SUPPLEMENTAL INFORMATION

Very-long-chain fatty acid metabolic capacity of 17-beta-hydroxysteroid dehydrogenase type 12 (HSD17B12) promotes replication of hepatitis C virus and related flaviviruses. Bassim Mohamed, Clément Mazeaud, Martin Baril, Donald Poirier, Aïssatou Aïcha Sow, Laurent Chatel-Chaix, Vladimir Titorenko and Daniel Lamarre.

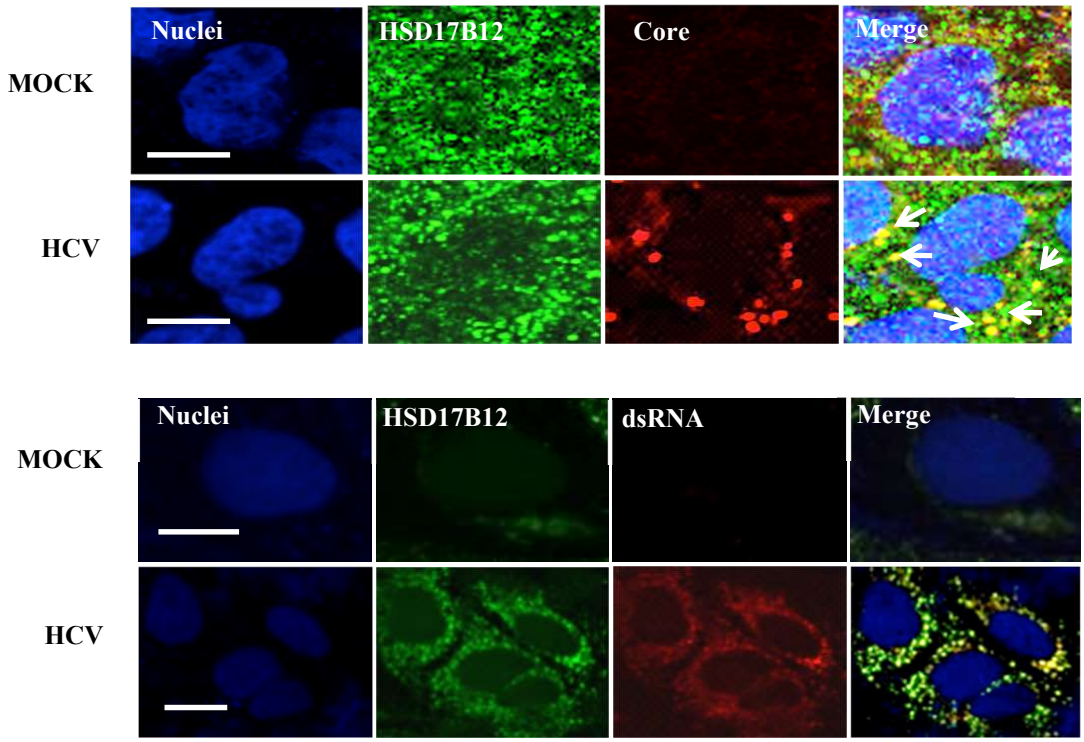

Figure S1. HSD17B12 overlaps with HCV RNA replication and assembly sites. Huh7.5 parental (Mock) and JFH-1-infected cells (HCV) were transfected with an N-terminal FLAG-tagged HSD17B12 fusion protein expressing vector. Cells were fixed with paraformaldehyde, permeabilized with 0.1% Triton X-100 and stained with anti-FLAG, anti-core and anti-dsRNA antibodies. Nuclei were stained with Hoechst. Images were obtained using a confocal laser scanning microscope. Co-localization is indicated by white arrows on merged images from the right column. Scale bars represent 20 μm.

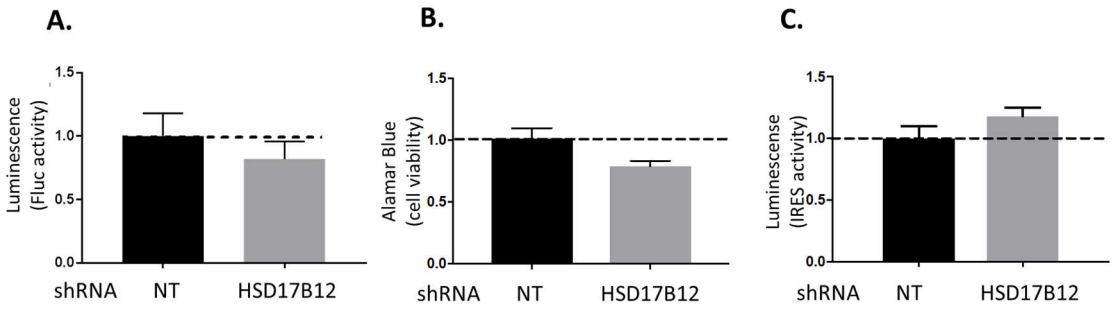

Figure S2. HSD17B12 KD has no effect on protein translation, cell survival and HCV IRES-mediated translation. Huh7 cells transduced with lentiviruses expressing shNT (NT) and shHSD17B12 (HSD17B12) were analyzed (A) for expression of a luciferase gene under an EF1-alpha promoter to assess general protein translation (B) for cell survival using an Alamar Blue assay and (C) for expression of HCV IRES-driven firefly luciferase normalized to CMV-driven renilla luciferase as a cap-dependent translation control. Error bars represent standard deviations from two experiments. No major differences were observed in all conditions.

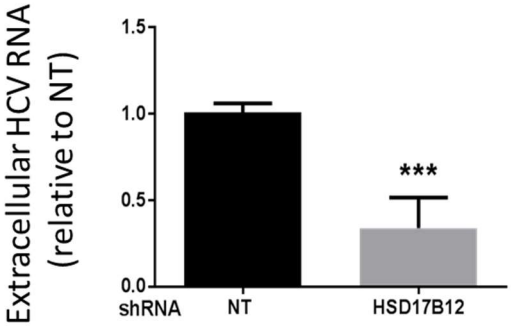

Figure S3. HSD17B12 KD decreases extracellular HCV RNA levels of HepG2 infected cells. HepG2 cells transduced with lentiviruses expressing shNT (NT) or shHSD17B12 (HSD17B12) were transfected with the JFH-1-expressing DNA plasmid for 4 days. Cell supernatants were analyzed for the extracellular HCV RNA levels using qRT-PCR and arbitrarily set to 1 for cells transduced with lentivirus expressing shNT. Error bars represent standard deviations from 3 biological replicates. P values < 0.001 (***) are indicated in comparison with shRNA NT treatment.

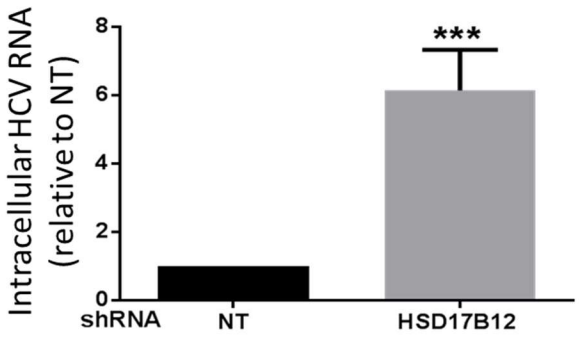

Figure S4. HSD17B12 KD increases intracellular HCV RNA levels of HepG2-infected cells. HepG2 cells transduced with lentiviruses expressing shNT (NT) and shHSD17B12 (HSD17B12) were transfected with the JFH-1-expressing DNA plasmid for 4 days. Cell extracts were then analyzed for the intracellular HCV RNA levels using qRT-PCR, normalized with actin RNA content and arbitrarily set to 1 for cells transduced with lentivirus expressing shNT. Error bars represent standard deviations from 3 biological replicates. P values < 0.001 (***) are indicated in comparison with shRNA NT treatment.

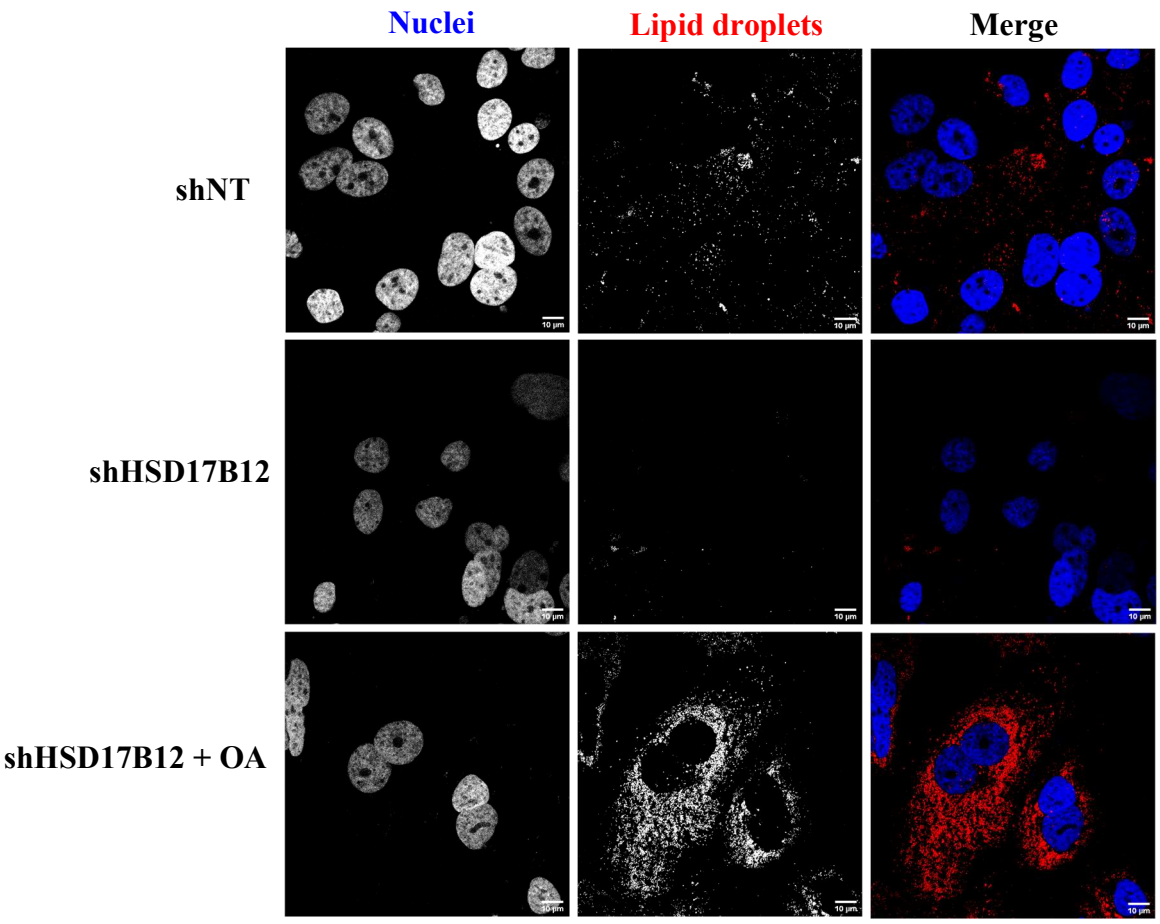

Figure S5. Oleic acid preparation rescues lipid droplets in HSD17B12 KD Huh7.5 cells. Three Huh7.5 cells conditions were prepared. A control condition transduced with shNT, and a test condition transduced with shHSD17B12, in addition to another test condition transduced with shHSD17B12 and treated with oleic acid preparation (50 µM). Five days post-transduction and 4 day-treatment with oleic acid, cells were stained with Lipidtox (LDs) and DAPI (nuclei) and were examined by fluorescence microscope for lipid droplets abundance. Scale bar = 10 µm.

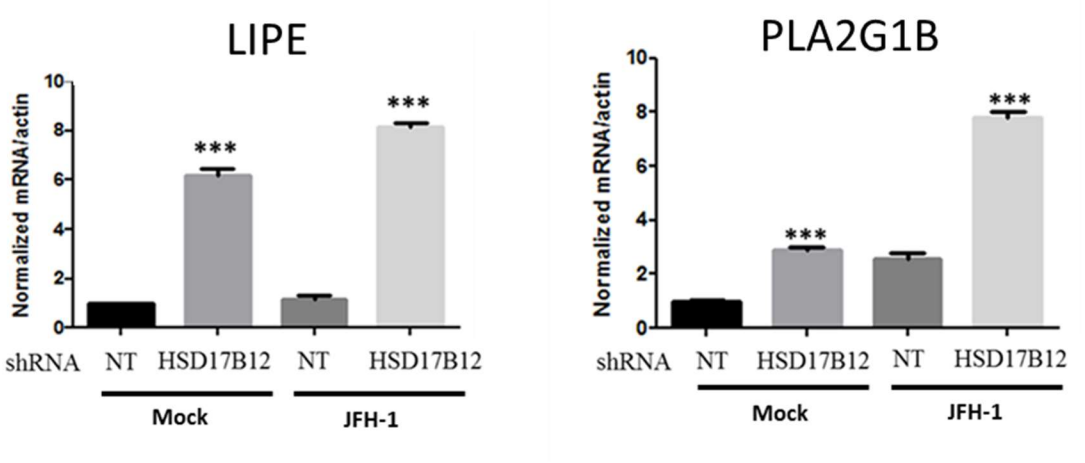

Figure S6. HSD17B12 KD increases expression of lipolysis genes. Huh7.5 cells transduced with lentiviruses expressing shNT (NT) or shHSD17B12 (HSD17B12), either uninfected (Mock) or transfected with JFH-1 DNA for four days are analyzed for mRNA levels of hormone sensitive lipase (LIPE) and phospholipase A2 (PLA2G1B) by RT-qPCR. Levels are normalized with actin RNA content and arbitrarily set to 1 for Mock NT cells. Error bars represent standard deviations from 3 biological replicates. P values < 0.001 (***) are indicated in comparison with shRNA NT treatment.

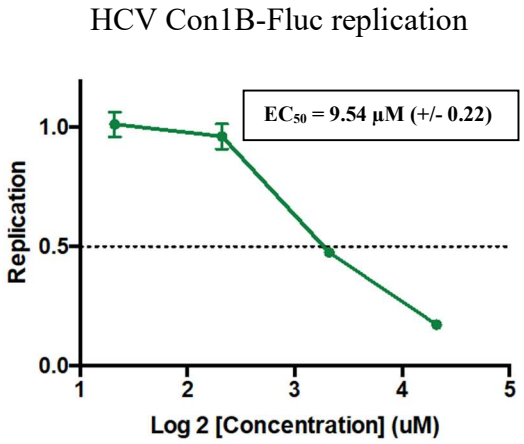

Figure S7. HSD17B12 inhibitor INH-12 decreases HCV subgenomic replication. The antiviral effects of treatment with various concentrations of small-molecule HSD17B12 inhibitor INH-12 were determined on HCV Con1b replicon-containing Huh7 cells using luciferase assays. INH-12 mean inhibitory concentration EC50 (+/- SEM) is indicated.