

Supplemental Materials

Supplemental Methods

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Supplemental Table 1. Oligonucleotide list for primers used in cloning and sequencing for CRISPR constructs and *ANTXR1* expression constructs.

Supplemental Methods

Reagents and Bacterial Strains

Polymerase chain reactions (PCRs) were carried out using a GeneAmp PCR System 9700 Thermocycler (Applied Biosystems). PCR fragments were purified using QIAquick PCR Purification Kit (Qiagen). Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen). Competent DH10B and Stbl3 cells were purchased from Invitrogen. Plasmids were isolated and purified from bacteria using QIAquick Spin Miniprep Kit (Qiagen). Sanger sequencing for individual clones and plasmids was performed by Genewiz, Inc. Nucleotide and protein sequence alignments were performed in Geneious Pro 4.7.6.

Cell Lines and Viruses

All cell culture media and solutions were produced by the Memorial Sloan Kettering Cancer Center (MSKCC) Media Prep core facility. HAP1 cells were purchased from Haplogen GmbH and maintained in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% fetal calf serum. TC-71 cells were obtained from Children's Oncology Group (COG) Cell Culture Repository and maintained in IMDM supplemented with 10% fetal bovine serum and 1× ITS supplement. All other cell lines used in this study were purchased from the American Type Culture Collection (ATCC). HEK 293T/17 cells were maintained in high glucose Dulbecco's Modified Eagle Medium (DMEM HG) supplemented with 10% fetal calf serum and 1 mM sodium pyruvate. All additional lines were maintained in RPMI 1640 supplemented with 10% fetal calf serum and 10 mM HEPES. All cell lines are routinely confirmed by STR analysis and confirmed mycoplasma negative by DDC Medical. Purified SVV was provided by Neotropix, Inc., (Malvern, PA). SVV and SVV-GFP reporter virus, which contains a fusion protein of GFP and a 2A sequence immediately following the native 2A sequence, was cultured, purified, and titered as previously described (1, 2).

Human GeCKO v2 Library

Plasmid DNA libraries were used as a template in a nested PCR to first amplify the section of the plasmid containing the sgRNA (Supplemental Table 1; primer #1-2) and subsequently to add Illumina sequencing adaptors and barcodes (primer #3-18). The nested PCR products were then sequenced for confirmation of sgRNA representation using an Illumina HiSeq2500 (primer #19; Supplemental Figure 1b).

Library Transfection

For the human GeCKO library, 20 15 cm² dishes (Corning) were seeded with HEK 293T/17 cells (7.0×10^6 per plate). The GeCKO libraries A and B were pooled 1:1 (54 µg each library) and co-transfected with psPAX2 (72 µg) and pMD2.G (36 µg) with 432 µL of 1 mg/mL PEI in 36 mL OptiMEM. The transfection mix was then divided equally among the 15 cm² dishes. Sixteen hours post transfection, media on each dish was changed and supplemented with 1 U/mL DNase I (New England Biolabs). The media lentiviral supernatant was harvested 72 h post transfection and filtered through a 0.45 µm Stericup PVDF filter (Millipore). The virus was then pelleted by

ultracentrifugation at 24,000 rpm for 2 h at 4°C. The virus pellet was resuspended in fresh DMEM and incubated overnight at 4°C. Lentivirus aliquots were stored at -80°C.

GeCKO Library Screen

The human GeCKO v2 library lentivirus was titered on parental HAP1 and H446 cells as described previously (3, 4). Parental cells were transduced with the lentiCas9-Blast lentivirus, allowing for constitutive expression of the DNA nuclease, Cas9. The lentiCas9-Blast plasmid was a gift from Feng Zhang (Addgene plasmid #52962). Transduced cells were selected with 6 µg/mL blasticidin. HAP1-Cas9 cells (2.0×10^8) were seeded equally in 70 15 cm² dishes. H446-Cas9 cells (1.5×10^8) were seeded into 50 15 cm² dishes. GeCKO lentivirus was thawed on ice, mixed in a total volume of 375 mL OptiMEM supplemented with 32 µg/mL polybrene, and divided equally among the HAP1-Cas9 or H446-Cas9 plates. Lentivirus was added at an MOI=0.4 transduction units/cell (TU/cell) for both screens. Additional media was added to each plate to bring the final polybrene concentration to 8 µg/mL. Media was changed 24 h post transduction to remove polybrene. The media was changed 48 h post transduction to select transduced H446-Cas9 or HAP1-Cas9 cells with 0.5 µg/mL or 1.0 µg/mL puromycin, respectively. Transduced cells were allowed to grow for 4 additional days to allow for complete knockdown of sgRNA-targeted genes. On day 7 post transduction in the HAP1 screen, 2.0×10^8 cells were plated at equal cell density in 40 15 cm² dishes and infected with SVV at a MOI=1,000 vp/cell the next day. On day 7 post transduction in the H446 screen, 1.5×10^8 cells were plated at equal cell density in 50 15 cm² dishes and infected with SVV at a MOI=1.0 vp/cell the next day. The remaining cells for each cell line were pooled, pelleted by centrifugation, and stored in -80°C as the corresponding Day 7 post transduction sample. For one week post SVV infection during the HAP1 screen, 15 mL media on the infected plates were exchanged every 3 days to resupply cells with fresh media. Surviving cells were pooled, pelleted by centrifugation, and stored at -80°C as the SVV resistant sample. For two weeks post SVV infection during the H446 screen, 10 mL media on the infected plates were exchanged every 3 days to resupply cells with fresh media. Visible colonies of surviving cells were collected by isolated trypsinization in cloning cylinders and seeded in 1 well of a 24 well plate (Corning). All colonies too small for isolation were harvested by trypsinization and pooled before expansion. Each isolated colony was ultimately expanded from a 24 well to a 75 cm² flask as the cells were propagated. Cells from each colony were pelleted by centrifugation and stored in -80°C.

Identification of sgRNAs

For the HAP1 screen, extracted genomic DNA from Day 7 post transduction and SVV resistant population was used as a template for the GeCKO v2 library nested PCR and analyzed for sgRNA representation by Illumina HiSeq as described above. Sequenced sgRNAs were imported from raw FASTQ files, normalized for library size, then converted to log counts per million reads (logCPM). Log fold change was then calculated between control and resistant samples. Based on the distribution of non-targeting sgRNAs, we focused on genes for which ≥ 2 unique sgRNAs had average logCPM>6 and logFC>5. Gene-wise testing was performed by the Mann-Whitney test. Computer code available upon request.

For the H446 screen, extracted genomic DNA from each SVV resistant colony was used as a PCR template to amplify the lentiviral insert containing the gene-targeting sgRNA (primer # 54).

The PCR product was purified then sequenced via Sanger sequencing. PCR products that contained multiple sgRNAs, as determined by Sanger sequencing, were ligated into the linearized pCR2.1 plasmid using the TA Cloning Kit (Invitrogen). The ligation reaction was transformed into DH10B cells (Invitrogen) and selected on LB agar plates supplemented 100 µg/mL carbenicillin (Fisher). Colonies from each transformation plate were isolated, amplified, and sequenced by Sanger sequencing.

Individual sgRNA Plasmids

The lentiCRISPR v2 plasmid, which expresses a single sgRNA under the hU6 promoter and the WT Cas9 nuclease under the EFS promoter, was a gift from Feng Zhang (Addgene plasmid #52961). Oligos containing the gene targeting sgRNA with 5' overhang BsmBI digestion sites were synthesized by Sigma Aldrich (primers #22-53). The oligos were annealed and inserted into the lentiCRISPR v2 backbone as described previously (3, 4). sgRNA sequences and plasmids were confirmed by Sanger sequencing.

CRISPR Secondary Screens

Individual targeting sgRNAs were cloned into the lentiCRISPRv2 plasmid as previously described (3, 4). The sgRNA plasmids were individually transfected into HEK 293T/17 cells and transduced in parental H446 or HAP1 cells as described above. Transduced H446 cells were selected with 0.5 µg/mL puromycin. Transduced HAP1 cells were selected with 1.0 µg/mL puromycin. Cells were allowed to grow for at least 7 days post transduction to allow for complete gene knockout. Cell viability was assessed by AlamarBlue fluorescent cell viability dye (ThermoFisher Scientific) as described below.

Identification of ANTXR1 indels in ANTXR1 KO lines

Extracted genomic DNA from parental H446 cells and ANTXR1 KO mutant clones was used as a PCR template to amplify the target of the *ANTXR1* sgRNA, exon 2 of the *ANTXR1* gene, using sequence specific primers (primer #55-56). The PCR product was ligated into the linearized pCR2.1 plasmid using the TA Cloning Kit (Invitrogen). The ligation reaction was transformed into DH10B cells (Invitrogen) and selected on LB agar plates supplemented 100 µg/mL carbenicillin (Fisher Scientific). Colonies from each transformation plate were isolated, amplified, and sequenced by Sanger sequencing. Exon 2 sequences from *ANTXR1* KO lines were compared to WT H446 exon 2 sequences and *ANTXR1* gene reference sequence (NG_012649.1; Pubmed) to identify indels in each cell line.

Lentivirus Production

The lentiviral packaging plasmids pMD2.G (Addgene plasmid #12259) and psPAX2 (Addgene plasmid #12260) were gifts from Didier Trono. All transfections of lentiviral plasmids were performed as follows unless otherwise stated: Lentiviral plasmids were transfected at a 3:2:1 DNA ratio of lentiviral plasmid:psPAX2:pMD2.G in 1 mg/mL polyethylinimine (PEI; Sigma Aldrich) at a 2:1 PEI:DNA ratio in OptiMEM (Gibco). Media was changed 16 h post transfection. Seventy-two hours post transfection the virus containing media was harvested and filtered through a 0.45 µm PDVF syringe filter (Millipore) to remove cell debris. Lentivirus aliquots were stored at -80°C.

Lentiviral Transductions

All lentiviral transductions were performed as follows unless otherwise stated: Cells (1.0×10^6) to be transduced were plated in a 75 cm² flask the day before transduction. Lentivirus was thawed on ice and added to OptiMEM supplemented with 32 µg/mL polybrene. After the virus-OptiMEM mix was added to the cells, additional media was added to bring the final polybrene concentration to 8 µg/mL. Media was changed 24 h after transduction to remove polybrene. Media supplemented with 0.5 µg/mL puromycin (Sigma Aldrich) or 6 µg/mL blasticidin (Fisher Scientific) was changed 48 h after transduction to select lentiCRISPRv2 or lentiCas9-Blast transduced cells, respectively. Transduced cells were maintained in media containing either puromycin or blasticidin. For Dox inducible ANTXR1 lentivirus (pInducer20-ANTXR1), the SCLC H69 and H146 cell lines were transduced and maintained in tetracycline-free (tet-free) media supplemented with 500 µg/mL G-418 (Thermo Fisher).

Inducible ANTXR1 Expression Plasmid Construction

To create an inducible *ANTXR1* expression lentiviral plasmid, we obtained a plasmid expressing a full-length *ANTXR1* cDNA as a generous gift from Drs. Stephen Leppla and Shihui Liu from NIH/NIAID. The plasmid (ANTXR1-HA), which constitutively expresses the *ANTXR1* cDNA fused to a C-terminal influenza virus hemagglutinin (HA) tag, was used as a template in PCR with primers incorporating Gateway attB1 and attB2 cloning sequences (primer # 57-58). The PCR product was purified and subsequently used in a BP reaction with the Gateway destination vector, pDONR221 (Invitrogen), transformed into DH10B cells, and selected on LB agar plates supplemented with 50 µg/ml kanamycin (Sigma Aldrich). The plasmid containing the PCR fragment was purified and used in an LR reaction with pInducer20, a gift from Stephen Elledge (Addgene plasmid #44012). The recombinant plasmid was transformed into Stb13 cells and selected on LB agar plates supplemented with 100 µg/ml carbenicillin (Sigma). The purified plasmid, containing a doxycycline (Dox) inducible *ANTXR1* cDNA with the HA fusion tag, was confirmed by Sanger sequencing and used to produce lentivirus for the creation of stable cell lines.

ANTXR1-KO Lines

All cell lines were transduced with *ANTXR1.3* sgRNA lentiCRISPRv2 lentivirus as described above. Transduced cells in each cell line were selected with 1.0 µg/mL puromycin. Cells were allowed to grow for at least 7 days post transduction to allow for complete gene knockdown. For the H446 *ANTXR1* KO mCherry cell line, a pLenti6 W118-mCherry lentiviral expression plasmid containing the fluorescent mCherry protein cDNA was used to produce lentivirus as described above. The H446 *ANTXR1* KO 4 clone isolated from the H446 GeCKO screen was transduced with the mCherry lentivirus as described previously. Forty-eight hours after transduction, transduced cells were isolated by positive mCherry fluorescence using fluorescent activated cell sorting (FACS) with a BD FACSAria (Becton Dickinson) and subsequently cultured as a stable cell line (H446 *ANTXR1* KO mCherry).

ANTXR1 Expression Experiments

For re-expression experiments in SCLC *ANTXR1* KO lines, cells were transiently co-transfected with ANTXR1-HA and pLenti6 W118-mCherry expression plasmids at a 10:1 molar ratio in 1 mg/mL PEI in OptiMEM with cells transfected with mCherry alone as controls. Cells were challenged with SVV-GFP at the TCID₅₀ for each cell line 48 h post transfection and harvested for analysis 6 hr post SVV-GFP infection. For expression experiments in non-permissive SCLC cell lines, pInducer20-ANTXR1 transduced H69 or H146 cells were seeded in 6-well plates 16 h prior to the start of the experiment. Cells were maintained in tet-free media alone or supplemented with 1 µg/mL doxycycline for 72 h prior to the addition of SVV-GFP. Cells were incubated with SVV-GFP for 6 h then harvested for analysis.

ANTXR1 Expression Experiments

Unless otherwise stated, cells were transfected and analyzed as follows: *ANTXR1* rescue experiments were performed with the ANTXR1-HA expression plasmid. Cells were plated in tissue culture treated 6-well plates 24 h prior to transfection. Cells were transiently co-transfected with ANTXR1-HA and pLenti6 W118-mCherry, which constitutively expresses the fluorescent protein mCherry, in 1 mg/mL PEI in OptiMEM with untransfected cells as controls. Media was changed 16 h post transfection. The cells were then harvested for Western blot analysis or challenged with SVV-GFP for flow cytometry analysis. For Western blot lysates, transfected cells were harvested 48 h post transfection and pelleted by centrifugation. Cell pellets were lysed in radioimmunoprecipitation assay (RIPA) buffer (Pierce) supplemented with 1X Halt Protease and Phosphatase Inhibitor Cocktail (Pierce) and subsequently clarified by centrifugation. Protein lysates were quantified using the BCA protein assay kit (Pierce) and prepared for Western blot analysis by boiling in for 10 min at 90 °C in NuPAGE sample reducing agent and LDS sample buffer (Invitrogen). Western blots analysis was performed as described in main text.

SVV-GFP Infections

For mixed culture *in vitro* experiments, parental H446 and H446 ANTXR1 KO mCherry cells were seeded as pure culture or seeded as a mixed culture with a 1:1 ratio. Cells were then challenged with SVV-GFP (MOI= 0.1 vp/cell) for 16 h at 37 °C and subsequently imaged. For blocking experiments, SVV-GFP (MOI=5) was incubated with 5 µg/mL ANTXR1-Fc or ANTXR2-Fc chimera, IgG-Fc (Sino Biological), or control (R&D Systems) on ice for 1 h and subsequently added to cells for 16 h at 37 °C. For one-step growth kinetics assay, parental H446, H446 ANTXR1 KO, or DMS79 cells were challenged with SVV-GFP (MOI=0.1 vp/cell) for 1 h at 37 °C. The virus containing media was then replaced with fresh media to remove free virus. Media samples were taken at 2 h intervals and frozen at -80 °C. Parental H446 cells were challenged with serial dilutions of media supernatant for 6 h at 37 °C. Cells were then processed and analyzed by flow cytometry. SVV-GFP infectious titers $\geq 10^4$ IU/mL were determined by flow cytometry data as previously described (5).

Co-immunoprecipitations (Co-IP)

For initial ANTXR1-Fc and ANTXR2-Fc co-IP experiments, serial dilutions of Fc chimera proteins (0.25 µg) in PBS, pH 7.4 were incubated with 1 µL of 30 mg/mL Dynabeads for 10 min at room temperature. Dynabead-Fc complexes were washed and subsequently incubated with SVV (2.0×10^{10} vp) for 2 h at 4 °C. Triplicate washes were repeated and dynabead-protein

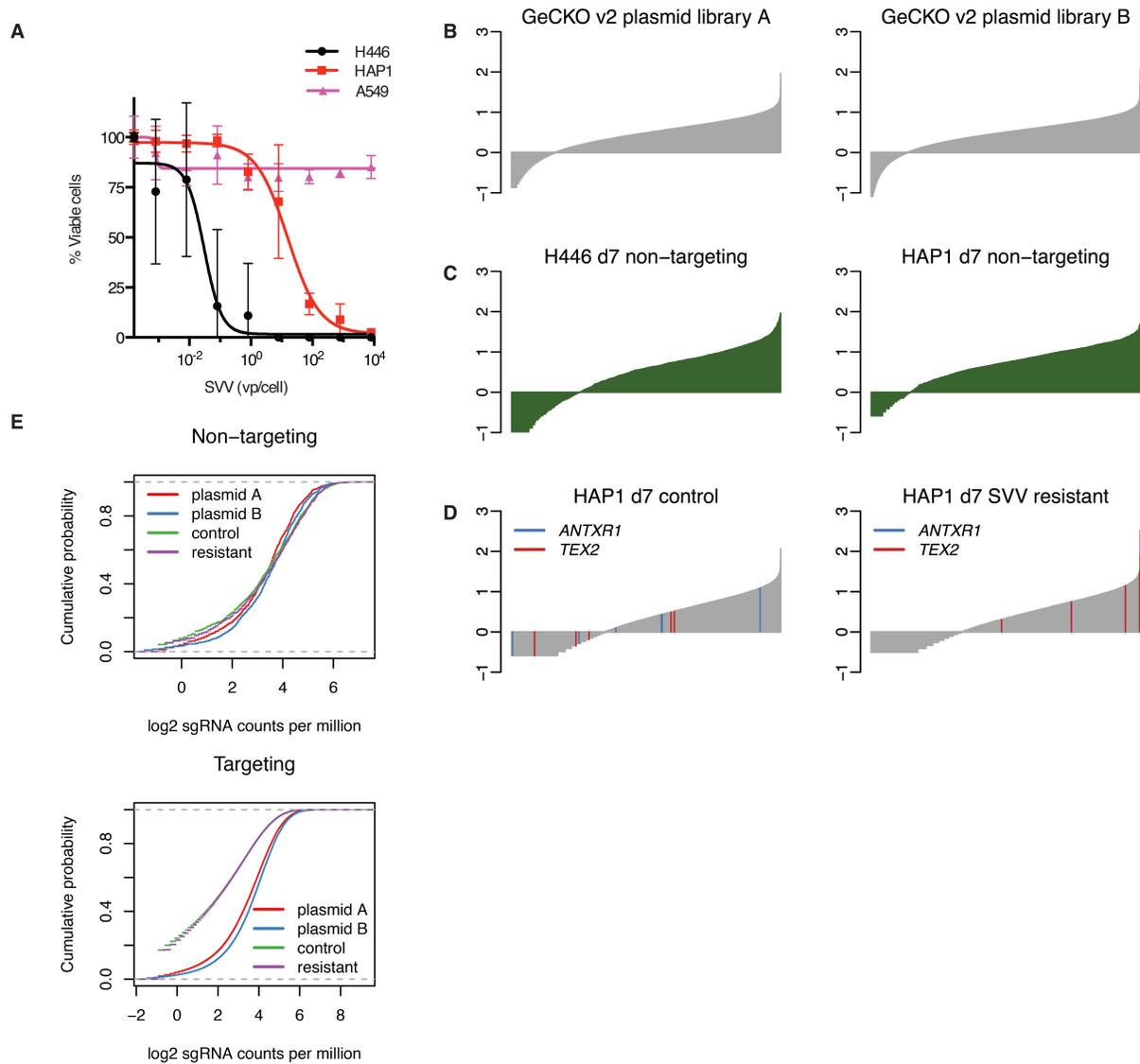
complexes were then subjected to protein elution. For co-IP using high stringency washes, PBS, pH 7.4 supplemented with 0.02% Tween-20 and increasing amounts of NaCl from 125 mM to 2 M were used to wash the Dynabead-protein complexes after SVV addition.

Flow Cytometry Analysis

Cells were harvested using Accutase enzyme cell detachment media (Gibco), pelleted by centrifugation, and washed with sterile PBS, pH 7.4 supplemented with 2% FCS and 0.5 mM EDTA (FACS buffer). Cells were stained with LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Invitrogen) for 30 min at 4 °C, washed with FACS buffer, then fixed in 4% paraformaldehyde solution for 10 min at 4 °C. For tumor samples, tumors were manually processed into single cell suspensions and subjected to ACK lysis buffer (Crystalgen) incubation to remove contaminating murine red blood cells. Cells were then stained with LIVE/DEAD Fixable Aqua Dead Cell Stain Kit and washed multiple times with FACS buffer. After a final wash with FACS buffer, cells were run on a BD LSR II Flow Cytometer (Becton Dickinson) using unstained cells and cells incubated with media alone as controls. For tumor samples, untreated pure parental and *ANTXR1* KO mCherry tumors were processed and used as controls. All experimental samples were collected and performed in triplicate. Data from tumor samples represents average of 4-5 individual tumors.

Supplemental Figures

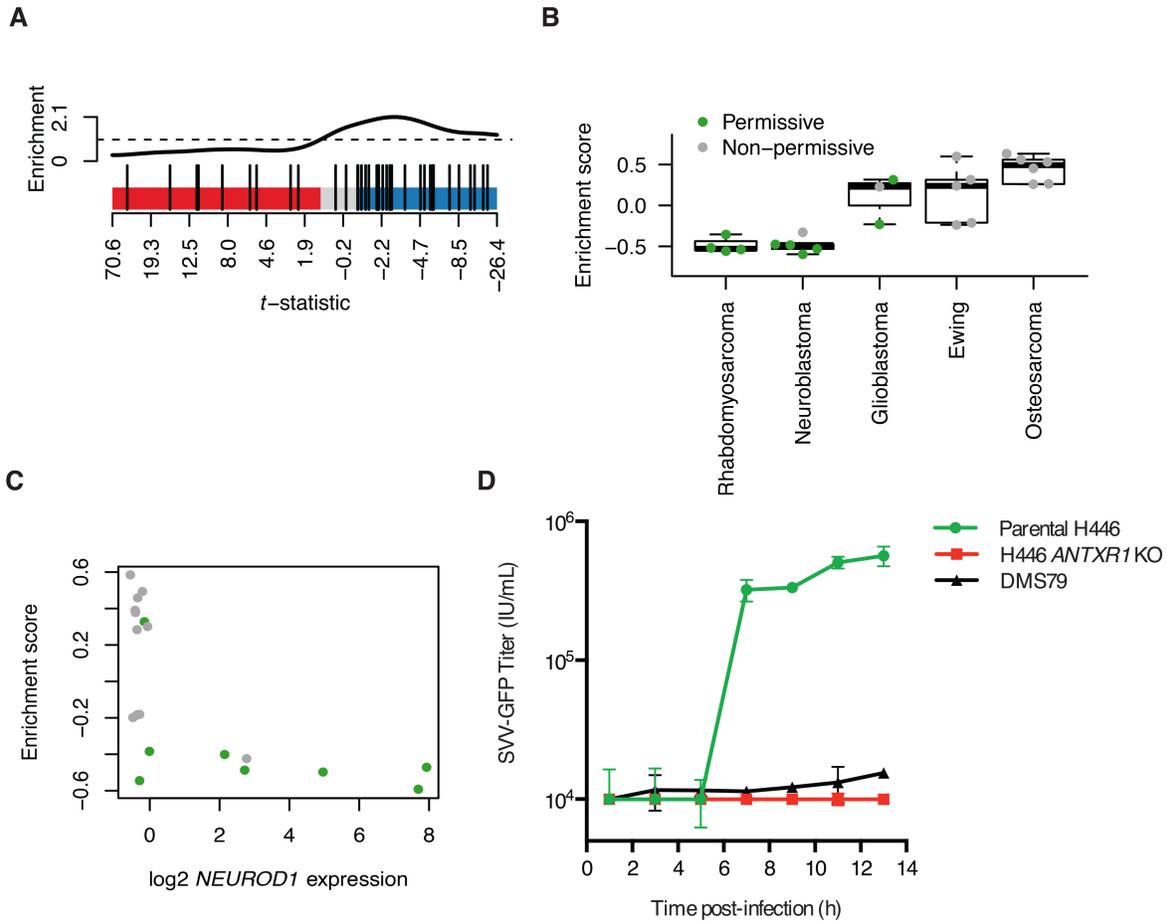
Supplemental Figure 1



Supplemental Figure 1. Cell lines and relative representation of each sgRNA in the Human GeCKO v2 library at various points during the screens. A) HAP1 cells were incubated with increasing MOIs of SVV for 72 h and cell viability assessed by AlamarBlue. H446 and A549 cells were assayed as positive and negative controls, respectively. Each data point represents the average of n=6 replicates with error bars representing standard deviation. **C)** Each plasmid half library was sequenced prior to lentiviral production. **C)** Representation of non-targeting sgRNAs in transduced H446 and HAP1 cells was maintained through the experiment. **D)** sgRNAs targeting both *ANTXR1* (blue) and *TEX2* (red) were enriched in the SVV resistant population compared to control. **E)** Cumulative distribution function plots of sgRNAs in the HAP1 screen demonstrates maintenance of library representation equivalent to the plasmid libraries for

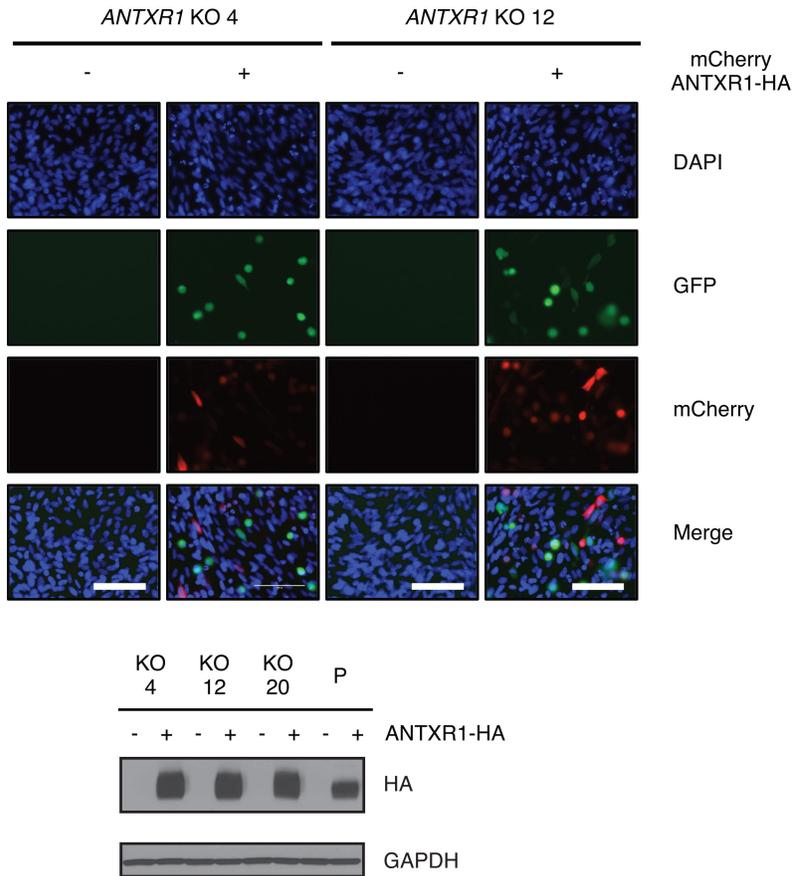
non-targeting sgRNAs (top panel), and loss of representation due to targeting of essential genes (bottom panel).

Supplemental Figure 2



Supplemental Figure 2. Sample-wise comparison of enrichment scores. **A)** An enrichment barcode plot depicting the negative enrichment of type I interferon signaling genes in permissive mouse xenografts of the Pediatric Preclinical Testing Program (PPTP). **B)** Sample-wise enrichment scores were calculated for the top enriched gene set and plotted based on the histology of the tumor of origin. Rhabdomyosarcoma and neuroblastoma have the highest fraction of permissive xenografts as well as the lowest baseline expression of type I interferon signaling genes. **C)** *NEUROD1* expressing SCLC cell lines are negatively enriched in type I interferon signaling genes. **D)** One-step growth kinetics of parental H446 (green), H446 *ANTXR1* KO (red), and DMS79 (black) cells challenged with SVV-GFP (MOI=0.1 vp/cell). Parental H446 cells were incubated with serial dilutions of media supernatants from multiple timepoints and subsequently analyzed by flow cytometry to determine viral titers. Each data point represents the average of n=3 replicates with error bars representing standard deviation.

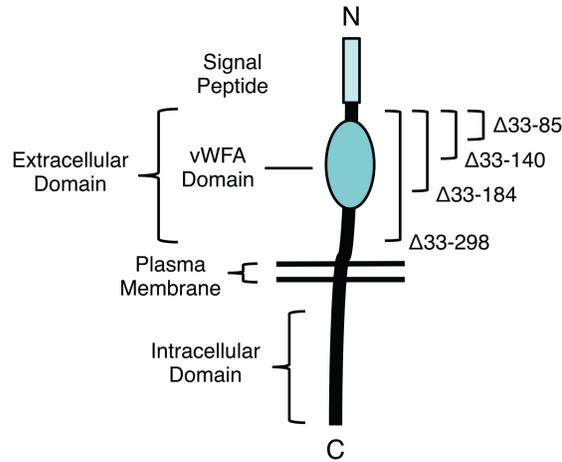
Supplemental Figure 3



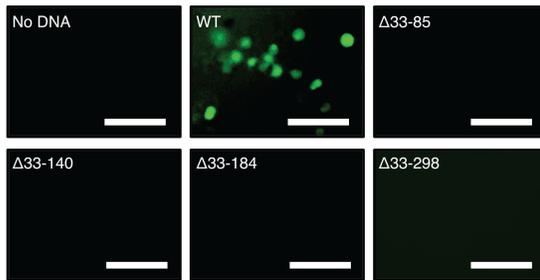
Supplemental Figure 3. Rescue of SVV permissivity by re-expression of ANTXR1-HA in additional H446 *ANTXR1* KO lines. Two additional H446 *ANTXR1* KO cell lines, KO 4 and KO 12, were transfected with the ANTXR1-HA expression plasmid and challenged with SVV-GFP. A co-transfected mCherry expression plasmid was used as a transfection control. Images representative of three independent experiments. ANTXR1-HA protein was confirmed via Western blot in each of the three transfected cell lines. Blot representative of two independent Westerns.

Supplemental Figure 4

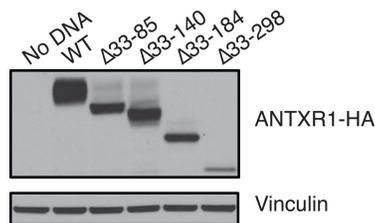
A



B



C



Supplemental Figure 4. Entire ANTXR1 extracellular domain required for *ANTXR1* KO rescue of SVV permissivity. **A)** Depiction of the full-length ANTXR1 protein and location of N-terminal truncations. Known proteins domains, such as the Von Willebrand factor type A domain (vWFA) are shown on the left. Amino acids in the ANTXR1 protein that are removed in each truncation are shown on the right. **B)** H446 *ANTXR1* KO cells were transfected with full-length ANTXR1 (WT) expression plasmid or N-terminal truncated ANTXR1 expression plasmids and challenged 24 h post-transfection with SVV-GFP for 16 h. Cells were co-transfected with mCherry expression plasmid to confirm transfection. Untransfected H446 *ANTXR1* KO cells were used as a negative control. Scale bars represent 100 μ m. Images representative of two independent experiments. **C)** Full-length and truncated ANTXR1-HA protein expression was confirmed via Western blot. Blot representative of two independent Westerns.

Tables

Name	Primer Sequence
1- lentiCRISPR v2 amplification F	5'-CTTTAGTTTGTATGTCTGTTGCTATTATGTCTACTATTCTTTCC-3'
2- lentiCRISPR v2 amplification R	5'-AATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCCG-3'
3- HiSeq F#1	5'- AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTC CGATCTTTCTTGTGGAAAGGACGAAACACCG-3'
4-HiSeq F#2	5'- AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTC CGATCTATTCTTGTGGAAAGGACGAAACACCG-3'
5-HiSeq F#3	5'- AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTC CGATCTGATTCTTGTGGAAAGGACGAAACACCG-3'
6-HiSeq F#4	5'- AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTC CGATCTCGATTCTTGTGGAAAGGACGAAACACCG-3'
7-HiSeq R#1	5'- CAAGCAGAAGACGGCATAACGAGATCGTGATGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTTCTACTATTCTTTCCCCTGCACTGT-3'
8-HiSeq R#2	5'- CAAGCAGAAGACGGCATAACGAGATACATCGGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTTCTACTATTCTTTCCCCTGCACTGT-3'
9-HiSeq R#3	5'- CAAGCAGAAGACGGCATAACGAGATGCCTAAGTACTGGAGTTCAGACGTGT GCTCTTCCGATCTTCTACTATTCTTTCCCCTGCACTGT-3'
10-HiSeq R#4	5'- CAAGCAGAAGACGGCATAACGAGATTGGTCAGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTTCTACTATTCTTTCCCCTGCACTGT-3'
11-HiSeq R#5	5'- CAAGCAGAAGACGGCATAACGAGATCACTGTGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTTCTACTATTCTTTCCCCTGCACTGT-3'
12-HiSeq R#6	5'- CAAGCAGAAGACGGCATAACGAGATATTGGCGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTTCTACTATTCTTTCCCCTGCACTGT-3'
13-HiSeq R#7	5'- CAAGCAGAAGACGGCATAACGAGATGATCTGGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTTCTACTATTCTTTCCCCTGCACTGT-3'
14-HiSeq R#8	5'- CAAGCAGAAGACGGCATAACGAGATTCAAGTGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTTCTACTATTCTTTCCCCTGCACTGT-3'
15-HiSeq R#9	5'- CAAGCAGAAGACGGCATAACGAGATCTGATCGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTTCTACTATTCTTTCCCCTGCACTGT-3'
16-HiSeq R#10	5'- CAAGCAGAAGACGGCATAACGAGATAAGCTAGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTTCTACTATTCTTTCCCCTGCACTGT-3'
17-HiSeq R#11	5'- CAAGCAGAAGACGGCATAACGAGATGTAGCCGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCTTCTACTATTCTTTCCCCTGCACTGT-3'
18-HiSeq R#12	5'-

	CAAGCAGAAGACGGCATAACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTACTATTCTTTCCCTGCACTGT-3'
19-HiSeq seq primer	5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3'
20-lentiGuide F	5'-AATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCCG-3'
21-lentiGuide R	5'-CTTTAGTTTGTATGTCTGTTGCTATTATGTCTACTATTCTTTCC-3'
22-ANTXR1.1 F	5'-CACCGGGAGACACTTACATGCATGA-3'
23-ANTXR1.1 R	5'-AAACTCATGCATGTAAGTGTCTCCC-3'
24-ANTXR1.2 F	5'-CACCGCAGGAAGTGTGCTGCACCAC-3'
25-ANTXR1.2 R	5'-AAACGTGGTGCAGCACACTTCCTGC-3'
26-ANTXR1.3 F	5'-CACCGCTATTACTTTGTGGAACAGT-3'
27-ANTXR1.3 R	5'-AAACACTGTTCCACAAAGTAATAGC-3'
28-G3BP1 F	5'-CACCGCGCACTCTTTGATCCCGCTG-3'
29-G3BP1 R	5'-AAACCAGCGGGATCAAAGAGTGCGC-3'
30-KCNJ1 F	5'-CACCGCGTGTCAAACACATTCCGAC-3'
31-KCNJ1 R	5'-AAACGTGCGAATGTGTTTGACACGC-3'
32-LSMEM2 F	5'-CACCGGAGTGAATCCCTGCGCATCC-3'
33-LSMEM2 R	5-AAACGGATGCGCAGGGATTCCTCC-3'
34-NR2C2 F	5'-CACCGAACTGACAGCCCCATAGTGA-3'
35-NR2C2 R	5'-AAACTCACTATGGGGCTGTCAGTTC-3'
36-NTHL1 F	5'-CACCGACAGCCCCGTGAAGCGTCCG-3'
37-NTHL1 R	5'-AAACCGGACGCTTCACGGGGCTGTC-3'
38-PLXNB2 F	5'-CACCGCTGCGGCTGGTGCCTCGTCG-3'
39-PLXNB2 R	5'-AAACCGACGACGCACCAGCCGCAGC-3'
40-PPBP F	5'-CACCGCAACTTACATCACTTCGACT-3'
41-PPBP R	5'-AAACAGTCGAAGTGATGTAAGTTGC-3'
42-SCAF8 F	5'-CACCGTAGCATACCTTGTTCATCCCC-3'
43-SCAF8 R	5'-AAACGGGGATGACAAGGTATGCTAC-3'
44-TACO1 F	5'-CACCGGCGACACACCTCTAAGATAT-3'
45-TACO1 R	5-AAACATATCTTAGAGGTGTGTCGCC-3'
46-TEX2.1 F	5'-CACCGTACCCCATTTGTATCGAGCT-3'
47-TEX2.1 R	5'-AAACAGCTCGATACAAATGGGGTAC-3'
48-TEX2.2 F	5'-CACCGCTGAATGTGTCAAAGTCGCA-3'
49-TEX2.2 R	5'-AAACTGCGACTTTGACACATTCAGC-3'
50-ZDHHC7 F	5'-CACCGGCACTTGTAGATGACTTCCC-3'
51-ZDHHC7 R	5'-AAACGGGAAGTCATCTACAAGTGCC-3'
52-ZNF101 F	5'-CACCGTGAAATCAGATCTCACGCGC-3'
53-ZNF101 R	5'-AAACGCGCGTGAGATCTGATTTAC-3'
54- sgRNA Seq F	5'-GACTATCATATGCTTACCGT-3'
55-ANTXR1 exon2 PCR F	5'-TGAGTCCAGTTATTGGAGAGGTC-3'
56-ANTXR1 exon2 PCR R	5'-CGAGATCTGAGAGCCCAACT-3'
57-attB1 cDNA F	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTAACCATGGCCACGGCGGAGCGGAGA-3'
58-attB2 cDNA R	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTATCATCAAGCGTAGTCTGGGACG-3'
59-ANTXR1 truncation Δ33-85 F	5'-GTTGTTCCCTCGGGTGGACCTGCGTCCCCCTTGCCCGGCGCA-3'
60- ANTXR1 truncation Δ33-85 R	5'-GGGCAAGGGGGACGCAGGTCCACCCGAGGAACAACCTTAAT-3'
61- ANTXR1 truncation Δ33-140 F	5'-GACGCTGGCTGTCCTGTACCTGCGTCCCCCTTGCCCGGCGC-3'

62- ANTXR1 truncation Δ33-140 R	5'-GGGCAAGGGGGACGCAGGTACAGGACAGCCAGCGTCATCAT-3'
63- ANTXR1 truncation Δ33-184 F	5'-AATCCGGGCCAGCTGTGTCCTGCGTCCCCCTGCCCCGGCGC-3'
64- ANTXR1 truncation Δ33-184 R	5'-GGGCAAGGGGGACGCAGGACACAGCTGGCCCCGGATTGCGGA-3'
65- ANTXR1 truncation Δ33-298 F	5'-TGCAGCTTTCATGCCAACCCCTGCGTCCCCCTGCCCCGGCGC-3'
66- ANTXR1 truncation Δ33-298 R	5'-GGGCAAGGGGGACGCAGGGTTGGCATGAAAGCTGCACTCCA-3'

Table 1. Oligonucleotide list for primers used in cloning and sequencing for CRISPR constructs and ANTXR1 expression constructs. Numbers correspond to oligonucleotide identity in the main text. Primer name describes the sgRNA gene target or product the primers will eventually create. Oligonucleotides shown from 5' to 3'.

Supplemental References

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