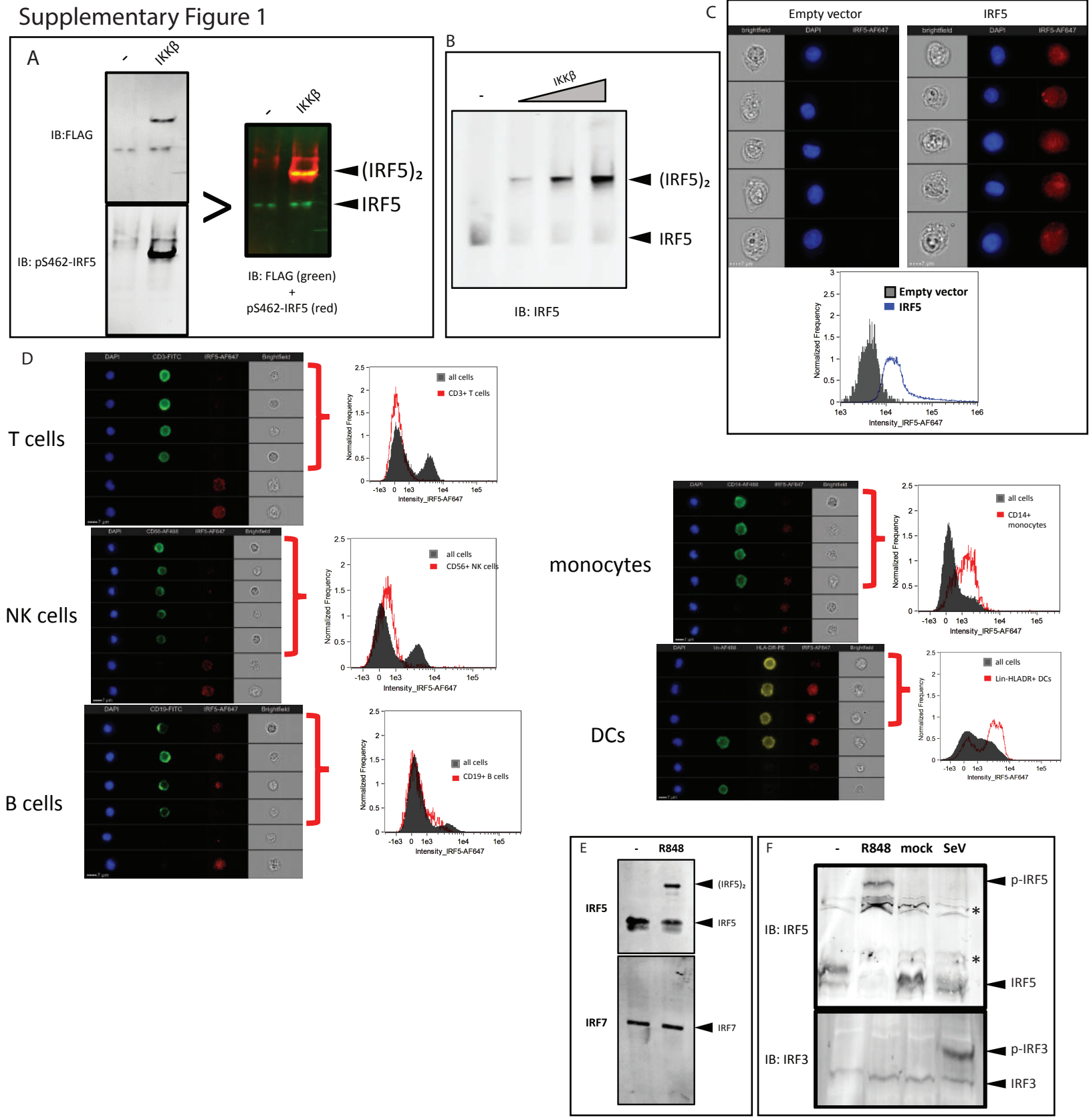

Figure S1. IRF5 activation in different cell types.
(a) Native PAGE immunoblot analysis of FLAG (IRF5) and phospho-IRF5 in HEK293T cells overexpressing IRF5 alone or IRF5+IKKβ. **(b)** Native PAGE immunoblot analysis of IRF5 in HEK293T cells overexpressing IRF5 and increasing amount of IKKβ. All data are representative of at least three independent experiments. **(c)** ImageStream analysis of HEK293T cells overexpressing empty vector control (left) or IRF5 (right). Brightfield, DAPI (blue), and IRF5-AF647 (red) channels are shown for 5 representative images. Histogram showing fluorescence intensity of IRF5-AF647 channel for empty vector control (grey solid) and IRF5 (blue line) is shown on the far right. All data are representative of at least two independent experiments. **(d)** ImageStream analysis of PBMCs labeled with CD3-FITC (T cells), CD56-AF488 (NK cells), CD19-FITC (B cells), CD14-AF488 (monocytes), or FITC-conjugated lineage panel together with HLA-DR-PE (DCs). DAPI (blue), marker (green), IRF5-AF647 (red) and brightfield channels are shown for representative images. Histograms showing fluorescence intensity of IRF5-AF647 channel for total cell population (grey solid) and cells gated on the indicated cell surface marker (red brackets indicate gated marker+ cells, corresponding to red line in histogram) is shown on the far right. **(e)** Native PAGE immunoblot analysis of IRF5 (top), or IRF7 (bottom) in CAL-1 cells treated with 1ug/ml R848 for 2 hours. **(f)** Phos-Tag immunoblot analysis of IRF5 (top) or IRF3 (bottom) CAL-1 cells untreated (-), treated with 1ug/ml R848 for 6 hours (R848), mock-infected (mock), or infected with SeV at 100 HAU/ml for 12 hours. Non-specific bands are marked with *. Immunoblot experiments in (a-c, e-f) have been performed at least 3 times. ImageStream experiment in (d) has been performed once with 3 donors.

Supplementary Figure 2

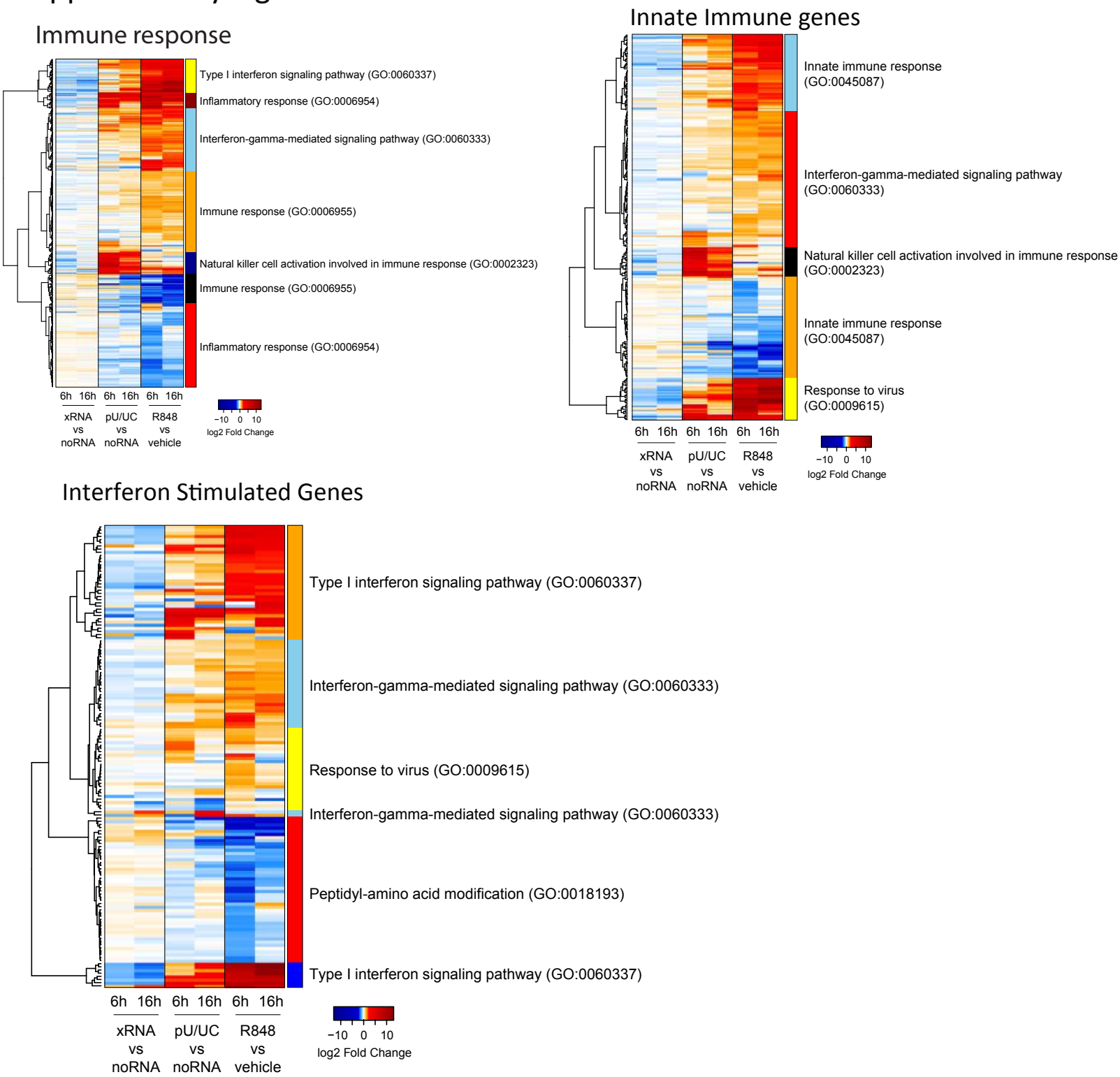

Figure S2. RLR/IRF3 and TLR7/IRF5 regulate different gene sets.
Gene expression profiles of genes in the "immune response", "innate immune genes", and "interferon stimulated genes" GO categories that are DE genes in in at least one comparison (R848/vehicle-6hrs, R848/vehicle-16hrs, pU/UC/xRNA-6hrs, and pU/UC/xRNA-16hrs), visualized by log2 fold change over control sample. RNAseq experiment was performed once with biological triplicates.

Supplementary Figure 3

Top 20 GO terms enriched in R848 vs vehicle treatment

GO term	Description	p-value	Genes
0060333	IFNg-mediated signaling pathway	4.51E-36	HLA-DQB1, HLA-DQB2, HLA-DRB1, PML, OAS3, OAS1, OAS2, B2M, VCAM1, TRIM5, CD44, HLA-DRB5, HLA-DPB1, CIITA, ICAM1, HLA-H, SP100, HCK, HLA-A, TRIM26, HLA-C, HLA-B, STAT1, HLA-E, TRIM22, HLA-DQA2, HLA-DQA1, TRIM21, HLA-G, HLA-F, IRF9, TRIM38, OASL, TRIM34, TRIM31, MT2A, IRF2, HLA-DPA1, JAK2, GBP2, GBP1, HLA-DRA
0006955	immune response	8.63E-35	IL16, IL18, SUSD2, TNFSF15, TNFSF14, TNFSF13, CLNK, TNFSF12, CXCL11, HLA-DMA, B2M, CXCL10, SEMA7A, IL4R, IL1B, FAS, LTA, CIITA, HLA-H, EXOSC9, IL18RAP, PTGER4, HLA-A, CCL4L1, HLA-C, CCL4L2, HLA-B, CD40, HLA-E, HLA-DQA2, HLA-DQA1, HLA-F, SERPINB9, TNFRSF9, LILRB2, CD86, CCR7, CCR6, CD36, TNFSF13B, HLA-DPA1, MAP3K14, GBP2, HLA-DRA, HLA-DQB1, HLA-DQB2, GPR183, CCL3, CCL2, HLA-DRB1, ENPP1, C3, ENPP2, IFITM3, ENPP3, CCR1, OAS3, CXCL9, TNFRSF8, CD70, OAS1, IL32, OAS2, CCL5, IL7R, CCL4, CHIT1, FTH1, TNFRSF1A, CCL22, HLA-DRB5, IL2RG, HLA-DPB1, HLA-DOA, HLA-DOB, IL18R1, TCF7, IL6, IL2RA, TNFSF4, IL7, SMAD3, TNFSF9, TRIM22, AIM2, IKBKE, TNFSF10, RGS1, CD274, IFI6, BMP6
0051607	defense response to virus	4.63E-28	IFI44L, APOBEC3G, PMAIP1, ISG20, APOBEC3D, CXCL10, APOBEC3B, TRIM5, APOBEC3A, NLRC5, ISG15, DDX60, DNAJC3, MX1, MX2, HERC5, IFI16, CD40, LILRB1, CD86, IFNB1, TRIM34, LYST, IL12B, EIF2AK2, GBP1, IFITM1, IFITM3, CXCL9, PML, OAS3, OAS1, OAS2, IL23A, ITGAX, C19ORF66, ZC3H12A, DHX58, PTPRC, IL6, STAT1, TRIM22, STAT2, IFIT3, IRF9, IFIT2, TRIM56, PLSCR1, IFIT1, OASL, IFIT5, ADAR
0006954	inflammatory response	2.28E-25	ELF3, IL18, NFKB1, NFKB2, CXCL11, MMP25, CXCL10, CCRL2, S1PR3, PTGIR, MYD88, TNFRSF11A, CCL3L1, SEMA7A, CCL3L3, TICAM2, IL1B, MGLL, FAS, ADAM8, TNIP1, TNIP3, CIITA, PIK3CG, NFKBIZ, SP100, IL18RAP, NLRP4, C4A, LYN, C4B, IL27, RELB, CHST2, CCL4L1, CCL4L2, IFI16, CD40, SIGLEC1, TNFRSF9, CCR7, RIPK2, NFE2L2, TNFAIP3, AOC3, CCL1, C3AR1, CCL3, CCL2, C3, CCR1, CXCL9, TNFRSF8, GPR68, CCL5, CCL4, TNFRSF1A, CCL22, IL23A, REL, ZC3H12A, HAVCR2, IL6, IL2RA, HCK, CYP4F11, AIM2, LGALS9, APOL3, CLEC7A, PLA2G4C, IGFBP4, F2R, BMP6
0060337	type I IFN signaling pathway	1.93E-24	IFITM1, IFITM3, OAS3, OAS1, OAS2, IFI35, ISG20, ISG15, XAF1, MX1, MX2, HLA-H, SP100, HLA-A, HLA-C, HLA-B, STAT1, HLA-E, HLA-G, HLA-F, STAT2, IFIT3, IRF9, IFIT2, OASL, IFIT1, IFI27, IFNB1, IRF2, GBP2, IFI6, ADAR
0009615	response to virus	3.65E-18	IFIH1, IFITM1, IFITM3, TBX21, OAS3, OAS1, OAS2, CCL5, CCL4, SRC, ISG20, CCL22, MYD88, DDX60, BCL3, MX1, MX2, DHX58, TNFSF4, CCL4L1, IFI44, PIM2, FOXP3, TRIM22, LILRB1, DDX58, IFIT3, IFIT2, IFIT1, OASL, IFNB1, GTF2F1, EIF2AK2, ADAR
0045071	negative regulation of viral genome replication	2.84E-15	IFITM1, IFITM3, OAS3, APOBEC3G, OAS1, IFI16, PARP10, CCL5, ISG20, PLSCR1, APOBEC3A, OASL, IFIT1, ISG15, IFNB1, C19ORF66, EIF2AK2, MX1, TNIP1, ADAR
0007165	signal transduction	2.39E-13	TNFSF15, TNFSF14, TNFSF13, TNFSF12, CXCL11, TNFSF18, CXCL10, CD48, MYD88, LILRA3, ANK3, PDE4B, CSF3R, IL15RA, IL1B, RAPGEF3, FAS, CHRNA6, MX1, RAPGEF1, MAP2K6, PKIG, CCL4L1, RPS6KC1, OPTN, STK4, TANK, LILRB1, VEGFC, LILRB2, CD38, CCR6, RIPK2, ASB2, EXT1, CD226, TRAF1, CCL1, CCL2, LITAF, ERBB3, CD70, SP110, CCL4, SRC, ALCAM, TAGAP, LGALS3BP, CD69, HLA-DOA, HLA-DOB, TRAF4, CD300C, VAV2, SLAMF1, P2RX5, APOL3, TNFSF10, RGS1, PDE7A, CD274, HIVEP2, RIT1, ARHGAP10, NAMPT, SORL1, NFKB1, SDC4, ARHGAP22, TNFRSF11A, EVI2A, IL4R, CSF2RB, CNTNAP1, NRG2, LTA, LYN, CLIC2, ARHGAP24, CD83, TNFSF13B, TXN, RIN2, INPP4B, INPP4A, ICOSLG, C3, PPP1R12B, CREM, CXCL9, TNFRSF8, SFN, IL7R, TNFRSF1A, CCL22, LPXN, DAPP1, IL2RG, TRIP10, RASA4, INPP5B, IL18R1, TNFSF4, IGF1, TNFSF9, RASSF3, RASSF4, PNOC, CHN1, JAK2, IGFBP4, IGFBP5, BCAR3
0045087	innate immune response	1.78E-11	NFKB1, APOBEC3G, NFKB2, APOBEC3D, B2M, APOBEC3B, APOBEC3A, TRIM5, NLRC5, NOD2, MYD88, DDX60, LILRA5, TICAM2, CLEC4A, MX1, MX2, AKIRIN2, PIK3CG, C4A, LYN, NCF2, C4B, IL27, RELB, HERC5, HLA-C, SERPING1, HLA-B, IFI16, HLA-E, TRIM31, RIPK2, PRDM1, EIF2AK2, FRK, IFIH1, PML, SRC, CYLD, IL23A, REL, DHX58, HAVCR2, LGALS3, TRIM26, SLAMF1, TRIM21, AIM2, DDX58, APOL1, IFIT5, JAK2, CLEC7A, JAK3, ADAR
0002504	antigen processing and presentation of peptide/polysaccharide antigen via MHC class II	2.42E-11	HLA-DQB1, HLA-DQB2, HLA-DRB1, HLA-DRB5, HLA-DPA1, HLA-DPB1, HLA-DOA, HLA-DMA, HLA-DQA2, HLA-DOB, HLA-DQA1, HLA-DRA
0071222	cellular response to LPS	7.95E-11	HAVCR2, ICAM1, IL6, CCL2, TNFSF4, LITAF, STAP1, NFKB1, CD40, ABCA1, SRC, B2M, CXCL10, LILRB1, LILRB2, CD86, CD36, CD80, PDE4B, TICAM2, TFPI, ZC3H12A, MAPK8, IL12B, TNFAIP3, TNIP3
0043123	positive regulation of IkB kinase/NFkB signaling	1.00E-10	LITAF, AKAP13, LPAR1, TNFRSF1A, TRIM5, NOD2, MYD88, REL, HMOX1, TICAM2, CFLAR, PELI1, PIM2, CD40, BIRC3, TRIM22, BIRC2, S100A13, LGALS9, TRAF3IP2, IKBKE, CASP10, TRIM38, APOL3, CCR7, TNFSF10, CD36, RIPK1, RIPK2, MAP3K14, F2R
0043547	positive regulation of GTPase activity	2.15E-10	DENND5A, NRG4, RP2, RASGEF1B, RAB3IP, RGL1, ARHGAP22, PTGIR, S1PR1, CCL3L1, CCL3L3, CSF2RB, RAPGEF3, DOCK10, NRG2, RAPGEF1, ICAM1, ARHGEF3, CCL4L1, ARHGEF17, CCL4L2, CD40, ARHGAP24, CDKL5, SEC61B, JUN, RIN2, WNT11, CCL1, FGD2, CCL3, CCL2, ERBB3, AGFG2, CYTH1, AKAP13, CCL5, CCL4, ADAP1, RGS10, TAGAP, CCL22, RASGRP3, PLEKHG6, IL2RG, TRIP10, RASA4, INPP5B, TBC1D3, IL2RA, MCF2, S100A10, RGS18, VAV2, RGS13, RGS1, ADAP2, RGS3, CHN1, JAK2, DENND4A, JAK3, ARHGAP10, BCAR3
0006915	apoptotic process	6.61E-10	IER3, SEPT4, CADM1, TNFSF14, TP63, NFKB1, PMAIP1, TNFSF12, MYD88, CDKN2A, CASP7, MAP3K8, IL1B, FAS, MX1, LTA, MAP2K6, SGK1, BCL2L14, PIM1, PIM2, STK4, BCL2L11, ELMO2, SERPINB9, TNFRSF9, CLIC4, RIPK1, RIPK2, GADD45B, TNFAIP3, PPP1R15A, CTSH, TRAF1, LITAF, PML, NFKBIA, RFFL, ZFP36L1, PEA15, PRUNE2, TRIM69, TCTN3, ZC3H12A, XAF1, BMF, TRAF4, PHLDA1, CFLAR, IL2RA, CARD6, TNFSF9, BIRC3, STAT1, S100A14, AIM2, NOTCH2, CASP10, PLSCR1, TNFSF10, PLK3, CSRNP1, JAK2
0032729	positive regulation of IFNg production	1.56E-09	HAVCR2, IL18R1, TNFSF4, IL18, HLA-A, IL23A, PDE4B, TICAM2, IL1B, RIPK2, BCL3, HLA-DPA1, HLA-DPB1, IL12B, CD226, LTA
0031295	T cell costimulation	2.67E-09	HLA-DQB1, HLA-DQB2, LYN, HLA-DRB1, TNFSF14, HLA-DQA2, HLA-DQA1, SRC, CD86, TNFSF13B, CD80, CD274, MAP3K8, HLA-DRB5, HLA-DPA1, HLA-DPB1, MAP3K14, CD5, ICOSLG, HLA-DRA
0019882	antigen processing and presentation	2.93E-09	HLA-DQB1, HLA-H, RAB8B, HLA-DRB1, RELB, HLA-A, HLA-C, HLA-B, HLA-E, HLA-DQA2, HLA-G, HLA-DQA1, HLA-DRB5, HLA-DPA1, HLA-DPB1, CTSH, HLA-DRA
0043065	positive regulation of apoptotic process	1.05E-08	BID, FGD2, SEPT4, CYP1B1, TGFB3, AKAP13, TNFRSF8, OSGIN1, BCL2L1, PMAIP1, LPAR1, SRC, CDKN2A, HMOX1, FAS, BMF, ARL6IP5, LTA, MAP2K6, ARHGEF3, IL6, MCF2, BCL2A1, ARHGEF17, VAV2, ST20, STK4, SLIT2, BCL2L11, LILRB1, IFIT2, TNFSF10, RIPK1, MLLT11, MNDA, RIPK2, MAPK8, WNT11, BIN1, GADD45B
0032496	response to LPS	1.42E-08	DUSP10, CXCL9, TNFRSF8, CNP, NFKB2, CXCL11, CXCL10, TRIB1, GCH1, VCAM1, TNFRSF1A, PTGIR, TNFRSF11A, IL10RA, CSF2RB, FAS, AKIRIN2, LTA, PELI1, PTGER4, CD40, S100A14, LGALS9, TNFRSF9, CCR7, JUN, JAK2, F2R
0070374	positive regulation of ERK1/2 cascade	1.47E-08	CCL1, GPR183, CCL3, GCNT2, CCL2, CCR1, CCL5, CCL4, SRC, CCL22, NOD2, CD44, CCL3L1, SEMA7A, CCL3L3, HAVCR2, ICAM1, IL6, CCL4L1, PRKCDBP, CCL4L2, SLAMF1, LGALS9, CCR7, CD36, GPR55, JUN, RIPK2, F2R

Top 20 GO terms enriched in pU/UC vs xRNA transfection

GO term	Description	p-value	Genes
0060337	type I IFN signaling pathway	8.86E-29	IFNA21, RSAD2, OAS1, OAS2, IFI35, ISG20, IFNA2, IFNA1, ISG15, IFNA7, IFNA5, IFNA4, IFNA8, MX2, HLA-H, IFNA10, HLA-A, STAT2, IFIT3, IFIT2, IFIT1, OASL, IFNB1, IRF2, IFNA14, IFNA13, IRF4, IFNA16, IFI6, IFNA17
0051607	defense response to virus	1.03E-23	IFNA21, ZC3HAV1, CXCL9, RSAD2, IFNW1, OAS1, OAS2, PMAIP1, TLR7, CXCL10, ISG20, IFNA2, APOBEC3A, IFNA1, IFNA7, ISG15, IFNE, IFNA5, DDX60, IFNA4, ZC3H12A, IFNA8, MX2, DHX58, IL6, IFNA10, HERC5, STAT2, IFIT3, IFIT2, OASL, IFIT1, IFNB1, IFNA14, IFNA13, IFNA16, GBP1, IFNA17
0006959	humoral immune response	2.96E-20	GPR183, IFNA21, IL6, CCL2, IFNA10, IFNW1, PAX5, TRAF3IP2, IFNA2, CD83, IFNA1, IFNA7, IFNB1, IFNE, IFNA5, IFNA4, IFNA14, IFNA13, IFNA8, TREM2, IFNA16, LTA, IFNA17
0002323	NK cell activation in immune response	9.92E-19	IFNA21, IFNA10, IFNW1, IFNA2, IFNA1, IFNA7, IFNE, IFNB1, IFNA5, IFNA4, IFNA14, IFNA8, IFNA13, IFNA16, IFNA17
0033141	positive regulation of STAT phosphorylation	9.92E-19	IFNA21, IFNA10, IFNW1, IFNA2, IFNA1, IFNA7, IFNE, IFNB1, IFNA5, IFNA4, IFNA14, IFNA8, IFNA13, IFNA16, IFNA17
0006954	inflammatory response	9.62E-18	CCL1, CCL3, CCL2, CXCL9, TNFRSF8, NFKB1, NFKB2, IL15, CCL5, CXCL11, CCL4, TLR7, CXCL10, CCRL2, IFNA2, FOS, S1PR3, TNFRSF1B, REL, CCL3L1, CCL3L3, TICAM2, ZC3H12A, TNIP1, CD27, CSF1R, TNIP3, NFKBIZ, IL6, DAB2IP, IL2RA, GBP5, IL18RAP, C4A, C4B, IL27, RELB, CCL4L1, CCL4L2, AIM2, PRKD1, TNFRSF9, CCR7, CAMK4, PLA2G4C, TNFAIP3, IGFBP4
0009615	response to virus	1.25E-17	IFIH1, TNFSF4, ZC3HAV1, ACTA2, CCL4L1, IFNW1, RSAD2, IFI44, OAS1, PIM2, OAS2, CCL5, CCL4, ISG20, IFIT3, DDX58, IFIT2, IFIT1, OASL, IFNA7, IFNB1, DDX60, IFNA4, BCL3, MX2, DHX58, IFNA17
0002286	T cell activation involved in immune response	2.52E-17	IFNA21, IFNA10, IFNW1, IFNA2, IFNA1, IFNA7, IFNE, IFNB1, IFNA5, IFNA4, IFNA14, IFNA8, IFNA13, IFNA16, IFNA17
0043330	response to exogenous dsRNA	9.22E-17	IFNA21, IFNA10, IFNW1, NFKBIA, DDX58, IFNA2, IFNA1, IFNA7, IFNB1, IFNE, IFNA5, IFNA4, IFNA14, IFNA8, IFNA13, IFNA16, IFNA17
0006955	immune response	6.72E-16	GPR183, CCL3, IL1R1, CCL2, ENPP2, CXCL9, TNFSF15, TNFRSF8, TNFSF14, CD70, OAS1, IL15, OAS2, IL7R, CCL5, CXCL11, CCL4, CXCL10, TNFRSF1B, XBP1, IL2RG, HLA-DOB, LTA, CD27, FYB, IL18R1, HLA-H, IL6, TCF7, IL18RAP, TNFSF4, IL2RA, PTGER4, HLA-A, CCL4L1, CCL4L2, GZMH, AIM2, IKBKE, TNFRSF9, CCR7, TNFSF10, CD36, TNFSF13B, LAX1, CD274, IFI6
0060338	regulation of type I IFN-mediated signaling pathway	6.83E-16	IFNA21, IFNA10, STAT2, IFNA2, USP18, IFNA1, IFNA7, IFNB1, IFNA5, IFNA4, IFNA14, IFNA8, IFNA13, IFNA16, IFNA17
0042100	B cell proliferation	9.23E-16	IFNA21, IFNA10, IFNW1, IL7R, IFNA2, IFNA1, IFNA7, IFNE, IFNB1, IFNA5, IFNA4, IFNA14, IFNA8, IFNA13, IFNA16, IFNA17
0019221	cytokine-mediated signaling pathway	1.08E-13	IFNA21, CCL2, IL6ST, IFNW1, IFNA2, IFNA1, IFNA7, CLCF1, IFNE, IL10RA, IFNA5, IFNA4, CSF3R, IL15RA, IFNA8, CSF1R, LRRC4, IL6, SOCS3, SOCS1, IFNA10, IFNA14, IFNA13, IFNA16, IFNA17
0030183	B cell differentiation	1.66E-11	IFNA21, IFNA10, IFNW1, IFNA2, IFNA1, IFNA7, IFNB1, CLCF1, IFNE, IFNA5, PLCG2, IFNA4, IFNA14, IFNA8, IFNA13, IFNA16, IFNA17
0045087	innate immune response	4.29E-11	IFNA21, IFIH1, ZC3HAV1, IFNW1, NFKB1, NFKB2, TLR7, IFNA2, APOBEC3A, IFNA1, IFNA7, REL, IFNE, IFNA5, DDX60, IFNA4, TICAM2, IFNA8, MX2, DHX58, CSF1R, SRPK2, DAB2IP, C4A, NCF2, C4B, IL27, IFNA10, RELB, HERC5, AIM2, DDX58, PRKD1, APOL1, IFNA14, IFNA13, TREML1, TREM2, IFNA16, IFNA17
0071347	cellular response to IL-1	5.82E-10	CCL1, DAB2IP, CCL3, IL6, CCL2, MYLK3, CCL4L1, NFKB1, CCL4L2, ANKRD1, CCL5, CCL4, PCK1, CCL3L1, CCL3L3, ZC3H12A
0045071	negative regulation of viral genome replication	5.97E-09	SRPK2, APOBEC3A, IFIT1, OASL, ISG15, ZC3HAV1, IFNB1, RSAD2, OAS1, CCL5, TNIP1, ISG20
0071222	cellular response to LPS	1.02E-08	ZFP36, DAB2IP, IL6, TNFSF4, CCL2, NFKB1, ANKRD1, CXCL10, TNFRSF1B, CD36, CD80, XBP1, PDE4B, CX3CR1, TICAM2, ZC3H12A, TNFAIP3, TNIP3
0002250	adaptive immune response	2.02E-08	GPR183, IFNA21, IFNA10, IFNW1, SLAMF7, IFNA1, BTN3A1, CAMK4, IFNA7, IFNB1, IFNE, LAX1, IFNA5, IFNA4, TAP1, IFNA14, IFNA13, IFNA8, IFNA16, IFNA17
0007596	blood coagulation	3.00E-08	IFNA21, IFNA10, IFNA2, IFNA1, CD36, IFNA7, P2RX1, IFNB1, IFNA5, IFNA4, COL1A2, IRF2, IFNA14, HBG1, SERPINA1, IFNA13, IFNA8, HBG2, SERPIND1, IFNA16, IFNA17, AP3B1

Figure S3. RLR/IRF3 and TLR7/IRF5 regulate genes with distinct functions.
Table showing top 20 most enriched GO terms in DE genes from R848/vehicle and pU/UC/xRNA. GO term ID number, description, p-value of enrichment, and DE genes that drive the enrichment are shown. RNAseq experiment was performed once with biological triplicates.

Supplementary Figure 4

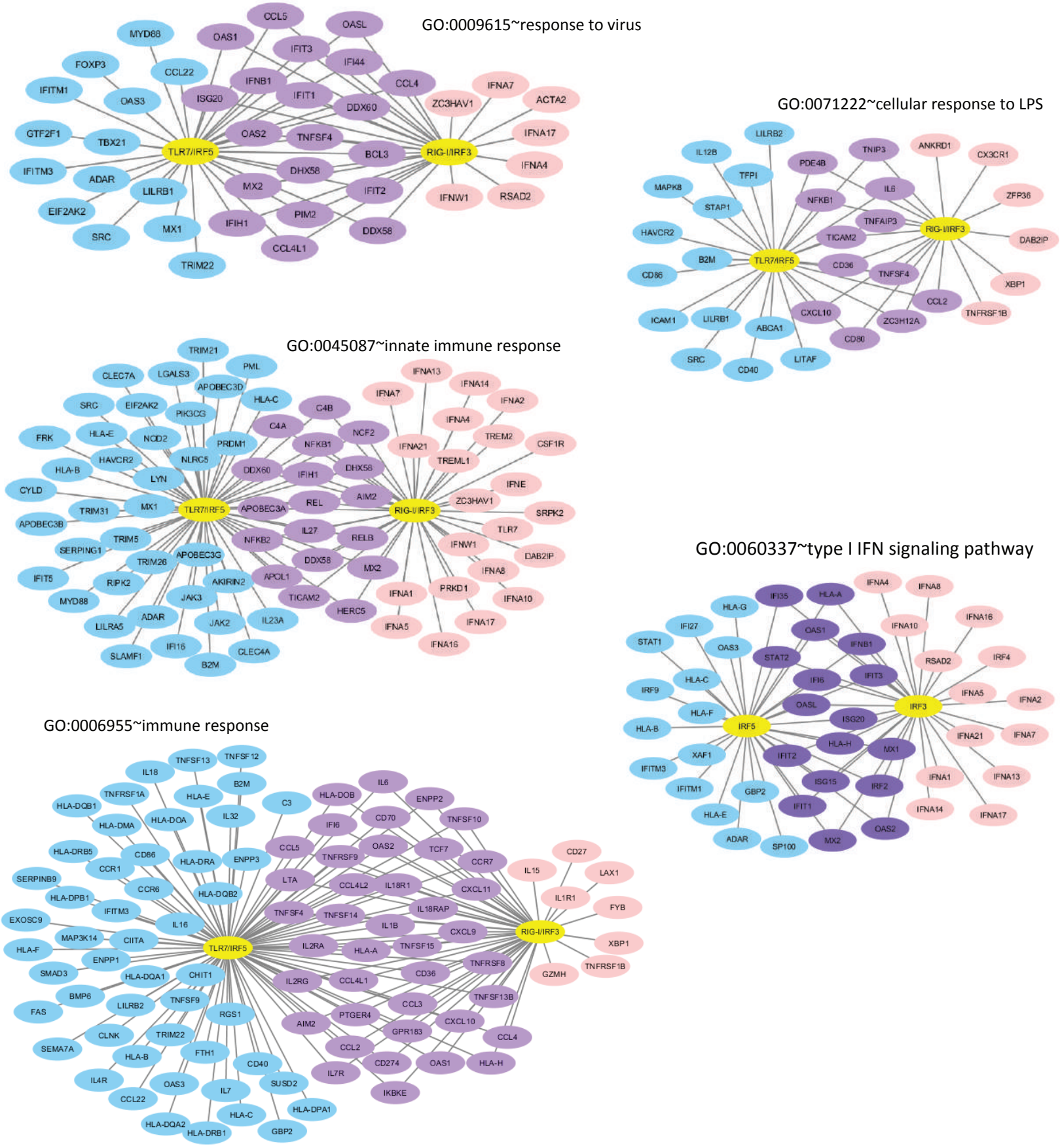

Figure S4. RLR/IRF3 and TLR7/IRF5 drive expression of different genes within same GO category.
Shared GO terms between genes DE in R848/vehicle and pU/UC/xRNA are displayed in networks. Blue denotes R848 treatment induced genes, pink denotes pU/UC transfection induced genes, purple denotes genes induced by both treatments.