

Expanded View Figures

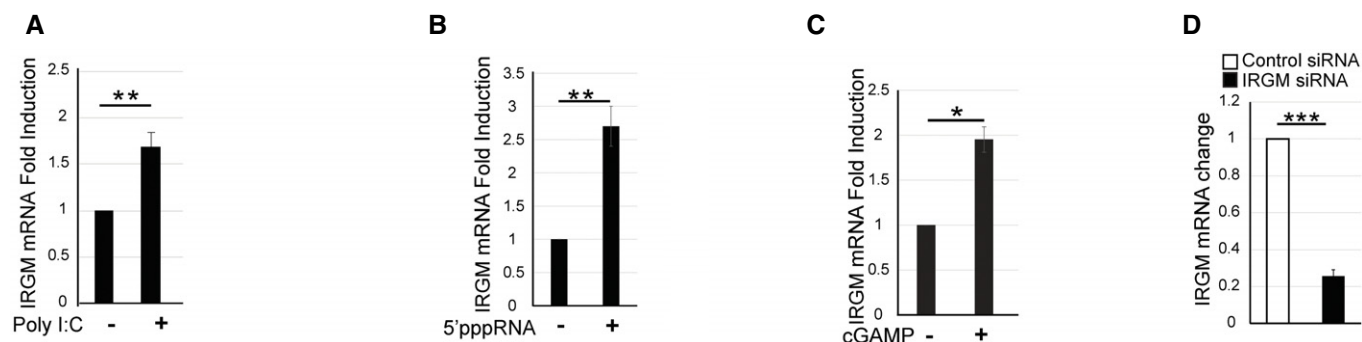


Figure EV1. Viruses and viral PAMP-induced IRGM expression suppress the interferon response.

- A THP-1 cells were untreated or treated with Poly I:C (1 μ g/ml) for 4 h and were subjected to qRT-PCR with IRGM ($n = 3$, mean $\pm$ SD, $**P \leq 0.005$ Student's unpaired t -test).
- B THP-1 cells untreated or treated with 5'ppp-dsRNA (1 μ g/ml) for 4 h and was subjected to qRT-PCR with IRGM ($n = 3$, mean $\pm$ SD, $**P \leq 0.005$, Student's unpaired t -test).
- C THP-1 cells untreated or treated with cGAMP (1 μ g/ml) for 4 h and was subjected to qRT-PCR with IRGM ($n = 3$, mean $\pm$ SD, $*P < 0.05$, Student's unpaired t -test).
- D The graph depicts the knockdown efficiency upon transfection of control and IRGM siRNA in THP-1 cells ($n = 4$, mean $\pm$ SD, $***P < 0.0005$, Student's unpaired t -test).

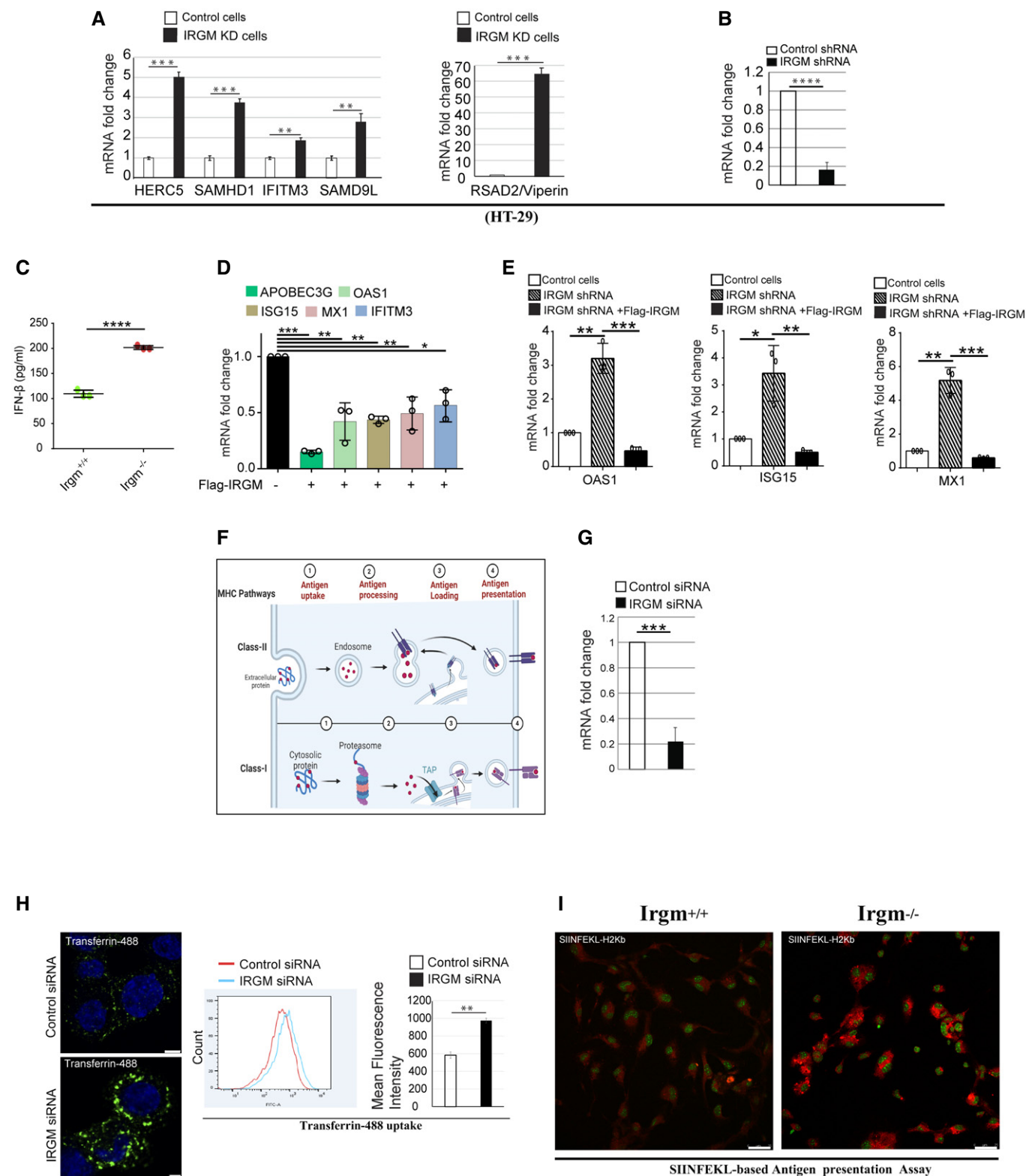


Figure EV2.

Figure EV2. Key antiviral mechanisms are upregulated upon IRGM depletion.

- A RNA isolated from control and IRGM knockdown HT-29 cells and subjected to qRT-PCR with indicated viral restriction factor genes ($n = 3$, mean $\pm$ SD, $**P < 0.005$, $***P < 0.0005$, Student's unpaired t -test).
- B The graph depicts the knockdown efficiency of control and IRGM shRNA stable HT-29 cells ($n = 3$, mean $\pm$ SD, $****P < 0.00005$, Student's unpaired t -test).
- C The graph depicts the IFN- β levels in serum of *Irgm1* wild-type and KO mice ($n = 3$ mice each group, mean $\pm$ SD, $****P < 0.00005$, Student's unpaired t -test).
- D The qRT-PCR analysis of APOBEC3G, OAS1, ISG15, MX1 and IFITM3 with RNA isolated from control or Flag IRGM overexpressing HT-29 cells ($n = 3$, Mean $\pm$ SD, $*P < 0.05$, $**P < 0.005$, $***P < 0.0005$, Student's unpaired t -test).
- E The qRT-PCR analysis of OAS1, ISG15 and MX1 with RNA isolated from control or IRGM shRNA or Flag IRGM complemented IRGM shRNA HT-29 cells. $n = 3$, Mean $\pm$ SD, $*P < 0.05$, $**P < 0.005$, $***P < 0.0005$, Student's unpaired t -test.
- F Pictorial representation of sequential events of antigen uptake, processing, and presentation via Class I and Class II MHC Pathways. Created using Biorender.com.
- G The graph depicts the knockdown efficiency upon transfection of control and si-IRGM in THP-1 cells ($n = 3$, mean $\pm$ SD, $***P < 0.0005$, Student's unpaired t -test).
- H Transferrin uptake assay shown by representative confocal images and flow cytometry analysis of control and si-IRGM transfected THP-1 cells treated with AF488 Transferrin (green) (10 μ g/ml, 30 min). Graph depicts the mean fluorescence intensity of transferrin uptake in control and si-IRGM transfected THP-1 cells treated with AF488 transferrin. Scale, 5 μ m (upper panel); Scale, 3 μ m, (lower panel).
- I Representative confocal images of H-2Kb-SIINFEKL (red) in *Irgm1*^{+/+} and *Irgm1*^{-/-} BMDMs treated with OVA (2 mg/ml, 3 h). Scale, 25 μ m.

Figure EV3. IRGM-depleted cells are resistant to viral infection.

- A Total RNA was isolated from mock HT-29 cells or CHIKV (MOI 1, 24 h) infected HT-29 cells or Flag IRGM overexpressing HT-29 cells and subjected to qRT-PCR with VSV specific primers to quantitate total viral load ($n = 3$, Mean $\pm$ SD, $*P < 0.05$, Student's unpaired t -test).
- B Total RNA was isolated from mock and VSV (MOI 2.5, 24 h) infected control and IRGM knockdown HeLa cells and subjected to qRT-PCR with VSV specific primers to quantitate total viral load ($n = 3$, mean $\pm$ SE, $***P < 0.0005$, Student's unpaired t -test).
- C Left panels, representative fluorescence microscopic images of control and IRGM knockdown HeLa (MOI 2.5) and THP-1 (MOI 5) cells infected with VSV-eGFP for 4 h. Right panel, the graph depicts quantitative analysis of percentage of cells that are VSV-eGFP positive ($n = 3$, mean $\pm$ SD, $**P < 0.005$, $***P < 0.0005$ Student's unpaired t -test).
- D Left panels, representative fluorescence microscopic images of control or IRGM KD or Flag IRGM complemented IRGM KD THP-1 cells infected with VSV-eGFP (MOI 5.0) for 4 h. Scale, 400 μ m. Right panel, the graph depicts VSV-eGFP viral mRNA fold change. $n = 3$, Mean $\pm$ SD, $*P < 0.05$, Student's unpaired t -test.
- E Meta-analysis using Metascape, comparing the transcriptome significantly induced in *Irgm1*^{-/-} mice (> 1.5 folds; $P < 0.05$) and the genes induced significantly upon SARS-CoV-2 infection (seven different conditions from four different studies). The gene expression from different conditions and studies cluster together along with the query gene list (marked with red).
- F Circos plot depicting the gene overlap analysis between the genes induced in *Irgm1*^{-/-} mice (> 1.5 folds; $P < 0.05$) and the genes induced significantly upon SARS-CoV-2 infection (seven different conditions from four different studies). On the outside, each arc represents the identity of each gene list. On the inside, each arc represents a gene list, where each gene has a spot on the arc. Dark orange color represents the genes that appear in multiple lists and light orange color represents genes that are unique to that gene list. Purple lines link the same gene that is shared by multiple gene lists. Blue lines link the different genes where they fall into the same ontology term (the term has to be statistically significantly enriched and with a size no larger than 100). The greater the number of purple links and the longer the dark orange arcs imply greater overlap among the input gene lists. Blue links indicate the amount of functional overlap among the input gene list.
- G The heatmap depicts statistically enriched terms (GO or KEGG or Reactome) obtained when induced genes in *Irgm1*^{-/-} mice (> 1.5 folds; $P < 0.05$) were compared with the genes induced upon SARS-CoV-2 infection (seven different conditions from four different studies). The heatmap cells are colored by their P -values (P -values are calculated based on the accumulative hypergeometric distribution, and q -values are calculated using the Benjamini-Hochberg procedure to account for multiple testings as described at <https://metascape.org/COVID/>), white cells indicate the lack of enrichment for that term in the corresponding gene list.
- H Amplification plot of qRT-PCR analysis conducted with SARS-CoV-2 nucleocapsid primers and RNA isolated from the supernatant of THP-1 cells infected with SARS-CoV-2 for 10 h and 20 h. The horizontal line (with values in red font) indicates threshold value of qPCR.
- I Representative images of the plaque assay in Vero E6 cells performed from the culture supernatant of uninfected and SARS-CoV-2 (MOI 1, 24 h) infected THP-1 cells.
- J Western blot analysis with cell lysates of control and si-IRGM transfected THP-1 and HT-29 cells and probed with the indicated antibodies.
- K qRT-PCR analysis showing the percentage of viral infection in control or IRGM siRNA transfected THP-1 cells untreated or treated with N-acetyl-L-cysteine (NAC, 1 mM, 2 h) followed by VSV-eGFP infection (MOI 5.0) for 4 h. Mean $\pm$ SD, $n = 3$ (biological replicates), $*P < 0.05$, Student's unpaired t -test.

Source data are available online for this figure.

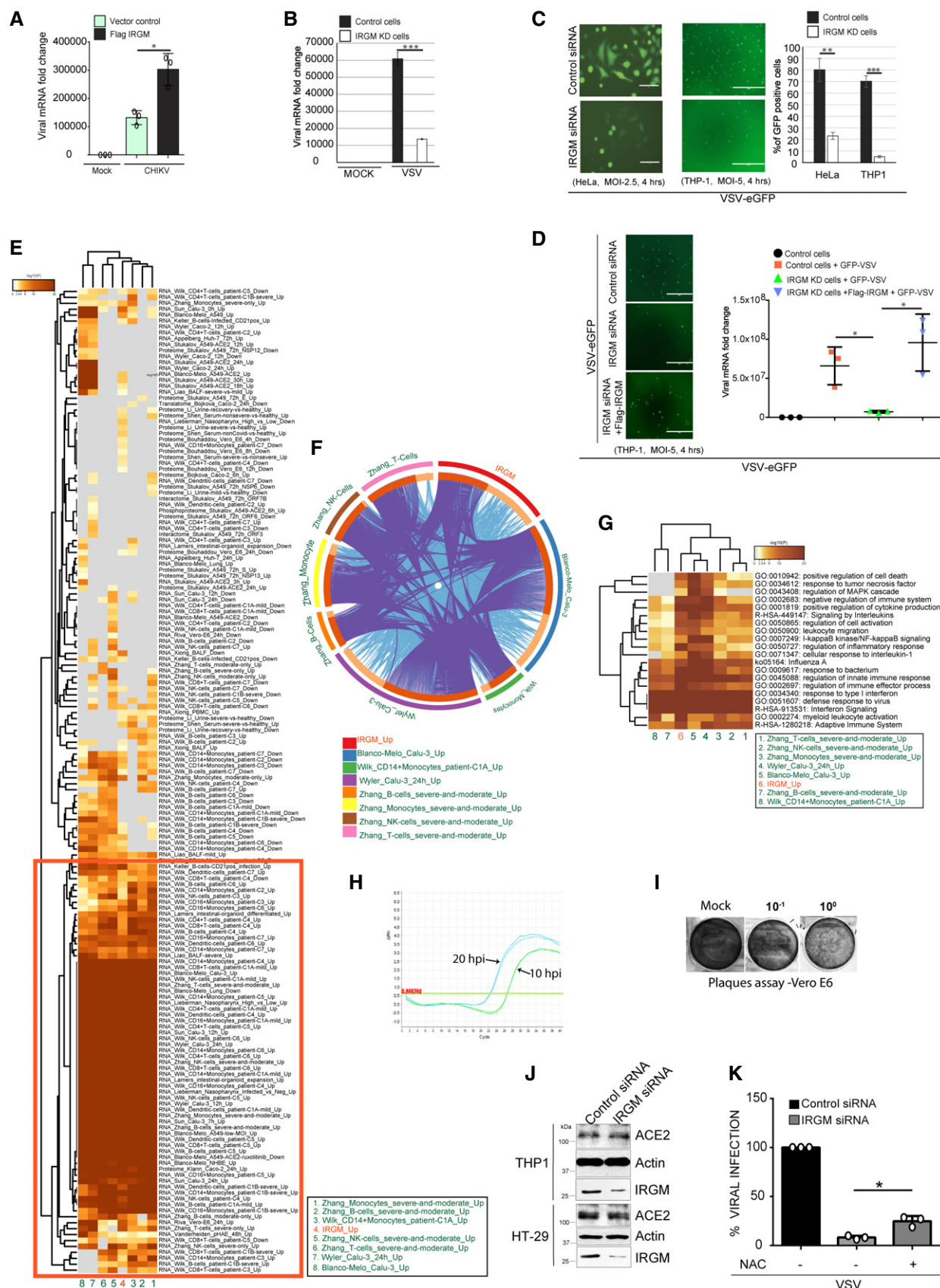


Figure EV3.