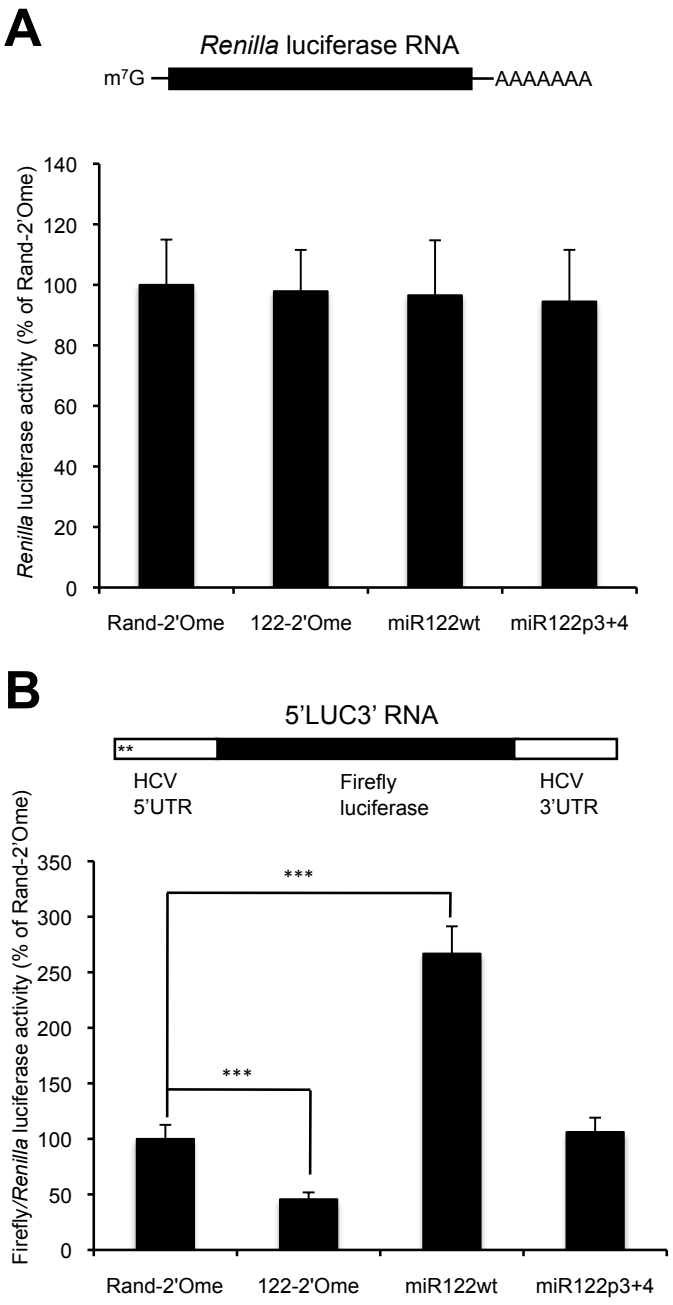
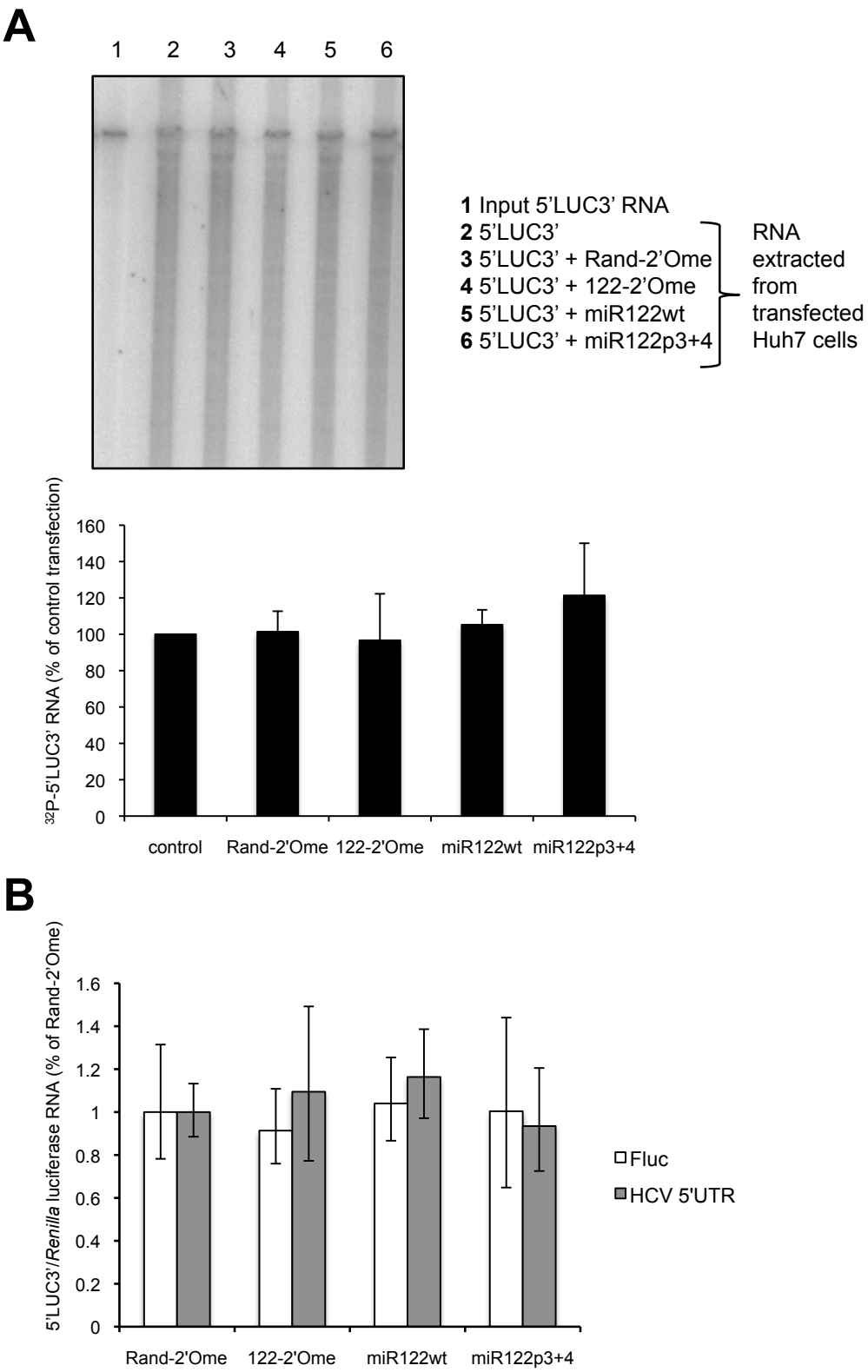
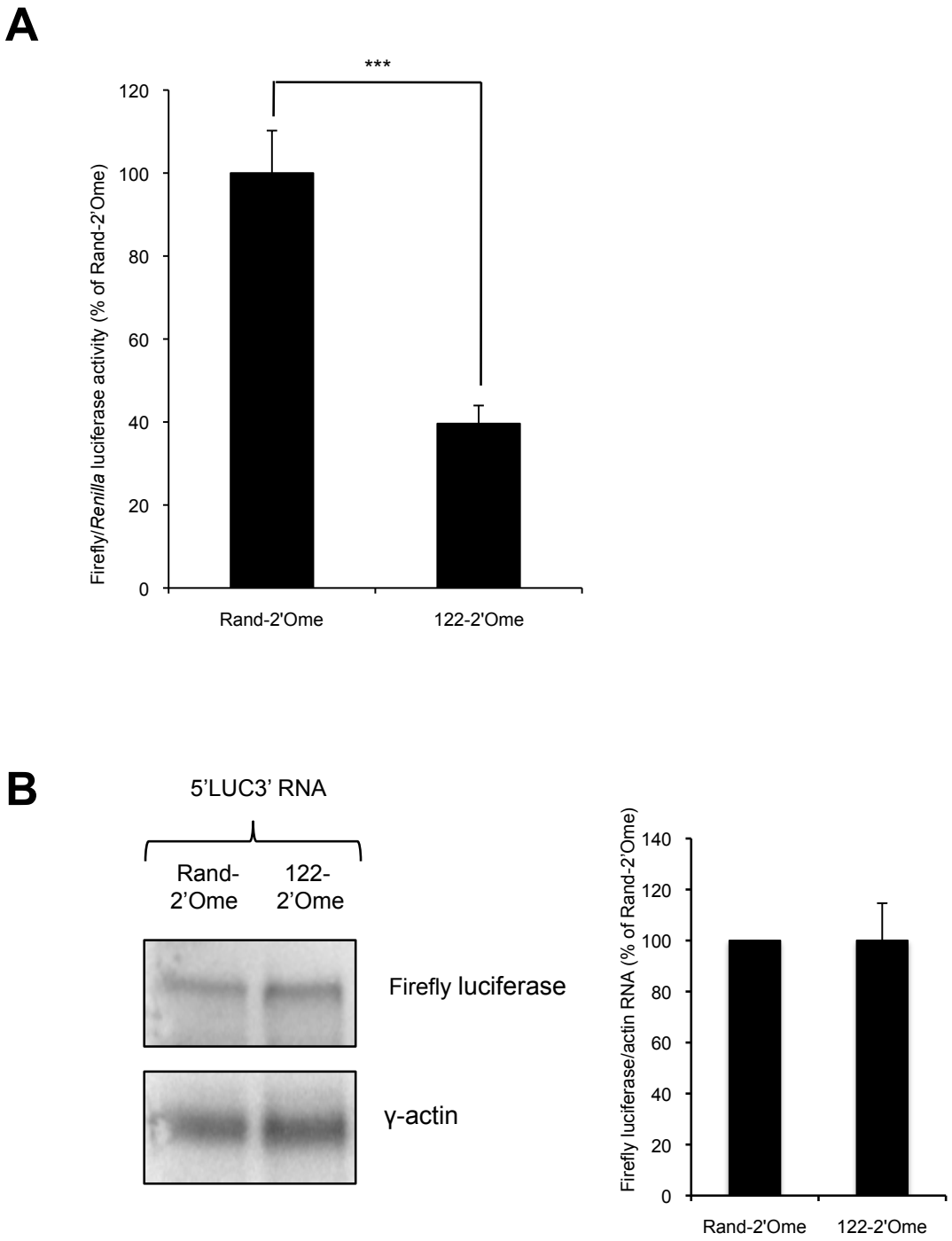


Supplementary Figure S1

Translation of 5'LUC3' reporter RNA is stimulated by miR-122 following RNA transfections. (A) *Renilla* luciferase activity was not significantly affected by miR-122 sequestration or overexpression and is therefore a suitable transfection control. Capped, polyadenylated *Renilla* luciferase RNA was introduced into Huh7 cells with one of the following molecules: Rand-2'Ome, a randomized control 2'-O-methylated molecule; 122-2'Ome, a 2'-O-methylated oligomer complementary to miR-122; miR122wt, a synthetic wildtype miR-122 duplex; miR122p3+4, a synthetic miR-122 duplex with positions 3 and 4 of the seed mutated to their complement. The average *Renilla* luciferase activity of five independent triplicate transfections was determined at 6h post transfection and is shown relative to Rand-2'Ome values. Error bars represent standard deviation. (B) 5'LUC3' RNA was introduced into Huh7 cells with the *Renilla* luciferase RNA transfection control and each of the molecules in (A). Average firefly/*Renilla* luciferase activity from three independent triplicate experiments is shown as a percentage of the values obtained with Rand-2'Ome. Error bars represent standard deviation. Luciferase activtiy from 5'LUC3' RNA was reduced by 122-2'Ome and increased by miR122wt (***, $P<0.0001$ compared to Rand-2'Ome), indicating that miR-122 stimulates luciferase expression.

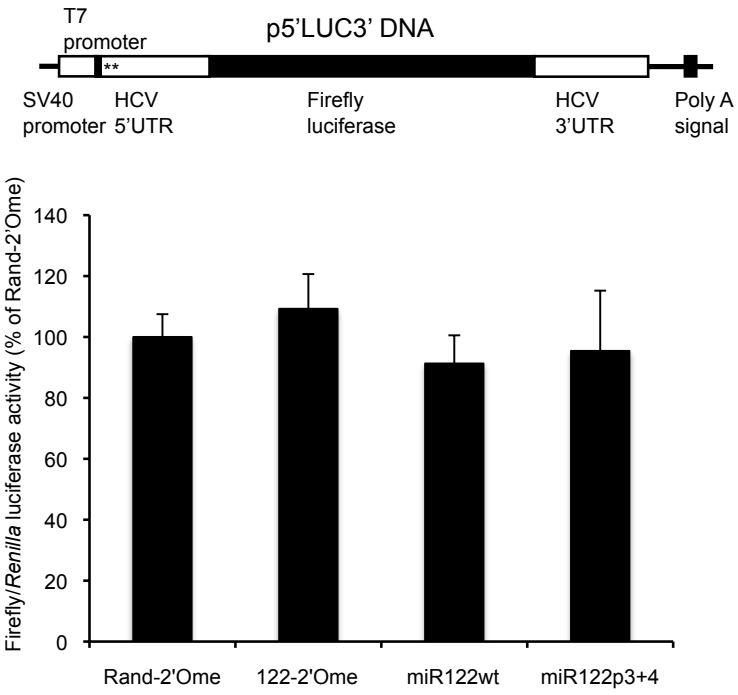


Supplementary Figure S2 5'LUC3' RNA stability is not affected by miR-122. (A) ^{32}P-labelled 5'LUC3' RNA was introduced into Huh7 cells with sequestration or overexpression of miR-122. Total RNA was harvested at 6h post transfection and separated by denaturing polyacrylamide gel electrophoresis. Densitometry data were quantified from three independent experiments. RNA stability and integrity were not significantly affected by miR-122. (B) qPCR using primers targeted to either the firefly luciferase coding region or the HCV 5'UTR was carried out following RNA transfection as in Supplementary Figure S1B. RNA levels are shown as $2^{-\Delta\Delta Ct}$ relative to the level of the *Renilla* luciferase transfection control RNA as a percentage of Rand-2'Ome values, and are an average of two independent experiments +/- standard deviation.

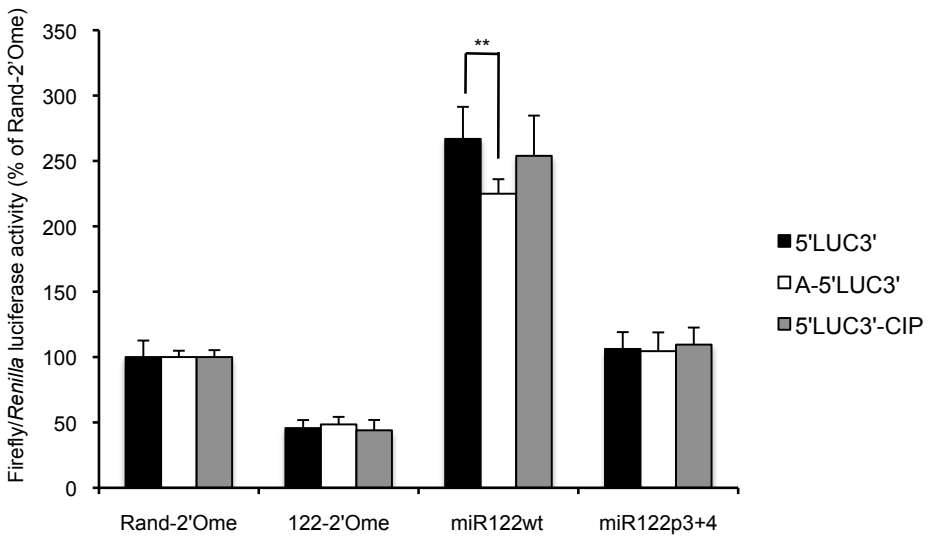


Supplementary Figure S3

The method of transfection does not affect the response to miR-122. (A) 5'LUC3' RNA was introduced into Huh7 cells by electroporation with capped, polyadenylated, *Renilla* luciferase RNA as a transfection control, and either Rand-2'Ome or 122-2'Ome. Luciferase activity was measured at 6h post electroporation, and average firefly/*Renilla* luciferase activity, plus standard deviation, is shown as a percentage of the Rand-2'Ome values across three independent experiments. Sequestration of miR-122 reduced firefly luciferase activity (***, $P<0.0001$). (B) Total RNA was extracted from the cells electroporated in (A), and firefly luciferase RNA levels determined by northern blotting. The membranes were re-probed for γ-actin as a loading control. Average firefly luciferase RNA levels quantified across two independent experiments are shown as a proportion of actin RNA relative to the Rand-2'Ome values, plus standard deviation. Sequestration of miR-122 did not affect firefly luciferase RNA levels.

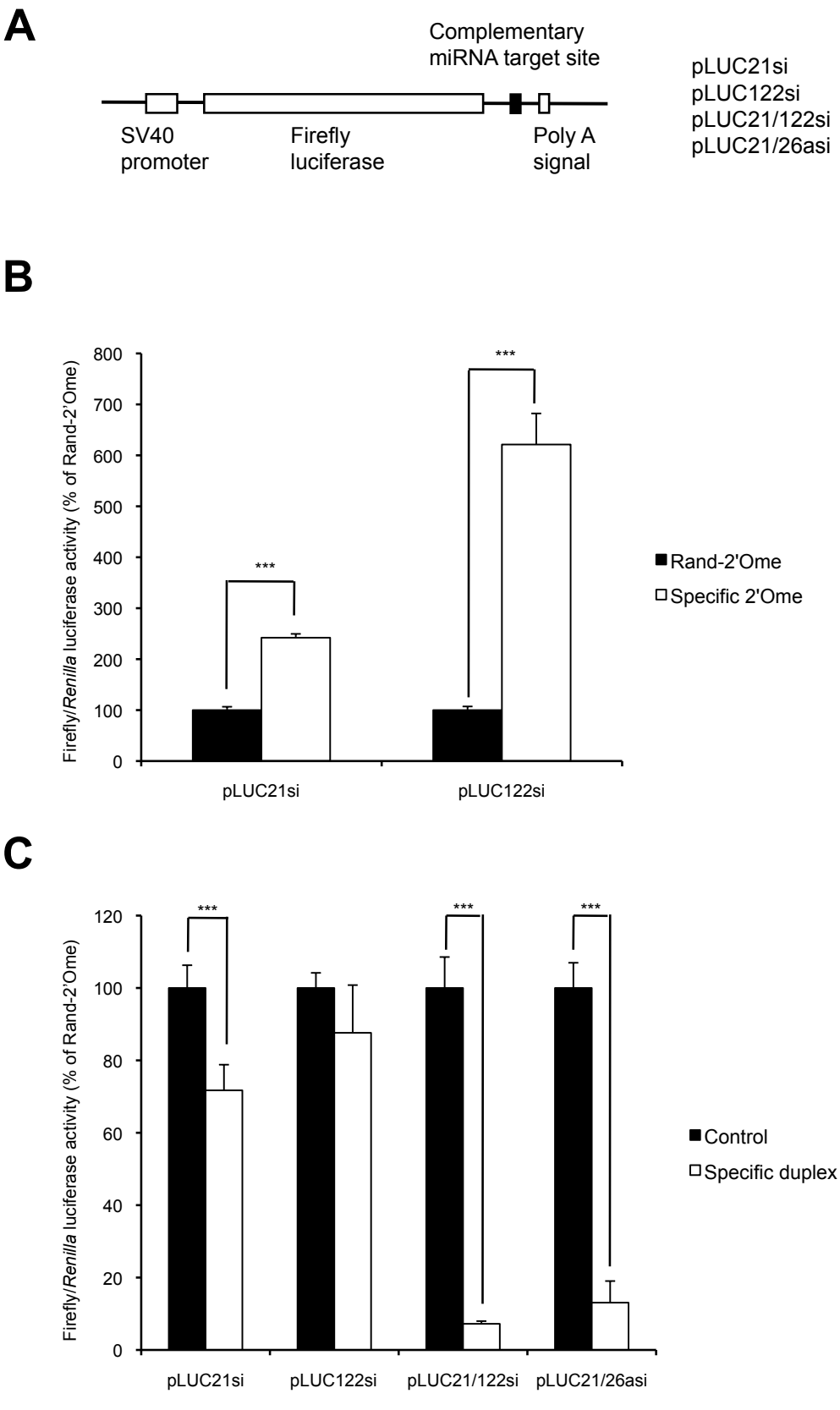


Supplementary Figure S4
miR-122 does not regulate HCV 5'UTR-driven translation following DNA transfection. The p5'LUC3' reporter was introduced into Huh7 cells by DNA transfection with sequestration or overexpression of miR-122, with pSV40-RL as a transfection control. Firefly/*Renilla* luciferase activity was determined as a percentage of Rand-2'Ome values of three independent triplicate experiments at 48h post transfection. Error bars represent standard deviation. miR-122 did not significantly affect firefly luciferase expression.



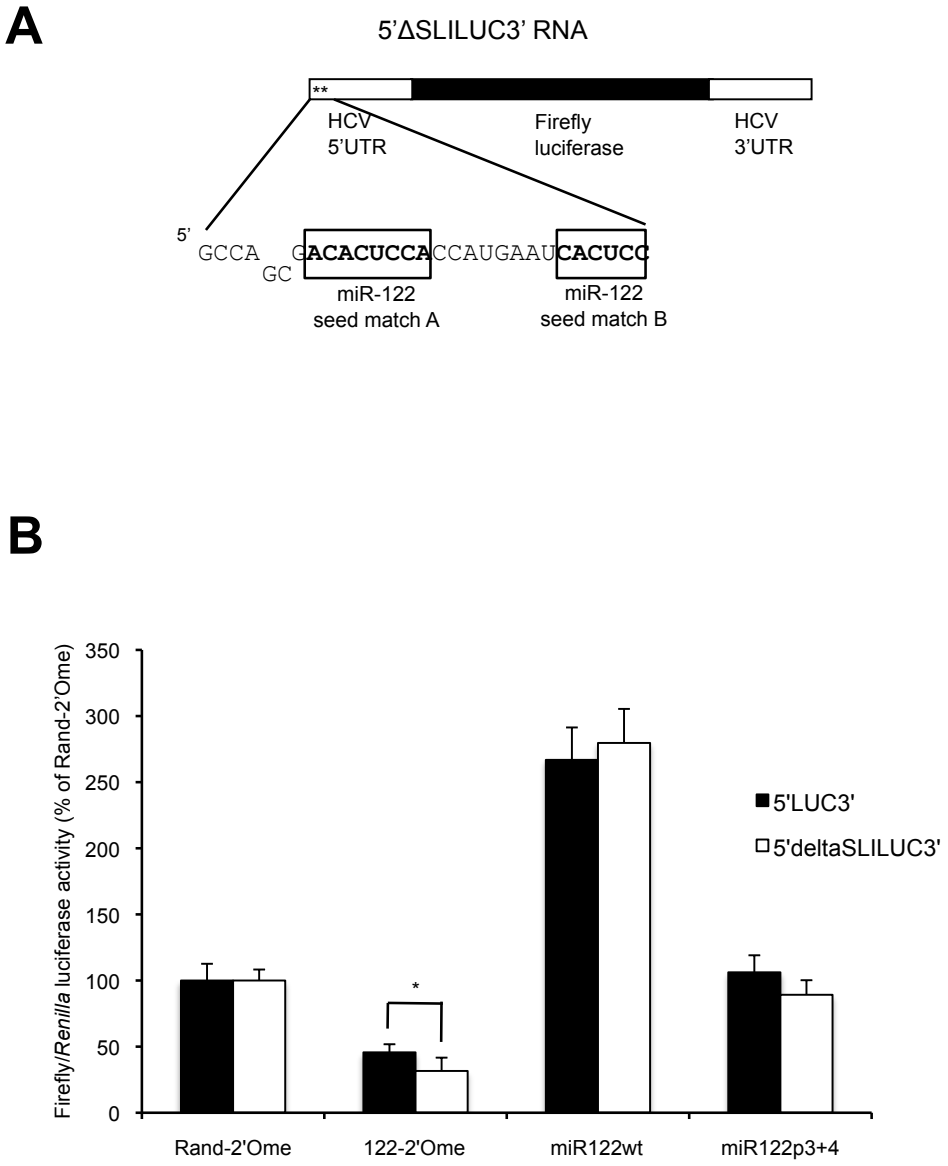
Supplementary Figure S5

Activation of translation by miR-122 binding to the HCV 5'UTR does not require a 5'-triphosphate on HCV 5'UTR RNA. 5'LUC3' RNA bearing an inactive 5' ApppG cap, or treated with calf intestinal alkaline phosphatase (CIP) to leave a monophosphorylated 5' end, was introduced into Huh7 cells with sequestration or overexpression of miR-122 and with a capped, polyadenylated *Renilla* luciferase RNA transfection control. Data represent firefly/*Renilla* luciferase activity as a percentage of Rand-2'Ome values for each RNA. Values are an average of three independent triplicate experiments, and error bars represent standard deviation. An A-cap slightly inhibited activation of translation by miR122wt (**, $P<0.001$), but did not affect repression by 122-2'Ome. Regulation of monophosphorylated 5'LUC3' RNA did not differ significantly from that of triphosphorylated 5'LUC3' RNA.


A
Complementary
miRNA target site
SV40
promoter
Firefly
luciferase
Poly A
signal
pLUC21si
pLUC122si
pLUC21/122si
pLUC21/26asi
B
Firefly/Renilla luciferase activity (% of Rand-2'Ome)
800
700
600
500
400
300
200
100
0
***
***
pLUC21si
pLUC122si
Rand-2'Ome
Specific 2'Ome
C
Firefly/Renilla luciferase activity (% of Rand-2'Ome)
120
100
80
60
40
20
0
***
***
***
pLUC21si
pLUC122si
pLUC21/122si
pLUC21/26asi
Control
Specific duplex

Supplementary Figure S6

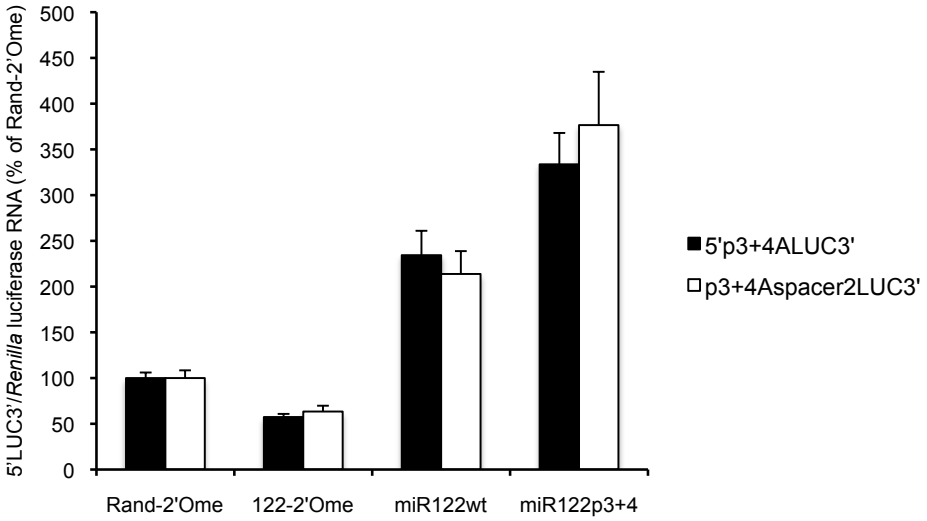
Luciferase reporters with exactly complementary binding sites for wildtype and chimeric miRNAs demonstrate that sequestration and overexpression are effective. (A) A single complementary site for miR21wt, miR122wt, miR21/122 or miR21/26a was introduced into the 3'UTR of a firefly luciferase reporter plasmid. The miRNA is expected to bind to its complementary site and act as an siRNA, resulting in cleavage and degradation of the reporter mRNA. (B) pLUC122si and pLUC21si vectors were introduced into Huh7 cells with a randomized control 2'-O-methylated oligomer (Rand-2'Ome) or a specific complementary 2'-O-methylated oligomer to sequester endogenous miR-21 or miR-122 (21-2'Ome or 122-2'Ome). Both oligomers effectively derepressed luciferase activity from the appropriate plasmid. miR-122 is expressed at higher levels than miR-21 in Huh7 cells, and therefore pLUC122si shows a greater response to sequestration of the corresponding miRNA. Firefly luciferase activity is shown relative to *Renilla* luciferase activity from a pSV40-RL transfection control as a percentage of Rand-2'Ome values for each plasmid. (C) Wildtype miR-122 or miR-21 or chimeric miR21/122 or miR21/26a duplexes were introduced into Huh7 cells with the appropriate luciferase sensor plasmids. Luciferase activity from the wildtype sensor plasmids was already effectively repressed by endogenous miRNAs and was not greatly affected by the transfected miRNA. The chimeric miRNAs strongly reduced luciferase activity from the corresponding sensor plasmids. Firefly luciferase activity is shown relative to *Renilla* luciferase activity from a pSV40-RL transfection control as a percentage of data obtained in the absence of miRNA overexpression for each plasmid. All data are an average of three independent triplicate experiments. Error bars represent standard deviation. (*** $P<0.0001$)



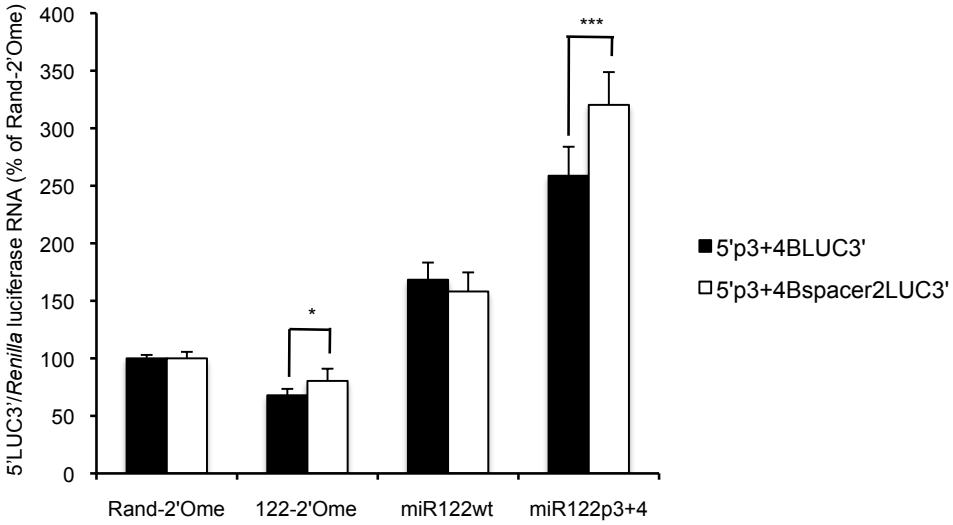
Supplementary Figure S7

Stem-loop I (SLI) is not required for miR-122 to activate translation via the HCV 5'UTR. (A) SLI, comprising nt 6-19 of HCV RNA, was deleted from the plasmid encoding 5'LUC3' RNA. (B) 5'ΔSLILUC3' RNA was introduced into Huh7 cells with a capped, polyadenylated *Renilla* luciferase RNA transfection control, with sequestration or overexpression of miR-122. Firefly/*Renilla* luciferase activity is shown as a percentage of Rand-2'Ome data. Values are an average of three independent triplicate experiments, plus standard deviation. Repression of translation by 122-2'Ome was slightly enhanced by the ΔSLI mutation (*, $P<0.01$) but activation by miR122wt was not significantly affected.

A



B



Supplementary Figure S8

Insertion of an additional 8nt spacer sequence downstream of seed match B does not consistently alter regulation of translation via either seed match. (A) 5'p3+4Aspacer2LUC3' was generated by insertion of an 8nt spacer sequence downstream of seed match B as in Figure 5C, except that seed match A was mutated at p3+4 to allow regulation via each of the seed matches to be distinguished. There were no changes in regulation via either seed match in the presence of spacer2. (B) As (A), except that seed match B was mutated at p3+4 and seed match A was wildtype. Spacer2 induced a small but significant decrease in the effects of 122-2'Ome, and an increase in translation activation by miR122p3+4 binding to seed match B (*, $P<0.01$; ***, $P<0.0001$). However, when the data in (A) and Figure 5C are taken into account, we conclude that there is no specific effect of the additional spacer on translation regulation via either seed match. Firefly luciferase activity is shown relative to *Renilla* luciferase activty from the transfection control as a percentage of Rand-2'Ome data. Values are an average of three independent triplicate experiments, plus standard deviation.

Supplementary methods

Electrophoresis of radiolabeled RNA

To determine whether miR-122 affects the integrity of HCV IRES RNA, radiolabeled 5'LUC3' RNA was synthesized by including α-^{32}P CTP in the transcription reaction. This RNA was used in lipofectamine 2000 transfections of Huh7 cells in a 6 well plate, and total RNA was isolated after 6h. The RNA was separated on a denaturing 4% polyacrylamide/7M urea/1x TBE gel. The gel was dried and visualized by autoradiography.

Supplementary Table S1: DNA oligonucleotide sequences

5'UTR F	GACGGCCAGTGCCAAGCTTACTTGGTCAG
5'UTR R	GTTGGTGTTACCCATGGTTTTTCTTTGAGG
3'UTR F	GCATCTACCTCCTCCCCAACTAGTGAAGG
3'UTR R	GGAGAATGCCATGCGAATTCACATGATCTGC
5'core R	GACGAATTCCAGTCTAGACTCCCTTAGCC
1-45 R	CAGTAGTTCCTCCATGGGGAGTGATTCATG
CSFV F	GATTTGGTCCATGGCACCCCTCCAG
CSFV R	CTGGAGGGCGCCATGGTTGGTTTTTG
FMDV F	GACACCCATGGTGCAACTTGAAACTCC
FMDV R	CAGTCAGTTGTATCCATGGGTTCAGTGG
Kpn F	CTCTATCGATAGGTACCGAGCTCTTAC
LUC R	GCAGTTGCTCTCCAGCGGTTCCATCTTCC
Bss F	GTGCAGCCTCCAGGCGCGCCCCTCC
Bss R	GGAGGGGCGCGCCTGGAGGCTGCAC
CSFVIII F	CTCCCGCGCGCACCCCTCCAGCGACG
m21A F	GATGGGGGCCATAAGCTACCATGAATCACTC
m21A R	GAGTGATTCATGGTAGCTTATCGCCCCCATC
m21B F	CCATGAATTAAGCTCCTGTGAGGAACTAC
m21B R	GTAGTTCCTCACAGGAGCTTAATTCATGG
H77Xmn F	GCTCATCATTGGAAAACGTTCTTCGGGGCG
H77Kpn R	GCCAAGGGTACCCGGGCTGAGCCCAGGTCC
p3+4A F	GATGGGGGCGACACAGCACCATGAATCACTC
p3+4A R	GAGTGATTCATGGTGCTGTGTCGCCCCCATC
p3+4B F	CCATGAATCACAGCCCTGTGAGGAACTAC
p3+4B R	GTAGTTCCTCACAGGGCTGTGATTCATGG
m2-4 F	CGACTCACTATAGGGTGCCCCCTGATG
m2-4 R	CATCAGGGGGCACCCTATAGTGAGTCG
m30-7 F	GCGACACTCCAGGTACTTACACTCCCCTGTG
m30-7 R	CACAGGGGAGTGTAAGTACCTGGAGTGTCGC
m21EXT F	CTAATACGACTCACTATAGCCAGCGATAAGCTACCATGAA TTAAGCTAGCCAGCCCCCTG
m21EXT R	CAGGGGGCTGGCTAGCTTAATTCATGGTAGCTTATCGCTGG CTATAGTGAGTCGTATTAG
wtEXT F	CTAATACGACTCACTATAGCCAGCGACACTCCACCATGAATCACTCCA GCCAGCCCCCTG
wtEXT R	CAGGGGGCTGGCTGGAGTGATTCATGGTGGAGTGCTGGCTATAGTG AGTCGTATTAG
spacer2 F	CATGAATCACTCCGGTACTTACCTGTGAGGAAC
spacer2 R	GTTCCACACAGGTAAGTACCGGAGTGATTCATG
p3+4Bspacer2 F	CATGAATCACAGCGGTACTTACCTGTGAGGAAC
p3+4Bspacer2 R	GTTCCTCACAGGTAAGTACCGCTGTGATTCATG
ΔSLI F	CGACTCACTATAGCCAGCGACACTCCACC
ΔSLI R	GGTGGAGTGTCGCTGGCTATAGTGAGTCG
mutLRA F	GGTGGCGGTCAGATCGTAGGAGGTGTTTAC
mutLRA R	GTAAACACCTCCTACGATCTGACCGCCACC
LUC F	GAAAGGCCCGGCGCCATTCTATCCGC
21si R	CGGGAATTCTAGCTTATCAGACTGATGTTGAGCGGCCGCACTAG
122si R	CGGGAATTCTGGAGTGTGACAATGGTGTTTGTGCGGCCGCACTAG
21/122si R	CGGGAATTCTAGCTTATGACAATGGTGTTTGTGCGGCCGCACTAG

Supplementary Table S1, continued

21/26asi R	CGGGAATTCTAGCTTATATCCAGGATAGGCTGCGGCCGCACTAG
Ago1 QF	CAGGGGGCTGGCTGGAGTGATTCATGGTGGAGTGCTGGCTATAGTG AGTCGTATTAG
Ago1 QR	CATTCGCCAGCTCACAATGGCT
Ago2 QF	CGCGTCCGAAGGCTGCTCTA
Ago2 QR	TGGCTGTGCCTTGTAAAACGCT
TNRC6A QF	CTCTGTGGATGCTCCTGAAAG
TNRC6A QR	TGCTTGGATTTAACCCTCCATTT
TNRC6B QF	GGAAATTGGAGGAATGTGAGTG
TNRC6B QR	GGATGTCTGACCTACTGTGCT
Dicer QF	TGGTCAACTCTGCAAACCAG
Dicer QR	CAAGGCGACATAGCAAGTCA
GAPDH QF	AAGGTGAAGGTCGGAGTCAA
GAPDH QR	GAAGATGGTGATGGGATTTC
Actin QF	AGCACAGAGCCTCGCCTTT
Actin QR	TCATCATCCATGGTGAGCTG
Fluc QF	TCGCCAGTCAAGTAACAAC
Fluc QR	ACTTCGTCCACAAACACAA
HCV 5'UTR QF	GCGACACTCCACCATGAAT
HCV 5'UTR QR	GGTTCCGCAGACCACTATG
Rluc QF	AACGCGGCCTCTTCTTATTT
Rluc QR	GTCTGGTATAATACACCGCG

Supplementary Table S2: 2'-O-methylated oligonucleotide sequences

122-2'Ome	ACAAACACCAUUGUCACACUCCA
Rand-2'Ome	GUGUAACACGUCUAUACGCCCAA
21-2'Ome	UCACAUCAGUCUGAUAAGCUA

Supplementary Table S3: RNA oligonucleotide sequences

miR122wt	UGGAGUGUGACAAUGGUGUUUGU
miR122p3+4	UGCUGUGUGACAAUGGUGUUUGU
miR122*	AAACGCCAUUAUCACACUAAAUA
miR21wt	UAGCUUAUCAGACUGAUGUUGA
miR21*	AACACCAGUCGAUGGGUUGUC
miR21/122	UAGCUUAUGACAAUGGUGUUUGU
miR21/122*	AAACGCCAUUAUCAUGGGUUGUC
miR21/26a	UAGCUUAUAUCCAGGAUAGGCU
miR21/26a*	CCUAUUCUUGGUAUGGGUUGUC
miR21p16	UAGCUUAUCAGACUGGUGUUGA
miR21/122p9,11	UAGCUUAUCAGAAUGGUGUUUGU
miR21/122p9,11*	AAACGCCAUUAUGAUGGGUUGUC
miR21/122p16	UAGCUUAUGACAAUGAUGUUUGU
miR21/122p16*	AAACGUCAUUAUCAUGGGUUGUC
miR21/122p21-3	UAGCUUAUGACAAUGGUGUUGA