Figure S1 Extended validation of TRPPC system and expanded utility to other viral systems, related to Figure 1.

A, TRPPCa in A549-CRISPRa cells of a luciferase reporter targeted by sgRNA expressed from transfected *NS* in the presence of the viral replication machinery. *Split NS* that lacks an sgRNA is included as a control.

B, TRPPCa functions with *NS* from primary isolates of IAV and IBV. Activation of a luciferase reporter targeted by sgRNA expressed from transfected CA07 or IBV *TRPPC-NS* in the presence of matched viral polymerase and NP.

C, TRPPC-inhibition (TRPPCi) suppresses gene expression. PR8 *TRPPC-NS* suppressed reporter gene expression when transfected into cells with the viral polymerase, NP, and dCas9-KRAB.

D, Example plaque morphologies for WT and engineered viruses.

E, TRPPC viruses replicate similar to WT in multiple cell lines. Multicycle replication kinetics of WT, split-NS, or TRPPCa-NS with a non-targeting sgRNA in MDCK and A549-CRISPRa cells (MOI = 0.01).

F, Engineered *NS* segment stability was measured over serial passages by assessing amplicon sizes by RT-PCR.

G, TRPPC targeting does not affect replication in cells lacking the CRISPRa machinery. Multicycle replication of TRPPC viruses targeting specified host genes in WT A549 cells inoculated at MOI = 0.01.

H, Diagram of genome engineering where ribozymes are used to process sgRNAs from the inflenza virus genome (left) or a U6 promoter is used to express sgRNAs from the adenovirus genome (right).

I, TRPPCa of a luciferase reporter in 293T-CRISPRa cells. Cell were transfected with vectors expressing viral genomic RNA *Split NS* that lacks an sgRNA, or *TRPCC Rz NS* targeting the reporter promoter.

J, Adenovirus TRPPCa: TRPPC activation of a luciferase reporter in 293T-CRISPRa cells transfected with an adenoviral subgenomic plasmid expressing an sgRNA targeting the reporter promoter (left) or infected with a virus expressing an sgRNA. Data are normalized to WT controls.

K, Adenovirus TRPPCi: TRPPC inhibition of a luciferase reporter in 293T expressing dCas9-KRAB-MeCP2, VP16-Gal4, and the UAS-Nluc-PEST reporter transfected with a adenoviral subgenomic plasmid (left) or infected with adenovirus (right) expressing an sgRNA targeting the UAS. Data are normalized to WT controls.

L, Replication of TRPPCa and TRPCCi viruses. A549 cells were infected with WT or sgRNA-expressing viruses (MOI = 5), samples were collected at the indicated times, and titered by plaque assay.

Data are shown as grand mean of 3 replicates ± SEM (C, D, F, J-L) mean ± s.d. (E, G, I). Unpaired T tests (C, D, F, I, J, K) or one-way ANOVA with post-hoc Dunnett's tests (E, G, J-L) were performed (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$).

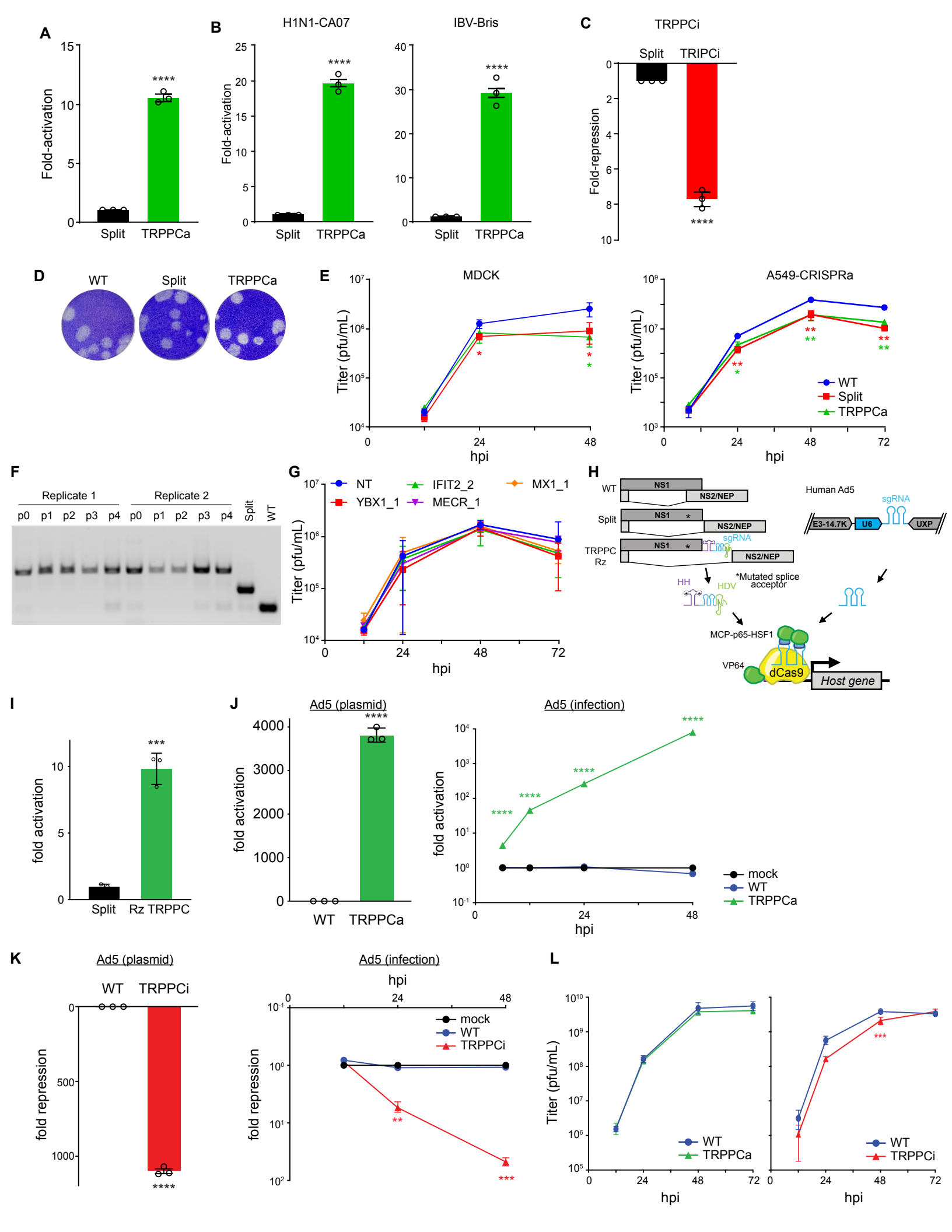

A
Fold-activation
Split
TRPPCa
B
H1N1-CA07
IBV-Bris
Fold-activation
Split
TRPPCa
C
TRPPCi
Split
TRIPCi
Fold-repression
D
WT
Split
TRPPCa
E
MDCK
A549-CRISPRa
Titer (pfu/mL)
hpi
WT
Split
TRPPCa
F
Replicate 1
Replicate 2
p0
p1
p2
p3
p4
Split
WT
G
NT
IFIT2_2
MX1_1
YBX1_1
MECR_1
Titer (pfu/mL)
hpi
H
WT
Split
TRPPC Rz
NS1
NS2/NEP
sgRNA
Human Ad5
E3-14.7K
U6
UXP
HH
HDV
*Mutated splice acceptor
MCP-p65-HSF1
VP64
dCas9
Host gene
I
fold activation
Split
Rz TRPPC
J
Ad5 (plasmid)
Ad5 (infection)
fold activation
WT
TRPPCa
hpi
mock
WT
TRPPCa
K
Ad5 (plasmid)
Ad5 (infection)
WT
TRPPCi
fold repression
hpi
mock
WT
TRPPCi
L
Titer (pfu/mL)
hpi
WT
TRPPCa
WT
TRPPCi

Figure S2 Characterization of TRPPC library and gene enrichment analysis, related to Figure 2.

A, Experimental workflow for the creation of a genome-wide TRPPC virus library.

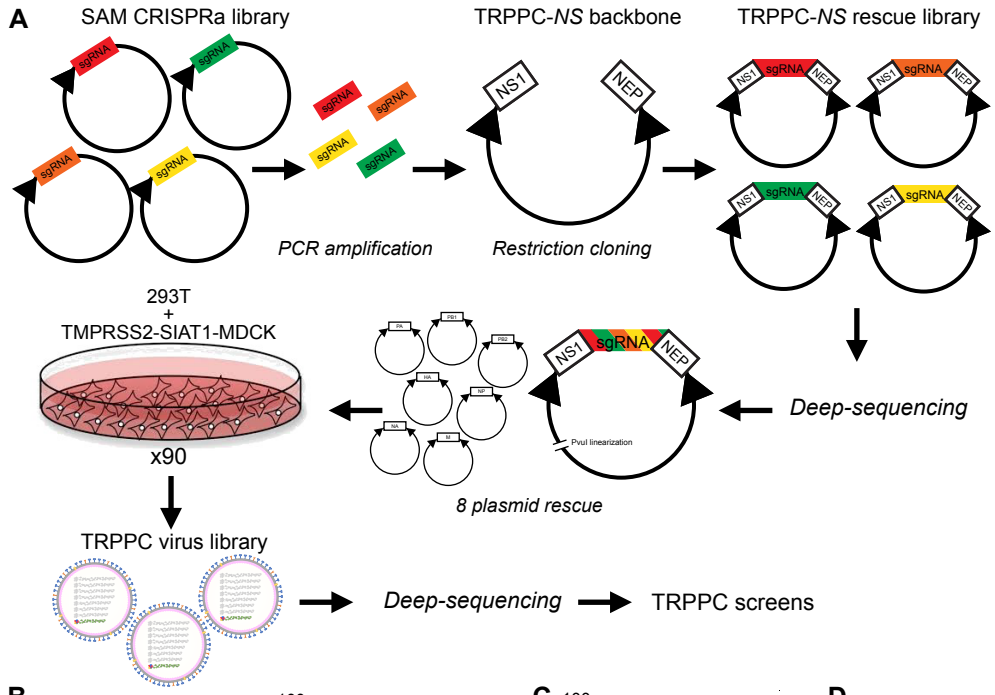
B, Distribution histogram and cumulative frequency plot of each member in the TRPPC virus library stock prior to selection.

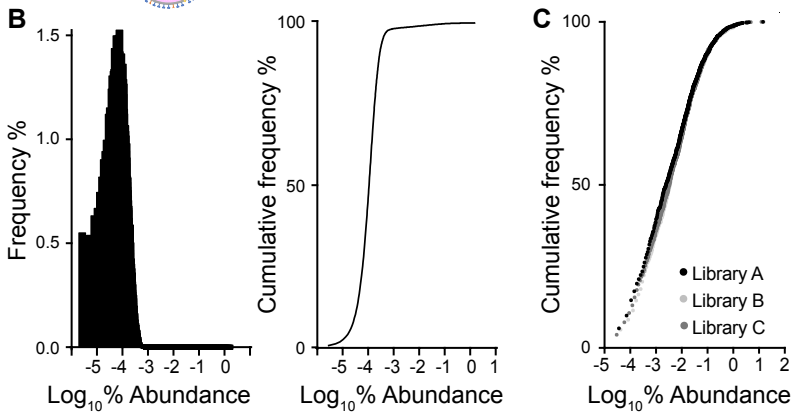
C, Cumulative frequency plot of each member in the three replicate screens after the fifth passage. Only sgRNAs detected at least once are included in the analysis.

D, Shannon's diversity indices (H') and richness of the viral populations at each passage during the 3 TRPPC screens.

E, GO analysis highlighting the molecular function pathways enriched among the top 100 selected genes based on MAGeCK scores.

F, Groupings of high-level gene functions conferred by the top 100 genes.





D

virus

Shannon Diversity (H')

plasmid

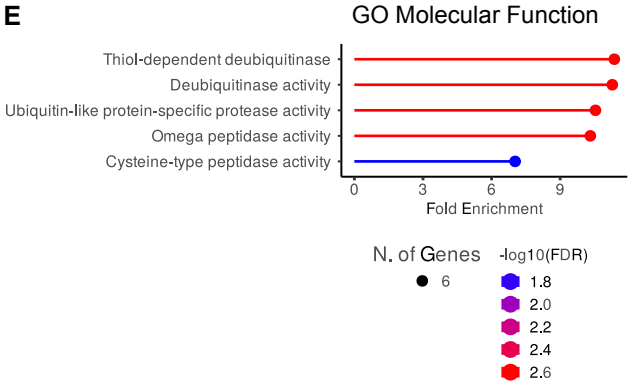
10.90131

p0	p1	p2	p3	p4	p5
6.506059	5.682367	5.282353	5.192416	5.068081	4.850333
6.506059	5.678302	5.305056	5.20663	5.122207	4.955211
6.506059	5.646149	5.202897	5.077303	4.987117	4.769359

richness

69179

p0	p1	p2	p3	p4	p5
69276	3742	1993	1768	1652	1565
69276	3656	1989	1750	1629	1555
69276	3512	1880	1661	1536	1419



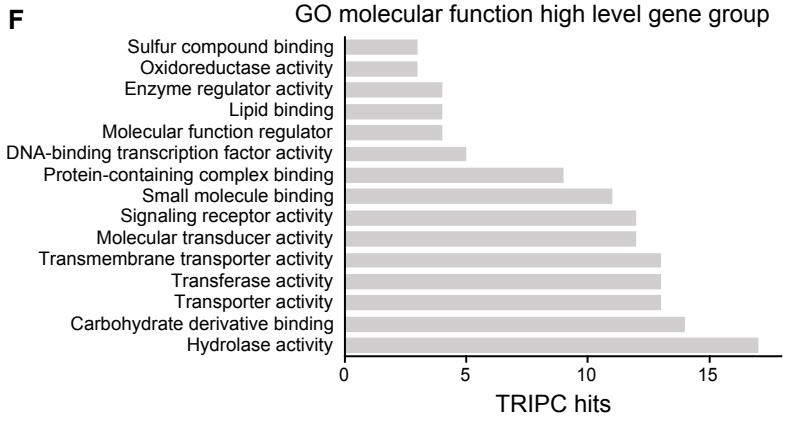


Figure S3 Further validation of TREX1 as a proviral host factor, related to Figure 3.

A, TREX1 expression was measured in WT A549 inoculated with *TREX1*-targeting or non-targeting control TRPPC viruses (MOI = 1) by RT-qPCR (top) and western blot (bottom).

B, Multicycle replication of *TREX1*- or non-targeting TRPPC viruses in WT A549 cells (MOI = 0.01). Titers determined by plaque assay.

C, Multicycle replication of a WSN influenza A reporter virus (MOI = 0.05) in A549 cells transfected with GFP-tagged TREX1, TREX1^{D18N} or a GFP-alone control.

D, Multicycle replication of a WSN influenza A reporter virus (MOI = 0.05) in A549 cells expressing GFP-tagged TREX1, TREX1^{D18N} or a GFP-alone control.

E, *TREX1* genotype of knockout cells. Sanger sequencing traces display CRISPR-Cas9 editing at the *TREX1* locus for 3 selected knockout clones. Edits compared to the WT genome are shown for 2 homozygous (B6, C8) and 1 heterozygous (G11) clones.

F, TREX1 knockout was confirmed by western blotting lysates from WT, clonal KO, and complemented A549 cell lines. Endogenous TREX1 and recombinant TREX1-V5-2A are indicated. * = non-specific bands. These cells lines are used throughout Fig 3-6.

Data are shown as grand mean of 3 replicates ± SEM (C-D) or mean ± s.d. (A-B). Unpaired T tests (D) or one-way ANOVA with post-hoc Dunnett's tests (A, C) were performed (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns = not significant).

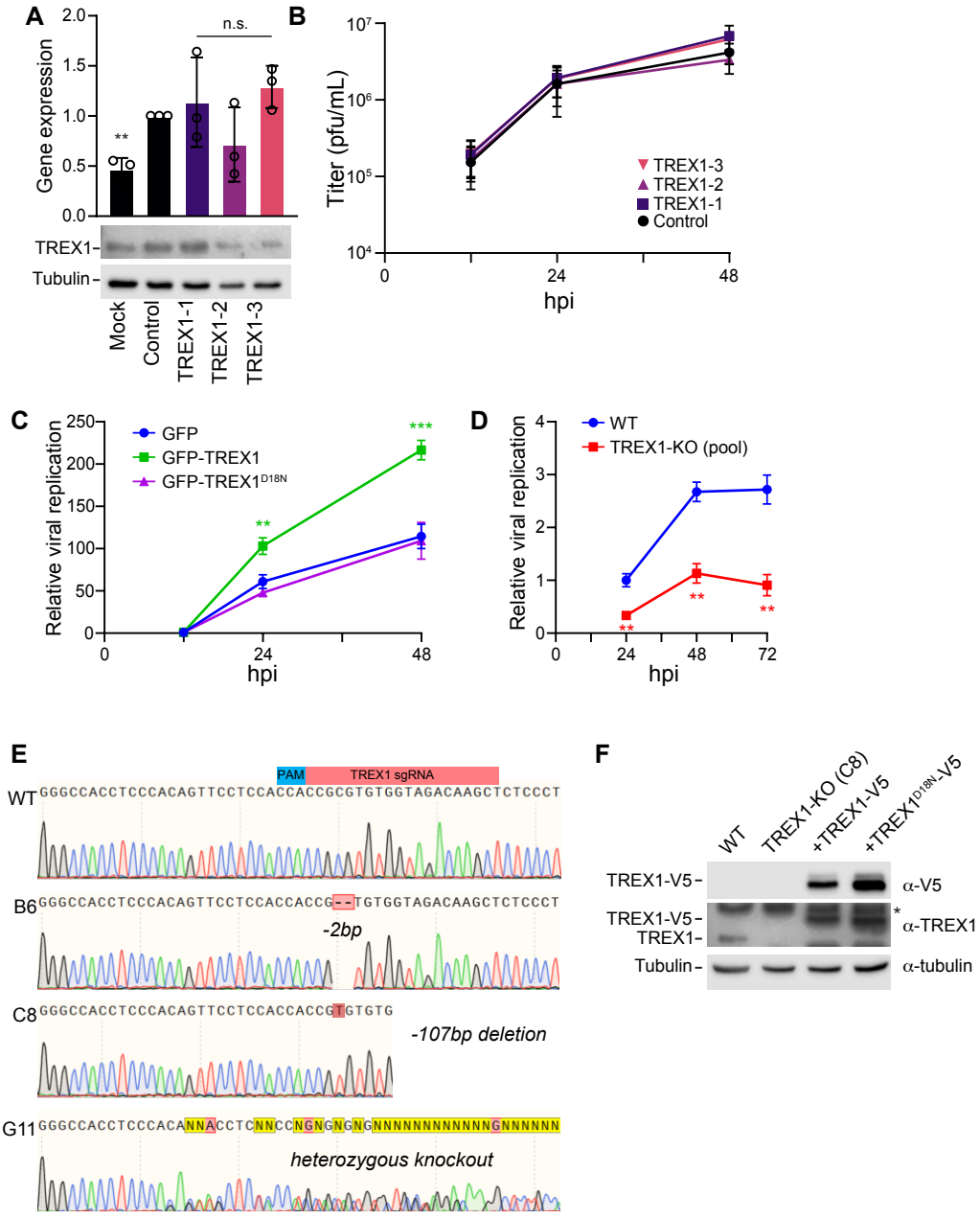

A
Gene expression
n.s.
**
TREX1
Tubulin
Mock
Control
TREX1-1
TREX1-2
TREX1-3
B
Titer (pfu/mL)
hpi
TREX1-3
TREX1-2
TREX1-1
Control
C
Relative viral replication
GFP
GFP-TREX1
GFP-TREX1D18N
hpi
D
Relative viral replication
WT
TREX1-KO (pool)
hpi
E
PAM
TREX1 sgRNA
WT
B6
-2bp
C8
-107bp deletion
G11
heterozygous knockout
F
WT
TREX1-KO (C8)
+TREX1-V5
+TREX1D18N-V5
TREX1-V5
TREX1
Tubulin
α-V5
α-TREX1
α-tubulin

Figure S4 Reporter cell line validation and IAV replication in MAVS-knockout, related to Figure 4.

A-B, IFN signaling and RNA sensing remain intact in TREX1-KO cells. ISRE induction in WT and TREX1-KO reporter cells treated with a)IFNβ or b) transfected with poly(I:C). ISRE activation is normalized to untreated and mock-transfected cells, respectively.

C, Sensing of foreign nucleic acids blocks IAV replication. Replication of influenza A virus (MOI = 0.05) in WT A549 cells treated with the indicated nucleic acid ligands.

D, Western blotting demonstrates STING expression in our lineage of A549 cells in both mock and influenza virus-infected conditions.

E, Infection in cells lacking RNA sensing for comparison. Multicycle replication of influenza A virus (MOI = 0.05) in WT and MAVS-KO A549 cells.

Data are shown as grand mean of 3 replicates ± SEM. Significance was tested with a two-way ANOVA with Šídák's mulitple comparisons (A-B) or a one-way ANOVA with post-hoc Dunnett's test (**p<0.01, ***p<0.001, ****p<0.0001, ns = not significant).

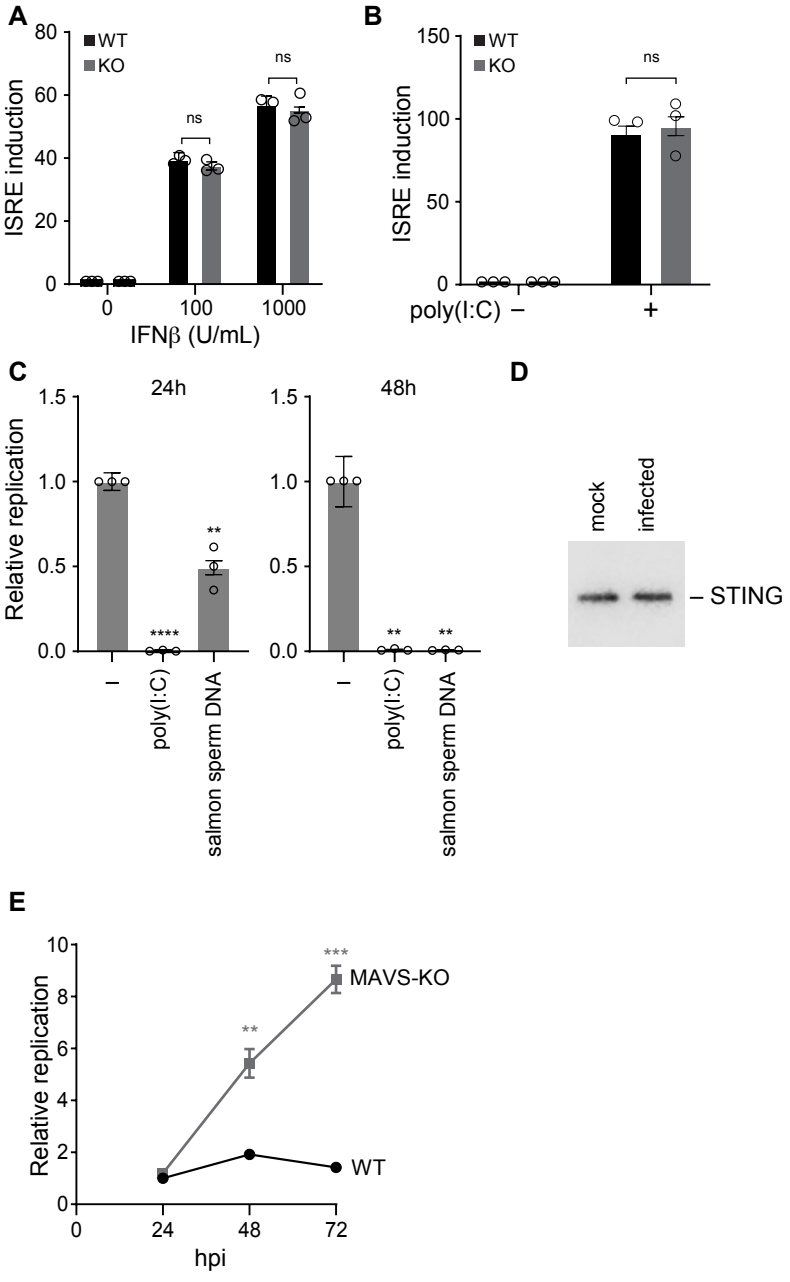

A
ISRE induction
WT
KO
ns
ns
IFNβ (U/mL)
B
ISRE induction
WT
KO
ns
poly(I:C)
C
24h
48h
Relative replication
poly(I:C)
salmon sperm DNA
D
mock
infected
STING
E
Relative replication
MAVS-KO
WT
hpi

Figure S5 Cytoplasmic DNA puncta and ISRE induction by untreated cytosolic extracts, related to Figure 5. A, Mean intensity of cytoplasmic puncta identified by staining for dsDNA were quantified at 0 hpi (left) and 6 hpi (right) for WT, TREX1-knockout (KO), or complemented cells (+TREX1) inoculated with influenza A virus. Representative micrographs for these experiments are in Fig 5A. Individual data points and mean are shown and analyzed by a one-way ANOVA with post-hoc Dunnett's tests (***$p<0.001$, ****$p<0.0001$).

B, ISRE induction was measured in WT and TREX1-KO reporter cells transfected with untreated cytosolic extracts derived from mock or infected cells. Bioluminescence values are normalized to untransfected cells.

Data are shown as grand mean of 3 replicates ± SEM.

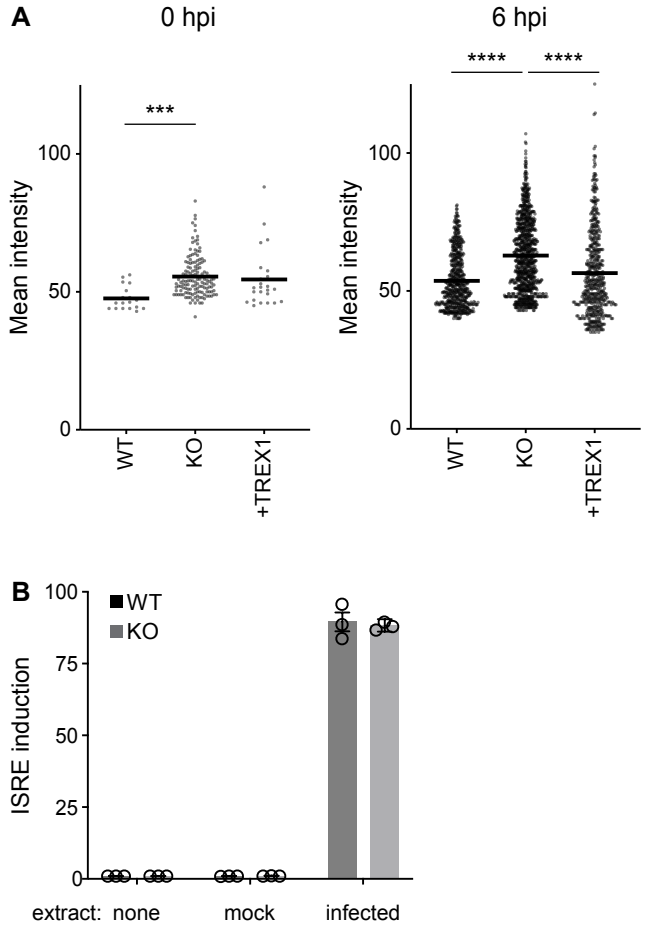
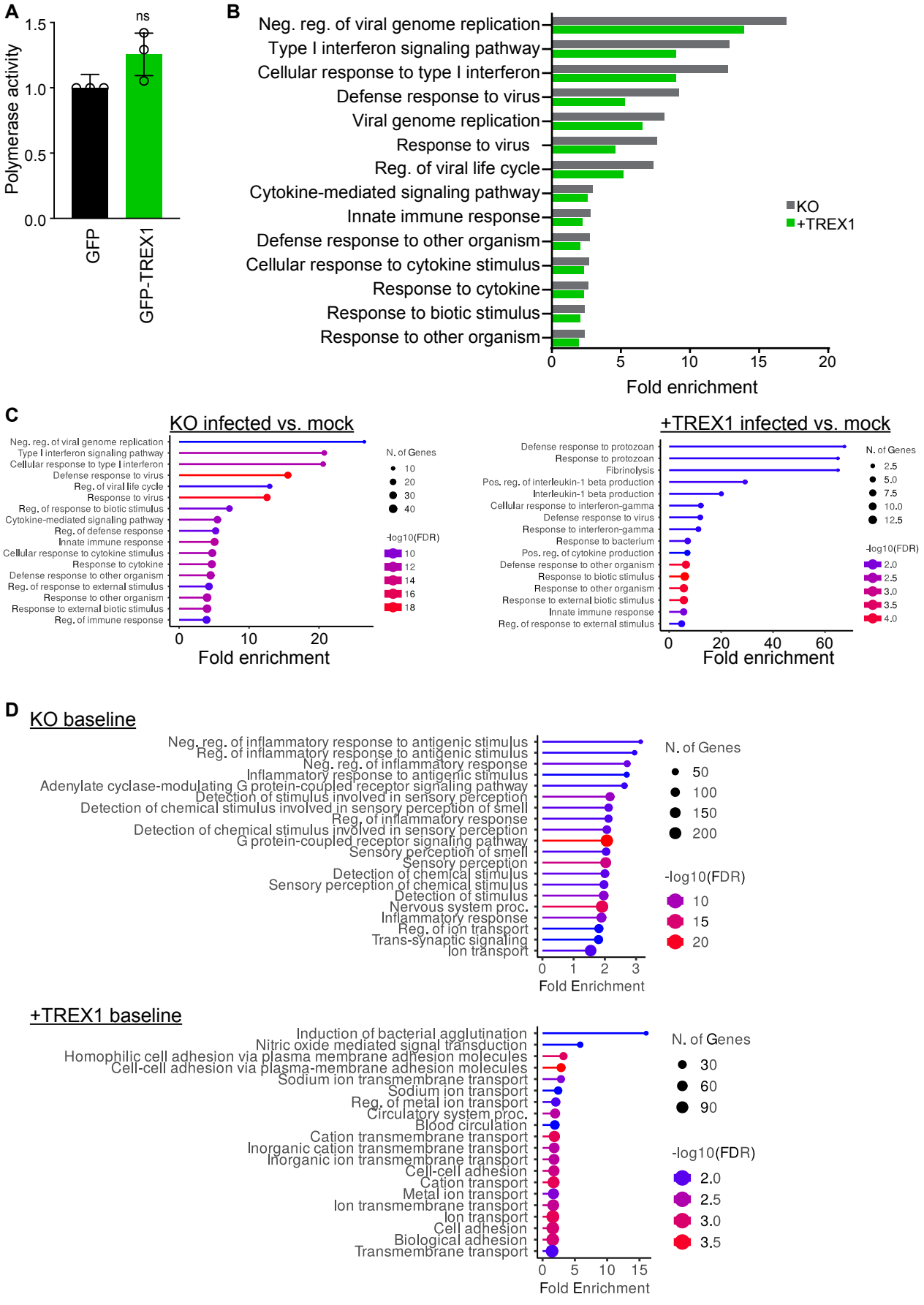

A
0 hpi
6 hpi
***
****
****
Mean intensity
100
50
0
WT
KO
+TREX1
B
WT
KO
ISRE induction
100
75
50
25
0
extract: none
mock
infected

Figure S6 TREX1 modulates host gene expression but does not alter viral polymerase activity, related to Figure 6.

A, Influenza polymerase activity was measured in a mini-replicon assay in the presence of exogenous GFP-TREX1 or vector control. Data are shown as means of 3 replicates ± SEM. Pairwise T-tests tests were performed (ns = not significant).

B-C, Loss of TREX1 amplifies innate immune responses. **B,** Gene enrichment analysis of all host genes upregulated >4-fold during infection in TREX1-KO or complemented cells compared to their matched mock-treated cells. GO biological processes are shown. Enrichment FDR $<10^{-7}$ for all biological categories. **C,** Gene enrichment analysis of ISGs plotted in Fig 6F. This includes only ISGs induced ≥2-fold during infection in TREX1 KO cells whose induction levels change by at least 50% different between cell lines. Significantly enriched biological processes are shown along with their enrichment values.

D, TREX1-KO cells exhibit a chronic inflammatory state. Genes differentially expressed at least 4-fold between uninfected TREX1-KO and complemented cells were subject to gene enrichment analysis. Significantly enriched biological processes are shown along with their enrichment values.


A
Polymerase activity
ns
GFP
GFP-TREX1
B
Neg. reg. of viral genome replication
Type I interferon signaling pathway
Cellular response to type I interferon
Defense response to virus
Viral genome replication
Response to virus
Reg. of viral life cycle
Cytokine-mediated signaling pathway
Innate immune response
Defense response to other organism
Cellular response to cytokine stimulus
Response to cytokine
Response to biotic stimulus
Response to other organism
KO
+TREX1
Fold enrichment
C
KO infected vs. mock
+TREX1 infected vs. mock
N. of Genes
-log10(FDR)
Fold enrichment
D
KO baseline
Neg. reg. of inflammatory response to antigenic stimulus
Reg. of inflammatory response to antigenic stimulus
Neg. reg. of inflammatory response
Inflammatory response to antigenic stimulus
Adenylate cyclase-modulating G protein-coupled receptor signaling pathway
Detection of stimulus involved in sensory perception
Detection of chemical stimulus involved in sensory perception of smell
Reg. of inflammatory response
Detection of chemical stimulus involved in sensory perception
G protein-coupled receptor signaling pathway
Sensory perception of smell
Sensory perception
Detection of chemical stimulus
Sensory perception of chemical stimulus
Detection of stimulus
Nervous system proc.
Inflammatory response
Reg. of ion transport
Trans-synaptic signaling
Ion transport
Fold Enrichment
+TREX1 baseline
Induction of bacterial agglutination
Nitric oxide mediated signal transduction
Homophilic cell adhesion via plasma membrane adhesion molecules
Cell-cell adhesion via plasma-membrane adhesion molecules
Sodium ion transmembrane transport
Sodium ion transport
Reg. of metal ion transport
Circulatory system proc.
Blood circulation
Cation transmembrane transport
Inorganic cation transmembrane transport
Inorganic ion transmembrane transport
Cell-cell adhesion
Cation transport
Metal ion transport
Ion transmembrane transport
Ion transport
Cell adhesion
Biological adhesion
Transmembrane transport
Fold Enrichment

Methods S1 Sequence of TRPPC cassettes, related to Figure 1 and Figure S1

PR8 TRPPC NS (plus-sense)

Genomic sequence of the engineered NS segment.

UTR
NS1
mutant splice acceptor
miR124
approximate Drosh processing sites (3′ of marked nt) (PMID20841420)
sgRNA 2.0 (*PMID 25494202)*
Shechner sgRNA target sequence (PMID 26030444)
MS2 hairpins
NEP (5′ exon in NS1 coding sequence
UTR

agcaaaagcagggtgacaaaaacataatggatccaaacactgtgtcaagctttcaggtagattgctttctttggcatgtccgcaaacga
gttgcagaccaagaactaggcgatgccccattccttgatcggcttcgccgagatcagaaatccctaagaggaaggggcagtactctcgg
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tggcctctgtacctgcgtcgcgttacctaactgacatgactcttgaggaaatgtcaagggactggtccatgctcatacccaagcagaaa
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gctggagactctaatattgctaagggctttcaccgaagagggagcaattgttggcgaaatttcaccattgccttctctc**ccgg**gacata
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agtttgaggtaccGGGCAGGGAGAAAATTATAGTAATAGTTGCAATGAGTCACTTGCTTCTAGATCAAGATCAGAG**A**CTCTGCTCTCCG
TGTTCACAGCGGACCTTGATTTAATGTCATACAATTAAGGCACGCGGTGAATGCCAAGAGCGGA**G**CCTACGGCTGCACTTGAAGGACAT
CCGAGAGAAGTTAGGAAGGGTGGGGAGAAACAATTCTAGAATGActcgag*ATCTAGATACGACTCACTAT*GTTTTAGAGCTAGGCCAAC
ATGAGGATCACCCATGTCTGCAGGGCCTAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGGCCAACATGAGGATCACCCATGTCT
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ggttgattgaagaagtgagacacaaactgaagataacagagaatagttttgagcaaataacatttatgcaagccttacatctattgctt
gaagtggagcaagagataagaactttctcgtttcagcttatttagtactaaaaaacacccttgtttctact

Ad5 TRPPC

Adenovirus TRPPC cassette.

Ad5 TRPPCa-SH (in pAd5-B6)
E3 (14.7K)
U6 promoter
NNN= sgRNA target sequence
sgRNA backbone, MS2 hairpins, and U6 terminator
UXP ORF fragment (antisense)

ATGACTGACACCCTAGATCTAGAAATGGACGGAATTATTACAGAGCAGCGCCTGCTAGAAAGACGCAGGG
CAGCGGCCGAGCAACAGCGCATGAATCAAGAGCTCCAAGACATGGTTAACTTGCACCAGTGCAAAAGGG
GTATCTTTTGTCTGGTAAAGCAGGCCAAAGTCACCTACGACAGTAATACCACCGGACACCGCCTTAGCTA
CAAGTTGCCAACCAAGCGTCAGAAATTGGTGGTCATGGTGGGAGAAAAGCCCATTACCATAACTCAGCAC
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CCCTGTGCGGTCTCAAAGATCTTATTCCCTTTAACTAATAAAAAAAAATAA*gagggcctatttcccatgattccttcatatttg*
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*gtagtttgcagttttaaaattatgttttaaaatggactatcatatgcttaccgtaacttgaaagtatttcgatttcttggctttatatatcttgtggaaaggac*gaaacac
cNNNNNNNNNNNNNNNNNNNNgttttagagctaggccaacatgaggatcacccatgtctgcagggcctagcaagttaaaataaggctagtcc
gttatcaacttggccaacatgaggatcacccatgtctgcagggccaagtggcaccgagtcggtgctttttttTAAAGCATCACTTACTTAAAATC
AGTTAGCAAATTTCTGTCCAGTTTATTCAGCA

Ad5 TRPPCi-CH (in pAd5-B6)
E3 (14.7K)
U6 promoter
sgRNA target sequence in UAS-Nluc-PEST
tracr-v2 backbone and U6 terminator
UXP ORF fragment (antisense)

ATGACTGACACCCTAGATCTAGAAATGGACGGAATTATTACAGAGCAGCGCCTGCTAGAAAGACGCAGGG
CAGCGGCCGAGCAACAGCGCATGAATCAAGAGCTCCAAGACATGGTTAACTTGCACCAGTGCAAAAGGG
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CAAGTTGCCAACCAAGCGTCAGAAATTGGTGGTCATGGTGGGAGAAAAGCCCATTACCATAACTCAGCAC
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*gtagtttgcagttttaaaattatgtttaaaatggactatcatatgcttaccgtaacttgaaagtatttcgatttcttggctttatatatcttgtggaaaggac*gaaacac
ctacctcatcaggaacatgtGTTTCAGAGCTACAGCAGAAATGCTGTAGCAAGTTGAAATAAGGCTAGTCCGTTATC
AACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTTAAAGCATCACTTACTTAAAATCAGTTAGCAAATT
TCTGTCCAGTTTATTCAGCA

PR8 TRPPC NS Rz (plus-sense)

Genomic sequence of the engineered NS segment where sgRNAs are flanked by ribozymes.
UTR
NS1
mutant splice acceptor
hammerhead ribozyme (complimentary sequence, approximate cleavage site 3′ of **bold** nt)(PMID 27606350)
sgRNA 2.0 (*PMID 25494202)*
Shechner sgRNA target sequence (PMID 26030444)
MS2 hairpins
HDV ribozyme (approximate cleavage site 3′ of **bold** nt)(PMID 27606350)
NEP (5′ exon in NS1 coding sequence)
UTR

agcaaaagcagggtgacaaaaacataatggatccaaacactgtgtcaagctttcaggtagattgctttctttggcatgtccgcaaacga
gttgcagaccaagaactaggcgatgccccattccttgatcggcttcgccgagatcagaaatccctaagaggaaggggcagtactctcgg
tctggacatcaagacagccacacgtgctggaaagcagatagtggagcggattctgaaagaagaatccgatgaggcacttaaaatgacca
tggcctctgtacctgcgtcgcgttacctaactgacatgactcttgaggaaatgtcaagggactggtccatgctcatacccaagcagaaa
gtggcaggccctctttgtatcagaatggaccaggcgatcatggataagaacatcatactgaaagcgaacttcagtgtgatttttgaccg
gctggagactctaatattgctaagggctttcaccgaagagggagcaattgttggcgaaatttcaccattgccttctctc**ccgg**gacata
ctgctgaggatgtcaaaaatgcagttggagtcctcatcggaggacttgaatggaatgataacacagttcgagtctctgaaactctacag
agattcgcttggagaagcagtaatgagaatgggagacctccactcactccaaaacagaaacgagaaatggcgggaacaattaggtcaga
agtttgaggtaccGCGATCGCCTGGCCCTGATGAGTCCGTGAGGACGAAACGGTAGGAATTCCTACCGT**C**GGCCAg*ATCTAGATACGAC*
*TCACTATG*TTTTAGAGCTAGGCCAACATGAGGATCACCCATGTCTGCAGGGCCTAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT
GGCCAACATGAGGATCACCCATGTCTGCAGGGCCAAGTGGCACCGAGTCGGTGCTTTTTgcggcctcga**g**GGCCGGCATGGTCCCAGCC
TCCTCGCTGGCGCCGGCTGGGCAACATTCCGAGGGGACCGTCCCCTCGGTAATGGCGAATGGGACTATCgcggccgcgatcaccattgc
cttctcttccaggacatactgctgaggatgtcaaaaatgcagttggagtcctcatcggaggacttgaatggaatgataacacagttcga
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caaataacatttatgcaagccttacatctattgcttgaagtggagcaagagataagaactttctcgtttcagcttatttagtactaaaa
aacacccttgtttctact