Supporting Information

Ploss et al. 10.1073/pnas.0915130107

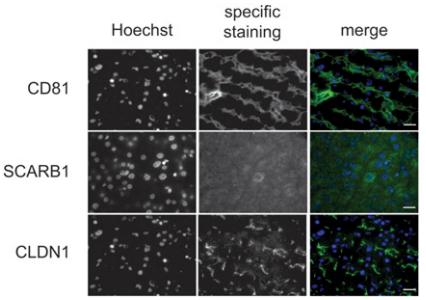

Fig. S1. HCV entry factor staining in normal human liver. Wide-field fluorescence images of fixed sections of human liver from normal uninfected donors stained nuclei (blue in merged image) and antigen-specific staining (green in merged image) for CD81 (*Upper*), SCARB1 (*Middle*) and CLDN1 (*Lower*). Scale bars: 30 μm.

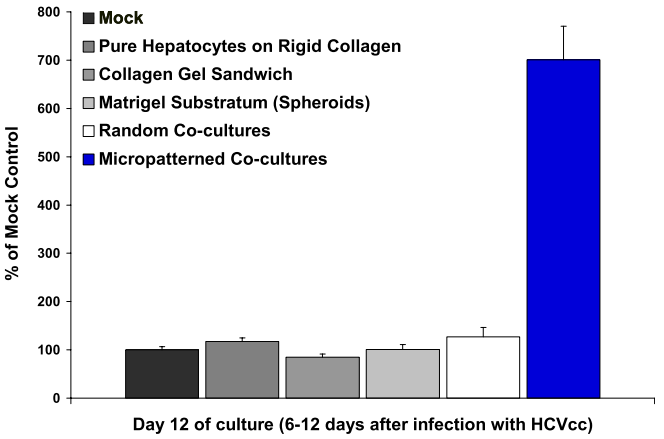

Fig. S2. Primary hepatocytes in MPCCs maintain HCVcc infection over longer periods of time than conventional hepatocyte systems. Conventional pure hepatocyte cultures, widely used in the pharmaceutical industry, and MPCCs were created from the same donors. Conventional cultures were infected with HCVcc within 24 h of plating, whereas MPCCs were infected once they achieved functional stability (6 days after plating). Luciferase activity in supernatants was monitored over 2 weeks postinfection. One representative time point (6-12 days postinfection) is shown. Luciferase activity is expressed as percent of mock control.

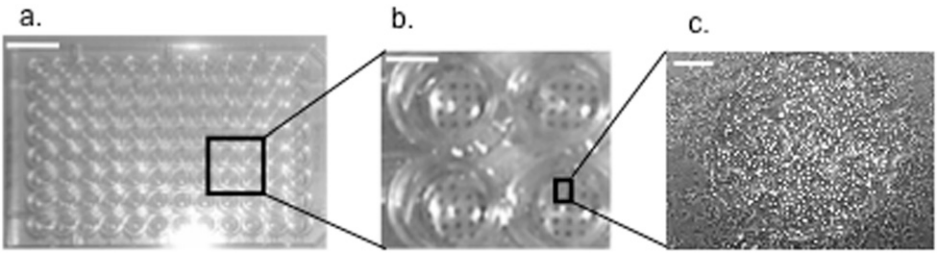

Fig. S3. Miniaturized 96-well primary hepatocyte MPCCs. (*A*) MPCCs were created in off-the-shelf tissue culture polystyrene plates in formats up to 96-well plates using soft lithographic techniques. (*B*) Each well of a 96-well plate contains 14–15 islands of hepatocytes that are 500 μm in diameter and spaced 1200 μm apart (center-to-center), and (*C*) surrounded by 3T3-J2 murine embryonic fibroblasts to create MPCCs. Scale bars: 2 cm (a), 4 mm (b), 100 μm (c).

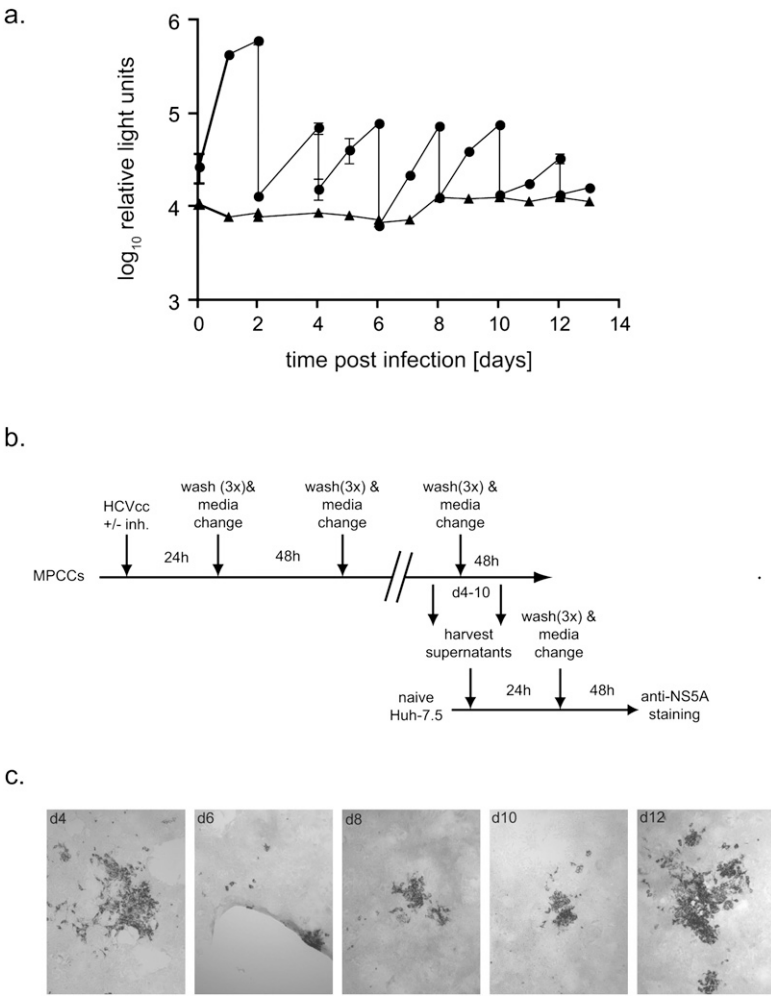

Fig. S4. Primary human hepatocytes in MPCCs produce infectious virus. (*A*) HCVcc infection kinetics in primary hepatocyte MPCCs. Primary hepatocytes in MPCCs were infected with Jc1FLAG2(p7-nsGluc2A) (circles) or mock infected (triangles). After 24 h, virus was removed and MPCC medium added; samples were taken daily and media replaced with washing three times every 48 h. (*B*) Schematic of the experimental set up. Supernatants collected pre- and postwash at days 4, 6, 8, 10, and 12 following infection were used to infect naïve Huh-7.5 cells. Twenty-four hours postinfection, media were replaced and NS5A staining was performed 72 h postinfection to visualize HCV infection. (*C*) HCV infection of Huh-7.5 cells was visualized by immunohistochemical staining for NS5A. Days indicate the time points when supernatants were taken from the infected MPCC cultures.