Supporting Information

Price et al. 10.1073/pnas.1422340112

Fig. S1. Design of the rgRNA. (*A* and *B*) Schematic of the interaction between the (*A*) *F. novicida* U112 CRISPR repeat sequence and the tracrRNA (red) or (*B*) the scaRNA (blue) and the tracrRNA. (*C*) Design of the rgRNA based on the interaction between the tracrRNA (red) and the scaRNA (blue) with the RNA targeting region (black).

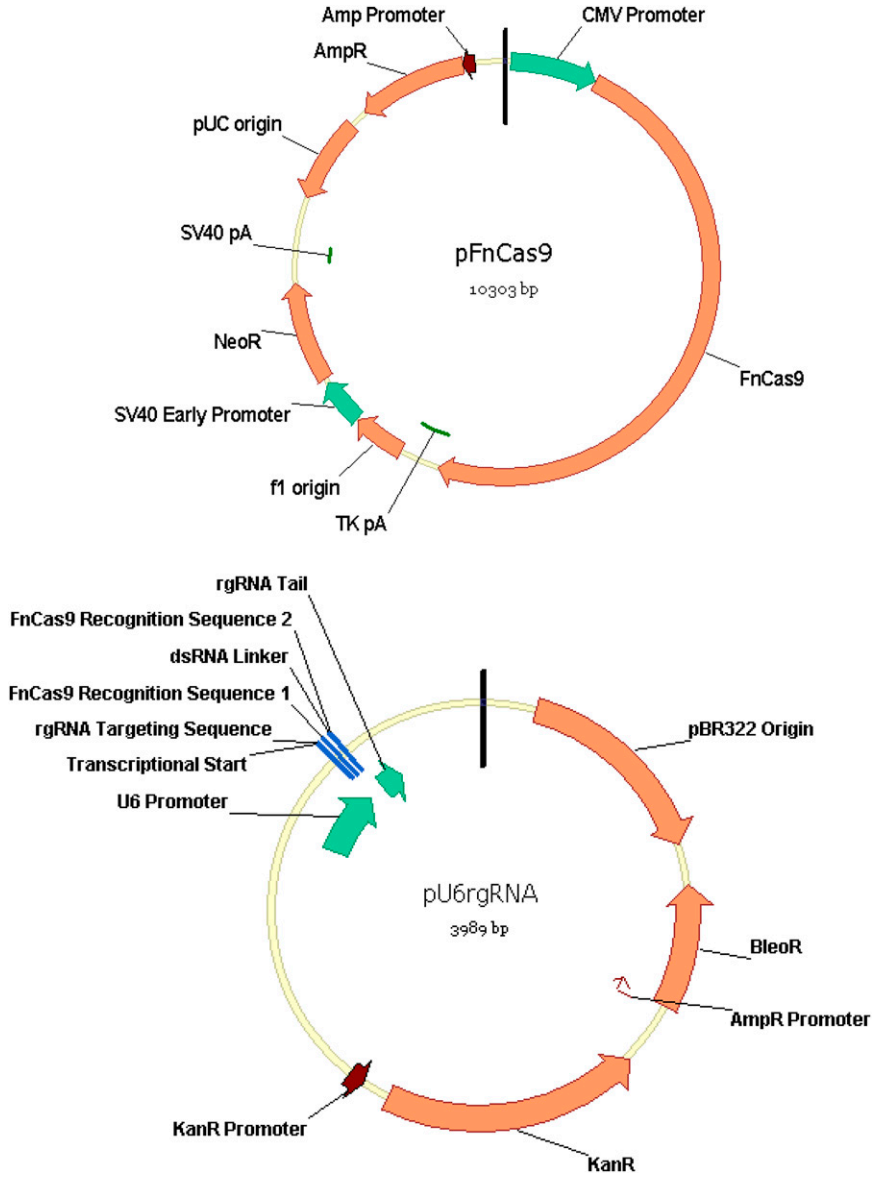

Fig. S2. Plasmid maps of the FnCas9 and rgRNA encoding vectors.

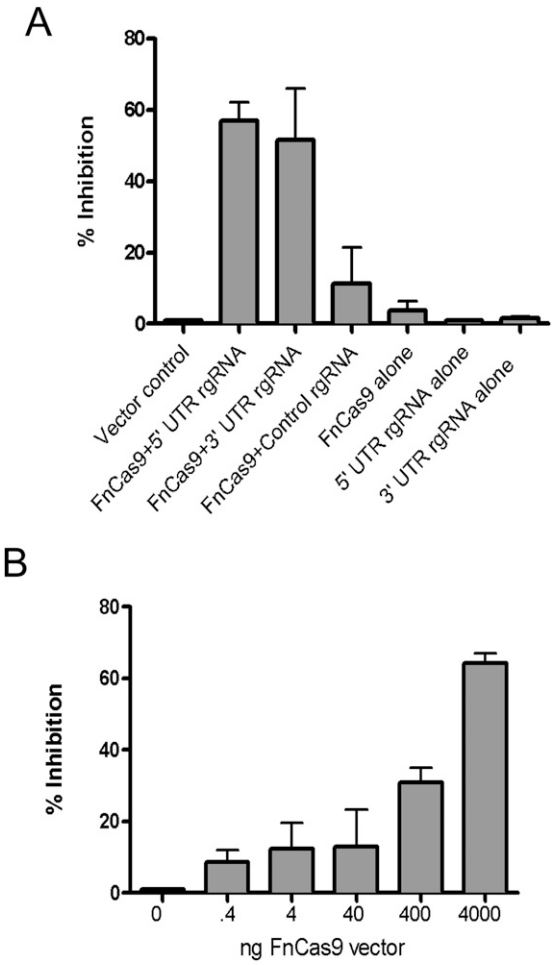

Fig. S3. HA epitope-tagged FnCas9 retains the ability to inhibit HCV luciferase production in a dose-dependent manner. (*A*) Huh-7.5 cells were transfected with the indicated combinations of HA epitope-tagged FnCas9 and rgRNA as well as HCV RNA. At 72 h postinfection, viral luciferase production was measured and displayed as percent inhibition compared with vector controls ($n = 3$; bars represent the SEM; data are representative of at least five experiments). (*B*) Experiments were performed as above, varying the concentration of FnCas9 ($n = 3$; bars represent the SEM; data are representative of at least two experiments).

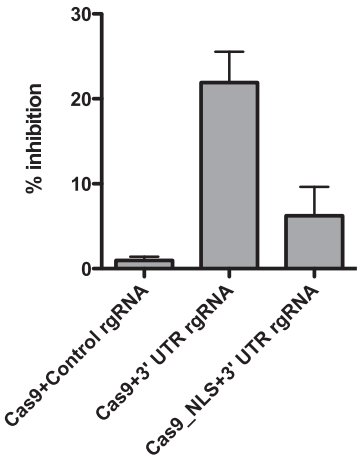

Fig. S4. NLS abrogates FnCas9 inhibition of HCV luciferase production. Huh-7.5 cells were transfected with the indicated combinations of FnCas9 (with or without an NLS) and rgRNA targeting the 3′ UTR as well as HCV RNA encoding *Renilla* luciferase. At 72 h postinfection, viral luciferase production was measured and displayed as percent inhibition compared with vector controls ($n = 3$; bars represent the SEM; data are representative of at least five experiments).

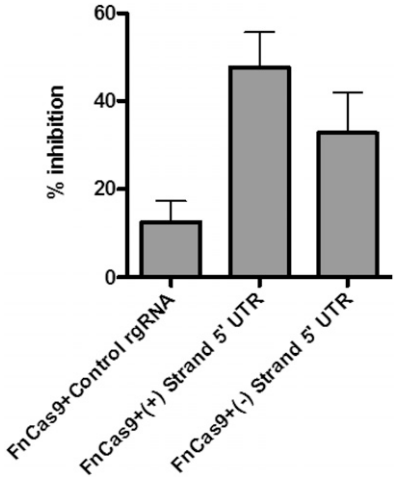

Fig. S5. FnCas9 can inhibit HCV through positive or negative sense strand targeting. Huh-7.5 cells were transfected with the indicated combinations of FnCas9 and an rgRNA targeting either the positive or negative sense strand of the 5′ UTR as well as HCV RNA encoding *Renilla* luciferase. Viral luciferase production was measured and displayed as percent inhibition compared with FnCas9 and control targeting rgRNA ($n = 3$; bars represent the SEM; data are representative of at least two experiments).

A

Target	5′ region	3′ region
5′ UTR	aaggc	gggcg
5′ UTR shifted	gaaca	cuugc
5′ UTR (-) strand	gcggg	uggua
3′ UTR	guggc	cggcu

B

dsDNA target:

(NG PAM +strand)
3′-agcggtagtgtgatattttcattagttcgaacggtaacagggcg-5′ (+strand)
5′-tccccatcacactataaaagtaatcaagcttgccattgtcccgc-3′ (-strand)
3′-uagugugauauuuucauuaguucgaacgguaaca-5′ **spacer1 crRNA**

(NG -strand)
3′-agccctagtgtgatattttcattagttcgaacggtaacaacccg-5′ (+strand)
5′-tcggcatcacactataaaagtaatcaagcttgccattgttgggc-3′ (-strand)
3′-uagugugauauuuucauuaguucgaacgguaaca-5′ **spacer1 crRNA**

C

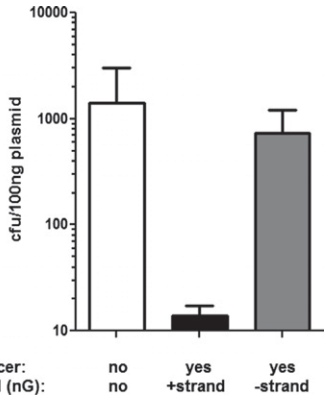

Fig. S6. FnCas9 targeting of DNA is PAM-dependent, whereas RNA targeting is PAM-independent. (*A*) Alignment of the adjacent sequences to both the 5′ and 3′ end of the regions targeted by the rgRNAs in this manuscript. (*B*) Schematic outline of the natural crRNA 1 and proto-spacer interaction in the context of the PAM sequence (–NG) (1). (*C*) Transformation efficiency (plotted as cfu per 100 ng of plasmid) of wild-type *F. novicida* U112 with either empty vector, a vector containing spacer 1 of the *F. novicida* crRNA array, and the predicted PAM on the nontargeted strand (which would be recognized by Cas9) or a predicted PAM on the targeted strand (which is not recognized by Cas9) ($n = 3$; bars represent the SEM; data are representative of at least two experiments).

1. Fonfara I, et al. (2014) Phylogeny of Cas9 determines functional exchangeablity of dual-RNA and Cas9 among orthologous type II CRISPR-Cas systems. *Nucleic Acids Res* 42(4):2577–2590.

a

S. pyogenes HCV 5′ UTR Targeting rgRNA

```
AGAUCGA--GAUUUUGGAACACCAUGACGGACUAUG-5
A ||||   :||||||:
 AUAGCAAGUUAAAAUA
               A
                GGCUAGUCCGUUAUCAACUUG-3′
```

b

S. thermophilus HCV 5′ UTR Targeting rgRNA

```
AGUAGAACUC-UCAUGUUUUUGGAACACCAUGACGGACUAUG-5
A |||||||| |  ||||||||
 AAUCUUGAGAAAGACAAAAACA
                     A
                      GGCUAGUCCGUUAUCAACUUG-3′
```

c

N. meningitidis HCV 5′ UTR Targeting rgRNA

```
AGAGUCUUUCCCUCGAUGUUGGAACACCAUGACGGACUAUG-5′
A |||||||||  | |||||||
 AUCAGAAAGGAAUCUACAACA
                    A
                     GGCUAGUCCGUUAUCAACUUG-3′
```

Fig. S7. rgRNAs for orthologous Cas9 proteins. Schematic of the rgRNAs generated to target the 5′ UTR of HCV RNA by orthologous Cas9 proteins from (*A*) *S. pyogenes*, (*B*) *S. thermophilus*, and (*C*) *N. meningitidis*.

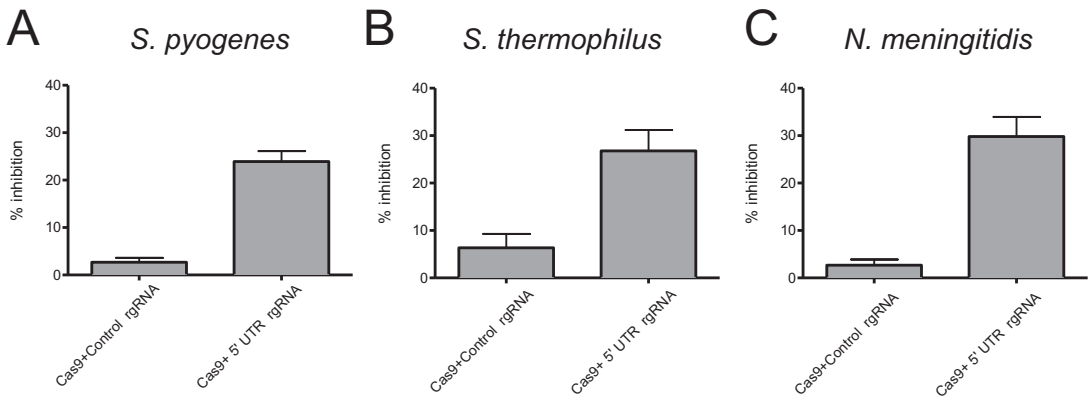

Fig. S8. Orthologous Cas9 proteins are capable of HCV inhibition. Huh-7.5 cells were transfected with Cas9 proteins from (*A*) *S. pyogenes*, (*B*) *S. thermophilus*, or (*C*) *N. meningitidis*, as well as a cognate control or 5′ UTR-targeting rgRNA (Fig. S7), and infected with HCV. At 72 h, viral luciferase was quantified. The percent inhibition compared with the control rgRNA is displayed ($n = 8$; data were compiled from three independent experiments).

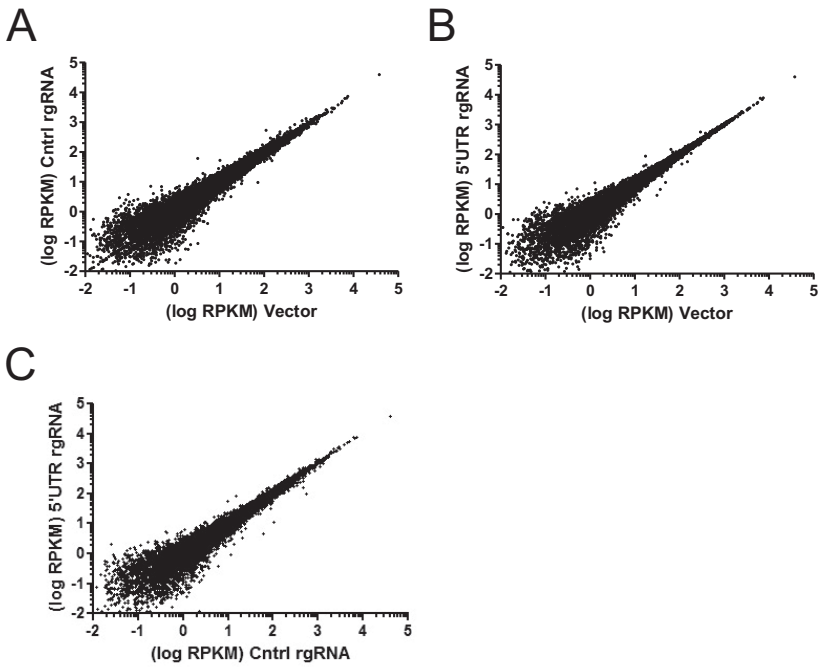

Fig. S9. Global gene expression of Huh-7.5 cells transfected with the FnCas9 machinery. Huh-7.5 cells were transfected with FnCas9 and either the non-targeting control rgRNA, 5′ UTR-targeting rgRNA, or an empty vector alone. RNA was extracted and sequenced to analyze gene expression. Data are plotted to compare the reads per kilobase per million mapped reads for each individual gene. (*A*) FnCas9 + control rgRNA compared with vector alone. (*B*) FnCas9 + 5′ UTR-targeting rgRNA compared with vector alone. (*C*) FnCas9 + control rgRNA compared with FnCas9 + 5′ UTR-targeting rgRNA.

Table S1. Primers used in this study

Primer name	Sequence
Cloning	
Cas9	
FnCas9SLIC-F1	ACCGGGACCGATCCAGCCTCCGGACTTCTAGAATGAACTTTAAGATCC
FnCas9SLIC-R1	GTTTCAGTTAGCCTCCCCCGTTTGTTTAAACTCATTACTAGGCGTAGTC
StCas9SLIC-F1	GACCGATCCAGCCTCCGGACTCTAGAGCCACCATGGGCGGTAGGCGTGTACGGTGG
StCas9SLIC-R1	TAGGCGTAGTCAGGCACATCATAAGGGTATCCGGAGCCGCCGTCAGCCCTGCTGAAGTC
SpCas9SLIC-F1	GACCGATCCAGCCTCCGGACTCTAGAGCCACCATGGACAAGAAGTACTCCATTGG
SpCas9SLIC-R1	GCGTAGTCAGGCACATCATAAGGGTATCCGGAGCCGCCGTCAGCCCTGCTGTCTCCAC
NmCas9SLIC-F1	GACCGATCCAGCCTCCGGACTCTAGAGCCACCATGGCTGCCTTCAAACCTAATTCAATC
NmCas9SLIC-R1	CGTAGTCAGGCACATCATAAGGGTATCCGGAGCCGCCACGGACAGGCGGGCGTTTTTTC
pcDNAcomCas9Rev	GTTTCAGTTAGCCTCCCCCGTTTAAACTCATTACTAGGCGTAGTCAGGCACATCATAAG
rgRNAs	
FnCas9-HCV5UTR-rev	AATTACGAGCTTGTGGTACTGCCTGATACGGTGTTTCGTCCTTTCCACAAGATATATAAAGCCAAG
FnCas9-HCV5UTR-fwd	GTACCACAAGCTCGTAATTAATAAACCATGAAAGTATGGTTTATTAGATTGTTGAAGGCTAGTCCGTTATCAAC
FnCas9-HCV3UTR-rev	AATTACGAGTCCATCTTAGCCCTAGTCACGGTGTTTCGTCCTTTCCACAAGATATATAAAGCCAAG
FnCas9-HCV3UTR-fwd	CTAAGATGGACTCGTAATTAATAAACCATGAAAGTATGGTTTATTAGATTGTTGAAGGCTAGTCCGTTATCAAC
FnCas9-control-1rev	AATTACGAGTGAATTTAATTCTGAGAAGACGGTGTTTCGTCCTTTCCACAAGATATATAAAGCCAAG
FnCas9-control-1fwd	AATTAAATTCACTCGTAATTAATAAACCATGAAAGTATGGTTTATTAGATTGTTGAAGGCTAGTCCGTTATC
HCV5UTR-5ntScramFwd	AGTACCTTGAGCTCGTAATTAATAAACCATGAAAGTATGGTTTATTAGATTGTTGAAGGCTAGTCCGTTATCAAC
HCV5UTR-5ntScramRev	CTCAAGGTACTGCCTGATACGGTGTTTCGTCCTTTCCACAAGATATATAAAGCCAAG
HCV5UTR-9ntScramFwd	AGCTATTAAACGCTCGTAATTAATAAACCATGAAAGTATGGTTTATTAGATTGTTGAAGGCTAGTCCGTTATCAAC
HCV5UTR-9ntScramRev	CGTTTAATAGCTGCCTGATACGGTGTTTCGTCCTTTCCACAAGATATATAAAGCCAAG
HCV5UTR-shiftedFwd	CAGGCAGTACCCTCGTAATTAATAAACCATGAAAGTATGGTTTATTAGATTGTTGAAGGCTAGTCCGTTATCAACTTG
HCV5UTR-shiftedRev	GGTACTGCCTGATAGGGCGCCGGTGTTTCGTCCTTTCCACAAGATATATAAAGCCAAGAAATC
HCV5UTR-negFwd	CTGCCTGATACTCGTAATTAATAAACCATGAAAGTATGGTTTATTAGATTGTTGAAGGCTAGTCCGTTATCAACTTG
HCV5UTR-negRev	TATCAGGCAGTACCACAAGCGGTGTTTCGTCCTTTCCACAAGATATATAAAGCCAAGAAATCG
5′5bpscrambleFWD	ATAGCGGCAGTACCACAAGCTCGTAATTAATAAACCATG
5′5bpscrambleREV	CTTGTGGTACTGCCGCTATCGGTGTTTCGTCCTTTCCACAAGATATATAAAGCC
3′6bpscrambleFWD	TATCAGGTAGTACTTAAACCTCGTAATTAATAAACCATG
3′6bpscrambleREV	GTTTAAGTACTACCTGATACGGTGTTTCGTCCTTTCCACAAGATATATAAAGCC
3′8bpscrambleFWD	TATCAGGCAGTTATTAAACCTCGTAATTAATAAACCATG
3′8bpscrambleREV	GTTTAATAACTGCCTGATACGGTGTTTCGTCCTTTCCACAAGATATATAAAGCC
5′3bpscrambleFWD	GATACAGGCAGTACCACAAGCTCGTAATTAATAAACCATG
5′3bpscrambleREV	GCTTGTGGTACTGCCTGTATCGGTGTTTCGTCCTTTCCAC
5′1T:AFwd	AATCAGGCAGTACCACAAGCTCGTAATTAATAAACCATG
5′1T:ARev	GCTTGTGGTACTGCCTGATTCGGTGTTTCGTCCTTTCCAC
5′2A:CFwd	TCTCAGGCAGTACCACAAGCTCGTAATTAATAAACCATG
5′2A:Crev	GCTTGTGGTACTGCCTGAGACGGTGTTTCGTCCTTTCCAC
5′3T:AFwd	TAACAGGCAGTACCACAAGCTCGTAATTAATAAACCATG
5′3T:AREV	GCTTGTGGTACTGCCTGTTACGGTGTTTCGTCCTTTCCAC
5′2bpscrambleFWD	ATTCAGGCAGTACCACAAGCTCGTAATTAATAAACCATGAAAGTATG
5′2bpscrambleREV	CTTGTGGTACTGCCTGAATCGGTGTTTCGTCCTTTCCACAAGATATATAAAGCC
5′3/2bpscrambleFWD	TTACAGGCAGTACCACAAGCTCGTAATTAATAAACCATGAAAGTATG
5′3/2bpscrambleREV	CTTGTGGTACTGCCTGTAACGGTGTTTCGTCCTTTCCACAAGATATATAAAGCC
5′3/1bpscrambleFWD	AAACAGGCAGTACCACAAGCTCGTAATTAATAAACCATGAAAGTATGG
5′3/1bpscrambleFWD	CTTGTGGTACTGCCTGTTTCGGTGTTTCGTCCTTTCCACAAGATATATAAAGCC
DNA targeting	
Spacer 1 FWD pBav (–strand nG)	CACTATAAAAGTAATCAAGCTTGCCATTGTTGGGCGGCCGCTTCTAGAGGAAATC
Spacer 1 REV pBav (–strand nG)	GCAAGCTTGATTACTTTTATAGTGTGATCCCGAATTCGACGTCAAATTCTATC
Spacer1 FWD pBav (+strand nG)	CACTATAAAAGTAATCAAGCTTGCCATTGTCCCGCGGCCGCTTCTAGAGGAAATC
Spacer1 REV pBav (+strand nG)	GCAAGCTTGATTACTTTTATAGTGTGATGGGGAATTCGACGTCAAATTCTATC
RT-PCR	
rgRNA-RTrev	CAAGTTGATAACGGACTAGCCTT
5UTR-RTfwd	TATCAGGCAGTACCACAAGC
Cntrl-RTfwd	TCTTCTCAGAATTAAATTCAC
HCV 5′ Fwd	AGY GTT GGG TYG CGA AAG
HCV 5′ Rev	CAC TCG CTT GCY CCC T
HCV 5′ Probe	6-FAM-CCT TGT GGT ACT GCC TGA-MGB NFQ
Gapdh-Fwd	GAAATCCCATCACCATCTTCCAGG
Gapdh-Rev	GAGCCCCAGCCTTCTCCATG

Other Supporting Information Files

Dataset S1 (DOCX)
Dataset S2 (XLSX)