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Supplemental Information

An Antiviral Branch of the IL-1 Signaling Pathway Restricts Immune-Evasive Virus Replication

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Supplemental Figures and Legends

Figures S1-S6.

Figure S1

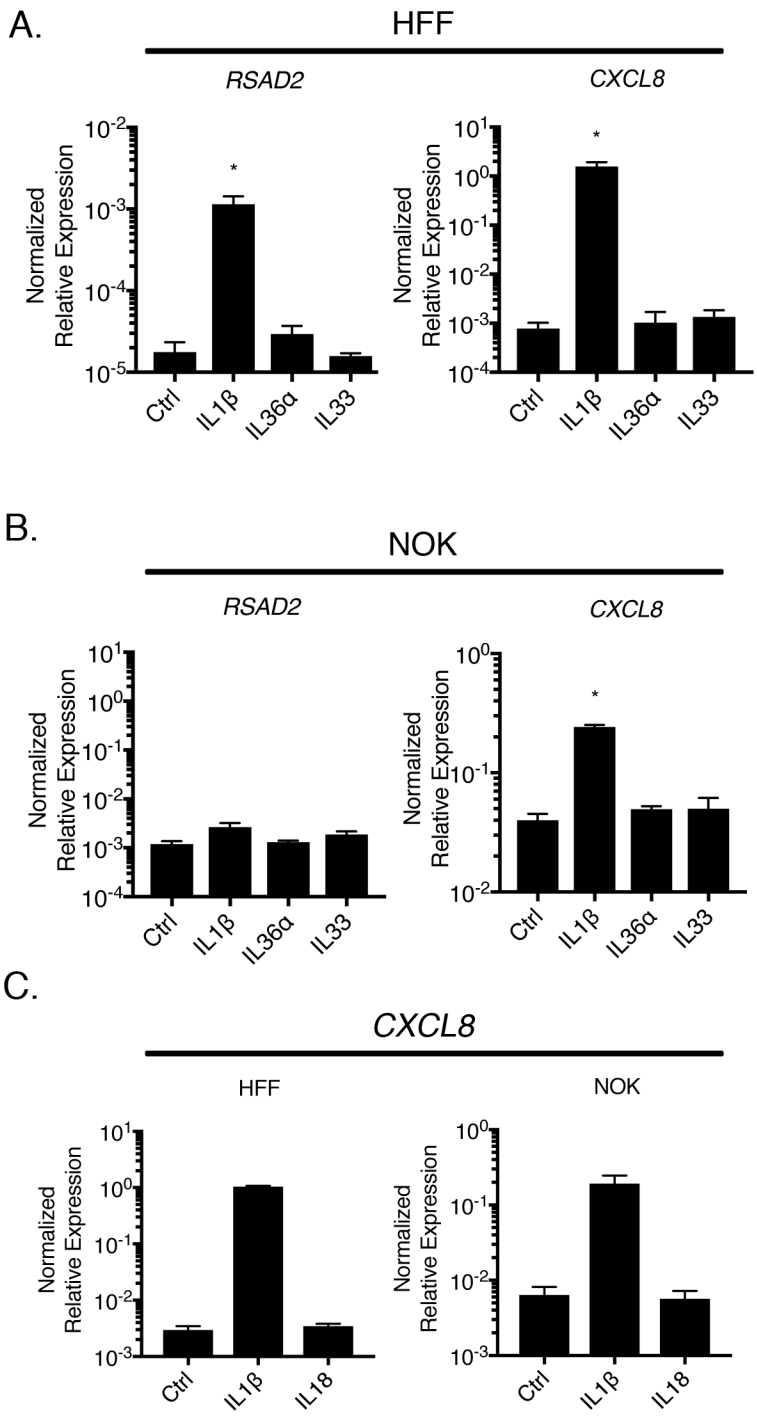
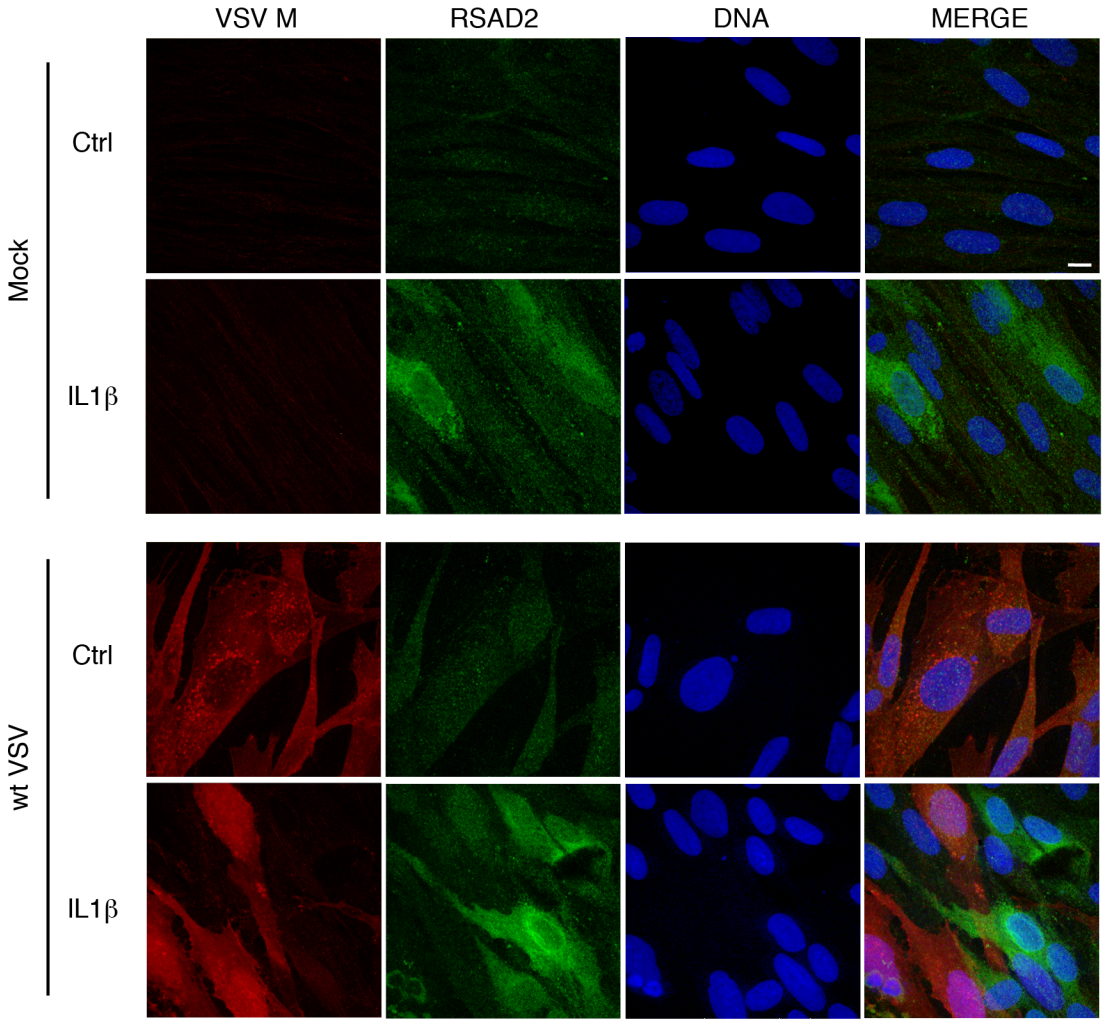

A.
HFF
RSAD2
CXCL8
Normalized Relative Expression
Ctrl
IL1β
IL36α
IL33
B.
NOK
RSAD2
CXCL8
Normalized Relative Expression
Ctrl
IL1β
IL36α
IL33
C.
CXCL8
HFF
NOK
Normalized Relative Expression
Ctrl
IL1β
IL18

Figure S2

A.


VSV M
RSAD2
DNA
MERGE
Mock
Ctrl
IL1β
wt VSV
Ctrl
IL1β

B.

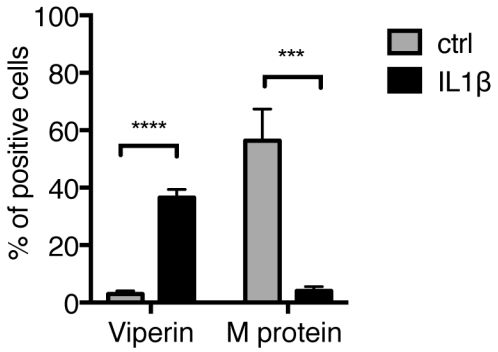
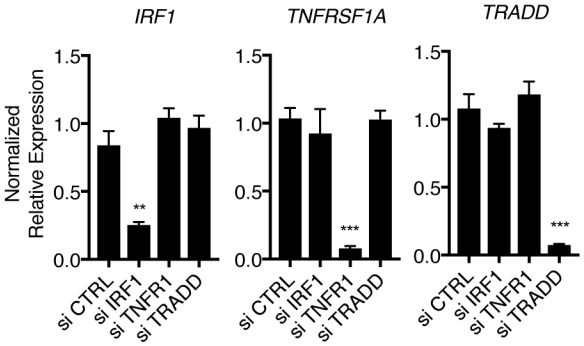
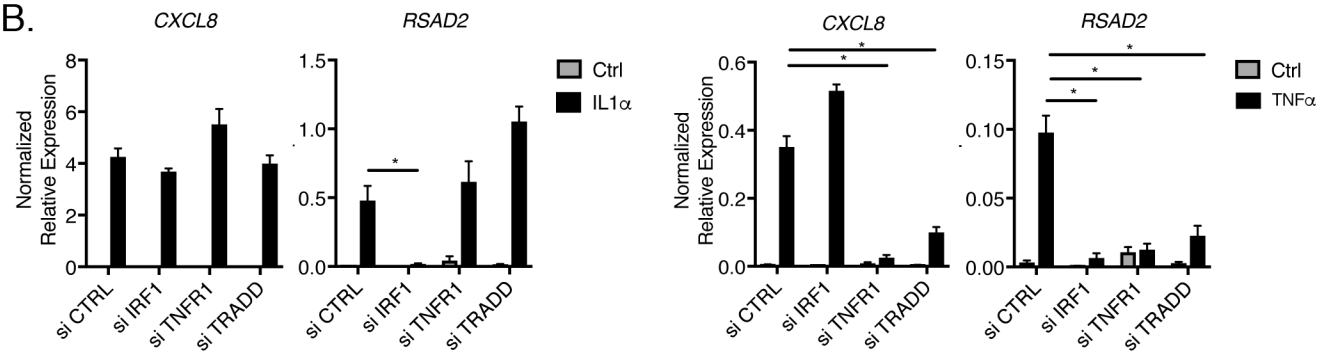

% of positive cells
100
80
60
40
20
0
****
***
ctrl
IL1β
Viperin
M protein

Figure S3

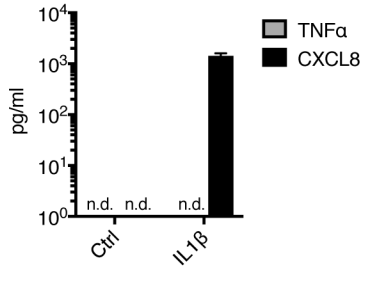
A.



B.



C.



D.

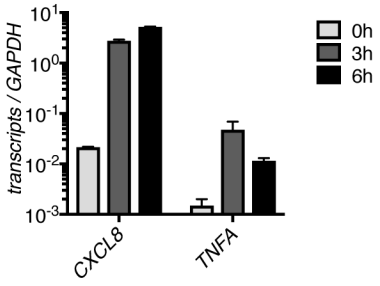


Figure S4

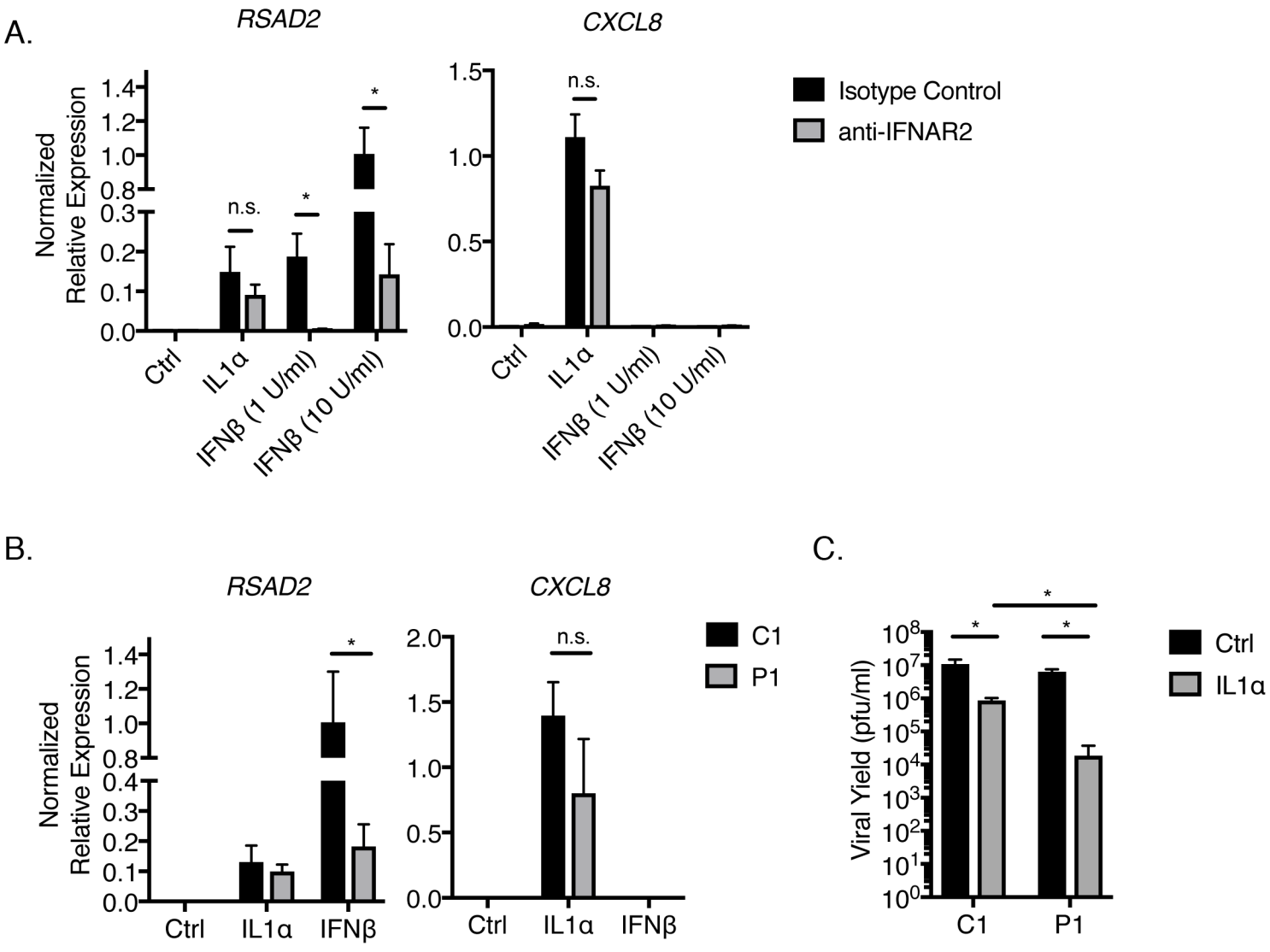

A.
RSAD2
CXCL8
Normalized Relative Expression
n.s.
*
Ctrl
IL1α
IFNβ (1 U/ml)
IFNβ (10 U/ml)
Isotype Control
anti-IFNAR2
B.
RSAD2
CXCL8
C1
P1
IFNβ
C.
Viral Yield (pfu/ml)
Ctrl
IL1α
C1
P1

Figure S5

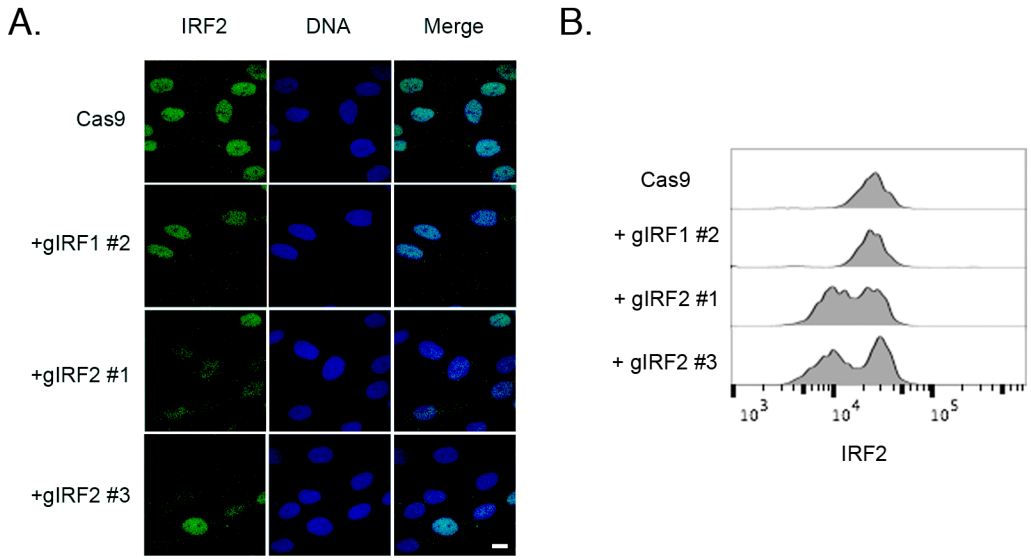
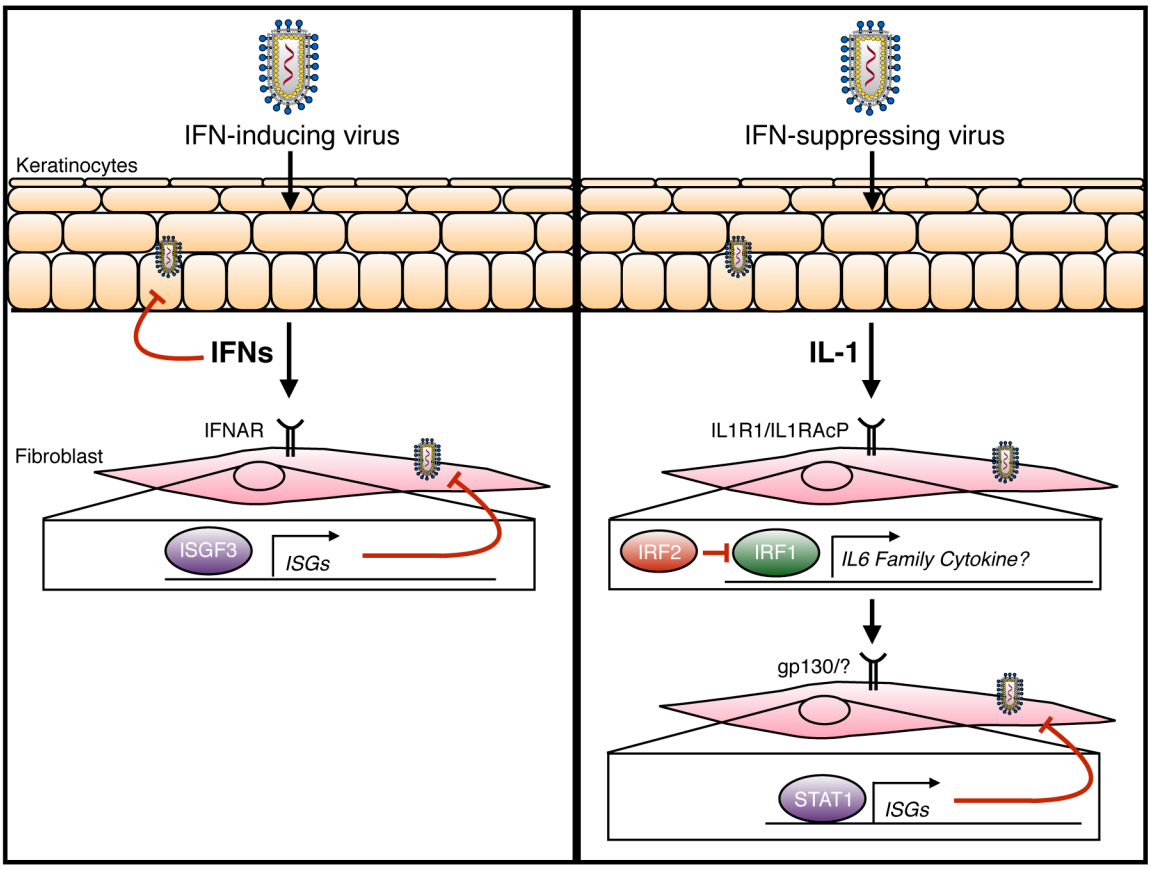

A.
IRF2
DNA
Merge
Cas9
+gIRF1 #2
+gIRF2 #1
+gIRF2 #3
B.
Cas9
+ gIRF1 #2
+ gIRF2 #1
+ gIRF2 #3
10^3
10^4
10^5
IRF2

Figure S6


IFN-inducing virus
IFN-suppressing virus
Keratinocytes
IFNs
IL-1
IFNAR
IL1R1/IL1RAcP
Fibroblast
ISGF3
ISGs
IRF2
IRF1
IL6 Family Cytokine?
gp130/?
STAT1
ISGs

Supplemental Figure Legends.

Figure S1. Antiviral transcriptional responses are specific to IL-1 subfamily cytokines, Related to Figure 2. HFFs (A) and NOKs (B) were stimulated with IL-1β (10 ng/ml), IL-36α (10 ng/ml), or IL-33 (10 ng/ml). Total cellular RNA was harvested at 6 h and quantified by qRT-PCR for *RSAD2* and *CXCL8* transcript abundance. C) HFF (*left)* and NOKs (*right)* were stimulated with IL-1β (10 ng/ml) or IL-18 (10 ng/ml) and *CXCL8* abundance was determined by qRT-PCR at 6 h. (A-C) Data are normalized to *GAPDH*. Data are an average of three independent experiments +/- SEM. Student's t test; p*<0.05, **p<0.01.

Figure S2. IL-1 treatment inhibits viral protein accumulation in VSV-infected cells, Related to Figure 4. A) HFFs were mock-infected or infected with wt VSV at a MOI of 0.1 in the presence or absence of IL1β (10 ng/ml). Cells were fixed and stained for VSV M protein and RSAD2 at 24 hpi using specific antibodies. Nuclei were counterstained with DRAQ5. Scale bar 10 μm. B) Two-hundred cells in each condition were examined for VSV M and RSAD2 protein. VSV M and RSAD2 positive cells are presented as a percentage of the total number of cells examined. Scale bar 10 μm. Results are the average of three-independent experiments +/- SEM. Student's t test; p***<0.001, ****p<0.0001.

Figure S3. TNF receptor signaling is not required for IL-1 mediated antiviral transcriptional responses, Related to Figure 6. (A-B) HFFs were transfected with ctrl siRNAs or siRNAs targeting *IRF1, TNFR1,* or *TRADD* for 72 h. A) The efficiency of siRNA-mediated depletion of indicated transcripts was demonstrated by qRT-PCR. B) siRNA-transfected cells were then treated with (left) IL-1α (100 pg/ml) or (right) TNFα (100 pg/ml) and total cellular RNA was isolated at 6 h. *RSAD2* and *CXCL8* transcripts were quantified by qRT-PCR and normalized to *GAPDH*. C) HFFs were stimulated with IL-1β (10 ng/ml) for 24 h. Cell free supernatants were isolated and analyzed by ELISA for TNFα and CXCL8 protein. D) HFFs were stimulated with IL-1β (10 ng/ml)

and total cellular RNA was isolated at 0, 3, and 6 h. Transcript abundance was determined by nCounter and normalized to *GAPDH.* Data are an average of three-independent experiments +/- SEM. Student's t test; p*<0.05, **p<0.01 ***p<0.001

Figure S4. IFNAR signaling is not required for IL-1 mediated antiviral responses, Related to Figure 6. A) HFFs were pre-treated with indicated antibodies (20 μg/ml) for 1 h prior to dilution with an equal volume of control media or media containing IL-1α (200 pg/ml) or IFNβ (2 U/ml or 20 U/ml). The final concentration of IL-1α and IFNβ were 100 pg/ml and 1 U/ml or 10 U/ml, respectively. B) Control fibroblasts (C1) or fibroblasts isolated from patients with mutations in *IFNAR1* (P1) were treated with IL-1α (100 pg/ml) or IFNβ (10 U/ml). Total cellular RNA was isolated at 6 h post-stimulation and analyzed by qRT-PCR for indicated transcripts. C) Control (C1) or patient (P1) fibroblasts were infected with VSV (MOI 0.1) in the presence of IL-1α (100 pg/ml). Infected-cell supernatants were isolated at 48 hpi and analyzed by plaque assay. Data are the average of at least three independent experiments +/- SEM. Student's t test *p<0.05,

Figure S5. IRF2 protein abundance is reduced in fibroblasts transduced with CRISPR/Cas9 lentivirus constructs, Related to Figure 7. hTERT-HFFs transduced with lentiviruses expressing Cas9 alone or in combination with indicated guide RNAs were fixed and examined by A) immunofluorescence or B) flow cytometry for IRF2 protein abundance. Scale bar 10 μm.

Figure S6. Model of the antiviral signaling pathway induced by IL-1 cytokines. **Related to Figures 1-7.** (*left*) Keratinocytes infected with non-immune evasive viruses transcriptionally upregulate IFNs, which act in an autocrine or paracrine manner to induce ISGs and restrict virus replication. By contrast, infection of keratinocytes with viruses that suppress IFN expression (*right*) results in release of pre-stored IL-1 cytokines. IL-1 cytokines stimulate fibroblasts to induce IRF1 transcriptional activity and expression of an antiviral factor. This factor may signal through gp130

on fibroblasts to activate STAT1 and initiate ISG expression. The ability of IRF1 to regulate this signaling pathway is inhibited by IRF2.