Supporting Information

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SI Materials and Methods

Gene Knockdown with siRNA. For knockdown of the sodium-taurocholate cotransporting polypeptide (NTCP) SLC10A1, the cells were sequentially transfected with human NTCP-specific siRNA #1 5′-CACAAGUGCUGUAGAAUUAdTdT-3 and/or siRNA #2 5′-GGACAAGGUGCCCUAUAAAdTdT-3′ using TransMessenger Transfection Reagent (Qiagen) according to the manufacturer's protocol. The control siRNA 5′-UUCUCCGAA-CGUGUCACGUdTdT-3′ has a scramble sequence with no known mammalian target sequence.

Primers for Human Hepatocyte-Specific Markers and Hepatitis C Virus Receptors. The following primers were used for human hepatocyte-specific markers and hepatitis C virus (HCV) receptors: albumin: 5′-ACTATCTATCCGTGGTCCTGA-3′ and 5′-TCT-TGATTTGTCTCTCCTTCT-3′; α-1 antitrypsin (AAT): 5′-GT-GCCTATGATGAAGCGTTT-3′ and 5′-AGACCTTAGTGAT-GCCCAGTT-3′; HNF4: 5′-GGCCAAGTACATCCCAGCTT-3′ and 5′-TCATTGCCTAGGAGCAGCAC-3′; cytochrome P450 3A4 CYP3A4: 5′-TCCATTCCTCATCCCAATTCTTGA-3′ and 5′-TCCACTCGGTGCTTTTGTGT-3′; human CD81: 5′-AC-CTCCTGTATCTGGAGCTGG-3′ and 5′-TTGGCGATCTGG-TCCTTGTTG-3′; scavenger receptor class B type I (SR-BI): 5′-TCGCAGGCATTGGACAAACT-3′ and 5′-CTCCTTATCC-TTTGAGCCCTTTT-3′; claudin-1 (CLDN1): 5′-GTGGAGGA-TTTACT CCTATGCCG-3′ and 5′-ATCAAGGCACGGGT-TGCTT-3′; occludin: 5′-TCAAA CCGAATCATTATGCAC-CA-3′ and 5′-AGATGGCAATGCACATCACAA-3′; apolipoprotein E (ApoE): 5′-CGCTTTTGGGATTACCTGCG-3′ and 5′-GGGGTCAGTTGTTCCTCCAG-3′.

Lamivudine Treatment of Immunodeficient Mice Inoculated with Hepatitus B Virus-Infected HLCZ01 Cells. All experiments using mice were reviewed and approved by the Institutional Animal Care and Use Committee of Hunan Provincial Tumor Hospital. Hepatitis B virus (HBV)-infected HLCZ01 cells (5×10^6) in 200 μL of PBS were injected s.c. into the lower right flank of 6-wk-old male NOD/SCID immunodeficient mice (Vital River). Beginning 20 d after cell inoculation (dpi), five mice were treated daily with lamivudine by gavage, and five mice were used as control. Sera were collected at the indicated time points, and HBV DNA was extracted using the QIAamp MiniElute Virus Spin Kit (Qiagen) according to the manufacturer's instructions. HBV DNA in the sera was analyzed by real-time PCR.

Western Blot Analysis. Protein was standardized using Lowry Protein Assay (Bio-Rad). Mouse monoclonal anti-AAT and anti-albumin (AL-01) antibodies were from Santa Cruz Biotechnology. Rabbit anti-NTCP antibody (EPR8606) and mouse monoclonal anti-HCV core antibody (HCcAg) (B2) were obtained from Abcam. Goat anti-mouse and rabbit HRP-conjugated secondary antibodies were purchased from Invitrogen.

Flow Cytometry. The cells recovered after trypsinization were resuspended in an appropriate volume of Flow Cytometry Staining Buffer (eBioscience) so that the final cell concentration was 2×10^7/mL The cells were fixed by the addition of 100 μL of IC Fixation Buffer (eBioscience) to 100 μL of the cell suspension described above and incubation for 30 min, followed by the addition of 2 mL of 1× Permeabilization Buffer (eBioscience) for permeabilization. After one washing with 2 mL of 1× Permeabilization Buffer, the cells were stained successively with primary antibody and fluorophore-conjugated secondary antibody (Invitrogen). After washing, the cells were resuspended with 0.5 mL of Flow Cytometry Staining Buffer and were analyzed on a FACS Caliber Cytometer (BD Pharmingen). The data were analyzed with CellQuest software.

Immunoelectron Microscopy. HBV-infected HLCZ01 cells were centrifuged, and cell pellets were fixed in 0.25% glutaraldehyde/4% paraformaldehyde/0.1 M sucrose buffered with 0.1 M sodium phosphate (pH 6.8) for 30 min at room temperature. After rinsing in 0.1 M sodium phosphate containing 0.1 M sucrose, the samples were dehydrated in a graded ethanol series and then were embedded in Epon 812 (SPI-CHEM). Semithin sections were made to identify areas of interest, and ultrathin sections were cut with diamond knife on a Leica Ultracut UCT. For immunogold localization, grids were floated (section side down) on drops of the indicated solutions at room temperature in the following order: (*i*) blocking in Tris-buffered saline (pH 8.0), 0.05% Tween (TBST) containing 1% fish gelatin (FG) for 20 min; (*ii*) 3-h incubation with mouse monoclonal anti-HBV surface antigen (HBsAg) antibody (1:50 dilution in TBST, 1% FG); (*iii*) washing three times in 5× TBST, 1% FG and in 1× TBST, 1% FG; (*iv*) 1-h incubation with goat anti-mouse IgG coupled to 10-nm gold particles (1:100 dilution in TBST, 1% FG) (Abcam); (*v*) washing three times in 1× TBST, 1% FG for 10 min in fixative buffer and for 10 min in distilled water. The samples were examined on an FEI Tecnai G2 12 transmission electron microscope (FEI). The supernatants were diluted 1:10, and 3 μL of each diluted sample was put on a formvar-coated 200-mesh nickel grid. The samples were semidried at room temperature for 15 min. A drop of TBS-BSA placed on parafilm was floated on the grid for 5 min in a moist chamber. Then a drop of TBS containing 3% gelatin was floated on the grid for 30 min. The samples were incubated with mouse monoclonal anti-HBsAg antibody solution (diluted 1: 50 in TBS-BSA) at room temperature for 60 min, followed by washing three times with TBS-BSA. Then the samples were incubated for 60 min with 10 nm gold-conjugated goat anti-mouse IgG solution (diluted in 1:100 in TBS-BSA) and washed three times with TBS-BSA. The samples were removed from the moist chamber, stained with uranyl acetate and lead citrate, and used for electron microscopy observation as described above.

Titration of Infectious HBV. The protocol for titration of infectious HBV was modified from published literature. Briefly, cell supernatants were serially diluted 10-fold in complete culture medium and used to infect 10^4 naive HLCZ01 cells per well in 96-well plates. The inocula were incubated with cells for 1 h at 37 °C and then were supplemented with fresh medium. The level of HBV infection was determined 5 d postinfection by immunofluorescence staining for HBsAg. The viral titer is expressed as focus-forming units per milliliter of supernatant (FFU/mL), determined by the average number of HBsAg-positive foci detected at the highest dilutions.

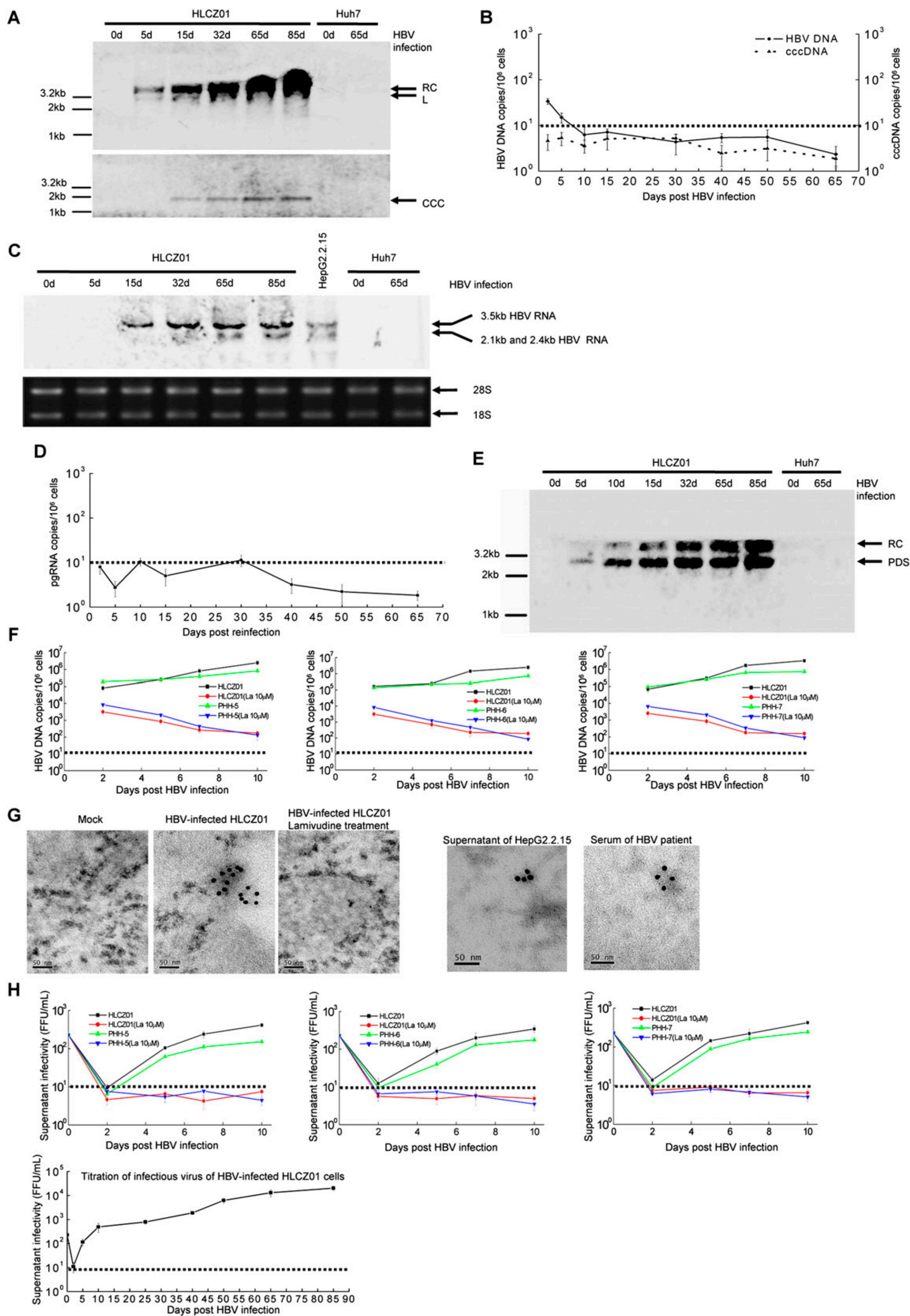

Fig. S1. Confirmation of HBV infection in HLCZ01 cells by Southern and Northern blot analysis. (*A*) Intracellular viral DNA and covalent closed circular DNA (cccDNA) measured by Southern blot in HLCZ01 cells inoculated with the supernatant of HepG2.2.15 cells. The supernatant from HepG2.2.15 cells was filtered and used to inoculate HLCZ01 cells. Total cellular DNA was isolated. Southern blotting for HBV DNA and cccDNA was performed. Huh7 cells were used as a negative control. The positions of the relaxed circular form (RC), linear HBV DNA (L), and covalently circular DNA (CCC) are indicated at the right. (*B*) Absence of intracellular viral DNA and cccDNA in Huh7 cells inoculated with the supernatant of HepG2.2.15 cells. The supernatant of HepG2.2.15 cells was filtered and used to inoculate Huh7 cells. The viral DNA or cccDNA measured by real-time PCR is shown as the number of HBV DNA or cccDNA copies per 10^6 cells, respectively.

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(*C*) The kinetics of viral pregenomic RNA determined by Northern blot within HLCZ01 cells inoculated with the supernatant of HepG2.2.15 cells. HLCZ01 cells were treated as described in *A*, and Northern blotting for HBV pregenomic RNA in the cells was performed. The 28S and 18S RNA were used as the loading controls for Northern blot analysis. Huh7 cells were used as a negative control. (*D*) Absence of viral pregenomic RNA within Huh7 cells inoculated with the supernatant of HepG2.2.15 cells. Huh7 cells were treated as described in *B*. HBV pregenomic RNA measured by real-time PCR is shown as number of the copies of HBV pregenomic RNA per 10^6 cells. (*E*) Extracellular viral DNA measured by Southern blotting in the supernatant of HBV-infected HLCZ01 cells. HLCZ01 cells were treated as described in *A*. Southern blotting for HBV DNA in the supernatant was performed. The positions of relaxed circular form (RC) and partially double-stranded (PDS) HBV DNA are indicated at the right. (*F*) Kinetics of intracellular viral DNA in primary human hepatocytes (PHH) and HLCZ01 cells inoculated with the supernatant of HepG2.2.15 cells. HLCZ01 cells and PHH were inoculated with the supernatant from HepG2.2.15 cells in the presence of 10 μM lamivudine. Intracellular HBV DNA measured by real-time PCR is shown as the number of HBV DNA copies per 10^6 cells. (*G*) HLCZ01 cells were inoculated with the supernatant from HepG2.2.15 cells at an MOI of 20 in the presence of 10 μM lamivudine. The cells were immunostained for observation under electron microscopy as described in *SI Materials and Methods*. (*Left*) Mock-treated HLCZ01 cells were used as negative control. (*Right*) The supernatants of HepG2.2.15 cells and sera from patients with hepatitis B were immunostained for observation under electron microscopy. Dense, dark particles are HBsAg-positive particles. (Scale bars, 50 nm.) (*H*) Titration of HBV preparations applied to and released from PHH and HLCZ01 cells inoculated with the supernatant of HepG2.2.15 cells. PHH and HLCZ01 cells were treated as described in *F*. The supernatant of the cells was harvested at the indicated time points and titered by FFU assay on naive HLCZ01 cells as described in *SI Materials and Methods*. Data shown are the mean ± SD from three independent experiments performed in triplicate. Horizontal dashed lines indicate the lower limit of quantification (LLOQ) of the assay.

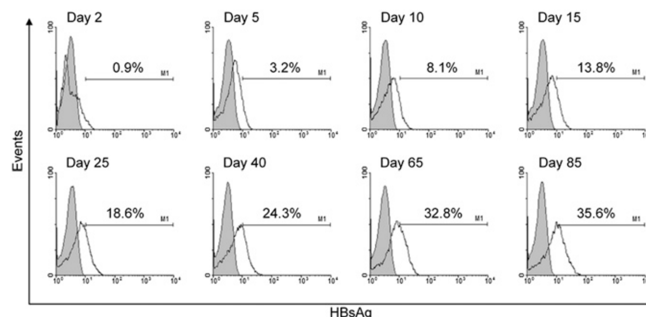

Fig. S2. Detection of HBV-infected HLCZ01 cells by flow cytometry. HLCZ01 cells were inoculated with the supernatant of HepG2.2.15 cells. After overnight exposure, cells were washed three times and were cultured at the indicated time points. The cells were collected for flow cytometry using anti-HBsAg antibody. Naive HLCZ01 cells were used as control (gray curve). The data shown are from one experiment that is representative of three independent experiments.

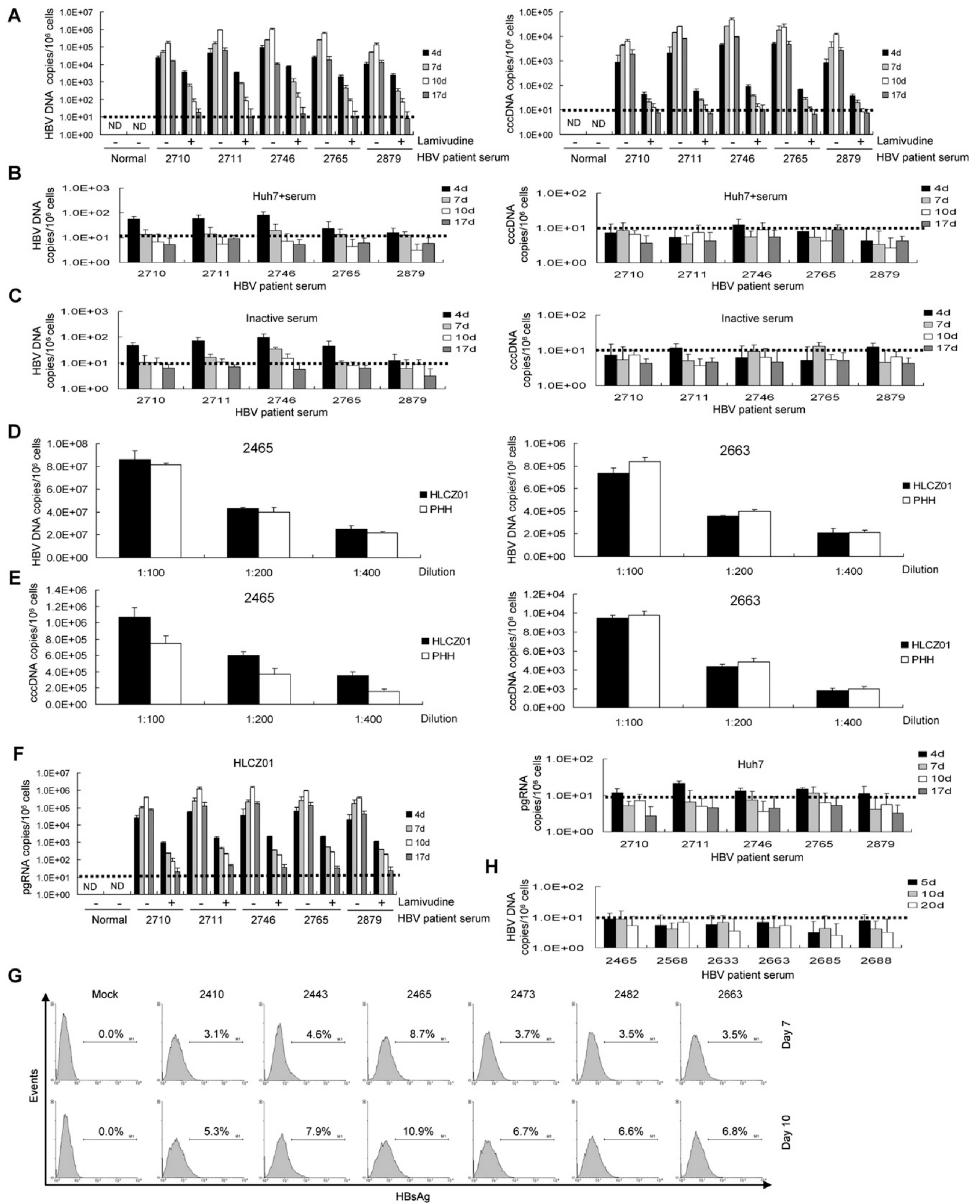

Fig. S3. Propagation of HBV clinical isolates in HLCZ01 cells. (*A* and *B*) HBV clinical isolates do not propagate in Huh7 cells. HLCZ01 (*A*) and Huh7 (*B*) cells were inoculated with sera from HBV-infected donors in the presence of 10 μM lamivudine. Real-time PCR for HBV DNA or cccDNA was performed. Viral DNA replication is shown as the number of HBV DNA or cccDNA copies per 10^6 cells. Normal sera were used as negative controls. ND, not detectable. (*C*) Inactivated HBV clinical isolates do not propagate in HLCZ01 cells. HLCZ01 cells were inoculated with heat-inactivated sera from hepatitis B patients. Real-time PCR for intracellular HBV DNA and cccDNA was performed. Viral DNA or cccDNA replication is shown as the number of HBV or cccDNA copies per 10^6 cells. (*D* and *E*) HBV clinical isolates propagate in HLCZ01 cells in a dose-dependent manner. HLCZ01 cells or PHH were inoculated with series of diluted sera from HBV-infected

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donors #2465 and #2663 in the presence of 10 μM lamivudine. Real-time PCR for intracellular HBV DNA (*D*) and cccDNA (*E*) was performed. Viral DNA or cccDNA replication is shown as the number of HBV or cccDNA copies per 10^6 cells. (*F*) Absence of viral pregenomic RNA within Huh7 cells inoculated with sera from hepatitis B patients. HLCZ01 or Huh7 cells were treated as described in *A*. Total cellular RNA was isolated, and real-time PCR for HBV pregenomic RNA was performed. The viral pregenomic RNA level is shown as the number of HBV pregenomic RNA copies per 10^6 cells. Normal sera were used as a negative control. ND, not detectable. (*G*) HLCZ01 cells were inoculated with sera from HBV-infected donors. The cells were collected for flow cytometry using anti-HBsAg antibody. Data are shown for one experiment that is representative of three independent experiments. (*H*) Viral particles in the supernatant of HLCZ01 cells infected with HBV sera could not be transferred to Huh7 cells. HLCZ01 cells were inoculated with sera from hepatitis B patients. The supernatant of HLCZ01 cells infected with HBV sera was collected and used to inoculate Huh7 cells. Intracellular HBV DNA in Huh7 measured by real-time PCR is shown as the number of HBV copies per 10^6 cells. Horizontal dashed lines indicate the LLOQ of the assay.

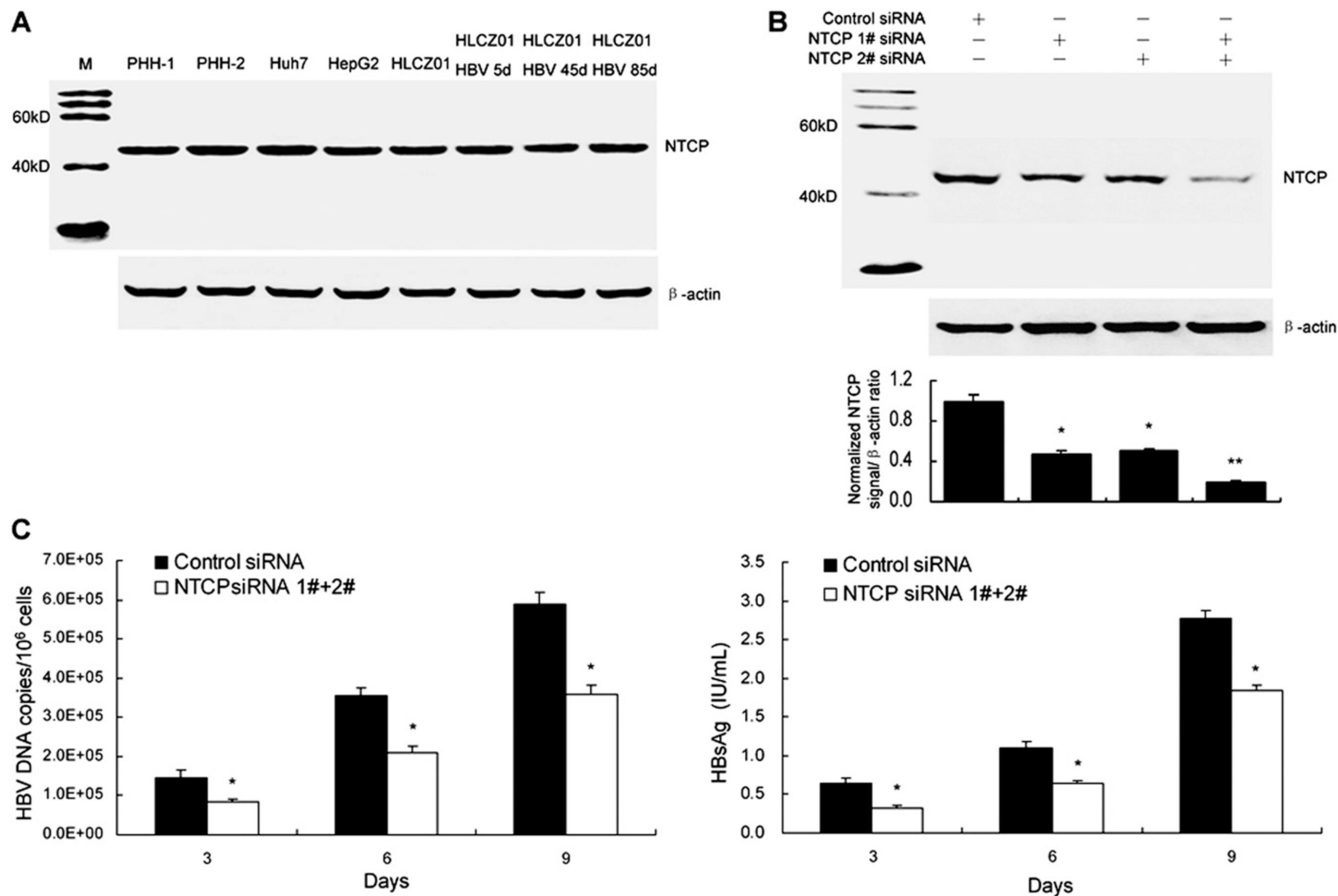

Fig. S4. NTCP is required for HBV entry. (*A*) Expression of NTCP in HLCZ01 cells and PHH. The supernatant of HepG2.2.15 cells was filtered and used to inoculate HLCZ01 cells. After overnight exposure, cells were washed three times and were cultured. Protein was isolated at the indicated times, and NTCP was detected by Western blot analysis. PHH isolated from two cases were labeled as PHH1 and PHH2. Huh7 and HepG2 cells were used as control. (*B*) Confirmation of silencing of NTCP in HLCZ01 cells. SiRNAs targeting two regions of NTCP were delivered into HLCZ01 cells by transfection. NTCP protein was detected with Western blot analysis (*Upper*) and quantified by densitometry in comparison with β-actin (*Lower*). (*C*) Silencing NTCP inhibits HBV infection in HLCZ01 cells. HLCZ01 cells were treated as described in *B*. Then the cells were inoculated with the filter supernatant of HepG2.2.15 cells. Total cellular DNA was isolated, and real-time PCR for HBV DNA was performed. (*Left*) The viral DNA replication is shown as the number of HBV copies per 10^6 cells. (*Right*) HBsAg in the culture supernatant was detected by ELISA. The results are the average of three independent experiments performed in triplicate. $^*P < 0.05$, $^{**}P < 0.01$ versus control.

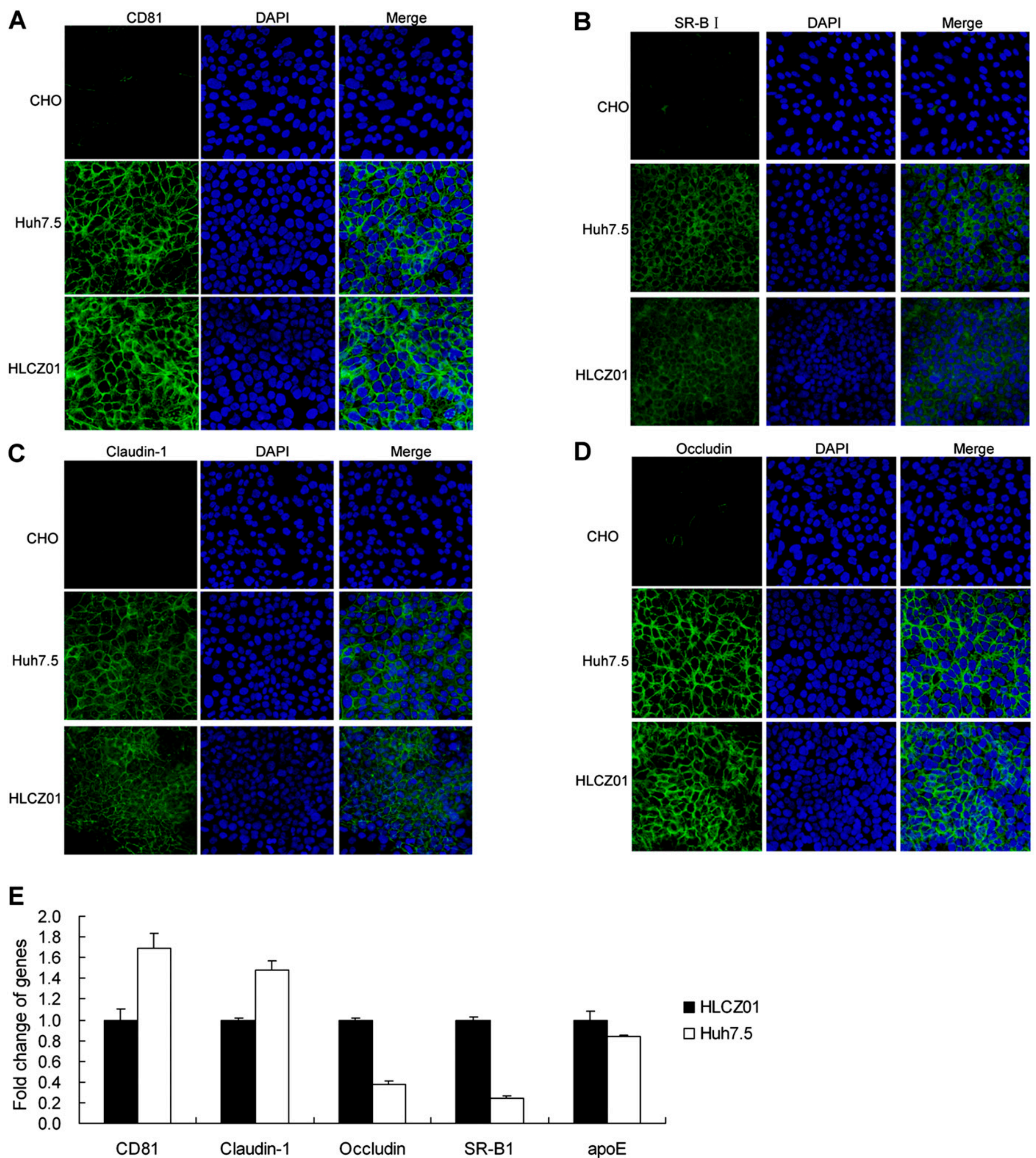

Fig. S5. HLCZ01 cells express HCV receptors. Immunofluorescence of human CD81 (*A*), SR-BI (*B*), CLDN1 (*C*), and occludin (OCLN) (*D*) in HLCZ01 and Huh7.5 cells. HLCZ01 and Huh7.5 cells were harvested for immunostaining using mouse monoclonal anti-human CD81, CLDN1, OCLN, or rabbit anti-human SR-BI antibodies, respectively. DAPI was used to counterstain nuclei. Identical settings were maintained for image capture. (*E*) Expression of CD81, SR-BI, CLDN1, OCLN, and ApoE mRNA in HLCZ01 and Huh7.5 cells. CD81, SR-BI, CLDN1, OCLN, and ApoE mRNA levels in HLCZ01 and Huh7.5 cells were detected by real-time PCR and normalized to GAPDH. The results are the average of three independent experiments performed in triplicate.

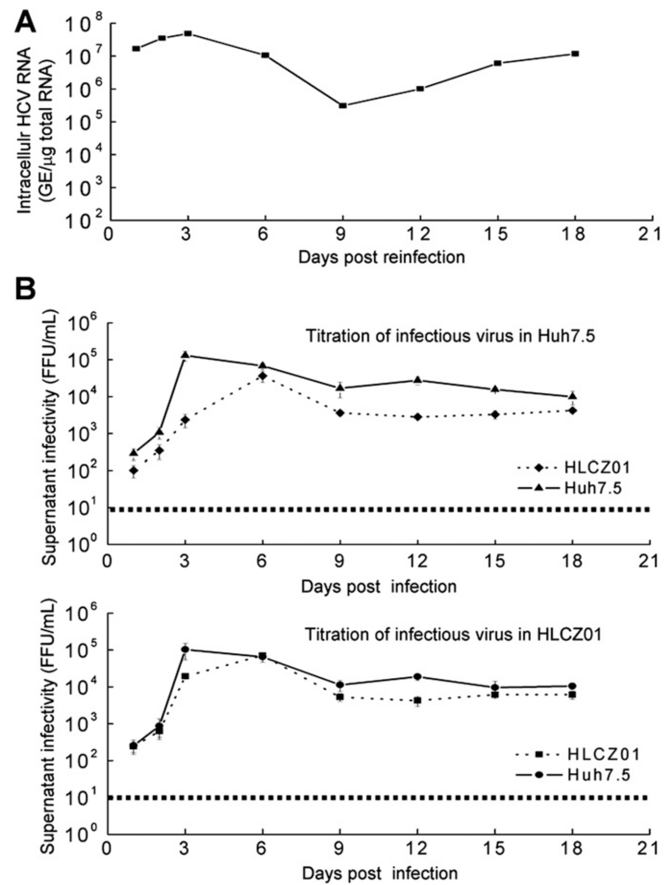

Fig. S6. Titration of infectious virus particles produced in HCV-infected HLCZ01 cells by FFU assay. (*A*) Release of infectious viruses from HCV-infected HLCZ01 cells. HLCZ01 cells were inoculated with the supernatant from HCV-infected HLCZ01 cells. Intracellular HCV RNA determined by real-time PCR analysis is expressed as the HCV RNA genomic equivalence (GE) per microgram of total cellular RNA. (*B*) A JFH1 virus suspension at an MOI of 0.1 was used to infect HLCZ01 and Huh7.5 cells. The supernatants of HLCZ01 and Huh7.5 cells were harvested at the indicated time points and titered by FFU assay on naive Huh7.5 (*Upper*) and HLCZ01 cells (*Lower*). Data shown are mean ± SD from three independent experiments performed in triplicate. Horizontal dashed lined indicate the LLOQ of the assay.

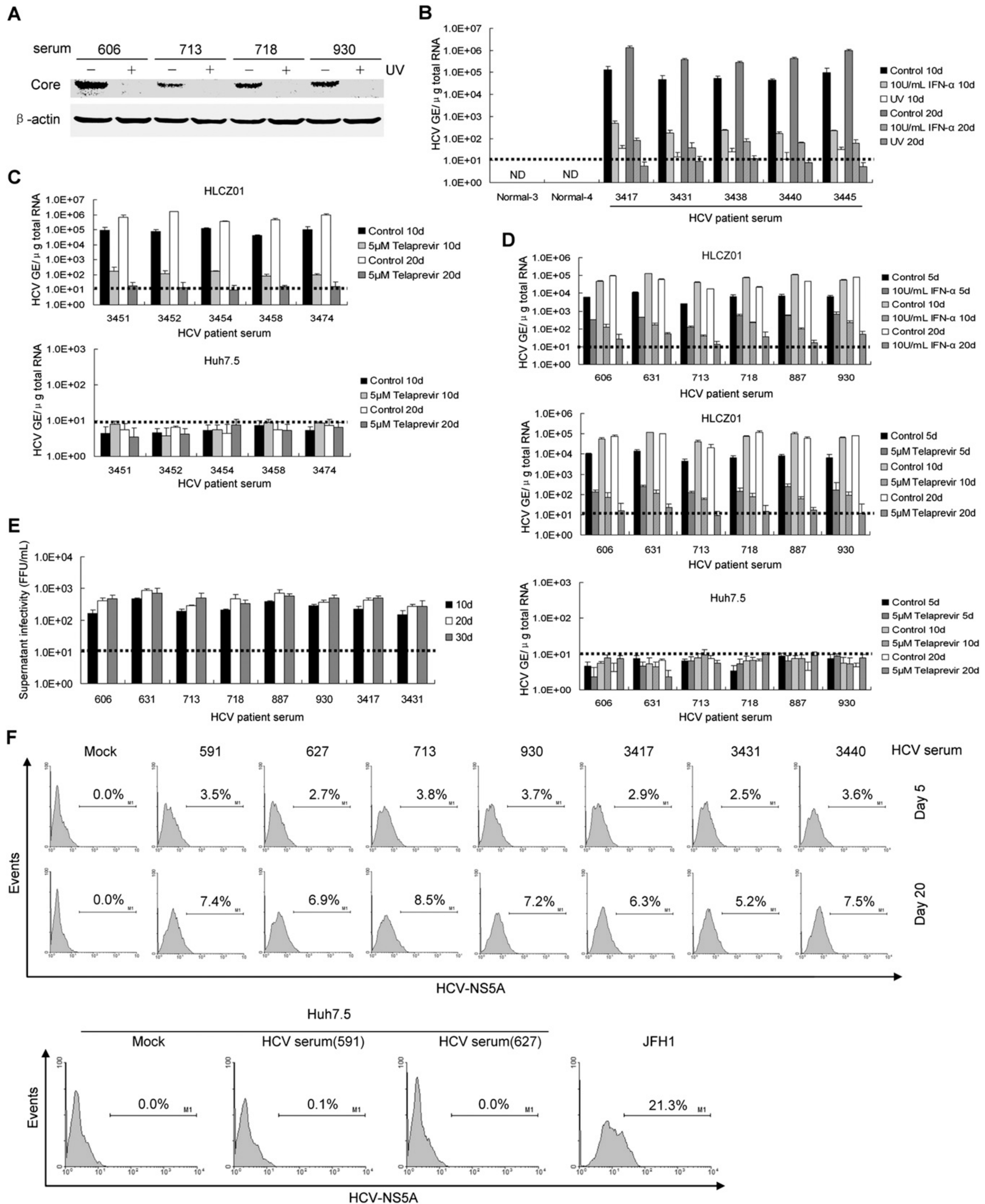

Fig. S7. HCV clinical isolates propagate in HLCZ01 cells. (*A*) HLCZ01 cells were inoculated with the different genotypes of sera from HCV-infected donors. Protein was isolated from the cells at 20 dpi for Western blotting using monoclonal anti-HCcAg. UV-inactivated sera from patients with hepatitis C were used as negative control. (*B*) The sera from HCV-infected donors were inactivated by UV and then were incubated with HLCZ01 cells. After overnight exposure, cells were washed three times and were cultured with or without 10 U/mL IFN-α. Intracellular viral RNA measured by real-time PCR is shown as the HCV RNA GE per microgram of total cellular RNA. (*C*) Telaprevir inhibits viral RNA replication in HLCZ01 cells inoculated with sera from HCV-infected patients. (*Upper*) HLCZ01 cells were inoculated with the different genotypes of sera from HCV-infected donors. After exposure, cells were washed three times and cultured in the

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presence of 5 μM telaprevir. The intracellular viral RNA measured by real-time PCR is shown as the HCV RNA GE per microgram of total cellular RNA. (*Lower*) Huh7.5 cells were used as negative control. (*D*) IFN-α or telaprevir inhibits the passage of sera-derived HCV from sera-inoculated HLCZ01 cells to naive HLCZ01 cells. Different genotypes of sera from patients with hepatitis C were inoculated with HLCZ01 cells. After overnight exposure, cells were washed three times and cultured. The supernatant was harvested and was incubated with naive HLCZ01 cells in the presence of IFN-α (*Top*) or telaprevir (*Middle*). The viral RNA level measured by real-time PCR is shown as the HCV RNA GE per microgram of total cellular RNA. Huh7.5 cells were used as negative control (*Bottom*). (*E*) Quantification of infectious units in HLCZ01 cells infected with HCV sera. HLCZ01 cells were inoculated with sera from HCV-infected donors. The supernatant of the cells was harvested at the indicated time points and titered by FFU assay on naive HLCZ01 cells. Data shown are the mean ± SD from three independent experiments performed in triplicate. (*F*) Detection by flow cytometry of HLCZ01 cells infected by HCV sera. HLCZ01 cells were inoculated with sera from HCV-infected donors. The cells were collected 5 and 20 dpi for flow cytometry analysis using anti-HCV NS5A antibody. Huh7.5 cells inoculated with HCV sera and JFH1-infected Huh7.5 cells were used as negative and positive controls, respectively. Data shown are from one experiment that is representative of three independent experiments. Horizontal dashed lines indicate the LLOQ of the assay.

Fig. S8. Induction of IFN-stimulated genes in HLCZ01 cells by HCV infection. HLCZ01 cells were infected by the JFH1 virus at an MOI of 0.1, and total cellular RNA was isolated. The expression of G1P3 (*A*) and 1-8U mRNA (*B*) was measured by real-time PCR and normalized with GAPDH. The results shown are the average of three independent experiments performed in triplicate. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus control.

Fig. S9. HBV/HCV coinfection in HLCZ01 cells. (*A*) Flow cytometry analysis of HLCZ01 cells coinfected with HBV and HCV. HLCZ01 cells were infected with HBV at an MOI of 20, 50, or 100 for 10 d. Then HBV-infected cells were infected with HCV at an MOI of 0.1, 0.2, or 0.5 for another 5 d. Cells were collected for flow cytometry using anti-HBsAg and anti-HCcAg. The frequencies of HCV-positive cells (*Top*), HBV-positive cells (*Middle*), and HBV/HCV double-positive cells (*Bottom*) are the means of three independent experiments. (*B* and *C*) Effect of telaprevir and lamivudine on simultaneous infection of HBV and HCV in HLCZ01 cells. HLCZ01 cells were infected with HBV at an MOI of 20 and/or HCV at an MOI of 0.1 in the presence of 5 μM telaprevir or 10 μM lamivudine. Intracellular HBV DNA (*B*) and HCV RNA (*C*) were examined by real-time PCR. Horizontal dashed lines indicate the LLOQ of the assay.

Table S1. Clinical characteristics of patients with hepatitis B infection

Patient ID no.	Sex	Viral DNA. copies/mL	Viral genotype	HBsAg	HBeAg
2410	M	6.87×10^7	B	+	−
2411	M	2.36×10^7	B	+	+
2435	M	5.46×10^8	C	+	+
2443	F	4.85×10^8	C	+	+
2444	M	1.8×10^8	B	+	+
2458	M	4.97×10^7	C	+	−
2465	F	6.47×10^9	D	+	+
2473	F	9.82×10^7	C	+	−
2482	M	2.46×10^8	C	+	−
2515	M	1.73×10^8	B	+	+
2568	M	3.52×10^9	D	+	+
2633	M	2.33×10^9	B	+	+
2663	M	1.6×10^8	C	+	+
2685	F	4.98×10^8	C	+	+
2688	M	1.99×10^8	C	+	−
2710	M	1.5×10^7	D	+	+
2711	M	1.62×10^7	B	+	−
2746	M	6.35×10^7	C	+	−
2765	F	8.0×10^7	B	+	−
2879	M	2.87×10^7	C	+	+

Table S2. Clinical characteristics of patients with hepatitis C infection

Patient ID no.	Sex	Viral RNA (copies/mL)	Viral genotype	Core
591	F	2.37×10^7	1b	+
606	F	1.56×10^7	2a	+
627	M	1.59×10^8	1a	+
631	M	3.07×10^7	1b	+
713	M	1.27×10^7	2b	+
718	F	7.19×10^7	3a	+
721	M	3.01×10^8	4a	+
887	M	2.19×10^7	1a	+
893	F	1.93×10^7	2b	+
898	M	1.4×10^7	2a	+
930	F	1.14×10^7	1b	+
987	F	1.45×10^7	6a	+
3417	M	3.12×10^6	1a	+
3431	M	4.5×10^6	2a	+
3438	F	3.18×10^6	1b	+
3440	M	1.84×10^6	2a	+
3445	F	3.21×10^6	3a	+