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Supplemental Information

Non-redundant ISGF3 Components Promote NK Cell Survival in an Auto-regulatory Manner during Viral Infection

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FIGURE S1

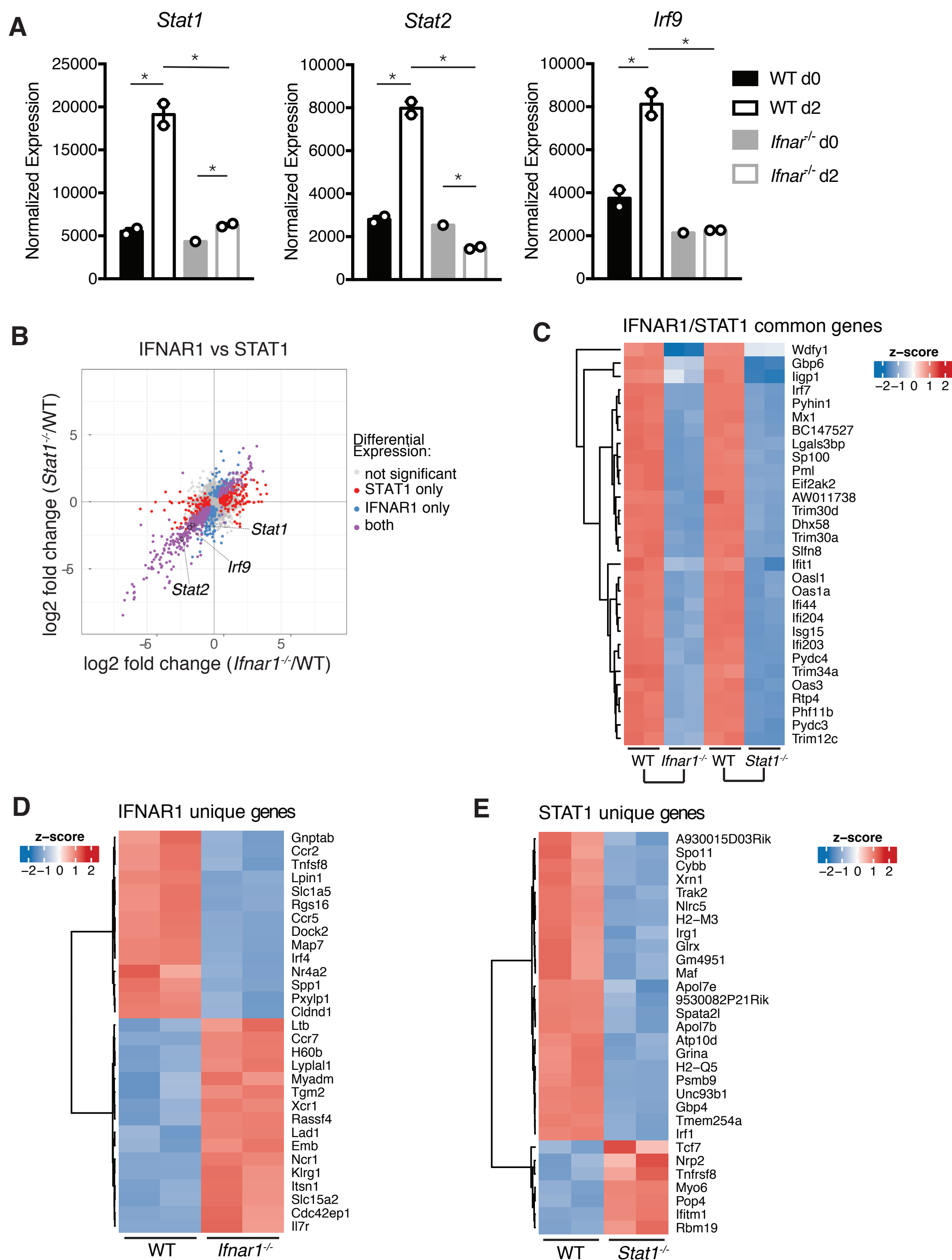


Figure S1. Autoregulation of ISGF3 components *in vivo* following MCMV infection. Related to Figure 2.
(**A**) Normalized counts of Stat1, Stat2, and Irf9 in WT and Ifnar1-/- NK cells from RNA-seq. Symbols represent biological replicates, and error bars show SEM (*P<0.05). (**B**) Scatter plot of RNA-seq data comparing gene-level log2 fold changes between Ifnar1-/- and WT NK cells versus Stat1-/- and WT NK cells at d1.5 PI. Red dots depict Stat1-/-/WT-unique expressed genes, blue dots Ifnar1-/-/WT-unique differentially expressed genes, and purple dots depict differentially expressed genes common to both. **C-E** Heatmaps of RNA-seq data as in B, depicting row-normalized log2-transformed counts of the top 30 differentially expressed genes ranked by adjusted p value, with base mean > 50, calculated by DESeq2. (**C**) Differentially expressed genes common to both Ifnar1-/- and Stat1-/-. Brackets indicate paired WT samples for Ifnar1-/- and Stat1-/-, respectively (see FIG 2A). (**D**) Ifnar1-/-/WT-unique differentially expressed genes. (**E**) Stat1-/-/WT-unique expressed genes.

FIGURE S2

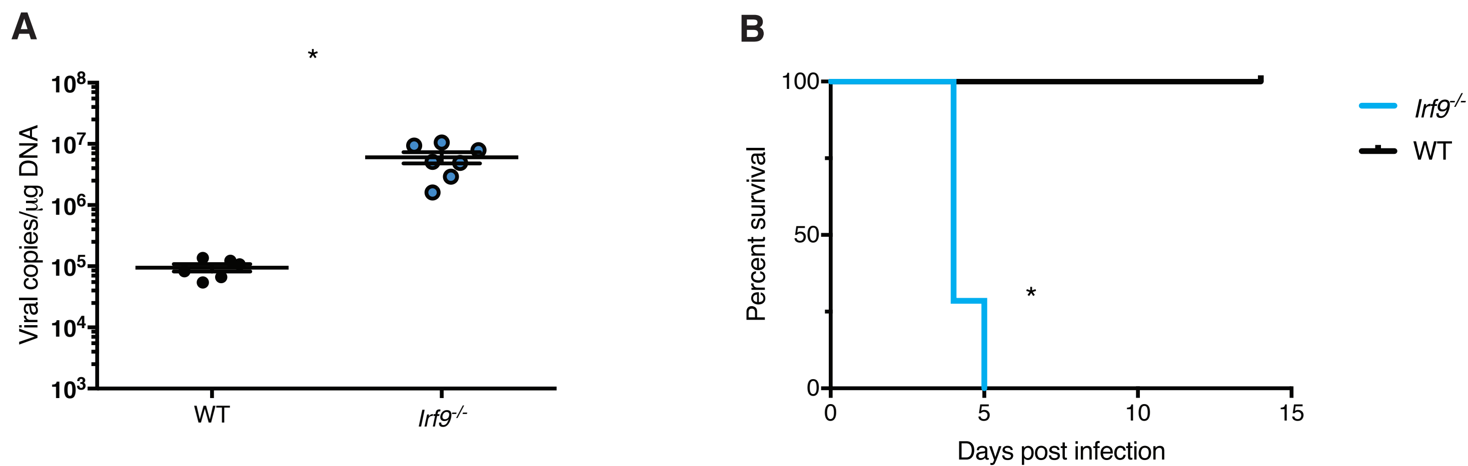


Figure S2. IRF9 is essential for control of MCMV. Related to Figure 3.
Peripheral blood viral titers at day 4 p.i. (**A**) and Kaplan-Meier survival curves (**B**) for WT (black line, n=6) and Irf9-/- mice (blue line, n=7) challenged IP with a lethal dose of MCMV. Data are representative of one (A) or two (B) independent experiments with n=5-7 mice per group. Symbols represent individual mice, and error bars show SEM (*P<0.05).

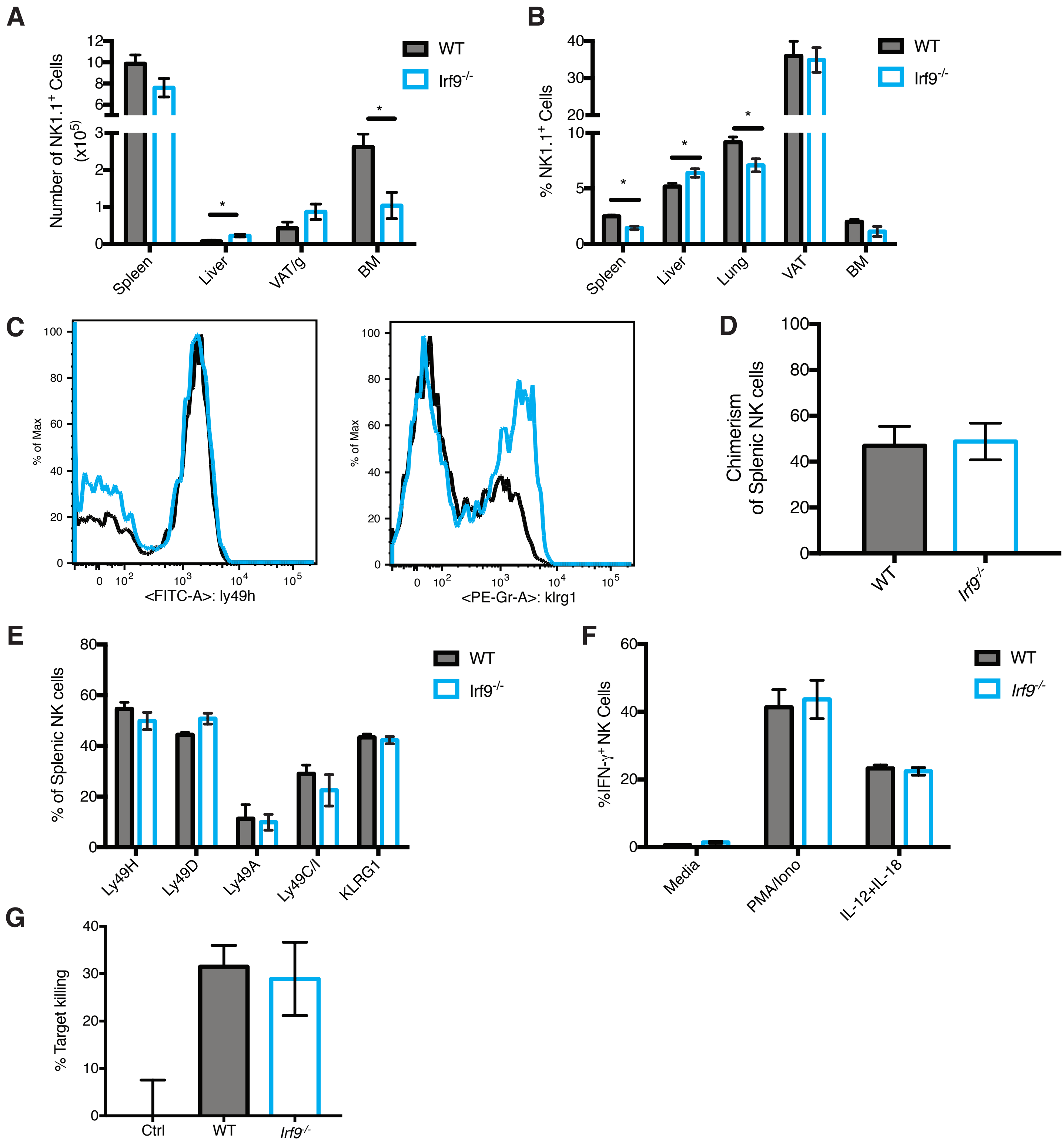
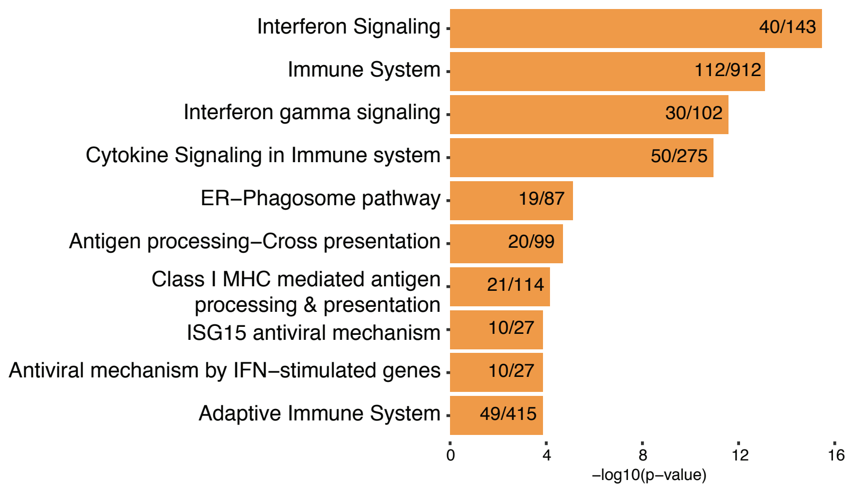


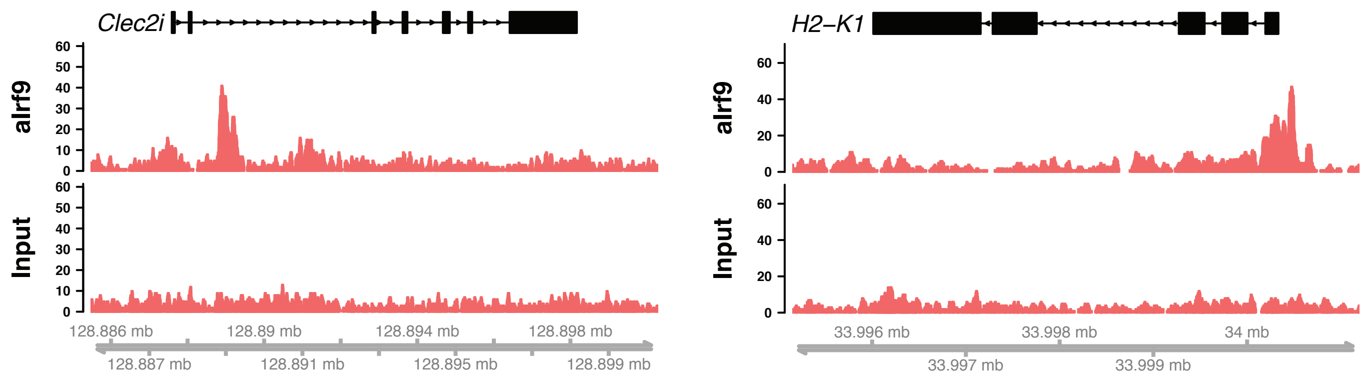
Figure S3. Cell-extrinsic requirement for IRF9 for the development of NK cells. Related to Figure 3.
Frequency (**A**) and absolute numbers (**B**) of NK cells in various organs of WT and *Irf9-/-* mice. (**C**) Representative histograms of Ly49H (left) and KLRG1 (right) expression of splenic NK cells in WT and *Irf9-/-* mice. **D-E** Lethally irradiated mice were injected with equal numbers of WT (CD45.1) and *Irf9-/-* (CD45.2) bone marrow cells. Following hematopoietic reconstitution, splenic NK cells were analyzed for contribution from each donor (**D**) and expression of activating and inhibitory receptors (**E**). (**F**) Percentage of splenic NK cells from mixed bone marrow chimeric mice producing IFN-γ following 4 hour ex vivo stimulation with PMA/ionomycin or IL-12 + IL-18. (**G**) Purified splenic NK cells from WT and *Irf9-/-* mice were incubated with Ba/F3-m157 target cells (CTVhi) and Ba/F3 control cells (CTVlo) at 10:1:1 ratio (effector:target:control) for 7.5 hours. Quantification of target cell killing by indicated NK cell populations compared to control wells lacking NK cells. Data are presented as mean ±SEM (*P<0.05), and are representative of one (A, B, E, G), two (D, F) or three (C) independent experiments with n=2-5 mice per group.

FIGURE S4

A



B



C

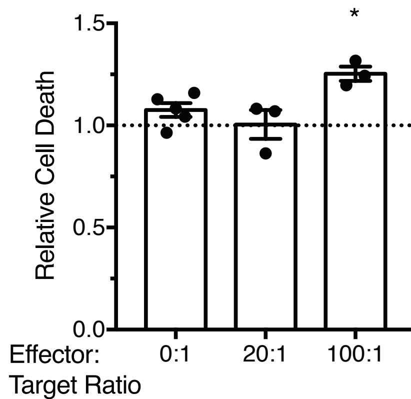


Figure S4. IRF9 regulates IFN signaling and expression of ligands for NK cell receptors. Related to Figure 4.
(**A**) Top ten enriched pathways as calculated by Reactome including the number of differentially expressed genes per pathway. Bar plots depict –log10 P values calculated by Reactome. (**B**) Representative gene tracks from IRF9 ChIP-Seq. (**C**) Congenically distinct "target" NK cells from WT and *Irf9-/-* mice were co-incubated with the indicated ratios of "effector" WT NK cells and pro-inflammatory cytokines for 16 hours. Cell death of *Irf9-/-* target NK cells is shown relative to cell death of WT target NK cells from the same well. Bar graphs show data as mean ±SEM, and are representative of two experiments with 3-5 replicates each. Means were compared to a hypothetical value of 1 using a one-sample t test (*P<0.05). RNA-Seq is 3 biological replicates. ChIP-Seq is 3 experiments of 20-25 pooled mice per experiment.