Supplemental Information

Figure S1

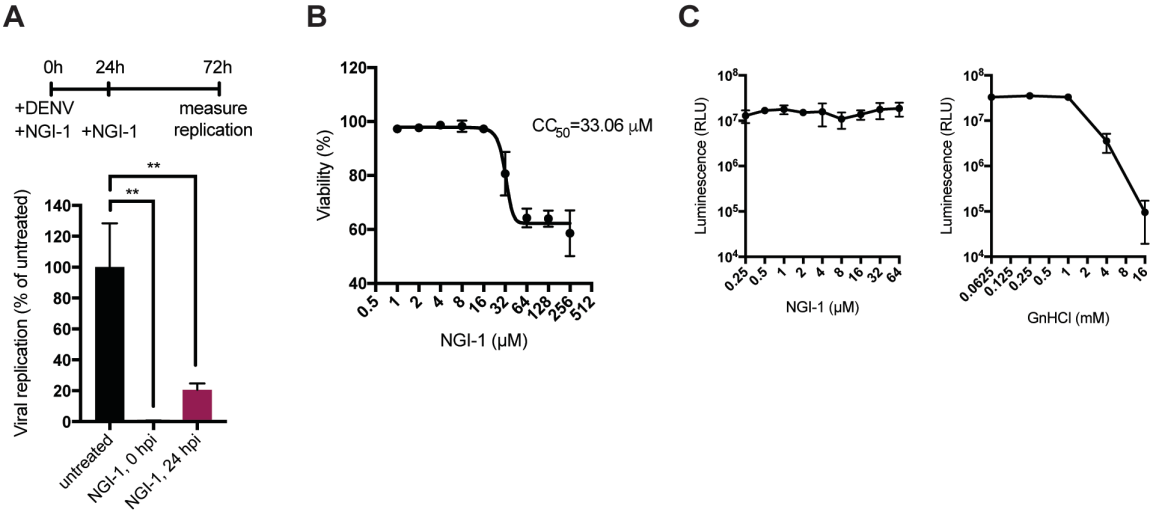


Figure S1: Antiviral effect of NGI-1 is specific to flavivirus infection and not due to cellular toxicity. Related to Figure 1.

(A) Post-infection treatment of DENV-infected cells with NGI-1. HEK293 cells were infected with DENV and subsequently treated with 8 μM NGI-1 at 0 or 24hpi. Viral replication was measured 72hpi.

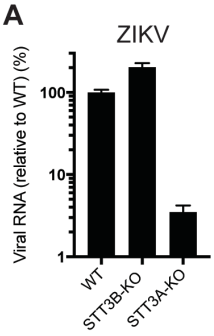
(B) Trypan Blue exclusion assay of HEK293 cells treated with NGI-1 for 48h.

(C) Dose-response curve of NGI-1 or guanidine hydrochloride (GnHCl) for Coxsackievirus B3 expressing Renilla luciferase in HEK293 cells 24hpi.

In all figures, the experiments were performed 2 times with 3 biological replicates each, and one representative is shown. Values are shown as mean ± SD.

Figure S2

A



B

	Genomic sequence	cDNA sequence (catalytic site)
Reference sequence	AAAAATATCCTGTTGGTGAATGCACTGGAG	GCATAGACAGTGTGTCGTTTGCAAGCAAGC
WT HUH7	AAAAATATCCTGTTGGTGAATGCACTGGAG	---
MAGT1 KO HUH7	AAAAATATCCTGTTGGTGAATG–ACTGGAG	---
MAGT1 KO HUH7+ wt MAGT1	AAAAATATCCTGTTGGTGAATG–ACTGGAG	GCATAGACAGTGTGTCGTTTGCAAGCAAGC
MAGT1 KO HUH7+ cat MAGT1	AAAAATATCCTGTTGGTGAATG–ACTGGAG	GCATAGACAGTCCGTCGTTTCTAAGCAAGC

Figure S2: ZIKV RNA replication requires STT3A but not STT3B, and genotyping of WT and MAGT1-KO Huh7 cells. Related to Figure 2.

(A) Quantitative RT-PCR for ZIKV RNA in WT, STT3A-KO or STT3B-KO HAP1 cells 48hpi. The experiment was performed with 3 biological replicates. Values are shown as mean ± SEM.

(B) Genomic DNA was isolated, genomic MAGT1 locus targeted by CRISPR-Cas9 as well as transduced MAGT1 cDNA were PCR amplified and then Sanger sequenced. Genomic sequence targeted by sgRNA and Cysteine-encoding nucleotides of MAGT1 catalytic site in complementing cDNA sequence are highlighted.

Figure S3

A

Frequency of base	Adaptive mutations				
	T4098C	T4472C	A6665G	T7169C	G7577A
A	0.0%	0.1%	99.9%	0.0%	0.1%
C	22.2%	0.5%	0.0%	0.2%	0.0%
G	0.0%	0.0%	0.1%	0.1%	99.9%
T	77.8%	99.5%	0.0%	99.7%	0.0%

TOTAL GENOMES=1280

B

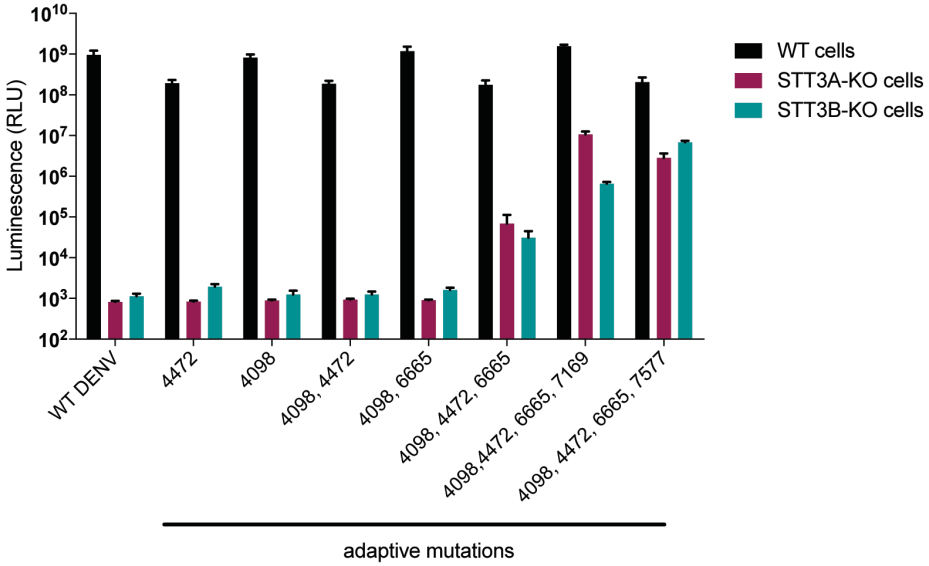


Figure S3: DENV requires introduction of all four adaptive mutations to efficiently replicate in STT3A- or STT3B-KO cells. Related to Figure 3.

(A) Frequency of acquired nucleotides present in the wild-type genomes of isolated DENV2 strains. T4098C is a synonymous mutation.

(B) Acquired mutations from passaging experiment (Figure 3A) were introduced individually or in combination into DENV luciferase reporter virus. WT, STT3A- or STT3B-KO Huh7 cells were infected with different generated reporter viruses and luminescence was measured 48hpi. The experiment was performed with 3 biological replicates. Values are shown as mean ± SD.

Supplemental Experimental Procedures

Cell culture

HEK293FT (Thermo Scientific), Huh7/Huh7.5.1 (gift from Dr. Frank Chisari), JEG-3 (ATCC) and NHDF (Promocell) cells were grown in DMEM supplemented with 10% fetal bovine serum, penicillin-streptomycin, non-essential amino acids and L-glutamine. HAP1 cells were cultured in IMDM supplemented with 10% fetal bovine serum, penicillin-streptomycin and L-glutamine. Raji-DC-SIGN cells (gift from Dr. Eva Harris) were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, penicillin-streptomycin and L-glutamine. Human iPSC derived Neural Progenitors (EMD Millipore) were cultured in fibronectin-coated tissue culture flasks using ENStem-A Neural Expansion Media (EMD Millipore) containing L-glutamine and 30 ng/ml FGF. Human monocyte-derived dendritic cells were derived from $CD14^+$ monocytes. Briefly, LRS chambers with white blood cell concentrate were obtained from the Stanford Blood Center and PBMCs were purified using Ficoll density gradient centrifugation. $CD14^+$ monocytes were isolated from the PBMCs using CD14 microbeads (Miltenyi Biotec) and subsequently differentiated into monocyte-derived dendritic cells by culturing in Mo-DC Differentiation Medium (Miltenyi Biotec) for 7 days. All cells were grown at 37C, 5% CO_2. Cell lines have not been authenticated. HEK293FT, Huh7, JEG-3 and Raji DC-SIGN cells have been tested negative for mycoplasma and other cell lines have not been tested.

Virus strains and serotypes

DENV-2 from infectious clone 16681 (gift from Dr. Karla Kirkegaard) was adapted to HAP1 cells through serial passaging and contains three coding mutations compared to the original clone 16681: Q399H in Envelope, L180F in NS2A and S238F in NS4B. DENV-1 (276RKI, BEI# NR-3782), DENV-2 (429557, BEI# NR-12216), DENV-3 (VN/BID-V1009/2006, BEI# NR-44088), and DENV-4 (BC287/97, BEI# NR-3806) were used as prototypic DENV serotypes in Figure 1G and obtained from BEI resources (NIH, NIAID). ZIKV Malaysian strain (P6-740) was kindly provided by Drs. Scott Weaver and Robert Tesh, ZIKV French Polynesia strain (H/PF/13) was a gift of Dr. Michael Diamond, and ZIKV Puerto Rico strain (PRVABC59, BEI# NR-50240) was ordered from BEI resources (NIH, NIAID). HAP1 adapted DENV-2 was grown on HAP1 cells and tittered on BHK cells using plaque-forming assay. STT3A- and STT3B-KO adapted DENV were grown on STT3A- and STT3B-KO HAP1 cells, respectively, and tittered on BHK cells. Prototypic DENV strains and ZIKV strains were grown on C6/36 cells and tittered on Huh7.5.1 cells. The infectious clone of the luciferase reporter ZIKV (based on strain FSS13025) was obtained from Dr. Pei-Yong Shi (Shan et al., 2016) and produced as follows: the plasmid pZIKV-Rluc was linearized using ClaI and linearized plasmid was used for in vitro transcription using MEGAscript T7 (Invitrogen) with the reaction containing 5mM $m^7G(5')ppp(5')G$ RNA Cap Structure Analog (NEB). For transfection 10 µg RNA was electroporated into 8 x 10^6 Vero cells using Bio-Rad Gene Pulser Xcell electroporator and the square wave protocol (0.85 kV, 25 µF, 3 pulses with 3 seconds intervals, cuvette= 4mm). ZIKV-Luc was harvested from supernatant 7 days post-electroporation. Yellow Fever Virus was generated by culturing the Yellow Fever Vaccine YF-VAX 17D-204 vaccine in HAP1 cells. West Nile Virus (Kunjin strain CH 16532) was a generous gift from Dr. John F. Anderson and was grown in BHK cells. Coxsackie Virus B3 Luciferase was a generous gift from Dr. Frank van Kuppeveld. Chikungunya virus (CHIKV 181/25) was a generous gift from Dr. Margaret Kielian and was propagated by infecting BHK-21 cells. Poliovirus was a generous gift from Dr. Hidde Ploegh. Venezuelan equine encephalitis virus (pVEEV/GFP) was a generous gift from Dr. Ilya Frolov.

Chemical compounds

NGI-1 was synthesized as previously described (Lopez-Sambrooks et al., 2016). Tunicamycin (from Streptomyces sp.) was purchased from Sigma and MK-0608 was purchased from Carbosynth (San Diego, CA).

Quantitative RT-PCR primer sequences

For quantitative RT-PCR experiments the following primer sequences were used:
Universal-DENV-F: GGTTAGAGGAGACCCCTCCC
Universal-DENV-R: GGTCTCCTCTAACCTCTAGTCC
ZIKV-PF13/PRVABC59-F: CCGCTGCCCAACACAAG
ZIKV-PF13/PRVABC59-R: CCACTAACGTTCTTTTGCAGACAT
ZIKV-P6-740-F: GCTCAACGAGCCAAAAAGTC
ZIKV-P6-740-R: TGCTGACTCCTATGCACCTG
Yellow Fever-F: GAAATGCCTGCCCTTTATGA
Yellow Fever-R: GCACATGGCAACAGAAGCTA
Kunjin-F: GCTTTGCCACCTCTCTTCAC
Kunjin-R: CGGTTGATGGTTTCCACTCT
CHIKV-F: CATGCCGTCACAGTTAAGGA
CHIKV-R: AGACGTCGCCTTTGTACACC
VEEV-F: CAGGACGATCTCATTCTCAC
VEEV-R: TCATTCACCTTGTACCGAACG
Polio-F: CAACCTCCCACTGGTGACTT
Polio-R: ATTTCCCCTGCTCAACCTTT
18S-F: AGAAACGGCTACCACATCCA
18S-R: CACCAGACTTGCCCTCCA

Generation of wild type and catalytic mutant MAGT1 cell lines

Isogenic MAGT1 KO Huh7 were previously generated (Marceau et al., 2016). To generate complemented cell lines with either wt or catalytically dead MAGT1-FLAG, geneblocks (Integrated DNA Technologies) were Gibson assembled into pLenti-CMV-DEST-Puro (digested with EcoRV releasing the DEST cassette) and sequence verified. The constructs contained either the wt MAGT1 coding sequence or catalytically dead MAGT1-FLAG mutations (CxxC to SxxS) (Cherepanova et al., 2014; Schulz et al., 2009). Lentivirus was produced in HEK293 cells, and Huh7 MAGT1 KO cells were transduced with different lentiviruses and subsequently selected using 4μg/ml puromycin.
For genotyping of knockout mutation genomic DNA was isolated and the region, which spans the sgRNA-targeting site, was amplified with intron-localized primers: F: ATAATGGTGGGACTCGTGCT and R: CCCTAACTTGGGATAAGGCTTC. Similarly, transduced cDNA sequences were specifically amplified to verify catalytic site mutation using the following primers: F: AGTCAGCTGATGGAATGGACTAAC and R: ATCTGAAATACATCAGAGCCTTCATC.

Construction of dengue luciferase reporter viruses

The construction of the luciferase reporter DENV-2 was previously described (Marceau et al., 2016). After identification of adaptation mutations by serial passage, individual mutations were introduced into luciferase reporter virus using the following methods. To generate DENV-Luc-4098 mutant reporter virus, WT DENV-Luc infectious clone was used as template to produce two PCR products using
5'-GAAGAGTGATGGTTATGGTAGGC-3' with
5'-GAGGGTTGTTAGGAAAATAGCTGTTGG-3' and
5'-CTATTTTCCTAACAACCCTCTCAAGAACC-3' with
5'-GCCTCACCCATCTCCACTCGAGTTG-3'.
Both PCR products were Gibson cloned using Gibson assembly reaction kit into NarI and XhoI cut DENV-Luc infectious clone.
To generate DENV-Luc-4472 mutant reporter virus WT DENV-Luc infectious clone was used as template to produce two PCR products using

5’-GAAGAGTGATGGTTATGGTAGGC-3’ with
5’-CTGCCGTGATTGGTGTTGATACAGG-3’ and
5’-CCTGTATCAACACCAATCACGGCAG-3’ with
5’-GCCTCACCCATCTCCACTCGAGTTG-3’.
Both PCR products were Gibson cloned into NarI and XhoI cut WT DENV-Luc infectious clone.
To generate DENV-Luc-4098,4472 mutant reporter virus DENV-Luc infectious clone containing 4098 point mutation was used as template to produce two PCR products using
5’-GAAGAGTGATGGTTATGGTAGGC-3’ with
5’-CTGCCGTGATTGGTGTTGATACAGG-3’ and
5’-CCTGTATCAACACCAATCACGGCAG-3’ with
5’-GCCTCACCCATCTCCACTCGAGTTG-3’.
Both PCR products were Gibson cloned into NarI and XhoI cut DENV-Luc infectious clone containing the 4098 point mutation.
To generate DENV-Luc-4098,6665 mutant reporter virus DENV-Luc infectious clone containing 4098 point mutation was used as template to produce two PCR products using
5’-GAAGAGTGATGGTTATGGTAGGC-3’ with
5’-CTGTATTTGTGCGCACCATAGGAGGATG-3’ and
5’-CATCCTCCTATGGTGCGCACAAATACAG-3’ with
5’-GTGATCTTCATTTAAGAATCCTAGGGCTTC-3’.
Both PCR products were Gibson cloned into NarI and AvrII cut DENV-Luc infectious clone containing the 4098 point mutation.
To generate DENV-Luc-4098, 4472, 6665 mutant reporter virus DENV-Luc infectious clone containing 4098 point mutation was used as template to produce three PCR products using
5’-GAAGAGTGATGGTTATGGTAGGC-3’ with
5’-CTGCCGTGATTGGTGTTGATACAGG-3’ and
5’-CCTGTATCAACACCAATCACGGCAG-3’ with
5’-CTGTATTTGTGCGCACCATAGGAGGATG-3’ and
5’-CATCCTCCTATGGTGCGCACAAATACAG-3’ with
5’-GTGATCTTCATTTAAGAATCCTAGGGCTTC-3’.
All three PCR products were Gibson cloned into NarI and AvrII cut DENV-Luc infectious clone containing the 4098 point mutation.
To generate DENV-Luc-4098, 4472, 6665 7169 mutant reporter virus DENV-Luc infectious clone containing 4098, 4472, 6665 point mutations was used as template to produce two PCR products using
5’-GAAGAGTGATGGTTATGGTAGGC-3’ with
5’-ATAATGTGCTGCCAATAAGAAAAGAGCTGCTG-3’ and
5’-GAAGGGGAACTGACAACATAGGAGAGACG-3’ with
5’-GTGATCTTCATTTAAGAATCCTAGGGCTTC-3’.
Both PCR products were Gibson cloned into NarI and AvrII cut DENV-Luc infectious clone containing 4098, 4472, 6665 point mutations.
To generate DENV-Luc-4098, 4472, 6665 7577 mutant reporter virus DENV-Luc infectious clone containing 4098, 4472, 6665 point mutations was used as template to produce two PCR products using
5’-GAAGAGTGATGGTTATGGTAGGC-3’ with
5’-CGTCTCTCCTATGTTGTCAGTTCCCCTTC-3’ and
5’-GAAGGGGAACTGACAACATAGGAGAGACG-3’ with
5’-GTGATCTTCATTTAAGAATCCTAGGGCTTC-3’.
Both PCR products were Gibson cloned into NarI and AvrII cut DENV-Luc infectious clone containing 4098, 4472, 6665 point mutations.
DENV-Luc were produced by linearizing the different plasmids using XbaI followed by in vitro transcription and transfection into BHK cells using lipofectamine 2000. Virus was harvested on day 7 and 8

post-transfection. The different DENV-Luc were normalized to an inoculum that yields 200,000 RLUs/well 24hpi in WT Huh7 cells.

Virus adaptation

DENV2 (16681) was serially passaged every two days onto newly plated STT3A-KO and STT3B-KO Huh7 cells by transfer of a 4-fold dilution of the supernatant of infected cells for the first 3 passages and then of a 200-fold dilution for the subsequent passages. Cytopathic effects were observed after approximately 16 passages. Adapted virus was then harvested and RNA extracted from infected cells. cDNA was generated using random heptamers and 2-3 kbp PCR products spanning the length of the DENV2 genome were amplified. PCR products were then sequenced by Sanger sequencing using primers spaced approximately 500bp apart. For STT3A-KO cells, 3 independently adapted viruses were isolated, all containing the same mutations. For STT3B-KO cells, one adapted virus was isolated. Adapted viruses were further propagated on their respective knockout cells to make viral stocks used for infection assays. To assess the frequency of the acquired nucleotides in naturally occurring DENV2 strains, 1280 complete genome sequences from NCBI were mapped to the DENV2 16681 strain containing adapted mutations using Geneious 10.0.7.

Supplemental References

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