nature microbiology

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41564-018-0244-1

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A CRISPR screen identifies IFI6 as an ER-resident interferon effector that blocks flavivirus replication

R. Blake Richardson[1], Maikke B. Ohlson[1], Jennifer L. Eitson[1], Ashwani Kumar[2], Matthew B. McDougal[1], Ian N. Boys[1], Katrina B. Mar[1], Pamela C. De La Cruz-Rivera[1], Connor Douglas[3], Genevieve Konopka[3], Chao Xing[2] and John W. Schoggins[1]*

[1]Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX, USA. [2]Bioinformatics Core, McDermott Center, University of Texas Southwestern Medical Center, Dallas, TX, USA. [3]Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX, USA. *e-mail: john.schoggins@utsouthwestern.edu

Supplementary Information for:

A CRISPR screen reveals IFI6 as an ER-resident interferon effector that blocks flavivirus replication

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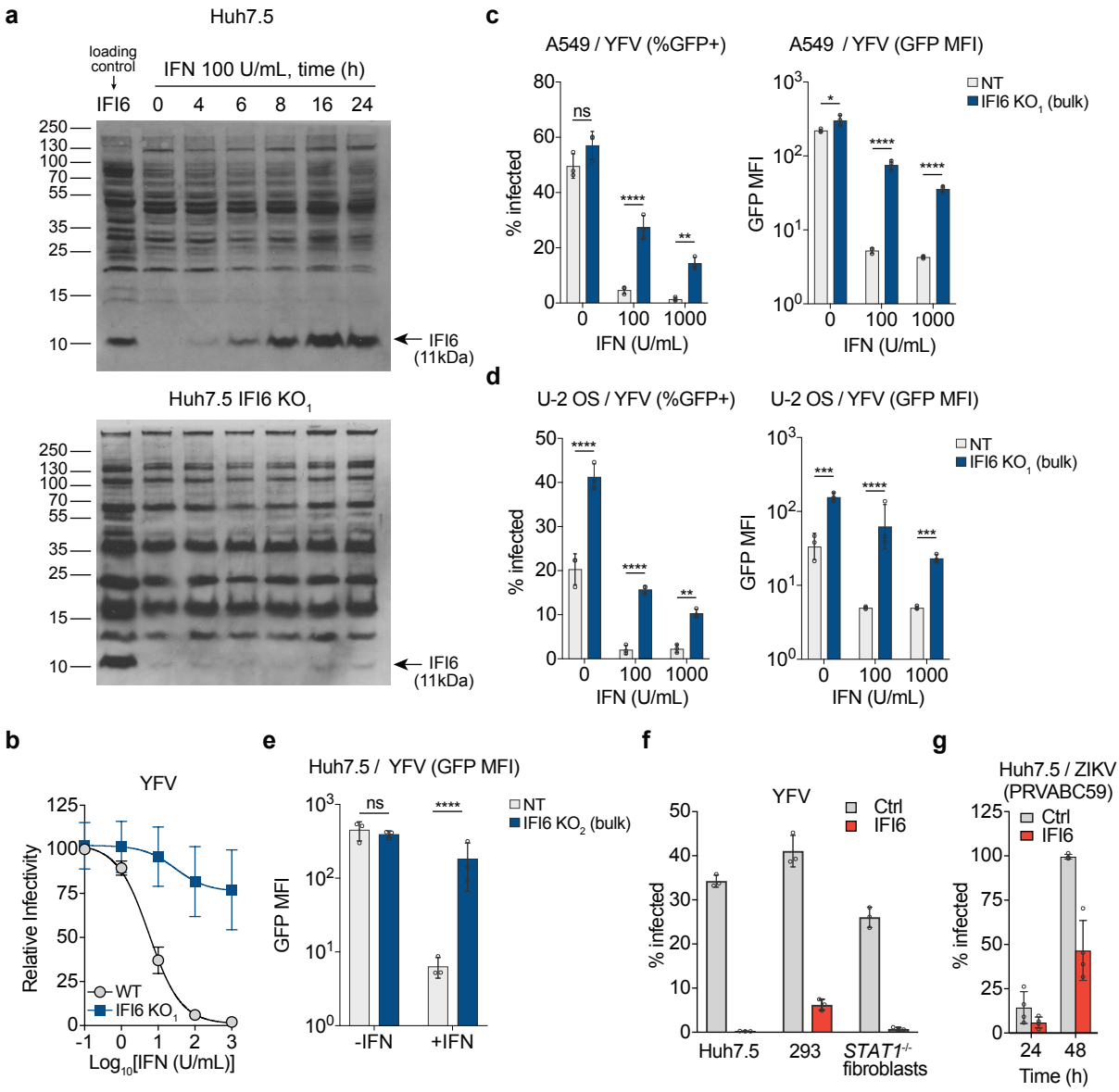
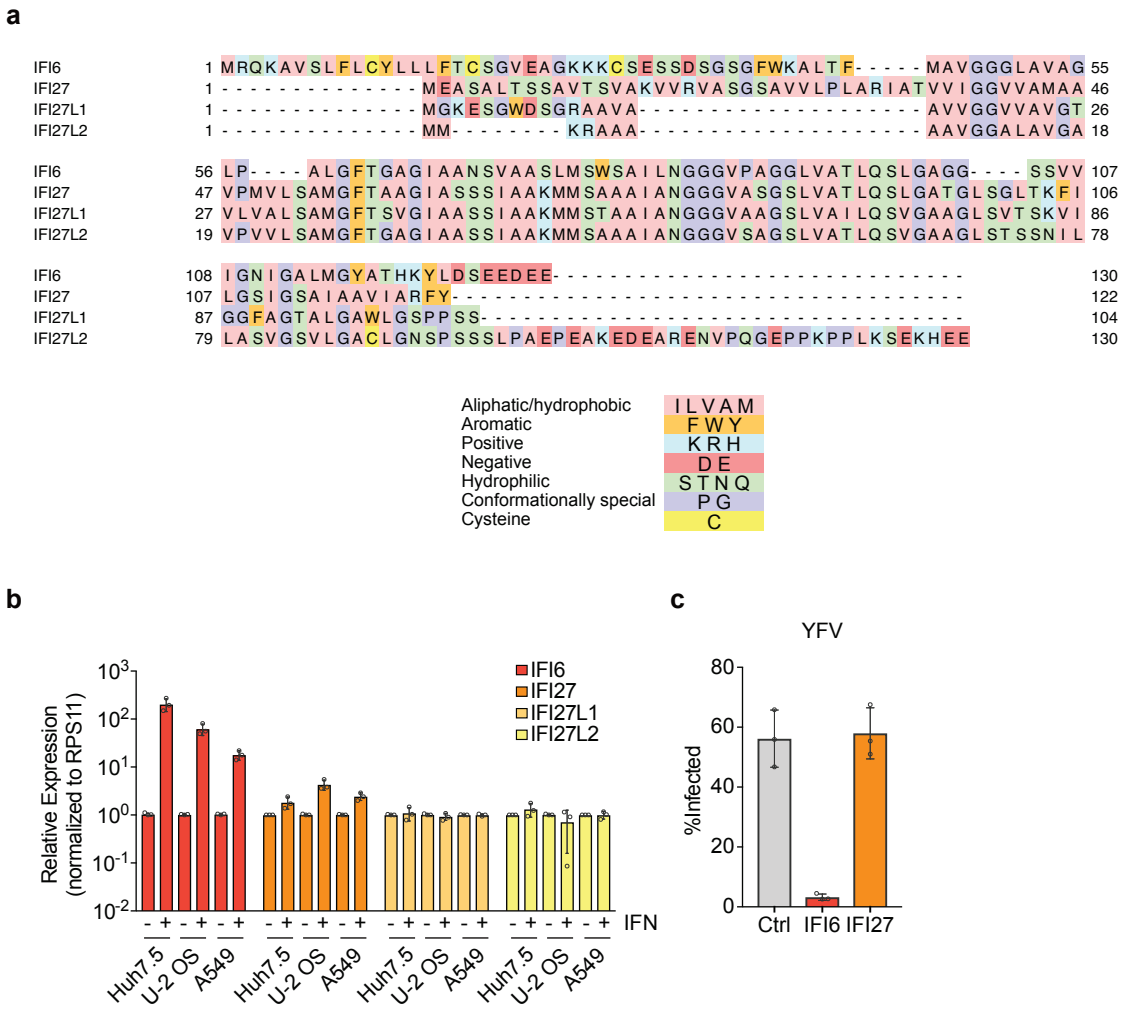
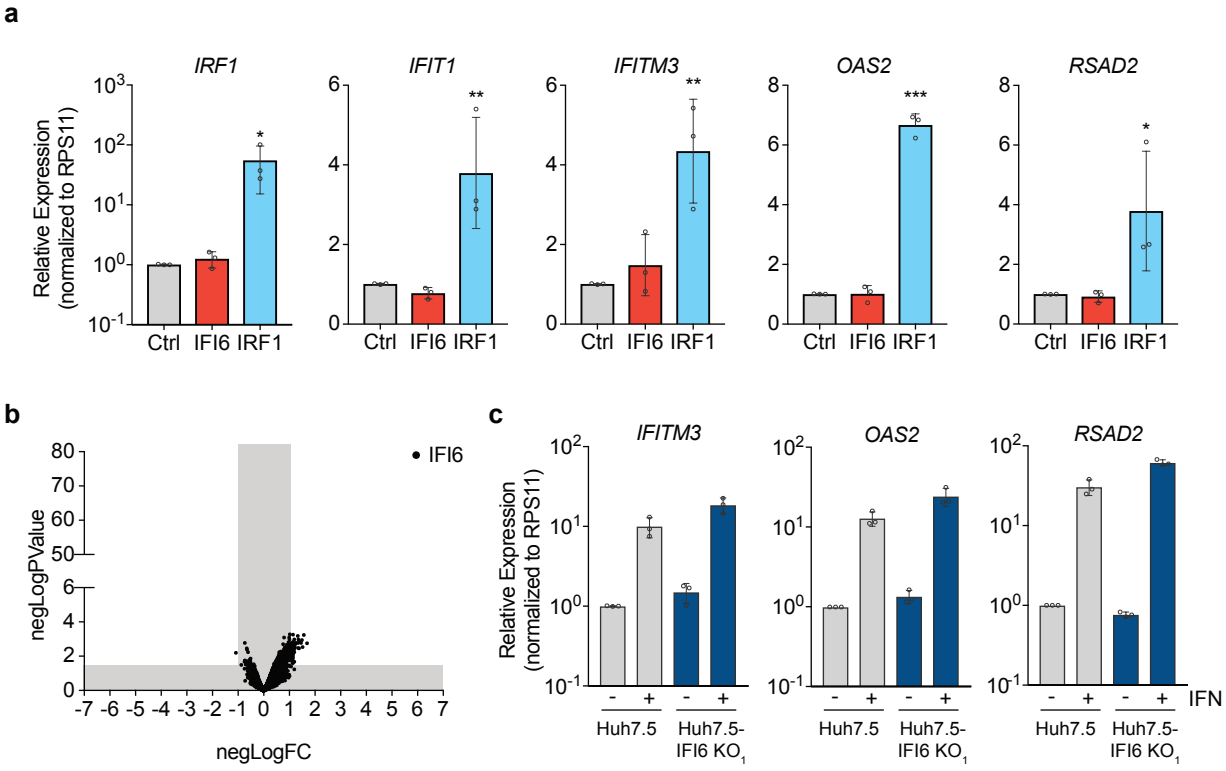


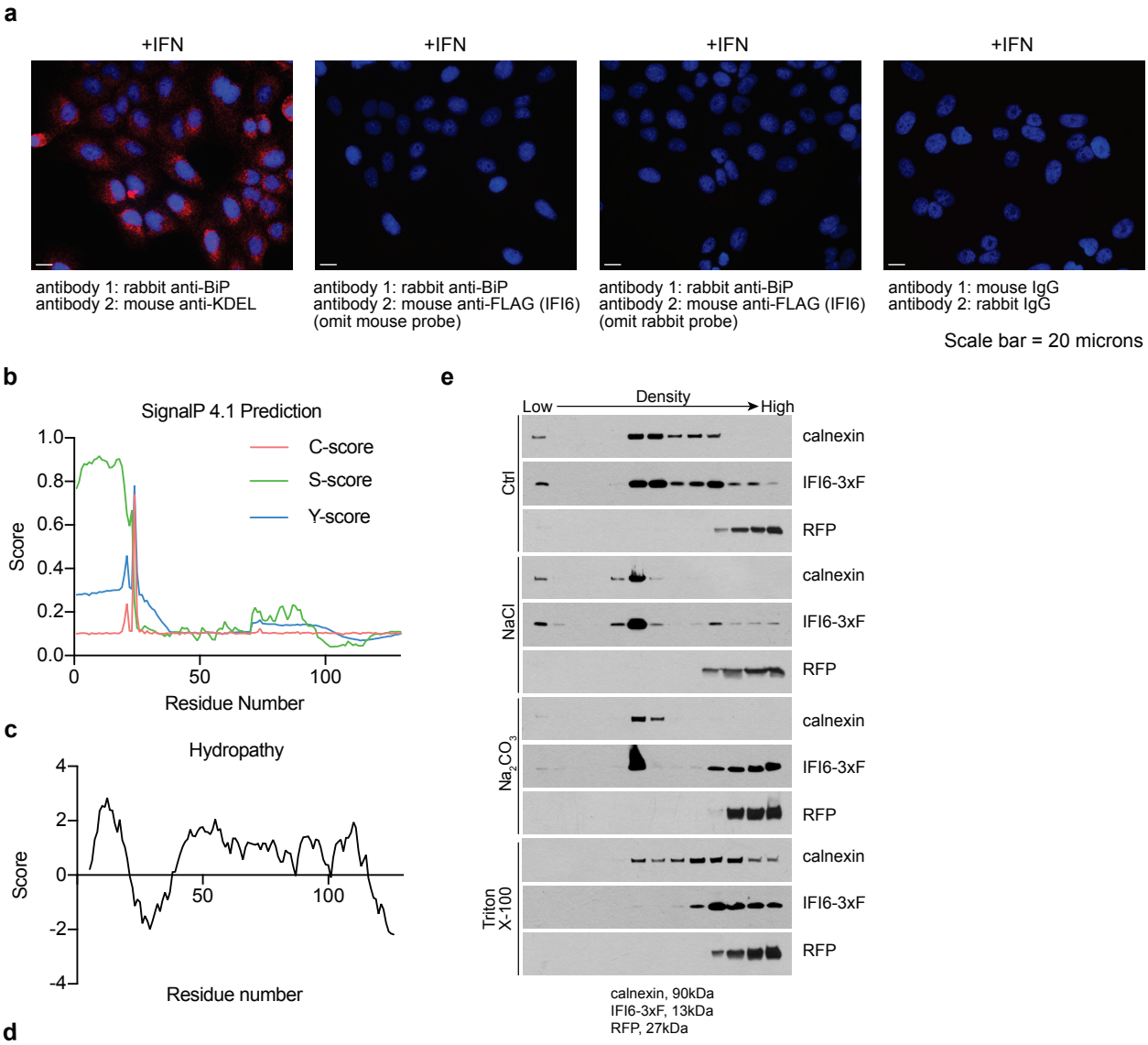
Supplementary Figure 1. IFI6 induction by IFN and inhibition of viral infection. a, Huh7.5 or Huh7.5-IFI6-KO cells were treated with IFNα over a time course and lysates were analysed by western blot using an in-house generated anti-IFI6 rabbit polyclonal antibody. Arrows point to specific induction of IFI6 relative to an ectopically expressed IFI6 control (lane 1). The molecular weight is consistent with a mature protein after cleavage of the putative signal peptide. The antibody also detects numerous non-specific bands, which confirm equal protein loading. **b,** IFNα dose response curves in Huh7.5 or Huh7.5-IFI6-KO$_1$ cells challenged with YFV-Venus, n=3. **c,d,** YFV-Venus infection in IFNα-treated A549 (**c**) or U-2 OS (**d**) cells targeted for IFI6 depletion by CRISPR. Infectivity (%GFP$^+$ cells) and replication (GFP mean fluorescence intensity) were monitored by FACS. In **c**, n=3: (left, %GFP) ** P=0.0017, **** P=9.7E-6; (right, MFI) * P=0.013, **** P=4.3E-12 (100 U/mL), **** P=6.5E-11 (1000 U/mL) (two-way ANOVA, Sidak post-test). In **d,** n=3: (left, %GFP) ** P=0.0011, **** P=7.6E-8 (untreated), **** P=7.9E-6 (100 U/mL); (right, MFI) *** P=0.00035, **** P=2.6E-6 (100U/mL) (two-way ANOVA, Sidak post-test). **e,** YFV replication in Huh7.5-NT or Huh7.5-IFI6-KO$_2$ cells with or without a single dose of IFNα (1000 U/mL) pre-treatment. n=3, **** P=1.3E-5 (two-way ANOVA, Sidak post-test). **f,** YFV-Venus infectivity in Huh7.5, 293, or *STAT1*$^{-/-}$ fibroblast cells transduced with lentivirus expressing IFI6 or an empty vector (Ctrl), n=3. **g,** Huh7.5 cells were stably transduced with lentivirus expressing IFI6 or an empty (Ctrl) vector. Cells were infected with ZIKV (PRVABC59) and infectivity was monitored by FACS, n=3.



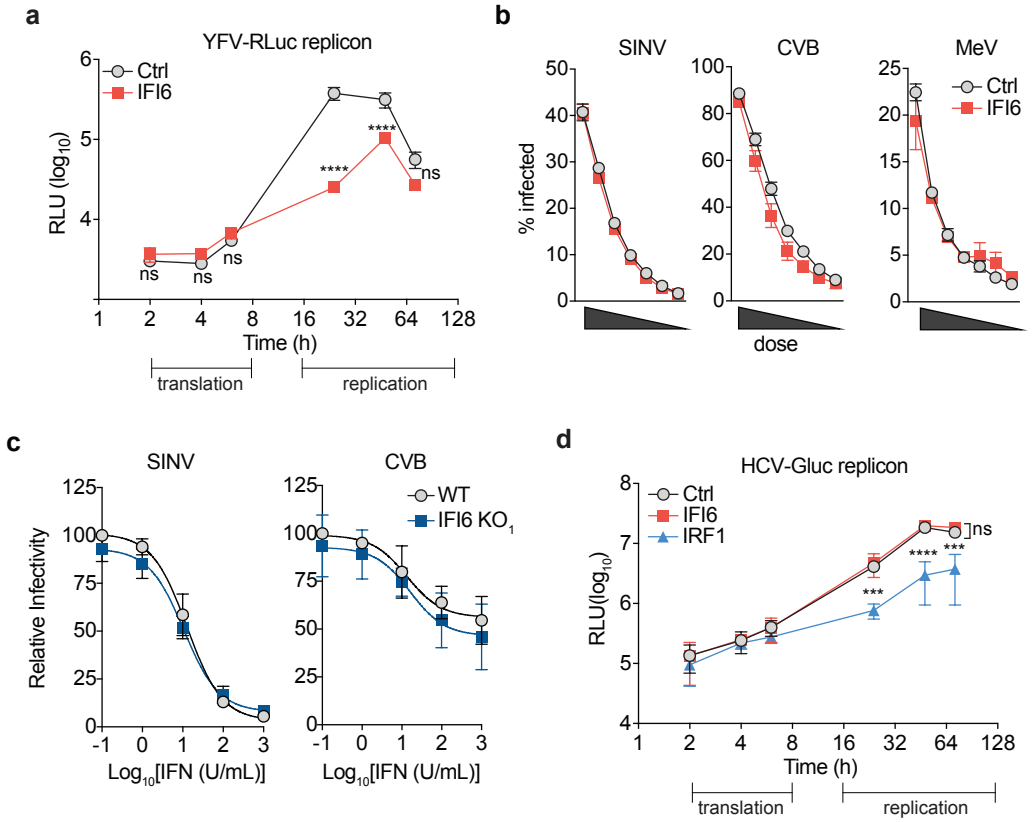
Supplementary Figure 2. Characterization of IFI-6-16 family. a, Multiple sequence alignment of human IFI-6-16 family members. Amino acid sequences (IFI6: NP_002029.3, IFI27: NP_001275885.1, IFI27L1: NP_996832.1, IFI27L2: NP_114425.1) were aligned using COBALT and graphically rendered with Jalview. Residues are colored according to physicochemical properties using a modified Zappo color scheme. **b,** Induction of *IFI6*, *IFI27*, *IFI27L1*, *IFI27L2* by 100 U/mL IFNα (6 h) in Huh7.5, U-2 OS, and A549 cells, measured by RT-qPCR, n=3. **c,** YFV-Venus infectivity in Huh7.5 cells transduced with lentivirus expressing IFI6, IFI27, or an empty vector (Ctrl), n=3.



Supplementary Figure 3. IFI6 does not modulate global antiviral gene expression. a, RT-qPCR analysis of *IRF1*, *IFIT1*, *IFITM3*, *OAS2*, and *RSAD2* mRNA expression in *STAT1*$^{-/-}$ fibroblasts transiently transduced with lentivirus expressing IFI6, an empty vector, or the antiviral transcription factor IRF1. n=3, * P=0.05 (IRF1), ** P=0.099 (IFIT1), ** P=0.006 (IFITM3), *** P=0.0001 (OAS2), * P =0.05 (RSAD2) (one-way ANOVA, Dunnett post-test). **b**, RNA-Seq analysis of global differential gene expression in *STAT1*$^{-/-}$ fibroblasts transiently transduced with lentivirus expressing IFI6 or Fluc as a control, n=3. Differential expression and statistical significance were determine by edge R software package. **c,** RT-PCR analysis of *IFITM3*, *OAS2*, and *RSAD2* mRNA expression in Huh7.5 or Huh7.5-IFI6-KO cells treated for 6 h with 100 U/mL IFNα. Results are presented as the mean of three independent experiments. Error bars represent SD.



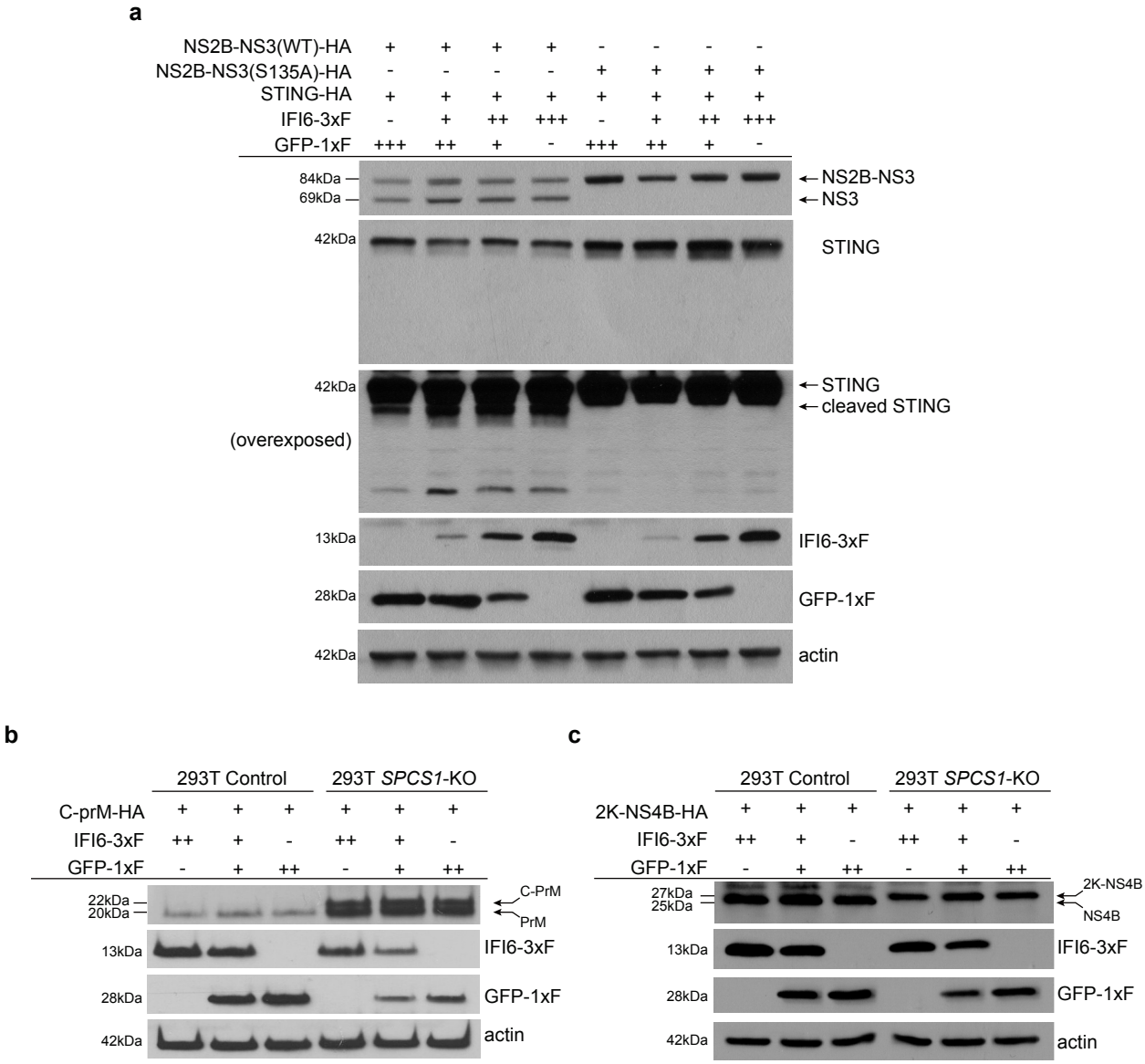
Supplementary Figure 4. Analyses supporting BiP-IFI6 interaction and localization of IFI6 to ER membranes. a, Proximity ligation assay controls to accompany **Fig 2j**. **b,** Signal peptide prediction using SignalP 4.1 server. C-score, raw cleavage site score; S-score, signal peptide score; Y-score, combined cleavage site score. For non-secreted proteins, all scores should be close to negative target value of 0.1. IFI6 has a signal peptide cleavage site after Ala-23. **c,** Kyte-Doolittle hydropathy plot of IFI6 demonstrating a highly hydrophobic stretch from amino acid 45-105. **d**, IFI6 transmembrane prediction using the TMHMM server. **e,** Similar to **Fig 3f**, membrane flotation assay of lysates from Huh7.5 cells co-expressing IFI6-3xF and RFP, using an iodixanol gradient. Fractions were analyzed by western blot with antibodies against calnexin, IFI6, or RFP.



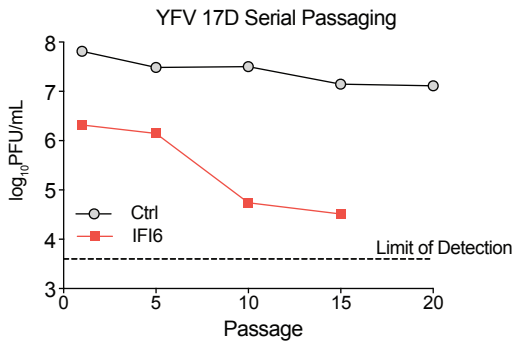
e

Species	family	genus	enveloped	genome	inhibit by IFI6
YFV, DENV, WNV, ZIKV	*Flaviviridae*	*Flavivirus*	yes	+ssRNA	yes
HCV	*Flaviviridae*	*Hepacivirus*	yes	+ssRNA	no
HCoV	*Coronaviridae*	*Betacoronavirus*	yes	+ssRNA	no
SINV	*Togaviridae*	*Alphavirus*	yes	+ssRNA	no
CVB	*Picornaviridae*	*Enterovirus*	no	+ssRNA	no
MV	*Paramyxoviridae*	*Morbillivirus*	yes	-ssRNA	no

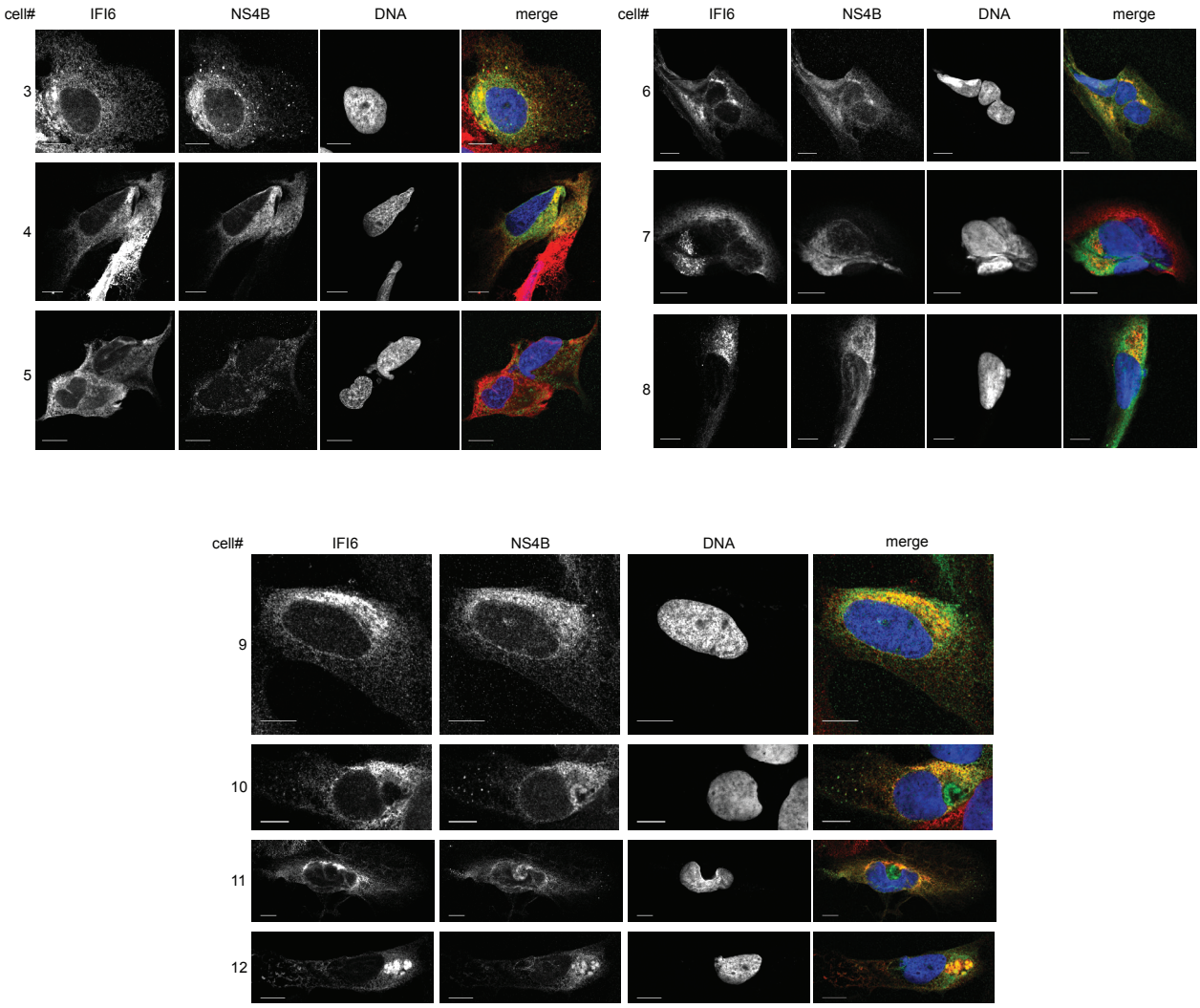
Supplementary Figure 5. IFI6 specifically inhibits flaviviruses. a, Control or IFI6-expressing Huh7.5 cells were transfected with YFV-Rluc replicon RNA and luciferase relative light units in cell lysates were measured. n=3, **** P=4E-13 (24 h), **** P=1.7E-8 (48 h) (two-way ANOVA on log-transformed data, Sidak post-test). **b,** Huh7.5 cells expressing IFI6, or an empty (Ctrl) vector were infected with the following viruses over a range of input doses: SINV-GFP (10 h), CVB-GFP infection (6 h), or MeV-GFP (24 h). **c**, IFNα dose response curves in Huh7.5 control or Huh7.5-IFI6-KO_1 cells challenged with SINV-GFP or CVB-GFP. **d,** Similar to **a**, but cells were transfected with HCV-Gluc replicon RNA. n=3, *** P=0.0014 (24 h), **** P=0.0001 (48 h), *** P=0.00026 (72 h) (two-way ANOVA on log-transformed data, Dunnett post-test). **e**, Grouping of viruses with respect to susceptibility to IFI6-mediated inhibition.



Supplementary Figure 6. IFI6 does not affect viral NS3-dependent or host SPCS1-dependent cleavage. **a.** Western blot analysis of 293T cells transfected with plasmids expressing DENV NS2B-N3 WT or protease-defective S135A mutant, in combination with STING and increasing amounts of IFI6 or EGFP as a control. NS2B-NS3 autocleavage and NS3-dependent STING cleavage products are denoted with arrows. **b,c,** Western blot analysis of 293T sgRNA control or 293T SPSC1-KO cells transfected with plasmids expressing C-prM-HA (**b**) or DENV 2K-NS4B-HA (**c**) and increasing amounts of IFI6 or EGFP as a control. Uncleaved and cleaved products are denoted with arrows. Data are representative of two (**a**) or three (**b,c**) independent replicates.



Supplementary Figure 7. Failure to rescue a viral mutant that overcomes IFI6. YFV was serially passaged every 2 days in Huh7.5 cells stably transduced with lentivirus expressing IFI6 or an empty vector. Supernatants were assayed for virus production by plaque assay on BHK-21 J cells.



Supplementary Figure 8. IFI6 colocalizes with DENV NS4B. Laser scanning confocal microscopy of Huh7.5 cells expressing IFI6-3xF and infected with DENV. Cells were stained with anti-FLAG antibody (red) or anti-NS4B antibody (green). With **Fig 4f**, data are representative images from three independent experiments.

Supplementary Figure 9. Uncropped Western blots

The following 14 pages contain images of uncropped Western blots used in main paper figures. Dotted boxes indicate lanes used from each scanned blot.

Figure 1n

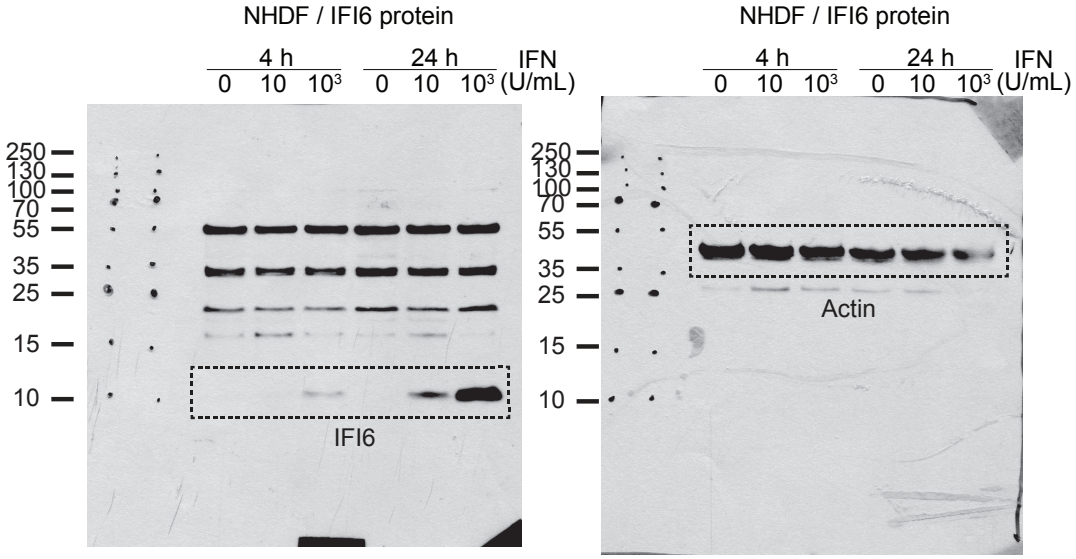


Figure 1o

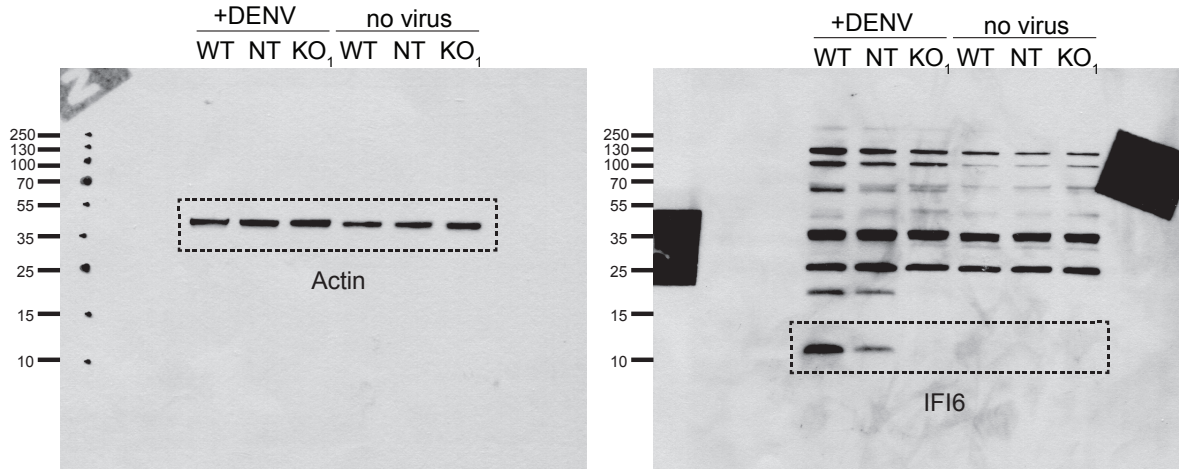
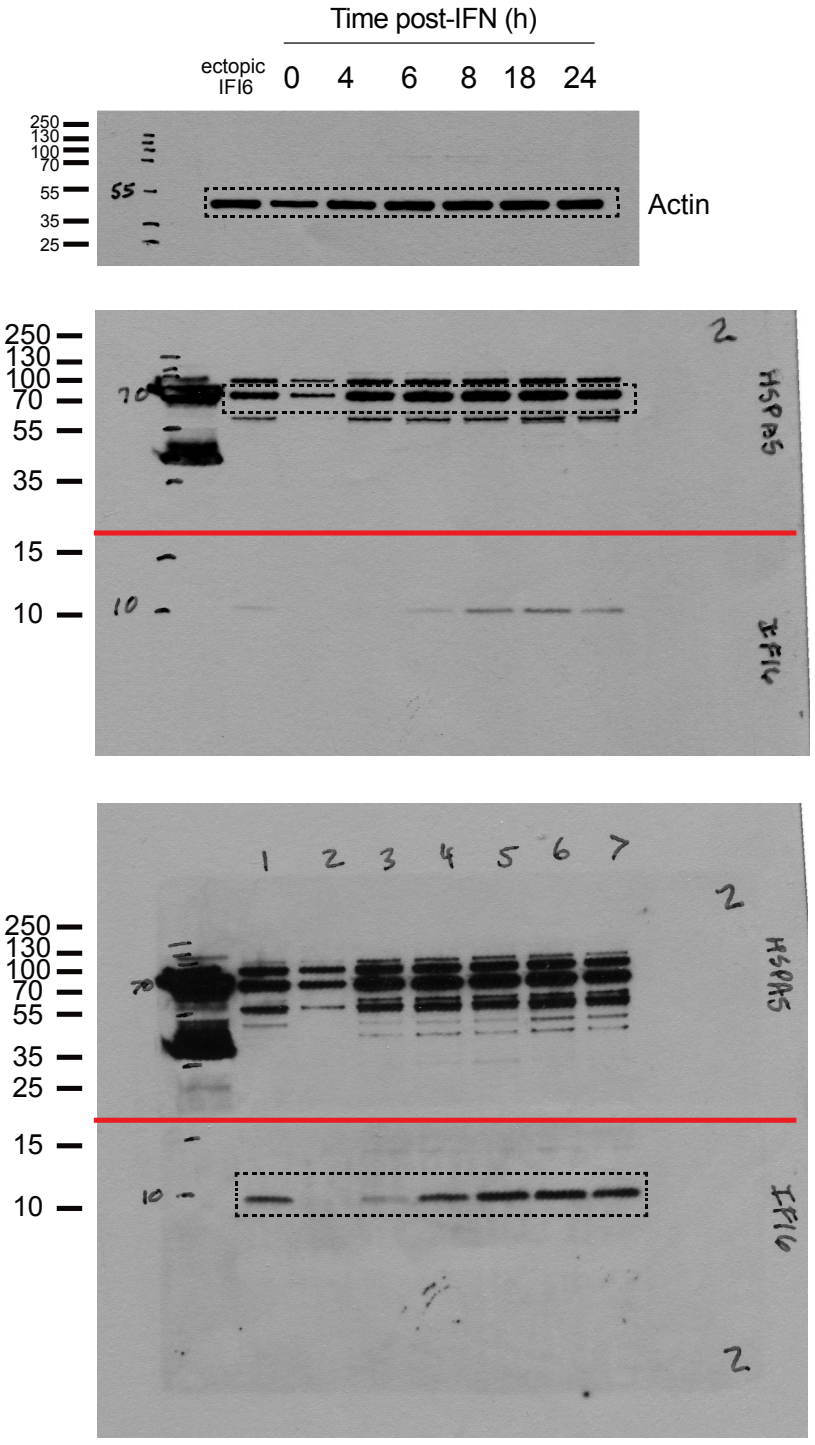
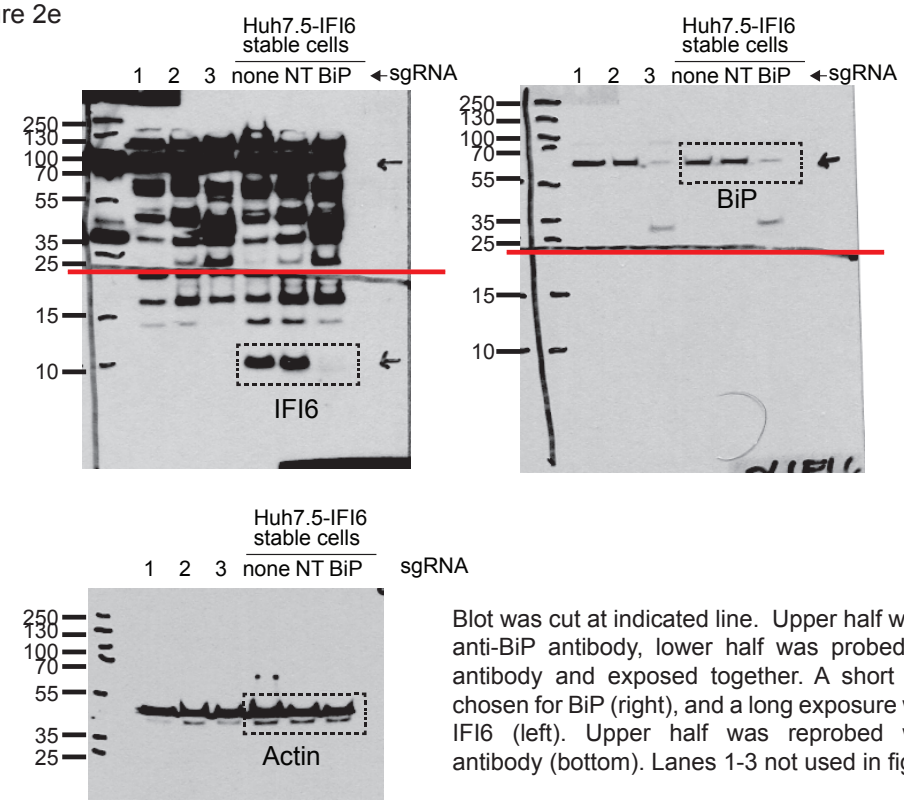


Figure 2a



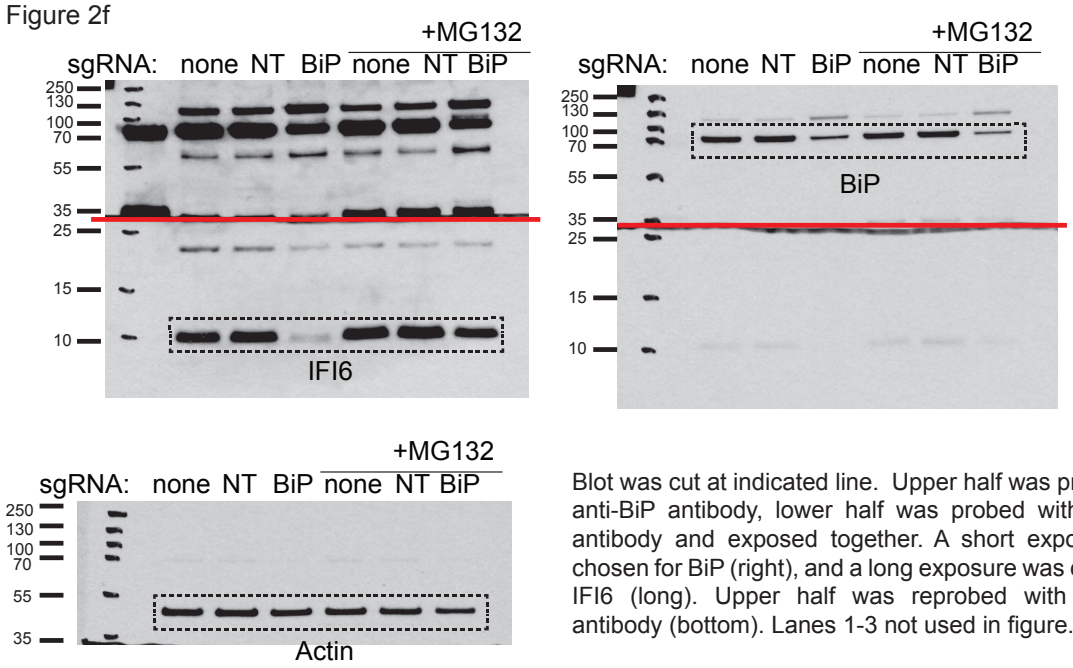
Blot was cut at indicated line. Upper half was probed with anti-BiP, lower half was probed with anti-IFI6 and exposed together. A short exposure was chosen for BiP (top), and a long exposure was chosen for IFI6 (bottom)`.

Figure 2e



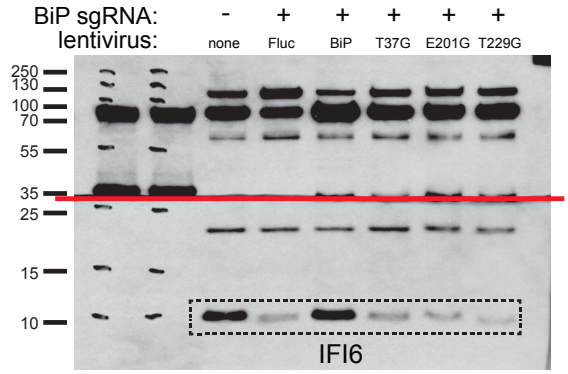
Blot was cut at indicated line. Upper half was probed with anti-BiP antibody, lower half was probed with anti-IFI6 antibody and exposed together. A short exposure was chosen for BiP (right), and a long exposure was chosen for IFI6 (left). Upper half was reprobed with anti-actin antibody (bottom). Lanes 1-3 not used in figure.

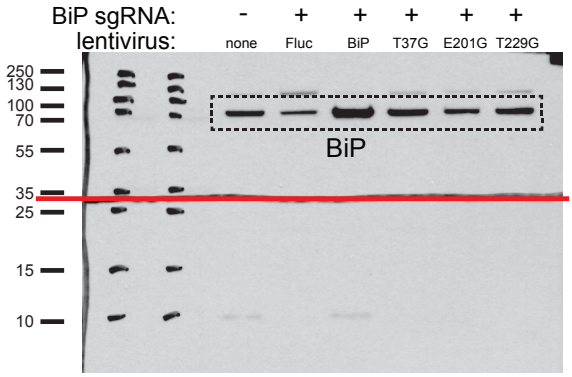
Figure 2f

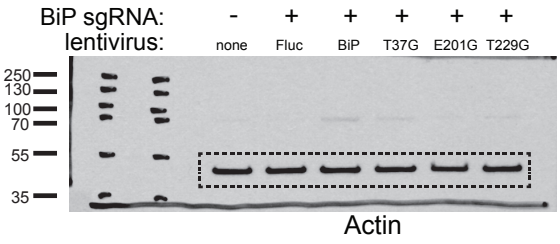


Blot was cut at indicated line. Upper half was probed with anti-BiP antibody, lower half was probed with anti-IFI6 antibody and exposed together. A short exposure was chosen for BiP (right), and a long exposure was chosen for IFI6 (long). Upper half was reprobed with anti-actin antibody (bottom). Lanes 1-3 not used in figure.

Figure 2g







Blot was cut at indicated line. Upper half was probed with HSPA5, lower half was probed with IFI6 and exposed together. A short exposure was chosen for BiP(right), and a long exposure was chosen for IFI6 (left). Upper half was reprobed for actin (bottom).

Figure 2h

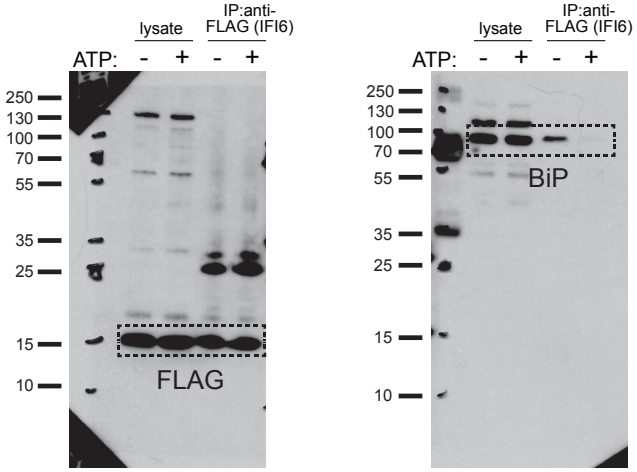


Figure 2i

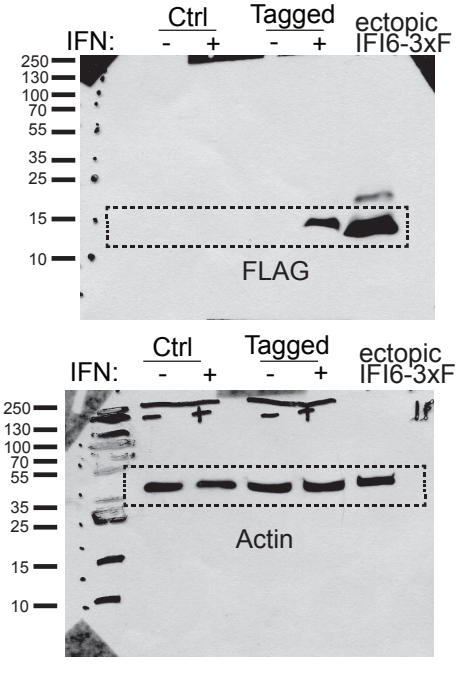


Figure 3f, anti-calnexin

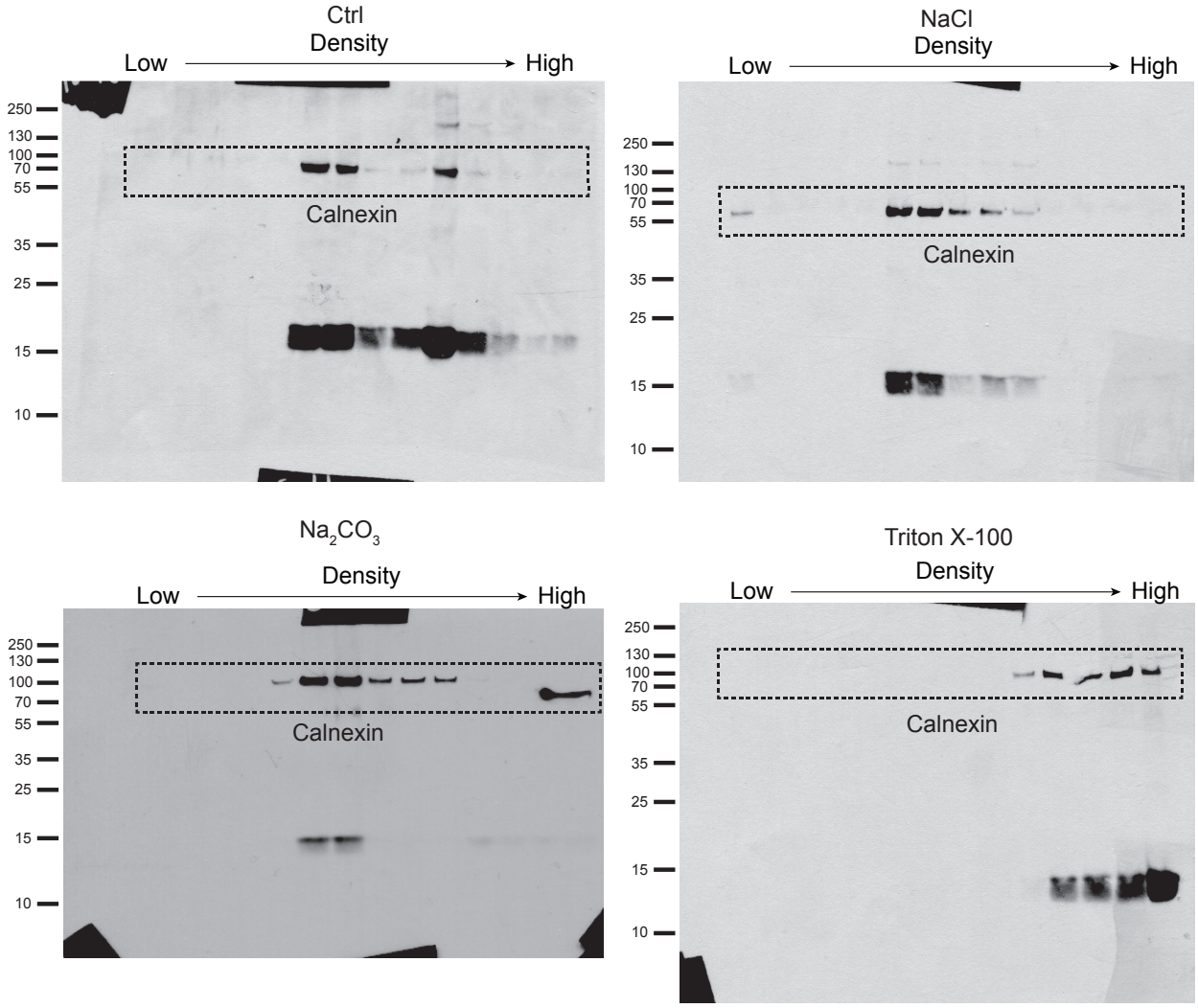


Figure 3f, anti-FLAG (IFI6)

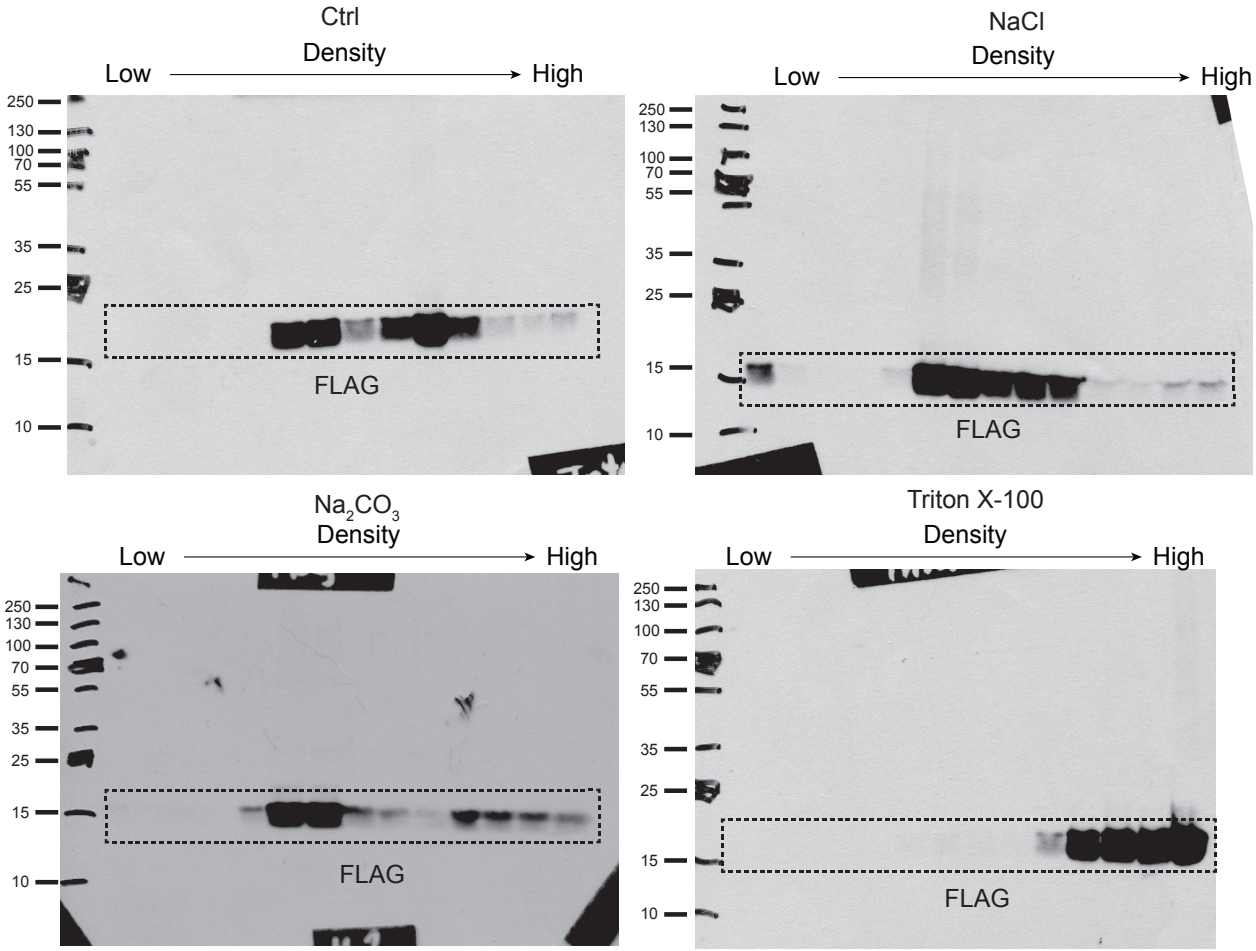


Figure 3g

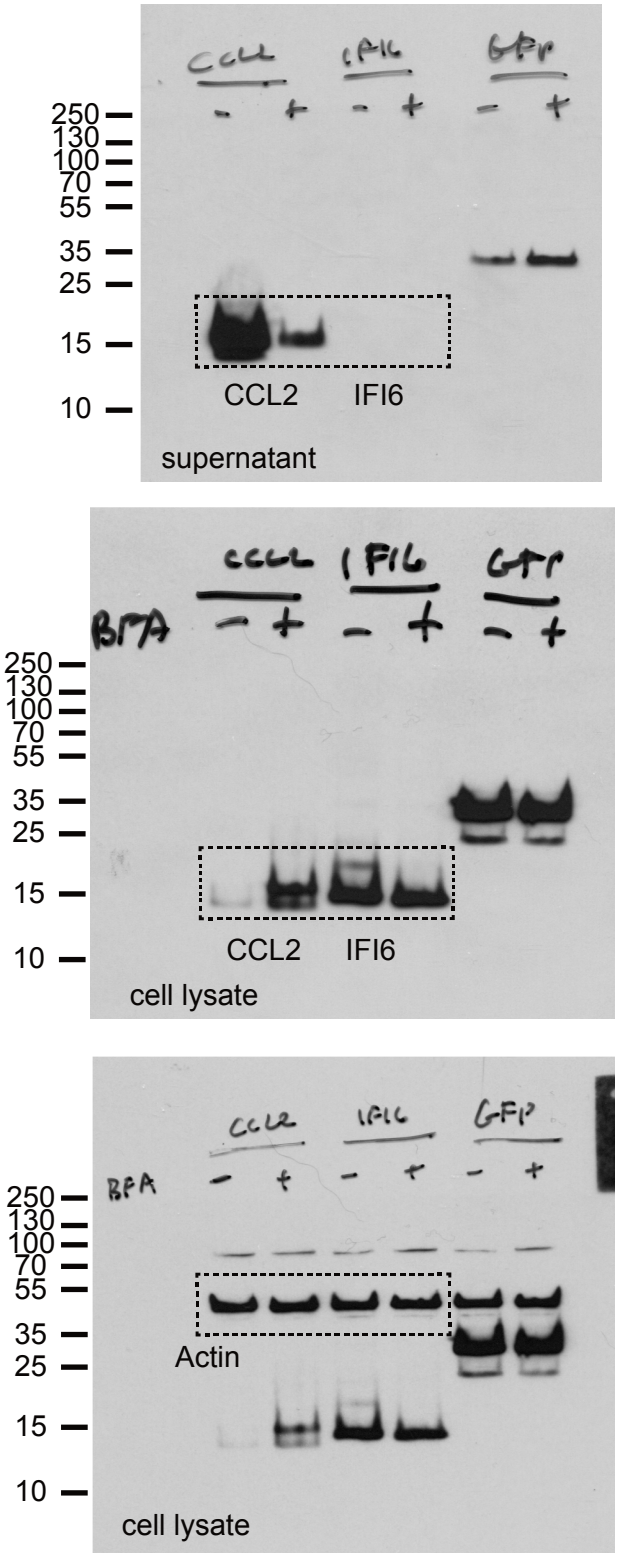
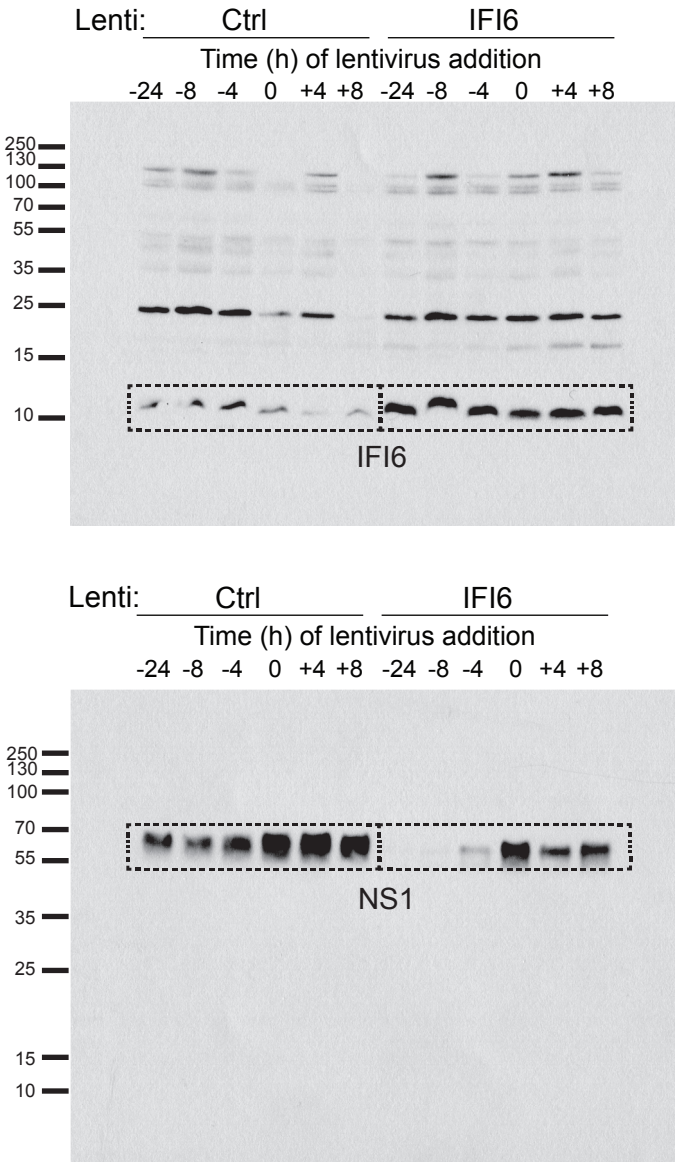

CCL2
IFI6
GFP
- + - + - +
250
130
100
70
55
35
25
15
10
CCL2
IFI6
supernatant
BFA
cell lysate
Actin
cell lysate

Figure 4e


Lenti: Ctrl IFI6
Time (h) of lentivirus addition
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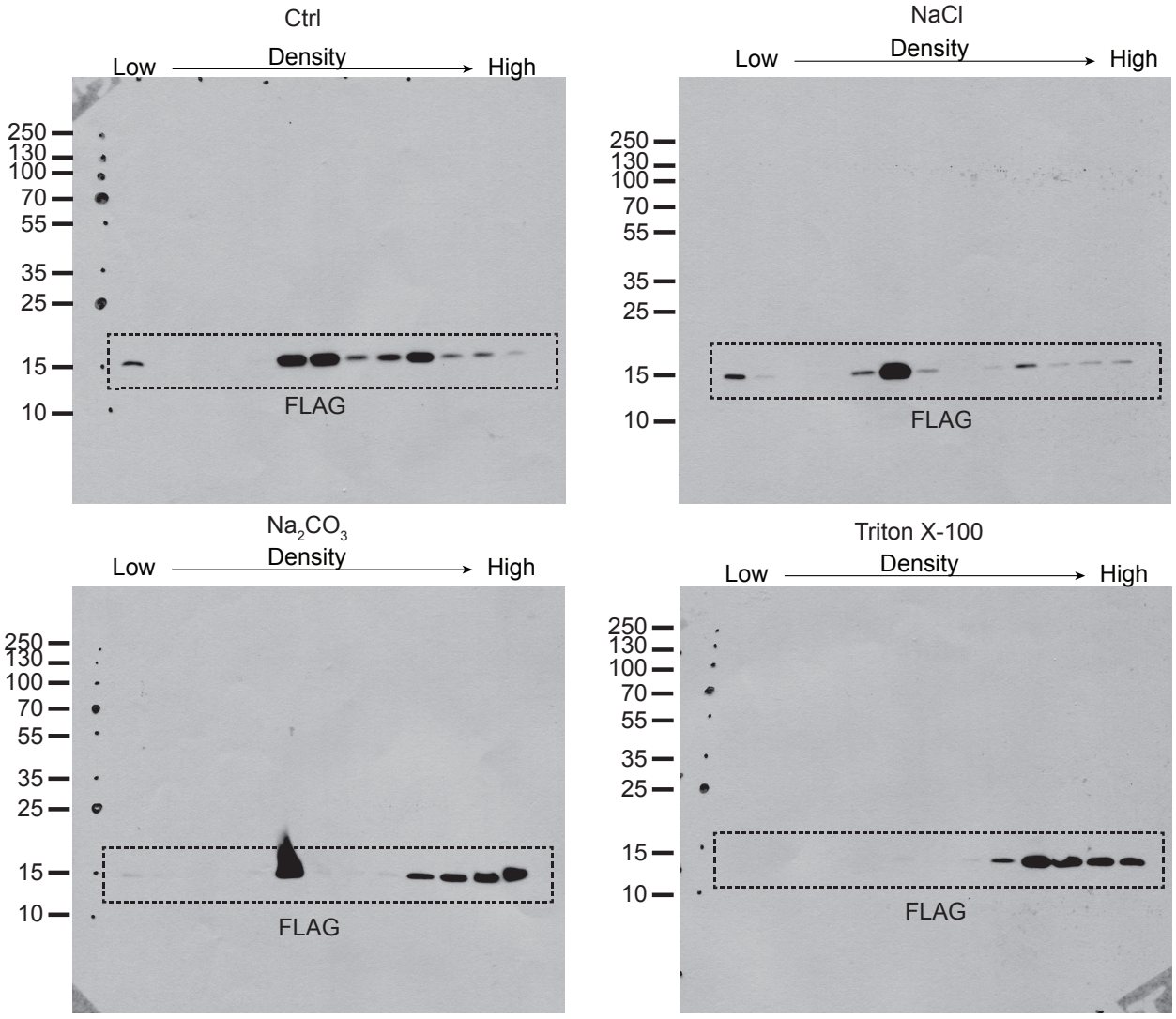
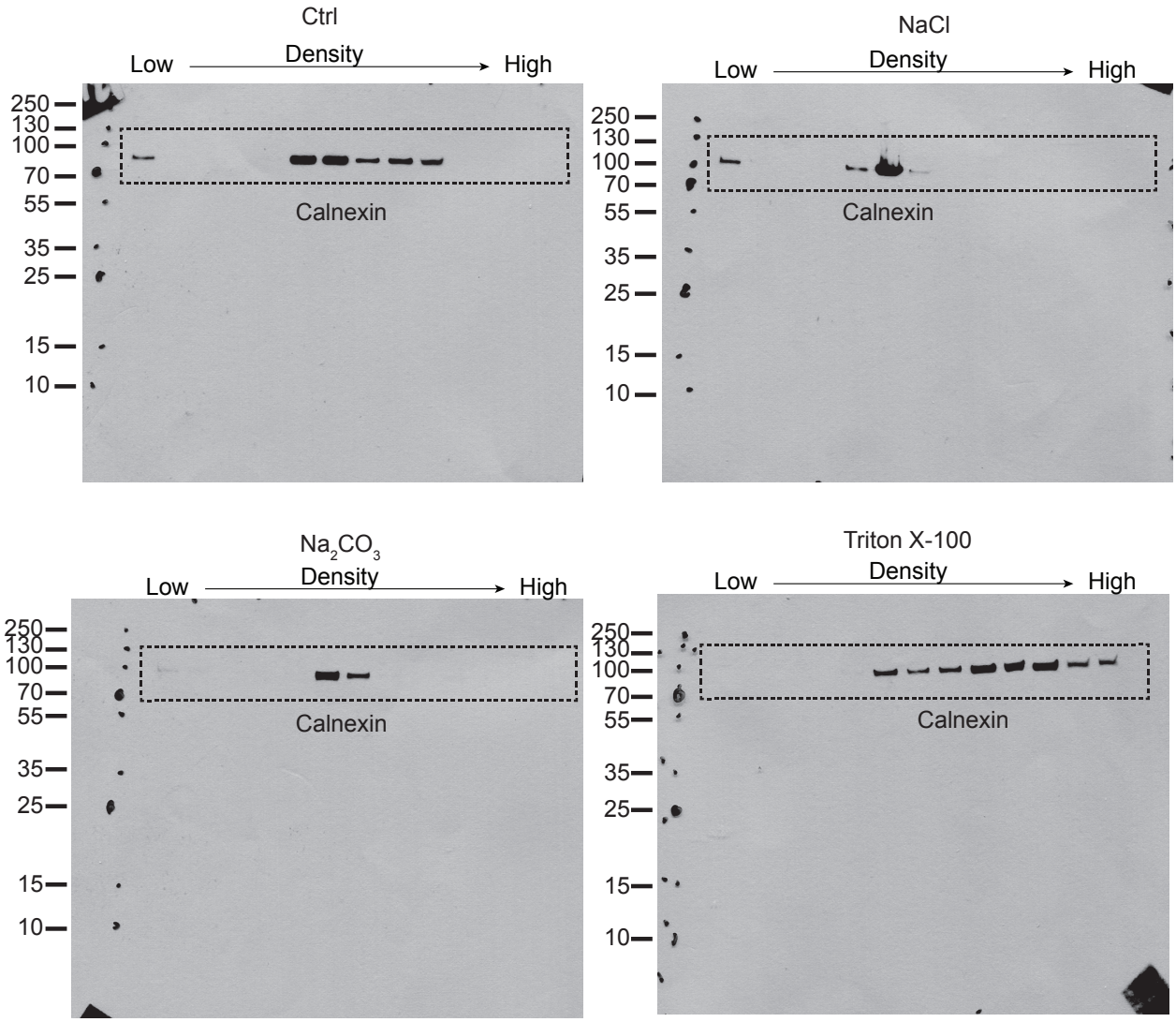
250
130
100
70
55
35
25
15
10
IFI6
Lenti: Ctrl IFI6
Time (h) of lentivirus addition
-24 -8 -4 0 +4 +8 -24 -8 -4 0 +4 +8
250
130
100
70
55
35
25
15
10
NS1

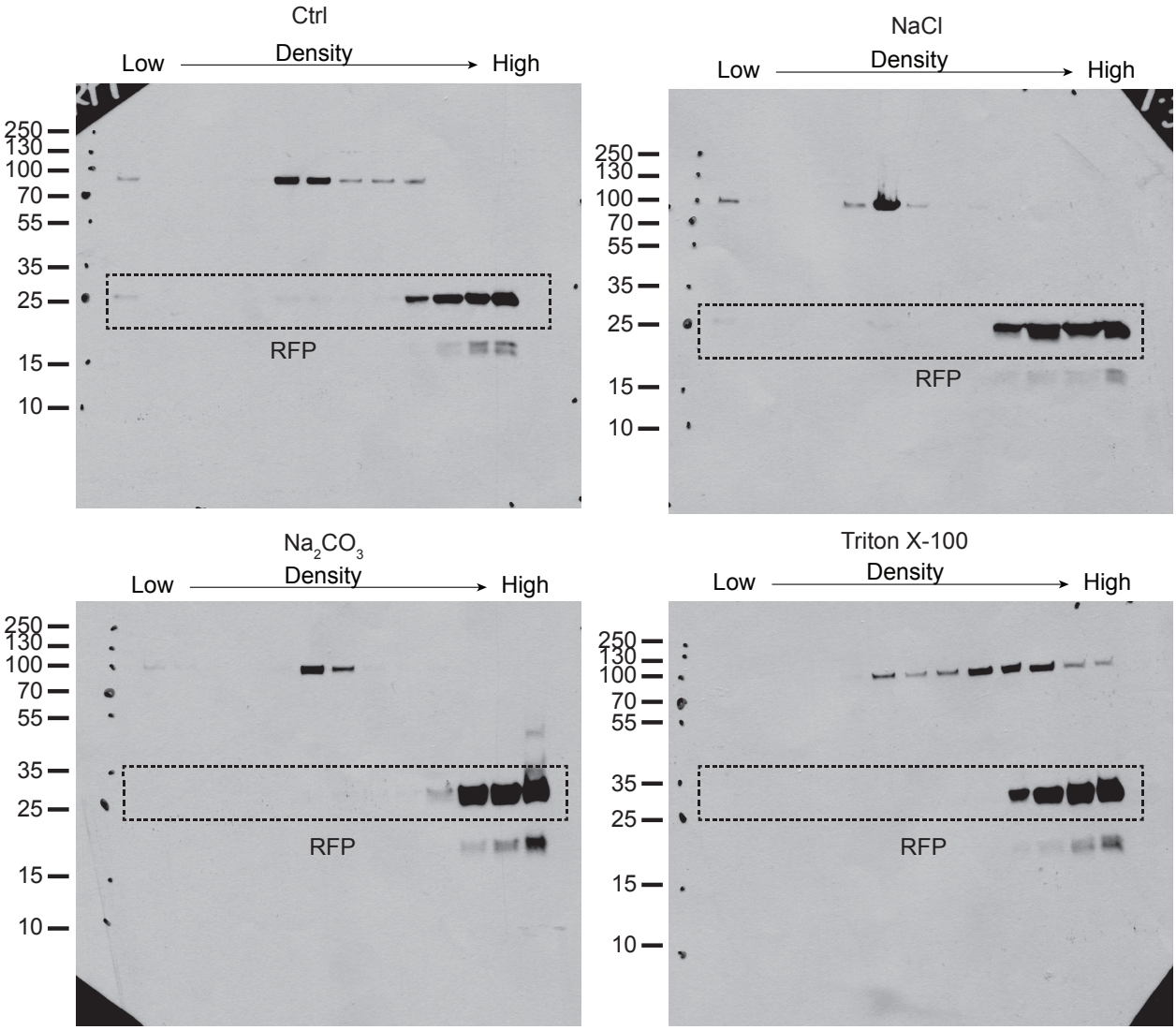
Supplementary Figure 4e, anti-FLAG (IFI6)



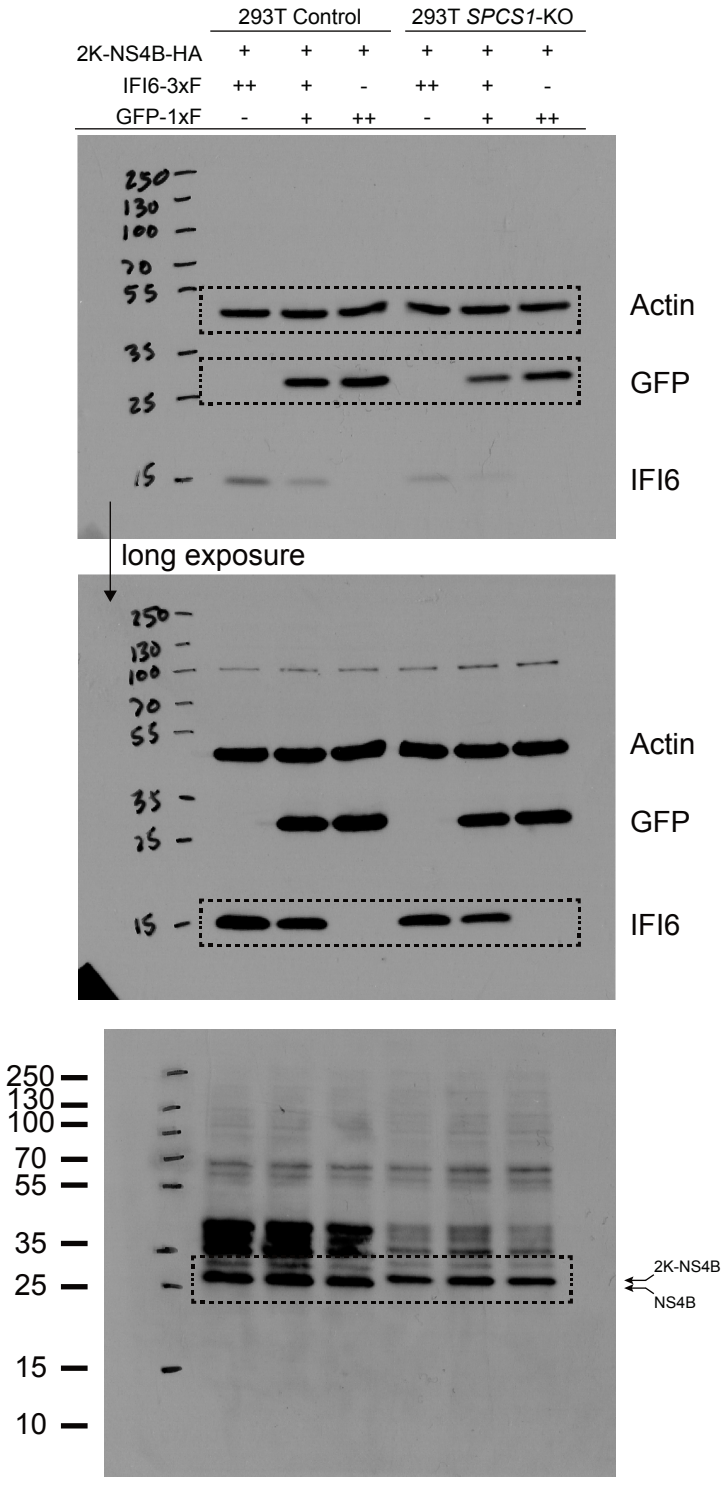
Supplementary Figure 4e, anti-calnexin



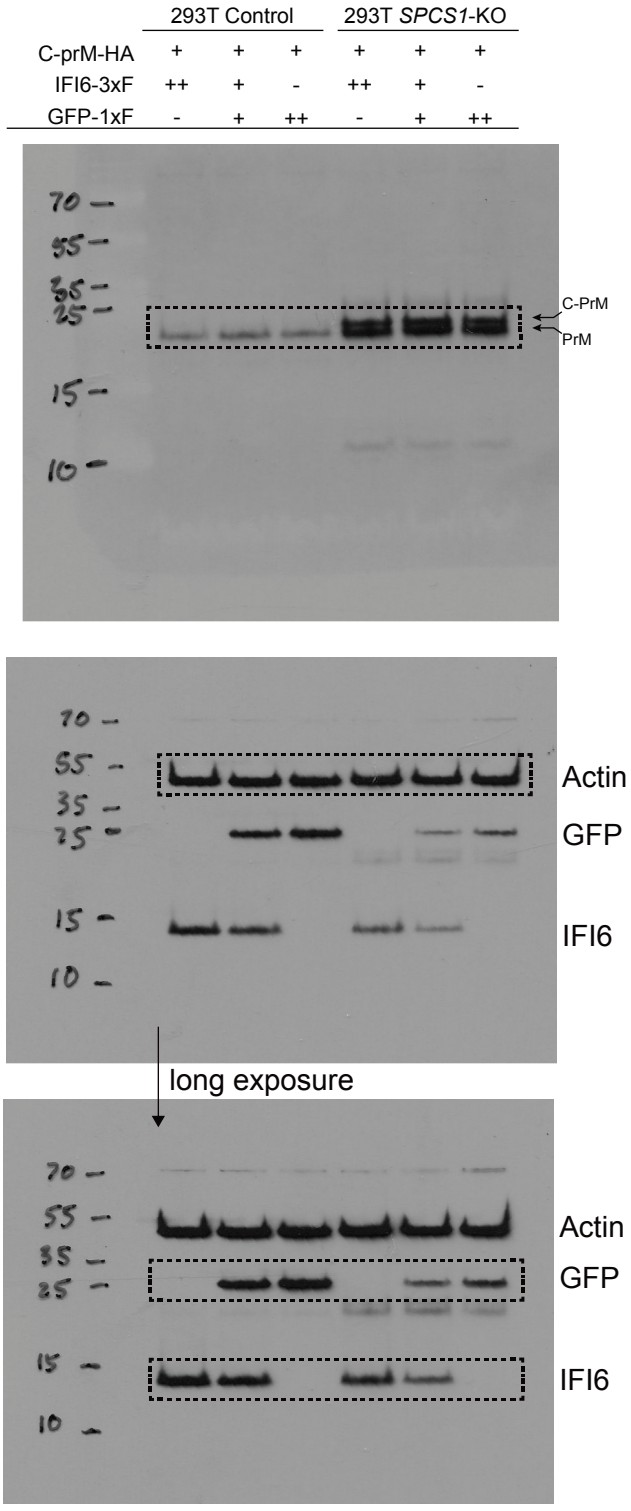
Supplementary Figure 4e, RFP



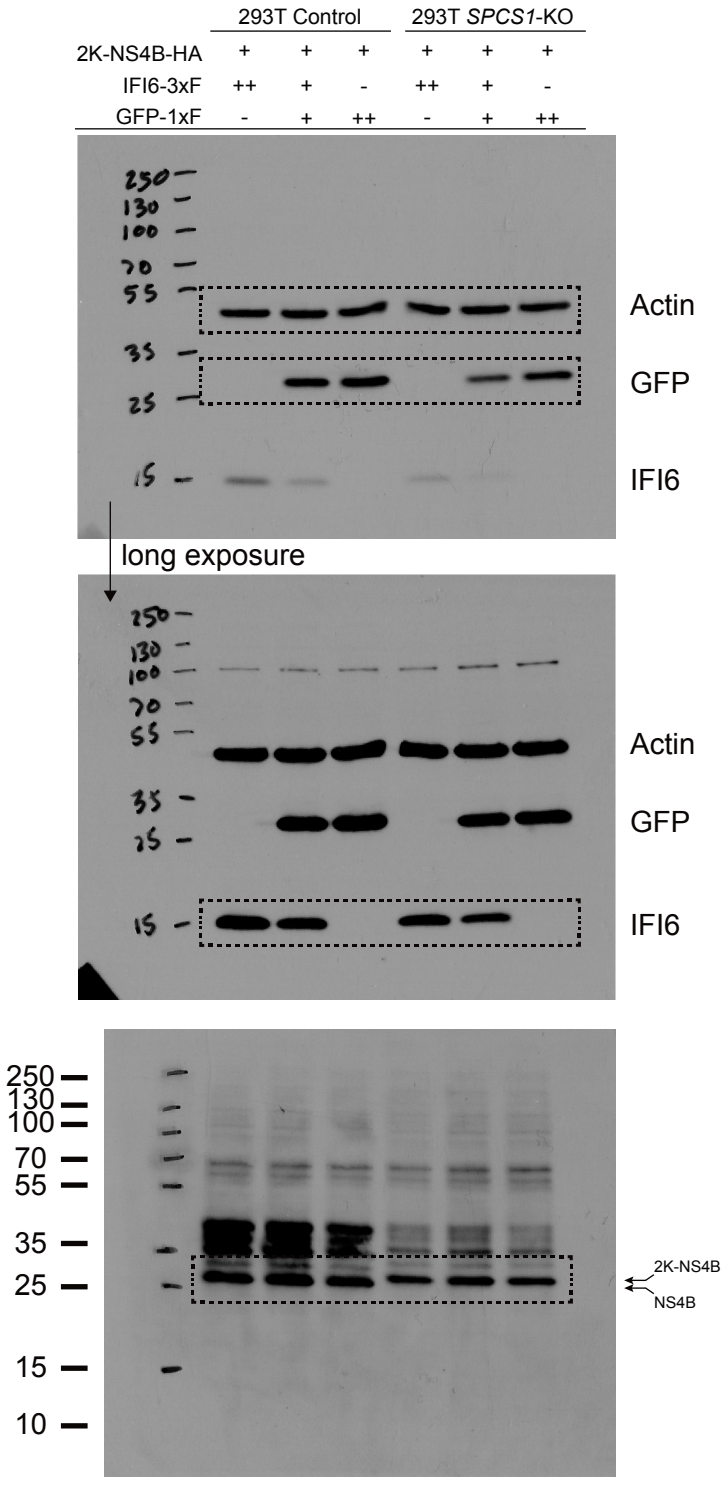
Supplementary Figure 6c



Supplementary Figure 6b



Supplementary Figure 6c



Supplementary Table 1. MAGeCK analysis output of CRISPR screen.

The accompanying Microsoft Excel (.xlsx) file contains the MAGeCK analysis output for the CRISPR screen shown in Figure 1. The screen was performed one time at a library coverage of approximately 900X. Statistical significance was assigned to genes with FDR < 0.01.

Supplementary Table 2. Oligos used in this study

Cloning and PCR oligos		
Primer name	**Application**	**5' to 3' sequence**
IFI6-3xFLAG forward 1	FLAG insertion by overlap PCR	gctagtggccacgCTGCAG
IFI6-3xFLAG reverse 1	FLAG insertion by overlap PCR	CACCGTCATGGTCTTTGTAGTCGGATCCct cctcatcctcctcactatcgagatactt
IFI6-3xFLAG forward 2	FLAG insertion by overlap PCR	aagtatctcgatagtgaggaggatgaggagGGATCCG ACTACAAAGACCATGACGGTG
IFI6-3xFLAG reverse 2	FLAG insertion by overlap PCR	TGCGGCCGCACTCGAGGAATTCCTACTTG TCATCGTCATCCTTGTAGTC
IFI6-HA forward	HA insertion by oligo annealing	GATCCTACCCATACGATGTTCCAGATTAC GCTTAGC
IFI6-HA reverse	HA insertion by oligo annealing	TCGAGCTAAGCGTAATCTGGAACATCGTA TGGGTAG
5-SacI-GFP	GFP fusion to IFI6 N terminus	catatagagctcgATGGTGAGCAAGGGCGAGG AG
3-GFP-XhoI	GFP fusion to IFI6 N terminus	gttttctcgagcTACTTGTACAGCTCGTCCATGC CG
OAS2-Forward	RT-PCR to detect OAS2	GAACACCATCTGTGACGTCCT
OAS2-Reverse	RT-PCR to detect OAS2	GAGCCACCTATGGCCACTCC
IFI6-Forward	RT-PCR to detect IFI6	GGTCTGCGATCCTGAATGGG
IFI6-Reverse	RT-PCR to detect IFI6	TCACTATCGAGATACTTGTGGGT
BiP T37G Forward	BiP site-directed mutagenesis	GCATCGACCTGGGGGGCACCTACTCCTG CG
BiP T37G Reverse	BiP site-directed mutagenesis	CGCAGGAGTAGGTGCCCCCCAGGTCGAT GC
BiP E201G Forward	BiP site-directed mutagenesis	GAGGATCATCAACGGGCCTACGGCAGCT GC
BiP E201G Reverse	BiP site-directed mutagenesis	GCAGCTGCCGTAGGCCCGTTGATGATCCT C
BiP T229G Forward	BiP site-directed mutagenesis	CCTGGGTGGCGGAGGCTTCGATGTGTCTC TTCTC
BiP T229G Reverse	BiP site-directed mutagenesis	GAGAAGAGACACATCGAAGCCTCCGCCAC CCAGG
DENV 2KNS4B HA Forward	PCR amplify DENV 2K-NS4B	accATGGGATCCACACCCCAAGACAAC

DENV 2KNS4B HA Reverse	PCR amplify DENV 2K-NS4B	GGTAgcccgcggcaccaccCCTTCTTGTGTTGGT
5 DENV-C-prM	PCR amplify DENV C-prM	ccATGGGATCCAGGAGACGCAGATCTGCAGG
3 DENV-C-prM	PCR amplify DENV C-prM	gtttcccgcggcaccaccTGTCATTGAAGGAGTGACAGCTG

CRISPR oligos		
Target gene	**Oligo**	**5' to 3' sequence**
IFI6	BR2 Guide1, oligo 1	caccgCTGCTGCTCTTCACTTGCAG
IFI6	BR2 Guide 1, oligo 2	aaacCTGCAAGTGAAGAGCAGCAGc
IFI6	IFI6 guide 1 (g1g2), oligo 1	caccgCGGCATCGCGGCCAACTCGG
IFI6	IFI6 guide 1 (g1g2), oligo 2	aaacCCGAGTTGGCCGCGATGCCGc
IFI6	IFI6 guide 2 (g1g2), oligo 1	caccgCTGACCTTCATGGCCGTCGG
IFI6	IFI6 guide 2 (g1g2), oligo 2	aaacCCGACGGCCATGAAGGTCAGc
IFI6 3xFLAG	FLAG Guide, oligo 1	caccgCCACAAGTATCTCGATAGTG
IFI6 3xFLAG	FLAG Guide, oligo 2	aaacCACTATCGAGATACTTGTGGc
BiP	Guide 1, oligo 1	caccgAATGGCAAGGAACCATCCCG
BiP	Guide 1, oligo 2	aaacCGGGATGGTTCCTTGCCATTc
BiP	Guide 2, oligo 1	caccgCAGACGGGTCATTCCACGTG
BiP	Guide 2, oligo 2	aaacCACGTGGAATGACCCGTCTGc
BiP	Guide 3, oligo 1	caccgCGACATAGGACGGCGTGATG
BiP	Guide 3, oligo 2	aaacCATCACGCCGTCCTATGTCGc
Non-targeting control 80255	Oligo 1	caccgGAACTCAACCAGAGGGCCAA
Non-targeting control 80255	Oligo 2	aaacTTGGCCCTCTGGTTGAGTTCc
Non-targeting control 80243	Oligo 1	caccgTGTTCTACTTTCGAAGTTAA
Non-targeting control 80243	Oligo 2	aaacTTAACTTCGAAAGTAGAACAc
IFI6 3xFLAG	FLAG Guide, oligo 1	caccgCCACAAGTATCTCGATAGTG
IFI6 3xFLAG	FLAG Guide, oligo 2	aaacCACTATCGAGATACTTGTGGc

CRISPR sequencing primers	
Primer	**5' to 3' sequence**
P5 0 nt stagger	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTTGTGGAAAGGACGAAACACCG
P5 1 nt stagger	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTGTGGAAAGGACGAAACACCG

P5 2 nt stagger	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCG ATCTGCTTGTGGAAAGGACGAAACACCG
P5 3 nt stagger	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCG ATCTAGCTTGTGGAAAGGACGAAACACCG
P5 4 nt stagger	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCG ATCTCAACTTGTGGAAAGGACGAAACACCG
P5 6 nt stagger	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCG ATCTTGCACCTTGTGGAAAGGACGAAACACCG
P5 7 nt stagger	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCG ATCTACGCAACTTGTGGAAAGGACGAAACACCG
P5 8 nt stagger	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCG ATCTGAAGACCCTTGTGGAAAGGACGAAACACCG
P7 barcode A01	CAAGCAGAAGACGGCATACGAGATCGGTTCAAGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTCCAATTCCCACTCCTTTCAAGACCT
P7 barcode A02	CAAGCAGAAGACGGCATACGAGATGCTGGATTGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTCCAATTCCCACTCCTTTCAAGACCT
P7 barcode A03	CAAGCAGAAGACGGCATACGAGATTAACTCGGGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTCCAATTCCCACTCCTTTCAAGACCT
P7 barcode A04	CAAGCAGAAGACGGCATACGAGATTAACAGTTGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTCCAATTCCCACTCCTTTCAAGACCT
P7 barcode A05	CAAGCAGAAGACGGCATACGAGATATACTCAAGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTCCAATTCCCACTCCTTTCAAGACCT
P7 barcode A06	CAAGCAGAAGACGGCATACGAGATGCTGAGAAGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTCCAATTCCCACTCCTTTCAAGACCT
P7 barcode A07	CAAGCAGAAGACGGCATACGAGATATTGGAGGGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTCCAATTCCCACTCCTTTCAAGACCT

Supplementary Methods

Genome-wide CRISPR screen

The human "Brunello" CRISPR knockout pooled library was purchased (#73179, addgene.org) and amplified according to instructions. The amplified pooled plasmid library was used to make infectious lentivirus from 293T cells. The day before transfection, 293T cells were seeded into twenty polylysine-coated 15 cm plates at a density of $6x10^6$ cells per plate. The day of transfection, the culture media was exchanged for 15mL DMEM supplemented with 3% FBS, 1X NEAA, and Pen-Strep, and pooled library plasmid was then co-transfected with lentiviral packaging plasmids expressing Gag-Pol and VSV-g at a ratio of 5:4:1 using X-tremeGENE 9 reagent (Roche) in transfection reactions containing 30 total ug of DNA per plate. Six h post-transfection, transfection complex-containing media was removed and replaced with 23 mL DMEM supplemented with 3% FBS, 1X NEAA, and Pen-Strep. Culture supernatant containing lentiviral particles was collected at 48 and 72 h post-transfection, pooled, and HEPES and polybrene was added to a final concentration of 20mM and 4ug/mL, respectively. To eliminate plasmid DNA carryover into NextGen sequencing reactions for subsequent screens[41], pooled library lentiviral supernatant was treated with Benzonase (Sigma) by adding 20X Benzonase buffer (1M Tris-HCl pH 8.0, 20mM $MgCl_2$, 2mg/mL BSA) to a final concentration of 1X, and 50U/mL Benzonase was added and the supernatant was incubated for 30 min at 37C. Treated lentiviral supernatant was filtered through a 0.45 micron filter and aliquots were stored at -80C. Lentiviral transduction efficiency was determined by transducing Huh7.5 cells with volumes of lentiviral supernatant ranging from 500µl to 10µl in 6w plates for 48 hrs, and the ratio (X100%) of puromycin-resistant to puromycin-sensitive cells following 3 days of incubation after splitting equal numbers of transduced cells into media with and without 4µg/ml puromycin was determined using CellTiter-Glo reagent (Promega). The volume of lentivirus that produced 30% transduction efficiency was used for all subsequent transductions in order to minimize the likelihood that any single cell would be infected with more than one lentivirus at a time during library screening.

To perform the IFNα CRISPR screen, Huh7.5 cells were seeded into ten 6-well plates at a density of $4x10^5$ cells per well and transduced the next day with library lentiviral supernatant in 1ml per well of DMEM supplemented with 3%FBS, 1X NEAA, 20mM HEPES, and 4ug/mL polybrene for 1 h, after which 2 mL per well of DMEM complete media was added. After 48 h, transduced cells from all wells were trypsinized, pooled, and re-plated into nine 15 cm plates containing DMEM supplemented with 10% FBS, 1x NEAA, and 4μg/ml puromycin and incubated for 3 days to select for transduction. Puromycin-selected cells were harvested with trypsin, pooled, counted, and reseeded into eight 15 cm plates at a density of $6.5x10^6$ cells per plate, corresponding to 675X library coverage. Cells were allowed to adhere to plates for 3 h, then were treated overnight with DMEM supplemented with 10%FBS, 1X NEAA and 100U/ml IFNα. The next day, each plate was infected with 0.8 mL YFV-17D-venus (MOI = 1) in 16ml of DMEM supplemented with 1% FBS and 1X NEAA for 3 h, then an additional 16 ml of DMEM complete media was added and the cells were incubated overnight. Twenty-four h post-infection the cells were trypsinized, pooled, pelleted and resuspended in FACS buffer (PBS, 2% FBS, 0.5 mM EDTA). Cells were filtered with a 100-micron cell strainer, stored on ice, then sorted at the Children's Medical Center Research Institute Flow Cytometry Facility by a FACSAria II (Becton Dickenson) flow cytometer while kept at 4°C. GFP-positive gated cells were collected in cell collection buffer (PBS, 50% FBS, 50 mM HEPES), and pelleted. Genomic DNA (gDNA) was extracted from isolated cell pellets following lysis in 500μl of tissue lysis buffer, containing 460μl of STE buffer (1 mM EDTA (pH 8.0), 10 mM Tris-HCl (pH 8.0), 100 mM NaCl) supplemented with 10μl of 0.5 M EDTA, 10μl of proteinase K (10 mg/ml in 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA), and 20μl of 10% SDS. Lysates were incubated overnight at 55°C while shaking at 550rpm on a Thermomixer (Eppendorf). The following day, 5μl of 2 mg/ml RNase A was added to each tube and incubated at 37°C for 1 h while shaking at 550rpm. Extractions were collected after mixing samples with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) twice, followed once by chloroform; each extraction was separated using MaXtract high density phase lock tubes (Qiagen). Twenty micrograms of glycoblue (Roche) and 1mL of 100% ethanol were

added to each sample and DNA was precipitated at −20°C for 1h followed by centrifugation at 18,000*g* for 10 minutes at 4°C. Pellets were washed with 1mL of 75% ethanol, dried, and resuspended in 50µL of water by incubating at 4°C overnight. Uninfected cell pellets of transduced cells were lysed and gDNA was extracted for controls. To amplify sgRNA sequences for Next Gen Sequencing, four parallel 100µl PCR reactions were run for each condition, and pooled. Each 100µl PCR reaction contained 6-10µg of gDNA, Ex Taq polymerase, pooled P5 and barcoded P7 primers (listed in supplementary table 1) as previously described[42]. DNA was purified for sequencing using AMPure XP beads (Agencourt) by mixing 300µl of pooled PCR with 150µl beads and incubated for 5 minutes to pre-clear genomic DNA. Magnetic separation was used to collect the supernatant. The supernatant was then mixed with 540µl of AMPure XP beads and incubated for 5 minutes to bind the PCR products. The supernatant was collected and discarded. Beads were washed twice with 1mL 70% ethanol and then dried for approximately 5 minutes. Bound DNA was eluted from the beads using 300µl sterile water. Before sequencing, all PCR DNA libraries were analyzed using a Bioanalyzer High Sensitivity DNA Analysis Kit (Agilent). Library concentration was determined by qPCR using a KAPA Library Quantification Kit for Illumina platforms. The samples were sequenced using Illumina NextSeq 500 with the read configuration as 75bp, single end. Each sample got approximately 10 million to 15 million reads. An in-house script was used to trim the adapter sequences from raw de-multiplexed FASTQ files and unique 20bp sgRNA sequences were processed for further downstream analysis. The reference sgRNA sequences for the human Brunello library were downloaded from Addgene (https://www.addgene.org/pooled-library/). Identical sgRNAs targeting the same protein coding genes were removed from the reference library. Software MAGeCK (v0.5.1) was used for data analysis. Sample reads were mapped to the reference sgRNA library with mismatch option as 0. Median normalization was performed to adjust for library sizes and read counts. Positively and negatively selected sgRNA and genes were identified with default parameters.

Supplementary References

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