Supporting Information

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SI Materials and Methods

Protein Expression. The DENV nonstructural genes were cloned into the pcDNA3.1 expression vector with V5 tags on the C termini. All genes were cloned to mimic the matured proteins with a methionine preceding the first amino acid of what would be the N terminus after cleavage from the polyprotein. All primers used in this study are listed in Table S3. For NS3 expression and purification, NS3 was PCR-amplified with the pcDNA3.1 primers and ligated into the pET151 bacterial expression vector (Invitrogen) with N-terminal V5 and His tags. Protein expression was induced with 250 μM isopropyl-β-D-thiogalactopyranoside (IPTG) at 16 °C for 16 h. NS3 was purified with the use of Ni-NTA beads (Qiagen) and stored in 10% (vol/vol) glycerol buffer until use.

Yeast Two-Hybrid Assay. Yeast strains BK100 (MATa ura3-52 ade2-101 trp1-901 leu2-3,112 his3-200 Gal4Δ gal80Δ GAL2-ADE2 LYS2::GAL1-HIS3 met2::GAL7-lacZ) and R2HMet (MATa ura3-52 ade2-101 trp1-901 leu2-3,112 his3-200 met2Δ::hisG gal4Δ gal80Δ) (1) were propagated according to standard protocols.

RNAi Analysis. A total of 1×10^6 Huh-7.5 cells in 0.05 mL of PBS (pH 7.4) was electroporated with 125 pM siRNA and 300 ng of DENV reporter replicon RNA for five pulses of 770 V for 99 μs with 1-s intervals on a BTX 830 electroporator with 96-well attachment. A total of 4×10^4 cells was plated into 96-well plates with DMEM supplemented with 10% (vol/vol) FBS. Cells were harvested at 6 and 48 h postelectroporation, and the level of DENV replication was determined via use of the Renilla Luciferase Assay System (Promega) per the manufacturer's instructions. The 48:6 h luciferase ratio represents DENV replication relative to input RNA. In parallel, cells were assayed for cellular ATP levels to assess viability using a CellTiter-Glo Luminescent Cell Viability Assay kit (Promega) per the manufacturer's instructions. Statistical significance was determined using an unpaired Student's *t* test.

DENV RNA Quantitation. DENV RNA was quantified using a custom-designed primer probe set: forward: 5′-TCC CAA ACG CAG TGA TAT TAC AA, reverse: 5′-TGA GAC CTT TGA TCG TCA ATG C, and probe: 5′-TGG TGT CCG TTT CCC CAC TGC TCT T. RNA was reverse-transcribed for 30 min at 50 °C, followed by an inactivation step (95 °C for 6 min). cDNA was then amplified at 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 15 s for 50 cycles. DENV RNA levels were normalized to an 18S ribosomal RNA internal control. Assays were performed on an ABI 7300 system and analyzed with SDS 1.3 software (Applied Biosystems).

Quantitation of Infectious DENV. Cellular supernatants containing released DENV particles were quantified via limiting dilution titer as previously described (2). Briefly, virus was serially diluted and plated in replicates of eight in a 96-well format. 48 hours postinfection cells were fixed and stained with antibodies to the DENV E protein (American Type Culture Collection; HB-112) to determine the number of wells positive for virus. The ID_{50} per milliliter of the sample was then calculated.

1. LaCount DJ, et al. (2005) A protein interaction network of the malaria parasite Plasmodium falciparum. *Nature* 438:103–107.

2. Randall G, et al. (2006) Silencing of USP18 potentiates the antiviral activity of interferon against hepatitis C virus infection. *Gastroenterology* 131:1584–1591.

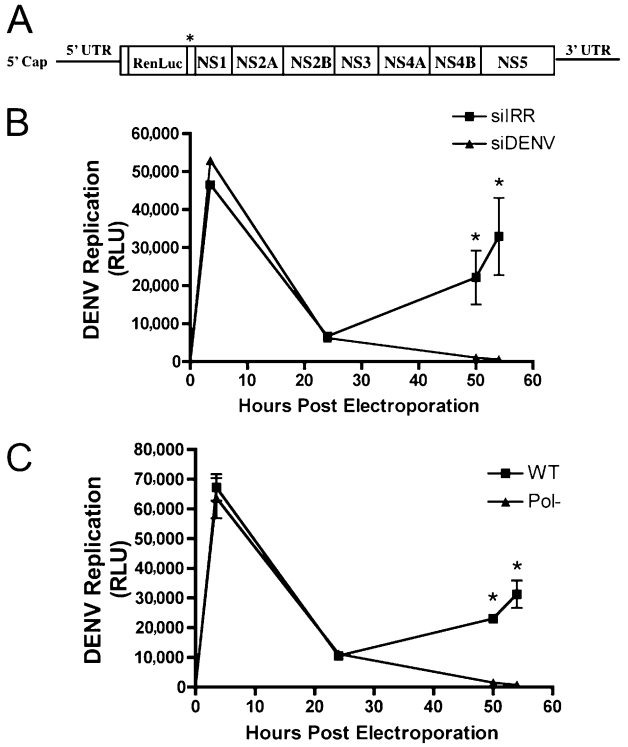

Fig. S1. Characterization of the DENV replicon. (*A*) Diagram of the DENV luciferase replicon. *Foot-and-mouth disease virus cleavage site. (*B*) Huh-7.5 cells were electroporated with the DENV replicon and either an irrelevant siRNA (siIRR) or a siRNA targeting the DENV genome (siDENV). Viral replication was determined by luciferase assay at the indicated times. (*C*) WT or polymerase-defective (Pol–) replicon RNAs were electroporated into Huh-7.5 cells, and viral replication was determined (*$P \leq 0.05$). RLU, relative luciferase units.

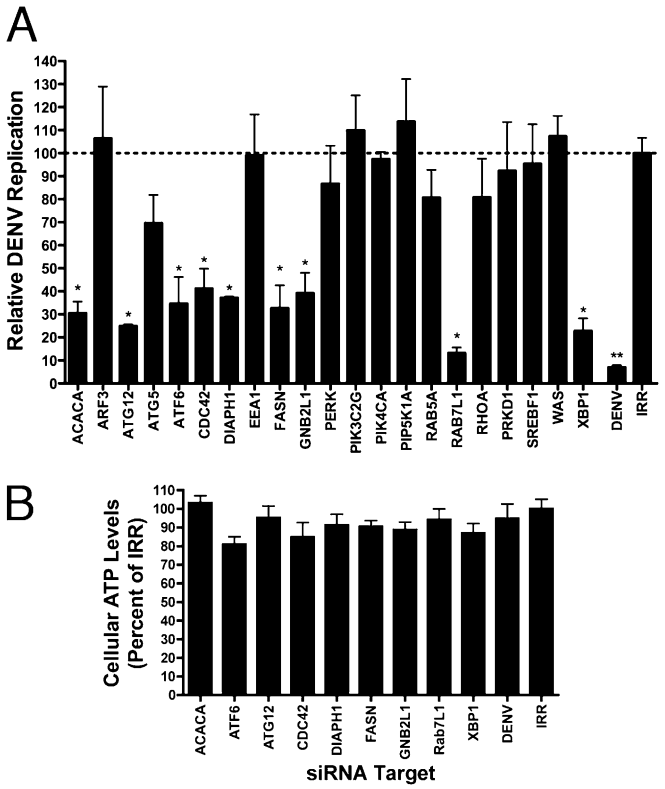

Fig. S2. siRNA knockdown of cellular genes inhibits DENV replication without compromising cellular viability. (*A*) Huh-7.5 cells were electroporated with siRNA pools targeting the indicated gene and DENV replicon RNAs. Graph represents the average ± SEM of DENV replication after silencing the indicated gene at 48 h postelectroporation. Genes tested are parts of the following biological pathways: lipid biogenesis, actin polymerization, autophagy and ER stress, phosphatidylinositol signaling, and vesicle trafficking. $*P \leq 0.05$; $**P \leq 0.001$. (*B*) All the genes that showed a significant reduction in viral replication when silenced were introduced into Huh-7.5 cells along with the DENV replicon. At 48 h postelectroporation, the cells were assayed for cellular ATP levels. No siRNA treatment resulted in a decrease in cellular viability that could account for the inhibition of viral replication. IRR indicates an siRNA targeting an irrelevant hepatitis C virus sequence; DENV indicates an siRNA targeting the DENV genomic RNA.

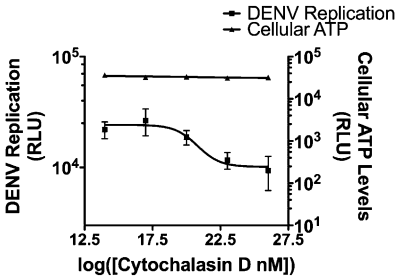

Fig. S3. Cytochalasin D inhibits DENV replication. Huh-7.5 cells were electroporated with the DENV replicon, and Cytochalasin D was applied at the indicated concentrations. DENV replication was assayed via luciferase assay 48 h postelectroporation. In parallel, cellular viability was also measured. RLU, relative luciferase units.

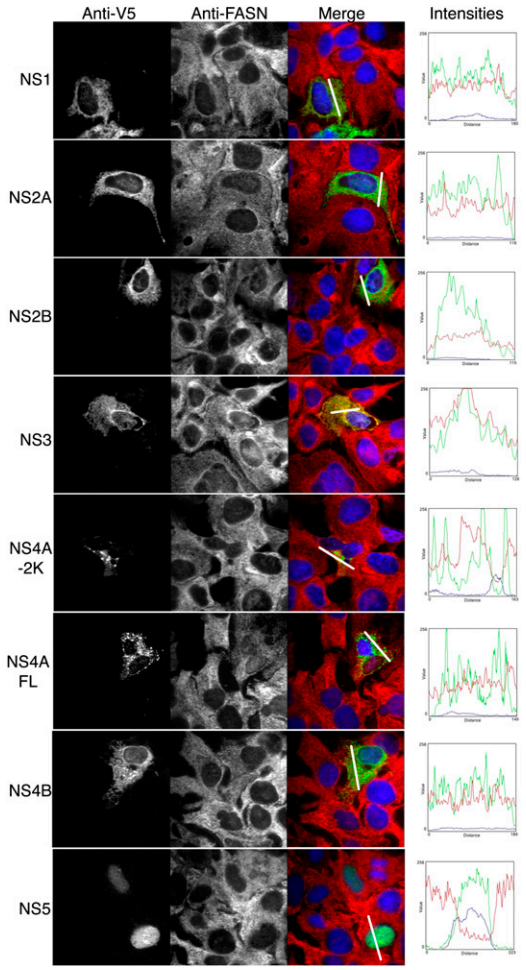

Fig. S4. NS3 is the only DENV nonstructural protein that colocalizes with FASN when transiently expressed. Huh-7.5 cells were transiently transfected with each of the DENV nonstructural proteins expressed as V5-tag fusions. Seventy-two hours posttransfection, cells were fixed and the localization of the V5-tagged proteins and their relationship to endogenous FASN were determined. The intensity plots represent the fluorescent intensities along the line indicated in the merge panel.

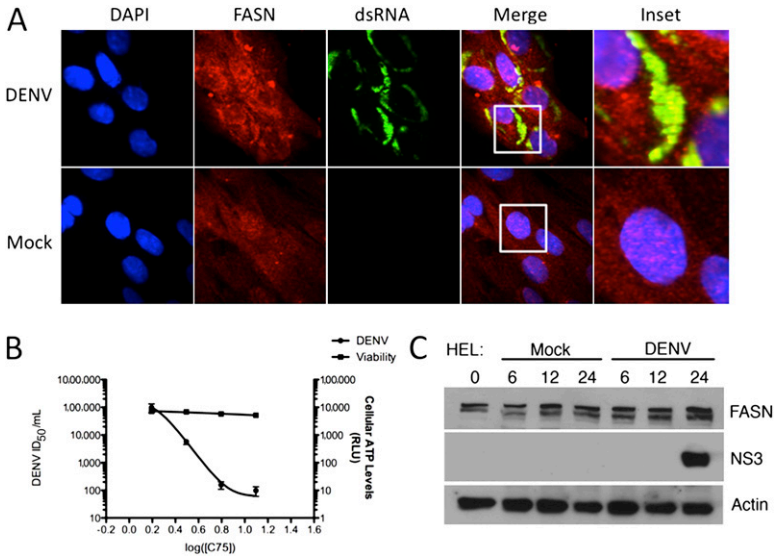

Fig. S5. FASN required and relocalized in HEL cells similar to Huh-7.5 cells. (*A*) HEL cells were mock- or DENV-infected at a multiplicity of infection (MOI) of 5 and fixed 36–48 h postinfection. Cells were probed with FASN (red) and dsRNA (green) antibodies, followed by the appropriate fluorescently conjugated secondary antibody. Insets reflect zoomed areas of the boxed regions. (Scale bar: 30 μm.) (*B*) HEL cells were DENV-infected at a MOI of 1 for 4 h, virus was removed, and medium containing the indicated concentration of C75 was applied in 1% DMEM. Cellular supernatants were harvested 24 h postinfection, and released virus was quantified via limiting dilution titer. (*C*) HEL cells were DENV-infected at a MOI of 5. At the indicated times postinfection, cells were lysed and protein was extracted. Western blot analysis was performed to determine the relative levels of FASN protein. The blot was stripped and reprobed with actin antibodies as a loading control or with NS3 antibodies to indicate DENV infection.

Table S1. siRNAs used in this study

ACACA	GCAGAUGACUUCCCUAAUC	*	*PIK3C2G*	GUAAAGCCUUGAAUGAUGA	
	GCAAUUAGAUUCGUUGUCA	*		GGACCGAGCUCCUUUCAUU	
	GAAAGCAGGUCAACUAUGA			GCACAAAUGUCAGCCAUAA	
	CAGCAAACCUGGAUUCUGA			GAAAGAAAUUGGCAGACUA	
ARF3	GGGAAGAGCUGAUGAGAAU		*PIK4CA*	GAGCAUCUCUCCCUACCUA	
	GGAAAGACCACCAUCCUAU			GUGAAGCGAUGUGGAGUUA	
	UAUGAACGCUGCUGAGAUC			CCACAGGCCUCUCCUACUU	
	GCAAUGAUCGGGAGCGAGU			GCAGAAAUUUGGCCUGUUU	
ATF6	GCAAAUGAGACGUAUGAAA	*	*PIP5K1A*	AGAAGCAGCUCCUCUUUAA	
	GGAGUGAGCUACAAGUGUA	*		GGAGAGACUUUGCAAAUGC	
ATG12	GCAGUAGAGCGAACACGAA	*		ACACAGUACUCAGUUGAUA	
	GCAGCUUCCUACUUCAAUU	*		AGUGAGGGCUCGCCUAUUC	
	GGGAAGGACUUACGGAUGU		*PRKD1*	GAAGAGAUGUAGCUAUUAA	
	GAACACCAAGUUUCACUGU			GAAAGAGUGUUUGUUGUUA	
ATG5	GGAAUAUCCUGCAGAAGAA			GAAUGCAGCUUUCAUGUAU	
	CAUCUGAGCUACCCGGAUA			UCGAAAUCACUACGGCAAA	
	GACAAGAAGACAUUAGUGA		*Rab5A*	GCAAGCAAGUCCUAACAUU	
	CAAUUGGUUUGCUAUUUGA			GGAAGAGGAGUAGACCUUA	
CDC42	UGGUGCUGUUGGUAAAACA	*		AGGAAUCAGUGUUGUAGUA	
	UGAGAUAACUCACCACUGU	*		GAAGAGGAGUAGACCUUAC	
	GGAGAACCAUAUACUCUUG		*Rab7L1*	CAGAUUGACCGGUUCAGUA	*
	GAUUACGACCGCUGAGUUA			GAGAACGGUUUCACAGGUU	*
	GAUGACCCCUCUACUAUUG			GGGACUACAUCAAUCUACA	
	CGGAAUAUGUACCGACUGU			CAACAAGUGUGAUCUGUCC	
DIAPH1	GGAGAUGGAUGACUUUAAU	*	*RHOA*	AUGGAAAGCAGGUAGAGUU	
	GAUAUGAGAGUGCAACUAA	*		GAACUAUGUGGCAGAUAUC	
	GAAGUGAACUGAUGCGUUU			GAAAGACAUGCUUGCUCAU	
	GAAGAGAGAGCAACUCAUA			GAGAUAUGGCAAACAGGAU	
EEA1	AGACAGAGCUUGAGAAUAA		*SREBF1*	UGACUUCCCUGGCCUAUUU	
	GAGAAGAUCUUUAUGCAAA			ACAUUGAGCUCCUCUCUUG	
	GAAGAGAAAUCAGCAGAUA			GCGCACUGCUGUCCACAAA	
	GAACAAGACUAUACUAAGU			ACACAGACGUGCUCAUGGA	
FASN	CCAUGGAGCGUAUCUGUGA	*	*WAS*	CGAGAACCAGCGACUCUUU	
	GCAACUCACGCUCCGGAAA	*		GCUGGCCACUGCAGUUGUU	
	GAGCGUAUCUGUGAGAAAC			GAACAUACCCUCCACCCUC	
	UGACAUCGUCCAUUCGUUU			CCUCUAAACUUAUCUACGA	
GNB2L1	CCAAGGAUGUGCUGAGUGU	*	*XBP1*	GCCUGUCUGUACUUCAUUC	*
	GAGAUAAGACCAUCAUCAU	*		CUCAUGGCCUUGUAGUUGA	*
	CAAAUACACUGUCCAGGAU		*DENV*	CGGGAAAGACGAAGAGAUAUU	
	GAUAAGACCAUCAUCAUGU		*IRR*	GGACUUCCAGAAGAACAUC	
PERK	GGUUUCGGUUGCUGACUGG			GGCGCUUGUGGACAUUCUG	
	CUGUAUAACGGUUUAUGUC				

*Individual siRNAs tested.

Table S2. RT-PCR assays used in this study

CDC42	Hs00741586_mH*
RAB7L1	Hs00187510_m1*
ACACA	Hs00167385_m1*
FASN	Hs00188012_m1*
DIAPH1	Hs00946556_m1*
ATF6	Hs00232586_m1*
XBP1	Hs00231936_m1*
ATG12	Hs01047860_g1*
GNB2L1	Hs00914568_g1*
GAPDH	4326317E*

*Applied Biosystems product number.

Table S3. Primers used in this study

DENV protein	Forward primer	Reverse primer
NS1	CACCATGGATAGTGGTTGCGTTGTG	AGCAGCTGTGACCAAGGAGTT
NS2A	CACCATGGGACATGGGCAGGTCGAC	AGCCCTTTTCTTGCTGGTTCT
NS2B	CACCATGAGCTGGCCATTAAATGAG	AGCCCGTTGTTTCTTCACTTC
NS3	CACCATGGCCGGAGTATTGTGGGAT	AGCCTTTCTTCCGGCTGCAAA
NS2B3	CACCATGAGCTGGCCATTAAATGAG	AGCCTTTCTTCCGGCTGCAAA
NS4A-2K	CACCATGTCTCTGACCCTGAACCTA	AGCTCTCTGTTTTTCAGGTTC
NS4A FL	CACCATGTCTCTGACCCTGAACCTA	AGCTGCCATGGTTGCGGCCAC
NS4B	CACCATGAACGAGATGGGTTTCCTA	AGCCCTTCTTGTGTTGGTTGT
NS5	CACCATGGGAACTGGCAACATAGGA	AGCCCACAGAACTCCTGCTTC