PNAS
www.pnas.org

Supplementary Information for

The RNA triphosphatase DUSP11 enables XRN-mediated restriction of hepatitis C virus

Rodney P. Kincaid, Victor L. Lam, Rachel P. Chirayil, Glenn Randall, Christopher S. Sullivan

Christopher S. Sullivan
Email: chris_sullivan@austin.utexas.edu

This PDF file includes:

Supplementary text
Figs. S1 to S6
Table S1

www.pnas.org/cgi/doi/10.1073/pnas.1802326115

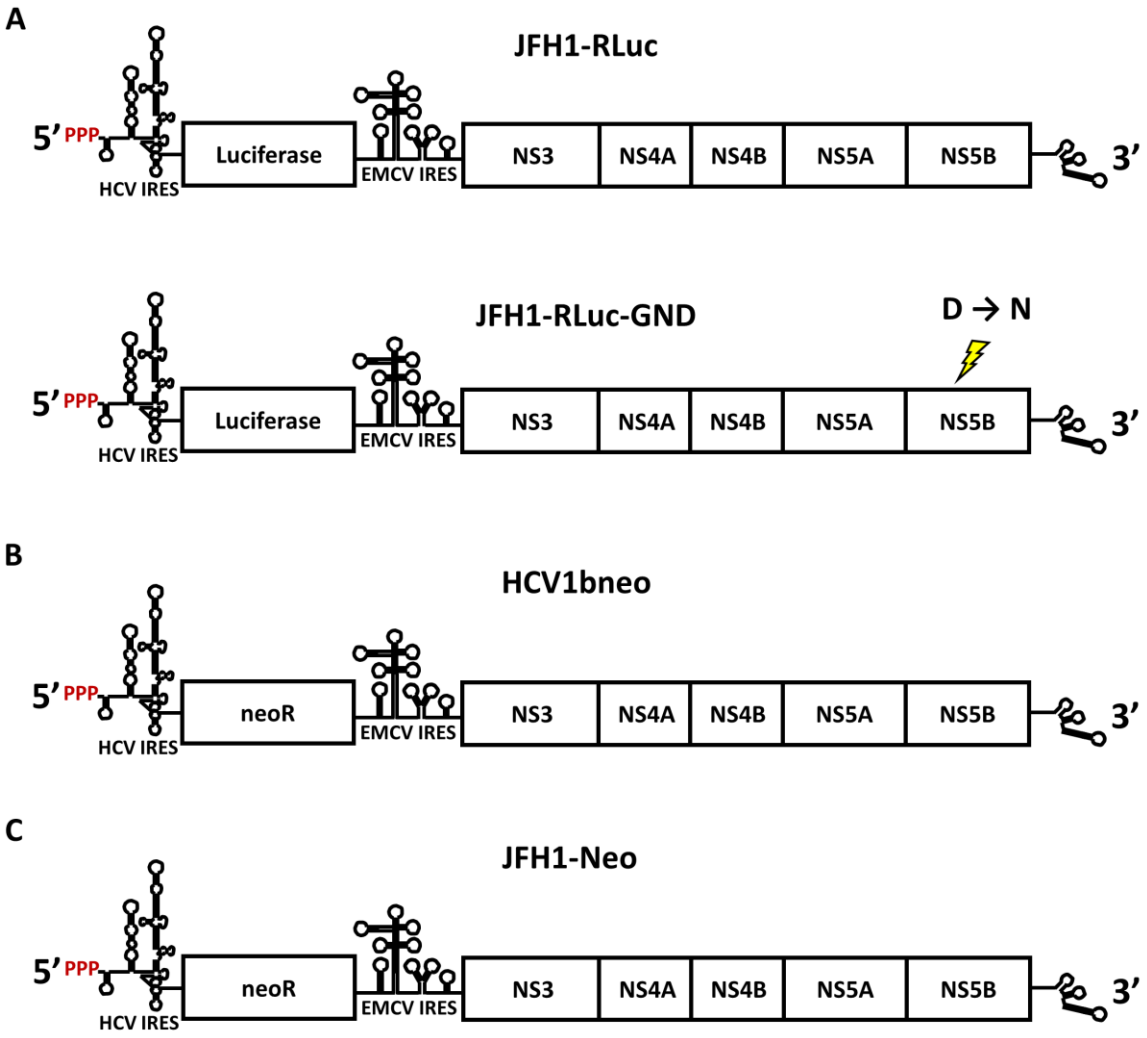

Figure S1. Schematics of HCV subgenomic replicons used in this study.

A. Schematic of JFH1 based *Renilla* luciferase subgenomic replicons. The JFH1-RLuc-GND bears a point mutation resulting in a single amino acid change in the virally encoded RNA dependent RNA polymerase rendering it inactive.
B. Schematic of neomycin resistance encoding subgenomic replicon maintained in GS4.1 cells.
C. Schematic of JFH1 neomycin resistance encoding subgenomic replicon.

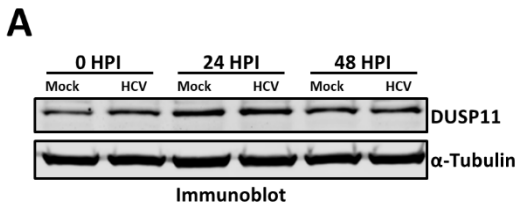

Figure S2. Immunoblot analysis of DUSP11 in HCV infected Huh7 cells.

A. Total protein was harvested from either HCVcc infected (HCV) or mock infected (Mock) Huh7 cells. Equal volumes of cell lysate was separated by SDS-PAGE and probed with the indicated antibodies.

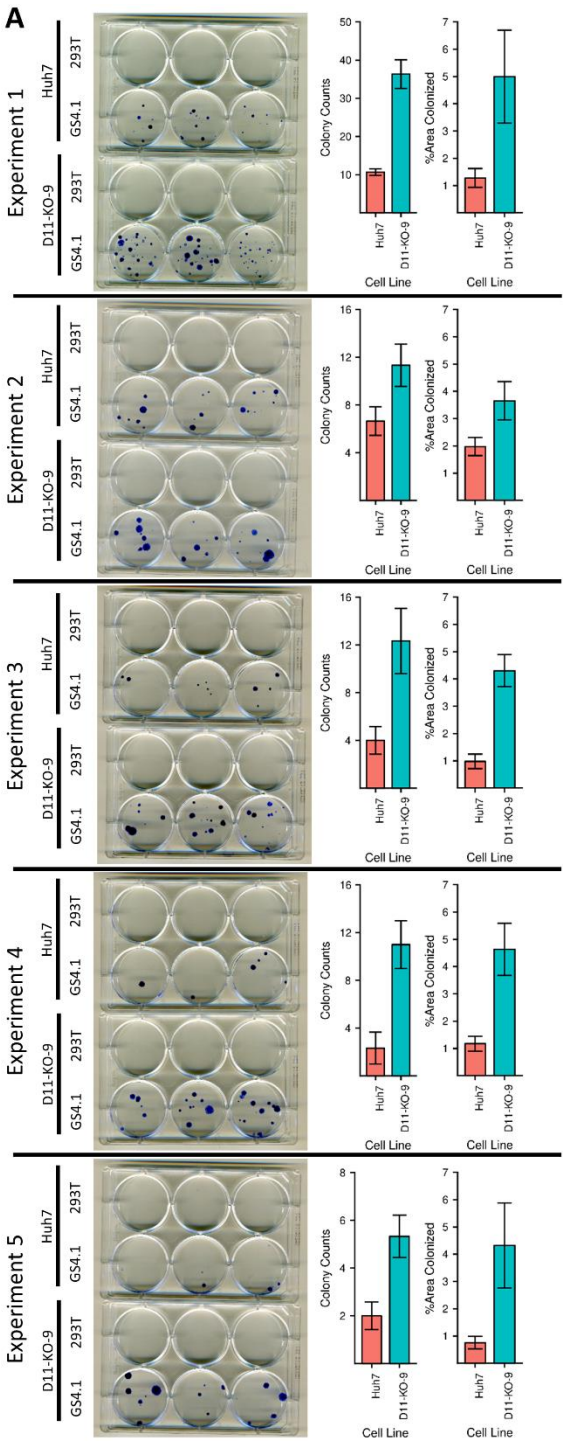

Figure S3. GS4.1 replicon colony formation assays in parental Huh7 cells and DUSP11 knockout cells (D11-KO-9).

A. Quantitation of area colonized and number of colonies with transfection of GS4.1 RNA is presented to the right of each experiments plates.

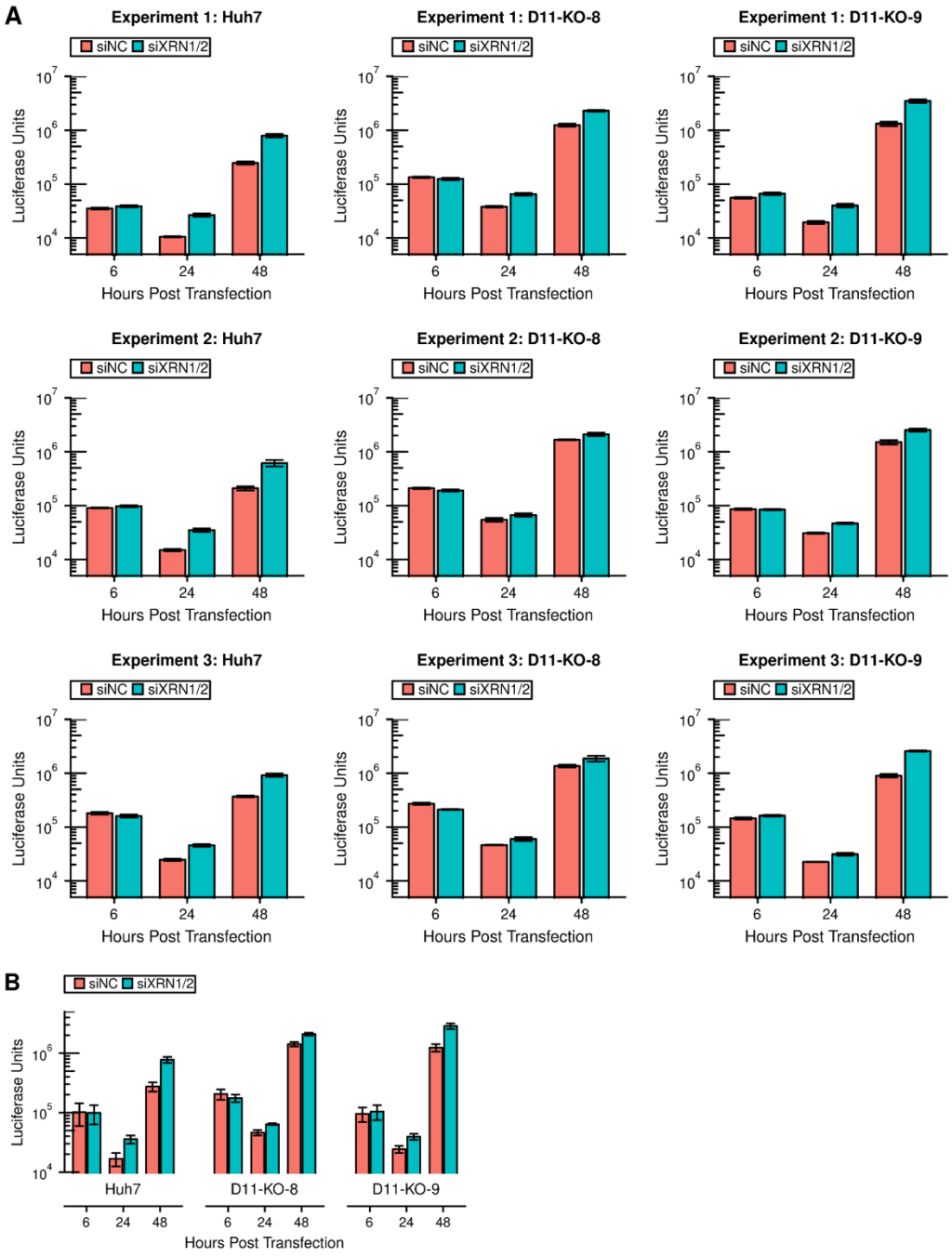

Figure S4. Raw un-normalized luciferase expression data for XRN siRNA replicon experiments in Figure 2C.

A. sgJFH1-RLuc replicon luciferase assay in parental Huh7 cells and two independent DUSP11 knockout clones (D11-KO-8 and D11-KO-9) treated with irrelevant control siRNA (siNC) or a pool of XRN1 and XRN2 specific siRNAs (siXRNs). Luciferase assays were performed at the indicated times post replicon RNA transfection. The un-normalized luciferase expression for each experiment is presented separately as the mean +/- SEM of the three wells assayed per condition.

B. Un-normalized means +/- SEM derived from the three experiments in panel A.

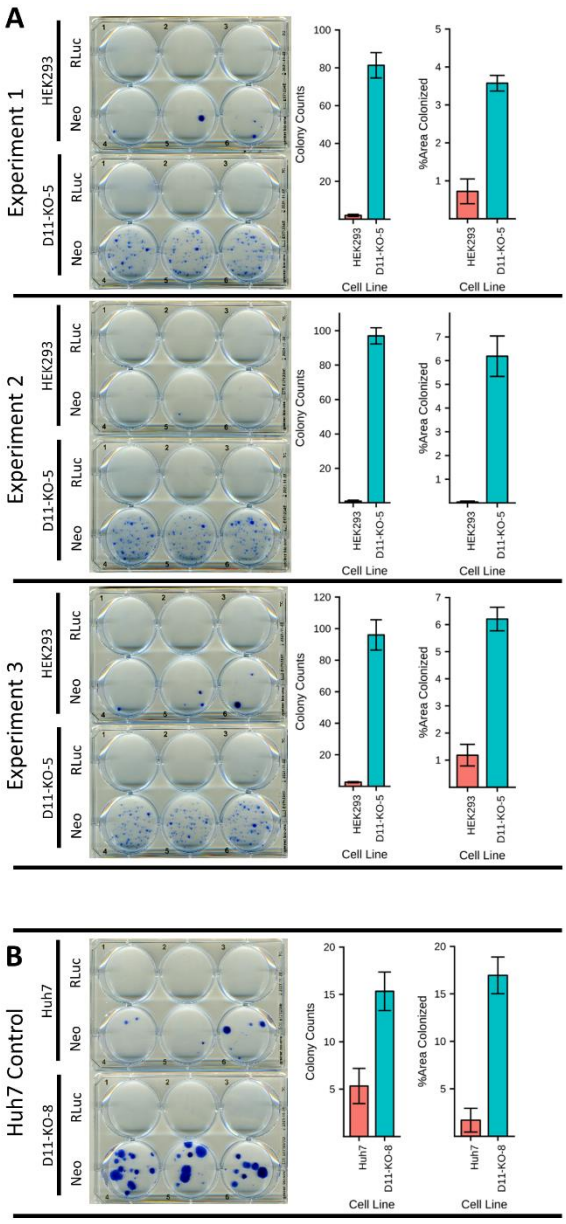

Figure S5. JFH1-Neo replicon colony formation assays in parental cells and DUSP11 knockout cells.

A. Quantitation of area colonized and number of colonies with transfection of *in vitro* transcribed JFH1-Neo RNA into HEK293 or HEK293-D11-KO-5 cells is presented to the right of each experiment's plates.

B. Quantitation of area colonized and number of colonies with transfection of *in vitro* transcribed JFH1-Neo RNA into Huh7 or Huh7-D11-KO-8 is presented to the right of each experiments plates.

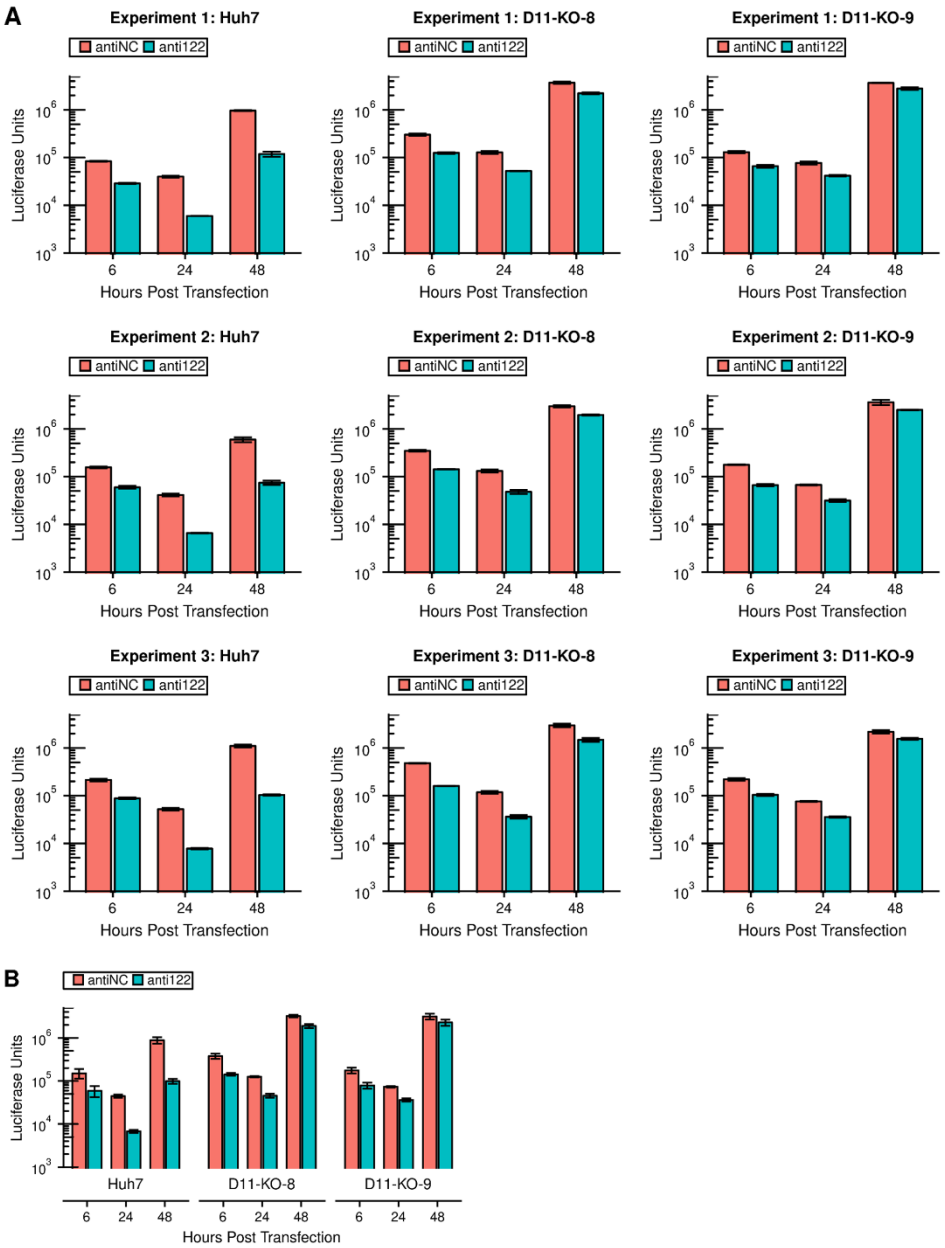

Figure S6. Raw un-normalized luciferase expression data for miR-122 antimir replicon experiments in Figure 3B.

A. sgJFH1-RLuc replicon luciferase assay in parental Huh7 cells and two independent DUSP11 knockout clones (D11-KO-8 and D11-KO-9) treated with irrelevant control antimiR (antiNC) or miR-122 specific antimiR (anti122). Luciferase assays were performed at the indicated times post replicon RNA transfection. The un-normalized luciferase expression for each experiment is presented separately as the mean +/- SEM of the three wells assayed per condition.

B. Un-normalized means +/- SEM derived from the three experiments in panel A.

Sequence Name	Sequence
HCV_5UTR_gBlock	ATCGATCGCTCGAGGCCGCTCGTAATACGACTCACTATAGCCAGCCCCCTGAT GGGGGCGACACTCCACCATGAATCACTCCCCTGTGAGGAACTACTGTCTTCAC GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGA CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGG AATTGCCAGGACGACCGGGTCCTTTCTTGGATAAACCCGCTCAATGCCTGGAG ATTTGGGCGTGCCCCCGCAAGACTGCTAGCCGAGTAGTGTTGGGTCGCGAAA GGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAGGTCTCG TAGACCGTGCACCATGAGCACGAATCCTAAACCTCAAAGAAAAACCAAACGT AACACCAACCGTCGCCCACATCTAGAATCGATCG
HCVT7F	GGCCGCTCGTAATACGACTCAC
HCVT7R	TGTGGGCGACGGTTGGTGTTAC

Table S1. Sequences of synthetic DNA and oligonucleotides.