SUPPLEMENTARY FIGURE AND TABLE LEGENDS

Figure S1. AAV vectors expressing HCV-miRNA-Cluster 5 prevent HCV spread.

(a) Huh-7.5 cells were co-infected with HCVcc (~0.2 IU/cell) and scAAV2-HCV-miR-Cluster 5, scAAV2-miR-Core, or scAAV2-eGFP at an AAV dose of $1x10^5$ vg/cell. Positive control wells included Huh-7.5 cells treated with IFN-α (200u/ml). Negative control wells consisted of Huh-7.5 cells that were infected with HCVcc but were not treated with a drug. Two hours later, the media was replaced and the cells were incubated for 48 hrs. Supernatants were serially passaged (total of six rounds) onto Huh-7.5 cells that had been transduced 24 hr previously with the corresponding AAV vector. After each round, total cellular RNA was isolated and HCVcc RNA levels were measured by QRT-PCR (limit of sensitivity= 0.05 HCV copies/cell). Data represent triplicate HCV RNA measurements of duplicate infections.

Figure S2. Southern blot analyses of mouse liver DNA.

Top panel: Copy number controls (generated by spiking liver genomic DNA from naïve mice with pscAAV -HCV-miR-Cluster 5 plasmid DNA) from 100-0.3 AAV copies/diploid genome (lanes 1-6), SphI-digested genomic liver DNA from mice injected with $2.5x10^9$ vg of pscAAV-HCV-miR-Cluster 5 (lanes 7-11), $2.5x10^{10}$ vg (lanes 12, 13), and uninjected mice (lane 14). Bottom panel: Copy number controls from 900-3 AAV copies/diploid genome (lanes 1-6), SphI-digested genomic liver DNA from mice injected with $2.5x10^{10}$ vg of pscAAV-HCV-miR-Cluster 5 (lanes 7-9), and $2.5x10^{11}$ vg (lanes 10-14).

Table S1. Sequence variation in miR-Core target site of HCV2a genome after various treatments (454/Roche PyroSequencing Analysis).

Huh-7.5 cells were co-infected with HCVcc (~0.2 IU/cell) and either scAAV2-HCV-miR-Cluster 5 or scAAV2-miR-Core at an AAV dose of $1x10^5$ vg/cell. Positive control wells included Huh-7.5 cells treated with IFN-α (200 u/ml) ad negative control wells consisted of Huh-7.5 cells that were infected with HCVcc, but were not treated with a drug. Following a 48 hr incubation period, supernatants were serially passed (total of 6 rounds) onto Huh-7.5 cells that had been transduced 24 hr previously with the corresponding AAV vector, or were retreated with IFN-α, or left untreated. After rounds 1, 3, and if possible, round 6, total cellular RNA (from Fig. S1)

was amplified using a forward primer that lies upstream of the miR-UTR1 target site and a reverse primer that lies downstream of the miR-Core target site. The 366 bp fragments were sequenced using 454/Roche pyrosequencing. Data in the table represent the percentage that a particular base occurred at a nucleotide position within the miR-Core target site (21 nt) . Cells highlighted in yellow indicate variation from the reference sequence (JFH; HCV 2a). Column labeled "Sequences" indicates the total number of DNA sequences analyzed.

SUPPLEMENTARY MATERIALS AND METHODS

HCV spread assay

Huh-7.5 cells were plated in 6 well plates at $2x10^5$ cells/well. Twenty-four hours later, cells were infected with either scAAV2-HCV-miR-Cluster 5, scAAV2-miR-Core, or scAAV2-eGFP at an AAV dose of $1x10^5$ vg/cell, and with JFH-1 HCVcc (~0.5 FFU/cell) (kindly provided by Dr. George Luo, University of Kentucky, Lexington, Kentucky). Positive control wells included Huh-7.5 cells treated with IFN-α (200 u/ml). Negative control wells consisted of Huh-7.5 cells that were infected with HCVcc but were not treated with a drug. Two hours later, the media was replaced, and the cells were incubated for 48 hrs. At this time, 1 ml of supernatant from each treatment group was used to infect HCVcc-naïve Huh-7.5 cells that had been transduced 24 hrs earlier with either scAAV2-HCV-miR-Cluster 5, scAAV2-miR-Core, or scAAV2-eGFP at a dose of $1x10^5$ vg/cell, or were treated with IFN-α or were left untreated. Total cellular RNA from the initially infected cells was isolated using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, and HCV RNA levels were quantified in triplicate by QRT-PCR. This procedure was continued for a total of six rounds. All infections were performed in duplicate.

HCV RNA isolation, multiplex PCR amplification and sequence determination.

Cellular RNA from HCVcc-treated Huh7.5 cells was PCR amplified with barcoded primers designed to generate a 300 bp fragment that included the target sites for miR-UTR1, miR-UTR2, miR-UTR3, and miR-Core. The PCR products were purified and subjected to pyrosequencing and bioinformatics analysis according to Wang et al [1].

Southern blot analysis of mouse liver DNA.

Genomic DNA was isolated from frozen ground livers using a DNeasy Blood and Tissue kit (Qiagen, Valencia CA) according to the manufacturer's instructions, and quantitated on a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE) . Two μg of mouse liver genomic DNA was digested with SphI and fractionated on a 1% agarose gel. Copy number controls were generated by spiking liver genomic DNA from naïve mice with pscAAV-ApoE/hAAT-HCV-miR-Cluster 5 plasmid DNA at 0.3 to 900 copies/diploid genome (1μg mouse genomic DNA is equivalent to $1.8x10^5$ diploid genomes), followed by digestion with SphI, and these were used to generate standard curves. The 1KB plus ladder was used as a molecular weight marker. The DNA was transferred to nylon membrane (Ambion, Austin, TX), and hybridization was carried out overnight in UltraHyb buffer (Ambion, Austin, TX) at 42°, using an α-P^{32}-CTP labeled 1074 bp probe, that was generated by SphI digestion of plasmid pscAAV-ApoE/HAAT-HCV-miR-Cluster 5. The membranes were subsequently washed twice with 2xSSC/0.1% SDS for 5 min. and twice with and 0.1xSSC/0.1% SDS for 20 min. at 42°C. The membranes were exposed to film, and developed using a Kodak processor. The membranes were also exposed to a phosphor screen, and imaged using a Typhoon 9400 imaging system. ImageQuant software (GE Healthcare Bio-Sciences Corp., Piscataway, NJ) was used to calculate the copy number of AAV genomes in injected animals.

Supplementary Reference.

1. Wang GP, Sherrill-Mix SA, Chang KM, Quince C, Bushman FD. Hepatitis C virus transmission bottlenecks analyzed by deep sequencing. *J Virol* 2010; **84**(12)**:** 6218-28.

Fig. S1

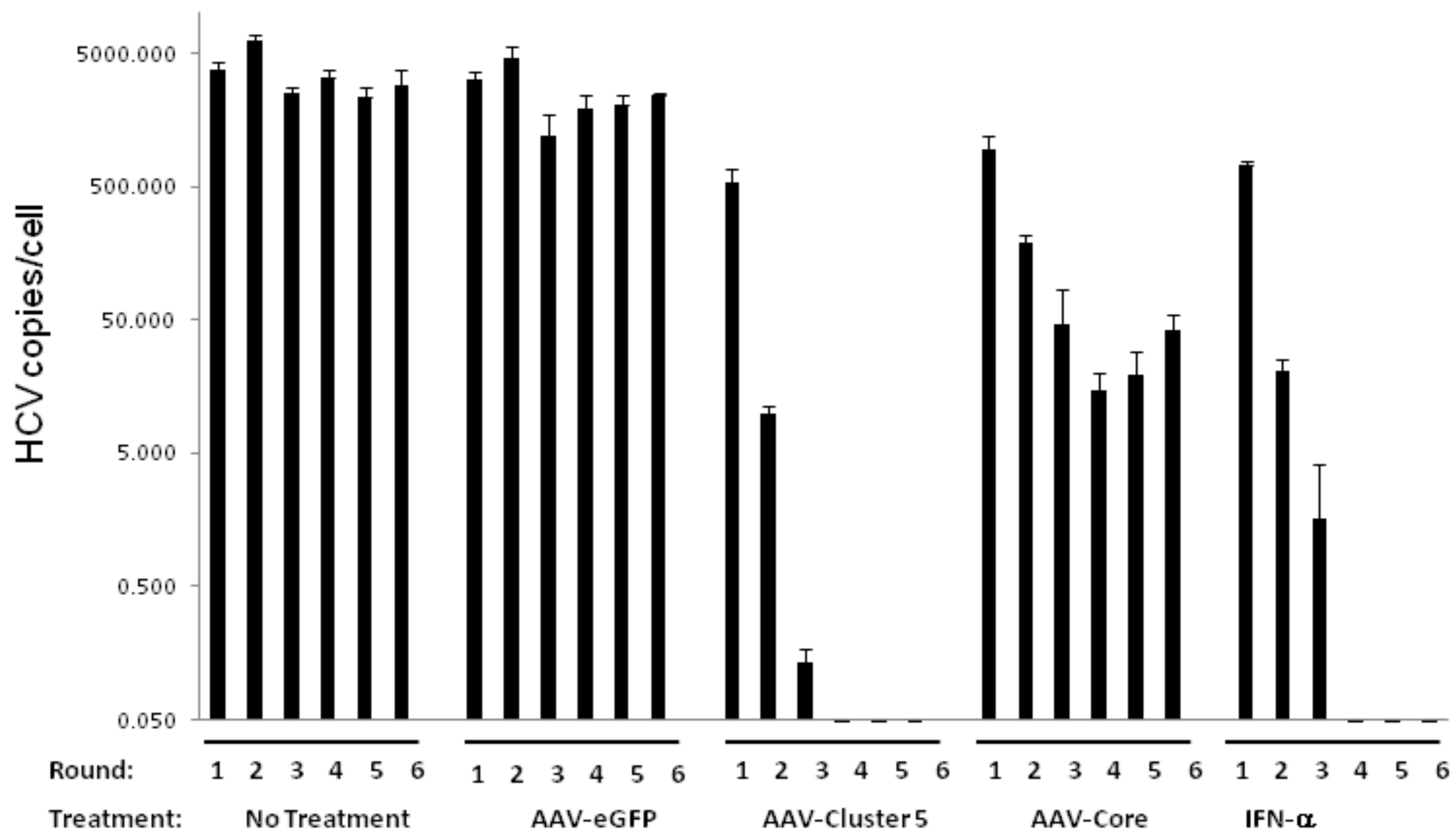

Fig. S2

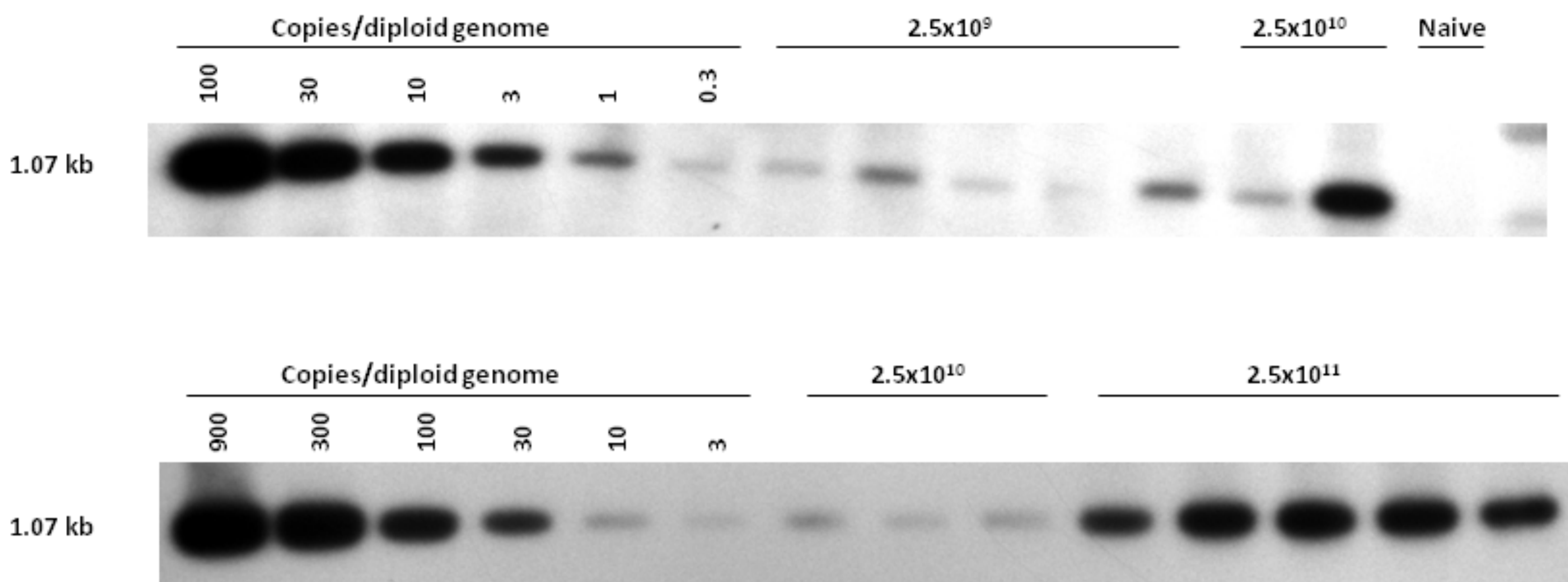

Table S1
Sequence variation in miR-Core target site of HCV2a genome after various treatments (454/Roche PyroSequencing Analysis)

Treatment	Round	Sequences	HCV2a																						Changes	Possible miR-Core R mutant
Cluster 5	1	9591	JFH nt	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299		
			Base	A	A	C	C	T	C	A	A	A	G	A	A	A	A	A	C	C	A	A	A	A	288: G to del increase	Yes
			A	100	100	0	0	0	0	100	100	100	0	100	100	100	99	37	0	0	100	100	100	56	293: A to del, stable	No, also in IFN and No Tx
			C	0	0	100	100	1	100	0	0	0	0	0	0	0	0	0	100	100	0	0	0	0	295: C to T increases	Yes
			G	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	1	299: A to G increases	No
			T	0	0	0	0	99	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	299: A to del increases then decreases	No, also in No Tx
			"-"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	63	0	0	0	0	0	43		
Cluster 5	3	8151	JFH nt	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299		
			Base	A	A	C	C	T	C	A	A	A	G	A	A	A	A	A	C	C	A	A	A	A		
			A	100	100	0	0	0	1	99	100	99	0	100	100	100	99	45	0	0	100	100	100	95		
			C	0	0	100	100	0	99	1	0	0	0	0	0	0	0	0	100	22	0	0	0	0		
			G	0	0	0	0	0	0	1	0	1	94	0	0	0	1	0	0	0	0	0	0	3		
			T	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	78	0	0	0	0		
			"-"	0	0	0	0	0	0	0	0	0	6	0	0	0	0	55	0	0	0	0	0	2		
Core	1	8977	JFH nt	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299		
			Base	A	A	C	C	T	C	A	A	A	G	A	A	A	A	A	C	C	A	A	A	A	284: C to A increase	Yes
			A	100	100	0	0	0	0	100	100	100	0	100	100	100	99	42	0	0	100	100	100	86	285: A to C increase	Yes
			C	0	0	100	100	0	100	0	0	0	0	0	0	0	0	0	100	97	0	0	0	0	288: G to del increases	Yes
			G	0	0	0	0	0	0	0	0	0	99	0	0	0	0	1	0	0	0	0	0	13	289: A to T increases	Yes
			T	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	292: A to del increases	Yes
			"-"	0	0	0	0	0	0	0	0	0	1	0	0	0	0	56	0	0	0	0	0	1	293: A to del increases, stabilizes	No
Core	3	9665	JFH nt	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	295: C to del increases then decreases	No
			Base	A	A	C	C	T	C	A	A	A	G	A	A	A	A	A	C	C	A	A	A	A	299: A to G increases then decreases	No
			A	100	100	0	0	0	4	95	100	99	0	99	100	100	85	45	0	0	100	100	98	94	299: A to del increases	No, also in No Tx
			C	0	0	100	100	0	96	4	0	0	0	0	0	0	0	1	100	58	0	0	0	0		
			G	0	0	0	0	0	0	1	0	0	91	0	0	0	1	0	0	0	0	0	2	3		
			T	0	0	0	0	99	0	0	0	0	0	0	0	0	10	0	0	42	0	0	0	0		
			"-"	0	0	0	0	1	0	0	0	1	9	0	0	0	4	55	0	0	0	0	0	3		
Core	6	11315	JFH nt	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299		
			Base	A	A	C	C	T	C	A	A	A	G	A	A	A	A	A	C	C	A	A	A	A		
			A	100	100	0	0	0	9	91	100	99	1	92	100	100	76	31	0	0	98	100	100	57		
			C	0	0	100	100	3	91	9	0	0	0	0	0	0	0	2	99	92	0	0	0	0		
			G	0	0	0	0	0	0	0	0	1	68	0	0	0	1	0	0	0	2	0	0	2		
			T	0	0	0	0	96	0	0	0	0	0	8	0	0	2	0	0	8	0	0	0	0		
			"-"	0	0	0	0	1	0	0	0	0	31	0	0	0	21	67	0	0	0	0	0	41		
IFN	1	10713	JFH nt	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299		
			Base	A	A	C	C	T	C	A	A	A	G	A	A	A	A	A	C	C	A	A	A	A	293: A to del stablizes	No
			A	100	100	0	0	0	0	100	100	100	0	100	100	100	100	41	0	0	100	100	100	100	298: A to C increase	No
			C	0	0	100	100	0	100	0	0	0	0	0	0	0	0	0	100	100	0	0	0	0		
			G	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0		
			T	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			"-"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	59	0	0	0	0	0	0		
IFN	3	7237	JFH nt	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299		
			Base	A	A	C	C	T	C	A	A	A	G	A	A	A	A	A	C	C	A	A	A	A		
			A	100	100	0	0	0	0	100	100	100	0	100	100	100	100	31	0	0	100	100	87	98		
			C	0	0	100	100	0	100	0	0	0	0	0	0	0	0	0	100	100	0	0	13	0		
			G	0	0	0	0	0	0	0	0	0	99	0	0	0	0	0	0	0	0	0	0	0		
			T	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			"-"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	69	0	0	0	0	0	2		
None	1	9488	JFH nt	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299		
			Base	A	A	C	C	T	C	A	A	A	G	A	A	A	A	A	C	C	A	A	A	A	293: A to del increases, stabilizes	No
			A	100	100	0	0	0	0	100	100	100	0	100	100	100	100	42	0	0	100	100	100	100	295: C to T increases then decreases	No
			C	0	0	100	100	0	100	0	0	0	0	0	0	0	0	0	100	96	0	0	0	0	299: A to del increases	No
			G	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0		
			T	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0		
			"-"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	58	0	0	0	0	0	0		
None	3	6218	JFH nt	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299		
			Base	A	A	C	C	T	C	A	A	A	G	A	A	A	A	A	C	C	A	A	A	A		
			A	100	100	0	0	0	0	100	100	100	0	100	100	100	100	60	0	0	100	100	100	100		
			C	0	0	100	100	0	100	0	0	0	0	0	0	0	0	0	100	100	0	0	0	0		
			G	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0		
			T	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			"-"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	40	0	0	0	0	0	0		
None	6	5881	Base	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299		
				A	A	C	C	T	C	A	A	A	G	A	A	A	A	A	C	C	A	A	A	A		
			A	100	100	0	0	0	0	100	100	100	0	99	100	100	100	47	0	1	100	100	100	92		
			C	0	0	100	100	0	100	0	0	0	0	0	0	0	0	1	100	99	0	0	0	0		
			G	0	0	0	0	0	0	0	0	0	100	1	0	0	0	0	0	0	0	0	0	0		
			T	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			"-"	0	0	0	0	0	0	0	0	0	0	0	0	0	0	52	0	0	0	0	0	8		

[a]Huh-7.5 cells were co-infected with HCVcc (~0.2 IU/cell) and either scAAV2-HCV-miR-Cluster 5 or scAAV2-miR-Core at an AAV dose of $1x10^5$ vg/cell.
Positive control wells included Huh-7.5 cells treated with IFN-a (200u/ml). Negative control wells consisted of Huh-7.5 cells that were infected with HCVcc but were not treated with a drug.
Following 48 hr incubation period, supernatants were serially passaged (total of six rounds) onto Huh-7.5 cells that had been tranduced 24 hr previously with the corresponding AAV vector.
After rounds 1,3, and in some cases, 6, total cellular RNA (from Suppl. Fig. 1) was amplified and sequenced using 454/Roche pyrosequencing.

[b]Sequences indicates the total number of sequences analzyed

[c]Data in table represent the percentage a particlar base occurred at that nt position, Cells highlighted in yellow indicate variation from reference sequence (JFH HCV2a).