Interferon-Inducible Cholesterol-25-Hydroxylase Inhibits Hepatitis C Virus Replication via Distinct Mechanisms

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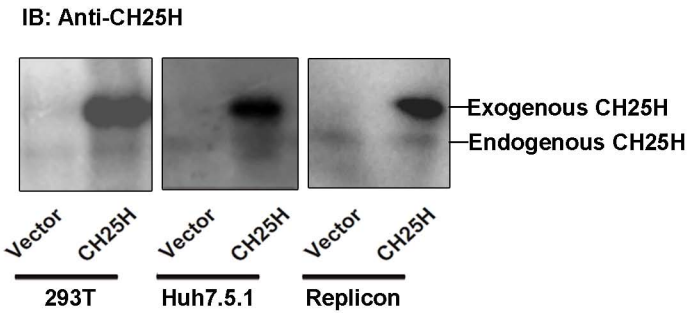
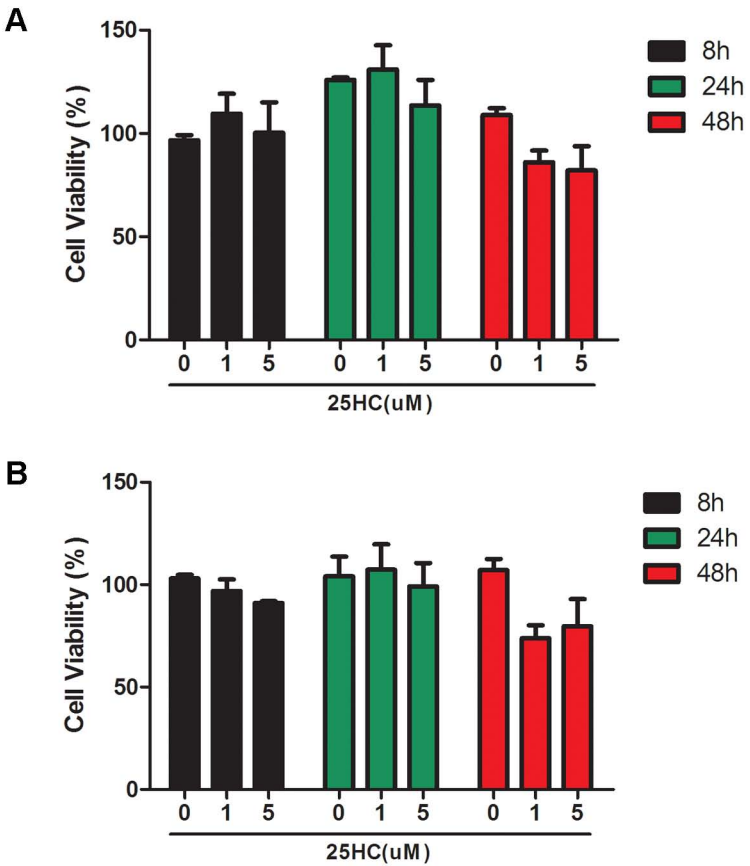
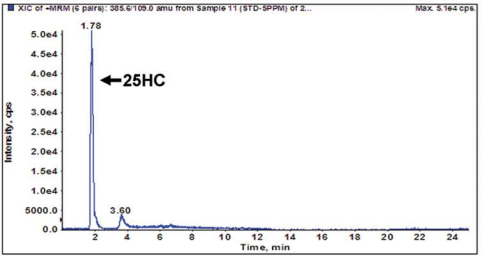


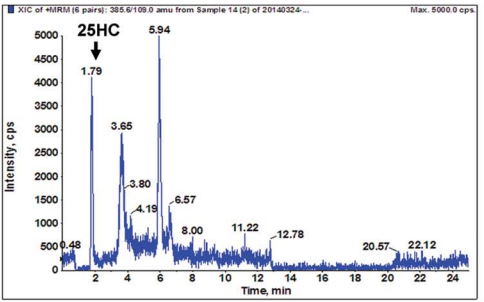
Supplementary Figure 1: Western blotting was performed to detect the expression of the CH25H. 293T cells, Huh7.5.1 cells or Replicon cells were transfected with vector or HA-CH25H, after 36h, the cells were harvested and lysed, and then the whole cell extracts were subjected to western blotting.



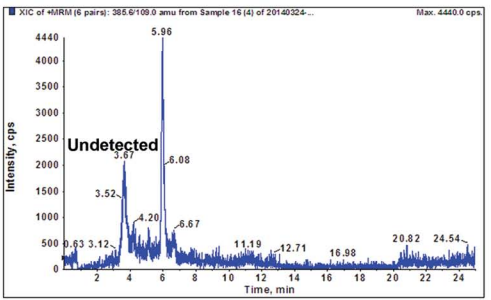
Supplementary Figure 2: Effect of 25HC on Huh7.5.1 (A) and Replicon (B) cell viability. Cells (5 × 103 cells/well) were first seeded into 96-well plates, incubated overnight and then treated with 25HC (0, 1, 5uM) for 8 h., 24h or 48h. As soon as the treatment was completed, 10 µl of the Cell Counting Kit-8 reagent was added to each well, and the cells were incubated for 2 h at 37°C. Finally, the spectrophotometric absorbance of each sample was measured with a microplate reader at 450 nm.



Standard

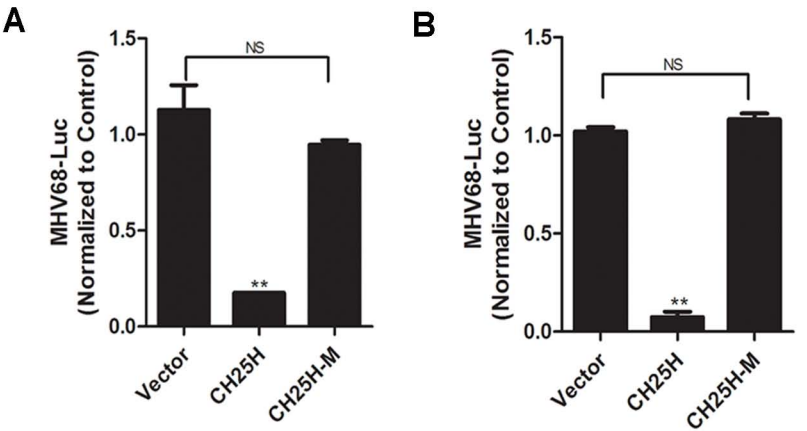


CH25H-WT

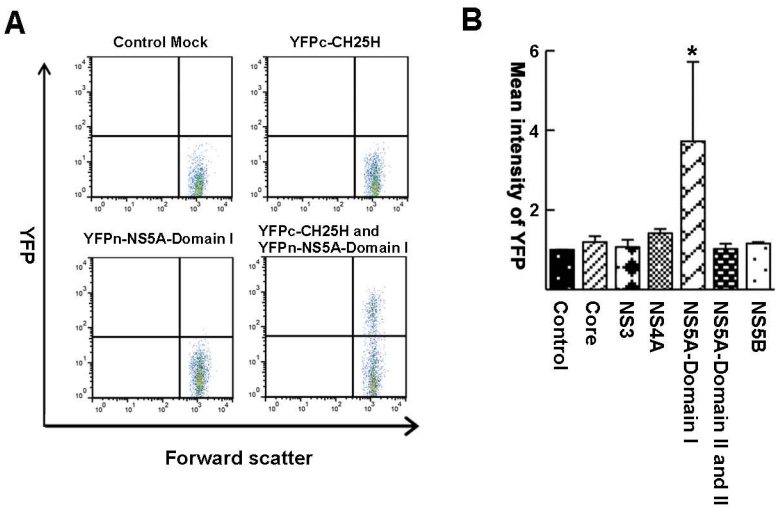


CH25H-M

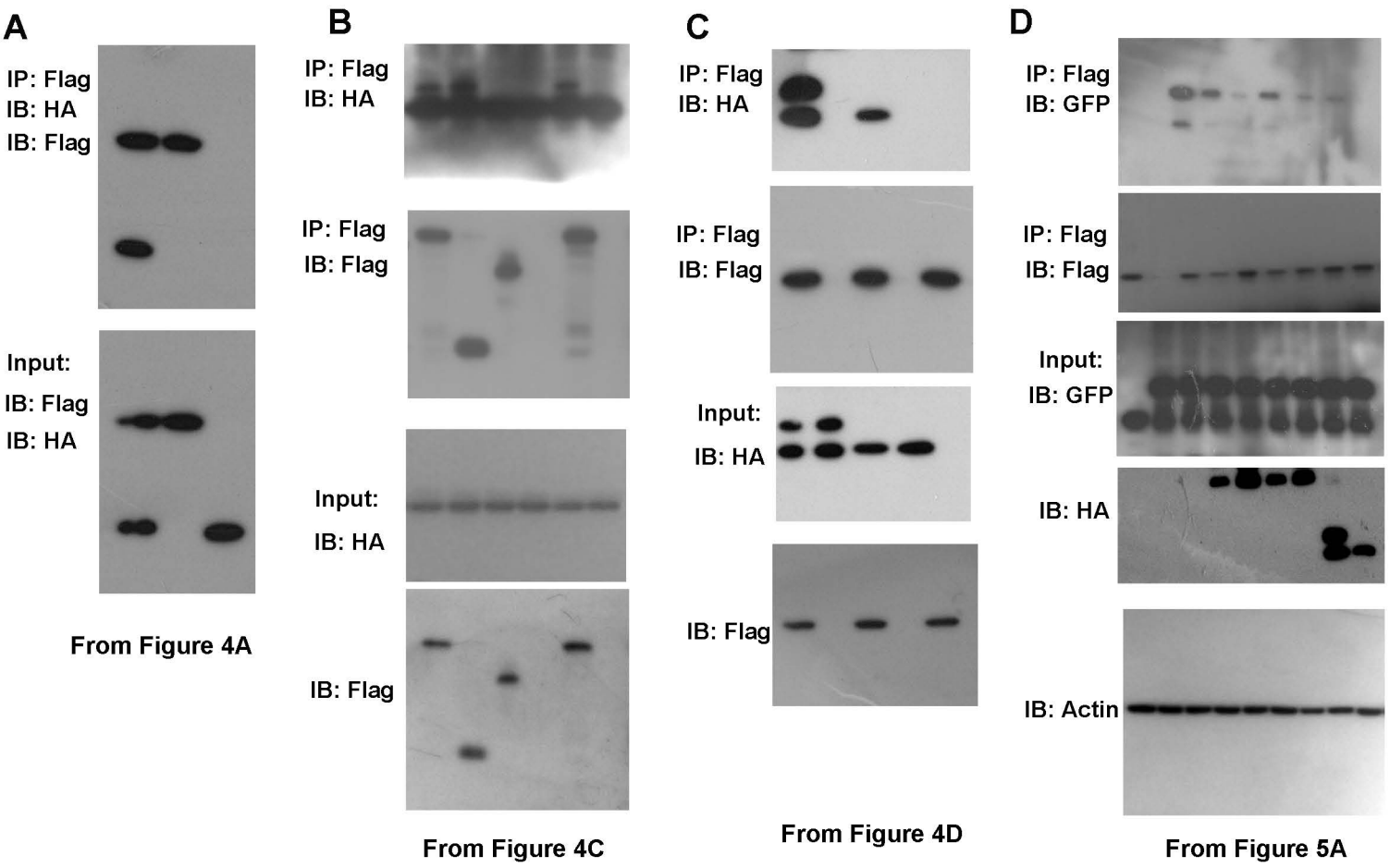
Supplementary Figure 3: Quantification of 25-Hydroxycholesterol in conditioned medium. The cell culture medium were collected from HEK293T cells after 48h transfection with CH25H or CH25H-M, then were analyzed by LC-MS. Experiments were done twice independently with similar results obtained.



Supplementary Figure 4: Effect of CH25H and CH25H-M on MHV-68 infection. HEK293T cells transfected with CH25H or CH25H-M were infected with MHV-68(M3FL), At approximately 24 hours post infection, the luciferase activity from HEK293T cells was measured (A), and the supernatant was transferred to fresh BHK21 and luciferase activity was measured at 9 hours after infection of BHK21 cells (B). All experiments were performed in triplicates and data shown are representative of three independent experiments with SEM indicated by error bars. **P < 0.01.



Supplementary Figure 5: Flow cytometry analysis of the BiFC assays of CH25H and HCV encoded proteins. A: 293T cells transiently co-transfeced with YFPc-CH25H and YFPn-NS5A-Domain I. 27 hours later, cells were harvested for flowcytometry. Mock 293T or transfected with YFPc-CH25H or YFPn-NS5A-Domain I alone were used as negative controls. B: 293T cells transiently co-transfected with YFPc-CH25H and the expression vectors for YFPn tagged HCV proteins, then the analysis were performed as A. All experiments were performed in triplicates and data shown are representative of three independent experiments with SEM indicated by error bars. *P < 0.05



Supplementary Figure 6. Original panel for each western blot

TABLE S1 Primers used for generation of target genes

	Primer (5'-3')	
Gene	Forward	Reverse
CH25H	AAAGAATTCAAATGAGCTGCCACAACTGC	AAAGGTACCTCACCGCGCTGGGACAGAT
CH25H-TM	AAAGAATTCAAATGAGCTGCCACAACTGC	AAAGGTACCTCAGAAGAGTAGCAGGCAGAA
CH25H-FA	AAAGAATTCAAGACATGGAGTTCTTCGTG	AAAGGTACCTCACCGCGCTGGGACAGAT
CH25H-M	TGGTGCACCACGACCTGCAACAGTCTCACTTTAA	CTGTTGCAGGTCGTGGTGCACCACACCCCCGTAC
CH25H-entry	CACCATGAGCTGCCACAACTG	CCGCGCTGGGACAGATGCAGTC
NS5A	CACCATGGGATCGTGGCTCCGCGACGTG	GGGGCTACAGGGAGTTATTAG
NS5A-Domain I	CACCATGGGATCGTGGCTCCGCGACGTG	TGCGTCGGGCTCAGGTTCAC
NS5A-Domain II/III	CACCATGGACGTATTGAGGTCCATGC	GGGGCTACAGGGAGTTATTAG
NS4A	CACCATGTGCGTTTCCATCATCGGCCG	GCATTCCTCCATCTCATC

The underlined nucleotides indicate restriction sites, GAATTC for EcoRI and GGTACC for KpnI