PNAS

Supporting Information for

A CRISPR-Cas9 Knockout Screening Identifies IRF2 as a Key Driver of OAS3/RNase L-Mediated RNA Decay During Viral Infection

Sunwoo Oh, Gisselle Santiago, Lavanya Manjunath, Junyi Li, Alexis Bouin, Bert L. Semler and Rémi Buisson

Rémi Buisson
Email: rbuisson@uci.edu

This PDF file includes:

Figures S1 to S5
Tables S1 to S5
Legend for Dataset S1

Other supporting materials for this manuscript include the following:

Dataset S1

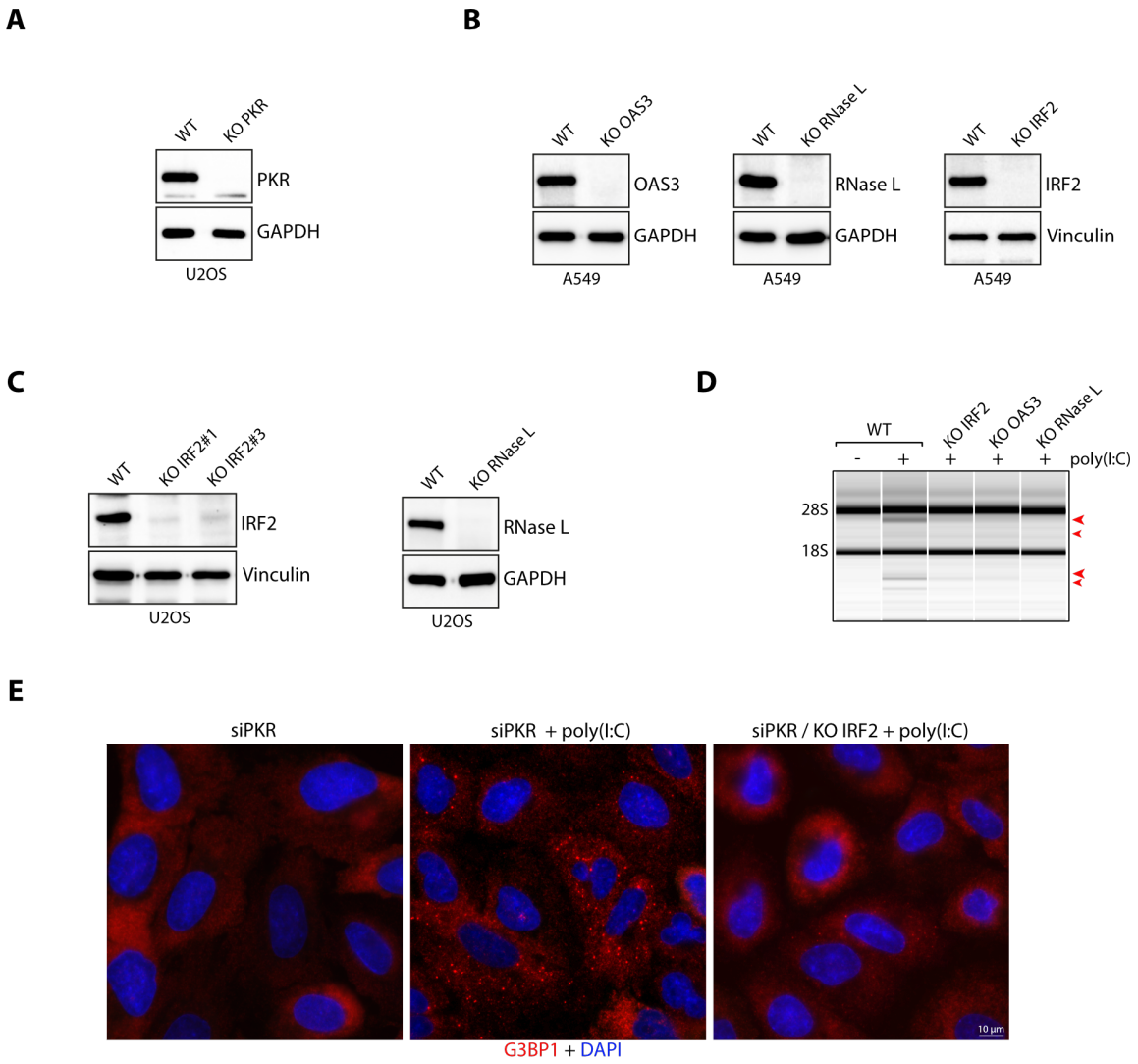

Fig. S1. A. The level of PKR and GAPDH was analyzed in U2OS WT and PKR KO cells by western blot. **B.** U2OS cells KO for OAS3, IRF2, or RNase L were analyzed by western blot using the indicated antibodies. **C.** U2OS cells KO for IRF2 or RNase L were analyzed by western blot using the indicated antibodies. **D.** Indicated U2OS cells were transfected with poly(I:C) (40ng/mL) for 4 hours and then analyzed for RNA integrity by bioanalyzer. The red arrows indicated ribosomal RNA cleavage products. **E.** Wider representative immunofluorescence for G3BP1 in A549 cells knocked down with PKR siRNA and transfected with poly(I:C) (25 ng/mL, 4h), as shown in Figure 1F.

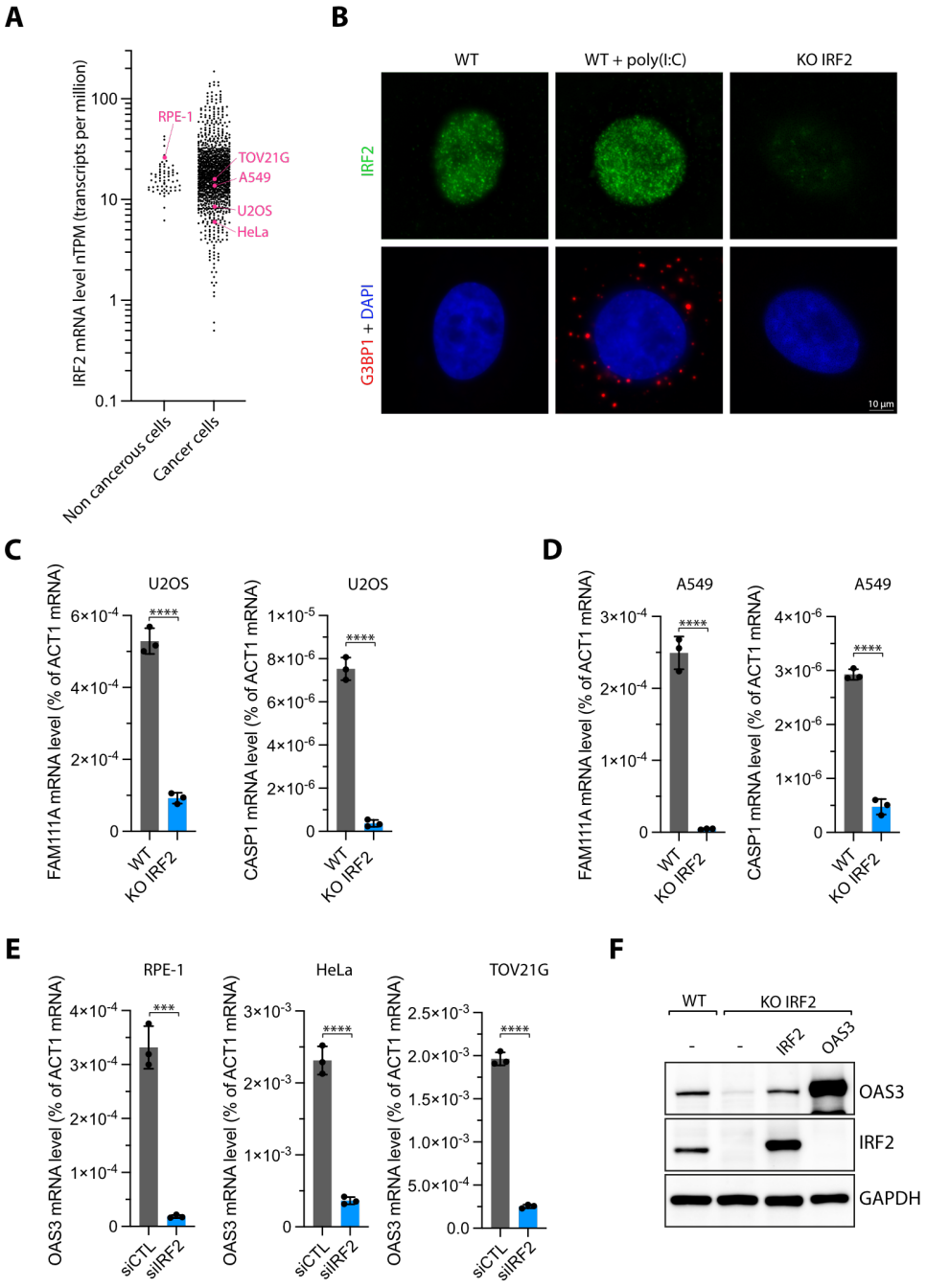

Fig. S2. A. IRF2 mRNA expression levels (nTPM; Transcripts per million) were obtained from The Human Protein Atlas and analyzed for non-cancerous and cancer cells. Cell lines validated in this study are highlighted in pink. **B.** Representative immunofluorescence for IRF2, G3BP1, and DAPI in A549 WT and A549 IRF2 KO cells. When indicated, cells were transfected with poly(I:C) (25 ng/mL) for 4h. **C-D.** The FAM111A and CASP1 mRNA levels were monitored by RT-qPCR in U2OS (**C**) or A549 (**B**) cell lines. Mean values ± SD (n = 3). ****P < 0.0001 (two-tailed t-test) **E.** RPE-1, HeLa, and TOV21G cells were transfected with siCTL or siIRF2 for 72 hours, and the OAS3 mRNA levels were monitored by RT-qPCR. Mean values ± SD (n = 3). ****P < 0.0001 (two-tailed t-test) **F.** A549 WT or IRF2 KO cells were complemented with wild-type IRF2 or OAS3. The protein levels of OAS3, IRF2, and GAPDH were analyzed by western blot.

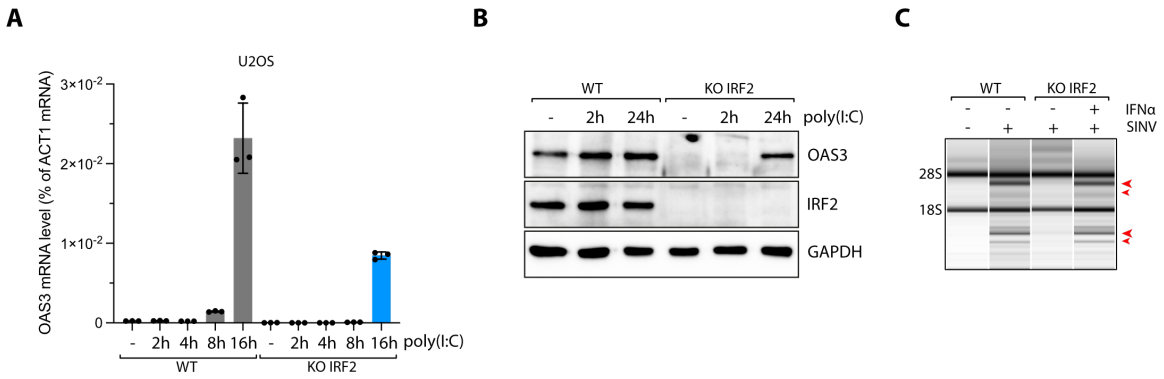

Fig. S3. A. U2OS WT and IRF2 KO cells were transfected with poly(I:C) (100 ng/mL). The level of OAS3 mRNA was monitored at indicated time points by RT-qPCR. Mean values ± SD. **B.** U2OS WT and IRF2 KO cells were transfected with poly(I:C) (100 ng/mL). The protein levels of OAS3, IRF2, and GAPDH were monitored at indicated time points by western blot. **C.** U2OS WT and IRF2 KO cells were pre-treated with IFNα (1000 U/mL) for 8 hours and infected with SINV (MOI=1) for 24 h. The RNAs were extracted and analyzed using bioanalyzer for RNA integrity. The red arrows indicate RNA cleavage products.

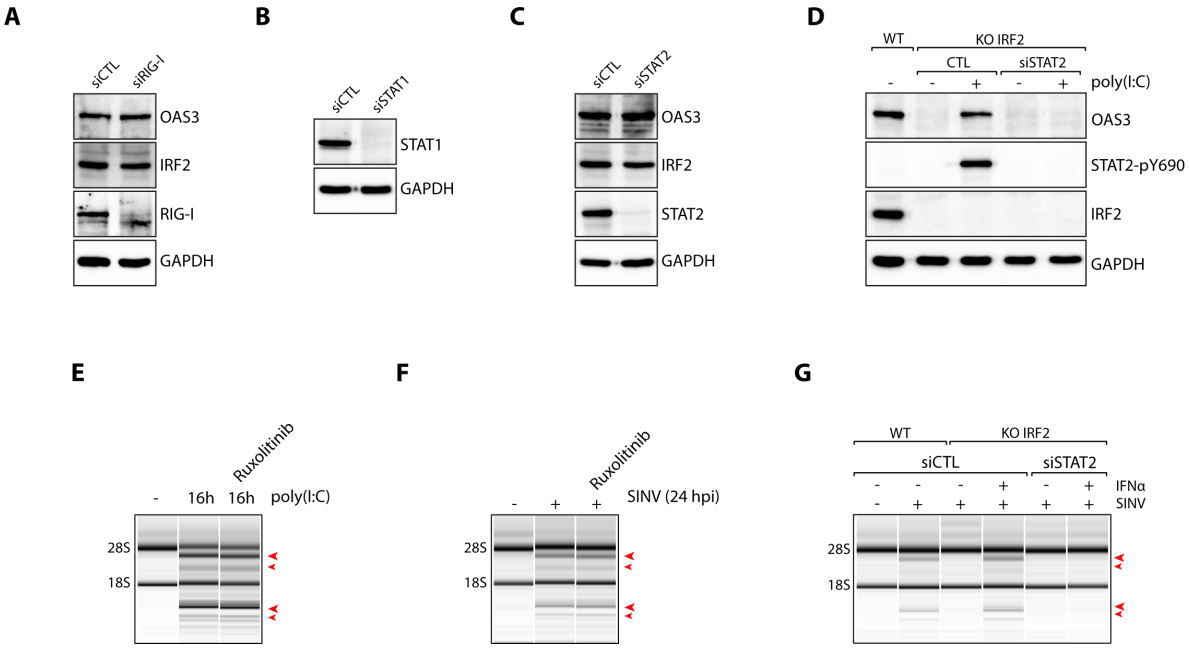

Fig. S4. A-C. A549 cells were knocked down for RIG-I (**A**), STAT1 (**B**), or STAT2 (**C**), and indicated protein levels were analyzed by western blot. **D.** U2OS IRF2 KO cells knocked down with either siCTL or siSTAT2 were transfected with poly(I:C) (100ng/mL) for 24h. The protein levels of OAS3, STAT2-pY690, IRF2, and GAPDH were analyzed by western blot. **E.** A549 WT cells transfected with poly(I:C) (10 ng/mL) for 16 hours were treated with Ruxolitinib (2 μM) and analyzed for RNA integrity using Bioanalyzer. The red arrows indicated ribosomal RNA cleavage products. **F.** U2OS WT cells infected with SINV (MOI=1) for 24h were treated with Ruxolitinib (2 μM). Total RNA was analyzed for integrity by bioanalyzer. The red arrows indicated ribosomal RNA cleavage products. **G.** U2OS WT or IRF2 KO cells transfected with either siCTL or siSTAT2 for 40 h. Cells were then treated with IFNα (1000 U/mL) for 8 hours and infected with SINV (MOI=1, 16 hpi). Total RNAs were isolated and analyzed for RNA integrity by bioanalyzer.

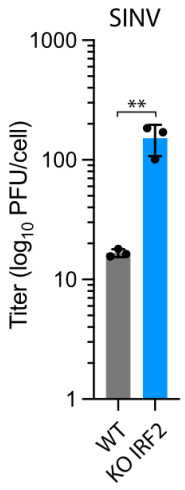

Fig. S5. A549 WT or IRF2 KO cells were infected with SINV (MOI =10, 24 hpi), and the titer (log_{10} PFU/cell) of infectious virus from the supernatant was quantified by plaque assay in Vero cells from three biological replicates. Mean values ± SD. **P < 0.01.

Table S1. CRISPR-Translate screening library primers

P5 Primers	**Sequence**
P5_0n	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG CTCTTCCGATCTTTGTGGAAAGGACGAAACACCG
P5_1n	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG CTCTTCCGATCTCTTGTGGAAAGGACGAAACACCG
P5_2n	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG CTCTTCCGATCTGCTTGTGGAAAGGACGAAACACCG
P5_3n	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG CTCTTCCGATCTAGCTTGTGGAAAGGACGAAACACCG
P7 Primers	**Sequence** (The unique barcode sequences are indicated in red)
P7_A1	CAAGCAGAAGACGGCATACGAGATCGGTTCAAGTGACTGGAGTTCAGACGTGTGC TCTTCCGATCTCCAATTCCCACTCCTTTCAAGACCT
P7_A2	CAAGCAGAAGACGGCATACGAGATGCTGGATTGTGACTGGAGTTCAGACGTGTGC TCTTCCGATCTCCAATTCCCACTCCTTTCAAGACCT
P7_A3	CAAGCAGAAGACGGCATACGAGATTAACTCGGGTGACTGGAGTTCAGACGTGTGC TCTTCCGATCTCCAATTCCCACTCCTTTCAAGACCT
P7_A4	CAAGCAGAAGACGGCATACGAGATTAACAGTTGTGACTGGAGTTCAGACGTGTGC TCTTCCGATCTCCAATTCCCACTCCTTTCAAGACCT
P7_A5	CAAGCAGAAGACGGCATACGAGATATACTCAAGTGACTGGAGTTCAGACGTGTGC TCTTCCGATCTCCAATTCCCACTCCTTTCAAGACCT
P7_A6	CAAGCAGAAGACGGCATACGAGATGCTGAGAAGTGACTGGAGTTCAGACGTGTGC TCTTCCGATCTCCAATTCCCACTCCTTTCAAGACCT

Table S2. siRNA sequences (Thermo Fisher Scientific)

siRNA	**Catalog**	**Sequence**
siCTL	4390843	Not disclosed by the company
siRIG-I	s223614	CAAGAAGAGUACCACUUAAtt
siSTAT1	s279	GGUUCACUAUAGUUGCGGAtt
siSTAT1	s278	CGGUUGAACCCUACACGAAtt
siSTAT2	s13528	GGCUCAUUGUGGUCUCUAAtt
siSTAT2	s13530	GGCCGAUUAACUACCCUAAtt
siIRF2	s7504	GAGGAAUAUUGAAGGCAAAtt
siIRF2	s7506	AGAACGGCCUUCUAAGAAAtt

Table S3. Antibodies

Targets	**Antibody Species**	**Company (Cat# number)**	**Antibody Dilution**
GAPDH	Rabbit polyclonal	EMD Millipore (#ABS16)	1/20,000 WB
RIG-I	Rabbit monoclonal	Cell signaling (#3743)	1/1000 WB
STAT1	Mouse monoclonal	Santa Cruz (#sc-464)	1/1000 WB
STAT2	Rabbit monoclonal	Cell Signaling (#72604)	1/1500 WB or 1/100 ChIP
STAT1-pY701	Rabbit monoclonal	Cell Signaling (#9167)	1/100 WB
STAT2-pY690	Rabbit monoclonal	Cell Signaling (#88410)	1/1500 WB
G3BP1	Rabbit monoclonal	BD Biosciences (#611127)	1/500 IF
PKR	Mouse monoclonal	BD Biosciences (#610764)	1/1000 WB
PKR-pT446	Rabbit monoclonal	Abcam (ab32036)	1/1000 WB
Puromycin	Mouse monoclonal	EMD Millipore (#MABE343)	1/500 IF
Vinculin	Mouse monoclonal	Sigma (V9264)	1/5000 WB
IRF2	Rabbit monoclonal	Cell Signaling (#59452)	1/1000 WB or 1/100 ChIP
OAS3	Rabbit monoclonal	Cell Signaling (#74906)	1/1000 WB
RNase L	Mouse monoclonal	Santa Cruz (sc-74405)	1/1000 WB

Table S4. gRNA sequences

Gene	Sequence (used for generating KOs with purified Cas9)
PKR	AATACATACCGTCAGAAGCA
RNase L	GCAATCGCTGCGAGGATAAA
Gene	**Sequence (used for generating KOs with PX458 system)**
IRF2	CAGCATTCGGTAGACCCTGA
OAS3	GACGGTCAACTATAGCACTG
RNase L	AGCTGTGAAGACGTTCTGTG
SLC38A6	TCACCTAAATCCAAAGACAC
ADSL	GGTATAAATTCCGGACATGG
NOP14	CTCTAACAGGATGAAGACGG

Table S5. qPCR primers

Target names	FORWARD	REVERSE
Actin	CCAACCGCGAGAAGATGA	CCAGAGGCGTACAGGGATAG
OAS3	CCGAACTGTCCTGGGCCTGATCC	CCCATTCCCCAGGTCCCATGTGG
IFN-β	ACACTGGTCGTGTTGTTGAC	GGAAAGAGCTGTCGTGGAGA
ISG15	CGCAGATCACCCAGAAGATCG	TTCGTCGCATTTGTCCACCA
IFIT2	TGGTGGCAGAAGAGGAAGAT	GTAGGCTGCTCTCCAAGGAA
DDX60	AAGGTGTTCCTTGATGATCTCC	TGACAATGGGAGTTGATATTCC
CASP1	CTGGGGACTCTCAGCAGATCA	ATAGCTGGGTTGTCCTGCAC
FAM111A	CTTCACAAAAAGGGCGCAA	ATCAACTGGCTGGGTGCTTT
OAS3 (ChIP-qPCR)	CAGGAAGTGGGTGTCAGGTC	CGGCCTTCGGATTTCTGGT

Dataset S1 (separate file). Ranking of all the genes detected with CRISPR-Translate from Figure 1B. RNase L, OAS3, and IRF2 were highlighted in red.