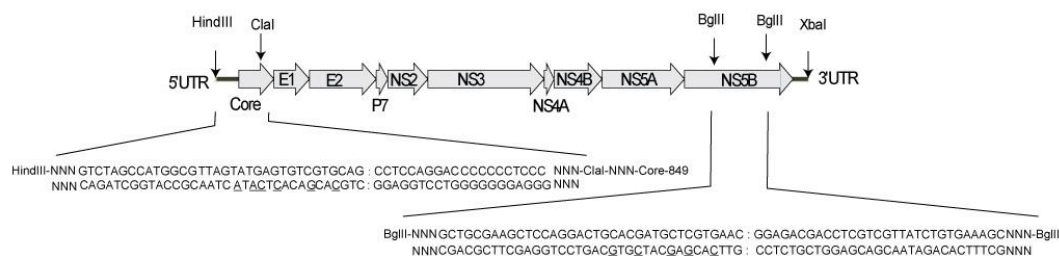


**Table S1. Primers used in HCV mutant construction and qPCR assays**

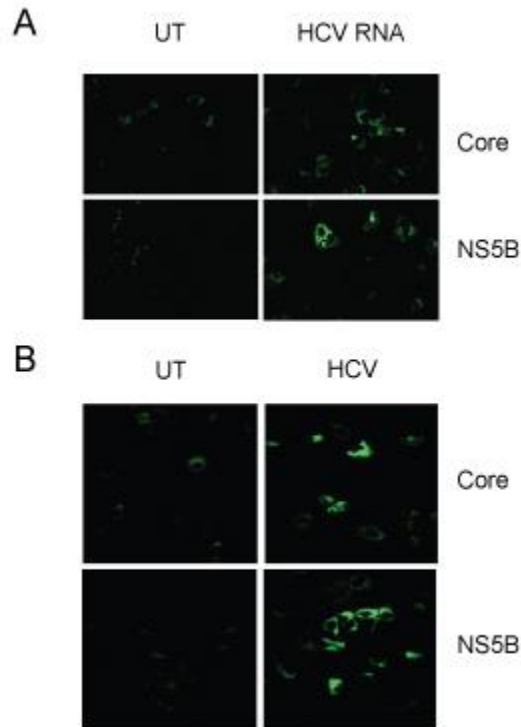
Primer <sup>1</sup>	Sequence <sup>2</sup>
<i>Hind</i> III-T7-5'UTR:1-19S	<b>AAGCTT</b> <u>GTAATACGACTCACTATAG</u> CCAGCCCCCGATTGGGGG
5'UTR:77-110LM AS	CTG <u>TCCACATTGGT</u> CCTAACGCCATGGCTAGAC
5'UTR: 77-110SM AS	CTG <u>TACCACACTCATACTA</u> ACGCCATGGCTAGAC
5'UTR: 111-130 Linker S	CCTCCAGGACCCCCCTCCC
HCV Core: 825-849 AS	GCAGATTCCTGTTGCATAGTTCAC
NS5B 8060-8084 S	TAAGCCAGCCCGCCTTATCGTATTC
NS5B 8511-8546 BM AS	GTT <u>GACCA</u> ACATGGT <u>ACAGT</u> CCTGGAGCTTCGCAGC
NS5B 8511-8546 SM AS	GTTACGAGCAT <u>GGTACAGT</u> CCTGGAGCTTCGCAGC
HCV NS5b(8547-8576) linker S	GGAGACGACCTCGTCGTTATCTGTGAAAGC
NS5B 9043-9066 AS	TGAGGCATGAAGCCACCCTATTGA
HCV 5'URT (130-146) S	CGGGAGAGCCATAGTGG
HCVUTR 272-290) AS	AGTACCACAAGGCCTTTCG
HCV NS5B (8374-8392) S	TAATACGACTCACTATAGGGCTTTATATCGGGGGTCC
Hcv ns5b (8657-8676) AS	AGTCGTATTCTGGTTGGGGC

<sup>1</sup> S, Sense; AS, Antisense.

<sup>2</sup> **AAGCTT**G, *Hind*III recognition size; GTAATACGACTCACTATAG, T7 RNA polymerase promoter; N, mutated nucleotide.



**Figure S1.** Map of HCV genome showing the *Hind* III-5'UTR-Core-*Cla*I and *Bgl*III-NS5B cloning cassettes. The nucleotides changed (N) within IRES domain II nt 95-110 and NS5B nt 8528 to 8543 and the 5' sequence of the blunt ligation partner (:) are shown and as outlined in Materials and methods, Section 2.2 Construction of HCV 1b mutant virus.



**Figure S2. HCV cell culture system.** Huh-7.5 cells were transfected or infected with full-length HCV RNA or HCV, respectively. (A) Immunofluorescence expression of HCV core antigen (top row) or NS5B antigen (bottom row) in mock-transfected (UT) or HCV 1b RNA-transfected Huh-7.5 cells as measured at 2 days post-transfection. (B) Immunofluorescence expression of HCV core antigen (top row) or NS5B antigen (bottom row) in mock-infected (UT) or HCV-infected Huh 7.5 cells as measured at 2 days post-infection.