Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
**Cell Reports**

**Interferon Regulatory Factors IRF1 and IRF7 Directly Regulate Gene Expression in Bats in Response to Viral Infection**

**Graphical Abstract**

**Highlights**
- IRF1, 3, and 7 are highly expressed in multiple bat tissues and control gene expression.
- Antiviral IRG expression in bat cells is largely IFN independent.
- IRF1 and 7 regulate distinct subsets and alter the kinetics/maintenance of IRGs.
- IRF1, 3, and 7 regulate antiviral responses to IAV/MERS/HSV-1/PRV3M in bat cells.

**Authors**
Aaron T. Irving, Qian Zhang, Pui-San Kong, ..., Justin H.J. Ng, Radoslaw M. Sobota, Lin-Fa Wang

**Correspondence**
aaronirving@intl.zju.edu.cn (A.T.I.), linfa.wang@duke-nus.edu.sg (L.-F.W.)

**In Brief**
Bats express high levels of antiviral genes in response to synthetic dsRNA, IFN, or virus by the transcription factors IRF1/3/7. Irving et al. show that this induction largely bypasses IFNα/β production and that this may be a method for limiting the inflammation induced by IFN signaling while still restricting virus infection.

Irving et al., 2020, Cell Reports 33, 108345
November 3, 2020 © 2020 The Author(s).
https://doi.org/10.1016/j.celrep.2020.108345
Interferon Regulatory Factors IRF1 and IRF7 Directly Regulate Gene Expression in Bats in Response to Viral Infection

Aaron T. Irving,1,2,3,8,* Qian Zhang,1,4,5 Pui-San Kong,1 Katarina Luko,1 Pritisha Rozario,1 Ming Wen,1 Feng Zhu,1 Peng Zhou,1,5 Justin H.J. Ng,1 Radoslaw M. Sobota,6,7 and Lin-Fa Wang1,*
1Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore, Singapore
2Zhejiang University-University of Edinburgh Institute, Zhejiang University School of Medicine, Zhejiang University International Campus, Haining, China
3Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China
4University of Chinese Academy of Sciences, Beijing, China
5Key Laboratory of Special Pathogens, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China
6Functional Proteomics Laboratory, Institute of Molecular and Cell Biology (A*STAR), Singapore, Singapore
7Institute of Medical Biology (IMB), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore
8Lead Contact
*Correspondence: aaronirving@intl.zju.edu.cn (A.T.I.), linfa.wang@duke-nus.edu.sg (L.-F.W.)
https://doi.org/10.1016/j.celrep.2020.108345

SUMMARY

Bat cells and tissue have elevated basal expression levels of antiviral genes commonly associated with interferon alpha (IFNα) signaling. Here, we show Interferon Regulatory Factor 1 (IRF1), 3, and 7 levels are elevated in most bat tissues and that, basally, IRFs contribute to the expression of type I IFN ligands and high expression of interferon regulated genes (IRGs). CRISPR knockout (KO) of IRF 1/3/7 in cells reveals distinct subsets of genes affected by each IRF in an IFN-ligand signaling-dependent and largely independent manner. As the master regulators of innate immunity, the IRFs control the kinetics and maintenance of the IRG response and play essential roles in response to influenza A virus (IAV), herpes simplex virus 1 (HSV-1), Melaka virus/Pteropine orthoreovirus 3 Melaka (PRV3M), and Middle East respiratory syndrome-related coronavirus (MERS-CoV) infection. With its differential expression in bats compared to that in humans, this highlights a critical role for basal IRF expression in viral responses and potentially immune cell development in bats with relevance for IRF function in human biology.

INTRODUCTION

Bats are unique mammals with physiological capabilities including elevated metabolism (McNab, 1989; O’Mara et al., 2017; Shen et al., 2010; Suarez and Welch, 2017; Thomas, 1975) and flight (Suarez et al., 2009a, 2009b; Thomas, 1975; Zhang et al., 2013). Some bats are capable of torpor/hibernation without drastic energy requirements (Bouma et al., 2010; Currie et al., 2018; Han et al., 2015). Bats from most species are long-lived with some having exceptional longevity quotients relative to their body size (Austad and Fischer, 1991; Brunet-Rossinetti, 2004; Brunet Rossinetti, 2004; Foley et al., 2018; Huang et al., 2016; Podlutsky et al., 2005). They share common features including minimal systemic inflammation, fever, or clinical disease upon viral infection (Cabrera-Romo et al., 2014; Davis et al., 2005; Paweska et al., 2016; Perea-Martinez et al., 2013; Reagan and Bruecker, 1952; Simpson and O’Sullivan, 1968; Stockmaier et al., 2015). Several groups have highlighted a heightened innate immune system, baseline interferon (IFN) signatures, and basal IFN-ligand expression (Baker et al., 2013; Fuchs et al., 2017; Pavlovich et al., 2018; Zhang et al., 2013; Zhou et al., 2016). Nonetheless, the adaptive immune responses, particularly antibody development and production, are somewhat limited (Davis et al., 2005; Herbold et al., 1983; Jones et al., 2019, 2015; Middleton et al., 2007; Obregón-Mo-roles et al., 2017; Paweska et al., 2016; Suu-Ire et al., 2017; Swaneepoel et al., 2007; Yong et al., 2018). Despite infection and shedding, bats do not succumb to most viral infections, even when experimentally infected with highly pathogenic zoonotic viruses. Studies have shown transient inflammation or mild disease that is soon resolved by the bat immune system (Jones et al., 2019).

Previously, we showed fruit bat cells deficient in part of the type-I IFN receptor IFNAR2 maintain the imprinted “antiviral signature” (Zhang et al., 2017), indicating the baseline interferon regulated gene (IRG) expression did not result from basal canonical IFN signaling. Although Interferon Regulatory Factors (IRFs) in humans control additional genes to type I IFN-ligands, their contribution to immunity is predominantly believed to be by IFN-ligand induction, rather than directly on IRGs (Andrilenas
et al., 2018; Ashley et al., 2019; Barnes et al., 2004; Cohen et al., 2014; Dery et al., 2018; Honda et al., 2005; Langlais et al., 2016; Eggenberger et al., 2018; Piya and Kim, 2018; Schmid et al., 2010; Shultz et al., 2009; Wang et al., 2017; Yu et al., 2019). IRF3 is more well-studied in this regard, although studies also suggest IRF1 and 7 contribute by inducing IFNs (Andrilenas et al., 2018; Kawai et al., 2004; Li et al., 2014a; Weiss et al., 2012; Zhou et al., 2012, 2015). IRF7 from the Australian black flying fox Pteropus alecto, has been highlighted as a potent regulator of interferon alpha (IFNα). Unlike the restriction to lymphoid cells/monocytes seen in most mammals, IRF7 is expressed in several tissues (Zhou et al., 2014). Bat IRF7 has a conserved DNA-binding domain, similar to that in humans, with an expanded myd88-binding region that from preliminary studies appears to function as per normal. Compared with that in humans, IRF7 in bats is considerably more potent in driving the IFN promoter. However, discrepancies over IFN expression across different bat species remain, with most species exhibiting limited IFN induction. Thus, understanding bat-specific IRF functions and regulation of this high-baseline antiviral activity in bats may be critical for controlling viral infection in humans. Here, we characterize IRF1/3/7 expression levels across tissues and the role of IRFs in gene induction, basally or post-induction. Additionally, we highlight the importance of IRFs in inducing IFN-independent antiviral responses in bat cells infected with herpes simplex virus 1 (HSV-1), Middle East respiratory syndrome-related coronavirus (MERS-CoV), influenza A virus (IAV), and Melaka virus/Pteropine orthohevovirus 3 Melaka (PRV3M).

RESULTS

High-Baseline IRG Signatures Are Not Induced by IFN Ligands

IFNAR2 CRISPR knockout (KO) was used to deplete the type-I IFN receptor in P. alecto PakiT03 cells (clones 4A, 9E). Although these clones no longer respond to the IFNα3 ligand, they still maintain a basal signature associated with IFN (Zhang et al., 2017). We treated these clones with the double-stranded RNA (dsRNA) mimic polyIC for 3 h to observe the induction of IRGs by next-generation sequencing (NGS) transcriptomics, independent of canonical type I IFN signaling (Table S1). A classic Interferon Stimulated Gene Factor 3 (ISGF3)-STAT1/STAT2/IRF9 transcription factor (TF) signature (Figure 1A), a common/pan anti-viral IRG signature (Figure 1B) (La Cruz-Rivera et al., 2017; Schoggins and Rice, 2011), and a recently published embryonic hematopoietic stem cell (eHSC) IRG signature (Figure 1C; Wu et al., 2018), or a u-ISGF3 unphosphorylated STAT1/2 signature (Figure 1D; Cheon et al., 2013; Sung et al., 2015; Zhou et al., 2016) were partially seen. These data show that IRG subsets are rapidly upregulated by polyIC, a synthetic dsRNA that mimics viral RNA, in the absence of type-I IFN signaling, at early time points. This response was not due to induction of IFNγ/α as the IFNγ ligand was not expressed and neither was the IFNα receptor IFNLR1 (Figure 1E). Similarly, the IRG signature associated with polyIC in IFNAR2-KO cells did not match that of IFNG from gene set enrichment analysis (GSEA) (Figure S1A), although IFNα treatment in wild-type (WT) cells did to some degree. Minimal IFNα ligand was detected basally and IFNβ was not. Some minor induction was observed with both IFNα3/polyIC treatment, although it was minimal, particularly compared to previous studies in humans/mice. It is, therefore, likely the direct up-regulation of IRGs is induced by regulators independent of IFN ligands. The basal expression of components of the IFN signaling cascade in clonal cell lines is largely consistent. This is, therefore, unlikely the result of u-ISGF3 signaling from a high expression of STAT1/STAT2 and/or IRF9 without induction. Notably, rapid upregulation of IRF1/7 was seen in response to IFNα and IRF1 to polyIC, respectively (Figure S1B).

To ensure the baseline IRG status is consistent in vivo, we examined the transcriptome from healthy adult P. alecto and E. spelaea spleen (wild-born) and compared them to publicly available data from middle-aged healthy humans and adult mice (prepared with the same pipeline). To minimize inter-species variation, the data were normalized to the geometric mean of 13 housekeeping genes. The hESC IRG genest signature is shown in Figure 1F. Although the expression varies among species, the trend is consistent with that of P. alecto with higher baseline IRG expression. Some notably higher genes include WARS, SERPINE1, MT2A (LOC102888224), SLC16A1, IFITM3, SERPING1, and PNR1. Although the anti-viral IRG genest had a similar trend, the average expression in an unstimulated state (Figure S1C) was insignificant for the whole signature (en-bulk), in vivo. E. spelaea was removed from the subsequent genest due to poor annotation of the genome. Significantly differential expression profiles were seen for the ISGF3 (Figures 1G and S1D) and u-ISGF3 signatures (Figures 1H and S1E). Additional IRGs differentially regulated, basally, between P. alecto and human spleen are also observed.
IRFs Are Highly Expressed across Bat Tissues In Vivo
To validate expression, IRF1 mRNA levels were examined using a cDNA panel generated from 10 tissues isolated from 3 bats/mice (Figure 2A). *P. alecto* tissue had 1 to 2 log higher expression in the brain, liver, spleen, lung, kidney, intestine, stomach, and salivary glands than mice. IRF1 activation is poorly understood, although high expression leads to spontaneous activation in the absence of stimuli (Garvin et al., 2019; Lin and Hiscott, 1999; Shultz et al., 2009). Similarly, IRF3 expression was more prominent in bats, except for the heart tissue (Figure 2B). Likewise, IRF7 expression was higher in most bat tissues (Figure 2C) including brain, liver, spleen, lung, kidney, stomach, and salivary gland. Compared with humans, NGS transcriptomic expression from bat tissues was higher in spleen, lung, and liver in both *P. alecto* and *E. spelaea* (normalized as per Figure 1t; Figure S2A). Although IRF7 is believed to be activated in a similar fashion to IRF3, by TBK1, IKKe, or IRAK1 and IKKe, its expression is largely limited to lymphoid cells and dendritic cell subsets in humans (reviewed in Genin et al., 2009). Although IRF3/7 have additional autoinhibitory motifs, alleviated in response to dsRNA by TBK1 serine phosphorylation, IRF3 has limited function without stimulation. IRF7 is known to have an additional element increasing both basal and virus-induced activity (Lin et al., 2000a) and is considered more promiscuous with its binding motif (Lin et al., 2000b). To this end, we observed spontaneous nuclear localization of IRF1 and partially for IRF7-GFP fusion constructs, which were used previously (Zhou et al., 2014), in PakiT03 cells after 12 h from expression alone. IRF3-GFP required 90 min of stimulation with polyIC for optimal nuclear localization (Figure S2B). IRF7-GFP can be seen in sub-nucleolar structures (speckles) both before and enhanced after polyIC stimulation. Compared to that of humans, bat IRF7 appears to have greater nuclear localization in the absence of stimulation (Lin et al., 2000a, 2000b). This matches the observation of IFNγ induction in response to IRF3 and 7 requiring polyIC induction (Figure 2D). We tested if IRF1 drove bat IFNα promoters, similar to IRF7 (Zhou et al., 2014). IRF1 was the strongest driver of all three characterized IFNα promoters in *P. alecto*, followed by IRF7 and IRF3 (Figure S2C); notably, this was in the absence of polyIC for which induction is solely by basal expression. Thus, the major limiting factor for IRF1/7 activity may be its lack of expression, although IRF7 can be enhanced by dsRNA.

IRF1 and IRF7 Regulate Antiviral Genes Basally
To examine IRF regulation on key IRGs during antiviral responses, we examined IFNα2 (Figure 2E), IFNγ (Figure 2D), MX1 (Figure 2F), and IFIT1 (Figure 2G) mRNA expression by using IFN1/3/7-GFP fusion constructs. To eliminate IFN-induced gene induction, IFNAR2 KO cells were treated with and without intracellular polyIC stimulation for 3 h (1 µg/ml). IFN3x expression was induced with and without polyIC by IRF1 although not by IRF3/7. IFNγ was induced by IRF1/7, with or without polyIC treatment, although it enhanced for IRF7 with polyIC. Conversely, IRF7 overexpression suppressed MX1 and IFIT1; however, this was partially alleviated by polyIC induction. IRF1 basal induced IFIT1 unexpectedly, whereas IRF3-expressing cells required polyIC induction to achieve full expression of IFIT1, fitting with the luciferase and nuclear localization observations. A mild IRF-suppressive effect was observed for BST2 and STAT1 but not for Rig-I (Figures S2D–S2F). This suppressive IRF effect on MDA5 and PKR was alleviated by polyIC stimulation (Figures S2G and 2H). To confirm IRF overexpression was not working by compensatory induction of IFNγ/λ, we treated the same cells with the Jak inhibitor ruxolitinib. Control and IFNα3x-stimulated cells showed a similar absence of IRG-induction profiles, as measured by a previously described nanostring panel (Irving et al., 2020), whereas ruxolitinib-treated cells with IFNα3 showed a slight increase, possibly indicating a degree of interference from IFNAR1 binding in the absence of IFNAR2. Fitting with the idea of Jak-STAT-signaling-independent, direct/indirect IRF-dependent induction, ruxolitinib actually enhanced IRF-overexpression-induced IRG induction, particularly with genes such
as XAF1, ZBP1, TLR6, RTP4, OASL, NLRC5, MX2, IFIT2/3, CXCL10, CIITA, and APOBEC3G (Figure S2I).

To examine the impact of IRF1/3/7 in antiviral responses, we generated IRF CRISPR KO cells. Multiple CRISPR clonal cell lines were sequenced (Key Resources Table) and screened for the expression of IRGs, including BST2, IFNα3, and STAT1 (Figures S2J–S2L). IRG induction was consistently affected for multiple clones of each IRF KO, indicating a consistent trend regardless of the individual clone line. Although both qPCR and NGS data validated decreased and partial transcripts, we were unable to verify the protein level due to the lack of specific antibodies to bat IRFs. We selected clones IRF1-g4-1D, IRF3-g4-2H, and IRF7-3C for the remainder of the study and referred to them as IRF1/3/7 CRISPR KO cells.

**IRF1/3/7 Alter the Response to Ligand or Infection with HSV-1, IAV, and MERS-CoV**

To interrogate this response with immuno-stimulation, we treated WT or IRF1/3/7 CRISPR KO cells with polyIC or IFNα3 (as previously mentioned). Basal expression of IFIT1, measured by qPCR, is maintained by high-baseline IRF1 and 7 levels (Figure 2H) IRF3 induction requires polyIC, whereas IFNα3 can induce IFIT1 in the absence of either IRF. In response to HSV-1 infection (multiplicity of infection [MOI], 0.1; 72 h), PakTI03 cells require IRF1, but not IRF3 or 7, to restrict HSV-1 viral load, as detected by titration of the supernatant (Figures 2I and 2J) and ICP0 mRNA expression (Figure 2K). In response to IAV infection (MOI, 0.1; 72 h; H1N1 A/NWS/33), IRF3 increases the production of infectious IAV particles; however, all three IRFs are required to minimize the IAV RNA amount (Figure S2M). Similar to IRF3 regulation (Banerjee et al., 2019), all three IRFs were required for full induction of IRGs, such as IFIT1 and MX2 (Figures 2L and 2M), during MERS-CoV infection (MOI, 0.1: 48 h). Partial induction of MX2 occurred in the IRF7 KO cells, suggesting an effect on a specific subset of IRGs. MERS-CoV N gene expression was reduced in the absence of all three IRFs (Figure 2N). However, this was confounded by abundant cell death indicated by GAPDH reduction (Figure S2N). Further studies on infection kinetics, regulation of pro- and antiviral genes, and subsequent viral loads would, therefore, be required for a complete analysis of each IRF within the context of infection.

**IRF-Mediated Regulation of Gene Expression in the Basal State**

IRF-regulated genes were detected in the basal/unstimulated state by NGS in each CRISPR KO cell line. Genes that were significantly changed (>2-fold, p < 0.05) for IRF1, 3, and 7 are shown in Figures 3A–3C. Differentially Expressed Gene (DEG) analysis by HTseq-count/EdgeR were performed by comparing transcript expression to the WT control. These findings demonstrate the specific and overlapping up- (Figure 3D) and downregulated (Figure 3E) genes (summarized in Table S2). Compared with basal WT expression, most IRGs in the antiviral IRG genset (Figure 3F) are affected by baseline IRF expression in bat cells, with the greatest effect in IRF1 and IRF7. This finding is consistent with earlier qPCR analysis (Figures 2H and S2J–S2L), which suggests this to be a common trend to the respective IRF KO; however, further validation in additional cell lines and different bat species would be ideal. Consequently, the hESC IRG-geneset (Figure S3A) and ISGF3 (Figure S3B) and u-ISGF3 genespets (Figure S3C) are all affected in a gene- and IRF-specific manner. Basally, there were limited changes to type I IFN ligand expression (<2-fold). Mild decreases in IFNα2/IFNα1 were observed in IRF1-deficient cells and with all IFNs except IFNκ/λ/ε (annotated as α4 in the current genome assembly) from IRF7 deficiency (Figure S3D). This result suggests that IRF1/7 drive IFN ligands basally, although complete removal of IRF1 or 7 suppresses IFNα4 and IFNα4/λ/ε expression, respectively. This suggests differential promoter regulation between the type I IFNs that may warrant further investigation. The majority of DEGs detected from basal regulation are unique compared to human biology, with only minimal overlap between known IRF1-transcriptional targets from the Encode database, chromatin immunoprecipitation (ChIP) assays performed in monocytes, or compared to other TFs in the CHEA 2016 database by EnrichR analysis (Figure S3E; Kuleshov et al., 2016). Likewise, there is minimal overlap between IRF3-KO DEGs and previously published IRF3-transcriptional targets in humans (Figure S3F), although limited information on IFN-independent, IRF3-dependent induction is available. IRF7 has no ChIP sequencing (ChIP-seq) data in the public databases, and yet, the DEGs from IRF7-KO cells show minimal overlap to a transcriptional profile seen from constitutively active IRF7 or SUZ12, a TF repressor identified by EnrichR with partial overlap to the IRF7 DEGs (Figure S3G). The unique gene profiles only partially correlate with other known TFs. The complete DEG heatmap with each IRF KO is shown in Figure S3H, indicating clusters of genes up- or downregulated.

Next, we analyzed the functional impact from each IRF by ingenuity pathway analysis (IPA) (Figure 3G). Very few pathways were directly associated with “antiviral responses”; rather, pathways identified involved basic metabolism, glycolysis, intracellular lipids, tryptophan metabolism, and reactive oxygen species/nitric oxide (ROS/NO) signaling. Although many pathways were significantly enriched by the DEGs (p value), the Z scores indicated that very few had an overall up- or downregulation. Collectively, this result suggests the pathways are dysregulated (i.e., some genes upregulated, some genes downregulated). Overall, the data indicate that although basal IRF expression contributes significantly to the antiviral state, a large number of DEGs invoke differential pathways to those previously observed in humans/mice. These pathways may no longer follow normal rules for regulation due to interference by high basal IRF expression.

**Activation of IFN/Antiviral Pathways and Non-antiviral Pathways by polyIC**

One of IRFs’ key functions is their immediate response to Pattern Recognition Receptors (PRRs) from pathogens. To examine this further, we stimulated the CRISPR KO cells with transfected polyIC (pic) and IFNα ligand. DEGs were selected as being differentially expressed (>1.5-fold change to WT, p < 0.05) if present at two time points post-treatment. IRF1/3/7, IFNAR2, or IF-NAR2/IRF7 double CRISPR KO cells were examined at 6 h or 9 h post-polyIC stimulation (Figure 4A). The DEG heatmap (>2-fold
change, graphed by row Z score) displays distinct clusters of genes associated with each regulator or in combination with IFNAR2 deficiency (Figures S4A and S4B). Overall, IRF1 had the largest transcriptional regulation capacity. However, there were specific IRF7-regulated genes in the presence/absence of intact IFN-signaling, highlighting IFN-dependent and -independent gene expression by IRF7. Similar pathways to basal expression (Figure 3G) were revealed by IPA at 6 h by using p value (significance; Figure 4B) or expression weighting (Z score; Figure 4C; Table S4). Additional pathways are also seen, including ILK/interleukin-8 (IL-8)/IL-12 signaling, dendritic cell (DC) maturation, immune cell migratory pathways, and IFN-independent IRF1/7 downregulation of NO/ROS production. Intact IFNAR2 upregulates IL-8, ILK, and CXCR4 signaling pathways, even with minimal IFN-ligand induction by polyIC (Figure S4C). GSEA scores for the signature “response to IFNx(4/4u2) shown a dependency of IRF3/IFNAR2 in response to polyIC (Figure 4D). Although the “IFN antiviral signature” showed a dependency only for IRF3 (Figure 4E), the “Interferon Regulated Genes induced from RSV ΔNS1 infection” displayed a dependency for IRF1/3 and IFNAR2 for significant enrichment of this geneset (Figure 4F). Intriguingly a similar enrichment score was observed for IRF1/3 and IFNAR2 for significant enrichment of this geneset (Figure 4F). GSEA scores for the signature “response to IFNx” showed a dependency of IRF3/IFNAR2 in response to polyIC (Figure 4D).

IRF-Mediated Responses to IFN Are Time Dependent
To examine if IRF1/3/7 are involved in positive feedback post-IFN-stimulation, the cells were analyzed by NGS at 3 h, 6 h, and 9 h. Compared with the WT, the IRF1/3/7 KO cells showed a unique set of genes differentially expressed (Figure 4H; p < 0.05, >2-fold induction, DEG analysis in 2 of 3 time points by EdgeR) throughout time (Figure S4I), with 486 DEGs significant across all three time points (Table S3). IPA revealed genes associated with IFN, death receptor, and IRF activation pathways (Figure 4I). There are several metabolic, intracellular lipid signaling, and ROS/NO production pathways that are downregulated post-stimulation, with downregulation exacerbated in IRF7 KO cells. Compared to the WT, additional pathways are affected independently by IRF1, 3, or 7. However, IRF7 is most notable where it maintains the IFN response at later time points, largely independent of the expression of IFNs (by positive feedback loop), as the absence of IRF1/3/7 had a minimal effect on IFN levels. Thus, these findings indicate redundancy among the IRF molecules for IFN induction, in contrast to human and mouse epithelia (Figure S4J).

Conversely the role of IRF3 is most significant at 6 h post-stimulation, suggesting it requires more time for polyIC-induced expression. However, IRF7 was necessary to sustain long-term (24 h) IFIT1 induction.
Correlation of Transcription to Total Protein Abundance

To determine the protein level dynamics of IRF regulation, cells were treated overnight, prior to quantification of total proteins by quantitative mass spectrometry. IPA revealed that although WT cells treated with high-dose IFNα upregulated IFN response proteins, only limited pathways were induced (Figure S4L; Table 1). Indeed, both IFNα and polyIC trigger a reduction in eIF2α and 4G relative to control cells, driving global translational inhibition by the eIF2 pathway (Figure S4L; Table S4). This effect was driven by IRFs, predominantly IRF7 in response to IFN or polyIC. Removal of IRFs, particularly IRF7, consequently further drove induction of largely the same pathways observed in the transcriptomic analysis. In response to IFN or polyIC, IRF7 suppresses cholesterol biosynthesis, pyrimidine deoxyribonucleotide synthesis, MAPK/ERK/IFN/LIF-/hypoaxia signaling pathways, and glycolysis. This is obviously in the absence of IFN ligands, as no IFNs were detected out of ~6,000 proteins (Table S4). A degree of caution must follow this methodology, however, as predicted protein spectra do not account for post-translational modifications, potentially obscuring peptide detection. Other pathways such as cell cycle, endocytic signaling, and protein ubiquitination are significantly altered and highly dysregulated.

IRF3 and IRF7 Are Essential for Antiviral and Non-Viral Pathways in Response to a Bat Orthoreovirus, PRV3M

Pteropine orthoreoviruses (PRVs) are bat viruses known to induce a potent IFN-type response in bat cells. To investigate how IRFs regulate the kinetics of PRV3M (Melaka virus), a virus known to cause zoonotic infections in humans (Chua et al., 2007; La Cruz-Rivera et al., 2017; Mok et al., 2015, 2017; Tan et al., 2017; Voon et al., 2015), transcriptome studies post-infection (MOI, 1) were used. They reveal a pro-viral role for IRF3 at early time points, with respect to viral RNA load. However, this was abrogated by 24 h (Table S5). IRF7 and IFNAR2 deficiency resulted in increased viral load at both 9 and 24 h, yielding a 2-fold increase for IRF7 and 4-fold increase for IFNAR2 at 24 h, whereas IRF1 was significant only at late time points (Figures 5A and S5A). This finding was reflected in the plaque assays (Figure S5A). An analysis of each genome segment revealed a ubiquitous increase in viral RNA across the PRV3M genome, indicating limited interference in the transcription of specific viral genes (Figure S5B). IRF- and IFNAR2-specific DEG analysis post-infection reveal that IRF1 had the largest effect (Figure 5G) and, yet, all three IRFs affected the amount of viral RNA.

The “IFN response” and “Death Receptor” pathways require intact IRF3/7 and IFNAR2 (Figure 5D). IRF1 deficiency enhances the IFN response. Other pathways affected by all three IRFs and IFNAR2 include oxidative phosphorylation, gluconeogenesis, and eIF2 signaling and pathways involved in sensing of NOS/ROS, which are actively downregulated. TREM1 signaling, citrulline biosynthesis, growth hormone, and TLR signaling pathways are switched on, and this is dependent on IRF1/3 and IFNAR2. The absence of IRF1 and IRF7 unexpectedly enhances p38 mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), Tec kinase, chemokine, phospholipase, ERK and VEGF signaling at the 9-h time point. IRF3 was critical for intracellular calcium, cyclic AMP (cAMP), GPCR, and intracellular phospholipid signaling pathways. IRF7 was required to turn off GPCR and phospholipase signaling later during infection, after an initial activation at 9 h.

Although the antiviral IRG geneset was robustly upregulated in both WT cells and IRF1-deficient cells, they were absent in IRF3/7/IFNAR2 KO cells (Figure 5E). However, specific genes like PARP15, UBE2L6, RNF213, and ETV7 were affected by IRF1. This result indicates that IRF7 plays a critical role early in viral infection, where it is activated in response to alternative TLR/PRR-stimuli by polyIC (Cao et al., 2008; Kawai et al., 2004; Weiss et al., 2012). Examination of “hypothetical regulators” matching the DEGs suggests IRF7 is de-activating SMARCA4, while activating multiple poorly characterized miRNAs (miRNAs) (Figure S5C). IRF3 was involved in de-activating ACKR2 while simultaneously driving a STAT1 signature of genes. Overall, the induction of the antiviral geneset (Figure 5E) correlated with infectious virus production, whereby IRF7 and IRF3 KO PakiT03 cells produced more virus, followed by IRF1, as visible by plaque assays on Vero E6 cells (Figure 5F). This finding indicates that all three IRFs contribute to the antiviral response to PRV3M and affect virus production. IRF7 sustained the antiviral geneset at later time points; however, this did not correlate with the production of IFN ligands (Figure S5D) and largely did not involve transcriptional control of known negative regulators of IFN signaling (Figure S5E). There was a mild regulation of
TRIM21 and USP18 by IRF3/7, although this is likely IFN dependent. IFNβ expression, most significantly changed in IRF3/7 KO cells, was induced by 9 h in IRF1-deficient cells, although this failed to induce the typical antiviral gene signature. The overall amplitude of IFN-ligand induction was quite low (Figure S5D) compared to a potent induction of IRGs (Figure 5E; Table S5).

IRF3/7 did contribute to higher levels of STAT1/STAT2/IRF9 at late time points, which may contribute to IFN-ligand IFN-independent signaling (Blaszczyk et al., 2015; Cheon et al., 2013; Nan et al., 2018; Sung et al., 2015). Although their induction was not observed in the absence of IFNAR2. The absence of classical antiviral IRG expression in IRF3/7 or IFNAR2 deficiency highlights the need for both intact IFN signaling and IRF-directed gene expression for the classical response. Multiple signaling pathways (e.g., mTOR, Sirtuin, PPAR, LXR, antioxidant, and RhoGDJ) are observed to be IFN-signaling independent and, yet, regulated by IRFs.

Reconstitution Alleviates IRF Deficiency

To ensure specificity of the clonal cell lines chosen, reconstitution of the relevant IRF KO cell line by overexpression of IRF1/3/7-GFP fusion constructs was performed to restore antiviral protection. Titration of viral supernatants confirms that the defect in each cell line was due to the absence of IRFs and not a clonal-line-compensatory mechanism (Figure 5G). Reconstitution of IRF1/3 and 7 significantly drove an antiviral effect and reduced PRV3M production. IRF7 reconstitution was also used to drive an antiviral phenotype in IFNAR2 KO cells, IFNAR2/IRF7 double KO cells, and an IRF1/IRF7 double KO clonal cell line (Figure S5F). This indicates that *P. alecto* IRF7 alone can suppress virus production in the absence type-I IFN signaling. This phenotype can be partially observed across species with *P. alecto* IRF7 functioning in *Myotis davidii* MdKi cells to suppress virus production, despite only minimal transfection efficiency (Figure S5G).

### Table 1. Top 15 Up- and Downregulated Proteins with Response to Treatment

| WT                  | IRF1 KO | IRF3 KO | IRF7 KO |
|---------------------|---------|---------|---------|
| IFN†                | pIC†    | IFN†    | IFN†    |
| OAS2                | FOXG1   | OAS2    | DPAGT1  | NAA15   | TPMT    | TBPL1   | TBPL1   |
| KCNA3               | HDH2    | SYNO2   | SYNO2   | TBPL1   | TBPL1   | TPMT    | CAMSAP3 |
| BST2                | QRich1  | TBPL1   | FMR1    | IFI6    | NAA15   | QT1     | OAS3    |
| STX3                | OAS2    | ISG15   | FOXG1   | TPMT    | IFI6    | FOSL1   | ZBTB34  |
| IFI6                | SUPT7L  | EXC6    | AQP1    | S100A8  | S100A8  | IFI6    | LY6H    |
| LY6H                | TANC1   | OAS1    | ARHGAP17| S100A9  | TACC3   | OAS3    | POLR2F  |
| SPTBN4              | CLINT1  | XAF1    | IRAQ2   | OAS1    | WIPF1   | ZBTB34  | IFI6    |
| UBE2L6              | LRSAM1  | IFIT3   | UBA7    | WIPF1   | DCXR    | STX11   | TRMT5   |
| ZB7B34              | ATG16L1 | PCBP3   | DMTN    | PIR     | S100A9  | IFIT3   | XAF1    |
| ISG15               | FMR1    | INOS0E  | INOS0E  | ECD     | QRT1    | AZI2    | TRAPP11  |
| LGALS9              | MRPL34  | TNFAIP2 | EXC6    | DCXR    | DDX58   | USP16   | EEF1A2  |
| NRF1                | SPTBN4  | VGLL2   | MYH14   | QRT1    | AZI2    | TRAPP11  | IFIT3   |
| OAS1                | FMR1    | INOS0E  | INOS0E  | ECD     | QRT1    | POLR2F  | DDX58   |
| TICRR               | TMEM168 | CLDN1   | OAS2    | PABPC1L | ZBP1    | ATM     | B2M     |
| USP18               | ZB7B34  | MAD2    | TBPL1   | BST2    | OAS1    | SLC25A4 | COMM1D  |
| IFN†                | pIC†    | IFN†    | pIC†    | IFN†    | pIC†    | IFN†    | IFN†    |
| USP16               | VTA1    | PTIPRC  | PTIPRC  | PTIPRC  | LRRC7   | LRRC7   | LRRC7   |
| VTA1                | ACY1    | C3      | C3      | LRC7    | PTIPRC  | SLC43A3 | SLC43A3 |
| ACY1                | SIPA1L3 | FBXO28  | FBXO28  | CERS4   | CERS4   | C3      | PTIPRC  |
| MPLKIP              | CIR1    | IFNAR1  | NOL12   | XPR1    | XPR1    | PAN3    | PAN3    |
| SIA1L3              | DES     | ERGIC1  | CSRP2   | PPM1E   | DES     | IFNAR1  | C3      |
| CIR1                | RCC1L   | DES     | IL6ST   | DES     | RBP1    | PTPRC   | POLR1D  |
| HOXA5               | INOS0E  | CSRP2   | MATN3   | RBP1    | PPM1E   | RASAL2  | MRPS34  |
| TMEM132A            | LAMB2   | AMFR    | ZBT14   | MYOC5   | NUDT12  | MRPS34  | IFNAR1  |
| WAC                 | RABL6   | ZBT14   | DES     | YIF1A   | YIF1A   | MAN2A1  | RASAL2  |
| PTER                | TMEM132A| ZC3H8   | ERGIC1  | ING5    | ING5    | GIT2    | RBM15B  |
| RCC1L               | UBAC1   | CERS4   | GGT7    | NUDT12  | TICRR   | POLR1D  | ZC3H8   |
| SAP130              | CIC     | MATN3   | ZC3H8   | LRP10   | MYOC5   | XPR1    | GIT2    |
| RABL6               | THBS2   | IL6ST   | ARHGAP21| TMEM35A | PCLO    | CEP290  | BR13B   |
| SESN2               | ATM     | LOXL4   | AMFR    | PCLO    | SPAT1   | RBM15B  | XPR1    |
| DOP1B               | PTER    | LRCC8A  | ARSI    | TAF1    | PEX5    | ZC3H8   | CEP290  |
A similar trend was observed for overexpression in HEK293T human cells (Figure S5H).

**DISCUSSION**

Using multiple assays in bat cell lines, we show that the high-baseline IFN-like signature in bat cells is further upregulated upon dsRNA treatment in a partially IFN-signaling-independent manner. IRFs, the master regulators of IRG responses, are also highly expressed across multiple tissues. This signature is confirmed across the spleen, liver, and lung tissues in two distant species, implicating the importance of this for innate defense mechanisms in bats. Intriguingly, IRF1/7 expression is reduced in the bat’s heart tissue, suggesting a lack of immune activation in the bat’s heart may be important for its physiology. Although there may be significant variation due to species diversity, a recent preprint for a bat single-cell atlas of *Rhinolophus sinicus* (Ren et al., 2020) suggests comparable IRF1/3/7 expression, with RNA expression observed across both lymphoid and non-lymphoid tissue, matching our own observations. Although healthy wild-born bats with no known current infections were used, compared to seemingly healthy humans and mice, temporary housing and handling may impact the natural microbiota and alter gene expression to some degree. Similarly, the previous infection history and microbiota status of the humans compared are also unknown.

Overexpression of IRFs induces low-level type I IFNs, with IRF1 being a more potent inducer than IRF7 in direct promoter assays. Additional IRGs were upregulated by IRF1/3/7, indicating basal regulation of IRG subsets by IRF1 and IRF7 in the absence of additional stimuli. This was confirmed by nuclear localization of IRF1/7 but not IRF3, prior to dsRNA/polyIC treatment. Whether bat IRF7 has unique basal-activation properties would require further investigation, ideally across multiple bat species. IRF3-induced genes were also largely dependent on dsRNA induction, consistent with the function of its human homolog. However, early time points also indicate IRF3-dependent and IFN-ligand-independent gene induction.

Generation of CRISPR KO cells deficient in IRF1/3/7 showed IRF1 has an antiviral role in HSV infection. An IRF1 phenotype has been previously implicated in human cells (Xie et al., 2018). We demonstrate that IRF3 is important for MERS-CoV activation of innate immunity in bat cells, as expected. Unexpectedly, both IRF1 and IRF7 also play key roles in MERS-CoV infection. IAV shows IRF1, 3, and 7 contribute equally to reducing IAV RNA in infected cells. Based on IRF TF expression in the lung, this suggests that all three IRFs regulate the dynamics of infection. Although many may be common response features, further investigation is needed to see whether this is true for other RNA viruses and for other β-CoVs, such as SARS-CoV-2. Deeper investigation into the transcriptomic studies reveals unique and overlapping IRF-regulated genes in the basal state, without stimuli or IFN ligand. While IRF-directed gene expression has been observed in other mammals it has been studied in the context of foreign RNA for activation. One study in hematopoietic stem cells suggests that IRF7 may control basal expression patterns, although few other studies have examined this scenario directly (Eggenberger et al., 2019). The key role for IRF7 in early time points appears to contrast that of human studies for which it is believed to play a role in amplification of an antiviral response rather than in primary detection and signaling.

Despite the minimal induction of type-I IFN ligands by IFN, polyIC, or virus, various IRGs known to inhibit viruses were regulated by IRF1 (IFIT2, CIITA, CXCL10, IFIT1, UBA7, CLEC7A, PLSCR1, CCL2, CYP1b1, CD274, PARP10, OAS3, RTP4, and BST2) or IRF7 (CIITA, CD274, MX1/2, B2M, CYP1b1, IFI1H, OAS2, and IFNL1). Genes such as PARP15, TRANK1, ZBP1, and APOBEC3BL are regulated by IRF7 in an IFN-independent manner and are further amplified with intact IFN signaling. Cells with intact IRF3 and IFNAR2 still required IRF7 to mount a full response. This result also highlights dynamic differences between basal expression and overexpression studies. Many of these pathways were consistently activated post-stimulation and suggests that IRF1 regulation is critical at early time points, which is likely IFN independent, whereas IRF7 regulation occurs at both early and later time points and is critical for the response to PRV3M infection. There were clear differences in responsiveness to synthetic dsRNA compared to IFN and live virus. This fits with previous literature in bat cells suggesting unique IFN-stimulated genes, dsRNA-induced genes, and virus-induced genes that, although partially overlapping, also have unique profiles. Both findings also support a previous report of temporal differences in IRG activation in bat cells (La Cruz-Rivera et al., 2017). Type-I IFNs are expressed basally (particularly IFNα2-like) and require both IRF1 and 7. IFN induction was limited after stimulation, however, compared to the potent induction of IRGs.

**Figure 5. Regulation of the Antiviral Response to Melaka Virus by IRF1, 3, and 7**

(A) PRV3M (Melaka virus) RNA load as measured by NGS transcriptome mean FPKM (all segments) at 9 h post-infection with an MOI of 1 (washed, 3 h post-infection). Cell lines as indicated including WT, IRF1/3/7, IFNAR2, and IFNAR2/IRF7 double CRISPR KO cells.

(B) As per (A) at 24-h time point.

(C) Venn diagram of overlapping and unique DEG analysis, compared to WT, from Edger in both time points after infection with PRV3M for cell lines as per (A).

(D) IPA of significantly changed genes (>2-fold change, p < 0.05) compared to untreated for all cell lines as per (A) (scale as indicated, fold-change, min/max).

Orange boxes highlight differences between WT and IRF clones. Blue boxes highlight similarities between WT and IFNAR2 KO cells (IFN independent).

(E) Heatmap of fold-change post-infection compared to untreated controls for the antiviral IRG subset, normalized to geometric mean of 13 housekeeping genes. A 4-color non-linear scale is used from blue-white-red-black (~1, unchanged), 20, 300-fold induction), as indicated.

(F) Viral titration on Vero E6 cells from supernatant 72 h post-infection with PRV3M at an MOI of 1; clonal cell line as indicated. Dilution series as indicated, in quadruplicate. Gaps in the monolayer occur from syncytia formation whereby syncytia are counted as a single plaque.

(G) Quantitation of viral production from titrated supernatants as per (F), including IRF1/3/7 KO cells restored with IRF1/3/7-GFP fusions constructs, respectively.

(H) IPA for the significant IFN-stimulated above GFP control, expressed as p value for significance of the pathway; −log10(p value) scale as indicated. Significance values for (A), (B), and (G) were determined by unpaired t test; ***p < 0.01, **p < 0.02, *p < 0.05 (n ≥ 3). All error bars are indicated as SEM.
suggesting negative regulation of IFN transcription. Known inhibitors of IFN signaling were not induced early in WT cells when some limited transcription was observed. Late time points suggest TRIM21, RCK1, USP18/25, NMI, and OASL may partially contribute in this regard. The apparent shutdown of protein translation in WT cells stimulated with IFN polyIC overnight suggests a mechanism to prevent excessive immune responses or to block excessive cytokine production. This phenomenon for skewing protein translation preferentially toward IRGs has been observed previously (Chitrakar et al., 2019), although further investigation is required to see if such is the case.

IRF7-deficient cells also failed to induce a proper antiviral IRG response post-infection with PRV3M. The large increase in virus production of IRF7 KO cells, with IRF3 and IFN signaling intact, suggests IRF3 and consequent IFN induction are not capable of a complete antiviral response against PRV3M. Most non-immune cells in mammals express minimal IRF7 and rely on IRF3 to detect viral RNAs. Combined with the strong antiviral effect of IRF7 in IFNAR2 KO cells, this suggests that the additional antiviral functions of the widely distributed IRF7 are essential for mounting a complete antiviral response in bats. This may be related to IRF7’s requirement to sustain IRG induction at later time points and may involve modulation of the initiation of protein translation. As IFNα1,4/ω2 ligands were still induced in IRF7-deficient cells, it is not likely to be due to the decreased IFNβ expression. The role of IRF7 at late time points possibly involves suppression of cAMP, p38/MAPK, AMPK, and GPCR/phospholipase signaling (the most significantly affected pathways). This supports our finding that IRF7 is a potent antiviral for PRV3M viral load/infectious titer, which can be conferred by P. alecto IRF7 overexpression in multiple species/cell types.

Many of these bat-specific, IRF-regulated genes contribute to pathways such as metabolism, glycolysis, intracellular lipids, GPCR, tryptophan metabolism, and ROS/NO signaling. As the master regulators of the immune system, IRFs are considered essential for the efficient development of immune cells. These pathways also suggest a deeper regulation of cell biology that may contribute to an IRF-regulated antiviral state in addition to the typical IFN-like signatures. Metabolism and ROS particularly are important for trained immunity. For example, IRF1 contributes to SMAD2 expression that may affect STAT3/IFNγ target genes and Th17/Treg development. The restriction on IFN production may also play a role in the development of immune cells and has been implicated previously for B cells, T cells, and monocytes (Gabrieli et al., 2004; Kavrochorianou et al., 2016; Silva-Barrios et al., 2016). IRF7 plays a role in microglia polarization (Tanaka et al., 2015), IRF1 in training macrophage responses (Cheng et al., 2019; Langlais et al., 2016), and both in PRR signaling in dendritic cells (Cohen et al., 2014; Honda et al., 2005; Hu et al., 2008; Li et al., 2014b; Robertson et al., 2014; Weiss et al., 2012); further investigations are warranted to examine the role for IRFs in immune cell development in bats.

In summary, both IRF1 and IRF7 regulate IFNs and basal expression of antiviral genes in P. alecto bats. IRF1/3/7 regulate genes at early time points independent of significant IFN induction, as opposed to primarily IRF3 in humans. Many of the IRF-regulated genes/pathways were not previously revealed in studies of IFR induction in humans/mice. Using dsRNA and an RNA virus, we highlight the importance of IRF3 and 7 post-induction. We demonstrate that bat cells have a prolonged IFN-like antiviral signature even in the absence of IFN ligands, which are minimally induced during early infection. Although careful examination of IRF master regulation will be needed across the numerous bat species and in vivo, given the unique tolerance of infection observed in bats, this study highlights IFN-, dsRNA-, and virus-induced genes and pathways not previously highlighted in other mammals. The higher basal expression of IRFs may contribute to this suppression and regulates HSV-1, IAV, MERS-CoV, and PRV3M infection, even in the absence of type I IFNs. This potentially influences the host’s ability to serve as a zoonotic reservoir and tolerance for viral infection. This work highlights key areas to focus on in not only bat innate immunity but also protection against viruses in other mammalian species. It also highlights the need for studying the conditions required for IRF1/7 expression in humans, post-viral infection, and any potential to alter their regulation.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
  - Lead Contact
  - Materials Availability
  - Data and Code Availability
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
  - Animals, viruses and cells
- **METHOD DETAILS**
  - RNA extraction and quantitative RT-PCR
  - RNaseq analysis
  - Viral plaque/titration assays
  - Viral infection/ligand stimulation assays
  - Mass spectrometry analysis
  - MS-data analysis
  - CRISPR Knockout cell line generation
  - IRF overexpression and luciferase studies
  - Pathway analysis
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2020.108345.

ACKNOWLEDGMENTS

This work was funded by the Singapore National Research Foundation grants (NRF2012NRF-CRP001-056 to L.-F.W. and NRF2016NRF-NSFC002-013 to L.-F.W. and P.Z.), National Medical Research Council of Singapore New Investigator’s Grant (NMRC/BNIG/2040/2015 to A.T.I.), and a Singapore National Research Foundation grant (ZRRF16006 to L.-F.W. and NRF2016NRF-NSFC002-013 to L.-F.W. and A.T.I.). This work is supported by the Biomedical Research Council (BMRC), Agency for Science, Technology and Research (A*STAR) core funding to R.M.S. Many thanks to the
following in helping with bat sample processing: Crameri Research Consulting, IH Mendenthal, Prof. Joanne Meers of UQ, the Queensland Animal Science Precinct (QASP) team led by Hume Field, and Duke-NUS team members from LEZV/LOVE labs for collection of bat samples. We thank DE Anderson and the Duke-NUS ABL3 staff and facility management for their expert advice and assistance.

AUTHOR CONTRIBUTIONS

A.T.I. designed the study, performed experiments, analyzed the data, and wrote the manuscript under supervision from L.-F.W. and with input from all authors. Q.Z., P.R., P.-S.K. and K.L. performed experiments and/or generated cell lines and analyzed data. J.H.J.N. generated tissue cDNA panels. K.L. and R.M.S. performed proteomics studies.

DECLARATION OF INTERESTS

We declare there is no conflict of interest.

Received: May 24, 2019
Revised: August 23, 2020
Accepted: October 13, 2020
Published: November 3, 2020

REFERENCES

Anders, S., Pyl, P.T., and Huber, W. (2015). HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics 31, 166–169.

Banerjee, A., Falzarano, D., Rapin, N., Lew, J., and Misra, V. (2019). Interferon-Independent Upregulation of Interferon-Stimulated Genes during Human Cytomegalovirus Infection is Dependent on IFR3 Expression. Viruses 11, 246.

Austad, S.N., and Fischer, K.E. (1991). Mammalian aging, metabolism, and ecology: Evidence from the bats and maruspius. J. Gerontol. 46, B47–B53.

Baker, M.L., Schoutz, T., and Wang, L.F. (2013). Antiviral immune responses of bats: a review. Zoonoses Public Health 60, 104–116.

Barnerjee, A., Falzarano, D., Rapin, N., Lew, J., and Misra, V. (2019). Interferon Regulatory Factor 3-Mediated Signaling Limits Middle-East Respiratory Syndrome (MERS) Coronavirus Propagation in Cells from an Insectivorous Bat. Viruses 11, 152.

Barnes, B.J., Richards, J., Mancl, M., Hanash, S., Beretta, L., and Pitta, P.M. (2004). Global and distinct targets of IRF-5 and IRF-7 during innate response to viral infection. J. Biol. Chem. 279, 45194–45207.

Bisazczczyk, K., Olejnik, A., Nowicka, H., Ozgyn, L., Chen, Y.-L., Chmielewski, S., Kostyroc, K., Wesoł, J., Balint, B.L., Lee, C.-K., and Blyussen, H.A. (2015). STAT2/IRF9 directs a prolonged ISGF3-like transcriptional response and antiviral activity in the absence of STAT1. Biochem. J. 466, 511–524.

Bouma, H.R., Carey, H.V., and Kroese, F.G.M. (2010). Hibernation: the immune system at rest? J. Leukoc. Biol. 88, 619–624.

Brunet-Rossini, A.K. (2004). Reduced free-radical production and extreme longevity in the little brown bat (Myotis lucifugus) versus two non-flying mammals. Mech. Ageing Dev. 125, 11–20.

Brunet Rossini, A.K. (2004). Testing the free radical theory of aging in bats. Ann. N Y Acad. Sci. 1019, 506–508.

Cabrera-Romo, S., Recio-Tótoro, B., Alcalá, A.C., Lanz, H., del Ángel, R.M., Sánchez-Cordero, V., Rodríguez-Moreno, Á., and Ludert, J.E. (2014). Experimental inoculation of Artibeus jamaicensis bats with dengue virus serotype 1 or 4 showed no evidence of sustained replication. Am. J. Trop. Med. Hyg. 97, 1227–1234.

Cao, W., Manicasamy, S., Tang, H., Kasturi, S.P., Pirani, A., Murthy, N., and Pulendran, B. (2008). Toll-like receptor-mediated induction of type I interferon in plasmacytoid dendritic cells requires the rapamy cin-sensitive PI3K/mTOR/p70S6K pathway. Nat. Immunol. 9, 1157–1164.

Caracausi, M., Piovesan, A., Antonaros, F., Stripoli, P., Vitale, L., and Pelleri, M.C. (2017). Systematic identification of human housekeeping genes possibly useful as references in gene expression studies. Mol. Med. Rep. 16, 2397–2410.

Cheng, Q., Behzadi, F., Sen, S., Ohta, S., Spreafico, R., Teles, R., Modlin, R.L., and Hoffmann, A. (2019). Sequential conditioning-stimulation reveals distinct gene- and stimulus-specific effects of Type I and II IFN on human macrophage functions. Sci. Rep. 9, 5288.

Cheon, H., Holvey-Bates, E.G., Schooggins, J.W., Forster, S., Hertzog, P., Imakara, N., Rice, C.M., Jackson, M.W., Junk, D.J., and Stark, G.R. (2013). IFNβ-dependent increases in STAT1, STAT2, and IRF9 mediate resistance to viruses and DNA damage. EMBO J. 32, 2751–2763.

Chitrakar, A., Rath, S., Donovan, J., Demarest, K., Li, Y., Sridhar, R.R., Weiss, S.R., Kotenko, S.V., Wingreen, N.S., and Korennykh, A. (2019). Real-time 2-SAT kinetics suggest that interferons β and λ evade global arrest of translation by RNAse L. Proc. Natl. Acad. Sci. USA 116, 2100–2111.

Chua, K.B., Crameri, G., Hyatt, A., Yu, M., Tompong, M.R., Rosli, J., McEachern, J., Crameri, S., Kumarasamy, V., Eaton, B.T., and Wang, L.F. (2007). A previously unknown reovirus of bat origin is associated with an acute respiratory disease in humans. Proc. Natl. Acad. Sci. USA 104, 11424–11429.

Cohen, M., Matcovitch, O., David, E., Barnett-Izhaki, Z., Keren-Shaul, H., Blecher-Gonen, R., Jaitin, D.A., Sica, A., Amit, I., and Schwartz, M. (2014). Chronic exposure to TGFβ1 regulates myeloid cell inflammatory response in an IFR7-dependent manner. EMBO J. 33, 2906–2921.

Crameri, G., Todd, S., Grimley, S., McEachern, J.A., Marsh, G.A., Smith, C., Tachedjian, M., De Jong, C., Virtue, E.R., Yu, M., et al. (2009). Establishment, immortalisation and characterisation of pteropid bat cell lines. PLoS One 4, e8266.

La Cruz-Rivera, P.C.D., Kanchwala, M., Liang, H., Kumar, A., Wang, L.-F., Xing, C., and Schooggins, J.W. (2017). The IFN response in bat cells consists of canonical and non-canonical ISGs with unique temporal expression kinetics. bioRxiv.https://doi.org/10.1101/167999.

Currie, S.E., Stavski, C., and Geiser, F. (2018). Cold-hearted bats: uncoupling of heart rate and metabolism during torpor at sub-zero temperatures. J. Exp. Biol. 221, jeb170894.

Davis, A., Bunning, M., Gordy, P., Panella, B., Biltvich, B., and Bowen, R. (2005). Experimental and natural infection of North American bats with West Nile virus. Am. J. Trop. Med. Hyg. 73, 467–469.

Dery, K.J., Silver, C., Yang, L., and ShivELY, J.E. (2018). Interferon regulatory factor 1 and a variant of heterogeneous nuclear ribonucleoprotein L coordinately silence the gene for adhesion protein CEACAM1. J. Biol. Chem. 293, 9277–9291.

Eggerberger, J., Blanco-Melo, D., Panis, M., Brennand, K.J., and tenOever, B.R. (2019). Type I interferon response impairs differentiation potential of pluripotent stem cells. Proc. Natl. Acad. Sci. USA 116, 1384–1393.

Eisenberg, E., and Levanon, E.Y. (2013). Human housekeeping genes, revisited. Trends Genet. 29, 569–574.

Foley, N.M., Hughes, G.M., Huang, Z., Jebb, D., Whelan, C.V., Pettit, E.J., Touzalin, F., Farcy, O., Jones, G., et al. (2018). Growing old, yet staying young: The role of telomeres in bats’ exceptional longevity. Sci. Adv. 4, eaao0926.

Fuchs, J., Hölder, M., Schilling, M., Patzina, C., Schoen, A., Hoener, T., Zimmer, G., Marz, M., Weber, F., Muller, M.A., et al. (2017). Evolution and antiviral specificity of interferon-induced Mrx proteins of bats against Ebola-, Influenza-, and other RNA viruses. J. Virol. 91, e00361-17.

Gabriele, L., Borghi, P., Rozera, C., Sestili, P., Andreotti, M., Guarini, A., Montefusco, E., Foà, R., and Belardelli, F. (2004). IFN-α promotes the rapid differentiation of monocyes from patients with chronic myeloid leukemia into...
activated dendritic cells tuned to undergo full maturation after LPS treatment. Blood 103, 980–987.

Garvin, A.J., Khalaf, A.H.A., Rettno, A., Xicluna, J., Butler, L., Morris, J.R., Heery, D.M., and Clarke, N.M. (2019). GSK3β-SCFFBXX7α mediated phosphorylation and ubiquitination of IRF1 are required for its transcription-dependen-
tent turnover. Nucleic Acids Res. 47, 4476–4494.

Génin, P., Vaccaro, A., and Civas, A. (2009). The role of differential expression of human interferon–α genes in antiviral immunity. Cytokine Growth Factor Rev. 20, 283–295.

Ghosh, S., and Chan, C.-K.K. (2016). Analysis of RNA-Seq Data Using TopHat and Cufflinks (Humana Press), pp. 339–361.

Glennon, N.B., Jabado, O., Lo, M.K., and Shaw, M.L. (2015). Transcriptome Profiling of the Virus-Induced Innate Response Memory in Pteropus vampyrus and Its Attenuation by Nipah Virus Interferon Antagonist Functions. J. Virol. 89, 7550–7566.

Han, Y., Zheng, G., Yang, T., Zhang, S., Dong, D., and Pan, Y.H. (2015). Adaptation of peroxisome proliferator-activated receptor alpha to hibernation in bats. BMC Evol. Biol. 15, 88.

Herbold, J.R., Heuschele, W.P., Berry, R.L., and Parsons, M.A. (1983). Reservoir of St. Louis encephalitis virus in Ohio bats. Am. J. Vet. Res. 44, 1889–1893.

Hölzer, M., Schoen, A., Wulle, J., Müller, M.A., Drosten, C., Marz, M., and Weber, F. (2019). Virus- and Interferon Alpha-Induced Transcriptomes of Cells from the Microbat Myotis daubentonii. Science 19, 647–661.

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

Hu, Y., Park-Min, K.-H., Yarilina, A., and Ishikawa, L.B. (2008). Regulation of STAT pathways and IRF1 during human dendritic cell maturation by TNF-alpha and pGE2. J. Leukoc. Biol. 84, 1353–1360.

Huang, Z., Zebb, D., and Teeling, E.C. (2016). Blood miRNomes and transcriptomes reveal novel longevity mechanisms in the long-lived bat, Myotis myotis. BMC Genomics 17, 906.

Irving, A.T., Wang, D., Vasilievski, O., Latchoumanin, O., Kozer, N., Clayton, A.H.A., Szczepny, A., Morimoto, H., Xu, D., Williams, B.R.G., and Sadler, A.H.A. (2019). GSK-3α and PGE2. J. Leukoc. Biol. 88, 8619–8633.

Kavrochorianou, N., Evangelidou, M., Markogiannaki, M., Tovey, M., Thyphronitis, G., and Haralabous, S. (2016). IFNAR signaling directly modulates T lymphocyte activity, resulting in milder experimental autoimmune encephalomyelitis development. J. Leukoc. Biol. 95, 175–186.

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

Kuleshov, M.V., Jones, M.R., Rouillard, A.D., Fernandez, N.F., Duan, O., Wang, Z., Kolev, S., Jenkins, S.L., Jagodnik, K.M., Lachmann, A., et al. (2016). Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 44, W90–W97.

Langlais, D., Barreiro, L.B., and Gros, P. (2016). The macrophage IFIRF/IFIR1 regulator is required for protection against infections and is associated with chronic inflammation. J. Exp. Med. 212, 585–603.

Law, C.W., Alhamdooosh, M., Su, S., Dong, X., Tian, L., Smyth, G.K., and Ritchie, M.E. (2016). RNA-seq analysis is easy as 1-2-3 with limma, Glimma and edgeR. F1000Res. 5, 1408.

Li, B., and Dewey, C.N. (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12, 323.

Li, W., Hofer, M.J., Jung, S.R., Lim, S.-L., and Campbell, LL. (2014a). IRF7-dependent type I interferon production induces lethal immune-mediated disease in STAT1 knockout mice infected with lymphocytic choriomeningitis virus. J. Virol. 88, 7578–7588.

Li, Y.-F., Lee, K.-G., Ou, X., and Lam, K.-P. (2014b). Bruton’s tyrosine kinase and protein kinase C α are required for TLR7/9-induced IFNα and IFN-α activa-
tion and interferon-β production in conventional dendritic cells. PLoS One 9, e105420.

Li, Y.-Z., Wu, L.J., Zhang, Q., Zhou, P., Wang, M.N., Yang, X.L., Ge, X.Y., Wang, L.F., and Shi, Z.L. (2015). Cloning, expression, and antiviral activity of interferon β from the Chinese microbat, Myotis davidii. Virol. Sin. 30, 425–432.

Lin, R., and Hiscott, J. (1999). A role for casein kinase II phosphorylation in the regulation of IRF-1 transcriptional activity. Mol. Cell. Biochem. 191, 169–180.

Lin, R., Mamane, Y., and Hiscott, J. (2000a). Multiple regulatory domains control IRF-7 activity in response to virus infection. J. Biol. Chem. 275, 34320–34327.

Lin, R., Génin, P., Mamane, Y., and Hiscott, J. (2000b). Selective DNA binding and association with the CREB binding protein coactivator contribute to differential activation of alpha/beta interferon genes by interferon regulatory factors 3 and 7. Mol. Cell. Biol. 20, 6342–6353.

McNab, B.K. (1989). Temperature Regulation and Rate of Metabolism in Three Bornean Bats. J. Mammal. 70, 153–161.

Middleton, D.J., Morriess, C.J., van der Heide, B.M., Russell, G.M., Braun, M.A., Westbury, H.A., Halpin, K., and Daniels, P.W. (2007). Experimental Nipah virus infection in pteropid bats (Pteropus poliocephalus). J. Comp. Pathol. 136, 266–272.

Mok, L., Wynne, J.W., Grimley, S., Shiell, B., Green, D., Monaghan, P., Pallister, J., Bacic, A., and Michalski, W.P. (2015). Mouse fibroblast L929 cells are less permissive to infection by Nelson Bay orthoreovirus compared to other mammalian cell lines. J. Gen. Virol. 96, 1787–1794.

Mok, L., Wynne, J.W., Tachedjian, M., Shiell, B., Ford, K., Matthews, D.A., Bacic, A., and Michalski, W.P. (2017). Proteomics informed by transcriptomics for characterising differential cellular susceptibility to Nelson Bay orthoreovirus infection. BMC Genomics 18, 615.

Nan, J., Wang, Y., Yang, J., and Stark, G.R. (2018). IRF9 and unphosphory-
lated STAT2 cooperate with NF-κB to drive IL6 expression. Proc. Natl. Acad. Sci. USA 115, 3906–3911.

O’Mara, M.T., Wikelski, M., Voigt, C.C., Ter Maat, A., Pollock, H.S., Burness, G., Desantis, L.M., and Dechmann, D.K. (2017). Cyclic bouts of extreme bradycardia counteract the high metabolism of frugivorous bats. eLife 6, e26866.

Obregón-Morales, C., Aguilar-Setién, Á., Perea Martinez, L., Galvez-Romero, G., Martinez-Martinez, F.O., and Arechiga-Ceballos, N. (2017). Experimental infection of Antilobus intermedius with a vampire bat rabies virus. Comp. Immun. Microbiol. Infect. Dis. 52, 43–47.

Pavlovic, S.S., Lovett, S.P., Koroleva, G., Guito, J.C., Arnold, C.E., Nagle, E.R., Kucsa, K., Lee, A., Thibaudeau-Nissen, F., Hume, A.J., et al. (2018). The
Egyptian Rousette Genome Reveals Unexpected Features of Bat Antiviral Immunity. Cell 173, 1098–1110.e18.

Paweska, J.T., Storm, N., Grobelaar, A.A., Markotter, W., Kemp, A., and Janse van Vuuren, P. (2016). Experimental Inoculation of Egyptian Fruit Bats (Rousettus aegyptiacus) with Ebola Virus. Viruses 8, 29.

Perea-Martínez, L., Moreno-Sandoval, H.N., Moreno-Altimarino, M.M., Salas-Rojas, M., García-Flores, M.M., Árchipiga-Ceballos, N., Tordo, N., Marianneau, P., and Aguilar-Setién, A. (2013). Experimental infection of Artibeus intermedium bats with serotype-2 dengue virus. Comp. Immunol. Microbiol. Infect. Dis. 36, 193–198.

Pyla, S., and Kim, T.-H. (2018). The Effect of Lipopolysaccharide on Noxa Expression Is Mediated through IRF1, 3, and 7. J. Microbiol. Biotechnol. 28, 491–497.

Podlutsky, A.J., Khritankov, A.M., Ovodov, N.D., and Austad, S.N. (2005). A new field record for bat longevity. J. Gerontol. A Biol. Sci. Med. Sci. 60, 1366–1368.

Reagan, R., and Brueckner, A.L. (1952). Studies of dengue fever virus in the cave bat (Myotus lucifugus). J. Infect. Dis. 97, 145–146.

Ren, L., Wu, C., Guo, L., Yao, J., Wang, C., Xiao, Y., Pisoo, A.O., Wu, Z., Lei, X., Liu, Y., et al. (2020). Single-cell transcriptional atlas of the Chinese horseshoe bat (Rhinolophus sinicus) provides insight into the cellular mechanisms which enable bats to be viral reservoirs. bioRxiv, 2020.06.30.175778.

Robertson, S.J., Lubick, K.J., Freedman, B.A., Carmody, A.B., and Best, S.M. (2014). Tick-borne flaviviruses antagonize both IRF-1 and type I IFN signaling to inhibit dendritic cell function. J. Immunol. 192, 2744–2755.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139–140.

Schoggins, J.W., and Rice, C.M. (2011). Interferon-stimulated genes and their quantification studies using common marmoset tissues. Mol. Biol. Rep. 38, 6747–6755.

Sharma, S., TenOever, B.R., Grandvaux, N., Zhou, G.P., Lin, R., and Hiscott, J. (2003). Triggering the interferon antiviral response through an IKK-related pathway. Science 300, 1148–1151.

Shen, Y.Y., Liang, L., Zhu, Z.H., Zhou, W.P., Irwin, D.M., and Zhang, Y.P. (2010). Adaptive evolution of energy metabolism genes and the origin of flight in bats. Proc. Natl. Acad. Sci. USA 107, 8666–8671.

Shimamoto, Y., Kitamura, H., Niimi, K., Yoshikawa, Y., Hoshi, F., Ishizuka, M., and Takahashi, E. (2013). Selection of suitable reference genes for mRNA quantification studies using common mammoset tissues. Mol. Biol. Rep. 40, 6499–6503.

Shultz, D.B., Rani, M.R., Fuller, J.D., Banshoff, R.M., and Stark, G.R. (2009). Roles of IKK-β, IRF1, and p65 in the activation of chemokine genes by interferon-γ. J. Interferon Cytokine Res. 29, 817–824.

Silva-Barrios, S., Smans, M., Duerr, C.U., Qureshi, S.T., Fritz, J.H., Descoteaux, A., and Stäger, S. (2016). Innate Immune B Cell Activation by Leishmania donovani Exacerbates Disease and Mediates Hypergammaglobulinaemia. Cell Rep. 15, 2427–2437.

Simpson, D.I., and O’Sullivan, J.P. (1968). Studies on arboviruses and bats (Chiroptera) in East Africa. I. Experimental infection of bats and virus transsion attempts in Aedes (Stegomyia) aegypti (Linnaeus). Ann. Trop. Med. Parasitol. 62, 422–431.

Stockmaier, S., Dechmann, D.K.N., Page, R.A., and O’Mara, M.T. (2015). No fever and leucocytosis in response to a lipopolysaccharide challenge in an insectivorous bat. Biol. Lett. 11, 20150576.

Su, S., Law, C.W., Ah-Cann, C., Asselin-Labat, M.-L., Blewitt, M.E., and Ritchie, M.E. (2017). Glimma: interactive graphics for gene expression analysis. Bioinformatics 33, 2050–2052.

Suarez, R.K., and Welch, K.C. (2017). Sugar Metabolism in Hummingbirds and Nectar Bats. Nutrients 9, 743.
Zhou, P., Cowled, C., Mansell, A., Monaghan, P., Green, D., Wu, L., Shi, Z., Wang, L.-F.F., and Baker, M.L. (2014). IRF7 in the Australian black flying fox, Pteropus alecto: evidence for a unique expression pattern and functional conservation. PLoS One 9, e103875.

Zhou, Q., Lavorgna, A., Bowman, M., Hiscott, J., and Harhaj, E.W. (2015). Aryl Hydrocarbon Receptor Interacting Protein Targets IRF7 to Suppress Antiviral Signaling and the Induction of Type I Interferon. J. Biol. Chem. 290, 14729–14739.

Zhou, P., Tachedjian, M., Wynne, J.W., Boyd, V., Cui, J., Smith, I., Cowled, C., Ng, J.H.J., Mok, L., Michalski, W.P., et al. (2016). Contraction of the type I IFN locus and unusual constitutive expression of IFN-α in bats. Proc. Natl. Acad. Sci. USA 113, 2696–2701.
## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Roche anti-GFP      | Sigma Aldrich | Cat# 11814460001; RRID:AB_390913 |
| **Bacterial and Virus Strains** |        |            |
| HSV-1 Kos           | ATCC   | VR-1493    |
| H1N1 IAV A/NWS/33   | ATCC   | VR-219     |
| PRV3M p3            | BK Chua, TLS | Prototype: taxonomy ID: 16867 |
| MERS-CoV (HCoV-EMC/2012) | EMC | JX869059.2 |
| **Biological Samples** |        |            |
| P. alecto tissue (cDNA panel) – South East Queensland, Australia. (F, M) | QASP/UQ/ABC/BRQ | N/A |
| M. muscus tissue (cDNA panel) – C57Bl6/J | Jackson laboratories | 000664 |
| E. spelaea (cDNA) – Singapore (F,M) | Duke-NUS | N/A |
| **Chemicals, Peptides, and Recombinant Proteins** |        |            |
| Pierce Protein A/G Magnetic Agarose Beads | Thermo scientific | 78609 |
| polyIC HMW          | InVivoGen | tlr-pic |
| IFNγ (P. alecto) – bacterial recombinant. | CSIRO/AAHL | N/A |
| Lipofectamine 3000  | Thermo Scientific | L3000015 |
| IFNγ (P. alecto) – mammalian-expressed, supernatant. | Duke-NUS | N/A |
| **Deposited Data**  |        |            |
| PakiT03 infection NGS (Gene Expression Omnibus) | Duke-NUS | GEO: GSE129390 |
| P. alecto NGS (Gene Expression Omnibus) | Duke-NUS | GEO: GSE129377 |
| E. spelaea NGS (Gene Expression Omnibus) | Duke-NUS | GEO: GSE129199 |
| Mass spectrometry data repository | https://repository.jpostdb.org/ | JPST000983 |
| **Experimental Models: Cell Lines** |        |            |
| PakiT03 (F) Kidney epithelial | CSIRO/AAHL | RRID: CVCL_DR89 |
| PakiT03-variants | Duke-NUS | 'Taxonomy ID: 9402 |
| Hek293T kidney epithelial | ATCC | RRID: CVCL_0063 |
| MdKı kidney epithelial | WIV, CAS, China | Taxonomy ID: 225400 |
| Vero E6            | Duke University | N/A |
| Vero B4            | CCLV   | RRID: CVCL_1912 |
| **Experimental Models: Organisms/Strains** |        |            |
| Pteropus alecto gouldii | South-East Queensland bat carers | N/A |
| Eonycteris spelaea | Singapore | N/A |
| Homo Sapiens       | Published data – SRA | N/A |
| **Oligonucleotides** |        |            |
| See Table S6       |        | N/A        |
| **Recombinant DNA** |        |            |
| pcDNA6.2/N-EmGFP-GW/TOPO | Invitrogen | K36020 |
| IRF1                | P.a. cDNA | GenBank: XM_006923152.3 |
| IRF3                | P.a. cDNA | GenBank: XM_025048312.1 |
| IRF7                | P.a. cDNA | GenBank: NM_001320278.1 |
| IRF5                | P.a. cDNA | GenBank: XM_006910531.3 |

(Continued on next page)
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Software and Algorithms** | | |
| Bowtie2/RSEM | GitHub | v2.3.4.2 |
| Cufflinks | GitHub | v2.2.1 |
| HTseq-count | GitHub | 0.6.1p1 |
| Tophat | GitHub | 2.1.1 |
| EdgeR | BioConductor | 3.24.3 |
| Glimma | BioConductor | 1.2.1 |
| Prism | Graphpad | 8 |
| Biovenn | http://www.biovenn.nl/ | N/A |
| Morpheus | https://clue.io:443/Broad | N/A |
| Ingenuity Pathway Analysis | QIAGEN | Summer/Fall 2019 |
| BioDBnet | https://biodbnet-abcc.ncifcrf.gov/db/db2db.php | |
| GSEA | https://www.gsea-msigdb.org/gsea/ | N/A |
| **Other** | | |
| IFNAR2-4A: 35bp, homozygous. | In-House CRISPR KO – Duke-NUS | PakiT03- IFNAR2-4A |
| ATGTTTTCAGATGAGCCTTTGACATTTTCGAG | | |
| ATAACTGTTAAGAAATTCGCGAAATTTTA | | |
| TCGTGGAAATTTTAAAGAC | | |
| CACTCCATTGTACACCTACATATACATTA | | |
| CAGTATGCAATCATGAG | | |
| IFNAR2-9E: 70bp, homozygous | In-House CRISPR KO – Duke-NUS | PakiT03- IFNAR2-9E |
| TTTACCATTTTCCTTTTCTCACTTTGGAC | | |
| GAGAAGATGTCATTACTGT | | |
| GAGGAAGGCATTACCATACACAGGTCATTAC | | |
| TGTGACCTGACAGATG | | |
| CAGTGGTGAAACATGTCTGAGCGTACACTCC | | |
| CAGGAGACTGTGACAGATG | | |
| ACCGGAGGAACAGGACGTGTCAGTCGACGTG | | |
| AGGGCGCGTTATCCT | | |
| CGTTAATGGAATGTGACTTGACCTCTCTCTTAT | | |
| CATCTCTGCCATTGTC | | |
| ATCAAGATCATCATTATTTTTCTCTTGGACAG | | |
| IFNAR2-9E: 44bp, homozygous. | In-House CRISPR KO – Duke-NUS | PakiT03- IFNAR2-9E |
| GGGGCGCAACTGGGCTGGCATTGGCGGCTG | | |
| CAAAGGCTAATGGTCTTC | | |
| TATCTTCCACCAGAGAAAGCTCAAGTCC | | |
| AGCCGAGACGTCAAG | | |
| ACCAGAGGAGGGAAGGGTACTGCGTGTC | | |
| CTGAGCAGCGCTGCCC | | |
| TGATCCCTGAGGACGGGGTGCCAGTGC | | |
| GAAGAAAGCAGCCT | | |
| ACGACAGCTGACCTACATACACAG | | |

(Continued on next page)
| REAGENT or RESOURCE SOURCE IDENTIFIER | REAGENT or RESOURCE NAME | SOURCE | IDENTIFIER |
|--------------------------------------|--------------------------|--------|------------|
| IRF1-g3-1A: – 23bp, 21 in exons 4, homozygous. GGGGCGAACCTGGGCTGCGATGCCCGTGCC CAAGGCTAATGTC CTTCTCTCCCCAGAGAAAGTCCAATGTC CAGCCGAGA GCCTAACAGGCAAGCCAGGAAGGTAAGTC CTGGTCCTGGCAGAGCTGGCCTAAGCTGTAAGCCTCAGAGGACTGGCCTGCGGCAGTGGCCCTGGGCCGTGGTGGGTGAGGAGGCAGGCTGGCCCTTCTCCGAGCACGTAAGGCAGGACTCCGAGGGCACTGGC |
| In-House CRISPR KO – Duke-NUS PakiT03- IRF1-g3-1A | IRF1-g3-4C: Large deletion, homozygous. 0 trace GAAAGTCTGGCTCAGACAGCTGAGCCTGGGCC CATCGACTGGCTGAGGAGCCACTGGCGCTTTCGCATCCCT TGGAAGAACGACGACTTCATGGAAGAGGAGAGCCAGAGCCTGACGCTGGCCCTGGGCCGTGGTGGGTGAGGAGGCAGGCTGGCCCTTCTCCGAGCACGTAAGGCAGGACTCCGAGGGCACTGGC |
| In-House CRISPR KO – Duke-NUS PakiT03- IRF1-g3-4C | IRF3-11C: +214, insert in exons 5, yellow region is inserted sequence. In-House CRISPR KO – Duke-NUS PakiT03- IRF3-11C |
| IRF3-2F: 9bp, homozygous. CGCAGGTTGGACCATGGCTACCCCAAGCCGAGGATCCCTGGCCTGGAGAAGGAGGCAGAGGACTGGCCTGAGCCTGGCCCTGGGCCGTGGTGGGTGAGGAGGCAGGCTGGCCCTTCTCCGAGCACGTAAGGCAGGACTCCGAGGGCACTGGC |
| In-House CRISPR KO – Duke-NUS PakiT03- IRF3-2F | IRF3-4B: +1, homozygous. CGCAGGTTGGACCATGGCTACCCCAAGCCGAGGATCCCTGGCCTGGAGAAGGAGGCAGAGGACTGGCCTGAGCCTGGCCCTGGGCCGTGGTGGGTGAGGAGGCAGGCTGGCCCTTCTCCGAGCACGTAAGGCAGGACTCCGAGGGCACTGGC |
| In-House CRISPR KO – Duke-NUS PakiT03- IRF3-4B |
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| IRF3-4G: --23bp, homozygous. | In-House CRISPR KO – Duke-NUS | PakiT03- IRF3-4G |
| CGCAGGTGGACCATGGACTCCTCCAAACCGCGAGGATCCT |        |            |
| GCCCTGGCTAGTGTCAGTGGACAGTGAGGGGACGCTTGGA |        |            |
| GGGCGTGCCATGCTGAAGAGAGCGACCCGGCAGCTTCTC |        |            |
| CATCCCTTGGAAGAAGCGGGCTGGGAGCAGATGGCCAGCGCAGG |        |            |
| AGGACTTTCCGCACTACTCCAGTGCGCAGGAGGCCAACAGA |        |            |
| CTGGGCAAACACGGGGCAGGCGGAGGACTCCGAGGAGACG |        |            |

| IRF7-3C: --17bp, homozygous. CGCCAATCCGCAAGAAGTGCACTCCGACCTCGGACGCTGCA | In-House CRISPR KO – Duke-NUS | PakiT03- IRF7-3C |
| CGCCAATCCGCAAGAAGTGCACTCCGACCTCGGACGCTGCA |        |            |
| ACAAACGCCACGACAATTCCGCAACCGGGGACGCTGCA |        |            |
| AGCCAGGTGGGCAGGAGTTCAAGTCCGAGGAGGAGGAGGAGG |        |            |
| GTGATGGACGCAGGAGAGGCAGGAGGAGGAGGAGGAGGAGG |        |            |
| GCACGCGCGATGCGCGGAGGAGGAGGAGGAGGAGGAGGAGG |        |            |
| ATGCGATGGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG |        |            |

| IRF7-2G: --15bp, homozygous. CGCCAATCCGCAAGAAGTGCACTCCGACCTCGGACGCTGCA | In-House CRISPR KO – Duke-NUS | PakiT03- IRF7-2G |
| CGCCAATCCGCAAGAAGTGCACTCCGACCTCGGACGCTGCA |        |            |
| ACAAACGCCACGACAATTCCGCAACCGGGGACGCTGCA |        |            |
| AGCCAGGTGGGCAGGAGTTCAAGTCCGAGGAGGAGGAGGAGG |        |            |
| GTGATGGACGCAGGAGAGGCAGGAGGAGGAGGAGGAGGAGG |        |            |
| GCACGCGCGATGCGCGGAGGAGGAGGAGGAGGAGGAGGAGG |        |            |
| ATGCGATGGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG |        |            |

| IRF7-5G: compound heterozygous, --37bp. --15bp. CGCCAATCCGCAAGAAGTGCACTCCGACCTCGGACGCTGCA | In-House CRISPR KO – Duke-NUS | PakiT03- IRF7-5G |
| CGCCAATCCGCAAGAAGTGCACTCCGACCTCGGACGCTGCA |        |            |
| ACAAACGCCACGACAATTCCGCAACCGGGGACGCTGCA |        |            |
| AGCCAGGTGGGCAGGAGTTCAAGTCCGAGGAGGAGGAGGAGG |        |            |
| GTGATGGACGCAGGAGAGGCAGGAGGAGGAGGAGGAGGAGG |        |            |
| GCACGCGCGATGCGCGGAGGAGGAGGAGGAGGAGGAGGAGG |        |            |
| ATGCGATGGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG |        |            |

(Continued on next page)
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| IRF7-6E: +407, homozygous. | In-House CRISPR KO – Duke-NUS PakiT03- | IRF7-6E |
| TGAGGCATTTTCGCAATTCCGGAAGTGCAGCTTGGAGGCCTGGGAACCCCGCCGACCGCTGGGAAGGGCCGAGGAGGCCGGACGGCCAGGGGAGAGGAGGTAAACAACGGGGAGGCAGCGGTCAGGGAGGGGTCAGGGAGGCCAGGACGGCCAGGGTAGCCGAGAGGAATGGCCTGCACATGCTGCTGGCACTTTGGTGGCAGCCGCTGGGTCAGG |
| IRF7-7C: −17bp, homozygous. | In-House CRISPR KO – Duke-NUS PakiT03- | IRF7-7C |
| CGCCAATTCGGGAAGTGCAGCTCCGAGCCGGCGCTGGGAAGGGCCGAGGAGGCCGGACGGCCAGGGGAGAGGAGGTAAACAACGGGGAGGCAGCGGTCAGGGAGGGGTCAGGGAGGCCAGGACGGCCAGGGTAGCCGAGAGGAATGGCCTGCACATGCTGCTGGCACTTTGGTGGCAGCCGCTGGGTCAGG |
| IFNAR2/IRF7-3D: \( C0 \) 38bp. | In-House CRISPR KO – Duke-NUS PakiT03-IFNAR2-4A/IRF7-3D | IRF7-3D |
| CGCCAATTCGGGAAGTGCAGCTCCGAGCCGGCGCTGGGAAGGGCCGAGGAGGCCGGACGGCCAGGGGAGAGGAGGTAAACAACGGGGAGGCAGCGGTCAGGGAGGGGTCAGGGAGGCCAGGACGGCCAGGGTAGCCGAGAGGAATGGCCTGCACATGCTGCTGGCACTTTGGTGGCAGCCGCTGGGTCAGG |
| IFNAR2/IRF7-2A: \( C0 \) 17bp, same with 4G. | In-House CRISPR KO – Duke-NUS PakiT03-IFNAR2-4A/IRF7-2A | IRF7-2A |
| CGCCAATTCGGGAAGTGCAGCTCCGAGCCGGCGCTGGGAAGGGCCGAGGAGGCCGGACGGCCAGGGGAGAGGAGGTAAACAACGGGGAGGCAGCGGTCAGGGAGGGGTCAGGGAGGCCAGGACGGCCAGGGTAGCCGAGAGGAATGGCCTGCACATGCTGCTGGCACTTTGGTGGCAGCCGCTGGGTCAGG |
| IFNAR2/IRF7-4F: \( C0 \) 39bp. | In-House CRISPR KO – Duke-NUS PakiT03-IFNAR2-4A/IRF7-4F | IRF7-4F |
| CGCCAATTCGGGAAGTGCAGCTCCGAGCCGGCGCTGGGAAGGGCCGAGGAGGCCGGACGGCCAGGGGAGAGGAGGTAAACAACGGGGAGGCAGCGGTCAGGGAGGGGTCAGGGAGGCCAGGACGGCCAGGGTAGCCGAGAGGAATGGCCTGCACATGCTGCTGGCACTTTGGTGGCAGCCGCTGGGTCAGG |

(Continued on next page)
RESOURCE AVAILABILITY

Lead Contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Lin-Fa Wang (linfa.wang@duke-nus.edu.sg).

Materials Availability
Plasmids generated for this study are available upon request with a simple MTA.
Virus strains used in this study are commonly available and also either available upon request with MTA (e.g., PRV3M) or purchased from ATCC (e.g., H1N1).
Monkey, human and bat cell lines and CrispR KO bat cell lines are available upon request with a simple MTA.
DNA and RNA samples from bat cells and tissue are available for collaboration upon request with MTA.

Data and Code Availability
The accession number for the data reported in this paper is in the NCBI GEO database GEO: GSE129390 (PakiT03), #GSE129377 (Pteropus alecto tissue) & GEO: GSE129199 (Eonycteris spelaea tissue). Mass spectrometry data are available under the accession number JPST000983 at the JPOST repository https://repository.jpostdb.org/.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals, viruses and cells
*E. spelaea* were captured with the ethics approval of National University of Singapore Institutional Animal Care and Use Committee (IACUC Permit # B01/12), and the National Parks permits NP/PR11-011-3a and NP/PR12-004-2. All experiments were performed in accordance with relevant guidelines and regulations. Capturing and processing of black flying foxes (*P. alecto*) in Australia was approved by the Queensland Animal Science Precinct & University of Queensland Animal Ethics Committee (AEC#SVS/073/16/USGMS), and the Australian Animal Health Laboratory (AAHL) Animal Ethics Committee (AEC#1389 and AEC#1557). Where possible, wild bats with irreparable physical damage (torn wings) already scheduled for euthanasia were utilized. Wild *P. alecto* bats were temporarily housed for attempted rehabilitation, adjusting to handling for several weeks. Wild-born, colony housed *E. spelaea* bats (several months) were adjusted to human handling and handling did not trigger any stress-markers/cortisol etc in the blood. Bats were age and sex-matched to the best of our ability (late juvenile for *E. Spelaea*, grown “young” adults for *P. Alecto*). Prior to processing, bats were transferred to a temperature-controlled facility, settled, euthanised and culled immediately by cardiac bleed to prevent any systemic effects from anesthesia. Processing of bats and the generation of PaKi cell lines was has been described previously (Crameri et al., 2009; Irving et al., 2019). PaKT03 (CVCL_DR89) and variants, Hek293T (CVCL_0063) and MdKi (Liang
et al., 2015) cell lines were all cultured in DMEM (GIBCO) with 10% (v/v) FBS. All tissue was preserved in RNA later: MERS-CoV was propagated in Vero B4 (CVCL_1912) cells in DMEM, 2% FBS. At a virus-induced CPE of 80–90%, viruses were harvested, clarified by centrifugation, and the virus containing supernatant was stored at –80°C. PRV3M/Melaka Virus (p3) was propagated in Vero E6 cells and clarified as previous. Viral titers were calculated upon infectivity Vero B4 cells, by plaque assay. Human H1N1 IA V strain A/WS/33 was purchased commercially (ATCC # VR-219), HSV-1 was propagated in HeLa cells. Human tissue data was publicly available (SAMEA2146236, SAMEA2153031, SAMEA2155751, SAMEA2159764, SAMEA2142363, SAMEA2144333, SAMEA2147920, SAMEA2155770, SAMEA2158569, SAMEA2145122, SAMEA215590, SAMEA2162895).

METHOD DETAILS

RNA extraction and quantitative RT-PCR
Harvested tissues of mice or bats were homogenized using silicon-carbide sharp particles (BioSpec Products) in the FastPrep-24 5G Homogenizer (MP Biomedicals). RNA was extracted using either the RNEasy micro kit (QIAGEN) for tissue or the EZNA total RNA kit I (Omega Bio-tek, Norcross, GA, USA) for cell culture. Extracted RNA (500 ng) was subsequently used for cDNA synthesis using the QuantiTect Reverse Transcription Kit (QIAGEN, Germany). Reactions of qPCR were setup using the SensiFAST SYBR No-ROX Kit (Bioline, London, UK) and analyzed on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, CA, USA) under the following cycling condition: 95°C for 5 min, followed by 40 cycles of 95°C for 5 s and 58°C for 30 s, and ending with a melt profile analysis. The fold change in mRNA expression was determined using the 2-ΔΔCt method relatively to the values in mock samples, after normalization to housekeeping genes (Geometric mean) GAPDH and SNRDP3. Samples were corrected for PCR efficiency by standard curves from sample serial dilution. Primer sequences are in the Key Resources Table.

RNaseq analysis
Total RNA was checked using the RNA 6000 LabChip Kit on the Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA). RNaseq libraries were prepared using Illumina Tru-Seq Stranded Total RNA with Ribo-Zero Gold kit following the manufacturer's instructions (Illumina, San Diego, California, USA). Libraries were validated with an Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA), diluted and applied to an Illumina flow cell using the Illumina cBOT system. Sequencing was performed on an Illumina HiSeq 3000 sequencer at the Duke-NUS Genome Biology Facility with the paired-end 150-bp read option.

After trimming and cleaning for quality assurance (including distribution of reads), all reads were mapped to the P. alecto reference genome (NCBI genome database: ASM32557v1, 1.01) with Bowtie and RSEM abundance estimation was performed (Li and Dewey, 2011). E. spelaea reads were denovo assembled with Tophat/Cufflinks (Ghosh and Chan, 2016) and the FPKM for both Bowtie/RSEM mapped datasets was calculated using Cufflinks followed by HTseq and edgeR (Anders et al., 2015; Robinson et al., 2010) being used to detect genes that were differentially expressed post-polyIC/PRV3M/IFN-α3 treatment, followed by visualization with the Glimma package (Law et al., 2016; Su et al., 2017). The cut-off for differentially expressed genes (DEGs) was set at > 2-fold change and p value ≤ 0.05. Significantly up/downregulated genes were calculated based upon Fishers test for variance of the sample compared to Geometric mean of the three wild-type replicates compared to an array based upon the geometric mean of all genes. Basal cell lines had 3 biological replicates per cell, each treatment time point was pooled RNA from 2 biological replicates (one sample per time point). As such the DEGs were calculated for genes enriched in at least 2 time points out of 3 for IFN treatment or both time points for polyIC and PRV3M infection.

Viral plaque/titration assays
Were performed as previously described (Irving et al., 2012) using a 2 h infection followed by rinsing and then adding a 2% Methylcel lulose (Sigma) overlay on PakTI03/Vero E6 cells in 2% FBS/DMEM for 2-4 days in triplicate in a 24-well plate with a 10-fold dilution series. 0.8% Carboxy-methyl-cellulose was used for PRV3M (Melaka virus, parental strain) with Vero B4 cells. PRV3M and MERS-CoV (EMC/2012) titers were determined by limiting dilution. In brief, tenfold serial diluted virus was added into a 96-well plate containing 1x10^4 Vero B4 cells per well. Cells were observed for cytopathic effect and the titers were expressed as TCID_{50} ml^{-1}. All work with live MERS-CoV was performed in BSL3 containment at SingHealth Experimental Medical Centre.

Viral infection/ligand stimulation assays
PolyIC stimulation (1mg/ml), IFNα3 treatment and infection with HSV/IAV were done as previously (Zhang et al., 2017). PRV3M/MERS-CoV infection was performed using the same method with an MOI of 0.1 or 1 (as detailed) for various time points. Supernatant was collected for titration as mentioned previously. Cells were lysed directly in TRK RNA lysis buffer.

Mass spectrometry analysis
WT and KO cells were treated with polyIC or P. alecto IFNα3, as previous, overnight (16h). Cells were lysed in Buffer (Irving et al., 2012) followed by precipitation and concentration and bound proteins were denatured directly in 8 M urea/50 mM Tris-HCl buffer pH 8.0. Proteins were reduced with 25 mM TCEP for 20 min at 25°C and alkylated with 55 mM 2-chloroacetamide (CAA) for 30 min, in the dark, at room temperature. Before digestion, samples were diluted with 100 mM triethylammonium bicarbonate buffer (TEAB). Protease digestion was carried out with LysC enzyme (Wako) for 4 h, followed by trypsin (Promega) treatment for 18 h at 25°C (1:100, en-
zyme:protein ratio). Subsequently, samples were acidified with 1% trifluoroacetic acid and peptides were desalted by Sep-Pak C18 cartridges (Waters). Elution of peptides was performed with 0.5% acetic acid, 80% acetonitrile followed by peptide concentration using a vacuum concentrator system (Eppendorf). For quantitative mass spectrometry samples were labelled with TMT isobaric mass tag reagent (Thermo). Labelling was performed according to the manufacturer instruction. Following labelling, combined peptides were fractionated using in-house prepared high pH reverse phase columns (Reposil-Pur Basic C18 10µm, Dr Maisch Gmbh). Samples were eluted in 11 fractions of increasing concentration of acetonitrile (7%, 10%, 12%, 15%, 17%, 20%, 22%, 25%, 27%, 30%, 50%) in 10mM ammonium formate. Peptides were washed with 70% acetonitrile in 0.1% Formic Acid twice. Vacuum dried peptides were subsequently analyzed on an EASY-n LC 1000 (Thermo) chromatography system coupled with Orbitrap Fusion mass spectrometer (Thermo). Each fraction was separated in 120 min gradient (0.1% formic acid in water and 99.9% acetonitrile with 0.1% formic acid) using a 50 cm × 75 µm inner diameter EASY-Spray Reverse Phase Column (C-18, 2 µm particles, Thermo).

For acquisition, an Orbitrap analyser with ion targets and resolution (OT-MS 4e5 ions 60k; OT-MS/MS 5e5 ions 50k) was used. Data was acquired in speed mode: cycle time 3 sec.

**MS-data analysis**

Thermo Proteome Discoverer software (v 2.2, Thermo Fisher Scientific) was used to generate peak lists followed by combined search using Mascot 2.6.1 engine (Matrix Science) against target-decoy Bat customized database with following parameters: Fixed modifications: Carbamidomethyl cysteine and TMT10-plex labelling on N-terminus peptide and Lysine. Variable modifications: Oxidated (M), Deamidated (NQ) and acetylated protein N-terminus were set as variable modifications. Mass accuracy for MS 20ppm, for fragment ions MS/MS 0.06Da. Enzyme: Trypsin/P with 3 missed cleavages allowed. FDR cut off for PSM and peptides was of 1% for high and 5% for medium confidence peptides.

**CRISPR Knockout cell line generation**

Guide RNA design, vector construction, transfection, single cell screening and validation were done as described previously (Zhang et al., 2017). Validated clones & sequences are in the Key Resources Table.

**IRF overexpression and luciferase studies**

IRF-GFP fusions constructs were generated in pCDNA6.2/emGFP-GW/D-Topo (Invitrogen) as described previously (Zhou et al., 2014), except for IRF1/5 that were cloned using the same methodology from PakiT03 cDNA (#XM_006923152.3, XM_006910531.3 respectively). Luciferase assays were as described previously in Hek293T cells (low baseline IRF expression). Cells were adhered to pre-cleaned glass confocal coverslips #1.5 in the bottom of 24-well plates or imaged directly on plastic tissue culture 24-well plates (for low magnification). Cells were treated as previously, rinsed with pre-warmed PBS (37°C), and fixed with 4% Paraformaldehyde 0.37% Glutaraldehyde (pre-warmed) for 20 minutes). Cells in plates were imaged directly in PBS, coverslips were mounted using Mowiol-4.88.

**Pathway analysis**

Pathway analysis was performed with Enrichr (https://maayanlab.cloud/Enrichr/) using significant DEGs as a list and the clustergram for CHEA and ENCODE consensus was used for TF/gene correlations. GSEA signatures were downloaded from m = MSigDB Hallmark/curated sets and gene lists were aligned, recording the k/K values and the FDR Q-value. Ingenuity Pathway Analysis was used by projecting bat gene ID onto human HGNC symbols (biodbnet) and average fold induction of genes (relative to untreated) was used in conjunction with P value or relative to WT cells (for proteomics). Only direct relationships were considered and highly significant/inferred data, excluding possible mutation analysis. Upstream regulator analysis included both genes and endogenous chemicals. Both Z-score for expression-weighted analysis and pure significance (P value) was collected and represented as indicated in the figure legends (complete values in supplemental tables).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Unless specifically mentioned in the figure legend all experiments were performed in a biological (n) triplicate with multiple replicates ≥ 3. Graphs for qPCR are combined for technical duplicates x 3-4 replicates, normalized across housekeeping geometric mean. Graphs were generated using Graphpad Prism and unless otherwise stated statistics is calculated using unpaired, two-tailed t test’s without correction for variance. Error is represented as SEM unless otherwise stated and p < 0.05, ** < 0.02, *** < 0.01. Heatmaps were generated in Morpheus (https://clue.io:443/ Broad Institute) and the scale is based upon Min/Med/Max, unless otherwise indicated, and displayed in each figure. Normalized heatmaps across species were calculated as fold expression relative to housekeeping for a Geometric mean of 13 housekeeping genes considered appropriate for cross-species/tissue comparison (Caracausi et al., 2017; Eisenberg and Levanon, 2013; Shimamoto et al., 2013), values are in the Supplemental tables. Venn Diagrams were generated by Glimma or BioVenn for weighted VennDiagrams.