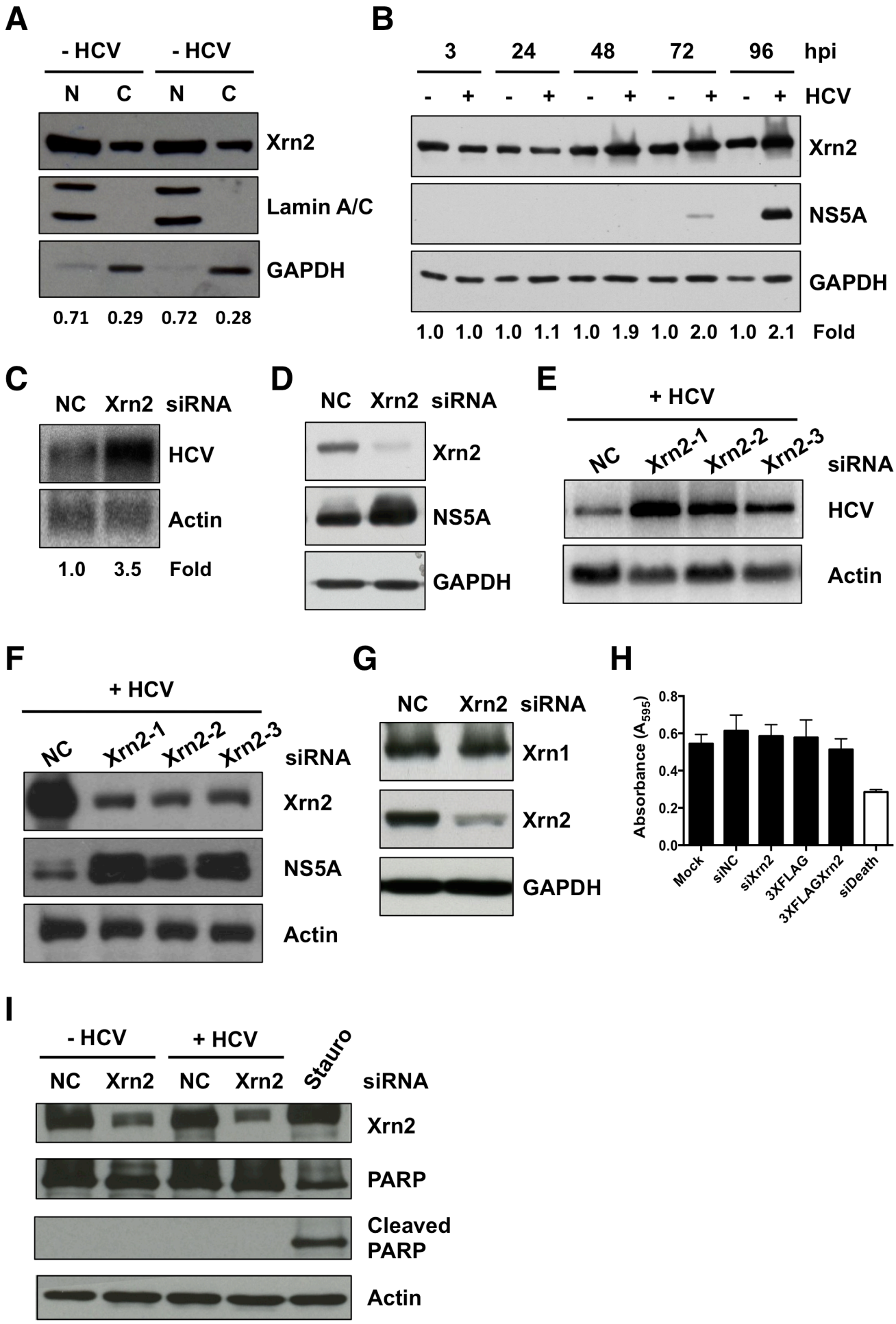

A
- HCV
- HCV
N
C
N
C
Xrn2
Lamin A/C
GAPDH
0.71
0.29
0.72
0.28
B
3
24
48
72
96
hpi
- + - + - + - + - + HCV
Xrn2
NS5A
GAPDH
1.0 1.0 1.0 1.1 1.0 1.9 1.0 2.0 1.0 2.1 Fold
C
NC
Xrn2
siRNA
HCV
Actin
1.0
3.5
Fold
D
NC
Xrn2
siRNA
Xrn2
NS5A
GAPDH
E
+ HCV
NC
Xrn2-1
Xrn2-2
Xrn2-3
siRNA
HCV
Actin
F
+ HCV
NC
Xrn2-1
Xrn2-2
Xrn2-3
siRNA
Xrn2
NS5A
Actin
G
NC
Xrn2
siRNA
Xrn1
Xrn2
GAPDH
H
Absorbance (A595)
0.8
0.6
0.4
0.2
0.0
Mock
siNC
siXrn2
3XFLAG
3XFLAGXrn2
siDeath
I
- HCV
+ HCV
NC
Xrn2
NC
Xrn2
Stauro
siRNA
Xrn2
PARP
Cleaved PARP
Actin

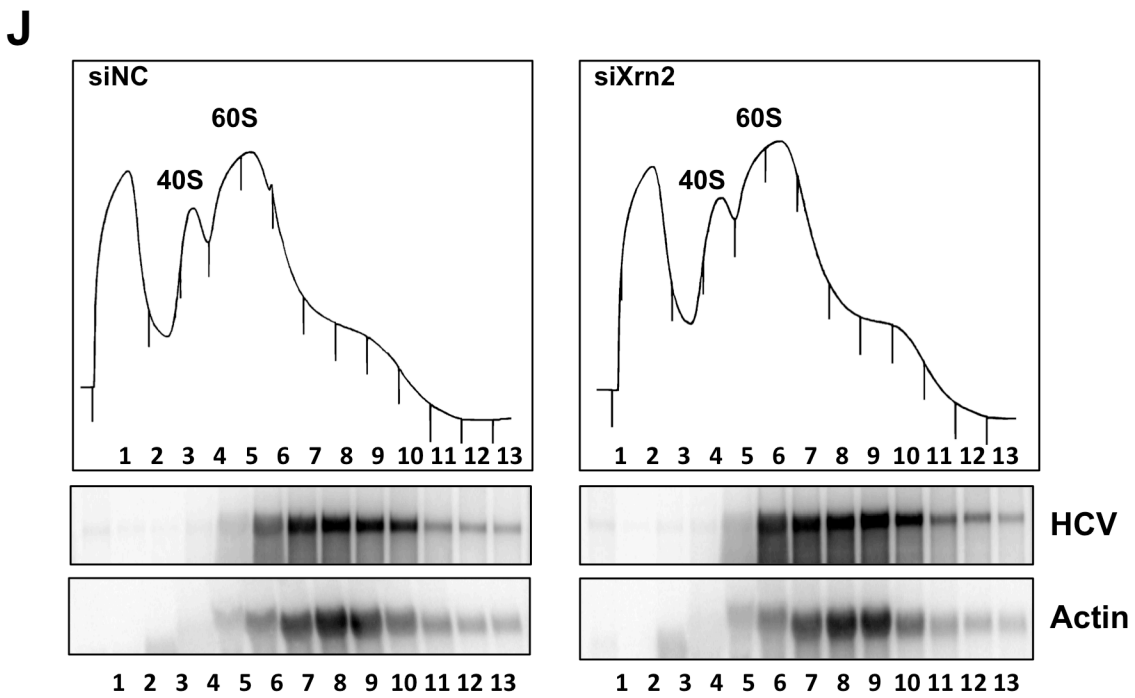


Figure S1. (Corresponds to Figure 1) Characterization of Xrn2 during HCV infection
(A) Western blot analysis of uninfected and HCV-infected subcellular fractionated lysates. Lamin (A/C) and GAPDH were used as nuclear and cytoplasmic markers, respectively. Immunoblot is representative of ta least three independent replicates. (B) Western blot analysis of Xrn2 and NS5A protein expression at indicated times post HCV-infection. GAPDH expression was examined as protein loading control. Xrn2 fold change is indicated. Immunoblot is representative of at least three independent experiments. Effects of Xrn2 depletion on HCV RNA and protein abundances in 5B replicons. (C) HCV RNA accumulation was measured by Northern blot analysis. HCV RNA abundances were normalized to γ-actin. Data from cells transfected with control siRNAs were set to 1. HCV RNA fold change is indicated. (D) Western blot analysis confirmed Xrn2 depletion and measured levels of HCV protein NS5A. GAPDH expression was examined as loading control. The data shown are representative of at least three independent experiments. Xrn2 depletion by additional siRNA duplexes. (E) HCV RNA accumulation was measured by Northern blot analysis. HCV RNA abundances were normalized to γ-actin. Data from cells transfected with control siRNAs were set to 1. HCV RNA fold change is indicated. (F) Western blot analysis confirmed Xrn2 depletion and measured levels of HCV protein NS5A. Actin expression was examined as loading control. The data shown are representative of at least three independent experiments. Effects of Xrn2 depletion on Xrn1 expression. (G) Indicated siRNAs were transfected two days prior to Western blot analysis of Xrn1 expression. GAPDH expression served as loading control. The data shown are representative of at least three independent experiments. Effects of Xrn2 depletion on cell viability. (H) Cell proliferation assay of Huh7 cells transfected with indicated siRNAs and 3XFLAG plasmids. The means of at least three independent experiments, performed in triplicate for each experiment were graphed. Error bars represent standard error of the mean. (I) siRNAs were transfected one day prior to infection, apoptosis was assessed by Western blot analysis of PARP cleavage three days post-infection. Xrn2 depletion was confirmed and actin expression served as loading control. Lysates from Huh7 cells treated with 1 μM Staurosporine (Stauro) for 3 hours were used as positive control. Immunoblot is representative of at least three independent replicates. Effects of Xrn2 depletion on puromycin density gradients. (J) HCV-infected, Xrn2-depleted (right panel) and negative control siRNA samples (left panel) treated with 2 mM puromycin and separated in 10-60% sucrose gradients. 40S and 60S ribosomal subunits are indicated. RNA was extracted from each gradient fraction and HCV RNA distribution and abundance were analyzed by Northern blotting. γ-actin expression was examined as loading control. Northern blots shown are representative of at least three independent replicates.

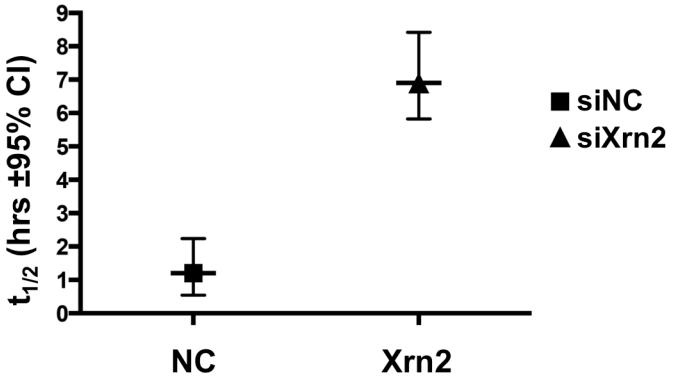
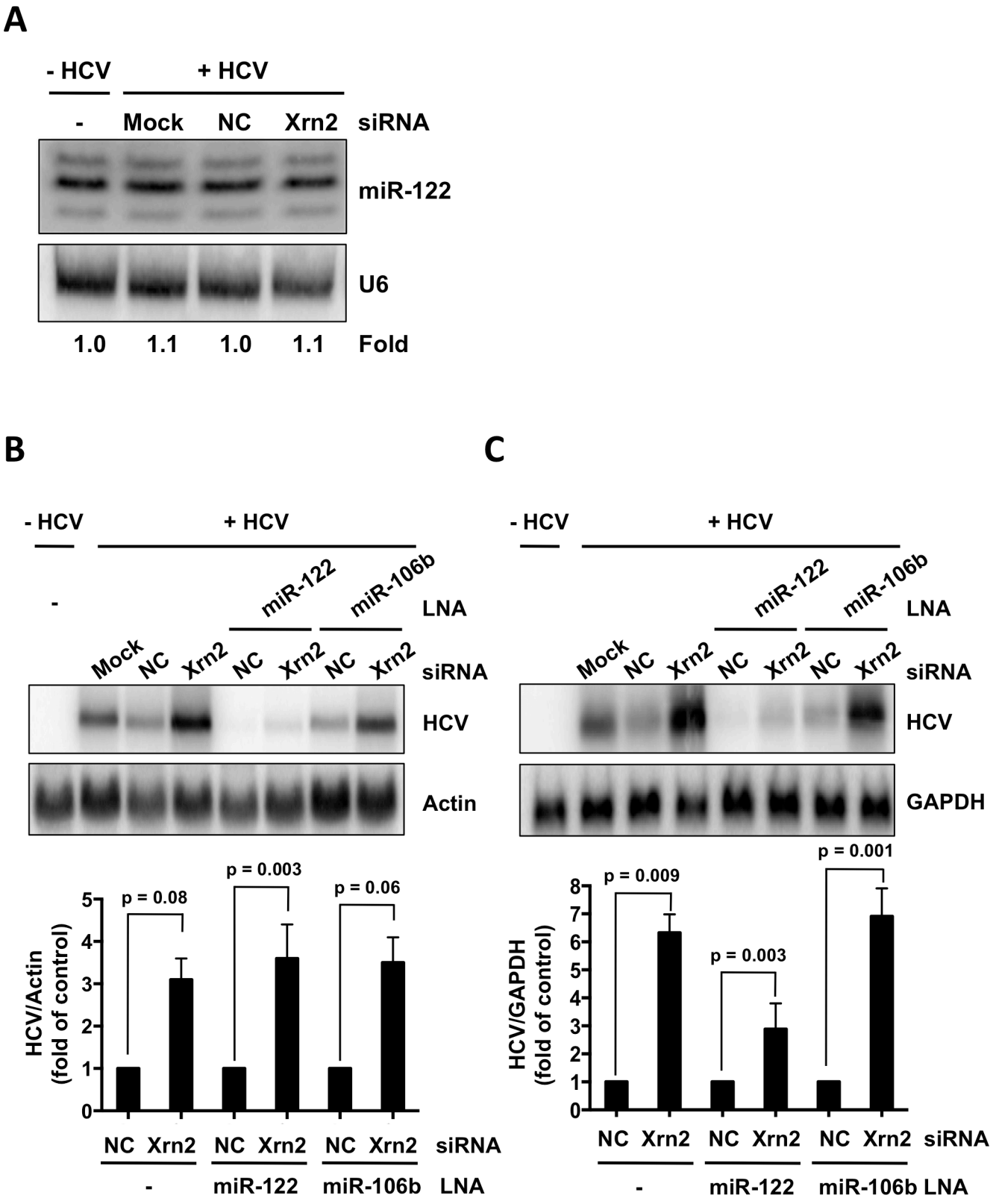


Figure S2. (Corresponds to Figure 2) Estimated half-life of HCV RNA
HCV RNA half-lives ($t_{1/2}$) ±95% confidence intervals (CI) under the conditions shown in Figure 3C. The data were fit to a one-phase decay model (R^2 = 0.895-0.946). The ±CI are 0.54-2.24 and 5.82-8.42 in hours for NC and Xrn2 respectively.


A
- HCV
+ HCV
-
Mock
NC
Xrn2
siRNA
miR-122
U6
1.0
1.1
1.0
1.1
Fold
B
- HCV
+ HCV
-
miR-122
miR-106b
LNA
Mock
NC
Xrn2
NC
Xrn2
NC
Xrn2
siRNA
HCV
Actin
p = 0.08
p = 0.003
p = 0.06
HCV/Actin
(fold of control)
NC
Xrn2
NC
Xrn2
NC
Xrn2
siRNA
-
miR-122
miR-106b
LNA
C
- HCV
+ HCV
-
miR-122
miR-106b
LNA
Mock
NC
Xrn2
NC
Xrn2
NC
Xrn2
siRNA
HCV
GAPDH
p = 0.009
p = 0.003
p = 0.001
HCV/GAPDH
(fold of control)
NC
Xrn2
NC
Xrn2
NC
Xrn2
siRNA
-
miR-122
miR-106b
LNA

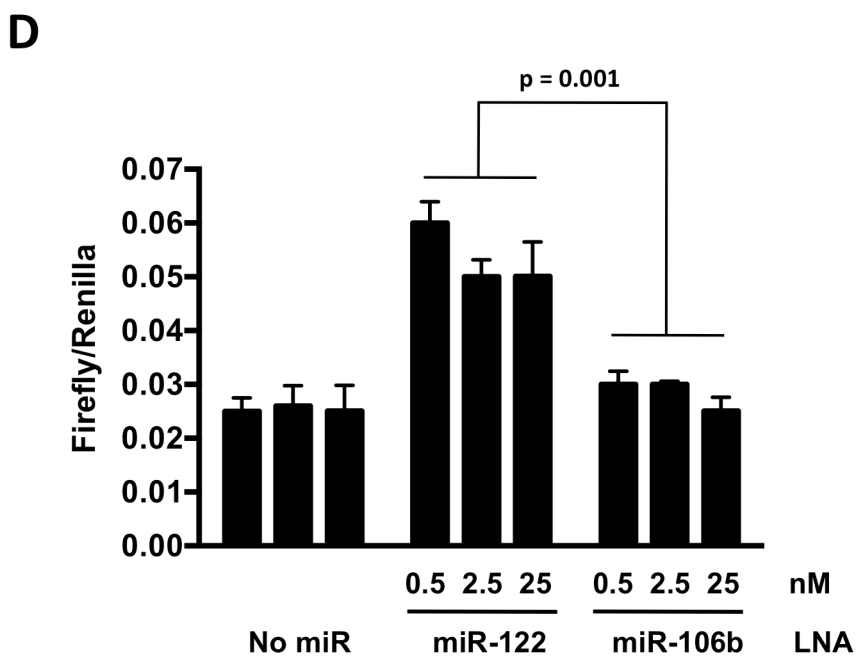


Figure S3. (Corresponds to Figure 4) HCV RNA abundance during sequestration of miR-122 and depletion of Xrn2

Effects of Xrn2 depletion on miR-122 abundance. (A) siRNAs were transfected one day prior to infection. Three days after infection RNA was extracted and miR-122 abundance was measured by Northern blot analysis. U6 snRNA served as loading control. Northern blot shown is representative of at least three independent replicates. Indicated siRNAs were transfected one day prior to infection. 25 nM (A) or 2.5 nM (B) miR-122 or miR-106b LNA was transfected one day post-infection. Three days post-infection RNA was extracted and HCV RNA abundance was measured by Northern blot analysis (top panels). Northern blots shown represent at least three independent replicates. Quantitation of HCV RNA abundance (bottom). HCV RNA abundances were normalized to γ-actin (A) or GAPDH (B) levels. Data from cells transfected with control siRNAs were set to 1. Error bars represent standard error of the mean. P-values were determined by Student's *t*-test. (C) Representative immunoblot of Xrn2 following siRNA transfection. Actin expression was examined as protein loading control. MicroRNA (luciferase) assay. (D) miR-122 or miR-106b LNA at 0.5, 2.5, and 25 nM concentration were transfected into Huh7 cells. One day post LNA transfection, pLuc122x2, a plasmid that expresses firefly luciferase, which contains four miR-122 seed match sites in its 3' noncoding region, was co-transfected with a Renilla reporter plasmid that served as a transfection control. Cells were harvested two days post-LNA transfection, and luciferase activities were measured. Data from miR-106b LNA-transfected cells were used as a negative control. Data are representative of at least three independent replicates and error bars represent standard error of the mean. P-values were determined by Student's *t*-test.

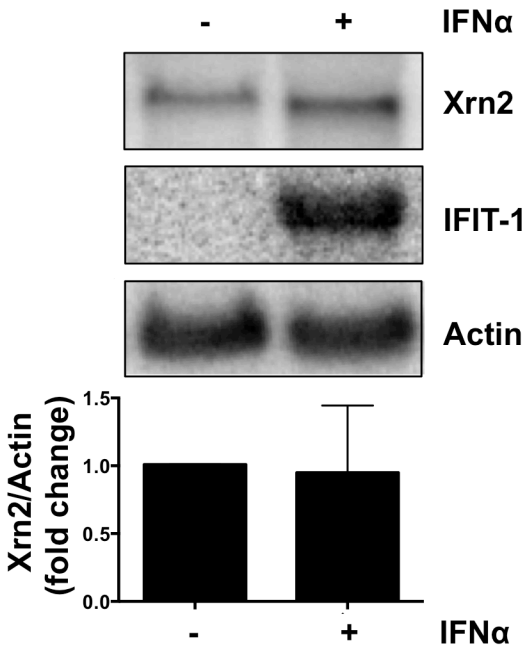


Figure S4. (Corresponds to Discussion) Xrn2 mRNA abundance during interferon alpha stimulation

Northern blot analysis of Xrn2 mRNA abundance in untreated and IFNα-treated cells (top). IFIT-1 expression was used as positive control for IFNα activation, actin expression was used loading control. Northern blot shown is representative of at least three independent replicates. Quantitation of Xrn2 mRNA abundance (bottom). Xrn2 mRNA abundance was normalized to γ-actin. Data from untreated cells were set to 1. Error bars represent standard error of the mean.