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IRF5 regulates unique subset of genes in dendritic cells during West Nile virus infection

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Abstract

Pathogen recognition receptor (PRR) signaling is critical for triggering innate immune activation and the expression immune response genes, including genes that impart restriction against virus replication. RIG-I-like receptors and Toll-like receptors are PRRs that signal immune activation and drive the expression of antiviral genes and the production of type I interferon (IFN) leading to induction of IFN-stimulated genes, in part through the Interferon Regulatory Factor (IRF) family of transcription factors. Previous studies with West Nile virus (WNV) showed that IRF3 and IRF7 regulate IFN expression in fibroblasts and neurons, whereas macrophages and dendritic cells (DCs) retained the ability to induce IFN β in the absence of IRF3 and IRF7 in a manner implicating IRF5 in PRR signaling actions. Here we assessed the contribution of IRF5 to immune gene induction in response to WNV infection in DCs. We examined IRF5-dependent gene expression and found that loss of IRF5 in mice resulted in modest and subtle changes in the expression of WNV-regulated genes. Anti-IRF5 chromatin immunoprecipitation with next-generation sequencing of genomic DNA coupled with mRNA analysis revealed unique IRF5 binding motifs within the mouse genome that are distinct from the canonical IRF binding motif and that link with IRF5-target gene expression. Using integrative bioinformatics analyses, we identified new IRF5 primary target genes in DCs in response to virus infection. This study provides novel insights into the distinct and unique innate immune and immune gene regulatory program directed by IRF5.

Graphical Abstract

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Authorship

KTC designed and performed experiments and wrote the manuscript; CD performed bioinformatics analyses and wrote the manuscript; YML and MK performed experiments; MG directed the research and edited the manuscript.

Conflicts of Interest Disclosure

The authors declare no competing financial interests.

Summary:

IRF5 regulates distinct gene regulation programs associated with diverse cellular pathways following West Nile Virus infection in mouse dendritic cells.

Keywords

IRF5; West Nile virus; bone marrow derived dendritic cells; ChIP-exo; RNA-seq; interferon signaling

Introduction

Interferon Regulatory Factors (IRFs) are transcription factors (TFs) that play key roles in regulating gene networks that coordinate appropriate and effective immune responses (1). Originally described as transcriptional regulators of the Type I interferon (IFN) system, IRFs play essential roles in a myriad of processes within innate and adaptive immunity and beyond (2, 3). Mammals have nine IRF family members that share a highly conserved N-terminal DNA binding domain and are predicted to regulate genes containing the IRF binding motif defined by the GAAA-containing nucleotide motif “GAAANNGAAA” (4, 5).

Within the IRF family, studies have focused extensively on IRF3 and IRF7 as the major IRFs that modulate antiviral gene expression. During infection with RNA viruses, such as the neurotropic flavivirus West Nile virus (WNV), cellular PRRs, including RIG-I-like receptors (RLRs) and Toll-like receptors (TLRs), recognize viral RNA motifs (6, 7). Signaling from PRRs leads to activation of downstream protein kinases that phosphorylate IRF3 and IRF7, resulting in the dimerization and nuclear translocation of these factors to induce target gene expression (8). Types I and III interferons (IFN), specific antiviral effector genes, and immunomodulatory genes, have been identified as IRF3 and IRF7 targets that coordinate an effective antiviral immune response (9, 10).

Beyond IRF3 and IRF7, recent studies of WNV infection in mouse and human cells suggest that additional factors are at play to orchestrate immune response against virus infection. *Irf3*^{-/-} *Irf7*^{-/-} double knockout (DKO) mice failed to control WNV replication, resulting in more rapid death when compared to the single gene KO mice (11, 12). However, DKO mice still produced type I IFN upon WNV infection, indicating that IFN production is regulated by additional factors at least in the absence of IRF3 and IRF7 (13). In addition, cell-type specific usage of specific IRF family members to induce antiviral gene expression has been observed. *Ex vivo* experiments with primary neurons and fibroblasts indicated that IFN β induction upon WNV infection was abrogated in the absence of IRF3 and IRF7, whereas IFN β production from dendritic cells (DCs) and macrophages after WNV infection was sustained in DKO cells (14). Additional studies utilizing primary cells from *Irf3*^{-/-} *Irf5*^{-/-} *Irf7*^{-/-} triple KO (TKO) mice indicate that IRF5 is responsible for triggering IFN and the production of other cytokines in DCs in response to WNV infection (15). However, whether IRF5 plays redundant or distinct roles from IRF3 and IRF7 in regulating antiviral innate immune response in DCs remains unclear.

Multiple studies have shown that IRF5 plays an immune protective role against infection by various viruses (16–19). However, in contrast to IRF3 and IRF7, the role of IRF5 in regulating antiviral defenses and viral infection is not well understood though studies of *Irf5*^{-/-} mice show that IRF5 participates in directing the production of IFN β and proinflammatory cytokines *in vivo* in response to virus infection and other inflammatory stimuli (20–23). Moreover, in humans, *IRF5* autoimmune risk haplotypes are correlated with elevated levels of IFN α (24–27), wherein DCs from these haplotype carriers produce elevated TNF α and IL-12 upon TLR stimulation (28, 29). Beyond cytokines, IRF5 regulates expression of genes essential for B cell differentiation, activation, and proliferation downstream of TLR9 and B cell receptor signaling (30, 31). These studies firmly established an important and versatile role of IRF5 in modulating both innate and adaptive immune responses through actions in various cell types. The precise function of IRF5 in regulating immune gene expression in response to virus infection remains to be elucidated.

We performed in-depth genomic analyses to identify *bona fide* IRF5 primary gene targets in mouse DCs *ex vivo*. We first assessed IRF5 dependent gene expression by examining global gene expression in WNV-infected bone marrow derived DCs (BMDCs) from *Irf5*^{-/-} (IRF5 KO) and wild type (WT) mice using next-generation RNA sequencing (RNA-seq). We then produced a high resolution genome-wide IRF5 occupancy map of mock- and WNV-infected BMDCs using chromatin immunoprecipitation combined with exonuclease digestion (ChIP-exo) followed by high-throughput sequencing (ChIP-seq) (32). Our data sets identify novel IRF5 binding motifs in addition to the canonical IRF binding motif, and reveal gene loci previously not shown to be regulated by IRF5. Integrative bioinformatics analyses revealed a high confidence IRF5 target gene set. Our study provides the first in-depth genome-wide analysis of IRF5 target genes and gene expression in DCs during acute WNV infection.

Materials and Methods

Cells and virus infection

Bone marrow (BM) cells were extracted from femurs of 6–8-week old wildtype B6 and *Irf5*^{−/−} (corrected for *Dock2* mutation) mice. Bone marrow derived dendritic cells (BMDCs) were obtained by culturing BM cells in complete RPMI supplemented with recombinant mouse IL-4 (40ng/ml, Peprotech) and GM-CSF (40ng/ml, Peprotech) for 7–9 days, changing media on day 3. Bone marrow derived macrophages (BMMs) were obtained by culturing BM cells in complete DMEM supplemented with recombinant mouse M-CSF (40ng/ml, Peprotech) for 7 days, changing media on day 3. Cells were kept at 37°C with 5% CO₂.

West Nile virus (WNV) Texas 2002 strain was propagated using previously described methods (33). Supernatants collected from infected Vero cells were titrated on BHK-21 cells and stored at 80°C.

For WNV infection, BMDCs or BMMs were infected in serum-free media with 2.5 MOI of WNV for 1.5 hours with gentle rocking. Complete media was added back and cells were harvested at the time points indicated. Biological triplicates were used for all experiments.

RNA extraction for RNAseq analysis

Total RNA was extracted using RNeasy mini kit (QIAGEN) with on-column DNase treatment. RNA quality was assessed using RNA 6000 nano kit on the 2100 Bioanalyzer (Agilent, USA). BMDC RNA with an RNA integrity number (RIN) of 8 was sent to Seattle Genomics (University of Washington) for cDNA library construction using the KAPA stranded RNA-seq kit with Ribo Erase and next-generation sequencing performed on the Illumina Nextseq 500 instrument for > 25 million 75 nt paired-end reads. BMM RNA was sent to the Genomics Core Laboratory at Benaroya Research Institute for cDNA construction with the TruSeq RNA Library Prep Kit v2 for 50 nt single-end sequencing with the Illumina HiSeq 2500 instrument. Raw reads were submitted to GEO under accession number GSE114993.

ChIP-exo

Chromatin was isolated using a truChIP chromatin shearing kit (Covaris) and sheared using M220 focused-ultrasonicator (Covaris). Sheared chromatin was run on an agarose gel and band corresponding to size 200–700bp was extracted. Immunoprecipitation was performed with validated IRF5 antibodies (Bethyl A303–385 and A303–386). Exonuclease processing of sheared chromatin and library construction was performed using ChIP-exo kit (Active Motif). Library size and quality was assessed using High Sensitivity DNA kit (Agilent) on the 2100 Bioanalyzer (Agilent). Next-generation sequencing was performed on the Illumina HiSeq 2500 instrument by the Genome Technology Access Center (Washington University). Raw reads were submitted to GEO (accession no. GSE114993).

Sequencing and data processing

Quality of both mRNA-seq and ChIP-exo reads were assessed using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>) and ribosomal sequences were filtered via alignments with Bowtie v2.1.0 (bowtie-bio.sourceforge.net/). Subsequently, mRNA-seq reads were mapped to the UCSC mm10 genome obtained from iGenomes using STAR v2.4.0j (https://github.com/alexdobin/STAR/releases/tag/STAR_2.4.0j) followed by HTSeq-count v0.6.0 (https://htseq.readthedocs.io/en/release_0.10.0/) to generate gene counts. BAM files generated by STAR were also assessed for additional QC metrics using the ChIPexoQual R package (34). ChIP-exo reads were mapped to the mm10 genome using Bowtie v1.1.1³⁸. Peaks were called using GEM v3.1 (35) with corresponding input-only reads as controls using a k-mer length range of 6–13 and the parameters “--smooth 3” and “--mrc 20” as suggested by the developers. To assess consistency between replicates following the Encode ChIP-seq guidelines (36), overlapping peaks between replicates were assessed by Irreproducible Discovery Rate (IDR) with a cutoff of <0.1.

Differential expression analysis

Genes with less than an average of 10 counts across all samples were removed to filter low-expressing genes. Counts were then normalized using edgeR (37) and voom (38), and outliers were assessed using principal component analysis and hierarchical clustering in the R statistical programming language v3.4.0 (<http://www.R-project.org/>). Differential expression analysis was performed using the limma Bioconductor package in R (39) with a cutoff of Benjamini-Hochberg-adjusted p-value < 0.05 and > 1.5-fold change compared to mock. An expression heatmap was generated with the heatmap.2 function in the gplots R package v3.0.1 (<https://CRAN.R-project.org/package=gplots>) and co-expressed genes were assigned to modules using Ward’s clustering. Modules were then functionally annotated with immune-related canonical pathways using Ingenuity Pathway Analysis (IPA) (40). Bubble plots and volcano plots were created using the ggplot2 (ggplot2.org/) and ggrepel (<https://CRAN.R-project.org/package=ggrepel>) R packages.

ChIP-exo analysis

Peak distances from transcription start sites (TSS’s) as well as nearest genes were extracted from GREAT v3.0.0 (41) using the “Basal plus extension” setting searching for proximal (5 kb upstream, 10 kb downstream) and distal (up to 1000 kb) gene regions. All motifs were identified *de novo* from combining replicate ChIP samples in GEM and filtering for motifs with a hypergeometric p-value < 0.001. Direct comparison of motifs was performed using TomTom (42). Shared and unique ChIP-exo peaks by experimental condition were identified with Venny (<http://bioinfogp.cnb.csic.es/tools/venny/index.html>). Enriched pathways of ChIP-exo peaks in each condition were annotated using canonical pathways in IPA. These genes were then functionally annotated for enriched canonical pathways and networks of protein and expression-level interactions were assembled using the network-building tools within IPA.

Public ChIP datasets

Peaks from other ChIP datasets were collected from ChIP-Atlas, a public database of ChIP experiments that have had peaks called using MACS2 (43). MACS2 scores for each peak were extracted from each SRA sample, averaged by cell type, and finally log-transformed prior to visualization.

Online Supplemental Material

Supplemental Figure 1 is a heatmap of NF- κ B associated gene expression. Supplemental Figure 2 is a heatmap of genes that are differentially expressed in at least time point in only WT or *Irf5*^{-/-} cells. Supplemental Figure 3 shows validation experiments for specificity and robustness of IRF5 ChIP antibodies. Supplemental Figure 4 shows a heatmap of gene expression of the IRF5 direct target genes, including the 24 hours post-infection timepoint. Supplemental Table 1 is a list of public ChIP datasets used to assess binding of IRF5 direct target genes by other IRF's. Lists of DE genes and ChIP peaks can be found here: <https://irf5genomics.galelab.org>.

Results

Loss of IRF5 results in subtle changes in transcriptional response of DCs during WNV infection

To identify IRF5-dependent WNV response genes, we infected BMDCs from WT and *Irf5*^{-/-} mice and assessed gene expression at 6, 12, and 24 hours post-infection. Compared to mock infection, WNV infection induced differential gene expression (DE) in 1,143 genes ($q < 0.05$, $lfc > 1.5$) across the time series in all conditions, with 835 genes differentially expressed in WT compared with 953 differentially expressed genes in the *Irf5*^{-/-}. A large majority of DE genes were observed at the 24 hours post-infection time point regardless of genotype, indicating that almost all significant cellular gene expression changes occurred after 12 hours post-infection (Fig. 1A).

Comparing gene expression profiles of WT and *Irf5*^{-/-} in response to WNV infection, global gene expression changes were mostly similar at the pathway level between WT and *Irf5*^{-/-} BMDCs. Co-expression modules of BMDCs revealed that IFN signaling and PRR genes were both highly induced/upregulated in response to infection, whereas IL-1 signaling was suppressed compared to mock at 24 hours post-infection both in the presence or absence of IRF5. The top most enriched immune pathways showed expression increases in both WT and *Irf5*^{-/-} cells at 24 hours post-infection, with a notable difference in NF- κ B signaling where 17 genes were DE in WT compared with 24 genes in *Irf5*^{-/-} (Fig. 1B). Further assessment of the NF- κ B genes revealed a greater induction of *Tlr7* and *Tlr8* expression in *Irf5*^{-/-} cells at 24h compared with WT, while *Il1b* gene expression is suppressed more strongly in WT cells than in *Irf5*^{-/-} cells upon WNV infection (Supplemental Figure 1). This suggests that lack of IRF5 during WNV infection can lead to dysregulation of gene expression associated with NF- κ B signaling.

To identify IRF5-responsive genes during WNV infection, we focused on genes that were only DE in *Irf5*^{-/-} or WT cells. The 307 genes that were DE only in *Irf5*^{-/-} cells included

the upregulated immune genes *Ii18*, *Tlr8*, *Aim2*, *Pycard*, *Nlrp10* and *Tnfrsf3* among others (Supplemental Figure 2), indicating that either expression of these genes is normally suppressed by IRF5 in BMDCs, or that chronic loss of IRF5 could lead to a decreased threshold for the induction of these genes upon virus infection. Furthermore, 189 genes were only DE in WT cells, including the cytokine related genes *Ii1b*, *Ii20rb* and the *Cd14* surface marker that were suppressed/downregulated, and *Ii10ra* which was upregulated (Supplemental Figure 2). The expression pattern of these genes suggests that IRF5 selectively represses certain immune regulatory genes while activating others, corroborating with previous findings that IRF5 can act as a transcriptional activator and repressor in different contexts (19). While the DE genes unique to WT and *Irf5*^{-/-} cells provide evidence for IRF5 function, it is important to note that their directional changes in expression were often the same between WT and *Irf5*^{-/-} cells. This indicates that IRF5 is not the only factor regulating these genes, but likely works cooperatively with other TFs such that the loss of IRF5 can be compensated in the context of immune gene induction.

IRF5 binding profiles change upon WNV infection

Because results from gene expression analysis revealed subtle changes in the absence of IRF5, we sought to identify *bona fide* IRF5 target genes during virus infection with ChIP-seq experiments on endogenous IRF5. We performed nucleotide-resolution ChIP-exo (32) analysis on mock- and WNV-infected WT and *Irf5*^{-/-} BMDCs at 6 and 12 hours post-infection to precisely identify IRF5 occupied loci and binding motifs. We validated the specificity of the ChIP-competent antibody and ChIP protocol prior to sequencing (Supplemental Fig 3), and ensured the quality of the sequencing data by the ChIPexoQual R package prior to peak calling (<https://irf5genomics.galelab.org>). We used the Genome wide Event finding and Motif discovery software (GEM) to identify peaks and motif sequences, and assessed peaks consistent across replicates compared to input controls for each respective condition (Irreproducible Discovery Rate (IDR) < 0.1). Overall, mock-infected samples contained more IRF5-binding peaks (153 peaks; 93 at 6 hours post-infection and 60 at 12 hours post-infection) than WNV-infected samples (109 peaks; 67 at 6 hours post-infection and 42 at 12 hours post-infection) (Fig. 2A). This could be due to the highly stochastic nature of virus infection, such that only a small number of peaks passed statistical threshold as high confidence IRF5 binding peaks within each replicate of primary cells. Genes associated with peaks proximal (-5 kb to + 10 kb) and distal (<1000 kb) from transcription start sites (TSSs) were then identified using the Genomic Regions Enrichment of Annotations Tool (GREAT) (41). Most peaks found were within 5 kb of TSSs in each experimental condition, although the proportion of these peaks dropped from 59% of all peaks to 38% from 6 hours to 12 hours in both mock and WNV infected samples, suggesting that IRF5 may regulate gene expression via mechanisms other than proximal promoter activation (Fig. 2B).

To identify IRF5-regulated genes, we used GREAT to identify genes associated with ChIP-exo peaks and compared the IRF5-occupied loci between infection state and time points (Fig. 3A). At 6 hours post-infection 24.4% of genes associated with peaks were shared between WNV and mock infections (49 genes in total), while at 12 hours 17.7% of genes were shared (29 genes in total) (Supplemental Fig 4). We found that IRF5 occupation at

many loci associated with protein ubiquitination and fatty-acid beta oxidation diminished upon WNV infection. At 12 hours post WNV infection there was a noticeable shift towards immune-related pathways including dendritic cell maturation, IL-1 mediation of RXR, and NFAT regulation of immune responses. Since the number of ChIP-exo peaks from individual replicates were too small for robust motif identification, we combined replicate samples and used GEM with otherwise identical parameters as above to search for *de novo* IRF5-binding motifs. We identified a *de novo* “GAAA” motif within peaks of all replicate-combined conditions using both GEM and HOMER (<https://irf5genomics.galelab.org/>), consistent with the previously identified canonical IRF family TF binding motif “GAAANNGAAA” (Fig. 3B) (4, 5). More than 60% of all peaks generated from replicate-combined conditions included a “GAAA” sequence (<https://irf5genomics.galelab.org/>). These observations also corroborate results from other studies where IRF5 was found to bind “GAAA” half sites, rather than the “GAAANNGAAA” full site as other IRF factors prefer (44). Another IRF5 ChIP study also identified composite (G)GAAA motifs (45). We note that this GAAA motif was not identified in the replicate-combined samples using MEME-ChIP or the individual replicates, due to low peak numbers (data not shown). We observed that the profile of *de novo* binding motifs changed over time, strongly supporting that IRF5 targets different genes over the time course of infection (Fig. 3C). A common pattern in the identified IRF5 binding motifs is the prevalence of G and C nucleotides (consisting of 71% of motif positions with strong base calls). While some motifs reappeared between infection states or time points, only the canonical IRF binding motif was present in all conditions, with all but the WNV 12 hour samples containing unique motifs. These results suggest that IRF5 not only recognizes the canonical IRF consensus sequence, but also binds other DNA motifs, or forms complexes with other factors that regulate loci harboring these other motifs, as suggested by previous studies (45).

Honing in on the novel enriched motifs bound by IRF5, we queried the JASPAR transcription factor binding profile database (46) for previously identified non-canonical IRF5 binding motifs. We found significant similarity between the “TCTCGCGAGA” motif in the mock-infected 6 hour samples with PB0139.1 (“ANCGAGA”) via the motif alignment program TomTom ($p = 7.57E-03$ and $p = 1.99E-02$, Euclidean distance and Sandelin-Wasserman function). This motif was identified in a universal protein binding microarray study in mice assessing binding patterns of multiple transcription factors (47), validating our motif discovery methodology. These DNA occupancy patterns reveal that IRF5 activity and function in regulating gene expression is dynamic through time during WNV infection.

Identification of IRF5 direct targets using a combinatorial approach linking transcriptional responses and DNA occupancy

To define an IRF5 primary target gene set, we combined RNA-seq and ChIP-exo analyses to identify genes that are both DE in WT BMDCs upon WNV infection, and with IRF5 binding within proximity of the loci. We used stringent criteria to filter out lowly expressed genes and only retained genes with high p-value amongst replicates to identify a *bona fide* set of IRF5 target genes with high confidence. In total, 12 genes fit the criteria of being bound by IRF5 in their promoter region while being DE in WT DCs upon WNV infection. Within this

gene set, several genes have known functions in the immune system, such as *Fcgr4* that encodes an Fc receptor for IgG and IgE antibodies, *Herc6* that encodes an E3 ubiquitin ligase for ISGylation, *Clic4* that encodes an ion channel shown to regulate IL-1 β expression and NLRP3 activation, *Ccl2* that encodes a chemokine, and *Oas1l* that negatively regulates IFN response but enhances RIG-I signaling response (Table 1). Altogether, these 12 primary target genes showed the largest differences in expression at the 6 and 12 hour post-infection time points (Fig. 4A), but were mostly similar by 24 hours post-infection (Supplemental Figure 5), suggesting that loss of IRF5 resulted in early dysregulation of gene expression that can be compensated at later time points by other factors.

To identify genes and pathways associated with IRF5 targets, we built an interaction network in Ingenuity Pathway Analysis (IPA) with the 12 IRF5 target genes identified in BMDCs. Genes in this IRF5-regulated network included innate immune signaling factors such as DHX58, ISG20, OASL1, OASL2, IFIT3, and IRF7 among others. Based on differences in expression levels of WT and *Irf5*^{-/-} cells, *Fcgr4* and *C130026I21Rik* are predicted to be activated by IRF5, while *Ccl2* is predicted to be suppressed. In the case of *Oas1l*, expression was higher in *Irf5*^{-/-} cells than WT at 12 hours post-infection, but lower at 24 hours post-infection, which suggests that IRF5 suppresses the expression of this gene earlier in infection and switches to activation or release of suppression by 24 hours. The remaining IRF5 target genes do not show differences in expression levels, suggesting compensation by other TFs. The additional gene interactions within the network notably include IRF5 as a central hub, thus providing further support from other studies that this network represents genes that are regulated by IRF5. However, as this network consists primarily of genes DE in both WT and *Irf5*^{-/-} cells, our data sets suggest that other TFs likely can compensate for the lack of IRF5 to drive DE gene expression, particularly in the case of associated innate immune genes (Fig. 4B).

To validate these IRF5 primary gene targets, we assessed their expression in WT or *Irf5*^{-/-} BMDCs using quantitative real-time PCR upon mock- or WNV-infection. As expected, we observed induction of *Fcgr4* in WT but not *Irf5*^{-/-} cells upon WNV infection (Figure 5A). Also consistent with our RNA-seq results, we observed a super-induction of *Ccl2* and *Oas1l* in the absence of IRF5 as compared to WT cells upon WNV infection (Figure 5B and 5C). We further assessed expression of genes associated with antiviral response. We observed dependence on IRF5 in *Ifnb* and *Ifit1* genes even though no evidence of IRF5 binding was found at these loci, indicating that IRF5 modulates expression of additional genes related to antiviral response beyond its primary target genes through secondary/indirect mechanisms (Figure 5D). We measured WNV genome copy number in WT and *Irf5*^{-/-} cells to ensure that the gene expression differences observed were not due to substantial differences in WNV infection rate (Figure 5E). We note that because of biological variability of primary DC cultures and the limits of qRT-PCR assays, the changes in gene expression are not all statistically significant based on stringent unpaired T-test using biological triplicates, but the trends are very consistent with RNA-seq results. Overall, these data imply that IRF5 regulates a specific subset of primary target genes, as well as other genes via other means to modulate and fine-tune an antiviral response.

IRF5 regulates overlapping and distinct target gene sets in different cell types

To assess whether IRF5 regulates similar set of genes in other cell types, we performed RNA-seq experiment in WT and *Irf5*^{-/-} WNV-infected bone marrow-derived macrophages (BMMs). Globally, BMMs had a more rapid and robust response to WNV infection than BMDCs. The two cell types showed largely overlapping DE gene expression patterns upon WNV infection with fine differences (Figure 6A). Focusing on the 12 IRF5 primary target genes identified in BMDCs at early infection time points where differences in WT and *Irf5*^{-/-} cells are clearest, 8 were also DE in BMMs. Specifically, *Oas11*, *Fcgr4*, *Herc6*, and *Aida* showed similar expression patterns between BMDCs and BMMs, while *Acer3*, *Frmd4a*, and *Ccl2* were divergent between the different cell types (Figure 6B). Overall, most but not all of these genes showed similar DE pattern between WT and *Irf5*^{-/-} cells within each cell type, indicating that IRF5 likely regulates overlapping and distinct gene targets in different cell types.

IRF family members regulate unique gene sets

IRF family members share the same consensus DNA binding motif. To assess whether IRF5 primary target genes are also regulated by other IRF family members, we examined binding scores from publicly available ChIP experimental data in human and mouse samples aggregated by IRF and cell type (Figure 7, Supplemental Table 1). Genes such as *Aida*, *Herc6*, *Acer3*, *Clic4* and *Oas11* showed evidence of binding consistently across multiple IRFs and cell types, while some other genes have a more specific binding profile (e.g. *Fcgr4* and *Tsku*). This suggests that despite sharing a consensus binding motif, each IRF member regulates different sets of genes in a cell type specific context.

Discussion

In this study we combined two orthogonal approaches to identify *bona fide* IRF5 primary target genes in BMDCs during WNV infection. We first performed gene expression analysis of WT and *Irf5*^{-/-} BMDCs upon WNV infection to identify IRF5-dependent genes. We found that overall changes in WNV-induced gene expression were subtle in the absence of IRF5, though some genes, including those in the NF- κ B pathway, showed stronger dependency on IRF5 for their expression. Thus, loss of IRF5 in DCs can likely be compensated by other factors in an infection state, such that the overall gene expression program does not drastically change in the absence of IRF5. Another possibility is that IRF5 serves to fine-tune the transcriptional response upon WNV infection, rather than a master regulator. When we performed ChIP-seq analysis to map the global binding pattern of endogenous IRF5 upon WNV infection, we found that IRF5-bound gene loci across all conditions (mock and WNV infection across time points) were enriched for the canonical IRF binding motif, providing strong evidence that other IRFs can compensate for the loss of IRF5 during infection.

Our study is the first to our knowledge to utilize the high-resolution ChIP-exo method to map genome-wide IRF5 occupancy, which allowed us to identify IRF5 binding motifs in near nucleotide resolution and high confidence. In addition to the canonical IRF binding motif, we also identified additional IRF5 binding sites, some uniquely found in virus

infection conditions. Compared with other traditional IRF5 ChIP-seq studies (30, 45), we identified lower number of peaks, likely due to the highly specific ChIP-exo protocol and statistical analyses for peak calling. We performed extensive quality control measures to ensure that background is negligible (IgG ChIP yielded no peak) and the ChIP reaction was specific (antibody did not IP any protein of mass corresponding to IRF5 in *Irf5*^{-/-} cells) (Supplemental Fig 3). The IRF5-bound genetic loci change over the time course of infection, highlighting the dynamic nature of IRF5-regulated genes. We found that IRF5-occupied gene loci during infection corresponded to multiple immune pathways, including DC maturation and NFAT pathways. Combining the RNA-seq and ChIP-seq datasets, we honed in on a novel subset of genes that we defined as IRF5 primary target genes with high confidence. We compared this gene set with DE genes found in macrophages upon WNV infection, and found that some but not all genes showed similar pattern, revealing unique IRF5 function in different cell types. We performed meta-analysis on publicly available datasets on the DNA occupancy of other IRF family members to gain further insights into the mode of gene regulation by IRFs. Our data suggest that while there are overlapping gene targets, each IRF regulates unique and distinct set of genes despite sharing a highly conserved DNA binding domain.

Other IRF5 ChIP-seq studies using traditional methods in different cell types have been published (30, 45). These studies found disparate IRF5-regulated gene sets, indicating that IRF5 has vastly different targets depending on the cellular contexts and experimental systems, e.g. B cells vs. macrophages; acute TLR4 stimulation vs. IgM crosslinking/TLR9 stimulation, etc. Our study focuses on IRF5 target genes during WNV infection over a time course in BMDCs, a WNV target cell type and one crucial for initiating a protective immune response against virus infection. Understanding the role of IRF5 in this context expands the current knowledge on IRF5 and elucidates its specific functions in BMDCs during virus infection.

We used an integrative approach to identify a set of novel and high confidence IRF5 target genes in response to WNV in DCs. Some of these genes have previously ascribed functions in immunity, including *Oas11*, *Herc6*, and *Ccl2*. Further, this gene set contains genes of diverse functions that likely contribute to DC functions during virus infection. For example, *Acer3* has been shown to mediate innate immune response by regulating the levels of ceramide and expression of proinflammatory cytokines (48). *Frmd4* regulates actin cytoskeleton dynamics and endocytic trafficking (49, 50), both important features of DC antigen presentation, activation, and migration in DCs. Other molecules, such as *Aida* and *Tsku*, regulate signal transduction and transcription events that are likely crucial for DC activation (51, 52). *Tcea1*, *C130026I2IRik* (homolog of SP140 in human), and *Ankle2* are important factors that regulate transcription (53–55), which likely accommodates the transcriptional burden that occurs during rapid DC activation. This gene set not only expands the repertoire of IRF5 targets, but also points to diverse cellular processes that regulate DC function during virus infection.

Previous studies have demonstrated that IRF5 is essential for protective immunity against WNV infection (15, 16). Consistent with our results, it was previously shown that the loss of IRF5 was associated with only small differences in the type I IFN response systemically and

in the draining lymph node during WNV infection (16), indicating that in the absence of IRF5 compensation in gene expression by other factors likely occurs. Instead, lower levels of other proinflammatory cytokines and chemokines, fewer and less activated immune cells, blunted WNV-specific antibody responses, and fewer antigen-specific memory B cells and long-lived plasma cells were found in *Irf5*^{-/-} mice (16). These observations and our data suggest that instead of being the master regulator of a specific immune pathway, IRF5 is rather a key factor that shapes and fine-tunes the antiviral immune response in a manner that is more nuanced and intricate, which impacts the spread of virus infection, mediated optimal innate immune regulation and the regulation of adaptive immunity.

Few studies have combined RNA-seq and ChIP-seq analyses to identify the dynamic immune response genes during virus infection. IRF5, in particular, is under-studied compared to other IRF family members, partly due to the limited availability of reliable reagents (56). We carefully tested reagents and developed protocols that allowed us to interrogate endogenous IRF5 in primary cells during WNV infection. Our in-depth analyses identified with high confidence a set of new IRF5 primary target genes, and revealed a role for IRF5 in directing immune gene expression in response to WNV infection in DCs. Further, IRF family members share a conserved DNA binding domain and are often presumed to have similar gene targets. Our study showed that despite the highly conserved nature of the DNA binding domain, IRFs regulate a disparate set of genes, likely due to cooperation with other TFs as illustrated by other studies (45, 57). Finally, due to their important role in immune response regulation, recently several IRFs have been the focus of targeted therapeutics (58, 59). Careful design and targeting is crucial as our study illustrated that compensation by other family members is highly likely upon the loss of function of a single IRF. Systemic interrogation of the IRF gene regulatory networks will lead to a full understanding of the intricate crosstalk between IRF family members.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BMDC	bone marrow derived dendritic cell
BMM	bone marrow derived macrophage
ChIP	chromatin immunoprecipitation
DC	dendritic cell
IRF	interferon regulatory factor

KO	knockout
PRR	pathogen recognition receptor
RLR	RIG-I-like receptor
TLR	Toll-like receptor
WNV	West Nile virus
WT	wildtype

References

1. Honda K, and Taniguchi T 2006 IRFs: master regulators of signalling by Toll-like receptors and cytosolic pattern-recognition receptors. *Nat Rev Immunol* 6: 644–658. [PubMed: 16932750]
2. Yanai H, Negishi H, and Taniguchi T 2012 The IRF family of transcription factors: Inception, impact and implications in oncogenesis. *Oncoimmunology* 1: 1376–1386. [PubMed: 23243601]
3. Taniguchi T, and Takaoka A 2002 The interferon-alpha/beta system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors. *Curr Opin Immunol* 14: 111–116. [PubMed: 11790540]
4. Takaoka A, Tamura T, and Taniguchi T 2008 Interferon regulatory factor family of transcription factors and regulation of oncogenesis. *Cancer Sci* 99: 467–478. [PubMed: 18190617]
5. Escalante CR, Yie J, Thanos D, and Aggarwal AK 1998 Structure of IRF-1 with bound DNA reveals determinants of interferon regulation. *Nature* 391: 103–106. [PubMed: 9422515]
6. Rossini G, Landini MP, Gelsomino F, Sambri V, and Varani S 2013 Innate host responses to West Nile virus: Implications for central nervous system immunopathology. *World J Virol* 2: 49–56. [PubMed: 24175229]
7. Suthar MS, Aguirre S, and Fernandez-Sesma A 2013 Innate immune sensing of flaviviruses. *PLoS Pathog* 9: e1003541. [PubMed: 24068919]
8. Kawai T, and Akira S 2008 Toll-like receptor and RIG-I-like receptor signaling. *Ann N Y Acad Sci* 1143: 1–20. [PubMed: 19076341]
9. Ning S, Pagano JS, and Barber GN 2011 IRF7: activation, regulation, modification and function. *Genes Immun* 12: 399–414. [PubMed: 21490621]
10. Hiscott J 2007 Triggering the innate antiviral response through IRF-3 activation. *J Biol Chem* 282: 15325–15329. [PubMed: 17395583]
11. Daffis S, Samuel MA, Keller BC, Gale M, Jr., and Diamond MS 2007 Cell-specific IRF-3 responses protect against West Nile virus infection by interferon-dependent and -independent mechanisms. *PLoS Pathog* 3: e106. [PubMed: 17676997]
12. Daffis S, Samuel MA, Suthar MS, Keller BC, Gale M, Jr., and Diamond MS 2008 Interferon regulatory factor IRF-7 induces the antiviral alpha interferon response and protects against lethal West Nile virus infection. *J Virol* 82: 8465–8475. [PubMed: 18562536]
13. Sato M, Suemori H, Hata N, Asagiri M, Ogasawara K, Nakao K, Nakaya T, Katsuki M, Noguchi S, Tanaka N, and Taniguchi T 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. [PubMed: 11070172]
14. Daffis S, Suthar MS, Szretter KJ, Gale M, Jr., and Diamond MS 2009 Induction of IFN-beta and the innate antiviral response in myeloid cells occurs through an IPS-1-dependent signal that does not require IRF-3 and IRF-7. *PLoS Pathog* 5: e1000607. [PubMed: 19798431]
15. Lazear HM, Lancaster A, Wilkins C, Suthar MS, Huang A, Vick SC, Clepper L, Thackray L, Brassil MM, Virgin HW, Nikolich-Zugich J, Moses AV, Gale M, Jr., Fruh K, and Diamond MS 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. [PubMed: 23300459]

16. Thackray LB, Shrestha B, Richner JM, Miner JJ, Pinto AK, Lazear HM, Gale M, Jr., and Diamond MS 2014 Interferon regulatory factor 5-dependent immune responses in the draining lymph node protect against West Nile virus infection. *J Virol* 88: 11007–11021. [PubMed: 25031348]
17. Proenca-Modena JL, Hyde JL, Sesti-Costa R, Lucas T, Pinto AK, Richner JM, Gorman MJ, Lazear HM, and Diamond MS 2016 Interferon-Regulatory Factor 5-Dependent Signaling Restricts Orthobunyavirus Dissemination to the Central Nervous System. *J Virol* 90: 189–205. [PubMed: 26468541]
18. Cevik O, Li D, Baljinnyam E, Manvar D, Pimenta EM, Waris G, Barnes BJ, and Kaushik-Basu N 2017 Interferon regulatory factor 5 (IRF5) suppresses hepatitis C virus (HCV) replication and HCV-associated hepatocellular carcinoma. *J Biol Chem* 292: 21676–21689. [PubMed: 29079574]
19. Feng D, Sangster-Guity N, Stone R, Korczeniewska J, Mancl ME, Fitzgerald-Bocarsly P, and Barnes BJ 2010 Differential requirement of histone acetylase and deacetylase activities for IRF5-mediated proinflammatory cytokine expression. *J Immunol* 185: 6003–6012. [PubMed: 20935208]
20. Takaoka A, Yanai H, Kondo S, Duncan G, Negishi H, Mizutani T, Kano S, Honda K, Ohba Y, Mak TW, and Taniguchi T 2005 Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. *Nature* 434: 243–249. [PubMed: 15665823]
21. Paun A, Reinert JT, Jiang Z, Medin C, Balkhi MY, Fitzgerald KA, and Pitha PM 2008 Functional characterization of murine interferon regulatory factor 5 (IRF-5) and its role in the innate antiviral response. *J Biol Chem* 283: 14295–14308. [PubMed: 18332133]
22. Purtha WE, Swiecki M, Colonna M, Diamond MS, and Bhattacharya D 2012 Spontaneous mutation of the Dock2 gene in *Irf5*^{-/-} mice complicates interpretation of type I interferon production and antibody responses. *Proc Natl Acad Sci U S A* 109: E898–904. [PubMed: 22431588]
23. Yasuda K, Nundel K, Watkins AA, Dhawan T, Bonegio RG, Ubellacker JM, Marshak-Rothstein A, and Rifkin IR 2013 Phenotype and function of B cells and dendritic cells from interferon regulatory factor 5-deficient mice with and without a mutation in DOCK2. *Int Immunol* 25: 295–306. [PubMed: 23291967]
24. Niewold TB, Kelly JA, Flesch MH, Espinoza LR, Harley JB, and Crow MK 2008 Association of the IRF5 risk haplotype with high serum interferon-alpha activity in systemic lupus erythematosus patients. *Arthritis Rheum* 58: 2481–2487. [PubMed: 18668568]
25. Niewold TB, Kelly JA, Kariuki SN, Franek BS, Kumar AA, Kaufman KM, Thomas K, Walker D, Kamp S, Frost JM, Wong AK, Merrill JT, Alarcon-Riquelme ME, Tikly M, Ramsey-Goldman R, Reveille JD, Petri MA, Edberg JC, Kimberly RP, Alarcon GS, Kamen DL, Gilkeson GS, Vyse TJ, James JA, Gaffney PM, Moser KL, Crow MK, and Harley JB 2012 IRF5 haplotypes demonstrate diverse serological associations which predict serum interferon alpha activity and explain the majority of the genetic association with systemic lupus erythematosus. *Ann Rheum Dis* 71: 463–468. [PubMed: 22088620]
26. Rullo OJ, Woo JM, Wu H, Hoftman AD, Maranian P, Brahn BA, McCurdy D, Cantor RM, and Tsao BP 2010 Association of IRF5 polymorphisms with activation of the interferon alpha pathway. *Ann Rheum Dis* 69: 611–617. [PubMed: 19854706]
27. Berggren O, Alexsson A, Morris DL, Tandré K, Weber G, Vyse TJ, Syvanen AC, Ronnblom L, and Eloranta ML 2015 IFN-alpha production by plasmacytoid dendritic cell associations with polymorphisms in gene loci related to autoimmune and inflammatory diseases. *Hum Mol Genet* 24: 3571–3581. [PubMed: 25779693]
28. Hedl M, and Abraham C 2012 IRF5 risk polymorphisms contribute to interindividual variance in pattern recognition receptor-mediated cytokine secretion in human monocyte-derived cells. *J Immunol* 188: 5348–5356. [PubMed: 22544929]
29. Graham RR, Kyogoku C, Sigurdsson S, Vlasova IA, Davies LR, Baechler EC, Plenge RM, Koeuth T, Ortmann WA, Hom G, Bauer JW, Gillett C, Burt N, Cunninghame Graham D. S., Onofrio R, Petri M, Gunnarsson I, Svenungsson E, Ronnblom L, Nordmark G, Gregersen PK, Moser K, Gaffney PM, Criswell LA, Vyse TJ, Syvanen AC, Bohjanen PR, Daly MJ, Behrens TW, and Altshuler D 2007 Three functional variants of IFN regulatory factor 5 (IRF5) define risk and protective haplotypes for human lupus. *Proc Natl Acad Sci U S A* 104: 6758–6763. [PubMed: 17412832]

30. De S, Zhang B, Shih T, Singh S, Winkler A, Donnelly R, and Barnes BJ 2017 B Cell-Intrinsic Role for IRF5 in TLR9/BCR-Induced Human B Cell Activation, Proliferation, and Plasmablast Differentiation. *Front Immunol* 8: 1938. [PubMed: 29367853]
31. Lien C, Fang CM, Huso D, Livak F, Lu R, and Pitha PM 2010 Critical role of IRF-5 in regulation of B-cell differentiation. *Proc Natl Acad Sci U S A* 107: 4664–4668. [PubMed: 20176957]
32. Rhee HS, and Pugh BF 2012 ChIP-exo method for identifying genomic location of DNA-binding proteins with near-single-nucleotide accuracy. *Curr Protoc Mol Biol* Chapter 21: Unit 21 24.
33. Keller BC, Fredericksen BL, Samuel MA, Mock RE, Mason PW, Diamond MS, and Gale M, Jr. 2006 Resistance to alpha/beta interferon is a determinant of West Nile virus replication fitness and virulence. *J Virol* 80: 9424–9434. [PubMed: 16973548]
34. Welch R, Chung D, Grass J, Landick R, and Keles S 2017 Data exploration, quality control and statistical analysis of ChIP-exo/nexus experiments. *Nucleic Acids Res* 45: e145. [PubMed: 28911122]
35. Guo Y, Mahony S, and Gifford DK 2012 High resolution genome wide binding event finding and motif discovery reveals transcription factor spatial binding constraints. *PLoS Comput Biol* 8: e1002638. [PubMed: 22912568]
36. Landt SG, Marinov GK, Kundaje A, Kheradpour P, Pauli F, Batzoglou S, Bernstein BE, Bickel P, Brown JB, Cayting P, Chen Y, DeSalvo G, Epstein C, Fisher-Aylor KI, Euskirchen G, Gerstein M, Gertz J, Hartemink AJ, Hoffman MM, Iyer VR, Jung YL, Karmakar S, Kellis M, Kharchenko PV, Li Q, Liu T, Liu XS, Ma L, Milosavljevic A, Myers RM, Park PJ, Pazin MJ, Perry MD, Raha D, Reddy TE, Rozowsky J, Shores N, Sidow A, Slatery M, Stamatoyannopoulos JA, Tolstorukov MY, White KP, Xi S, Farnham PJ, Lieb JD, Wold BJ, and Snyder M 2012 ChIP-seq guidelines and practices of the ENCODE and modENCODE consortia. *Genome Res* 22: 1813–1831. [PubMed: 22955991]
37. Robinson MD, McCarthy DJ, and Smyth GK 2010 edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26: 139–140. [PubMed: 19910308]
38. Law CW, Chen Y, Shi W, and Smyth GK 2014 voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol* 15: R29. [PubMed: 24485249]
39. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, and Smyth GK 2015 limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* 43: e47. [PubMed: 25605792]
40. Kramer A, Green J, Pollard J, Jr., and Tugendreich S 2014 Causal analysis approaches in Ingenuity Pathway Analysis. *Bioinformatics* 30: 523–530. [PubMed: 24336805]
41. McLean CY, Bristor D, Hiller M, Clarke SL, Schaar BT, Lowe CB, Wenger AM, and Bejerano G 2010 GREAT improves functional interpretation of cis-regulatory regions. *Nat Biotechnol* 28: 495–501. [PubMed: 20436461]
42. Gupta S, Stamatoyannopoulos JA, Bailey TL, and Noble WS 2007 Quantifying similarity between motifs. *Genome Biol* 8: R24. [PubMed: 17324271]
43. Zhang Y, Liu T, Meyer CA, Eeckhoutte J, Johnson DS, Bernstein BE, Nusbaum C, Myers RM, Brown M, Li W, and Liu XS 2008 Model-based analysis of ChIP-Seq (MACS). *Genome Biol* 9: R137. [PubMed: 18798982]
44. Andrienas KK, Ramlall V, Kurland J, Leung B, Harbaugh AG, and Siggers T 2018 DNA-binding landscape of IRF3, IRF5 and IRF7 dimers: implications for dimer-specific gene regulation. *Nucleic Acids Res* 46: 2509–2520. [PubMed: 29361124]
45. Saliba DG, Heger A, Eames HL, Oikonomopoulos S, Teixeira A, Blazek K, Androulidaki A, Wong D, Goh FG, Weiss M, Byrne A, Pasparakis M, Ragoussis J, and Udaloa IA 2014 IRF5:RelA interaction targets inflammatory genes in macrophages. *Cell Rep* 8: 1308–1317. [PubMed: 25159141]
46. Khan A, Fornes O, Stigliani A, Gheorghe M, Castro-Mondragon JA, van der Lee R, Bessy A, Cheneby J, Kulkarni SR, Tan G, Baranasic D, Arenillas DJ, Sandelin A, Vandepoele K, Lenhard B, Ballester B, Wasserman WW, Parcy F, and Mathelier A 2018 JASPAR 2018: update of the open-access database of transcription factor binding profiles and its web framework. *Nucleic Acids Res* 46: D1284. [PubMed: 29161433]

47. Badis G, Berger MF, Philippakis AA, Talukder S, Gehrke AR, Jaeger SA, Chan ET, Metzler G, Vedenko A, Chen X, Kuznetsov H, Wang CF, Coburn D, Newburger DE, Morris Q, Hughes TR, and Bulky ML 2009 Diversity and complexity in DNA recognition by transcription factors. *Science* 324: 1720–1723. [PubMed: 19443739]
48. Hu W, Xu R, Sun W, Szulc ZM, Bielawski J, Obeid LM, and Mao C 2010 Alkaline ceramidase 3 (ACER3) hydrolyzes unsaturated long-chain ceramides, and its down-regulation inhibits both cell proliferation and apoptosis. *J Biol Chem* 285: 7964–7976. [PubMed: 20068046]
49. Yan X, Nykanen NP, Brunello CA, Haapasalo A, Hiltunen M, Uronen RL, and Huttunen HJ 2016 FRMD4A-cytohesin signaling modulates the cellular release of tau. *J Cell Sci* 129: 2003–2015. [PubMed: 27044754]
50. Ikenouchi J, and Umeda M 2010 FRMD4A regulates epithelial polarity by connecting Arf6 activation with the PAR complex. *Proc Natl Acad Sci U S A* 107: 748–753. [PubMed: 20080746]
51. Rui Y, Xu Z, Xiong B, Cao Y, Lin S, Zhang M, Chan SC, Luo W, Han Y, Lu Z, Ye Z, Zhou HM, Han J, Meng A, and Lin SC 2007 A beta-catenin-independent dorsalization pathway activated by Axin/JNK signaling and antagonized by aida. *Dev Cell* 13: 268–282. [PubMed: 17681137]
52. Niimori D, Kawano R, Niimori-Kita K, Ihn H, and Ohta K 2014 Tsukushi is involved in the wound healing by regulating the expression of cytokines and growth factors. *J Cell Commun Signal* 8: 173–177. [PubMed: 25159578]
53. Jensen A, and Mullenders LH 2010 Transcription factor IIS impacts UV-inhibited transcription. *DNA Repair (Amst)* 9: 1142–1150. [PubMed: 20729154]
54. Yap KL, and Zhou MM 2010 Keeping it in the family: diverse histone recognition by conserved structural folds. *Crit Rev Biochem Mol Biol* 45: 488–505. [PubMed: 20923397]
55. Asencio C, Davidson IF, Santarella-Mellwig R, Ly-Hartig TB, Mall M, Wallenfang MR, Mattaj IW, and Gorjanacz M 2012 Coordination of kinase and phosphatase activities by Lem4 enables nuclear envelope reassembly during mitosis. *Cell* 150: 122–135. [PubMed: 22770216]
56. Li D, De S, Li D, Song S, Matta B, and Barnes BJ 2016 Specific detection of interferon regulatory factor 5 (IRF5): A case of antibody inequality. *Sci Rep* 6: 31002. [PubMed: 27481535]
57. Krausgruber T, Saliba D, Ryzhakov G, Lanfrancotti A, Blazek K, and Udalova IA 2010 IRF5 is required for late-phase TNF secretion by human dendritic cells. *Blood* 115: 4421–4430. [PubMed: 20237317]
58. Bedard KM, Wang ML, Proll SC, Loo YM, Katze MG, Gale M, Jr., and Iadonato SP 2012 Isoflavone agonists of IRF-3 dependent signaling have antiviral activity against RNA viruses. *J Virol* 86: 7334–7344. [PubMed: 22532686]
59. Green RR, Wilkins C, Patabhi S, Dong R, Loo Y, and Gale M, Jr. 2016 Transcriptional analysis of antiviral small molecule therapeutics as agonists of the RLR pathway. *Genom Data* 7: 290–292. [PubMed: 26981429]

Figure 1: Differential gene expression analysis following WNV infection in WT and *Irf5*^{-/-} BMDCs.

A. Heatmap of log₂ fold changes in differentially expressed genes relative to mock and WNV-infected cells at 6, 12, and 24 hours post-infection. Colored bars and tree indicate co-expression modules generated by Ward's clustering. Modules were annotated with significantly enriched ($p < 0.05$) canonical pathways by IPA.

B. Significantly enriched ($p < 0.05$) canonical pathways identified by IPA from differentially expressed genes at 24 hours post-infection in WT and *Irf5*^{-/-} cells.

Figure 2: IRF5 peak locations

A. Number of IRF5 ChIP-exo peaks between experimental conditions.

B. Number of IRF5 ChIP-exo peaks binned by absolute distance to associated TSS's across experimental conditions.

Figure 3: Identification of IRF5 binding sites and associated genes during WNV infection

A. Pathway enrichment of genes associated with IRF5 ChIP-exo peaks by condition. Significance of enrichment represented by size of bubble.

B. Motifs identified in IRF5 ChIP-exo experiments by GEM ($p < 0.001$) which show similarity with the canonical “GAAANNGAAANN” IRF binding motif. Aligned according to site similarity and inferred consensus sequence from all samples in bottom row.

C. Novel motifs identified within IRF5 peaks by GEM ($p < 0.001$). Motifs above the dashed line are shared between multiple experimental conditions, while motifs below are unique to a single condition. Canonical IRF binding motif excluded from figure.

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Figure 4: Identification of IRF5 target genes by combining RNA-seq and ChIP-exo

A. Heatmap of log2 fold changes in differentially expressed genes of WNV-infected cells relative to mock-infected cells that were also assigned peaks that passed the IDR threshold. Co-expression modules in heatmap were defined by Ward's clustering.

B. Interaction network built within IPA using the IRF5 target genes as seeds. Nodes represent genes that are labeled by gene name, and nodes that are connected with IRF5 by dashed lines are part of the 12 IRF5 targets. Arrow colors highlight the type of interaction specified by the legend. Node colors indicate predicted role of IRF5 regulation, with grey

showing *Oas1l* was increased in *Irf5*^{-/-} cells at 12 hours post-infection, but increased in WT cells at 24 hours post-infection.

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Figure 5: qRT-PCR of IRF5 target genes

A. WT and *Irf5*^{-/-} BMDCs were mock- or WNV-infected, and expression of *Fcgr4* was assessed at 12 and 24 hour time points. p-value from unpaired T-test of biological triplicates is shown.

B. WT and *Irf5*^{-/-} BMDCs were mock- or WNV-infected, and expression of *Ccl2* was assessed at 24 hour time point. p-value from unpaired T-test of biological triplicates is shown.

C. WT and *Irf5*^{-/-} BMDCs were mock- or WNV-infected, and expression of *Oas1* was assessed at 12 hour time point. p-value from unpaired T-test of biological triplicates is shown.

D. WT and *Irf5*^{-/-} BMDCs were mock- or WNV-infected, and expression of *Ifnb* and *Ifit1* was assessed at 24 hour time point. p-value from unpaired T-test of biological triplicates is shown.

E. WT and *Irf5*^{-/-} BMDCs were mock- or WNV-infected, and WNV genome copy number was assessed at 24 hour time point. p-value from unpaired T-test of biological triplicates is shown.

Figure 6: Global and IRF5 target gene expression in WT and *Irf5*^{-/-} BMMs

A. Heatmap of log2 fold changes of the union of all differentially expressed genes in WNV-infected WT and *Irf5*^{-/-} BMDCs and BMMs relative to mock-infected cells. Co-expression modules within the heatmap were defined by Ward's clustering, and modules functionally annotated with IPA canonical pathway analysis.

B. Heatmap of log2 fold changes of IRF5 target genes in BMDCs that were also differentially expressed in BMMs.

Figure 7: Comparison of IRF5 target genes with public ChIP datasets
A. Heatmap of log-average MACS2 ChIP binding scores from select public IRF ChIP experiments in the ChIP-atlas database. Higher scores (dark shading) indicates higher confidence of binding.

Table 1:

List of IRF5-dependent genes, and their known/predicted functions.

Gene symbol	Gene name	Known functions	References
Frmd4a	FERM domain containing 4A	Regulates actin cytoskeleton dynamics and membrane/endocytic trafficking	(49, 50)
Aida	Axin Interactor, Dorsalization Associated	Blocks Axin-mediated JNK activation	(51)
C130026I21Rik	RIKEN cDNA C130026I21 gene	SP140 nuclear body protein family member with no known function. Contains several chromatin related modules such as plant homeodomain (PHD), bromodomain (BRD) and SAND domain, which suggests a role in chromatin-mediated regulation of gene expression. Human Sp140 is an interferon inducible nuclear leukocyte-specific protein that may be involved in the pathogenesis of acute promyelocytic leukemia and viral infection.	(52, 53)
Fcgr4	Fc receptor, IgG, low affinity IV	Receptor for IgG and IgE, mediates immune cell activation and initiates phagocytosis, antigen presentation and production of proinflammatory mediators	(54–56)
Herc6	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase Family Member 6	Mediates protein ISGylation	(57, 58)
Ankle2	Ankyrin Repeat And LEM Domain Containing 2	Facilitates mitotic nuclear envelope reassembly by promoting dephosphorylation of BAF/BANF1 protein by PP2A; regulates thymic T cell development	(59, 60)
Clic4	Chloride Intracellular Channel 4	Host response to LPS; induces IL-1 β transcription and activation of NLRP3 inflammasome; promotes metastases of MCPyV induced cancer by promoting cell motility and invasion	(61–64)
Tcea1	Transcription Elongation Factor A1	Regulates myeloid cell proliferation; helps Pol II bypass blocks to transcription elongation to enable efficient transcription; mediates transcript cleavage and resumption of transcription elongation in UV-inhibited transcription	(65, 66)
Acer3	Alkaline Ceramidase 3	Hydrolyzes unsaturated long-chain ceramides; anti-inflammatory via suppression of C18:1-ceramide	(67–70)
Ccl2	C-C Motif Chemokine Ligand 2	Promotes migration of monocytes, memory T cells, dendritic cells, basophils and NK cells to inflammatory sites	(71)
Tsku	Tsukushi, Small Leucine Rich Proteoglycan	Regulates TGF- β 1 signaling and wound healing	(72, 73)
Oas1l	2'–5' Oligoadenylate synthetase-like 1	Inhibits translation of IRF7 mRNA and negatively regulates type I IFN response; traps viral RNAs in stress granules, enhances RIG-I-like receptor signaling response	(74–77)