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Supplementary Information for

Toxoplasma gondii effector TgIST blocks type I Interferon signaling to promote infection

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This PDF file includes:

Figs. S1 to S7
Tables S1 to S2

Other supplementary materials for this manuscript include the following:

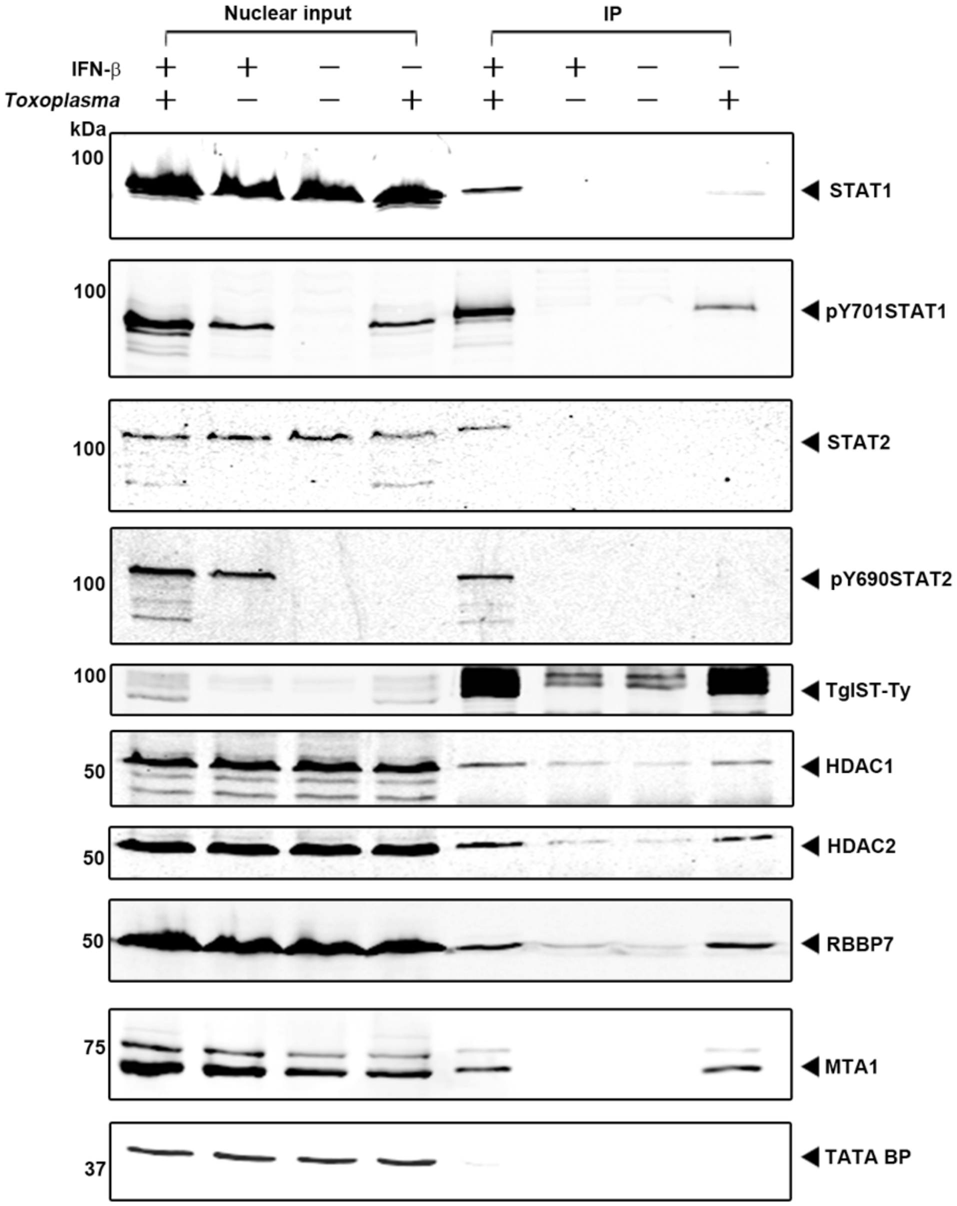
Dataset S1

Expression table of genes expressed at mean RPKM > 1 in at least one of six samples in the RNASeq dataset. (Dataset_S1.csv file).

Dataset S2

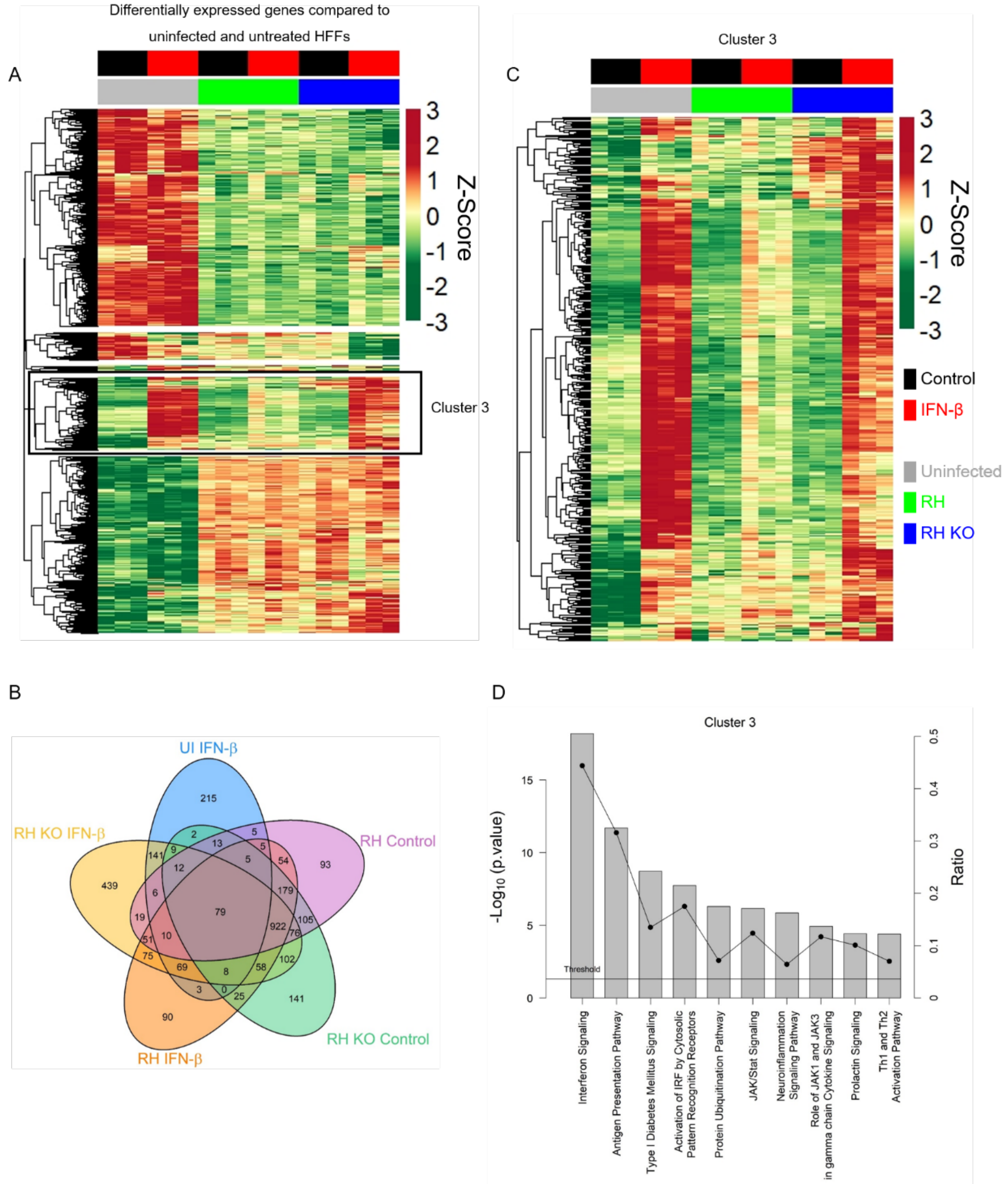
Table of differentially expressed genes with $\mathrm{Log}_2(|\text{Fold Change}|) > 1$ and $-\mathrm{Log}_{10}(P\ \text{Value}) > 1.3$ in all samples compared to untreated and uninfected HFFs. (Dataset_S2.csv file).

Figure S1



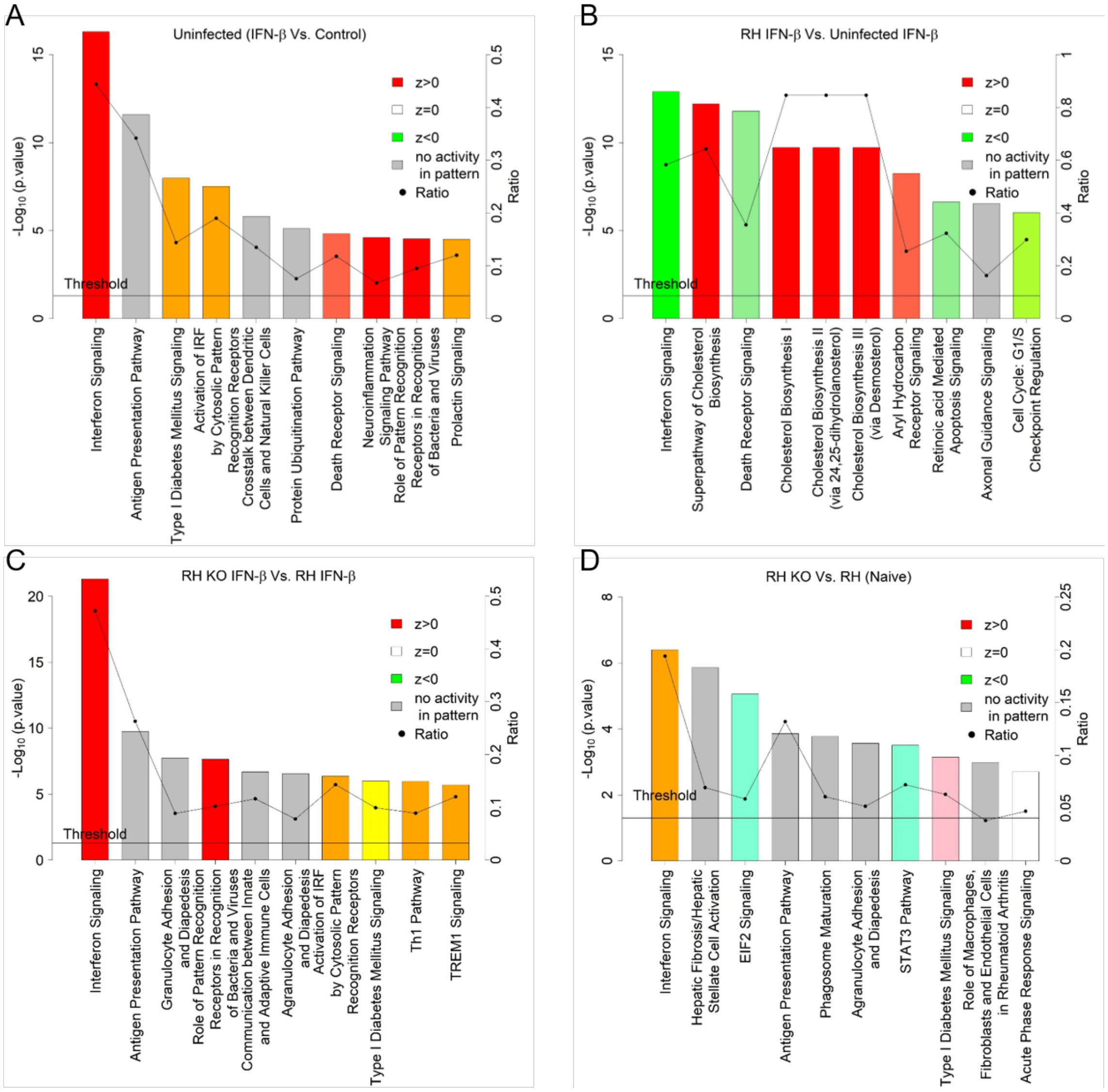
Western blot analysis of IP for TgIST-Ty from nuclear extracts of U3A-STAT1 cells infected with *Toxoplasma gondii* RH strains for 16 h and treated with IFN-β for 1h. Uninfected and untreated cells were used as controls. Samples were separated on 10%-12% SDS-PAGE gels and blotted with primary antibodies listed on the right panel.

Figure S2



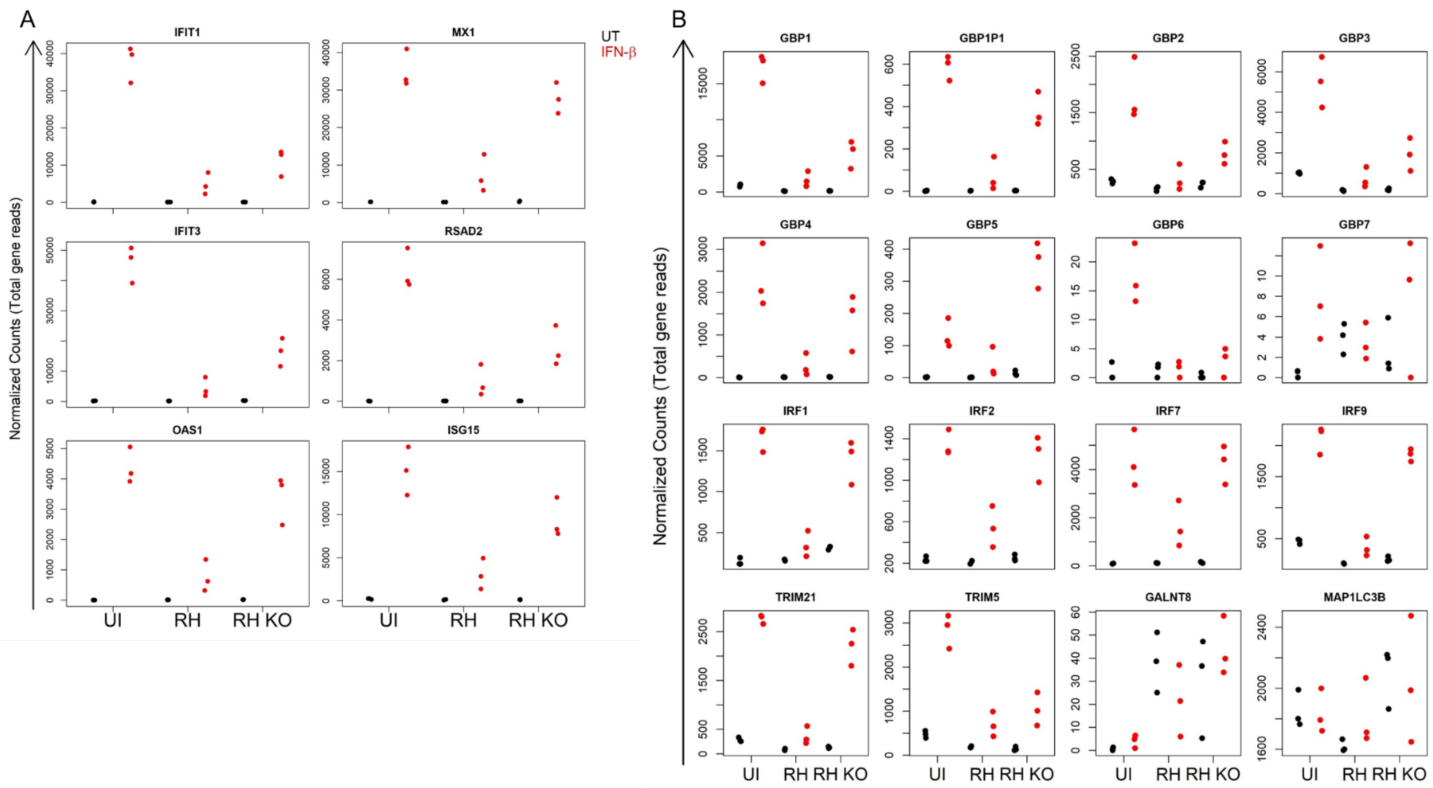
(A) Heatmap of differentially expressed genes in all samples (in triplicates) compared to uninfected control HFFs. The genes were hierarchically clustered using Euclidean distance and complete linkage on normalized Log_2(Fold Change). The genes were divided into 5 clusters 1-5 comprising of 1306, 1063, 437, 36 and 169 genes respectively. (B) Venn diagram of differentially expressed genes in different samples compared to uninfected control HFFs. (C) Expanded cluster 3 showing normalized expression of genes induced by IFN-β in uninfected control and TgIST knockout infected HFFs, whereas, wild type RH infection repressed their expression (D) Canonical pathway analysis using IPA show interferon signaling as the top significantly enriched canonical pathway for genes in cluster 3. The plotted data show level of significance (- Log_{10}(p.value), bars) calculated using Fisher’s exact test and ratio (fraction of differentially expressed genes in a pathway, black points with connected line) of the top 10 enriched pathways.

Figure S3



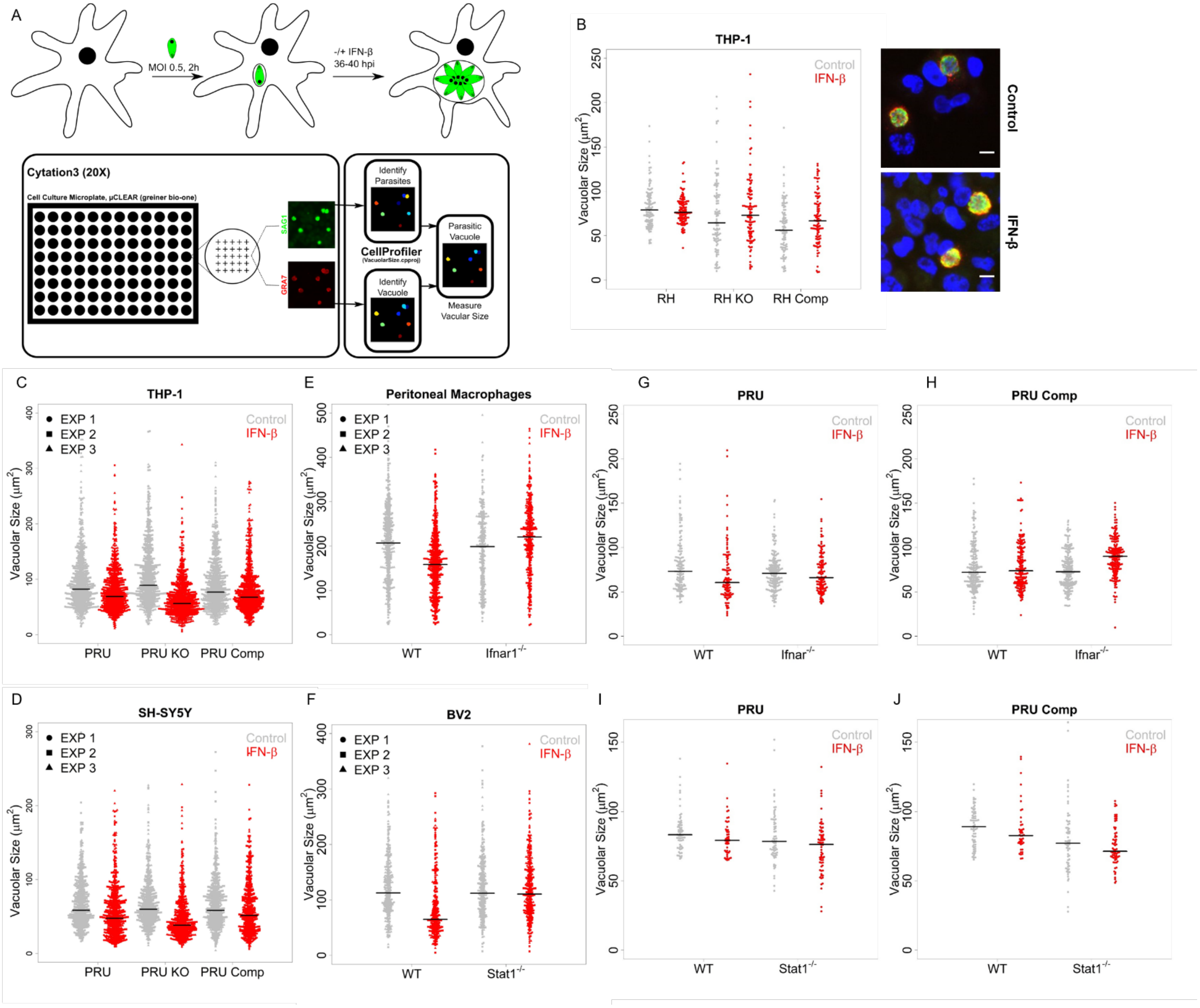
(A-C) Canonical pathway analysis of differentially expressed genes in Fig. 3A-3C using IPA. Bar color represents the z-score (red for positive to green for negative) or the statistical measure of the match between expected and observed gene expression relationship direction for that pathway. Grey bars represent ineligibility of IPA to predict any relationship direction. (D) Canonical pathway analysis of differentially expressed transcripts in naïve HFFs infected with TgIST knockout RH compared to wild type RH.

Figure S4



Normalized gene expression (Total gene reads) of (A) representative genes for cellular type I IFN activation and (B) genes involved in cellular defense against pathogens in the RNASeq dataset. Most of the genes that show upregulation by IFN-β stimulation of uninfected HFFs also showed increased expression in HFFs infected with TgIST knockout RH compared to wild type RH parasites.

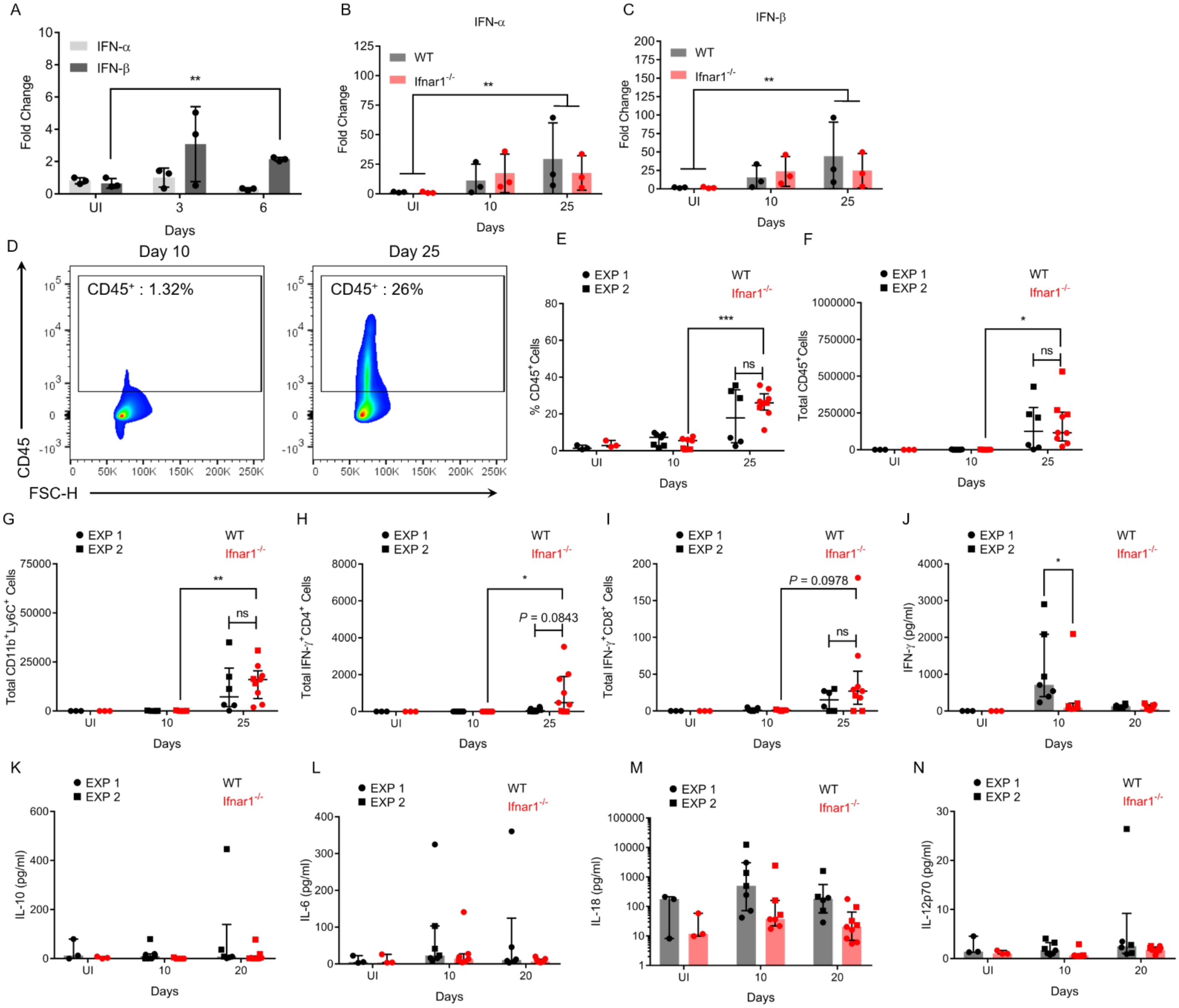
Figure S5



(A) Pipeline used for automated plate-based image acquisition in Cytation3 Multi-Mode plate-based imager. Raw TIFF files in different emission channels were acquired and imported into CellProfiler 2.1.1 (Downloaded from http://cellprofiler.org/releases/). Images were analyzed using custom designed CellProfiler project template. (B) Vacuolar growth of RHWT, RH KO and RH Comp parasites in PMA differentiated THP-1 cells after 36 h of infection. Scatter plot for vacuolar growth of type II wild type parasites (PRU), ΔTgist knockout (PRU KO) or complemented lines (PRU Comp) after 40 h of infection in (C)

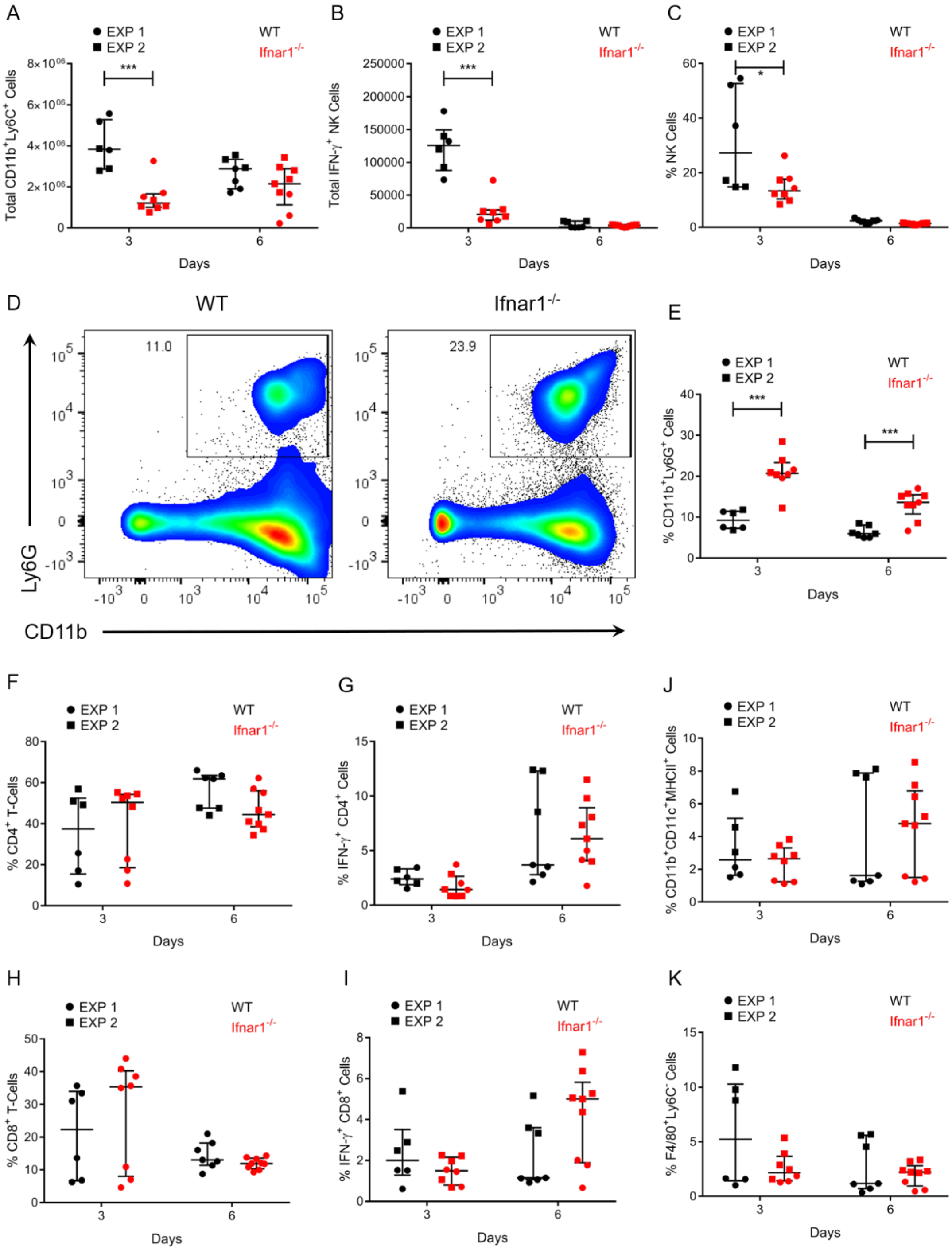
Differentiated THP-1 human macrophages and (D) SH-SY5Y human neuroblastoma cells. ∆Tgist knockout (PRU KO) infected (E) wild type (WT) and Ifnar1$^{-/-}$ peritoneal macrophages and (F) mouse microglial wild type (WT) and Stat1$^{-/-}$ BV2 cells. Vacuolar growth of PRU WT and PRU Comp after 40 h of infection in peritoneal macrophages from wild type and Ifnar1$^{-/-}$ mice (G,H) or wild type and Stat1$^{-/-}$ BV2 cells (I, J). Each dot represents the mean vacuolar size of parasitophorous vacuole (µm^2) of an image with black line representing the median within an experiment. The cells were either treated with TNF-α alone (Control, 10 ng/ml) or in combination with IFN-β (100 Units/ml).

Figure S6



(A) Real time PCR showing mRNA expression of IFN-α and IFN-β in peritoneal cells of uninfected mice (UI) or mice infected with 200 tachyzoites of ME49 at 3 days and 6 days post-infection. mRNA expression is plotted as mean fold difference ± S.D. compared to uninfected controls. Each dot represents single mouse from each group. ** represent significant difference at $P < 0.01$ between compared samples using unpaired two-tailed Student's *t*-test. (B-C) Real time PCR showing mRNA expression of (B) IFN-α and (C) IFN-β in brain tissues of uninfected mice or ME49 (5 cyst) infected wild type and Ifnar1-/- mice at 10 days and 25 days post-infection. mRNA expression is plotted as mean fold difference ± S.D. compared to uninfected wild type control mice. ** represents significant difference at $P < 0.01$ between uninfected and ME49 infected mice (wild type and Ifnar1$^{-/-}$ combined) at 25 days post-infection using non-parametric Kruskal-Wallis test. (D) Representative plot showing live CD45^{+} mononuclear cells in the brain of Ifnar1$^{-/-}$ mice at 10 days and 25 days post-infection with ME49 cyst. (E) Percentages and (F) total numbers of CD45+ cells in brain of uninfected and ME49 infected wild type and Ifnar1$^{-/-}$ mice. Total numbers of (G) CD11b^{+}Ly6C^{+} cells, (H) IFN-γ^{+} CD4^{+} T-cells and (I) IFN-γ^{+} CD8^{+} T-cells in brain of uninfected or ME49 infected wild type and Ifnar1$^{-/-}$ mice at 10 days and 25 days post-infection. (J-N) Serum levels of IFN-γ, IL-10, IL-6, IL-18 and IL-12p70 in uninfected controls and ME49 infected wild type and Ifnar1$^{-/-}$ mice. (E-N) shows cumulative data from 2 independent biological replicates plotted as median ± I.Q.R. with * and *** representing significant difference at $P < 0.05$ and $P < 0.01$ between compared groups using Ordinary One-Way ANOVA.

Figure S7



Flow cytometric measurements of different cells in wild type and Ifnar1$^{-/-}$ mice at day 3 and 6 post infection with 10^5 PRU KO parasites. Total number of (A) CD11b$^+$Ly6C$^+$ monocytes and (B) IFN-γ$^+$ NK cells in the peritoneal exudate. (C) Percentage of total NK cells (NK1.1$^+$NKp46$^+$). (D) Representative flow plot at day

3 post infection and (E) percentage of neutrophils ($CD11b^+Ly6G^+$). Percentage of (F) total $CD4^+$ T-Cells, (G) IFN-γ^+ $CD4^+$ T-Cells, (H) total $CD8^+$ T-cells, (I) IFN-γ^+ $CD8^+$ T-Cells, (J) dendritic cells ($CD11b^+CD11c^+MHCII^+$) and (K) resident macrophages ($F4/80^+Ly6C^-$). Cumulative data from 2 independent experiments is shown in C-I. Quantitative data is plotted as median ± I.Q.R. * and *** represents significant difference at $P < 0.05$ and $P < 0.005$ respectively between compared groups using multiple Student's *t*-test with Holm-Sidak correction.

Table S1

List of genes associated with activated STAT1 signaling and differentially expressed in IFN-β stimulated HFFs infected with TgIST knockout RH compared to wild type.

ID	Genes in dataset	Prediction (based on measurement direction)	Expr Log Ratio	Findings
ENSG00000132530	XAF1	Activated	1.533	Upregulates (1)
ENSG00000140105	WARS	Activated	2.265	Upregulates (2)
ENSG00000184979	USP18	Activated	1.913	Upregulates (1)
ENSG00000213886	UBD	Activated	3.128	Upregulates (1)
ENSG00000132109	TRIM21	Activated	2.576	Upregulates (1)
ENSG00000135148	TRAFD1	Activated	1.478	Upregulates (1)
ENSG00000168394	TAP1	Activated	2.338	Upregulates (14)
ENSG00000170581	STAT2	Activated	1.596	Upregulates (2)
ENSG00000115415	STAT1	Activated	1.121	Upregulates (74)
ENSG00000135899	SP110	Activated	1.496	Upregulates (1)
ENSG00000184557	SOCS3	Activated	3.537	Upregulates (13)
ENSG00000185338	SOCS1	Activated	1.252	Upregulates (14)
ENSG00000101347	SAMHD1	Activated	1.627	Upregulates (1)
ENSG00000177409	SAMD9L	Activated	1.414	Upregulates (1)
ENSG00000134321	RSAD2	Activated	1.416	Upregulates (1)
ENSG00000173821	RNF213	Activated	1.615	Upregulates (1)
ENSG00000240065	PSMB9	Activated	2.358	Upregulates (17)
ENSG00000204264	PSMB8	Activated	2.057	Upregulates (2)
ENSG00000205220	PSMB10	Activated	1.457	Upregulates (1)
ENSG00000141682	PMAIP1	Activated	1.787	Upregulates (1)
ENSG00000135114	OASL	Activated	1.743	Upregulates (1)
ENSG00000089127	OAS1	Activated	2.134	Upregulates (3)
ENSG00000140853	NLRC5	Activated	2.336	Upregulates (1)
ENSG00000185499	MUC1	Activated	1.79	Upregulates (1)
ENSG00000187608	ISG15	Activated	1.557	Upregulates (5)
ENSG00000213928	IRF9	Activated	2.317	Upregulates (11)
ENSG00000185507	IRF7	Activated	1.293	Upregulates (8)
ENSG00000168310	IRF2	Activated	1.227	Upregulates (1)
ENSG00000125347	IRF1	Activated	1.933	Upregulates (57)
ENSG00000115594	IL1R1	Activated	1.224	Upregulates (2)
ENSG00000134470	IL15RA	Activated	1.355	Upregulates (1)
ENSG00000142089	IFITM3	Activated	1.216	Upregulates (1)
ENSG00000185201	IFITM2	Activated	1.087	Upregulates (2)
ENSG00000185885	IFITM1	Activated	2.209	Upregulates (6)
ENSG00000119917	IFIT3	Activated	1.852	Upregulates (5)
ENSG00000185745	IFIT1	Activated	1.122	Upregulates (4)
ENSG00000115267	IFIH1	Activated	2.012	Upregulates (1)
ENSG00000126709	IFI6	Activated	2.04	Upregulates (4)
ENSG00000068079	IFI35	Activated	2.279	Upregulates (2)
ENSG00000216490	IFI30	Activated	2.015	Upregulates (1)
ENSG00000090339	ICAM1	Activated	1.615	Upregulates (19)

ENSG00000204592	HLA-E	Activated	1.445	Upregulates (1)
ENSG00000138642	HERC6	Activated	1.799	Upregulates (1)
ENSG00000140511	HAPLN3	Activated	2.384	Upregulates (1)
ENSG00000162654	GBP4	Activated	2.2	Upregulates (1)
ENSG00000117226	GBP3	Activated	1.346	Upregulates (3)
ENSG00000162645	GBP2	Activated	1.206	Upregulates (11)
ENSG00000225492	GBP1P1	Activated	2.39	Upregulates (1)
ENSG00000117228	GBP1	Activated	1.581	Upregulates (3)
ENSG00000138685	FGF2	Activated	-1.003	Downregulates (3)
ENSG00000137628	DDX60	Activated	1.595	Upregulates (1)
ENSG00000169245	CXCL10	Activated	3.275	Upregulates (35)
ENSG00000163131	CTSS	Activated	1.794	Upregulates (1)
ENSG00000134326	CMPK2	Activated	2.579	Upregulates (1)
ENSG00000101017	CD40	Activated	1.193	Upregulates (28)
ENSG00000120217	CD274	Activated	1.417	Upregulates (8)
ENSG00000137752	CASP1	Activated	1.191	Upregulates (6)
ENSG00000125730	C3	Activated	1.274	Upregulates (2)
ENSG00000113916	BCL6	Activated	1.646	Upregulates (1)
ENSG00000168062	BATF2	Activated	3.099	Upregulates (2)
ENSG00000221963	APOL6	Activated	2.623	Upregulates (1)
ENSG00000239713	APOBEC3G	Activated	1.239	Upregulates (1)
ENSG00000175899	A2M	Activated	1.225	Upregulates (2)
ENSG00000169429	CXCL8	Inhibited	-2.353	Upregulates (4)
ENSG00000164400	CSF2	Inhibited	-1.537	Upregulates (1)
ENSG00000115009	CCL20	Inhibited	-1.083	Upregulates (2)
ENSG00000135744	AGT	Affected	1.389	Regulates (3)
ENSG00000089685	BIRC5	Affected	-1.243	Regulates (1)
ENSG00000137496	IL18BP	Affected	1.266	Regulates (1)
ENSG00000157601	MX1	Affected	1.845	Regulates (1)
ENSG00000111335	OAS2	Affected	2.381	Regulates (1)
ENSG00000138496	PARP9	Affected	1.579	Regulates (1)
ENSG00000137193	PIM1	Affected	2.002	Regulates (1)
ENSG00000164342	TLR3	Affected	1.277	Regulates (1)
ENSG00000102524	TNFSF13B	Affected	1.832	Regulates (1)
ENSG00000132274	TRIM22	Affected	1.596	Regulates (1)
ENSG00000025708	TYMP	Affected	1.231	Regulates (1)

Table S2

List of transcripts associated with activated STAT1 signaling and differentially expressed in naïve HFFs infected with TgIST knockout RH compared to wild type.

ID	Genes in dataset	Prediction (based on measurement direction)	Expr Log Ratio	Findings
ENSG00000140105	WARS	Activated	1.772	Upregulates (2)
ENSG00000132109	TRIM21	Activated	1.339	Upregulates (1)
ENSG00000168394	TAP1	Activated	1.341	Upregulates (14)
ENSG00000184557	SOCS3	Activated	1.8	Upregulates (13)
ENSG00000159216	RUNX1	Activated	1.279	Upregulates (1)
ENSG00000240065	PSMB9	Activated	1.213	Upregulates (17)
ENSG00000204264	PSMB8	Activated	1.002	Upregulates (2)
ENSG00000185499	MUC1	Activated	1.42	Upregulates (1)
ENSG00000125347	IRF1	Activated	1.246	Upregulates (57)
ENSG00000115594	IL1R1	Activated	2.449	Upregulates (2)
ENSG00000185885	IFITM1	Activated	1.458	Upregulates (6)
ENSG00000068079	IFI35	Activated	1.058	Upregulates (2)
ENSG00000090339	ICAM1	Activated	1.282	Upregulates (19)
ENSG00000140511	HAPLN3	Activated	1.591	Upregulates (1)
ENSG00000026103	FAS	Activated	1.538	Upregulates (6)
ENSG00000113916	BCL6	Activated	1.169	Upregulates (1)
ENSG00000221963	APOL6	Activated	1.922	Upregulates (1)
ENSG00000100985	MMP9	Inhibited	1.306	Downregulates (5)
ENSG00000138685	FGF2	Inhibited	2.054	Downregulates (3)
ENSG00000169429	CXCL8	Inhibited	-2.197	Upregulates (4)
ENSG00000182326	C1S	Inhibited	-1.426	Upregulates (1)
ENSG00000157601	MX1	Affected	1.787	Regulates (1)

Table S3

List of real time PCR primers

Gene	Forward (5' → 3')	Reverse (5' → 3')
IFIT1	ATGTATTACCACATGGGCAGACTGG	CATCCTTCCTCACAGTCTATTTCTGG
MX1	TCGGGCAAGAGCTCCGTGTTG	CAAGTTTCTTCAGTTTCAGCACCAGC
IFIT3	CAAGGAAGACAGTGTCTCAAGGGATC	ACTCTTCAGCTTGCCGTAAGCATTCC
RSAD2	GTGAGGTTCTGCAAAGTAGAGTTGC	TTCCTCGTCAAAGCTGTCACAGG
OAS1	TGCTTCCGAGGTAGCTCCTACC	ACTGAGGAAGACAACCAGGTCAGC
ISG15	ACTCATCTTTGCCAGTACAGG	CAGCTCTGACACCGACATG
YWHAZ	ACTACCGTTACTTGGCTGAGGTTGC	CCAGTCTGATAGGATGTGTTGGTTGC
mIFN-α	CTTCCACAGGATCACTGTGTACCT	TTCTGCTCTGACCACCTCCC
mIFN-β	CGAGCAGAGATCTTCAGGAAC	TCACTACCAGTCCCAGAGTC
mActin	ACCTTCTACAATGAGCTGCG	CTGGATGGCTACGTACATGG