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Supplemental Information

Virus-Intrinsic Differences and Heterogeneous IRF3 Activation Influence IFN-Independent Antiviral Protection

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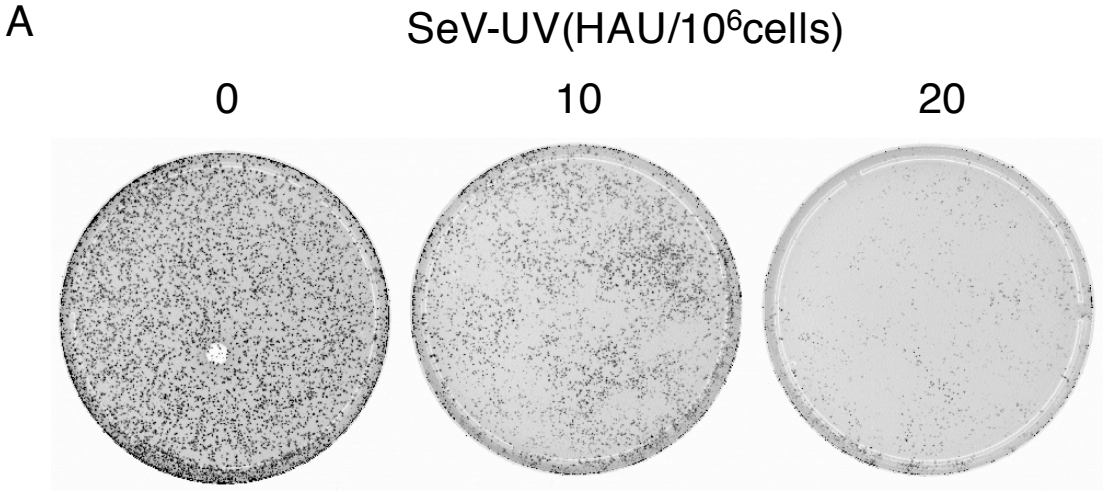
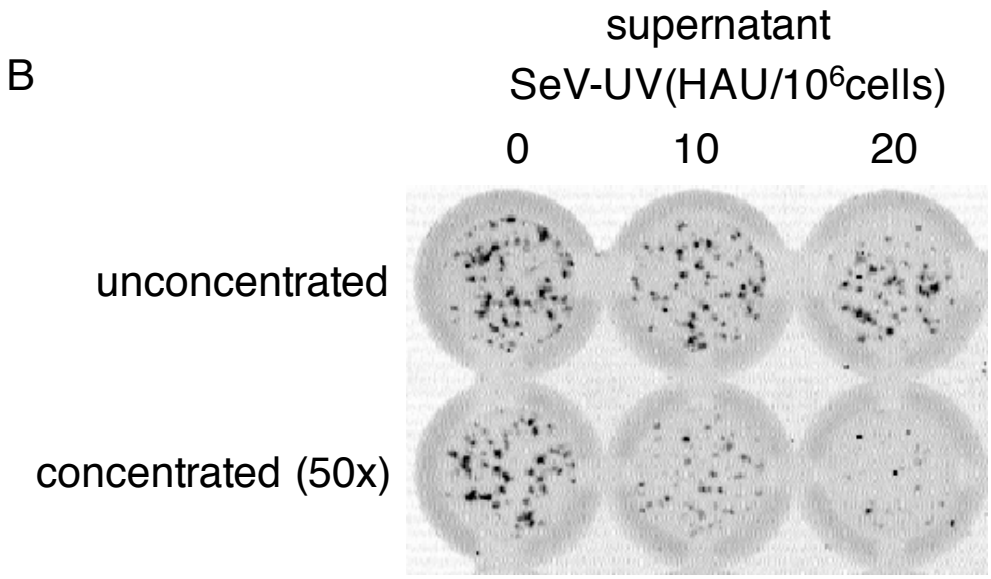
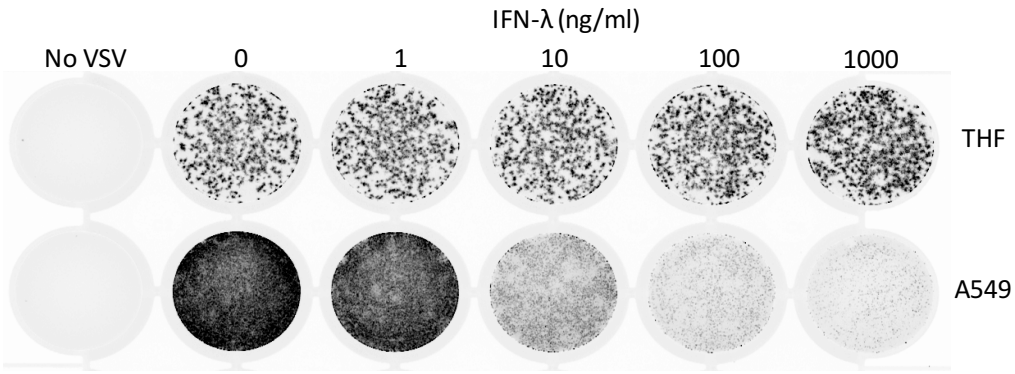




Supplementary figure 1 - IFN detected in concentrated supernatants from SeV-UV infected THFs (Related to Figure 1). 1 x 10^6 THFs were treated with the indicated amount of SeV-UV and incubated for 16 hours before concentrating the supernatant 50-fold and transferring concentrated or non-concentrated supernatants in 100 µl to 1 x 10^4 THFs and performing a plaque reduction assay. THFs were challenged with VSV-GFP either 16 hours after SeV-UV treatment (A) or 6 hours after supernatant transfer (B).



Supplementary figure 2 - A549 but not THFs protected following treatment with recombinant IFN-λ (Related to Figure 1). THFs or A549 were treated with the indicated concentration of IFN-λ and challenged with VSV-GFP 6 hours later in a plaque reduction assay.

Supplementary Table 1 - Differential expression of genes significantly upregulated in HCMV-UV or SeV-UV treated wildtype or IFNAR1 KO THFs (Related to Figure 2)

Gene	Fold change				Gene	Fold change			
	wildtype		IFNAR KO			wildtype		IFNAR KO	
	HCMV-UV	SeV-UV	HCMV-UV	SeV-UV		HCMV-UV	SeV-UV	HCMV-UV	SeV-UV
OASL	**303.44**	**64.35**	**215.92**	7.28	RNF19B	**1.52**	**1.53**	1.13	1.05
CXCL10	**120.79**	**112.51**	2.89	**9.60**	SLFN5	**1.51**	**2.31**	1.16	1.14
CXCL11	**109.44**	**42.17**	2.33	1.40	LAP3	**1.51**	**1.68**	1.01	-1.05
IFIT2	**85.17**	**21.80**	**72.92**	1.83	RGAG1	1.50	-1.33	**2.06**	1.07
CH25H	**46.06**	1.73	**27.37**	1.37	SOX13	**1.50**	1.04	1.24	-1.05
IDO1	**27.33**	**11.02**	3.66	1.00	TMEM126A	**1.50**	1.09	-1.15	1.09
IFIT3	**24.11**	**10.90**	**16.89**	1.30	SERPINB2	**1.49**	**1.61**	1.20	1.35
GBP5	**19.69**	**10.79**	**4.39**	-1.23	SKIDA1	1.48	1.30	**2.09**	1.29
IFIT1	**18.69**	**12.57**	**10.90**	1.11	VSIG10L	1.48	**1.61**	-1.07	-1.05
LOC100288911	**14.45**	**14.57**	3.26	2.03	OGFR	**1.48**	**1.76**	1.10	1.02
ISG15	**14.33**	**9.91**	**8.28**	1.21	XKR8	**1.47**	1.07	1.29	1.07
IFI44L	**12.17**	**39.63**	1.05	1.43	RPS6KC1	**1.46**	1.08	1.31	1.08
RTP4	**12.01**	**7.75**	**12.47**	-1.20	FAM26E	**1.46**	1.10	1.34	1.03
OAS2	**10.95**	**13.91**	**9.80**	1.44	ELF1	**1.45**	**1.48**	1.09	1.02
OAS1	**10.66**	**14.83**	**2.00**	1.66	STAT2	**1.45**	**1.85**	1.27	1.08
HERC5	**10.25**	**3.48**	**9.66**	1.03	RASGRP3	1.44	**1.87**	-1.10	-1.09
BATF2	**9.38**	**12.16**	1.28	-1.09	DDX60L	**1.44**	**2.43**	1.07	-1.12
IFIH1	**9.26**	**8.05**	**6.11**	1.18	APOBEC3G	1.43	**1.69**	1.21	1.28
GBP4	**9.17**	**5.42**	**4.03**	1.13	FNDC3A	**1.43**	1.09	1.23	1.06
ATF3	**8.21**	1.57	**5.31**	1.22	LCP2	**1.43**	-1.00	1.24	1.11
DDX58	**7.57**	**7.70**	**3.94**	1.11	TMEM38A	**1.43**	1.12	-1.06	**-1.45**
ISG20	**7.15**	**2.93**	**4.01**	1.43	TMEM51	**1.42**	1.05	1.19	-1.03
CD7	**6.23**	**2.85**	1.43	-1.40	HMOX1	1.42	1.65	1.50	**1.78**
AIM2	**6.08**	2.00	**4.10**	-3.02	ZBTB42	**1.41**	**1.48**	-1.03	-1.02
XAF1	**5.99**	**11.98**	1.29	1.23	DNAJB5	**1.41**	-1.09	1.25	-1.08
IFI44	**5.93**	**4.98**	**6.65**	1.30	SP100	**1.41**	**1.83**	-1.00	-1.04
RARRES3	**5.93**	1.18	**3.04**	-1.17	LYSMD2	**1.40**	1.33	-1.08	-1.16
THEMIS2	**5.83**	**6.74**	1.60	-1.87	RP6-109B7.3	**1.39**	-1.08	1.05	1.15
CD48	**5.65**	2.35	1.38	1.35	TBXA2R	**1.39**	1.14	-1.02	1.02
USP18	**5.65**	**8.37**	1.87	-1.26	TMEM62	**1.39**	**1.39**	1.02	1.06
RHEBL1	**5.63**	1.39	**4.15**	-1.02	KLF9	**1.38**	1.27	**1.44**	**1.44**
NFKBIZ	**5.41**	1.37	**4.53**	1.03	MTERFD3	**1.38**	1.11	-1.23	-1.07
DDX60	**5.28**	**3.08**	**3.44**	-1.01	WARS	**1.37**	1.23	1.10	1.07
TNFSF10	**5.26**	**6.41**	1.75	1.39	IFITM3	**1.37**	**1.40**	1.03	1.05
NCF2	**5.07**	1.07	**2.33**	-1.38	KIAA0040	**1.37**	1.24	1.06	1.01
IRF1	**4.94**	**2.89**	**1.89**	1.08	PRKD2	**1.37**	**1.70**	1.07	1.06
HELZ2	**4.63**	**8.05**	1.11	1.17	PHF11	1.36	**1.73**	1.01	-1.00
KLF4	**4.58**	1.24	**2.91**	-1.07	B4GALT5	**1.36**	1.02	1.14	-1.01
MX2	**4.49**	**7.86**	**1.58**	1.02	GBP3	**1.36**	**1.48**	1.08	1.04
RANBP3L	**4.26**	-1.10	1.19	-1.03	PNPT1	1.36	**2.22**	1.00	1.00
IL6	**3.93**	1.15	**9.44**	1.95	GTPBP1	**1.36**	1.23	1.21	1.00
SNPH	**3.90**	1.06	**3.00**	-1.06	FENDRR	**1.36**	1.17	**1.39**	1.13
HERC6	**3.75**	**3.06**	**2.21**	-1.03	MASTL	**1.35**	**1.61**	1.01	-1.01
ZC3HAV1	**3.67**	**2.44**	**2.55**	1.06	GCA	**1.35**	1.20	-1.17	-1.10
SAMD9	**3.62**	**3.96**	**1.67**	1.00	BTN3A3	**1.34**	1.16	-1.04	-1.03
CEACAM1	**3.49**	1.70	**2.44**	1.07	IFNAR2	**1.34**	1.03	1.13	-1.08
ADAP1	**3.43**	-1.12	**2.50**	1.26	TRIM56	**1.33**	**1.57**	1.18	1.03
SLC15A3	**3.39**	**4.68**	1.47	1.22	RBM43	1.33	**1.52**	1.07	1.06
APOL6	**3.35**	**2.88**	**2.37**	1.10	FBXO30	**1.33**	1.05	**1.44**	1.17
DTX3L	**3.31**	**4.57**	1.22	-1.04	ZNF107	**1.33**	**1.41**	1.12	1.09
DHX58	**3.27**	**2.97**	**2.52**	1.10	ADAR	**1.33**	**1.71**	1.01	-1.02
PTGS2	**3.24**	1.14	1.21	1.42	CASP7	**1.33**	**1.52**	1.00	1.02
NEDD9	**3.15**	1.15	**2.16**	-1.05	MCL1	**1.32**	1.18	1.25	-1.00

IFI6	**3.13**	**3.84**	1.34	1.01	FAM76A	**1.32**	**1.36**	1.28	1.23
KRT17	**3.11**	1.78	1.45	1.06	GALM	**1.31**	1.09	-1.03	-1.01
PMAIP1	**3.09**	1.19	**2.44**	-1.05	STAMBPL1	**1.31**	-1.20	1.21	-1.06
APOL1	**3.09**	**1.87**	**2.31**	1.09	CNP	**1.30**	**1.37**	1.00	-1.01
TMEM229B	**3.02**	**1.83**	**2.26**	1.00	TMEM219	**1.30**	1.10	1.13	-1.05
PARP9	**2.95**	**4.90**	1.17	-1.09	BTN3A1	**1.30**	1.15	1.13	1.01
TMEM140	**2.93**	**2.97**	1.81	1.18	MOB3C	**1.30**	**1.43**	-1.01	1.06
IFITM1	**2.80**	**4.16**	1.24	-1.40	MAP4K4	**1.30**	-1.00	**1.22**	-1.01
MX1	**2.80**	**4.53**	1.38	1.02	IL1R1	**1.30**	1.07	**1.32**	1.09
FAM46A	**2.77**	**1.83**	**1.63**	-1.01	COX7B2	**1.30**	1.21	-1.00	1.03
DAPP1	2.68	**3.54**	1.21	1.21	CYTH1	**1.29**	**1.37**	1.05	-1.07
GRIP2	**2.63**	1.71	1.58	1.07	TRIM22	1.29	**1.97**	1.13	-1.02
ZNF43	**2.62**	1.47	2.25	1.10	RNF31	**1.29**	**1.49**	1.07	-1.01
IFIT5	**2.60**	**2.88**	1.21	-1.05	SHISA5	**1.29**	**1.28**	1.02	-1.00
ZNFX1	**2.57**	**2.47**	**1.88**	1.06	OBFC1	**1.28**	-1.06	1.08	1.05
APOL2	**2.56**	**1.75**	**1.89**	1.08	TAF8	**1.27**	1.09	**1.30**	1.10
CCRN4L	**2.53**	1.15	**2.22**	1.10	BCL2L13	**1.27**	**1.32**	-1.03	1.04
PLEKHA4	**2.52**	**2.38**	1.52	-1.03	SERTAD1	**1.27**	**1.29**	1.06	1.11
PLSCR1	**2.50**	**3.81**	1.01	-1.12	CCDC6	**1.27**	1.03	1.15	-1.06
PTGER4	**2.48**	1.08	1.81	-1.09	BLZF1	1.27	**1.50**	-1.08	-1.01
PARP10	**2.37**	**3.65**	1.19	1.08	TIPARP	**1.26**	1.20	**1.29**	1.22
IRF9	**2.37**	**3.02**	1.13	1.07	UBA7	1.26	**1.63**	-1.04	1.01
SECTM1	**2.37**	**1.86**	1.33	1.09	DCP1A	**1.26**	**1.40**	1.13	1.09
G0S2	**2.35**	1.29	1.44	1.19	PCGF5	**1.25**	1.11	1.08	1.00
TRIM21	**2.33**	**2.68**	1.10	1.01	PANX1	**1.25**	**1.21**	1.14	1.01
LOC102724224	**2.32**	1.89	1.37	1.03	PLEKHA3	**1.25**	1.05	1.07	-1.02
SAMD9L	**2.29**	**3.26**	**1.40**	1.08	PHACTR4	1.25	**1.38**	1.09	1.03
ASPHD2	**2.27**	1.37	**1.60**	1.09	LGALS3BP	**1.24**	**1.24**	1.02	-1.01
REC8	**2.24**	**2.71**	1.09	1.26	COA6	**1.24**	1.14	-1.04	1.06
USP32P1	2.23	**3.20**	1.88	1.34	GPBP1	**1.23**	1.19	1.14	1.06
APOL3	**2.22**	**1.89**	**2.05**	-1.10	ASNA1	**1.23**	1.05	-1.05	-1.01
NRG2	**2.22**	1.44	1.15	1.09	PELO	**1.23**	1.02	1.11	1.08
FGF2	**2.21**	1.01	**2.26**	1.05	PSME2	**1.23**	**1.22**	-1.01	1.04
ZFP36L2	**2.18**	1.03	**2.11**	1.03	SPATS2L	**1.23**	1.19	1.05	-1.02
PARP14	**2.18**	**3.64**	**1.48**	1.20	MLKL	1.22	**1.56**	1.00	1.10
PPM1K	**2.17**	**2.07**	**1.77**	1.06	CHMP5	**1.22**	**1.29**	-1.03	-1.02
PARP12	**2.16**	**2.80**	1.04	-1.10	SLC35C2	**1.22**	1.08	-1.00	-1.03
TRANK1	**2.15**	**2.08**	**1.98**	1.01	GCLM	1.21	1.20	1.17	**1.29**
BAMBI	**2.09**	-1.00	**2.41**	1.04	B2M	**1.21**	1.09	-1.01	-1.06
NEURL1	**2.08**	**1.83**	1.24	1.30	PSME1	**1.20**	**1.18**	1.03	-1.01
RNF149	**2.07**	1.12	**1.65**	1.06	SLC2A13	**1.20**	1.09	1.08	1.04
SOCS1	**2.04**	**1.69**	1.05	1.08	PRDX2	**1.20**	1.08	1.11	1.00
TNFAIP3	**2.04**	1.06	**1.75**	1.02	DNAJA1	**1.20**	**1.22**	1.02	-1.01
SLC25A28	**2.02**	**2.39**	-1.02	1.04	MRPL22	**1.20**	1.16	**-1.19**	-1.10
FAM65B	**2.02**	1.11	-1.00	1.02	SHOX2	1.20	**1.45**	-1.03	1.05
TRPC4	**2.00**	1.01	1.20	-1.01	TRIM5	1.20	**1.53**	1.08	1.12
SP110	**1.97**	**2.95**	1.06	-1.09	ATP5SL	**1.20**	1.09	-1.01	-1.03
OAS3	**1.97**	**2.71**	1.17	1.02	FMR1	1.19	**1.26**	1.09	-1.00
GBP1	**1.95**	**2.29**	1.09	1.01	GPR180	1.18	**1.32**	1.04	1.10
PIK3AP1	**1.93**	1.40	1.38	1.23	EHD4	1.18	**1.36**	1.07	1.01
GMPR	**1.92**	**2.93**	1.32	1.23	TRIQK	**1.18**	1.05	1.04	1.03
ZCCHC2	**1.92**	**2.37**	1.09	1.08	RBCK1	1.18	**1.30**	1.07	1.01
COL24A1	**1.92**	**2.00**	-1.12	1.13	STAMBP	**1.18**	1.05	-1.05	-1.01
LOC102724927	**1.91**	1.62	-1.08	-1.15	NUP54	**1.18**	1.08	-1.05	-1.03
SAMHD1	**1.91**	**2.40**	1.17	-1.03	SPCS1	**1.16**	1.06	-1.04	-1.02
UBE2L6	**1.91**	**2.16**	1.25	1.11	CMTR1	1.16	**1.39**	1.05	-1.01
ARID5A	**1.90**	1.47	1.14	-1.22	HOXD10	1.16	**1.28**	-1.04	-1.08
LOC728769	**1.88**	1.62	1.02	-1.09	ETS2	1.16	**1.39**	-1.06	1.01
HES4	**1.87**	1.78	1.50	-1.15	CCDC113	1.16	1.04	1.92	**2.60**

TRIM14	**1.85**	**2.55**	1.25	1.03	GTPBP2	1.16	**1.41**	-1.04	1.01
N4BP1	**1.84**	**1.90**	1.25	1.04	FHL3	**1.15**	1.10	-1.10	-1.05
CD274	**1.82**	**1.65**	1.12	-1.07	DUSP16	1.14	**1.29**	1.13	-1.00
TLR3	1.82	**2.44**	-1.19	1.04	TNFRSF21	1.14	1.05	**1.27**	1.07
BST2	**1.81**	**2.12**	1.11	1.04	SEMA3A	1.14	-1.07	**1.53**	-1.03
MYD88	**1.81**	**2.46**	-1.05	-1.08	IER2	1.13	**1.29**	-1.13	-1.05
TRIM25	**1.80**	**2.57**	-1.02	1.01	MED25	1.12	**1.20**	1.05	-1.07
SIX2	**1.79**	1.11	1.32	-1.35	ZNF844	1.12	**1.47**	1.10	1.01
C19orf66	**1.79**	**2.64**	1.00	1.04	KIAA0226	1.11	**1.24**	1.09	-1.03
TDRD7	**1.79**	**2.26**	1.22	1.07	APLP1	1.10	**1.28**	-1.08	1.02
SQRDL	**1.79**	1.16	**1.58**	1.12	TBX15	1.10	**1.26**	-1.05	1.01
TTC39B	**1.78**	**1.48**	**1.39**	1.17	NLRC5	1.09	**1.81**	-1.07	1.01
ZBED5-AS1	**1.78**	1.02	1.28	1.42	C6orf62	1.09	**1.18**	-1.03	-1.01
MSX1	**1.77**	**1.45**	1.36	1.13	ATP10A	1.08	**1.61**	1.06	1.08
CDK5R2	**1.76**	1.26	1.10	-1.04	ZGLP1	1.06	1.06	**1.82**	1.41
IFI35	**1.74**	**2.34**	-1.07	1.01	S1PR3	1.06	**1.25**	-1.06	1.08
EIF2AK2	**1.73**	**2.56**	1.03	-1.08	ETV6	1.06	**1.20**	1.01	1.05
HIP1R	**1.73**	1.00	1.37	1.04	RICTOR	1.05	**1.29**	1.10	1.06
PML	**1.71**	**2.05**	1.02	1.01	ANKFY1	1.05	**1.26**	1.01	-1.00
SIX1	**1.70**	1.24	1.14	-1.03	SOX9	1.05	**1.28**	**-1.29**	-1.13
TREX1	**1.69**	**1.77**	-1.04	-1.07	GNB4	1.05	**1.28**	-1.06	-1.05
FOXF1	**1.68**	1.07	**1.57**	1.09	BAZ1A	1.05	**1.24**	-1.00	1.00
CYP2J2	**1.67**	1.55	-1.15	-1.12	LOC100506714	1.04	1.28	1.25	**1.40**
ZFP42	**1.64**	**1.67**	1.10	1.17	ADAT2	1.04	-1.07	**1.55**	-1.00
NMI	**1.63**	**1.94**	1.10	-1.00	REEP2	1.04	1.15	**1.58**	1.27
SLAMF7	**1.63**	1.10	1.18	1.02	ZFP36	1.03	1.09	1.12	**1.22**
HIAT1	**1.62**	-1.03	1.17	-1.07	LETM2	1.02	-1.04	1.25	**1.41**
C18orf56	**1.62**	1.59	-1.43	-1.27	EBF4	-1.00	1.02	**3.36**	1.41
SPRY2	**1.62**	1.15	1.21	1.14	DCLRE1C	-1.00	**1.26**	-1.01	-1.06
IFI16	**1.60**	**1.96**	1.06	1.04	SLC30A4	-1.02	1.03	**1.25**	1.09
ZNF232	**1.60**	1.06	1.27	-1.25	KCNN3	-1.06	-1.05	**2.64**	**2.54**
SRGAP3	**1.59**	1.01	1.08	-1.00	INO80B	-1.06	-1.39	**1.92**	1.48
PIK3R3	**1.59**	1.07	**1.33**	-1.01	CDC14A	-1.09	-1.13	**1.30**	1.16
FZD4	**1.58**	-1.08	**1.55**	1.08	PHF10	-1.10	-1.10	**1.36**	1.09
VEGFC	**1.58**	1.18	1.32	1.05	ABCA3	-1.11	1.14	**5.12**	4.00
IRF2BPL	**1.58**	1.15	1.23	1.09	C4orf36	-1.13	-1.08	**1.65**	1.38
STAT1	**1.58**	**2.15**	-1.01	-1.01	AP1S3	-1.16	-1.02	1.23	**1.48**
TSKU	**1.57**	1.15	1.29	-1.04	LOC102724023	-1.17	1.84	**2.38**	1.24
GIMAP2	**1.57**	**1.57**	1.00	1.09	ADAMTS9	-1.19	-1.08	**1.92**	1.28
ZC3H12C	**1.56**	1.20	1.41	1.09	TMEM176A	-1.20	-1.22	1.43	**3.15**
PNP	**1.56**	1.06	1.33	1.03	OLFM4	-1.22	-1.10	**3.96**	-1.01
TRIM38	**1.54**	**1.80**	1.03	1.07	LOC100506258	-1.38	-1.08	2.22	**3.30**
IRF2	**1.52**	**1.55**	1.09	-1.00	NDP	-1.39	-1.67	**3.36**	1.43

Fold change calculated relative to mock infected wildtype or IFNAR KO cells

Values in bold are significantly different from mock

IFNAR: IFN-α/β receptor, HCMV-UV: UV-inactivated human cytomegalovirus, SeV-UV: UV-inactivated Sendai virus

Transparent Methods

Cells and viruses

Telomerized human fibroblasts (THFs) and THF IFNAR1 KOs (from Victor DeFilippis) were immortalized through expression of hTERT in BJ fibroblasts (Bresnahan et al., 2000). THF IRF3 KO and RelA KO were generated by transducing THF with lentivirus encoding Cas9 and gene specific gRNA, followed by selection of single cell clones lacking protein expression for the gene of interest. THF IFN-β-GFP cells were generated by transducing THF with lentivirus encoding green fluorescence protein (GFP) under the control of the IFN-β promoter region (Cellomics). Human embryonic lung (HEL) fibroblasts and NuLi-1 immortalized bronchiole epithelial cells were obtained from ATCC. THP-1 monocytes (from Dawn Bowdish) were differentiated into adherent macrophage-like cells by treatment with 100 nM PMA (Sigma-Aldrich) for 72 hours. SeV (Charles River) Cantell strain was produced in eggs and titred by plaque assay on CV-1 cells, HCMV strain AD169 (from Theresa Compton) was propagated and titred in human embryonic lung (HEL) fibroblasts and vesicular stomatitis virus expressing GFP (VSV-GFP) was propagated in Vero cells. Virus particles were counted by tunable resistive pulse sensing with a qViro-X particle counter (Izon). Virus inactivation was carried out in a stratolinker UV-crosslinker using working concentration of virus with the amount of energy optimized to yield a 5-log reduction in infectious titre. Treatments were done in minimal serum-free media for 1 hour at 37^{o}C with periodic rocking. E1000 dsDNA and dsRNA derived from the WNV genome was transcribed in vitro and purified as previously described (DeWitte-Orr et al., 2009). Transfections were carried out with lipofectamine 3000 (ThermoFisher) according to the manufacturer's instructions. B18R (Millipore) was used to block IFN at 50 ug/ml in serum-free media and incubated with cells for 30 minutes prior to treatment. Cycloheximide (Sigma) was used at 50 uM for a 30 minute pre-treatment and subsequent steps to inhibit protein synthesis.

Plaque reduction assay

Cells were conditioned with virus or other treatment and incubated 16 hours at 37^{o}C before challenge with VSV-GFP infection and an overlay containing 1% methyl-cellulose to restrict plaques. Immediately before VSV-GFP challenge, supernatants from treated cells were transferred to naive cells and allowed to sit for 6 hours before the supernatant conditioned cells

were also challenged with VSV-GFP. Plates were scanned with a Typhoon fluorescence scanner 24 hours post-infection with VSV-GFP to assay antiviral protection.

Transcriptome sequencing and analysis

RNA was extracted using an RNeasy RNA extraction kit (Qiagen) and treated with DNAase (Ambion) according to the manufacturer's instructions. cDNA libraries were created by polyA enrichment using NEBNext poly(A) magnetics isolation module (NEB) and reverse transcribed using NEBNext ultra II directional RNA library prep kit (NEB) according to the manufacturer's instructions. cDNA libraries were sequenced using an Illumina HiSeq rapid V2 (1 x 50 bp sequence reads) at the Farncombe Metagenomics Facility (McMaster University). Sequencing yielded ~1 x 10^7 reads/sample.

First, reads were filtered by quality (at least 90% of the bases must have a quality score of 20 and higher). Then the mapping of the remaining reads was performed using *HISAT2* (Kim et al., 2015) with hg38 (UCSC) reference genome; reads were counted by using *HTSeq count* (Anders et al., 2015). Genes showing less than 10 counts in more than 30% of the samples per group were removed using *filterByExpr* function in *EdgeR* package (McCarthy et al., 2012; Robinson et al., 2010) in R, resulting in 13,134 genes. These remaining count values were normalized with *TMM* normalization method (Robinson and Oshlack, 2010) and then transformed with *voom* transformation (Law et al., 2014). Next, batch effect was removed using ComBat (Johnson et al., 2007), with experiment date used as the batch information. *Limma* package (Ritchie et al., 2015) in R was used to examine differential expression between the groups of interest; p-values obtained from the analysis were corrected with BH correction for multiple testing (Benjamini and Hochberg, 1995), and corrected values <0.05 were considered to be significant.

Quantitative RT-PCR

RNA was extracted using TRIzol reagent (Invitrogen) or an RNeasy RNA extraction kit (Qiagen) and treated with DNAase (Ambion) according to the manufacturers' instructions. 500 ng of RNA was reversed transcribed using SuperScript II Reverse Transcriptase (Invitrogen) and random hexamer primers or with an iScript cDNA synthesis kit (BioRad) as per the manufacturer's instructions. Quantitative PCR reactions contained Taqman probes and Universal PCR Master Mix (Applied Biosystems) or primers and SsoFast EvaGreen Supermix (BioRad)

were used as indicated, along with PCR amplification on a StepOnePlus Q-PCR instrument (Applied Biosystems). Ct values were calculated and GAPDH was used as an endogenous control to calculate individual ΔΔCt values. ΔΔCt values of samples were compared with mock treated samples to calculate fold change. Taqman probes for human GAPDH (Hs02758991_g1), IFIT1 (Hs03027069_s1), ISG15 (Hs00192713_m1) and CXCL10 (Hs00171042_m1) and PCR primers for human GAPDH (F-5'-GGAGCGAGATCCCTCCAAAAT-3' and R-5'-GGCTGTTGTCATACTTCTCATGG-3'), genomic SeV (F-5'-GACCAGGAAATAAAGAGTGCA-3' and R-5'-CGATGTATTGGCATATAGCGT-3') and SeV DVG-546 (F-5'-TCCAAGACTATCTTTATCTATGTCC-3' and R-5'-GGTGAGGAATCTATACGTTATAC-3') were used.

Immunofluorescence

Cells were fixed on glass coverslips using 10% formalin, permeabilized in 0.2% Triton-X 100 in phosphate buffered saline (PBS) and blocked in 3% fetal bovine serum (FBS), 3% goat serum, 0.02% Tween-20 in PBS for 1 hour. The following antibodies were used for 1 hour at the indicated dilution in blocking buffer: anti-IRF3 (Millipore)(1:400), AlexaFluor488-conjugated anti-GFP (Invitrogen)(1:1000), anti-ISG15 (gift from Dr. EC Borden)(1:10), anti-SeV (1:2000), AlexaFluor488-conjugated anti-rabbit (Invitrogen)(1:400) and AlexaFluor594-conjugated anti-mouse (1:50). Hoechst 33258 (Invitrogen) was diluted 1:5000 in PBS and added to cells for 15 minutes. A Leica DM IRE2 microscope was used and IRF3 positive nuclei were calculated as a percentage of total nuclei using OpenLab software (Leica).

Flow cytometry

Cells were fixed and permeabilized using a cytofix/cytoperm fixation/permeabilization kit (BD Biosciences) according to the manufacturer's instructions. Cells were stained with anti-ISG15 (diluted 1:10) and APC-conjugated anti-mouse (Biolegend) (diluted 1:400) for 30 minutes each in perm/wash buffer (BD Biosciences). Flow cytometry of fixed cells was carried out in 1% BSA, 5mM EDTA in PBS using a MoFlow XDP cell sorter (Beckman Coulter). Cell populations were analyzed using FlowJo.

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