Supporting Information

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SI Materials and Methods.

Oligonucleotides and DNA Constructs. The sequences of guide and passenger strands used in small interfering RNA-knockdown were as follows: control (GL2, luciferase), 5′-UCG AAG UAU UCC GCG UAC GUU-3′ and 5′-CGU ACG CGG AAU ACU UCG AUU-3′ Argonaute (Ago)1, 5′-CCU GUC CAU UGC CUU CAU AUU-3′ and 5′-UAU GAA GGC AAU GGA CAG GUU-3′; and Ago2, 5′-CAG CCA GCA UCG AAC AUG AUU-3′ and 5′-UCA UGU UCG AUG CUG GCU GUU-3′. Guide and passenger strands were annealed before transfection.

To generate the plasmid pLuc-122x2p3, nucleotides 1–45 from plasmid pH77ΔE1/p7 S1 + S2: p3 (1) were amplified by PCR using the primers 5′-CTA ATA CGA CTC ACT AGT GCC AGC CCC CTG-3′ and 5′-GCT TTC TGC GTG AAT TCA GTA GTT CCT CAC-3′. The insertion was ligated into the plasmid pLuc-122x2 (2) between the *SpeI* and *EcoRI* sites. A second insertion consisting of nucleotides 1–45 from the same pH77ΔE1/p7 S1 + S2: p3 plasmid was amplified using the primers 5′-CAG CGG CCG CGA ATT CGA CTC ACT ATA GCC-3′ and 5′-CAG TAG TTC CCC GCG GGG CAG TGA TTC ATG-3′. This insertion was ligated into the same plasmid between the *EcoRI* and *SacII* sites to generate the pLuc-122x2p3 plasmid. The final plasmid contained mutations at all four miR-122 seed match sites, mutating the position in the seed match sequence that binds to the nucleotide in the third position of miR-122 from a C to a G.

Site-directed mutagenesis (QuikChange II XL, Stratagene) was used to create position 16 mutations for enhanced GFP-G_3U_{16} (5′-GTA GGC GGC CGC ACA AAC ATC ATT GTC ACA CTG CAT AGA T-3′ and 5′-ATC TAT GCA GTG TGA CAA TGA TGT TTG TGC GGC CGC CTA C-3′), EGFP-G_3A_{16} (5′-GTA GGC GGC CGC ACA AAC AAC ATT GTC ACA CTG CAT AGA T-3′ and 5′-ATC TAT GCA GTG TGA CAA TGT TGT TTG TGC GGC CGC CTA C-3′), and EGFP-G_3G_{16} (5′-GTA GGC GGC CGC ACA AAC AGC ATT GTC ACA CTG CAT AGA T-3′ and 5′-ATC TAT GCA GTG TGA CAA TGC TGT TTG TGC GGC CGC CTA C-3′).

Cell Culture and Transfection. For miRNA (luciferase) assays, DNA constructs and oligonucleotides were transfected into HeLa cells in six-well plates using Lipofectamine 2000 (Invitrogen), according to the manufacturer's instructions. Firefly luciferase and Renilla luciferase (pRL-SV40) plasmids were transfected at 2 μg and 0.2 μg per well, respectively. miR-122 duplexes were transfected at a concentration of 50 nM. The Renilla luciferase plasmid was used as a control for transfection efficiency and samples were normalized to Renilla luciferase expression. Transfected cells were cultured for 24 h and harvested in 1× Passive Lysis Buffer (Promega). Luciferase assays were performed according to the manufacturer's instructions.

For control (GL2, luciferase), Ago1 and Ago2 siRNA-mediated depletion, cells were transfected with the corresponding siRNA duplexes at a concentration of 25 nM 1 d prior to and 2 d postelectroporation. Four days after electroporation, cells were supplemented with miR-122 duplexes at 50 nM. Cells were washed and scraped in PBS 5 d after electroporation. Each sample was used for RNA analysis and protein analysis as described.

Western Blotting. Protein samples were obtained by harvesting cells in radio-immunoprecipitation assay (RIPA) buffer, containing 100 mM Tris-HCl, pH 7.4, 0.1% sodium dodecylsulfate, 150 mM NaCl, 1% sodium deoxycholic acid, 1% Triton X-100 supplemented with complete EDTA-free protease inhibitor cocktail (Roche). Protein was separated by 8% SDS-PAGE and transferred to Immobilon–P membrane (Millipore). Membranes were probed with antibodies directed against Ago1 and Ago2 (a gift from Dr. Günter Meister, Ulm, Germany) or with an antibody directed against actin (Sigma).

1. Jopling CL, Schutz S, Sarnow P (2008) Position-dependent function for a tandem microRNA miR-122-binding site located in the hepatitis C virus RNA genome. *Cell Host Microbe* 4:77–85.

2. Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P (2005) Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science* 309:1577–1581.

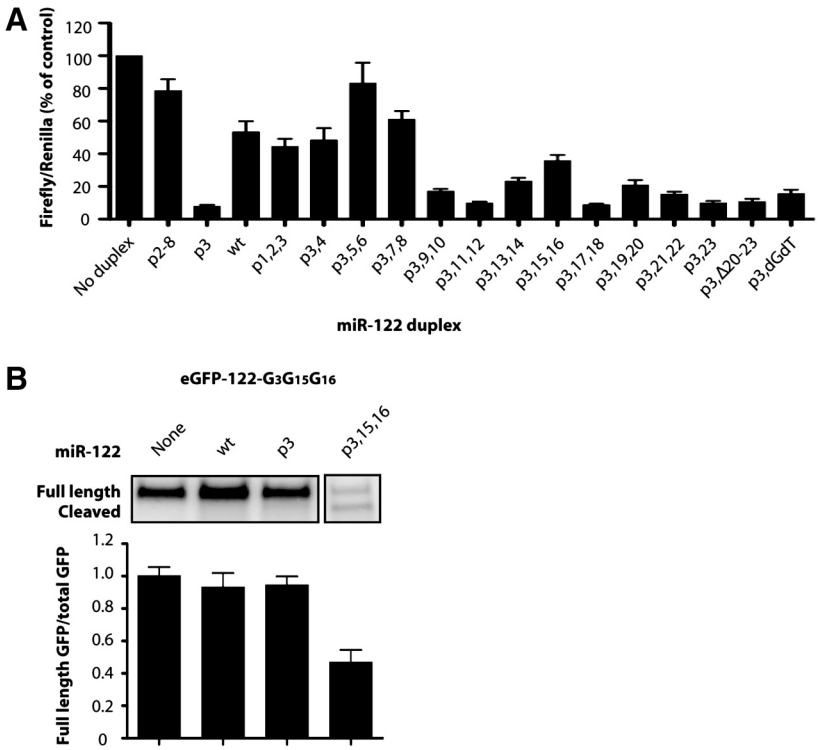

Fig. S1. MicroRNA (luciferase) assays and siRNA-mediated GFP cleavage assays with mutated miR-122 duplexes. (*A*) Mutated miR-122 duplexes were transfected into HeLa cells concurrently with pLuc122x2p3, a plasmid that expressed firefly luciferase, which contains four miR-122 p3 seed match sites in its 3′ noncoding region, and a Renilla reporter plasmid as a transfection control. Twenty-four hours posttransfection, cells were harvested and luciferase activities were measured. Data from mock-transfected cells (no duplex) were used as a negative control. (*B*) Mutant miR-122 duplexes were transfected into HeLa cells concurrently with a plasmid expressing GFP with a perfectly complementary site for miR-122 p3,15,16 in its 3′ noncoding region. Cells were harvested after 24 h and Northern blot analysis was performed to assess GFP RNA cleavage. Data are representative of at least three independent replicates and error bars represent standard error of the mean.

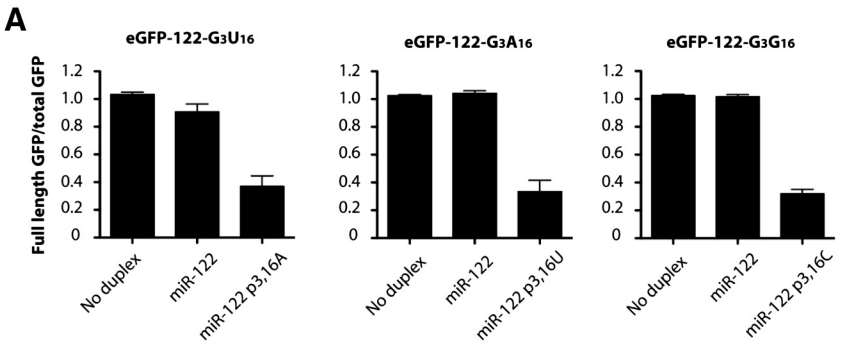

Fig. S2. Effects of nucleotide 16 in miR-122 on siRNA-mediated cleavage of GFP reporter RNA. MiR-122 p3 duplexes containing an "A," "U," or "C" at position 16 were cotransfected into HeLa cells with a plasmid expressing GFP that contained a perfectly complementary site to the mutant miR-122 molecule in its 3′ NCR. GFP mRNA was measured by Northern blot analysis. Full length and cleaved GFP mRNA are indicated. Quantitation is shown as the ratio of full length GFP mRNA to total (full length plus cleaved) GFP mRNA and represents at least three independent replicates. Error bars represent standard error of the mean.

Fig. S3. Effects of Ago1 and 2 depletion on miR-122 augmentation of hepatitis C virus (HCV) RNA abundance. (*A*) Cells were transfected with siRNA duplexes targeting a control or Ago1 and 2, electroporated with wild-type HCV, and transfected with either wild-type or p3 miR-122 duplexes as described in *SI Materials and Methods*. Northern blots are representative of four independent replicates. (*B*) Western blots of samples shown in (*A*). (*C*) Fold change of HCV RNA in the presence of wild-type miR-122 compared to p3 miR-122. Data represent four independent replicates. Error bars represent standard error of the mean.

Table S1. Mutant miR-122 guide and passenger strands

Guide strands	Sequence (5′–3′)
miR-122 (WT)	UGGAGUGUGACAAUGGUGUUUGU
p3	UG**C**AGUGUGACAAUGGUGUUUGU
p1,2,3	**ACC**AGUGUGACAAUGGUGUUUGU
p3,4	UG**CU**GUGUGACAAUGGUGUUUGU
p3,5,6	UG**CA**GUGACAAUGGUGUUUGU
p3,7,8	UG**C**AGU**CA**GACAAUGGUGUUUGU
p3,9,10	UG**C**AGUGU**CU**CAAUGGUGUUUGU
p3,11,12	UG**C**AGUGUGA**GU**AUGGUGUUUGU
p3,13,14	UG**C**AGUGUGACA**UA**GGUGUUUGU
p3,15,16	UG**C**AGUGUGACAAU**CC**UGUUUGU
p3,17,18	UG**C**AGUGUGACAAUGG**AC**UUUGU
p3,19,20	UG**C**AGUGUGACAAUGGUG**AA**UGU
p3,21,22	UG**C**AGUGUGACAAUGGUGUU**AC**U
p3,23	UG**C**AGUGUGACAAUGGUGUUUG**A**
p3,15A	UG**C**AGUGUGACAAU**A**GUGUUUGU
p3,15U	UG**C**AGUGUGACAAU**U**GUGUUUGU
p3,15C	UG**C**AGUGUGACAAU**C**GUGUUUGU
p3,16A	UG**C**AGUGUGACAAUG**A**UGUUUGU
p3,16U	UG**C**AGUGUGACAAUG**U**UGUUUGU
p3,16C	UG**C**AGUGUGACAAUG**C**UGUUUGU
p2-8	U**AAUCACA**GACAAUGGUGUUUGU
p3,Δ20–23	UG**C**AGUGUGACAAUGGUGU
p3,dGdT	UG**C**AGUGUGACAAUGGUGUUU**dGdT**
Passenger strands	**Sequence (5′–3′)**
WT*	AAACGCCAUUAUCACACUAAAUA
p3,5,6*	AAACGCCAUUAUCAC**UG**UAAAUA
p3,15,16*	AAACG**GG**AUUAUCACACUAAAUA
p3,17,18*	AAA**GU**CCAUUAUCACACUAAAUA
p3,19,20*	A**UU**CGCCAUUAUCACACUAAAUA
p3,21,22*	**U**AACGCCAUUAUCACACUAAAUA
p3,15A*	AAACGC**U**AUUAUCACACUAAAUA
p3,15U*	AAACGC**A**AUUAUCACACUAAAUA
p3,15C*	AAACGC**G**AUUAUCACACUAAAUA
p3,16A*	AAACG**U**CAUUAUCACACUAAAUA
p3,16U*	AAACG**A**CAUUAUCACACUAAAUA
p3,16C*	AAACG**G**CAUUAUCACACUAAAUA
p2-8*	AAACGCCAUUAUC**UGUGAGG**AUA
p3,Δ20–23*	GCCAUUAUCACACUAAAUA

Table S2. Comparison of 5′ NCR sequences (nucleotides 1–49) of HCV genotypes 1–6

Genotype	Subtype	Accession #	5'-Noncoding region
1	a	AF009606(H77)	-GCCAGCCCCCUGAUGGGG-GCG**ACACUCC**ACCAUGAAU**CACUCC**CCUGUG
	b	M58335	- - - - - - - - - - C . . . . . - . . . . . AG . . . . .
	c	D14853	- . . . . . - . . . . . G . . . . .
2	a	D00944	- A . . C . . . . . U A A U A . . . . – . . . . . G . . . . .
	b	D10988	- . . . C . . . . . - . . . . . G . . . . .
	c	D50409	- A . . C . . . . . U A A . A . . . . – . . . . . G . . . . .
	k	AB031663	U . . . C . . . . . U A A . . . . . – . . . . . G . . . . .
3	a	D17763	- A . . U . . . U.U . A C G A . . C — . . . . . G. . . . .
	b	D49374	- . . . U . . . U.U . U C G A . . C — . . . . .
	k	D63821	- . . . U . . . U.U . U C G A . . C - - - . . . . .
4	a	DQ295833*	- - - - - - - - - - - - - - . . . . . - . . . . . A G . . . . .
5	a	D50466*	- A. .C . . . . . U. A U . . . . . - . . . . . - . . . . .
6	a	D88476*	- . . . . . U. A. C . . . . - . . . . . G. . . .U - . . . . .
	b	D84262	- . . . . . U. A. C . . . . - . . . . . - . . . . .
	d	D84264	- . . . . . UAAU - . . . . - . . . . . - . . . . .
	f	D63822	- . . . . . U. AC - . . . . - . . . . . - . . . . .
	h	D84265	- . . . . . UAAU - . . . . - . . . . . - . . . . .
	k	D84264	- . . . . . UAAU - . . . . - . . . . . - . . . . .

Representative 5′ NCRs of HCV clones belonging to each of the confirmed genotypes/subtypes found at euHCVdb: http://euhcvdb.ibcp.fr/euHCVdb/jsp/nomen_tab1.jsp (1). The confirmed sequences for genotype 4a, 5a, and 6a are truncated at their 5′ end, and hence provisionally assigned sequences were retrieved for analysis as in [Lanford et al. (2); DQ295833*, D88473*, and D50466*].

1. Combet C, et al. (2007) euHCVdb: The European hepatitis C virus database. *Nucleic Acids Res* 35:D363–D366.
2. Lanford, et al. (2010) Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science* 327:198–201.