Supplementary Data

Figure S1. Characterization of HEK293 reporter cell lines. HEK293 luciferase reporter cell lines were left unstimulated or stimulated with 100 U/ml IFN-γ, 100 U/ml IFN-β, 20 ng/ml TNF-α, or 50 ng/ml IL-4. Cells were lysed 6-20 hours later and luciferase activity was measured and normalized to levels in unstimulated cells. **A.** HEK293 ISRE reporter cell line. Data and standard deviation from one experiment are shown. Unstimulated, IFN-γ, and IFN-β conditions have been repeated four times with similar results. **B.** HEK293 STAT1 reporter cell line. Average luciferase induction from three experiments is shown and error bars represent s.e.m.

Figure S2. CHX treatment prevents the expression of IFN-γ induced IRF1. HFFs on coverslips were pre-treated with 50 μg/ml CHX for one hour and 40 minutes and subsequently stimulated with 100 U/ml IFN-γ for two and a half hours. Cells were also left untreated and/or unstimulated. Cells were fixed, permeabilized, and stained for IRF1 (red) and with Hoechst dye (nucleus, blue).

Figure S3. CHX qPCR data normalized to NFE2L1. RT-qPCR data from Fig. 5A,B are shown normalized to a different control gene, *NFE2L1*.

Figure S4. Infection of cells for ChIP inhibits IRF1 expression and activates phosphorylation of STAT1. Coverslips were placed in 15 cm dishes prior to plating of HFFs for the ChIP assay. HFFs were infected with an RH or RH Δ*rop16* strain for five hours, or left uninfected (UI), with 100 U/ml IFN-γ added for the last hour of infection, or cells were left

unstimulated (US). After fixation, coverslips were removed from the plates and the remaining HFFs were used for ChIP assays (Fig. 2A). Coverslips were then permeablized and stained for IRF1 and phospho-STAT1^{Tyr}. The intensity of IRF1 (A) and phospho-STAT1^{Tyr} (B) nuclear staining was quantified in at least 30 cells, regardless of infection status. Each dot represents one cell and black bars represent average staining. This experiment was performed for all three biological replicates of the ChIP assay with similar results, data shown are from one experiment.

Figure S5. IFNb qPCR data normalized to NFE2L1. RT-qPCR data from one experiment from Fig. 3B are shown normalized to two different control genes, *ACTB* and *NFE2L1*.

Table S1. RT-qPCR primers. Primers were designed to amplify 100-300 bp near the 3' ends of genes, spanning intron-exon boundaries if possible. Primer pair efficiencies were calculated using Real-time PCR Miner (1).

Table S2. STAT1 ChIP-qPCR primers. Primers were designed to amplify 150-180 bp regions of STAT1 binding sites in the promoters of IFN-γ induced genes as well as a negative control region where STAT1 is not known to bind. Published STAT1 ChIP-seq data was used to determine regions of STAT1 binding (2). Primer pair efficiencies were calculated using Real-time PCR Miner (1).

Table S3. Mass spectrometry results for STAT1 IPs. Total number of STAT1 peptides, number of unique STAT1 peptides, and percent coverage of STAT1 detected in mass

spectrometry experiments are shown for each immunoprecipitation experiment, including each sample and each fraction.

References

1. **Zhao S, Fernald RD**. 2005. Comprehensive algorithm for quantitative real-time polymerase chain reaction. Journal of computational biology : a journal of computational molecular cell biology **12**:1047–64.

2. **Robertson G, Hirst M, Bainbridge M, Bilenky M, Zhao Y, Zeng T, Euskirchen G, Bernier B, Varhol R, Delaney A, Thiessen N, Griffith OL, He A, Marra M, Snyder M, Jones S**. 2007. Genome-wide profiles of STAT1 DNA association using chromatin immunoprecipitation and massively parallel sequencing. Nature methods **4**:651–657.

Fig. S1

A

Fold induction luciferase activity

0 1 2 3 4 5 6

ISRE

B

Fold induction luciferase activity

0 1 2 3 4 5 6 7 8 9 10

STAT1

US
IFN-γ
IFN-β
TNF-α
IL-4

Fig. S2

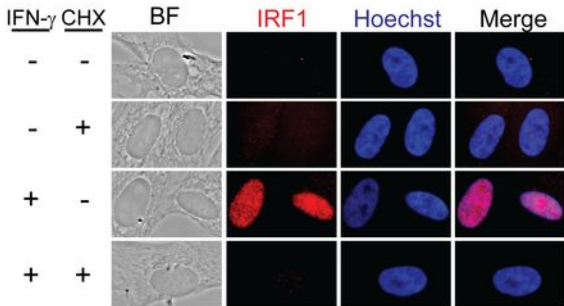

Fig. S3

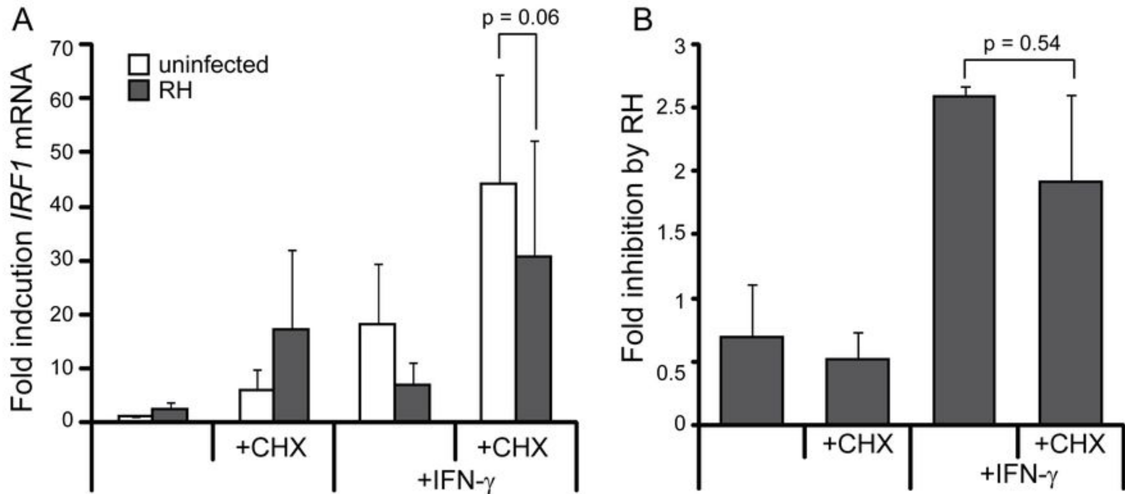

Fig. S4

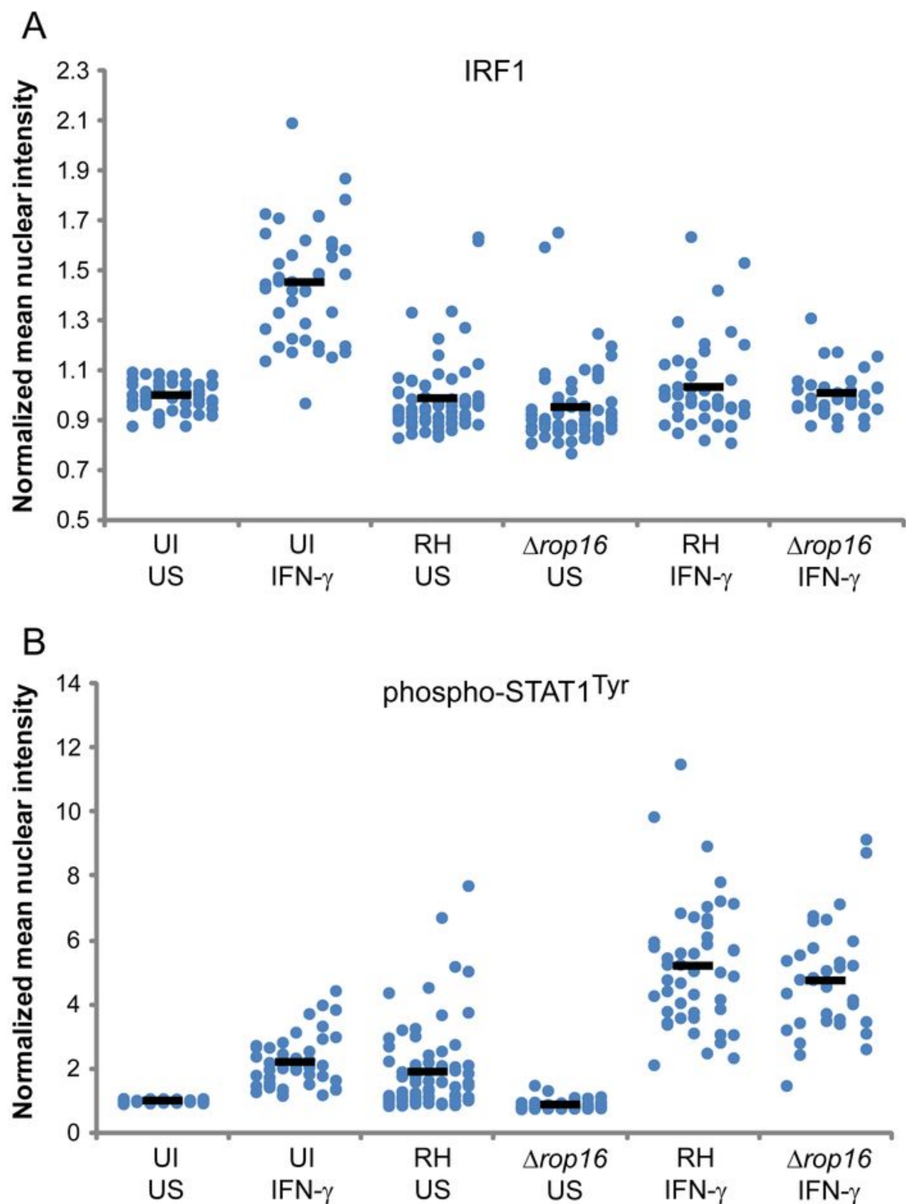

Fig. S5

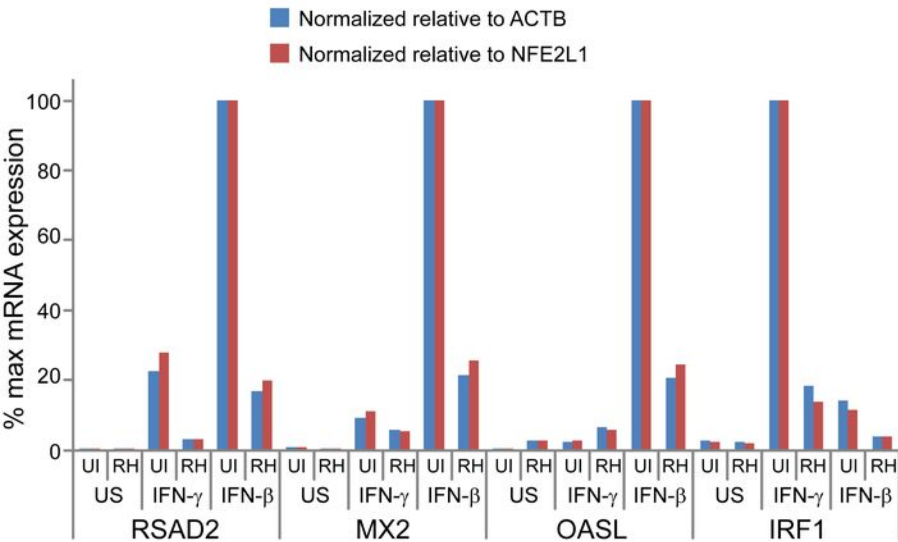

Table S1. RT-qPCR primers

	Forward primer (5' to 3')	Reverse primer (5' to 3')	Calculated Primer Pair Efficiency
IRF1	GGATTCCAGCCCTGATACCT	CCTGCTCCACCTCCAAGTC	100.50%
RSAD2	GAGCGCCACAAAGAAGTGTC	TCCTTCCGTCCCTTTCTACA	104.44%
MX2	GCATCCACCTGAATGCCTAC	GCTTTCTGCAAGGAGTCACC	93.34%
OASL	AGGGTACAGATGGGACATCG	AAGGGTTCACGATGAGGTTG	98.25%
ACTB	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT	95.41%
NFE2L1	AGCAGCTCTGAAGGCAGTTC	AGCTGGATCCTGGTAGCTCA	99.22%

Table S2. ChIP-qPCR primers

	Forward primer (5' to 3')	Reverse primer (5' to 3')	Calculated Primer Pair Efficiency
IRF1	TTGCCTCGACTAAGGAGTGG	TTCGCCGCTAGCTCTACAAC	89.50%
SOCS3	GGAGCAGGGAGTCCAAGTC	GCGCTCAGCCTTTCTCTG	105.46%
GBP1	TGGACAAATTCGTAGAAAGACTCA	GCACAAAAACTGTCCCCAAC	95.73%
CIITA	ACATTTTTGCCCATGAGGTC	CTCACATCCTAAGGGCCAGA	105.44%
CXCL10	AAAGGAACAGTCTGCCCTGA	GCCCTGCTCTCCCATACTTT	106.46%
IDO1	CACAGTCATTGTATTCTCTTTGCTG	GCATATGGCTTTCGTTACAGTC	107.48%
ICAM1	CCCTGTCAGTCCGGAAATAA	CCATAGCGAGGCTGAGGTT	100.73%
CCND2	TCACCCCCTCCCTTATTTTT	ACCCTCCAACTTTGGCTTCT	107.60%

Table S3. Mass spectrometry results for STAT1 IPs

	Strain and MOI	Sample	Fraction	Total STAT1 peptides	Unique STAT1 peptides	% Coverage of STAT1
IP #1	RH MOI~8	Uninfected, US	cytoplasmic	26	21	35
			nuclear extract	0		
			chromatin	0		
		Uninfected + IFNy	cytoplasmic	26	22	36
			nuclear extract	3	3	5
			chromatin	0		
		Infected, US	cytoplasmic	20	16	25
			nuclear extract	14	13	21
			chromatin	18	15	22
		Infected + IFNy	cytoplasmic	16	14	22
			nuclear extract	21	15	28
			chromatin	18	17	28
IP #2	RH MOI~1.5	Uninfected, US	cytoplasmic	81	24	43
			nuclear extract	0		
			chromatin	0		
		Uninfected + IFNy	cytoplasmic	71	29	48
			nuclear extract	14	12	21
			chromatin	6	5	9
		Infected, US	cytoplasmic	48	22	39
			nuclear extract	4	4	6
			chromatin	2	2	4
		Infected + IFNy	cytoplasmic	79	28	42
			nuclear extract	26	15	25
			chromatin	35	25	37
IP #3	RH Δ*rop16* MOI~5	Uninfected, US	cytoplasmic	88	35	59
			nuclear extract	0		
			chromatin	0		
		Uninfected + IFNy	cytoplasmic	90	36	59
			nuclear extract	21	16	28
			chromatin	11	9	15
		Infected, US	cytoplasmic	78	31	51
			nuclear extract	5	5	9
			chromatin	2	2	3
		Infected + IFNy	cytoplasmic	83	38	51
			nuclear extract	43	27	47
			chromatin	43	28	46