Supporting Information

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SI Materials and Methods

Cell Cultures and Virus Stocks. The human hepatoma cell lines Huh-7.5 and Huh-7.5.1 were cultured as described (1). Deidentified human fetal livers (16–24 wks gestation) were procured through Advanced Bioscience Resources or the Human Fetal Tissue Repository of The Albert Einstein College of Medicine. Human fetal liver cells (HFLCs) were isolated and cultured in hepatocyte-defined medium (HDM; BD Biosciences) as described (2). All protocols were reviewed and approved by the Rockefeller University Institutional Review Board. Cell culture–derived hepatitis C virus (HCVcc) stocks were produced by electroporation of hepatoma cells and titrated by limiting dilution as described (1). To generate primary cell-derived HCV stocks, HFLCs were plated at $10^5/cm^2$ in 24-well plates and transduced with lentiviral pseudoparticles encoding the tagRFP-nls-IPS fusion protein. This is a live-cell reporter system capable of monitoring HCV infection in real time. It is based on the knowledge that the HCV protease NS3-4A cleaves the host protein IPS. The tagRFP-nls-IPS construct encodes a red fluorescent protein (RFP) fused to IPS such that upon HCV infection, cleavage of IPS results in the exposure of a nuclear localization sequence (nls) and re-localization of the RFP from the mitochondria to the nucleus (3). After 5–6 d of culture, cells were pretreated overnight with 1 μM of the tank binding kinase-1 (TBK-1) inhibitor, BX795 (Invivogen), then transfected with HCV genomic RNA by incubation for 2 h with 300 μL Opti-MEM (Invitrogen) containing 20 μL/mL DMRIE-C transfection reagent (Invitrogen) and 1 μg/mL HCV RNA. The cells were washed once and maintained in HDM containing 1 μM BX795. Supernatants were harvested and pooled every 2–3 d, concentrated ∼10-fold over Amicon-Ultra centrifugal units (100-kDa MWCO; Millipore) and stored at −80 °C.

Generation of Tagged HCV. The HCV genome Clone 2 has been previously described and is referred to in this study as WT HCV (4). A tagged Clone 2 derivative encoding a duplication of amino acids 384 and 385 of the HCV polyprotein, a 6-histidine repeat (6xHis), and a One-STrep-tag (OST), consisting of two copies of the streptavidin tag II (Strep II) [AG**WSHPQFEK**$(GGGS)_2$GGG-**WSHPQFEK**GA; Strep II residues in bold] was generated by overlapping PCR using standard procedures. Plasmid and primer sequences are available upon request. The resulting plasmid, Clone 2-E2/6xHis/OST, was confirmed by enzymatic digestion and sequencing and is referred to in this study as tag-HCV.

Virus Capture Assay. For coupling of antibodies to Bio-Adembeads Protein G (Ademtech), 20 μL of resin was incubated overnight at 4 °C with 2 μg of α-HCV (AR4A), α-His, α-HIV (B6), or α-apoE (Academy Biomedical) antibodies in 500 μL of PBS. Bead-antibody complexes were washed three times with PBS and incubated with 500 μL of HCV-containing supernatant (2×10^7 viral genome copies) for 1 h at room temperature. Captured virions were pelleted for 1 min on a magnet and washed six times with 1 mL PBS. Virion-coated beads were applied to copper EM grids with a continuous carbon film (EMS) and subjected to negative staining or lysed in RNA lysis buffer. HCV RNA was extracted and quantified by RT-quantitative PCR as described (2).

Virion Pull-Down and Immunoblot Analysis. Fractionated WT- and tag-HCV samples were dialyzed to PBS and incubated with Dynabeads His-tag (Invitrogen) or MagStrep type 2HC beads (IBA) for 1 h at room temperature in the presence of protease inhibitors. For His-tag pull-downs, 20 mM imidazole was added during the binding step. Beads were washed six times with PBS and heated at 70 °C for 10 min in 1× SDS loading buffer or bound virions were eluted with 0.9 M imidazole. To prepare cell lysates, 72 h post-electroporation (post-ep), Huh-7.5.1 cells were lysed [50 mM Tris·HCl (pH 8.0), 150 mM NaCl, 1% (vol/vol) Triton X-100, and Mini EDTA-free Protease Inhibitor Mixture (Roche)] for 30 min at 4 °C and lysates were clarified by centrifugation at 16,000 × *g* for 10 min. A total of 30 μg of protein lysate or bead-eluted virions was separated on 4–12% Bis/Tris NuPage polyacrylamide gels (Invitrogen) and transferred to PVDF membranes (Millipore). Blots were probed with primary antibodies against HCV E2 and core (1:500; Austral Biologicals), 6xHis (1:500; Qiagen), Strep-tag (1:500; IBA), apoE and apoA-I (1:1,000; Academy Biomedical), apoB100 (1:1,000; Calbiochem), or β-actin (1:10,000; Sigma) followed by HRP-conjugated secondary antibodies (1:10,000; Jackson Immuno Research) and developed using Super Signal West Pico substrate (Thermo Scientific).

Negative Staining and Immunogold Labeling. To capture WT-HCV particles, Protein A BioGrids (copper, 400 mesh; Dune Sciences, Inc.) were preincubated with an HCV-specific human IgG (AR4A; 1 μg) according to the manufacturer's instructions. An HIV-specific human IgG (B6) (5) was used as a negative control. A total of 50 μL of 4% (vol/vol) paraformaldehyde (PFA)-fixed HCV-containing media [in 1.5% (vol/vol) FBS; titers ranging from 0.8 to 6×10^6 50% tissue culture infectious dose ($TCID_{50}$)/mL] were incubated on grids for 30 min. To capture tag-HCV particles, affinity grids were prepared as previously described, using grids with a continuous carbon film (copper, 400 mesh; Electron Microscopy Sciences), chloroform reconstituted 1,2-Dilauryl-sn-glycero-3-phosphatidylcholine, and 1,2-dioleoyl-sn-glycero-3-[N(5-amino-1-carboxypentyl) iminodi acetic acid] succinyl-nickel salt (Ni-NTA lipid; Avanti Polar lipids) (6). Two percent (vol/vol) Ni-NTA affinity grids were used for transmission EM (TEM) studies and floated onto 25–50 μL of PFA-fixed tag-HCV containing 20 mM imidazole for 15–30 min at room temperature. For immunogold labeling, grids were blocked for 10 min in 3% (wt/vol) BSA-PBS, pH7.4, incubated for 20 min with the following primary antibodies diluted in blocking buffer: α-E2 (1:15; 3/11), α-apoE (1:5; Academy Biomedical), α-apoA-I (1:45; Academy Biomedical), α-apoB (1:5; Calbiochem), α-His (1:20; Qiagen), and α-Strep-tag (1:20; IBA), then with secondary antibodies conjugated to colloidal gold (1:20; Jackson Immuno Research) for 20 min. Grids were negatively stained with 2% (vol/vol) uranyl acetate (EMS) and examined in a 100CX JEOL transmission electron microscope (JEOL TEM) with digital imaging system (XR41-C, Advantage Microscopy Technology Corp.) or in a TECNAI Bio-twin spirit G2 (FEI) with a built-in Gatan UltraScan 4000 camera (Gatan Inc.). Electron micrographs were analyzed with Digital Micrograph or Advantage Microscopy Technology software.

1. Lindenbach BD, et al. (2005) Complete replication of hepatitis C virus in cell culture. *Science* 309(5734):623–626.
2. Andrus L, et al. (2011) Expression of paramyxovirus V proteins promotes replication and spread of hepatitis C virus in cultures of primary human fetal liver cells. *Hepatology* 54 (6):1901–1912.
3. Jones CT, et al. (2010) Real-time imaging of hepatitis C virus infection using a fluorescent cell-based reporter system. *Nat Biotechnol* 28(2):167–171.
4. Walters KA, et al. (2009) Genomic analysis reveals a potential role for cell cycle perturbation in HCV-mediated apoptosis of cultured hepatocytes. *PLoS Pathog* 5(1): e1000269.

5. Giang E, et al. (2012) Human broadly neutralizing antibodies to the envelope glycoprotein complex of hepatitis C virus. *Proc Natl Acad Sci USA* 109(16):6205–6210.
6. Kelly DF, Abeyrathne PD, Dukovski D, Walz T (2008) The Affinity Grid: A pre-fabricated EM grid for monolayer purification. *J Mol Biol* 382(2):423–433.

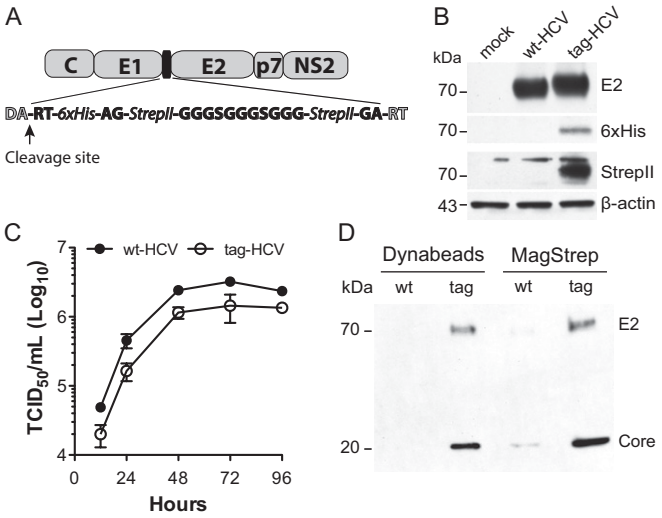


Fig. S1. Functional characterization of tag-HCV. (*A*) Schematic representation of the region of the HCV polyprotein containing the tag with the amino acid sequence of the insertion shown in black. At the N terminus of envelope glycoprotein E2, a duplication of residues 384–385 of E2, *6xHis*, and an OST, which contains two copies of the streptavidin tag II (StrepII; peptide sequence: WSHPQFEK) separated by spacers and linker have been inserted. The protease cleavage site at the E1/E2 junction is indicated (arrow). (*B*) Immunoblot for E2, 6xHis, and StrepII tags and β-actin expression in Huh-7.5.1 cells electroporated with no RNA (mock), WT-HCV, or tag-HCV RNA. Cells were harvested 72 h post-ep and 30 μg total cell lysate was loaded per lane. (*C*) Kinetics of virus production in Huh-7.5.1 cells. The amount of infectious virus released in the supernatant ($TCID_{50}$) was measured at the indicated time points post-ep by limiting dilution assay. Means and SD from three independent experiments are plotted. (*D*) Immunoblot analysis for E2 and core of affinity-purified virions. WT- and tag-HCV extracellular virions were precipitated using magnetic beads directed against either 6xHis (Dynabeads) or StrepII (MagStrep) tags, and eluted proteins were loaded onto SDS/PAGE gel.

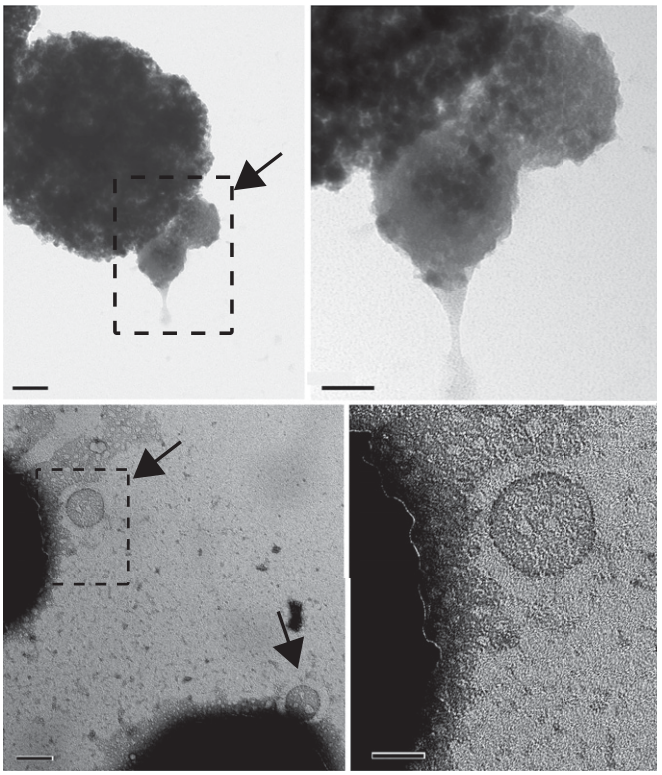


Fig. S2. Images of negatively stained HCV virions bound to Dynabeads. (*Left*) Bead-bound HCV particles are indicated by arrows and (*Right*) the framed boxes correspond to the enlarged images. (Scale bars: *Left*, 50 nm; *Right*, 20 nm.)

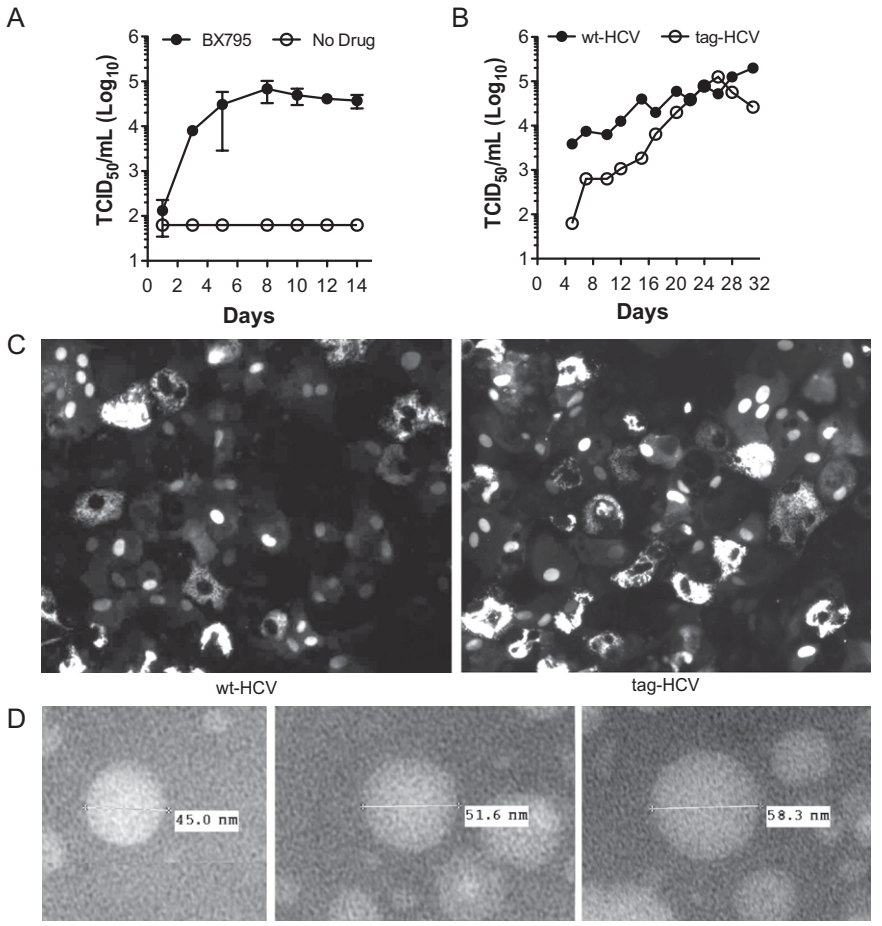


Fig. S3. Impact of TBK-1 inhibitor on HCV titers in HFLC. (*A*) Infectious virus production by HFLC transfected with WT-HCV RNA in the presence or absence of TBK-1 inhibitor (BX795). Cells were pretreated with 1 μM BX795, transfected as described in *SI Materials and Methods* and maintained in the continued presence of the drug. Culture medium was replaced every 2–3 d. Infectious virus in supernatants was assessed at the indicated time points (days) post-transfection by titration on Huh-7.5 cells. (*B*) Infectious virus production by HFLC transfected with WT- or tag-HCV RNA in the presence of BX795. (*C*) Representative images for HFLC transduced with lentiviral vector encoding tagRFP-nls-IPS at day 26 posttransfection with WT- or tag-HCV. RFP nuclear translocation is a signature of productive infection. (*D*) TEM images of WT-HCV virions from HFLC supernatant harvested at day 26 posttransfection, concentrated 10-fold, and captured using protein A EM grids coated with α-HCV antibody.

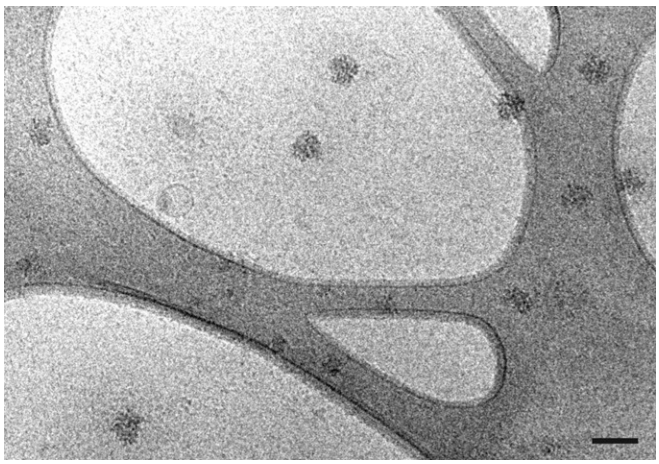
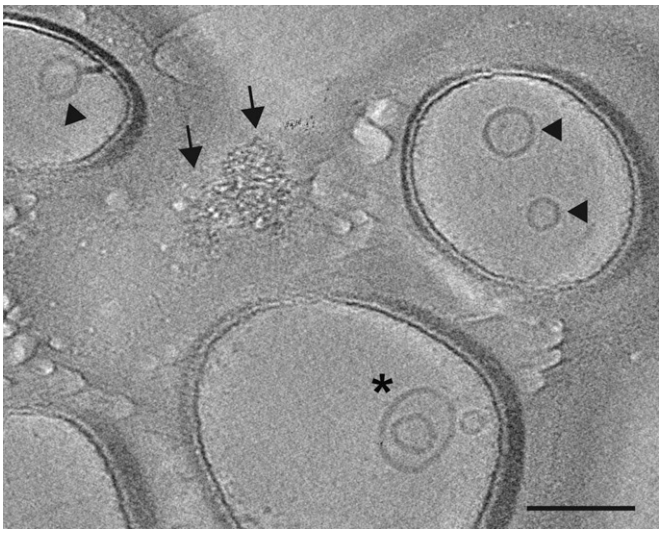
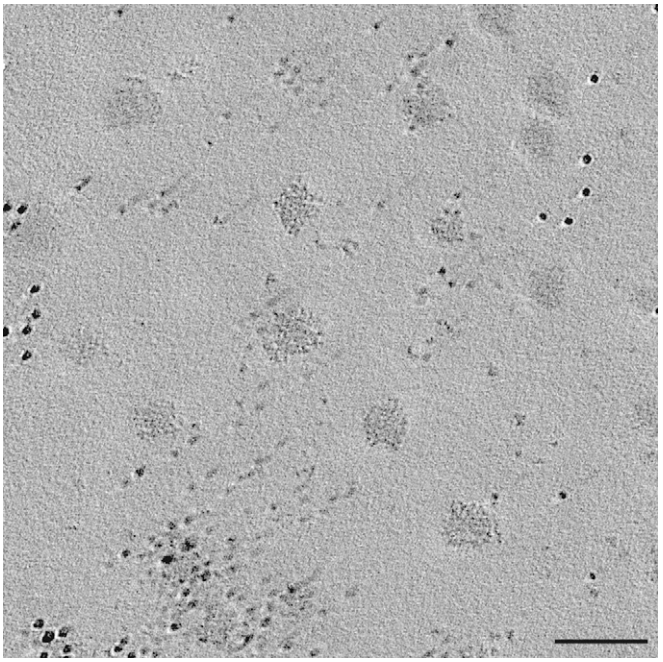


Fig. S4. Low magnification cryo-EM image of tag-HCV virions from Huh-7.5.1 cultures [in 1.5% (vol/vol) FBS] purified on 20% (vol/vol) Ni-NTA affinity grids. (Scale bar: 100 nm.)



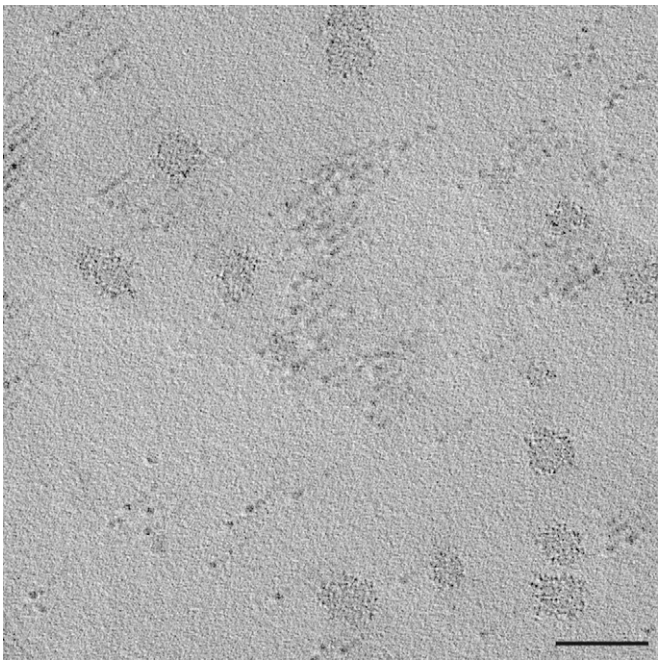
Movie S1. Cryo-ET of HCV-containing supernatant showing HCV virions on the carbon film and exosome-like structures inside the holes.

Movie S1



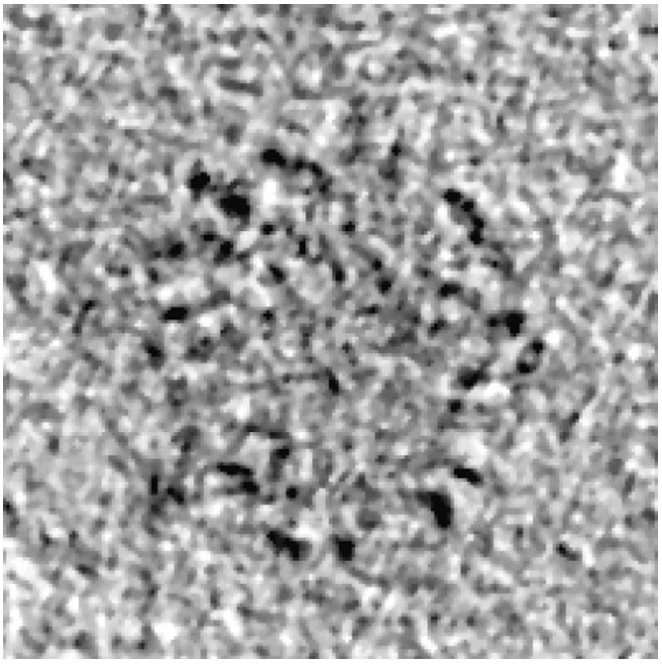
Movie S2. Cryo-ET of purified, highly infectious HCV. Animations through sequential *z*-slices of a reconstructed tomogram show the general morphology of HCV virions.

Movie S2



Movie S3. Reconstructed tomogram of an additional field containing purified HCV particles.

Movie S3



Movie S4. Reconstructed tomograms of a particle with visible striations in the viral envelope.

Movie S4