicate variation and predicted cell cycle phase (Tirosh, 2016). At each timepoint, cells were clustered using PCA reduction with the first 20 principal components at 0.6 resolution and tSNE analysis was performed to visually identify homogenous IL-1β-treated and mock-treated clusters. Differentially expressed genes between IL-1β-treated and mock-treated clusters were identified using the Wilcoxon rank sum test ($q < 0.05$) where genes showed expression in >25% of all cells in each treatment group. Predicted transcriptional activators were identified using the Upstream Regulators analysis in Ingenuity Pathway Analysis (IPA; (Krämer, 2014)).

Statistical analysis. All statistical analyses were performed as indicated in figure legends using GraphPad Prism 7.04 software, where *n* represents the number of independent experiments, except in Figures 1F, 1G, and 7D where *n* represents the number of independent blood donors.

4. Concluding remarks

Early work on the modulatory effects of IL-1 described its ability to induce IFN and confer antiviral protection (Van Damme, 1983, 1985, 1987). Due to the weak induction of IFN by IL-1, it was concluded that this was not the primary biological function of IL-1. Indeed, the authors postulated that some cells may constitutively express low levels of IFN, and exposure to TNF or IL-1 may enhance the production and response to IFN for antiviral protection (Van Damme, 1987). Few studies have since focused on IL-1R signaling in cell-autonomous defense (Copenhaver, 2015; Mayer-Barber, 2014; Orzalli, 2018). Our work is the first to mechanistically link IL-1β to the activation of a cGAS-STING-IRF3 axis via intrinsic sensing of mitochondrial stress. This program serves to prime cells for antimicrobial defense through several mechanisms. IRF3 activation basally elevates ISG expression, including the expression of PRRs and key intermediary signaling molecules, for sensitization to PAMP and DAMP recognition. IL-1R signaling also activates the AP-1 and NF-κB transcription factor families, which can cooperate with IRFs and STATs to enhance expression of IFN, antimicrobial effectors and inflammatory genes (Wathelet, 1998; Wienerroither, 2015). Our work, and that of Pilli *et al*., demonstrates that IL-1R signaling can also drive the maturation of autophagosomes, which can serve to eliminate intracellular microbes (xenophagy), dampen inflammation, and facilitate antigen processing and presentation to adaptive immune cells (Deretic, 2013; Pilli, 2012).

The involvement of mitochondria in this cell-intrinsic defense program is curious but not unique. A growing body of literature implicates mitochondria in the response to infection and in the cause of disease (Krysko, 2011; Nakahira, 2015; West, 2017). It is unclear whether this is a maladaptation intrinsic to the bacterial ancestry of mitochondria or if sensing of mitochondrial stress has been co-opted as an indirect mechanism to detect infection. As many microbes actively avoid and suppress PRR signaling, this program could prove to be a critical component of host defense. Mitochondria, apart from their bioenergetic role, participate in intracellular signaling that is vital to cell survival and function (Chandel, 1999). Therefore, it is essential that cells balance mitochondrial biogenesis with disposal and recycling of dysfunctional mitochondria. These processes are modulated by fusion and fission events, wherein fission generates one daughter

mitochondrion with intact membrane potential and a high probability of subsequent fusion and another mitochondrion with decreased membrane potential that will likely be removed by mitophagy (Twig, 2008). Our data indicate that IL-1β treatment results in increased mitochondrial mass and decreased mitochondrial membrane potential, leading us to speculate that IL-1R signaling halts mitophagy, drives fission, or a combination of the two. Any of these processes could be implicated in the mechanism by which mitochondrial DNA is exposed to cGAS. Inflammatory stimuli have been shown to drive mitochondrial fission (Baker, 2014; Katoh, 2017), which can induce a metabolic shift from oxidative phosphorylation to glycolysis (Nair, 2019). This switch to glycolysis is shown to produce metabolites that facilitate cytokine synthesis and ROS production (Kelly, 2015; Mills, 2016). Additionally, we question whether the differentiation status of a cell, which determines metabolic needs and mitochondrial biogenesis, could be a determining factor for IL-1-induced mitochondrial dysfunction. For example, we have demonstrated that terminally differentiated dendritic cells and macrophages take 24-36 hours for appreciable activation of IRF3 via sensing of mitochondrial DAMPs, while actively proliferating fibroblasts and epithelial cells respond within 3 hours of IL-1β treatment.

While our data demonstrate that IL-1β induces IRF3 activation in murine and human cells of myeloid, stromal and epithelial origin, this program is not universal across cell types. Liu *et al*. did not observe phosphorylation of IRF3 in mouse embryonic fibroblasts (MEFs) treated with IL-1β for 15 minutes (Liu, 2015), while Orzalli *et al*. did not observe appreciable ISG expression in MEFs treated with IL-1β for 3 or 6 hours (Orzalli, 2018). In contrast, a report by Rivieccio *et al*. showed that cultured human fetal astrocytes respond to IL-1β with IRF3 activation and induction of ISGs within 3 hours (Rivieccio, 2005). Early studies of cytokine-mediated protection against cytomegalovirus (CMV) in stromal cells identified an antiviral role for IL-1 *in vitro* (Iwata, 1999). This group went on to demonstrate that IL-1-mediated inhibition of CMV spread was due to the induction of IFN (Randolph-Habecker, 2002). Indeed, blocking antibodies against IFN or IFNAR ablated the protective effects of IL-1. Of interest, another group found that pre-administration of TNF, IL-1β and IFNγ all inhibited CMV replication in primary human astrocytes (Cheeran,

2000). Although the mechanisms of protection were not elucidated, the authors demonstrate that the suppressive effect of cytokine treatment occurred after viral entry to affect viral DNA synthesis and translation. Our current study and findings by Rivieccio *et al*. may provide the mechanistic link between these independent observations (Rivieccio, 2005). Additionally, Orzalli *et al*. recently defined an IRF1-dependent antiviral program elicited by IL-1R signaling in primary human fibroblasts and endothelial cells (Orzalli, 2018). These cell-specific programs, and the time it takes to initiate them, offer an interesting illustration of the dynamic response to IL-1β and resultant immune activation. It is unclear whether the cell-type specificity is dependent upon expression of IL-1R and DNA sensing components, or the activation of regulatory mechanisms that prevent or promote this pathway. Of note, cells with demonstrable IL-1R-to-IRF signaling provide important protection at barrier surfaces and this will likely prove to be a critical pathway of innate immune priming at these sites.

It is now appreciated that inflammasome-activating pathogens and immune adjuvants operate at the interface of innate and adaptive immunity such that they can promote adaptive immune responses against specific microbes (Evavold, 2018; Ichinohe, 2009; Pang, 2013; Ramos, 2012). We identified the upregulation of several genes involved in antigen processing and presentation by RNA-seq analysis of IL-1β-treated murine dendritic cells and scRNA-seq analysis of human epithelial cells. Our results using primary murine and human dendritic cells also show that IL-1R signaling can induce IRF3 target genes, IFN and ISGs in DCs, which may include critical costimulatory molecules and receptors for functional interactions with T lymphocytes. In fact, other studies have demonstrated that exogenous IL-1β can induce expression of costimulatory CD86 on DCs but the regulation thereof was not explored (Copenhaver, 2015; Pang, 2013). In this respect, it is likely that IL-1β-induced IFN directs DC maturation during migration to the lymph node or throughout the T cell priming phase within lymphoid tissues (Kadowaki, 2000; Mempel, 2004; Pang, 2013; Sallusto, 1995).

It is intriguing that IL-1R signaling engages cGAS and STING for IRF3 activation, rather than directly activating IRF3 as has been shown for TLR-MyD88-IRF7 signaling in plasmacytoid dendritic cells (Honda, 2005; Kawai, 2004). However, this response is not universal across cell types and may be limited to cells that express competent STING or other unidentified factors. We also noted that IL-1β-induced IRF3

phosphorylation and ISG expression are entirely STING-dependent in macrophages, while these processes are not completely ablated in cGAS-deficient cells. This may reflect an incomplete knockout of cGAS, in which case cGAS-competent cells can signal to neighboring cells with the production of cGAMP (Ablasser, 2013). Alternatively, this may indicate that mtDNA-independent processes are also involved in IL-1β-mediated activation of STING (Holm, 2016). Aside from IFN responses, activation of STING also drives the formation of autophagosomes with proven antibacterial and antiviral capacity (Costa Franco, 2018; Liu, 2018; McFarlane, 2011; Moretti J., 2017; Rasmussen, 2011; Watson, 2012). Autophagy can limit microbial replication, deliver PAMPs to endosomal PRRs or antigens to MHC class II loading compartments (Deretic, 2013; Lee, 2007; Nakagawa, 2004; Schmid, 2007; Singh, 2006). Autophagy can also limit inflammatory responses by sequestering the stimulus from recognition or by degrading essential signaling components (Takahama, 2018).

PAMP recognition and cytokine signaling are not acting in isolation *in vivo* such that integrative signaling can have important effects on disease outcome. Here we have observed a synergistic response to IL-1β and diverse PAMPs, with differential effects on NF-κB and IRF signaling. Indeed, a study by Chiliveru *et al*. has described a role for inflammatory cytokines TNF and IL-1β in licensing DNA-driven immune responses in keratinocytes (Chiliveru, 2014). This study implies that inflammatory environments can lead to breakdown of tolerance for DNA in the skin. While we reveal a protective role of IL-1β in cytokine crosstalk against flavivirus infection, we acknowledge that both IL-1β and IFN are implicated in autoimmune disorders in which chronic cytokine production is linked with inflammatory disease and interferonopathies (Crow, 2011; Hall, 2010; Lopalco, 2015). Indeed, anti-IL-1β treatment has been effective against several autoimmune disorders (Lopalco, 2015), but the specific influence of IL-1β on the dysregulation of IFN has not been investigated in this context. Autoinflammatory diseases are characterized by chronic inflammation and, importantly, a rapid cessation upon treatment with IL-1 blocking agents (Dinarello, 2009b). Hereditary syndromes with mutations in proteins that affect inflammasome activation are commonly treated with therapies against IL-1R signaling, but the therapeutic mechanisms of IL-1 antagonists in some syndromes are unclear (Sims, 2010). Of note, hyperactivity of STING is implicated in several autoinflammatory diseases (Ahn, 2012; Gall, 2012; Gao, 2015; Jeremiah, 2014), as is mtDNA in inflammatory pathology (Nakahira, 2015; West, 2017). Our identification of another mechanism of STING activation and mtDNA-

dependent inflammatory signaling is of great importance in understanding autoinflammatory disorders for which the etiology is not fully defined.

5. Abbreviations

AIM2	Absent in melanoma 2
ALR	AIM2-like receptor
AP-1	Activator protein 1
ASC	Apoptosis-associated speck-like doman containing a CARD
ATP	Adenosine triphosphate
B2M	Beta-2-microglobulin
BCL	B Cell CLL/Lymphoma
BMDC	Bone marrow-derived dendritic cell
BMM	Bone marrow-derived macrophage
CARD	Caspase recruitment domain
CCL	C-C motif chemokine ligand
CD86	Cluster of differentiation 86
cGAMP	Cyclic GMP-AMP
cGAS	Cyclic GMP-AMP synthase
CHX	Cyclohexamide
CLR	C-type lectin receptor
CMV	Cytomegalovirus
CNS	Central nervous system
COX	Cyclooxygenase
ctDNA	calf thymus DNA
CXCL	C-X-C motif chemokine ligand
DAI	DNA-dependent activator of IRFs
DAMP	Damage-associated molecular pattern
DC	Dendritic cell
DDX	DEAD-box helicase
DENV2	Dengue virus 2
DHX	DEAH-box helicase
D-loop	Displacement loop
DUBA	Deubiquitinating enzyme A
ER	Endoplasmic reticulum
ERGIC	ER-golgi intermediate compartment
ESR1	Estrogen receptor 1
FcRγ	Fc receptor gamma-chain
FIIND	Function-to-find domain
FOXO3	Forkhead box O3
GBP	Guanylate-binding protein
HCV	Hepatitis C virus

HFF	Human foreskin fibroblasts
IAP	Inhibitor of apoptosis protein
IFI16	Interferon gamma inducible protein 16
IFIT	Interferon induced protein with tetratricopeptide repeats
IFN	Interferon
IFNAR	IFNαβ receptor
IFNB1	Interferon beta 1
IFNL1	Interferon lambda 1
IκB	Inhibitor of NF-κB
IKK	IκB kinase
IL	Interleukin
IL-1R	Interleukin-1 receptor
IL-1RAcP	Interleukin-1 receptor accessory protein
IL1RN	Interleukin-1 receptor antagonist
IRAK1	IL-1R-associated kinase 1
IRF	Interferon regulatory factor
IRGB10	Immunity-related GTPase family member B10
ISG	Interferon-stimulated gene
ISGF3	ISG factor 3 gamma
ISRE	IFN-sensitive response element
ITAM	Immunoreceptor tyrosine-based activation motif
JAK	Janus kinase
LC3	Microtubule-associated proteins 1A/1B light chain 3A
LPS	Lipopolysaccharide
LRR	Leucine-rich repeat
MAPK	Mitogen-activated protein kinase
MAVS	Mitochondrial antiviral signaling protein
MDA5	Melanoma differentiation-associated protein 5
MMP	Mitochondrial membrane potential
moDC	Monocyte-derived dendritic cell
MOMP	Mitochondrial outer membrane permeabilization
MT-ATP6	Mitochondrially encoded ATP synthase membrane subunit 6
Mtb	Mycobacterium tuberculosis
MT-CO2	Mitochondrially encoded cytochrome c oxidase II
mtDNA	Mitochondrial DNA
MTG	MitoTracker Green
MT-ND1	Mitochondrially encoded NADH:Ubiquinone oxidoreductase core subunit 1
MX1	Myxovirus dynamin like GTPase 1
MyD88	Myeloid differentiation factor 88
NAD(P)H	Nitrate reductase
NEMO	NF-κB essential modulator
NF-kB	Nuclear factor kappa B
NLR	NOD- and LRR-containing receptor

NLRC	NLR family CARD domain containing
NLRP	NLR family pyrin domain containing
NOD	Nucleotide-binding oligomerization domain
NR3C1	Nuclear Receptor Subfamily 3 Group C Member 1
PAMP	Pathogen-associated molecular pattern
PGE2	Prostaglandin E2
PI3K	Phosphoinositide-3-Kinase
Poly(I:C)	Polyinosinic-polycytidylic acid
PRR	Pattern recognition receptor
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
RD	Repressor domain
redox	Reduction-oxidation
RIG-I	Retinoic acid-inducible gene I
RLR	RIG-I-like receptor
RNA-seq	RNA sequencing
ROS	Reactive oxygen species
RPL13A	Ribosomal protein L13a
scRNA-seq	Single cell RNA sequencing
SOCS	Supressor of cytokine signaling
STAT	Signal transducer and activator of transcription
STING	Stimulator of interferon genes
TAB	TAK1-binding protein
TAK1	TGFβ-activated kinase 1
TANK	TRAF family member-associated NF-κB activator
TBK1	TANK-binding kinase 1
TGF	Transforming growth factor
TIR	Toll/IL-1 receptor
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TOM20	Translocase of outer mitochondrial membrane 20
TRAF	TNF receptor associated factor
TRIF	Toll/IL-1 receptor domain-containing adaptor inducing IFNβ
VDAC	Voltage dependent anion channel 1
WNV	West Nile virus
WT	Wildtype

6. References

Aarreberg, L.D., C. Wilkins, H. Ramos, R. Green, M.A. Davis, K. Chow, M. Gale Jr. (2018). Interleukin-1β Signaling in Dendritic Cells Induces Antiviral Interferon Responses. MBio *9*, e00342-00318.

Abe, T., G.N. Barber (2014). Cytosolic-DNA-Mediated, STING-Dependent Proinflammatory Gene Induction Necessitates Canonical NF-κB Activation through TBK1. J Virol *88*, 5328-5341.

Ablasser, A., J. L. Schmid-Burgk, I. Hemmerling, G. L. Horvath, T. Schmidt, E. Latz, V. Hornung (2013). Cell intrinsic immunity spreads to bystander cells via the intercellular transfer of cGAMP. Nature *503*, 530-534.

Aguirre, S., A. Fernandez-Sesma (2017a). Collateral Damage during Dengue Virus Infection: Making Sense of DNA by cGAS. J Virol *91*, e01081-01016.

Aguirre, S., P. Luthra, M.T. Sanchez-Aparicio, A.M. Maestre, J. Patel, F. Lamothe, A.C. Fredericks, S. Tripathi, T. Zhu, J. Pintado-Silva et al. (2017b). Dengue virus NS2B protein targets cGAS for degradation and prevents mitochondrial DNA sensing during infection. Nat Microbiol *2*.

Ahn, J., D. Gutman, S. Saijo, G.N. Barber (2012). STING manifests self DNA-dependent inflammatory disease. Proc Natl Acad Sci *109*, 19386-19391.

Akira, S., K. Takeda, and T. Kaisho (2001). Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol *2*, 675-680.

Allen, I.C., J.E. Wilson, M. Schneider, J.D. Lich, R.A. Roberts, J.C. Arthur, R.M. Woodford, B.K. Davis, J.M. Uronis, H.H. Herfarth, C. Jobin, A.B. Rogers, J.P. Ting (2012). NLRP12 suppresses colon inflammation and tumorigenesis through the negative regulation of noncanonical NF-κB signaling. Immunity *36*, 742-754.

Almine, J.F., C.A. O'Hare, G. Dunphy, I.R. Haga, R.J. Naik, A. Atrih, D.J. Connolly, J. Taylor, I.R. Kelsall, A.G. Bowie, P.M. Beard, L. Unterholzner (2017). IFI16 and cGAS cooperate in the activation of STING during DNA sensing in human keratinocytes. Nat Commun *8*.

Anand, P.K., R.K. Malireddi, J.R. Lukens, P. Vogel, J. Bertin, M. Lamkanfi, T.D. Kanneganti (2012). NLRP6 negatively regulates innate immunity and host defence against bacterial pathogens. Nature *488*, 389-393.

Baker, B., U. Maitra, S. Geng, L. Li (2014). Molecular and Cellular Mechanisms Responsible for Cellular Stress and Low-grade Inflammation Induced by a Super-low Dose of Endotoxin. J Biol Chem *289*, 16262-16269.

Basu, A., J.K. Krady, and S.W. Levison (2004). Interleukin-1: a master regulator of neuroinflammation. J Neurosci Res *78*, 151-156.

Beg, A.A., T.S. Finco, P.V. Nantermet, A.S. Baldwin Jr. (1993). Tumor necrosis factor and interleukin-1 lead to phosphorylation and loss of I kappa B alpha: a mechanism for NF-kappa B activation. Mol Cell Biol *13*, 3301-3310.

Ben-Sasson, S.Z., Caucheteux, S., Crank, M., Hu-Li, J., and Paul, W.E. (2011). IL-1 acts on T cells to enhance the magnitude of in vivo immune responses. Cytokine *56*, 122-125.

Bonizzi, G., J. Piette, M.P. Merville, V. Bours (2000). Cell type-specific role for reactive oxygen species in nuclear factor-kappaB activation by interleukin-1. Biochem Pharmacol *59*, 7-11.

Boyden, E.D., W.F. Dietrich (2006). Nalp1b controls mouse macrophage susceptibility to anthrax lethal toxin. Nat Genet *38*, 240-244.

Brierley, M.M., E.N. Fish (2002). Review: IFN-alpha/beta receptor interactions to biologic outcomes: understanding the circuitry. J Interferon Cytokine Res *22*, 835-845.

Brigelius-Flohé, R., A. Banning, M. Kny, G.F. Böl (2004). Redox events in interleukin-1 signaling. Arch Biochem Biophys *423*, 66-73.

Brubaker, S.W., K.S. Bonham, I. Zanoni, J.C. Kagan (2015). Innate Immune Pattern Recognition: A Cell Biological Perspective. Annu Rev Immunol *33*, 257-290.

Bürckstümmer, T., C. Baumann, S. Blüml, E. Dixit, G. Dürnberger, H. Jahn, M. Planyavsky, M. Bilban, J. Colinge, K.L. Bennett, G. Superti-Furga (2009). An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. Nat Immunol *10*, 266-272.

Burdette, D.L., K.M. Monroe, K. Sotelo-Troha, J.S. Iwig, B. Eckert, M. Hyodo, Y. Hayakawa, R.E. Vance (2011). STING is a direct innate immune sensor of cyclic di-GMP. Nature *478*, 515-518.

Butler, A., P. Hoffman, P. Smibert, E. Papalexi, R. Satija (2018). Integrating single-cell transcriptomic data across different conditions, technologies, and species. Nat Biotechnol *36*, 411-420.

Cao, Z., J. Xiong, M. Takeuchi, T. Kurama, D.V. Goeddel (1996). TRAF6 is a signal transducer for interleukin-1. Nature *383*, 443-446.

Chandel, N.S., P.T. Schumacker (1999). Cells depleted of mitochondrial DNA (p0) yield insight into physiological mechanisms. FEBS Letters *454*, 173-176.

Chang, D.M., M.F. Shaio (1994). Production of Interleukin-1 (IL-1) and IL-1 Inhibitor by Human Monocytes Exposed to Dengue Virus. J Infect Dis *170*, 811-817.

Chatzinikolaou, G., I. Karakasilioti, G.A. Garinis (2014). DNA damage and innate immunity: links and trade-offs. Trends Immunol *35*, 429-435.

Cheeran, M.C., S. Hu, G. Gekker, J.R. Lokensgard (2000). Decreased cytomegalovirus expression following proinflammatory cytokine treatment of primary human astrocytes. J Immunol *164*, 926-933.

Chen, E.Y., C.M. Tan, Y. Kou, Q. Duan, Z. Wang, G.V. Meirelles, N.R. Clark, A. Ma'ayan (2013). Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics *14*, 128.

Chen, M., Q. Meng, Y. Qin, P. Liang, P. Tan, L. He, Y. Zhou, Y. Chen, J. Huang, R.F. Wang, J. Cui (2016). TRIM14 Inhibits cGAS Degradation Mediated by Selective Autophagy Receptor p62 to Promote Innate Immune Responses. Mol Cell *64*, 105-119.

Chiliveru, S., S.H. Rahbek, S.K. Jensen, S.E. Jørgensen, S.K. Nissen, S.H. Christiansen, T.H. Mogensen, M.R. Jakobsen, L. Iversen, C. Johansen, S.R. Paludan (2014). Inflammatory Cytokines Break Down Intrinsic Immunological Tolerance of Human Primary Keratinocytes

to Cytosolic DNA. J Immunol *192*, 2395-2404.

Chun, K.H., S.Y. Seong (2010). CD14 but not MD2 transmit signals from DAMP. Int Immunopharmacol *10*, 98-106.

Clark, K., L. Plater, M. Peggie, P. Cohen (2009). Use of the pharmacological inhibitor BX795 to study the regulation and physiological roles of TBK1 and IkappaB kinase epsilon: a distinct upstream kinase mediates Ser-172 phosphorylation and activation. J Biol Chem *284*, 14136-14146.

Clark, K., M. Peggie, L. Plater, R.J. Sorcek, E.R. Young, J.B. Madwed, J. Hough, E.G. McIver, and P. Cohen (2011). Novel cross-talk within the IKK family controls innate immunity. Biochem J *434*, 93-104.

Cline, M.S., Smoot, M., Cerami, E., Kuchinsky, A., Landys, N., Workman, C., Christmas, R., Avila-Campilo, I., Creech, M., Gross, B.*, et al.* (2007). Integration of biological networks and gene expression data using Cytoscape. Nat Protoc *2*, 2366-2382.

Copenhaver, A.M., C.N. Casson, H.T. Nguyen, M.M. Duda and S. Shin (2015). IL-1R signaling enables bystander cells to overcome bacterial blockade of host protein synthesis. Proc Natl Acad Sci *112*, 7557-7562.

Costa Franco, M.M., F. Marim, E.S. Guimarães, N.R.G. Assis, D.M. Cerqueira, J. Alves-Silva, J. Harms, G. Splitter, J. Smith, T.D. Kanneganti, N.M.G.P. de Queiroz, D. Gutman, G.N. Barber, S.C. Oliveira (2018). Brucella abortus Triggers a cGAS-Independent STING Pathway To Induce Host Protection That Involves Guanylate-Binding Proteins and Inflammasome Activation. J Immunol *200*, 607-622.

Coulombe, F., J. Jaworska, M. Verway, F. Tzelepis, A. Massoud, J. Gillard, G. Wong, G. Kobinger, Z. Xing, C. Couture, P. Joubert, J.H. Fritz, W.S. Powell, and M. Divangahi (2014). Targeted prostaglandin E2 inhibition enhances antiviral immunity through induction of type I interferon and apoptosis in macrophages. Immunity *40*, 554-568.

Couper K.N., D.G.B., and E.M. Riley (2008). IL-10: The Master Regulator of Immunity to Infection. J Immunol *180*, 5771-5777.

Crow, Y.J. (2011). Type I interferonopathies: a novel set of inborn errors of immunity. Ann N Y Acad Sci *1238*, 91-98.

Crowl, J.T., E.E. Gray, K. Pestal, H.E. Volkman, D.B. Stetson (2017). Intracellular Nucleic Acid Detection in Autoimmunity. Annu Rev Immunol *35*, 313-336.

Das, S., Mishra, M.K., Ghosh, J., and Basu, A. (2008). Japanese Encephalitis Virus infection induces IL-18 and IL-1beta in microglia and astrocytes: correlation with in vitro cytokine responsiveness of glial cells and subsequent neuronal death. J Neuroimmunol *195*, 60-72.

Davis, L.E., DeBiasi, R., Goade, D.E., Haaland, K.Y., Harrington, J.A., Harnar, J.B., Pergam, S.A., King, M.K., DeMasters, B.K., and Tyler, K.L. (2006). West Nile virus neuroinvasive disease. Ann Neurol *60*, 286-300.

De Nardo, D. (2015). Toll-like receptors: Activation, signalling and transcriptional modulation. Cytokine *74*, 181-189.

DeFilippis, V.R., D. Alvarado, T. Sali, S. Rothenburg, K. Früh K (2010). Human cytomegalovirus induces the interferon response via the DNA sensor ZBP1. J Virol *84*, 585-598.

Deretic, V., T. Saitoh, S. Akira (2013). Autophagy in infection, inflammation and immunity. Nat Rev Immunol *13*, 722-737.

DiDonato, J.A., M. Hayakawa, D.M. Rothwarf, E. Zandi, and M. Karin (1997). A cytokine-responsive IkappaB kinase that activates the transcription factor NF-kappaB. Nature *388*, 548-554.

Dinarello, C.A. (1996). Biologic basis for interleukin-1 in disease. Blood *87*, 2095-2147.

Dinarello, C.A. (2009a). Immunological and inflammatory functions of the interleukin-1 family. Annu Rev Immunol *27*, 519-550.

Dinarello, C.A. (2009b). Interleukin-1beta and the autoinflammatory diseases. N Engl J Med *360*, 2467-2470.

Dinarello, C.A. (2011). A clinical perspective of IL-1β as the gatekeeper of inflammation. Eur J Immunol *41*, 1203-1217.

Dobbs, N., N. Burnaevskiy, D. Chen, V.K. Gonugunta, N.M. Alto, N. Yan (2015). STING Activation by Translocation from the ER Is Associated with Infection and Autoinflammatory Disease. Cell Host Microbe *18*, 157-168.

Durrant, D.M., M.L. Robinette, and R.S. Klein (2013). IL-1R1 is required for dendritic cell-mediated T cell reactivation within the CNS during West Nile virus encephalitis. J Exp Med *210*, 503-516.

Errett, J.S., Suthar, M.S., McMillan, A., Diamond, M.S., and Gale, M., Jr. (2013). The essential, nonredundant roles of RIG-I and MDA5 in detecting and controlling West Nile virus infection. J Virol *87*, 11416-11425.

Evavold, C.L., J.C. Kagan (2018). How Inflammasomes Inform Adaptive Immunity. J Mol Biol *430*, 217-237.

Faustin, B., L. Lartigue, J.M. Bruey, F. Luciano, E. Sergienko, B. Bailly-Maitre, N. Volkmann, D. Hanein, I. Rouiller, J.C. Reed (2007). Reconstituted NALP1 inflammasome reveals two-step mechanism of caspase-1 activation. Mol Cell *25*, 713-724.

Fernandes-Alnemri, T., J.W. Yu, P. Datta, J. Wu, E.S. Alnemri (2009). AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. Nature *458*, 509-513.

Fitzgerald, K.A., D.C. Rowe, B.J. Barnes, D.R. Caffrey, A. Visintin, E. Latz, B. Monks, P.M. Pitha, D.T. Golenbock (2003a). LPS-TLR4 signaling to IRF-3/7 and NF-kappaB involves the toll adaptors TRAM and TRIF. J Exp Med *198*, 1043-1055.

Fitzgerald, K.A., S.M. McWhirter, K.L. Faia, D.C. Rowe, E. Latz, D.T. Golenbock, A.J. Coyle, S.M. Liao, and T. Maniatis (2003b). IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway. Nat Immunol *4*, 491-496.

Fredericksen, B.L., Keller, B.C., Fornek, J., Katze, M.G., and Gale, M., Jr. (2008). Establishment and maintenance of the innate antiviral response to West Nile Virus involves both RIG-I and MDA5 signaling through IPS-1. J Virol *82*, 609-616.

Fremond, C.M., D. Togbe, E. Doz, S. Rose, V. Vasseur, I. Maillet, M. Jacobs, B. Ryffel, V.F. Quesniaux (2007). IL-1 receptor-mediated signal is an essential component of MyD88-dependent innate response to Mycobacterium tuberculosis infection. J Immunol *179*, 1178-1189.

Frey, H., N. Schroeder, T. Manon-Jensen, R.V. Iozzo, L. Schaefer (2013). Biological interplay between proteoglycans and their innate immune receptors in inflammation. FEBS J *280*, 2165-2179.

Fujita, T., L.F. Reis, N. Watanabe, Y. Kimura, T. Taniguchi, and J. Vilcek. (1989). Induction of the transcription factor IRF-1 and interferon-beta mRNAs by cytokines and activators of second-messenger pathways. Proc Natl Acad Sci *86*, 9936-9940.

Fullam, A., M. Schröder (2013). DExD/H-box RNA helicases as mediators of anti-viral innate immunity and essential host factors for viral replication. Biochim Biophys Acta *1829*, 854-865.

Gall, A., P. Treuting, K.B. Elkon, Y.M. Loo, M. Gale Jr, G.N. Barber, D.B. Stetson (2012). Autoimmunity initiates in nonhematopoietic cells and progresses via lymphocytes in an interferon-dependent autoimmune disease. Immunity *36*, 120-121.

Gao, D., J. Wu, Y.T. Wu, F. Du, C. Aroh, N. Yan, L. Sun, Z.J. Chen (2013). Cyclic GMP-AMP synthase is an innate immune sensor of HIV and other retroviruses. Science *341*, 903-906.

Gao, D., T. Li, X.D. Li, X. Chen, Q.Z. Li, M. Wight-Carte, Z.J. Chen (2015). Activation of cyclic GMP-AMP synthase by self-DNA causes autoimmune diseases. Proc Natl Acad Sci *112*, E5699-5705.

Geijtenbeek, T.B., S.I. Gringhuis (2009). Signalling through C-type lectin receptors: shaping immune responses. Nat Rev Immunol *9*, 465-479.

Gentleman, R.C., Carey, V.J., Bates, D.M., Bolstad, B., Dettling, M., Dudoit, S., Ellis, B., Gautier, L., Ge, Y., Gentry, J.*, et al.* (2004). Bioconductor: open software development for computational biology and bioinformatics. Genome Biol *5*, R80.

Gonzalez-Navajas, J.M., Law, J., Nguyen, K.P., Bhargava, M., Corr, M.P., Varki, N., Eckmann, L., Hoffman, H.M., Lee, J., and Raz, E. (2010). Interleukin 1 receptor signaling regulates DUBA

expression and facilitates Toll-like receptor 9-driven antiinflammatory cytokine production. J Exp Med *207*, 2799-2807.

Goodridge, H.S., C.N. Reyes, C.A. Becker, T.R. Katsumoto, J. Ma, A.J. Wolf, N. Bose, A.S. Chan, A.S. Magee, M.E. Danielson, A. Weiss, J.P. Vasilakos, D.M. Underhill (2011). Activation of the innate immune receptor Dectin-1 upon formation of a 'phagocytic synapse'. Nature *472*, 471-475.

Goritzka, M., Durant, L.R., Pereira, C., Salek-Ardakani, S., Openshaw, P.J., and Johansson, C. (2014). Alpha/Beta Interferon Receptor Signaling Amplifies Early Proinflammatory Cytokine Production in the Lung during Respiratory Syncytial Virus Infection. J Virol *88*, 6128-6136.

Grandvaux, N., M.J. Servant, B. tenOever, G.C. Sen, S. Balachandran, G.N. Barber, R. Lin, and J. Hiscott. (2002). Transcriptional profiling of interferon regulatory factor 3 target genes: direct involvement in the regulation of interferon-stimulated genes. J Virol *76*, 5532-5539.

Grandvaux N., M.J.S., B. tenOever, G.C. Sen, S. Balachandran, G.N. Barber, R. Lin R, and J. Hiscott. (2002). Transcriptional profiling of interferon regulatory factor 3 target genes: direct involvement in the regulation of interferon-stimulated genes. J Virol *76*, 5532-5539.

Gray, E.E., P.M. Treuting, J.J. Woodward, D.B. Stetson (2015). Cutting Edge: cGAS Is Required for Lethal Autoimmune Disease in the Trex1-Deficient Mouse Model of Aicardi-Goutières Syndrome. J Immunol *195*, 1939-1943.

Greenfeder S.A., P.N., L. Kwee, M. Labow, R.A. Chizzonite, G. Ju (1995). Molecular Cloning and Characterization of a Second Subunit of the Interleukin 1 Receptor Complex. J Biol Chem *270*, 13757-13765.

Guarda, G., Braun, M., Staehli, F., Tardivel, A., Mattmann, C., Forster, I., Farlik, M., Decker, T., Du Pasquier, R.A., Romero, P.*, et al.* (2011). Type I interferon inhibits interleukin-1 production and inflammasome activation. Immunity *34*, 213-223.

Gubler, D.J. (2007). The continuing spread of West Nile virus in the western hemisphere. Clin Infect Dis *45*, 1039-1046.

Guo, J., K.L. Peters, and G.C. Sen. (2000). Induction of the human protein P56 by interferon, double-stranded RNA, or virus infection. Virology *267*, 209-219.

Häcker, H., M. Karin (2006). Regulation and function of IKK and IKK-related kinases. Sci STKE *2006*, re13.

Hacker, H., Redecke, V., Blagoev, B., Kratchmarova, I., Hsu, L.C., Wang, G.G., Kamps, M.P., Raz, E., Wagner, H., Hacker, G.*, et al.* (2006). Specificity in Toll-like receptor signalling through distinct effector functions of TRAF3 and TRAF6. Nature *439*, 204-207.

Hagenbuchner, J., M.J. Ausserlechner (2013). Mitochondria and FOXO3: breath or die. Front Physiol *4*.

Hall, J.C., A. Rosen (2010). Type I interferons: crucial participants in disease amplification in autoimmunity. Nat Rev Rheumatol *6*, 40-49.

Harada, H., T. Fujita, M. Miyamoto, Y. Kimura, M. Maruyama, A. Furia, T. Miyata and T. Taniguchi (1989). Structurally similar but functionally distinct factors, IRF-1 and IRF-2, bind to the same regulatory elements of IFN and IFN-inducible genes. Cell *58*, 729-739.

Hashiguchi, K., Q.M. Zhang-Akiyama (2009). Establishment of human cell lines lacking mitochondrial DNA. Methods Mol Biol *554*, 383-391.

Hayes, E.B., Komar, N., Nasci, R.S., Montgomery, S.P., O'Leary, D.R., and Campbell, G.L. (2005). Epidemiology and transmission dynamics of West Nile virus disease. Emerg Infect Dis *11*, 1167-1173.

Hemann, E.A., M. Gale Jr., R. Savan (2017). Interferon Lambda Genetics and Biology in Regulation of Viral Control. Front Immunol *8*.

Hirahara, K., A. Onodera, A.V. Villarino, M. Bonelli, G. Sciumè, A. Laurence, H.W. Sun, S.R. Brooks, G. Vahedi, H.Y. Shih, G. Gutierrez-Cruz, S. Iwata, R. Suzuki, Y. Mikami, Y. Okamoto, T. Nakayama, S.M. Holland, C.A. Hunter, Y. Kanno, J.J. O'Shea (2015). Asymmetric Action of STAT Transcription Factors Drives Transcriptional Outputs and Cytokine Specificity. Immunity *42*, 877-889.

Hisaeda, K., Inokuchi, A., Nakamura, T., Iwamoto, Y., Kohno, K., Kuwano, M., and Uchiumi, T. (2004). Interleukin-1beta represses MRP2 gene expression through inactivation of interferon regulatory factor 3 in HepG2 cells. Hepatology *39*, 1574-1582.

Holm, C.K., S.H. Rahbek, H.H. Gad, R.O. Bak, M.R. Jakobsen, Z. Jiang, A.L. Hansen, S.K. Jensen, C. Sun, M.K. Thomsen, A. Laustsen, C.G. Nielsen, K. Severinsen, Y. Xiong, D.L. Burdette, V. Hornung, R.J. Lebbink, M. Duch, K.A. Fitzgerald, S. Bahrami, J.G. Mikkelsen, R. Hartmann, S.R. Paludan (2016). Influenza A virus targets a cGAS-independent STING pathway that controls enveloped RNA viruses. Nat Commun *7*.

Honda, K., A. Takaoka, T. Taniguchi (2006). Type I Interferon Gene Induction by the Interferon Regulatory Factor Family of Transcription Factors. Immunity *25*, 849.

Honda, K., Y. Ohba, H. Yanai, H. Negishi, T. Mizutani, A. Takaoka, C. Taya, T. Taniguchi (2005). Spatiotemporal regulation of MyD88-IRF-7 signalling for robust type-I interferon induction. Nature *434*, 1035-1040.

Honda K., H.Y., H. Negishi, M. Asagiri, M. Sato, T. Mizutani, N. Shimada, Y. Ohba, A. Takaoka, N. Yoshida, and T. Taniguchi (2005). IRF-7 is the master regulator of type-I interferon-dependent immune responses. Nature *434*, 772-777.

Hornung, V., A. Ablasser, M. Charrel-Dennis, F. Bauernfeind, G. Horvath, D.R. Caffrey, E. Latz, K.A. Fitzgerald (2009). AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature *458*, 514-518.

Hornung, V., J. Ellegast, S. Kim, K. Brzózka, A. Jung, H. Kato, H. Poeck, S. Akira, K.K. Conzelmann, M. Schlee, S. Endres, G. Hartmann (2006). 5'-Triphosphate RNA Is the Ligand for RIG-I. Science *314*, 994-997.

Hu, M.M., Q. Yang, X.Q. Xie, C.Y. Liao, H. Lin, T.T. Liu, L. Yin, H.B. Shu (2016). Sumoylation Promotes the Stability of the DNA Sensor cGAS and the Adaptor STING to Regulate the Kinetics of Response to DNA Virus. Immunity *45*, 555-569.

Hu, X., Ho, H.H., Lou, O., Hidaka, C., and Ivashkiv, L.B. (2005). Homeostatic role of interferons conferred by inhibition of IL-1-mediated inflammation and tissue destruction. J Immunol *175*, 131-138.

Huang, J., X. Gao, S. Li, Z. Cao (1997). Recruitment of IRAK to the interleukin 1 receptor complex requires interleukin 1 receptor accessory protein. Proc Natl Acad Sci *94*, 12829-12832.

Ichikawa, T., Nakao, K., Nakata, K., Yamashita, M., Hamasaki, K., Shigeno, M., Abiru, S., Ishikawa, H., Ishii, N., and Eguchi, K. (2002). Involvement of IL-1beta and IL-10 in IFN-alpha-mediated antiviral gene induction in human hepatoma cells. Biochem Biophys Res Commun *294*, 414-422.

Ichim, G., J. Lopez, S.U. Ahmed, N. Muthalagu, E. Giampazolias, M.E. Delgado, M. Haller, J.S. Riley, S.M. Mason, D. Athineos et al. (2015). Limited mitochondrial permeabilization causes DNA damage and genomic instability in the absence of cell death. Mol Cell *57*, 860-872.

Ichinohe, T., H.K. Lee, Y. Ogura, R. Flavell, A. Iwasaki (2009). Inflammasome recognition of influenza virus is essential for adaptive immune responses. J Exp Med *206*, 79-87.

Ishii, K.J., T. Kawagoe, S. Koyama, K. Matsui, H. Kumar, T. Kawai, S. Uematsu, O. Takeuchi, F. Takeshita, C. Coban, S. Akira (2008). TANK-binding kinase-1 delineates innate and adaptive immune responses to DNA vaccines. Nature *451*, 725-729.

Ishikawa, H., Z. Ma, G.N. Barber (2009). STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature *461*, 788-792.

Ishitani, T., G. Takaesu, J. Ninomiya-Tsuji, H. Shibuya, R.B. Gaynor, K. Matsumoto (2003). Role of the TAB2-related protein TAB3 in IL-1 and TNF signaling. EMBO J *22*, 6277-6288.

Ivashkiv, L.B., and Donlin, L.T. (2014). Regulation of type I interferon responses. Nat Rev Immunol *14*, 36-49.

Iwata, M., J. Vieira, M. Byrne, H. Horton, B. Torok-Storb (1999). Interleukin-1 (IL-1) Inhibits Growth of Cytomegalovirus in Human Marrow Stromal Cells: Inhibition Is Reversed Upon Removal of IL-1. Blood *94*, 572-578.

Jeremiah, N., B. Neven, M. Gentili, I. Callebaut, S. Maschalidi, M.C. Stolzenberg, N. Goudin, M.L. Frémond, P. Nitschke, T.J. Molina et al. (2014). Inherited STING-activating mutation underlies a familial inflammatory syndrome with lupus-like manifestations. J Clin Invest *124*, 5516-5520.

Jønsson, K.L., A. Laustsen, C. Krapp, K.A. Skipper, K. Thavachelvam, D. Hotter, J.H. Egedal, M. Kjolby, P. Mohammadi, P. Prabakaran, L.K. Sørensen, C. Sun, S.B. Jensen, C.K. Holm, R.J. Lebbink, M. Johannsen, M. Nyegaard, J.G. Mikkelsen, F. Kirchhoff, S.R. Paludan, M.R. Jakobsen (2017). IFI16 is required for DNA sensing in human macrophages by promoting production and function of cGAMP. Nat Commun *8*.

Jorgensen, I., E.A. Miao (2015). Pyroptotic cell death defends against intracellular pathogens. Immunol Rev *265*, 130-142.

Jorgensen, I., M. Rayamajhi, E.A. Miao (2017). Programmed cell death as a defence against infection. Nat Rev Immunol *17*, 151-164.

Kabeya, Y., N. Mizushima, T. Ueno, A. Yamamoto, T. Kirisako, T. Noda, E. Kominami, Y. Ohsumi, T. Yoshimori (2000). LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. EMBO J *19*, 5720-5728.

Kadowaki, N., S. Antonenko, J.Y. Lau, Y.J. Liu (2000). Natural interferon alpha/beta-producing cells link innate and adaptive immunity. J Exp Med *192*, 219-226.

Kagan, J.C., T. Su, T. Horng, A. Chow, S. Akira, R. Medzhitov (2008). TRAM couples endocytosis of Toll-like receptor 4 to the induction of interferon-beta. Nat Immunol *9*, 361-368.

Kang, D.C., R.V. Gopalkrishnan, L. Lin, A. Randolph, K. Valerie, S. Pestka, P.B. Fisher PB. (2004). Expression analysis and genomic characterization of human melanoma differentiation associated gene-5, mda-5: a novel type I interferon-responsive apoptosis-inducing gene. Oncogene *23*, 1789-1800.

Kanneganti, T.D. (2010). Central roles of NLRs and inflammasomes in viral infection. Nat Rev Immunol *10*, 688-698.

Karin, M., Y. Ben-Neriah (2000). Phosphorylation Meets Ubiquitination: The Control of NF-κB Activity. Annu Rev Immunol *18*, 621-663.

Katoh, M., B. Wu, H.B. Nguyen, T.Q. Thai, R. Yamasaki, H. Lu, A.M. Rietsch, M.M. Zorlu, Y. Shinozaki, Y. Saitoh, S. Saitoh, T. Sakoh, K. Ikenaka, S. Koizumi, R.M. Ransohoff, N. Ohno (2017). Polymorphic regulation of mitochondrial fission and fusion modifies phenotypes of microglia in neuroinflammation. Sci Rep *7*, 4942.

Kawai, T., K. Takahashi, S. Sato, C. Coban, H. Kumar, H. Kato, K.J. Ishii, O. Takeuchi, S. Akira (2005). IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. Nat Immunol *6*, 981-988.

Kawai, T., S. Sato, K.J. Ishii, C. Coban, H. Hemmi, M. Yamamoto, K. Terai, M. Matsuda, J. Inoue, S. Uematsu, O. Takeuchi, S. Akira (2004). Interferon-α induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. Nat Immunol *5*, 1061-1068.

Kelly, B., L.A. O'Neill (2015). Metabolic reprogramming in macrophages and dendritic cells in innate immunity. Cell Res *25*, 771-778.

Kim, B.H., A.R. Shenoy, P. Kumar, R. Das, S. Tiwari, J.D. MacMicking (2011). A family of IFN-γ-inducible 65-kD GTPases protects against bacterial infection. Science *332*, 717-721.

Kim, T., S. Pazhoor, M. Bao, Z. Zhang, S. Hanabuchi, V. Facchinetti, L. Bover, J. Plumas, L. Chaperot, J. Qin, Y.J. Liu (2010). Aspartate-glutamate-alanine-histidine box motif (DEAH)/RNA helicase A helicases sense microbial DNA in human plasmacytoid dendritic cells. Proc Natl Acad Sci *107*, 15181-15186.

King, N.J., Getts, D.R., Getts, M.T., Rana, S., Shrestha, B., and Kesson, A.M. (2007). Immunopathology of flavivirus infections. Immunol Cell Biol *85*, 33-42.

Kishore, N., Q.Y. Huynh, S. Mathialagan, T. Hall, S. Rouw, D. Creely, G. Lange, J. Caroll, B. Reitz, A. Donnelly et al. (2002). IKK-i and TBK-1 are enzymatically distinct from the homologous enzyme IKK-2: comparative analysis of recombinant human IKK-i, TBK-1, and IKK-2. J Biol Chem *277*, 13840-13847.

Klionsky, D.J., Abdalla, F. C., Abeliovich, H., Abraham, R. T., Acevedo-Arozena, A., Adeli, K., … Zuckerbraun, B. (2012). Guidelines for the use and interpretation of assays for monitoring autophagy. Autophagy *8*, 445-544.

Kofoed, E.M., R.E. Vance (2011). Innate immune recognition of bacterial ligands by NAIPs determines inflammasome specificity. Nature *477*, 592-595.

Konno, H., K. Konno, G.N. Barber (2013). Cyclic dinucleotides trigger ULK1 (ATG1) phosphorylation of STING to prevent sustained innate immune signaling. Cell *155*, 688-698.

Kowalinski, E., T. Lunardi, A.A. McCarthy, J. Louber, J. Brunel, B. Grigorov, D. Gerlier, S. Cusack (2011). Structural basis for the activation of innate immune pattern-recognition receptor RIG-I by viral RNA. Cell *147*, 423-435.

Kowaltowski, A.J., A.E. Vercesi (1999). Mitochondrial damage induced by conditions of oxidative stress. Free Radic Biol Med *26*, 463-471.

Krämer, A., J. Green, J. Pollard Jr, S. Tugendreich (2014). Causal analysis approaches in Ingenuity Pathway Analysis. Bioinformatics *30*, 523-530.

Krow-Lucal, E., N.P. Lindsey, J. Lehman, M. Fischer, and J.E. Staples (2017). West Nile Virus and Other Nationally Notifiable Arboviral Diseases - United States, 2015. MMWR Morb Mortal Wkly Rep *66*, 51-55.

Krysko, D.V., P. Agostinis, O. Krysko, A.D. Garg, C. Bachert, B.N. Lambrecht, P. Vandenabeele (2011). Emerging role of damage-associated molecular patterns derived from mitochondria in inflammation. Trends Immunol *32*, 157-164.

Latz, E., T.S. Xiao, A. Stutz (2013). Activation and regulation of the inflammasomes. Nat Rev Immunol *13*, 397-411.

Lazear, H.M., Daniels, B.P., Pinto, A.K., Huang, A.C., Vick, S.C., Doyle, S.E., Gale, M., Jr., Klein, R.S., and Diamond, M.S. (2015). Interferon-lambda restricts West Nile virus neuroinvasion by tightening the blood-brain barrier. Sci Transl Med *7*, 284ra259.

Lazear, H.M., Lancaster, A., Wilkins, C., Suthar, M.S., Huang, A., Vick, S.C., Clepper, L., Thackray, L., Brassil, M.M., Virgin, H.W.*, et al.* (2013). IRF-3, IRF-5, and IRF-7 coordinately regulate the type I IFN response in myeloid dendritic cells downstream of MAVS signaling. PLoS Pathog *9*, e1003118.

Lazear, H.M., Pinto, A.K., Vogt, M.R., Gale, M., Jr., and Diamond, M.S. (2011). Beta interferon controls West Nile virus infection and pathogenesis in mice. J Virol *85*, 7186-7194.

Lee, H.K., J.M. Lund, B. Ramanathan, N. Mizushima, A. Iwasaki (2007). Autophagy-dependent viral recognition by plasmacytoid dendritic cells. Science *315*, 1398-1401.

Li, X.D., J. Wu, D. Gao, H. Wang, L. Sun, Z.J. Chen (2013). Pivotal roles of cGAS-cGAMP signaling in antiviral defense and immune adjuvant effects. Science *341*, 1390-1394.

Lippmann, J., S. Rothenburg, N. Deigendesch, J. Eitel, K. Meixenberger, V. van Laak, H. Slevogt, P.D. N'guessan, S. Hippenstiel, T. Chakraborty, A. Flieger, N. Suttorp, B. Opitz (2008). IFNbeta responses induced by intracellular bacteria or cytosolic DNA in different human cells do not require ZBP1 (DLM-1/DAI). Cell Microbiol *10*, 2579-2588.

Liu, H.M., Y.M. Loo, S.M. Horner, G.A. Zornetzer, M.G. Katze, M. Gale Jr (2012). The mitochondrial targeting chaperone 14-3-3ε regulates a RIG-I translocon that mediates membrane association and innate antiviral immunity. Cell Host Microbe *11*, 528-537.

Liu, S., J. Chen, X. Cai, J. Wu, X. Chen, Y.T. Wu, L. Sun, Z.J. Chen (2013). MAVS recruits multiple ubiquitin E3 ligases to activate antiviral signaling cascades. eLife *2*.

Liu, S., X. Cai, J. Wu, Q. Cong, X. Chen, T. Li, F. Du, J. Ren, Y.T. Wu, N.V. Grishin, Z.J. Chen (2015). Phosphorylation of innate immune adaptor proteins MAVS, STING, and TRIF induces IRF3 activation. Science *347*, aaa2630.

Liu, Y., B. Gordesky-Gold, M. Leney-Greene, N.L. Weinbren, M. Tudor, S. Cherry (2018). Inflammation-induced, STING-dependent autophagy restricts Zika Virus Infection in the Drosophila Brain. Cell Host Microbe *24*, 57-68.

Loo, Y.M., M. Gale Jr. (2011). Immune signaling by RIG-I-like receptors. Immunity *34*, 680-692.

Lopalco, G., L. Cantarini, A. Vitale, F. Iannone, M.G. Anelli, L. Andreozzi, G. Lapadula, M. Galeazzi, D. Rigante (2015). Interleukin-1 as a common denominator from autoinflammatory to autoimmune disorders: premises, perils, and perspectives. Mediators Inflamm *2015*.

Lopez de Padilla, C.M., and Niewold, T.B. (2016). The type I interferons: Basic concepts and clinical relevance in immune-mediated inflammatory diseases. Gene *576*, 14-21.

Luo, D., S.C. Ding, A. Vela, A. Kohlway, B.D. Lindenbach, A.M. Pyle (2011). Structural insights into RNA recognition by RIG-I. Cell *147*, 409-422.

Lynn, D.J., Chan, C., Naseer, M., Yau, M., Lo, R., Sribnaia, A., Ring, G., Que, J., Wee, K., Winsor, G.L.*, et al.* (2010). Curating the innate immunity interactome. BMC Syst Biol *4*, 117.

Malik, A., T.D. Kanneganti (2017). Inflammasome activation and assembly at a glance. J Cell Sci *130*, 3955-3963.

Mamane, Y., C. Heylbroeck, P. Génin, M. Algarté, M.J. Servant, C. LePage, C. DeLuca, H. Kwon, R. Lin, and J. Hiscott (1999). Interferon regulatory factors: the next generation. Gene *237*, 1-14.

Man, S.M., R. Karki, M. Sasai, D.E. Place, S. Kesavardhana, J. Temirov, S. Frase, Q. Zhu, R.K.S. Malireddi, T. Kuriakose, J.L. Peters, G. Neale, S.A. Brown, M. Yamamoto, T.D. Kanneganti (2016). IRGB10 Liberates Bacterial Ligands for Sensing by the AIM2 and Caspase-11-NLRP3 Inflammasomes. Cell *167*, 382-396.

Man, S.M., R. Karki, R.K. Malireddi, G. Neale, P. Vogel, M. Yamamoto, M. Lamkanfi, T.D. Kanneganti (2015). The transcription factor IRF1 and guanylate-binding proteins target activation of the AIM2 inflammasome by Francisella infection. Nat Immunol *16*, 467-475.

Margolis, S.R., S.C. Wilson, R.E. Vance (2017). Evolutionary Origins of cGAS-STING Signaling. Trends Immunol *38*, 733-743.

Marichal, T., K. Ohata, D. Bedoret, C. Mesnil, C. Sabatel, K. Kobiyama, P. Lekeux, C. Coban, S. Akira, K.J. Ishii et al. (2011). DNA released from dying host cells mediates aluminum adjuvant activity. Nat Med *17*, 996-1002.

Marié, I., J.E. Durbin, D.E. Levy (1998). Differential viral induction of distinct interferon-α genes by positive feedback through interferon regulatory factor-7. EMBO J *17*, 6660-6669.

Martin, M.U., H. Wesche (2002). Summary and comparison of the signaling mechanisms of the Toll/interleukin-1 receptor family. Biochem Biophys Acta *1592*, 265-280.

Martinon, F., K. Burns, J. Tschopp (2002). The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell *10*, 417-426.

Mayer-Barber, K.D., and B Yan (2017). Clash of the Cytokine Titans: counter-regulation of interleukin-1 and type I interferon-mediated inflammatory responses. Cell Mol Immunol *14*, 22-35.

Mayer-Barber, K.D., Andrade, B.B., Barber, D.L., Hieny, S., Feng, C.G., Caspar, P., Oland, S., Gordon, S., and Sher, A. (2011). Innate and adaptive interferons suppress IL-1alpha and IL-1beta production by distinct pulmonary myeloid subsets during Mycobacterium tuberculosis infection. Immunity *35*, 1023-1034.

Mayer-Barber, K.D., B.B. Andrade, S.D. Oland, E.P. Amaral, D.L. Barber, J. Gonzales, S.C. Derrick, R. Shi, N.P. Kumar, W. Wei et al. (2014). Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. Nature *511*, 99-103.

McFarlane, S., J. Aitken, J.S. Sutherland, M.J. Nicholl, V.G. Preston, C.M. Preston (2011). Early induction of autophagy in human fibroblasts after infection with human cytomegalovirus or herpes simplex virus 1. J Virol *85*, 4212-4221.

Mempel, T.R., S.E. Henrickson, U.H. Von Andrian (2004). T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. Nature *427*, 154-159.

Mercurio, F., H. Zhu, B.W. Murray, A. Shevchenko, B.L. Bennett, J. Li, D.B. Young, M. Barbosa, M. Mann, A. Manning, A. Rao (1997). IKK-1 and IKK-2: Cytokine-Activated IκB Kinases Essential for NF-κB Activation. Science *278*, 860-866.

Meunier, E., M.S. Dick, R.F. Dreier, N. Schürmann, D. Kenzelmann Broz, S. Warming, M. Roose-Girma, D. Bumann, N. Kayagaki, K. Takeda, M. Yamamoto, P. Broz. (2014). Caspase-11 activation requires lysis of pathogen-containing vacuoles by IFN-induced GTPases. Nature *509*, 366-370.

Meunier, E., P. Wallet, R.F. Dreier, S. Costanzo, L. Anton, S. Rühl, S. Dussurgey, M.S. Dick, A. Kistner, M. Rigard, D. Degrandi, K. Pfeffer, M. Yamamoto, T. Henry, P. Broz (2015). Guanylate-

binding proteins promote activation of the AIM2 inflammasome during infection with Francisella novicida. Nat Immunol *16*, 476-484.

Mills, E.L., B. Kelly, A. Logan, A.S.H. Costa, M. Varma, C.E. Bryant, P. Tourlomousis, J.H.M. Däbritz, E. Gottlieb, I. Latorre, S.C. Corr, G. McManus, D. Ryan, H.T. Jacobs, M. Szibor, R.J. Xavier, T. Braun, C. Frezza, M.P. Murphy, L.A. O'Neill (2016). Succinate Dehydrogenase Supports Metabolic Repurposing of Mitochondria to Drive Inflammatory Macrophages. Cell *167*, 457-470.

Minamikawa, T., D.A. Williams, D.N. Bowser, P. Nagley (1999). Mitochondrial permeability transition and swelling can occur reversibly without inducing cell death in intact human cells. Exp Cell Res *246*, 26-37.

Moreira, L.O., D.S. Zamboni (2012). NOD1 and NOD2 Signaling in Infection and Inflammation. Front Immunol *3*.

Moretti J., S.R., D. Bozec, J. Martinez, J.R. Chapman, B. Ueberheide, D.W. Lamming, Z.J. Chen, T. Horng, G. Yeretssian, D.R. Green, J.M. Blander (2017). STING Senses Microbial Viability to Orchestrate Stress-Mediated Autophagy of the Endoplasmic Reticulum. Cell *171*, 808-823.

Mori, M., M. Yoneyama, T. Ito, K. Takahashi, F. Inagaki and T. Fujita. (2004). Identification of Ser-386 of interferon regulatory factor 3 as critical target for inducible phosphorylation that determines activation. J Biol Chem *279*, 9698-9702.

Muzio, M., J. Ni, P. Feng, V.M. Dixit (1997). IRAK (Pelle) Family Member IRAK-2 and MyD88 as Proximal Mediators of IL-1 Signaling. Science *278*, 1612-1615.

Nair, S., K.S. Sobotka, P. Joshi, P. Gressens, B. Fleiss, C. Thornton, C. Mallard, H. Hagberg (2019). Lipopolysaccharide-induced alteration of mitochondrial morphology induces a metabolic shift in microglia modulating the inflammatory response in vitro and in vivo. Glia.

Nakagawa, I., A. Amano, N. Mizushima, A. Yamamoto, H. Yamaguchi, T. Kamimoto, A. Nara, J. Funao, M. Nakata, K. Tsuda, S. Hamada, T. Yoshimori (2004). Autophagy defends cells against invading group A Streptococcus. Science *306*, 1037-1040.

Nakahira, K., S. Hisata, A.M. Choi (2015). The Roles of Mitochondrial Damage-Associated Molecular Patterns in Diseases. Antioxid Redox Signal *23*, 1329-1350.

Negash, A.A., Ramos, H.J., Crochet, N., Lau, D.T., Doehle, B., Papic, N., Delker, D.A., Jo, J., Bertoletti, A., Hagedorn, C.H.*, et al.* (2013). IL-1beta production through the NLRP3 inflammasome by hepatic macrophages links hepatitis C virus infection with liver inflammation and disease. PLoS Pathog *9*, e1003330.

Ninomiya-Tsuji, J., K. Kishimoto, A. Hiyama, J. Inoue, Z. Cao, K. Matsumoto (1999). The kinase TAK1 can activate the NIK-IκB as well as the MAP kinase cascade in the IL-1 signalling pathway. Nature *398*, 252-256.

Novikov, A., Cardone, M., Thompson, R., Shenderov, K., Kirschman, K.D., Mayer-Barber, K.D., Myers, T.G., Rabin, R.L., Trinchieri, G., Sher, A.*, et al.* (2011). Mycobacterium tuberculosis triggers host type I IFN signaling to regulate IL-1beta production in human macrophages. J Immunol *187*, 2540-2547.

O'Neill, L.A., A.G. Bowie (2007). The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat Rev Immunol *7*, 353-364.

Ohmori, Y., T.A. Hamilton (1995). The interferon-stimulated response element and a kappa B site mediate synergistic induction of murine IP-10 gene transcription by IFN-gamma and TNF-alpha. J Immunol *154*, 5235-5244.

Orzalli, M.H., A. Smith, K.A. Jurado, A. Iwasaki, J.A. Garlick, J.C. Kagan (2018). An antiviral branch of the IL-1 signaling pathway restricts immune-evasive virus replication. Mol Cell *71*, 825-540.

Oshiumi, H., M. Matsumoto, K. Funami, T. Akazawa, T. Seya (2003a). TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induction. Nat Immunol *4*, 161-167.

Oshiumi, H., M. Sasai, K. Shida, T. Fujita, M. Matsumoto, T. Seya (2003b). TIR-containing adapter molecule (TICAM)-2, a bridging adapter recruiting to toll-like receptor 4 TICAM-1 that induces interferon-beta. J Biol Chem *278*, 49751-49762.

Osterlund, P.I., T.E. Pietilä, V. Veckman, S.V. Kotenko, I. Julkunen (2007). IFN regulatory factor family members differentially regulate the expression of type III IFN (IFN-lambda) genes. J Immunol *179*, 3434-3442.

Paludan, S.R., A.G. Bowie (2013). Immune sensing of DNA. Immunity *38*, 870-880.

Pang, I.K., T. Ichinohe, A. Iwasaki (2013). IL-1R signaling in dendritic cells replaces pattern-recognition receptors in promoting $CD8^+$ T cell responses to influenza A virus. Nat Immunol *14*, 246-253.

Patin, E.C., A. Thompson, S.J. Orr (2018). Pattern recognition receptors in fungal immunity. Semin Cell Dev Biol *S1084-9521*, 30541-30544.

Paz, S., Q. Sun, P. Nakhaei, R. Romieu-Mourez, D. Goubau, I. Julkunen, R. Lin, J. Hiscott (2006). Induction of IRF-3 and IRF-7 phosphorylation following activation of the RIG-I pathway. Cell Mol Biol (Noisy-le-Grand) *52*, 17-28.

Perwitasari O., H.C., M.S. Diamond and M. Gale, Jr. (2011). Inhibitor of κB kinase epsilon (IKK(epsilon)), STAT1, and IFIT2 proteins define novel innate immune effector pathway against West Nile virus infection. J Biol Chem *286*, 44412-44423.

Pilli, M., J. Arko-Mensah, M. Ponpuak, E. Roberts, S. Master, M.A. Mandell, N. Dupont, W. Ornatowski, S. Jiang, S.B. Bradfute, J.A. Bruun, T.E. Hansen, T. Johansen, V. Deretic (2012). TBK-1 promotes autophagy-mediated antimicrobial defense by controlling autophagosome maturation. Immunity *37*, 223-234.

Pisetsky, D.S. (2014). The translocation of nuclear molecules during inflammation and cell death. Antioxid Redox Signal *20*, 1117-1125.

Podolin, P.L., J.F. Callahan, B.J. Bolognese, Y.H Li, K. Carlson, T.G. Davis, G.W. Mellor, C. Evans, and A.K. Roshak (2005). Attenuation of Murine Collagen-Induced Arthritis by a Novel, Potent, Selective Small Molecule Inhibitor of IκB Kinase 2, TPCA-1 (2-[(Aminocarbonyl)amino]-5-

(4-fluorophenyl)-3-thiophenecarboxamide), Occurs via Reduction of Proinflammatory Cytokines and Antigen-Induced T Cell Proliferation. J Pharmacol Exp Ther *312*, 373-381.

Pomerantz, J.L., D. Baltimore (1999). NF-kappaB activation by a signaling complex containing TRAF2, TANK and TBK1, a novel IKK-related kinase. EMBO J *18*, 6694-6704.

Prabakaran, T., C. Bodda, C. Krapp, B.C. Zhang, M.H. Christensen, C. Sun, L. Reinert, Y. Cai, S.B. Jensen, M.K. Skouboe, J.R. Nyengaard, C.B. Thompson, R.J. Lebbink, G.C. Sen, G. van Loo, R. Nielsen, M. Komatsu, L.N. Nejsum, M.R. Jakobsen, M. Gyrd-Hansen, S.R. Paludan (2018). Attenuation of cGAS-STING signaling is mediated by a p62/SQSTM1-dependent autophagy pathway activated by TBK1. EMBO J *37*.

Price, A.E., K. Shamardani, K.A. Lugo, J. Deguine, A.W. Roberts, B.L. Lee, G.M. Barton (2018). A Map of Toll-like Receptor Expression in the Intestinal Epithelium Reveals Distinct Spatial, Cell Type-Specific, and Temporal Patterns. Immunity *49*, 560-575.

R Development Core Team (2012). R: A Language and Environment for Statistical Computing (Vienna, Austria, R Foundation for Statistical Computing).

Ramos, H.J., M.C. Lanteri, G. Blahnik, A. Negash, M.S. Suthar, M.M. Brassil, K. Sodhi, P.M. Treuting, M.P. Busch, P.J. Norris, M. Gale, Jr. (2012). IL-1beta signaling promotes CNS-intrinsic immune control of West Nile virus infection. PLoS Pathog *8*, e1003039.

Randolph-Habecker, J., M. Iwata, A.P. Geballe, S. Jarrahian, B. Torok-Storb (2002). Interleukin-1-Mediated Inhibition of Cytomegalovirus Replication Is Due to Increased IFN-b Production. J Interferon Cytokine Res *22*, 765-772.

Rasmussen, S.B., K.A. Horan, C.K. Holm, A.J. Stranks, T.C. Mettenleiter, A.K. Simon, S.B. Jensen, F.J. Rixon, B. He, S.R. Paludan (2011). Activation of autophagy by α-herpesviruses in myeloid cells is mediated by cytoplasmic viral DNA through a mechanism dependent on stimulator of IFN genes. J Immunol *187*, 5268-5276.

Redford, P.S., Mayer-Barber, K.D., McNab, F.W., Stavropoulos, E., Wack, A., Sher, A., and O'Garra, A. (2014). Influenza A virus impairs control of Mycobacterium tuberculosis coinfection through a type I interferon receptor-dependent pathway. J Infect Dis *209*, 270-274.

Riley, J.S., G. Quarato, C. Cloix, J. Lopez, J. O'Prey, M. Pearson, J. Chapman, H. Sesaki, L.M. Carlin, J.F. Passos, A.P. Wheeler, A. Oberst, K.M. Ryan, S.W. Tait (2018). Mitochondrial inner membrane permeabilisation enables mtDNA release during apoptosis. EMBO J, e99238.

Rivieccio, M.A., G.R. John, X. Song, H.S. Suh, Y. Zhao, S.C. Lee, C.F. Brosnan (2005). The cytokine IL-1beta activates IFN response factor 3 in human fetal astrocytes in culture. J Immunol *174*, 3719-3726.

Rongvaux, A., R. Jackson, C.C. Harman, T. Li, A.P. West, M.R. de Zoete, Y. Wu, B. Yordy, S.A. Lakhani, C.Y. Kuan et al. (2014). Apoptotic caspases prevent the induction of type I interferons by mitochondrial DNA. Cell *159*, 1563-1577.

Saito, T., D.M. Owen, F. Jiang, J. Marcotrigiano, M. Gale Jr. (2008). Innate immunity induced by composition-dependent RIG-I recognition of hepatitis C virus RNA. Nature *454*, 523-527.

Saito, T., R. Hirai, Y.M. Loo, D. Owen, C.L. Johnson, S.C. Sinha, S. Akira, T. Fujita, M. Gale Jr (2007). Regulation of innate antiviral defenses through a shared repressor domain in RIG-I and LGP2. Proc Natl Acad Sci *104*, 582-587.

Saitoh, T., N. Fujita, T. Hayashi, K. Takahara, T. Satoh, H. Lee, K. Matsunaga, S. Kageyama, H. Omori, T. Noda et al. (2009). Atg9a controls dsDNA-driven dynamic translocation of STING and the innate immune response. Proc Natl Acad Sci *106*, 20842-20846.

Sallusto, F., M. Cella, C. Danieli, A. Lanzavecchia (1995). Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. J Exp Med *182*, 389-400.

Samuel, M.A., and Diamond, M.S. (2006). Pathogenesis of West Nile Virus infection: a balance between virulence, innate and adaptive immunity, and viral evasion. J Virol *80*, 9349-9360.

Sato, M., H. Suemori, N. Hata, M. Asagiri, K. Ogasawara, K. Nakao, T. Nakaya, M. Katsuki, S. Noguchi, N. Tanaka, T. Taniguchi (2000). Distinct and essential roles of transcription factors IRF-3 and IRF-7 in response to viruses for IFN-alpha/beta gene induction. Immunity *13*, 539-548.

Sato, M., N. Hata, M. Asagiri, T. Nakaya, T. Taniguchi, N. Tanaka (1998). Positive feedback regulation of type I IFN genes by the IFN-inducible transcription factor IRF-7. FEBS Letters *441*, 106-110.

Satoh, T., H. Kato, Y. Kumagai, M. Yoneyama, S. Sato, K. Matsushita, T. Tsujimura, T. Fujita, S. Akira, O. Takeuchi (2010). LGP2 is a positive regulator of RIG-I– and MDA5-mediated antiviral responses. Proc Natl Acad Sci *107*, 1512-1517.

Schaefer, L. (2014). Complexity of danger: the diverse nature of damage-associated molecular patterns. J Biol Chem *289*, 35237-35245.

Schmid, D., C. Munz (2007). Innate and adaptive immunity through autophagy. Immunity *27*, 11-21.

Schneider, W.M., M.D. Chevillotte, C.M. Rice (2014). Interferon-stimulated genes: a complex web of host defenses. Annu Rev Immunol *32*, 513-545.

Schoggins, J.W., D.A. MacDuff, N. Imanaka, M.D. Gainey, B. Shrestha, J.L. Eitson, K.B. Mar, R.B. Richardson, A.V. Ratushny, V. Litvak, R. Dabelic, B. Manicassamy, J.D. Aitchison, A. Aderem, R.M. Elliott, A. García-Sastre, V. Racaniello, E.J. Snijder, W.M. Yokoyama, M.S. Diamond, H.W. Virgin, C.M. Rice (2014). Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. Nature *505*, 691-695.

Sejvar, J.J., Haddad, M.B., Tierney, B.C., Campbell, G.L., Marfin, A.A., Van Gerpen, J.A., Fleischauer, A., Leis, A.A., Stokic, D.S., and Petersen, L.R. (2003). Neurologic manifestations and outcome of West Nile virus infection. JAMA *290*, 511-515.

Sellin, M.E., A.A. Müller, B. Felmy, T. Dolowschiak, M. Diard, A. Tardivel, K.M. Maslowski, W.D. Hardt (2014). Epithelium-intrinsic NAIP/NLRC4 inflammasome drives infected enterocyte expulsion to restrict Salmonella replication in the intestinal mucosa. Cell Host Microbe *16*, 237-248.

Sen, J.C. (2001). Viruses and interferons. Annu Rev Microbiol *55*, 255-281.

Seth, R.B., L. Sun, C.K. Ea, Z.J. Chen (2005). Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. Cell *122*, 669-682.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., and Ideker, T. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res *13*, 2498-2504.

Sharma, S., B.R. tenOever, N. Grandvaux, G.P. Zhou, R. Lin, and J Hiscott (2003). Triggering the Interferon Antiviral Response Through an IKK-Related Pathway. Science *300*, 1148-1151.

Shi, C.S., K. Shenderov, N.N. Huang, J. Kabat, M. Abu-Asab, K.A. Fitzgerald, A. Sher, J.H. Kehrl (2012). Activation of autophagy by inflammatory signals limits IL-1b production by targeting ubiquitinated inflammasomes for destruction. Nat Immunol *13*, 255-263.

Shimada, T., T. Kawai, K. Takeda, M. Matsumoto, J. Inoue, Y. Tatsumi, A. Kanamaru, S. Akira (1999). IKK-i, a novel lipopolysaccharide-inducible kinase that is related to IkappaB kinases. Int Immunol *11*, 1357-1362.

Shrestha, B., Zhang, B., Purtha, W.E., Klein, R.S., and Diamond, M.S. (2008). Tumor necrosis factor alpha protects against lethal West Nile virus infection by promoting trafficking of mononuclear leukocytes into the central nervous system. J Virol *82*, 8956-8964.

Sims, J.E., and D.E. Smith (2010). The IL-1 family: regulators of immunity. Nat Rev Immunol *10*, 89-102.

Singh, S.B., A.S. Davis, G.A. Taylor, V. Deretic (2006). Human IRGM induces autophagy to eliminate intracellular mycobacteria. Science *313*, 1438-1441.

Smyth, G.K. (2004). Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol *3*, Article3.

Stehlik, C., R. de Martin, I. Kumabashiri, J.A. Schmid, B.R. Binder, J. Lipp (1998). Nuclear factor (NF)-kappaB-regulated X-chromosome-linked iap gene expression protects endothelial cells from tumor necrosis factor alpha-induced apoptosis. J Exp Med *188*, 211-216.

Stetson, D.B., and R Medzhitov (2006). Type I Interferons in Host Defense. Immunity *25*, 373-381.

Sun, B., K.B. Sundström, J.J. Chew, P. Bist, E.S. Gan, H.C. Tan, K.C. Goh, T. Chawla, C.K. Tang, E.E. Ooi (2017). Dengue virus activates cGAS through the release of mitochondrial DNA. Sci Rep *7*, 3594.

Sun, L., J. Wu, F. Du, X. Chen, Z.J. Chen (2013). Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science *339*, 786-791.

Suthar, M.S., Brassil, M.M., Blahnik, G., McMillan, A., Ramos, H.J., Proll, S.C., Belisle, S.E., Katze, M.G., and Gale, M., Jr. (2013a). A systems biology approach reveals that tissue tropism to West Nile virus is regulated by antiviral genes and innate immune cellular processes. PLoS Pathog *9*, e1003168.

Suthar, M.S., Diamond, M.S., and Gale, M., Jr. (2013b). West Nile virus infection and immunity. Nat Rev Microbiol *11*, 115-128.

Suthar, M.S., Ma, D.Y., Thomas, S., Lund, J.M., Zhang, N., Daffis, S., Rudensky, A.Y., Bevan, M.J., Clark, E.A., Kaja, M.K.*, et al.* (2010). IPS-1 is essential for the control of West Nile virus infection and immunity. PLoS Pathog *6*, e1000757.

Suzuki, N., S. Suzuki, G.S. Duncan, D.G. Millar, T. Wada, C. Mirtsos, H. Takada, A. Wakeham, A. Itie, S. Li, J.M. Penninger, H. Wesche, P.S. Ohashi, T.W. Mak, W.C. Yeh (2002). Severe impairment of interleukin-1 and Toll-like receptor signalling in mice lacking IRAK-4. Nature *416*, 750-756.

Tait, S.W., D.R. Green (2013). Mitochondrial regulation of cell death. Cold Spring Harb Perspect Biol *5*.

Tait, S.W., M.J. Parsons, F. Llambi, L. Bouchier-Hayes, S. Connell, C. Muñoz-Pinedo, D.R. Green (2010). Resistance to caspase-independent cell death requires persistence of intact mitochondria. Dev Cell *18*, 802-813.

Takaesu, G., S. Kishida, A. Hiyama, K. Yamaguchi, H. Shibuya, K. Irie, J. Ninomiya-Tsuji, K. Matsumoto (2000). TAB2, a Novel Adaptor Protein, Mediates Activation of TAK1 MAPKKK by Linking TAK1 to TRAF6 in the IL-1 Signal Transduction Pathway. Mol Cell *5*, 649-658.

Takahama, M., S. Akira, T. Saitoh (2018). Autophagy limits activation of the inflammasomes. Immunol Rev *281*, 62-73.

Takaoka, A., Z. Wang, M.K. Choi, H. Yanai, H. Negishi, T. Ban, Y. Lu, M. Miyagishi, T. Kodama, K. Honda, Y. Ohba, T. Taniguchi (2007). DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. Nature *448*, 501-505.

Takeuchi, O., S. Akira (2010). Pattern Recognition Receptors and Inflammation. Cell *140*, 805-820.

Taniguchi, T., K. Ogasawara, A. Takaoka, and N. Tanaka (2001). IRF family of transcription factors as regulators of host defense. Annu Rev Immunol *19*, 623-655.

Tarassishin, L., Suh, H.S., and Lee, S.C. (2011). Interferon regulatory factor 3 plays an anti-inflammatory role in microglia by activating the PI3K/Akt pathway. J Neuroinflammation *8*, 187.

tenOever B.R., S.L.N., M.A. Chua, S.M. McWhirter, A. Garcia-Sastre and T. Maniatis (2007). Multiple functions of the IKK-related kinase IKKepsilon in interferon-mediated antiviral immunity. Science *315*, 1274-1278.

Thannickal , V.J., B.L. Fanburg (2000). Reactive oxygen species in cell signaling. Am J Physiol Lung Cell Mol Physiol *279*, L1005-1028.

Thanos, D., and T. Maniatis (1992). The high mobility group protein HMG I(Y) is required for NF-kappa B-dependent virus induction of the human IFN-beta gene. Cell *71*, 777-789.

Tirosh, I., B. Izar, S.M. Prakadan, M.H. Wadsworth 2nd, D. Treacy, J.J. Trombetta, A. Rotem, C. Rodman, C. Lian, G. Murphy et al. (2016). Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. Science *352*, 189-196.

Tseng, P.H., Matsuzawa, A., Zhang, W., Mino, T., Vignali, D.A., and Karin, M. (2010). Different modes of ubiquitination of the adaptor TRAF3 selectively activate the expression of type I interferons and proinflammatory cytokines. Nat Immunol *11*, 70-75.

Twig, G., A. Elorza, A.J. Molina, H. Mohamed, J.D. Wikstrom, G. Walzer, L. Stiles, S.E. Haigh, S. Katz, G. Las, J. Alroy, M. Wu, B.F. Py, J. Yuan, J.T. Deeney, B.E. Corkey, O.S. Shirihai (2008). Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. EMBO J *27*, 433-446.

Unterholzner, L. (2013). The interferon response to intracellular DNA: Why so many receptors? Immunobiology *218*, 1312-1321.

Unterholzner, L., S.E. Keating, M. Baran, K.A. Horan, S.B. Jensen, S. Sharma, C.M. Sirois, T. Jin, E. Latz, T.S. Xiao, K.A. Fitzgerald, S.R. Paludan, A.G. Bowie (2010). IFI16 is an innate immune sensor for intracellular DNA. Nat Immunol *11*, 997-2004.

Van Damme, J., A. Billiau, M. De Ley, P. De Somer (1983). An interferon-beta-like or interferon-inducing protein released by mitogen-stimulated human leukocytes. J Gen Virol *64*, 1819-1822.

Van Damme, J., G. Opdenakker, A. Billiau, P. De Somer, L. De Wit, P. Poupart, J. Content (1985). Stimulation of Fibroblast Interferon Production by a 22K Protein from Human Leukocytes J Gen Virol *66*, 693-700.

Van Damme, J., M. De Ley, J. Van Snick, C.A. Dinarello, A. Billiau (1987). The role of interferon-beta 1 and the 26-kDa protein (interferon-beta 2) as mediators of the antiviral effect of interleukin 1 and tumor necrosis factor. J Immunol *139*, 1867-1872.

Vance, R.E. (2016). Cytosolic DNA Sensing: The Field Narrows. Immunity *45*, 227-228.

Vance, R.E., R.R. Isberg, D.A. Portnoy (2009). Patterns of Pathogenesis: Discrimination of Pathogenic and Nonpathogenic Microbes by the Innate Immune System. Cell Host Microbe *6*, 10-21.

Volkman, H.E., D.B. Stetson (2014). The enemy within: endogenous retroelements and autoimmune disease. Nat Immunol *15*, 415-422.

von Moltke, J., N.J. Trinidad, M. Moayeri, A.F. Kintzer, S.B. Wang, N. van Rooijen, C.R. Brown, B.A. Krantz, S.H. Leppla, K. Gronert, R.E. Vance (2012). Rapid induction of inflammatory lipid mediators by the inflammasome in vivo. Nature *490*, 107-111.

Wang, C., L. Deng, M. Hong, G.R. Akkaraju, J. Inoue, Z.J. Chen (2001). TAK1 is a ubiquitin-dependent kinase of MKK and IKK. Nature *412*, 346-351.

Wang, C.Y., M.W. Mayo, R.G. Korneluk, D.V. Goeddel, A.S. Baldwin Jr (1998). NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. Science *281*, 1680-1683.

Wang, Z., M.K. Choi, T. Ban, H. Yanai, H. Negishi, Y. Lu, T. Tamura, A. Takaoka, K. Nishikura, T. Taniguchi (2008). Regulation of innate immune responses by DAI (DLM-1/ZBP1) and other DNA-sensing molecules. Proc Natl Acad Sci *105*, 5477-5482.

Wathelet, M.G., C.H. Lin, B.S. Parekh, L.V. Ronco, P.M. Howley, T. Maniatis (1998). Virus infection induces the assembly of coordinately activated transcription factors on the IFN-beta enhancer in vivo. Mol Cell *1*, 507-518.

Watson, R.O., P.S. Manzanillo, J.S. Cox (2012). Extracellular M. tuberculosis DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. Cell *150*, 803-815.

Wesche, H., W.J. Henzel, W. Shillinglaw, S. Li, Z. Cao (1997). MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. Immunity *7*, 837-847.

West, A.P., G.S. Shadel (2017). Mitochondrial DNA in innate immune responses and inflammatory pathology. Nat Rev Immunol *17*, 363-375.

West, A.P., I.E. Brodsky, C. Rahner, D.K. Woo, H. Erdjument-Bromage, P. Tempst, M.C. Walsh, Y. Choi, G.S. Shadel, S. Ghosh (2011). TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. Nature *472*, 476-480.

West, A.P., W. Khoury-Hanold, M. Staron, M.C. Tal, C.M. Pineda, S.M. Lang, M. Bestwick, B.A. Duguay, N. Raimundo, D.A. MacDuff et al. (2015). Mitochondrial DNA stress primes the antiviral innate immune response. Nature *520*, 553-557.

White, M.J., K. McArthur, D. Metcalf, R.M. Lane, J.C. Cambier, M.J. Herold, M.F. van Delft, S. Bedoui, G. Lessene, M.E. Ritchie et al. (2014). Apoptotic caspases suppress mtDNA-induced STING-mediated type I IFN production. Cell *159*, 1549-1562.

Wienerroither, S., P. Shukla, M. Farlik, A. Majoros, B. Stych, C. Vogl, H. Cheon, G.R. Stark, B. Strobl, M. Müller, and T. Decker. (2015). Cooperative Transcriptional Activation of Antimicrobial Genes by STAT and NF-κB Pathways by Concerted Recruitment of the Mediator Complex. Cell Rep *12*, 300-312.

Wilkins, C., and Gale, M., Jr. (2010). Recognition of viruses by cytoplasmic sensors. Curr Opin Immunol *22*, 41-47.

Wong P.K., P.J.E., B.A. Croker, K.S. O'Donnell, N.A. Sims, S. Drake, H. Kiu, E.J. McManus, W.S. Alexander, A.W. Roberts, and I.P. Wicks (2006). SOCS-3 negatively regulates innate and adaptive immune mechanisms in acute IL-1-dependent inflammatory arthritis. J Clin Invest *116*, 1571-1581.

Wu, M.F., S.T. Chen, A.H. Yang, W.W. Lin, Y.L. Lin, N.J. Chen, I.S. Tsai, L. Li, S.L. Hsieh (2013a). CLEC5A is critical for dengue virus–induced inflammasome activation in human macrophages. Blood *121*, 95-106.

Wu, M.F., S.T. Chen, S.L. Hsieh (2013b). Distinct regulation of dengue virus-induced inflammasome activation in human macrophage subsets. J Biomed Sci *20*.

Yamamoto, M., S. Sato, H. Hemmi, S. Uematsu, K. Hoshino, T. Kaisho, O. Takeuchi, K. Takeda, S. Akira (2003). TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. Nat Immunol *4*, 1144-1150.

Yarilina, A., K.H. Park-Min, T. Antoniv, X. Hu, L.B. Ivashkiv (2008). TNF activates an IRF1-dependent autocrine loop leading to sustained expression of chemokines and STAT1-dependent type I interferon-response genes. Nat Immunol *9*, 378-387.

Yin, Q., Y. Tian, V. Kabaleeswaran, X. Jiang, D. Tu, M.J. Eck, Z.J. Chen, H. Wu (2012). Cyclic di-GMP Sensing via the Innate Immune Signaling Protein STING. Mol Cell *46*, 735-745.

Yoneyama, M., M. Kikuchi, K. Matsumoto, T. Imaizumi, M. Miyagishi, K. Taira, E. Foy, Y.M. Loo, M. Gale Jr, S. Akira, S. Yonehara, A. Kato, T. Fujita (2005). Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. J Immunol *175*, 2851-2858.

Yount, J.S., T.M. Moran, C.B. López (2007). Cytokine-independent upregulation of MDA5 in viral infection. J Virol *81*, 7316-7319.

Zaheer, R.S., D. Proud (2010). Human rhinovirus-induced epithelial production of CXCL10 is dependent upon IFN regulatory factor-1. Am J Respir Cell Mol Biol *43*, 413-421.

Zandi, E., D.M. Rothwarf, M. Delhase, M. Hayakawa, M. Karin (1997). The IkappaB kinase complex (IKK) contains two kinase subunits, IKKalpha and IKKbeta, necessary for IkappaB phosphorylation and NF-kappaB activation. Cell *91*, 243-252.

Zhang, Z., B. Yuan, M. Bao, N. Lu, T. Kim, Y.J. Liu (2011). The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. Nat Immunol *12*, 959-965.

Zhao, Y., J. Yang, J. Shi, Y.N. Gong, Q. Lu, H. Xu, L. Liu, F. Shao (2011). The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. Nature *477*, 596-600.

Zhong, Z., A. Umemura, E. Sanchez-Lopez, S. Liang, S. Shalapour, J. Wong, F. He, D. Boassa, G. Perkins, S.R. Ali, M.D. McGeough, M.H. Ellisman, E. Seki, A.B. Gustafsson, H.M. Hoffman, M.T. Diaz-Meco, J. Moscat, M. Karin (2016). NF-kB Restricts Inflammasome Activation via Elimination of Damaged Mitochondria. Cell *164*, 896-910.

Zhou, R., A.S. Yazdi, P. Menu, J. Tschopp (2011). A role for mitochondria in NLRP3 inflammasome activation. Nature *469*, 221-225.

Zorov, D.B., C.R. Filburn, L.O. Klotz, J.L. Zweier, S.J. Sollott (2000). Reactive Oxygen Species (ROS)-induced ROS Release: A New Phenomenon Accompanying Induction of the Mitochondrial Permeability Transition in Cardiac Myocytes. J Exp Med *192*, 1001-1014.