Supplemental Information

Long-term Hepatitis B Virus Infection of Engineered Cultures of Induced Pluripotent Stem Cell-derived Hepatocytes

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Contains: Supplemental Materials and Methods, Figs. S1–S11, and Supplemental Table 1

Supplemental Materials and Methods

Cell culture

The human induced pluripotent stem cell-derived hepatocyte-like cells (**iHep**) were commercially sourced from Fujifilm Cellular Dynamics International (Madison, WI): iCell 2.0 (donor 1) human iHeps were from a Caucasian female and programmed from dermal fibroblasts; myCell (donor 2) human iHeps (#01279) were from a Caucasian male and programmed from a blood mononuclear cell; and, human iHeps (donor 3, #01177) - the gift of Dr. Paul Watkins of the University of North Carolina at Chapel Hill - were from a female (unknown race) and programmed from a blood mononuclear cell, also by the same vendor as above. The iHep vials were thawed at 37°C for 3 minutes without shaking the vial and diluted with 10 mL of prewarmed thawing media, the formulation of which is described below. The cell suspension was spun at 200 xg for 3 minutes. The supernatant was discarded, and the cells were resuspended in iHep plating medium at a density of 800K cells/mL, and 50 µL was added to each well of a 96-well plate (40K total cells per well) that was pre-coated with 25 µg/mL of rat tail collagen I (Corning Life Sciences, Tewksbury, MA) to generate iHep-containing conventional confluent (**iCC**) monocultures.

The thawing media, plating media, and maintenance media for iHeps included the same base medium composed of Rosewell Park Memorial Institute 140 (RPMI 140, Corning Life Sciences,), 5% knockout serum (Gibco, Waltham, MA), 4 mM L-glutamine (Corning Life Sciences), 1X B27 supplement (Invitrogen, Waltham, MA), 15 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES, Corning Life Sciences), 1% insulin-transferrin-selenous acid-bovine serum albumin-linoleic acid (ITS+) supplement (Corning Life Sciences), 1% vol/vol penicillin-streptomycin (Corning Life Sciences), 100 nM dexamethasone (Sigma-Aldrich, St. Louis, MO), 7 ng/mL glucagon (Sigma-Aldrich), 2.5 ng/mL Oncostatin M (OSM, R&D Systems, Minneapolis, MN), and 10 µM FPH2 small molecule (Sigma-Aldrich), previously shown to enhance hepatic functions (1). An inhibitor (Y-27632, Sigma-Aldrich) of Rho-associated, coiled-coil containing protein kinase (ROCK) was added at 5 µM only to the plating medium. In contrast, 3',5'-cyclic adenosine monophosphate (cAMP, Alfa Aesar, Tewksbury, MA) was added only to the maintenance medium at different concentrations per the experimental design.

The iHeps were seeded at 800K cells/mL density in 50 µL of the plating medium in each well of a 96-well plate (40K total cells per well). The cells were allowed to attach to the micropatterned collagen domains overnight in a plating medium (see formulation above) in a 37°C incubator. The unattached cells were washed with a plating medium, leaving ~ 4,500 iHeps spread over 14 islands. iHeps were cultured in maintenance media (see formulation above) for 5 days to differentiate them further on the islands per the manufacturer's instructions. Next, 15,000 3T3-J2 murine embryonic fibroblasts were seeded on day 5 around the iHep colonies in a medium containing Dulbecco's Modified Eagle's Medium (DMEM, Corning Life Science), 10% vol/vol bovine calf serum (Gibco), 15 mM HEPES, 1% vol/vol ITS+ supplement, 1% vol/vol penicillin-streptomycin, 100 nM dexamethasone, 7 ng/mL glucagon, 2.5 ng/mL OSM, 10 µM FPH2, and cAMP, the concentration of which was adjusted according to the experimental design. Four days following fibroblast seeding, the serum in the DMEM-based maintenance medium above for iHep-containing micropatterned co-cultures (**iMPCC**) was reduced to 1% vol/vol to prevent fibroblast overgrowth.

The plating medium for primary human hepatocytes (**PHHs**) contained Williams E base (Sigma-Aldrich), 15 mM HEPES, 1% vol/vol ITS+ supplement, 1% vol/vol penicillin-streptomycin, 100 nM dexamethasone, and 7 ng/mL glucagon. For PHH-fibroblast micropatterned coculture (PHH-MPCC), plates were patterned as mentioned above, and the seeding density of PHH was 666K cells/mL in 50µL of plating medium in each well of a 96-well plate (33,300 total cells per well). After 4-6 hours of attachment to the micropatterned collagen domains, the unattached PHHs were washed with DMEM, and the medium was replaced with that containing 10% vol/vol fetal bovine serum (Gibco) with other medium components being the same as the plating medium above. The next day, 15,000 3T3-J2 fibroblasts were seeded atop PHH islands in a medium with a similar formulation as above, except that the base was changed to DMEM, and 10% bovine serum was used.

Gene expression analysis

Total RNA was extracted from cultures using the Total RNA Lysis Solution (Invitrogen) and purified with the PureLink RNA Mini Kit (Invitrogen). The genomic DNA was digested by using the Optizyme^{TM} recombinant DNase-I digestion kit (Fisher BioReagents, Pittsburgh, PA) per the manufacturer's instructions. Purified RNA was then reverse transcribed into complementary DNA (cDNA) using the high-capacity cDNA reverse transcription kit (Applied Biosystems, Waltham, MA) on a MasterCycler RealPlex 2 (Eppendorf North America, Hauppauge, NY). About 1000 ng of cDNA was added to each quantitative polymerase chain reaction (qPCR) along with the SYBR Green master mix (Applied Biosystems) and primers (**Table S1**). The primer sets were selected to be human-specific without cross-reactivity to mouse DNA, and included glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), hepatocyte nuclear factor 4-alpha (*HNF4α*), *HNF1α*, *NTCP* (also known as SLC10A1), asialoglycoprotein receptor 1 (*ASGR1*), albumin (*ALB*), α-feto protein (*AFP*), cytochrome P450 3A4, 3A7 (*CYP3A4, 3A7*), retinoid X receptor-alpha (*RXRα*), peroxisome proliferator-activated receptor-alpha (*PPARα*), cAMP response element-binding protein (*CREB*), and apolipoprotein B mRNA-editing enzyme catalytic polypeptide-Like 3B (*APOBEC3B*). Hepatic gene expression was normalized to *GAPDH*.

HBV-infected and non-infected iMPCC and PHH-MPCC lysates were collected for RNA sequencing on post-infection day (**PID**) 12. Cell samples were lysed with TRIzol (Invitrogen) per the manufacturer's instructions. The samples were purified with RNeasy kit (Qiagen Sciences, Germantown, MD). Genomic DNA was removed via DNAse-I treatment (Millipore Sigma) per the manufacturer's instructions. RNA sequencing was performed at the University of Chicago Genomics Facility. Following the manufacturer's protocol, strand-specific libraries were prepared via the TruSeq Stranded mRNA kit (Illumina, San Diego, CA). Library quality and quantity were assessed via the Agilent bio-analyzer. Libraries were sequenced on the Illumina NovaSeq 6000. To identify human mRNA reads and exclude mRNA from 3T3-J2 mouse embryonic fibroblasts, we first used the Xenome analysis platform (2) developed explicitly for species-specific identification of mRNA reads using the GRCh38.p10 Ensembl human genome and GRCm38.p6 Ensembl mouse genome as reference genomes. Subsequent analyses focused on the human mRNA using The STAR aligner (version 2.7.6a) (3) with default parameter settings for alignment. The raw counts table was generated by the featureCounts function in Subread package (version 2.0.1) (4). The R package limma (version 3.46.0) was used for counts normalization and differential expression analysis (5). The P value was adjusted using the Benjamini-Hochberg method and 0.05 was applied as the significance threshold. The samples were corrected for donor bias with R package sva (version 3.38.0) (6).

Gene Ontology (GO) enrichment analysis was performed on the differentially expressed genes (DEGs) with R package clusterProfiler (version 3.18.0) (7) and the database org.Hs.eg.db (version 3.12.0). The P value was adjusted by Benjamini-Hochberg method and 0.05 was applied as the significance threshold. The gene-concept networks depict the linkages of genes and the biological functions and pathways. The function cnetplot in R package clusterProfiler (version 3.18.0) (7) was used to generate the gene-concept networks.

HBV DNA assays

DNA was extracted from the collected supernatants using the QIAamp DNA MinElute Virus Spin kit (Qiagen). DNA was extracted according to the manufacturer's protocol, and the final product was eluted in 80 µL of AE buffer. Five microliters were taken for quantitative PCR (qPCR). SYBR Green master mix was used during qPCR along with the HBV primers, forward: CCG TCT GTG CCT TCT CAT CTG, reverse: AGT CCA AGA GTC CTC TTA TGT AAG ACC TT. Quantitative PCR was performed using the MasterCycler RealPlex 2 with the following conditions: (i) denaturation at 50°C for 2 minutes followed by 95°C for 2 minutes (one cycle); (ii) qPCR at 95°C for 15 seconds, 56°C for 15 seconds, and 72°C for 1 minute (40 cycles) based on manufacturer's instructions. HBV DNA quantification was done via the Diagnostic kit to quantify HBV DNA (Kehua, Shanghai, China). The number of viral particles was quantified by using a standard curve composed of four working standards over a range of $3.7x10^4$ to $3.7x10^7$ IU/mL. The total intracellular DNA was purified from HBV-infected cells by using the QIAamp DNA mini kit (Qiagen). The DNA was eluted with 50 µL elution buffer, and 5 µL DNA samples were used to run qPCR as described above. The procedure of qPCR was the same as that for extracellular HBV DNA. HBV DNA expression was normalized to housekeeping gene prion protein (PRNP).

Twenty-five microliters of isolated intracellular DNA sample were treated with 1 µL T5 exonuclease (NEB, Frankfurt, Germany) for 30 minutes at 37°C. The reaction was terminated by

adding 9.9 µL 50mM ethylenediaminetetraacetic acid (EDTA). DNA was purified using the protocol in the DNA purification and concentrator kit (ZYMO Research, Irvine, CA). Briefly, 90 µL of DNA binding solution was added to each sample and mixed well. Then, the solution was transferred to a DNA-binding column; after centrifugation and washing, 50 µL of elution buffer was used to elute and concentrate the DNA. For relative quantification of covalently closed circular DNA (cccDNA), 5 µL of the above processed sample was mixed with SYBR Green Mastermix and cccDNA primers, forward: GTC TGT GCC TTC TCA TCT GC, reverse: AGT AAC TCC ACA GTA GCT CCA AAT T. Quantitative PCR was performed using the MasterCycler RealPlex 2 using the following conditions: (i) denaturation at 50°C for 2 minutes followed by 95°C for 2 minutes (one cycle); (ii) qPCR at 95°C for 15 seconds, 56°C for 15 seconds, and 72°C for 1 minute (50 cycles) based on manufacturer's instructions. To quantify relative cccDNA expression, mitochondrial cytochrome oxidase subunit 3 (*MT-CO3*) was used as a reference gene.

HBV antigen assays

Secreted HBeAg and HBsAg were quantified using enzyme-linked immunosorbent assay (ELISA) kits (Kehua). Briefly, 20 µL of cell culture supernatants were diluted in 30 µL of 1X phosphate-buffered saline (PBS) and the mixture was added to anti-HBeAg or anti-HBsAg antibody-coated wells. For HBeAg ELISA, 50 µL of horseradish peroxidase (HRP)-conjugated anti-HBeAg antibody solution was added to each well with samples and incubated at 37°C for 30 minutes. After washing, a color development procedure per the manufacturer's protocol was executed, and the optical density (OD) of each sample at 450 nm was determined using the Synergy H1 multi-mode plate reader (BioTek, Winooski, VT). The HBsAg ELISA was performed similarly, except samples were incubated at 37°C for 60 minutes before incubation with HRP-conjugated anti-HBsAg antibody solution for 30 minutes, and OD was determined at 450 nm.

Hepatocyte functional assessments

Culture supernatants were assayed for albumin levels using a sandwich ELISA assay (Bethyl Laboratories, Montgomery, TX) with horseradish peroxidase detection and 3,3',5,5'-tetramethylbenzidine (TMB, Rockland Immunochemicals, Boyertown, PA) as the substrate (8). Urea concentration in culture supernatants was assayed using a colorimetric assay utilizing diacetyl monoxime with acid and heat (Stanbio Labs, Boerne, TX) (8). Absorbance values were quantified using the Synergy H1 multimode plate reader. Cytochrome P450 (CYP450) 3A4 enzyme activity was measured by incubating the cultures with luciferin-IPA (Promega, Madison, WI) for 3 hours (9, 10). According to the manufacturer's protocols, luciferin generated by CYP450 metabolism was quantified via luminescence on the Synergy H1 multi-mode reader.

Immunofluorescence (IF) staining

For HBV core antigen (HBcAg) IF, HBV-infected iMPCCs were fixed with 4% paraformaldehyde for 10 minutes at room temperature. After washing, the cells were permeabilized with 0.5% PBS/Triton-100. A 10% bovine serum albumin (BSA) solution was used to block samples at 37°C for 30 minutes. A mouse anti-HBcAg antibody (Novus Biologicals, Littleton, CO) at 1:100 dilution was added to each well and incubated at 4°C overnight. After washing three times with 1X PBS, a secondary anti-mouse antibody labeled with Alexa fluor 488 (Invitrogen) at 1:100

dilution was used to stain HBcAg at room temperature for 1 hour. At the last 15 minutes of secondary antibody staining, 300 nM of 4′,6-diamidino-2-phenylindole (DAPI) was introduced to each well to stain the cell nuclei. After 3 washes with 1X PBS, cells were incubated with 1X PBS and imaged using an EVOS FL Imaging System (Invitrogen).

For albumin IF, HBV-infected iCC or iMPCC on PID 7 were fixed with 4% paraformaldehyde for 20 minutes at room temperature. After washing, a 5% donkey serum solution was used to block samples at 37°C for 45 minutes. A goat anti-albumin antibody (Bethyl Laboratories, Montgomery, TX) at 1:200 dilution was added to each well and incubated at 4°C overnight. After washing three times with 1X PBS, a secondary anti-goat antibody labeled with Alexa fluor 488 (Invitrogen) at 1:200 dilution was used to stain albumin at room temperature for 1 hour. DAPI staining was as described above. For fluorescently labeling collagen islands, micropatterned wells were incubated with 20 μg/mL Alexa Fluor 488 carboxylic acid NHS ester (Invitrogen). The immunofluorescence staining images were taken using an Olympus IX83 epifluorescent microscope (Evident Scientific, Tokyo, Japan).

Supplemental Figures

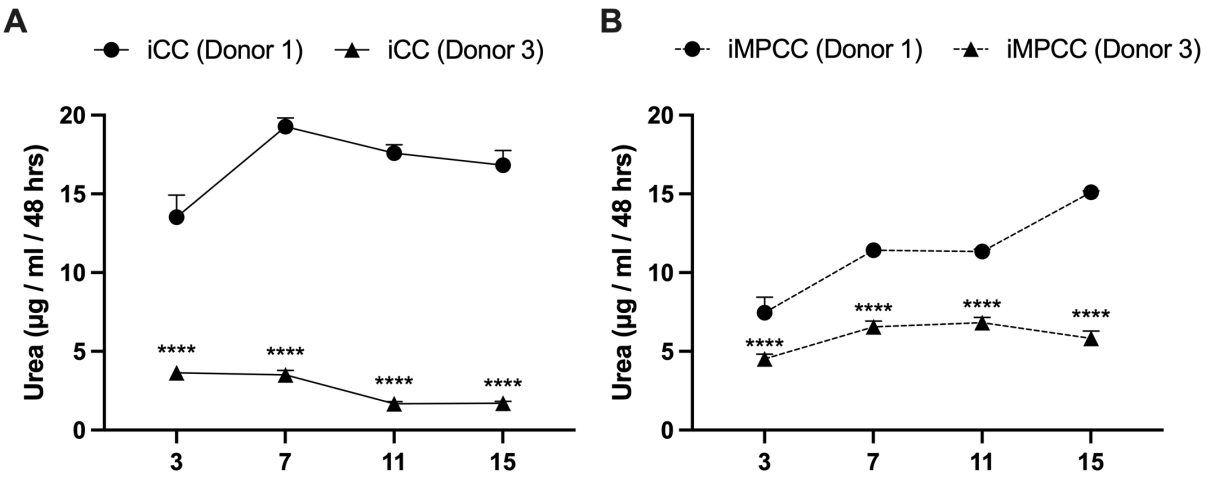

Fig. S1. Urea secretions in iCC and iMPCC. (A) Comparison of urea secretion in iCCs created using donor 1 and donor 3 iHeps. (B) Comparison of urea secretion in iMPCCs created using the same donor 1 and donor 3 iHeps. ****p<0.0001.

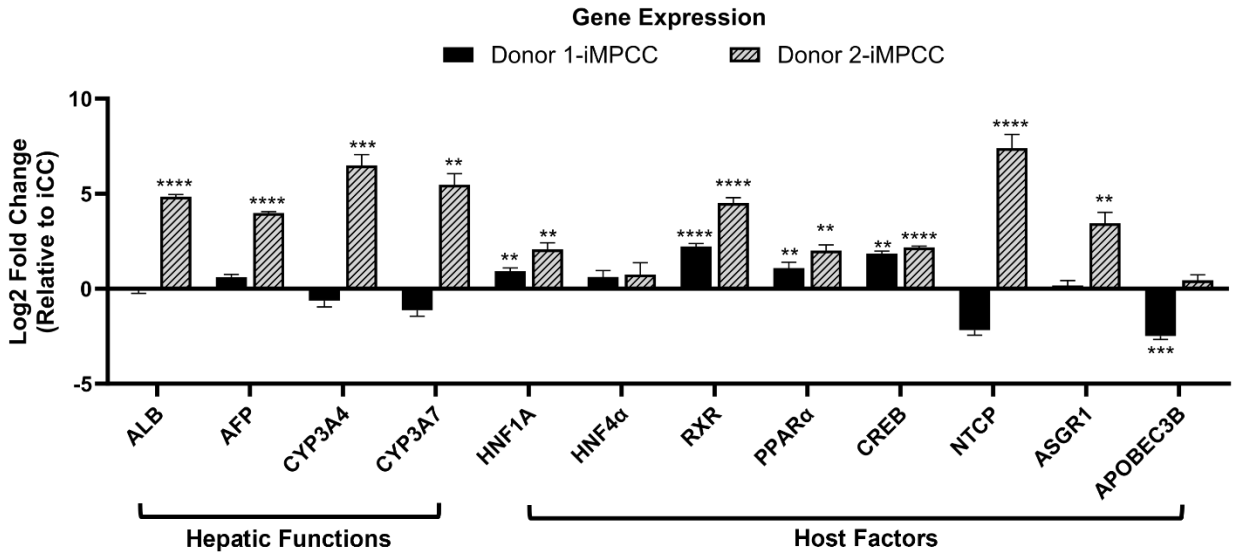

Fig. S2. Expression of genes related to hepatic functions and those implicated as host factors for HBV infection in iCCs and iMPCCs. Both culture platforms were lysed on day 12 following the initiation of culture. The expression of each gene in iMPCC was first normalized to GAPDH and then to the corresponding value in iCC; thus, line 0 indicates expression levels in iCC for each of the two iHep donors (donors 1 and 2). Statistical significance is displayed for iMPCC gene expression relative to iCC for the indicated iHep donor. **p<0.01, ***p<0.001, ****p<0.0001.

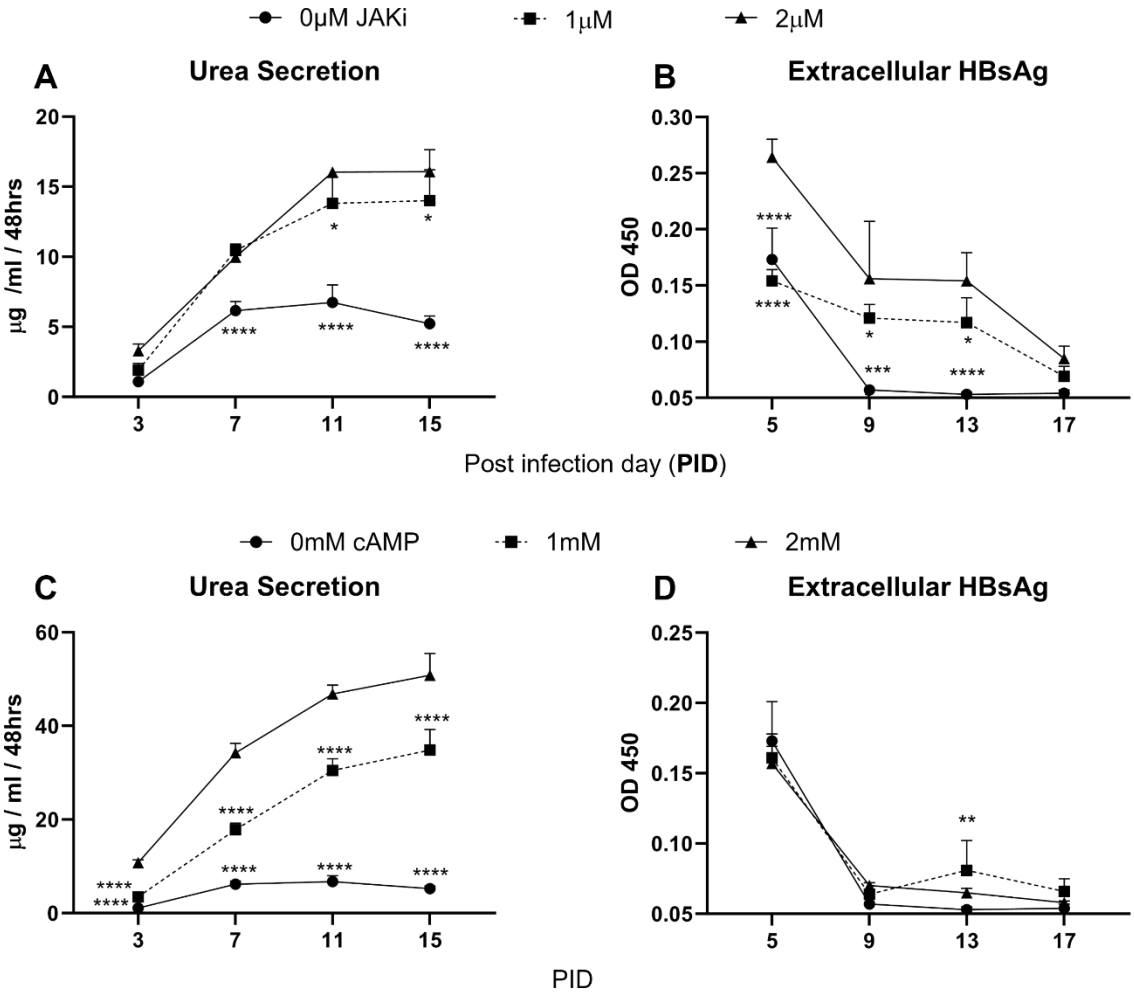

Fig. S3. Effects of Janus kinase inhibitor (JAKi) and cAMP on HBV infection in iMPCCs. Time course of (A) urea secretion and (B) HBsAg secretion in infected iMPCCs (donor 2 iHeps) with or without treatment with JAKi. OD 450 is the optical density at 450 nm. Mock-treated iMPCCs showed values of 0.05 (start of the y-axis) for the HBsAg ELISA. Statistical significance is displayed for secretion levels relative to the 2 μM JAKi treated group (C, D) Similar results as for panels A and B except cultures were treated with different concentrations of cAMP. Statistical significance is displayed for secretion levels relative to the 2 mM cAMP-treated group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

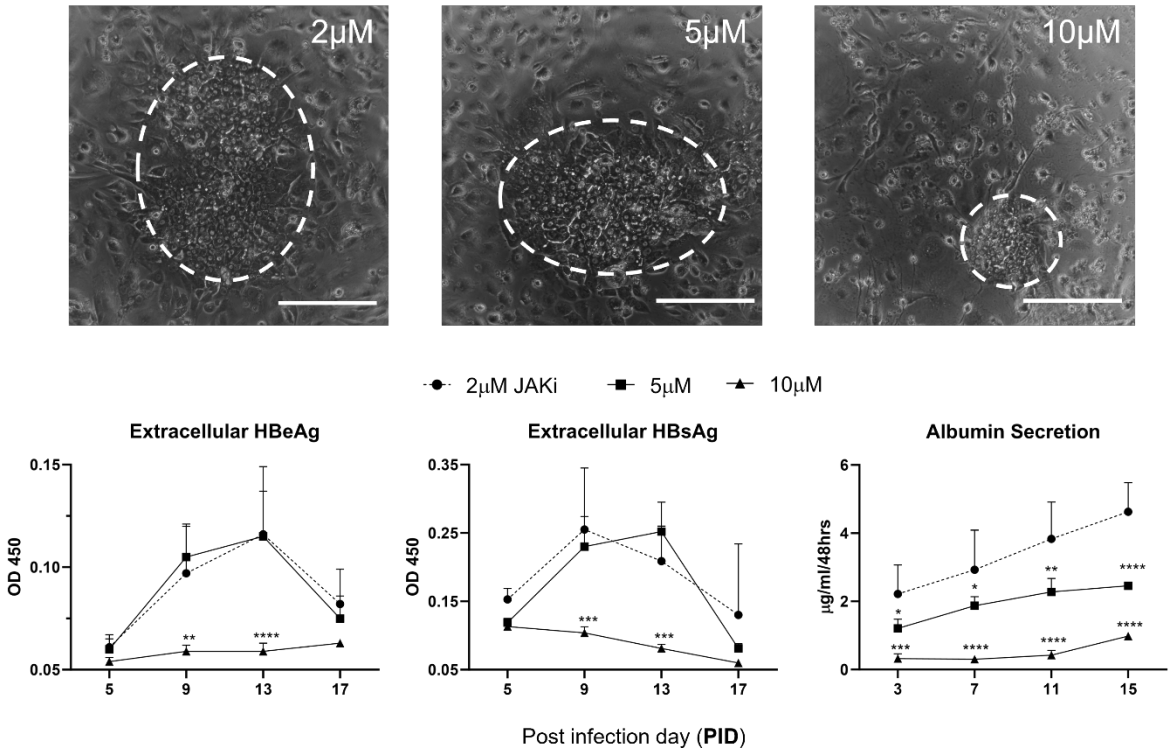

Fig. S4. Effects of escalating concentrations of JAKi on HBV infection in iMPCCs. (A) Phase contrast images of infected iMPCCs (donor 2 iHeps) on PID 7 following treatment with escalating JAKi concentrations. Treatment with no JAKi led to a similar morphology as the 2 μM JAKi condition. Scale bars are 200 μm. (B) HBeAg and (C) HBsAg levels in supernatants of infected iMPCCs treated with escalating concentrations of JAKi. OD 450 is the optical density at 450 nm from the ELISA. Mock-treated iMPCC showed values of 0.05 (start of the y-axis) for the HBeAg and HBsAg ELISAs. (D) Albumin level in supernatants of infected iMPCCs treated with escalating concentrations of JAKi. Statistical significance in panels (B, C, and D) is displayed for secretion levels relative to the 2 μM JAKi-treated group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

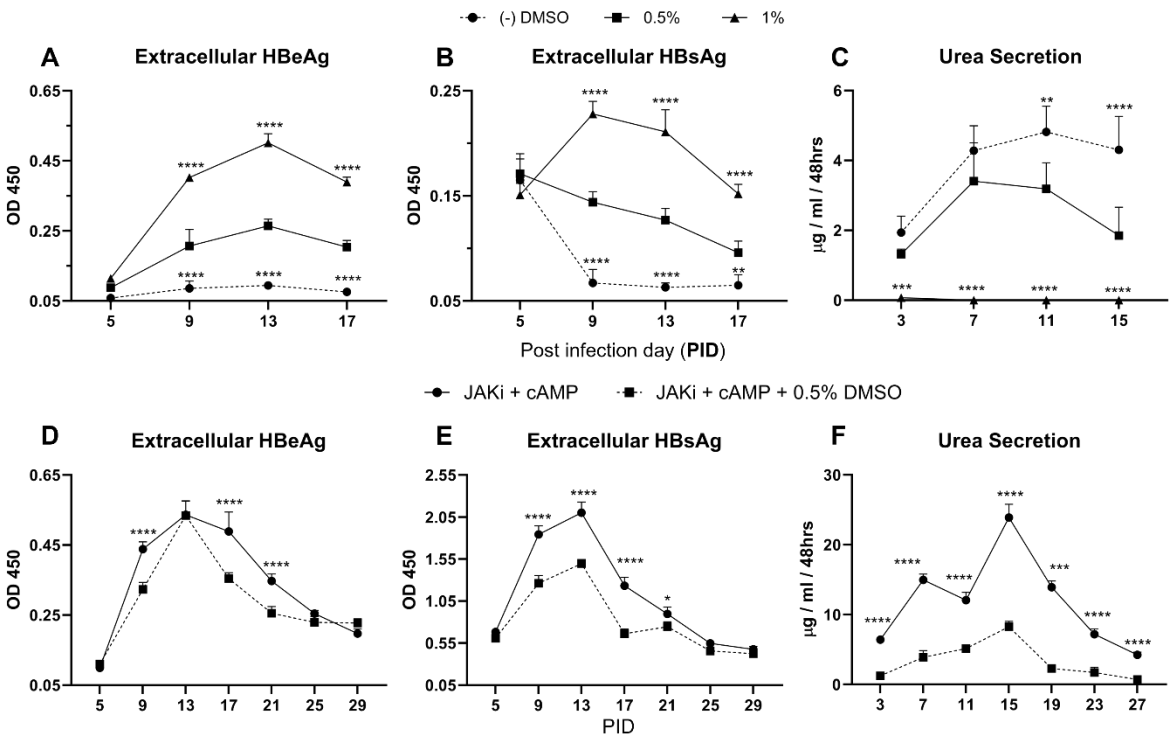

Fig. S5. Effects of dimethyl sulfoxide (DMSO) on HBV infection in iMPCCs. The effects of increasing concentrations of DMSO on secretions of (A) HBeAg, (B) HBsAg, and (C) urea from infected iMPCCs (donor 1 iHeps). OD 450 is the optical density at 450 nm from the ELISA. Mock-treated iMPCC showed values of 0.05 (start of the y-axis) for the HBeAg and HBsAg ELISAs. Statistical significance (panels A, B, and C) is displayed for secretion levels relative to the 0.5% DMSO-treated group. The effect of adding 0.5% DMSO to the combination of JAKi (2 µM) and cAMP (2 mM) on (D) HBeAg secretion, (E) HBsAg secretion, and (F) urea secretion from infected iMPCCs. Statistical significance (panels D, E, and F) is displayed for secretion levels relative to the 0.5% DMSO-treated group. *p<0.05, **p<0.01, ****p<0.0001.

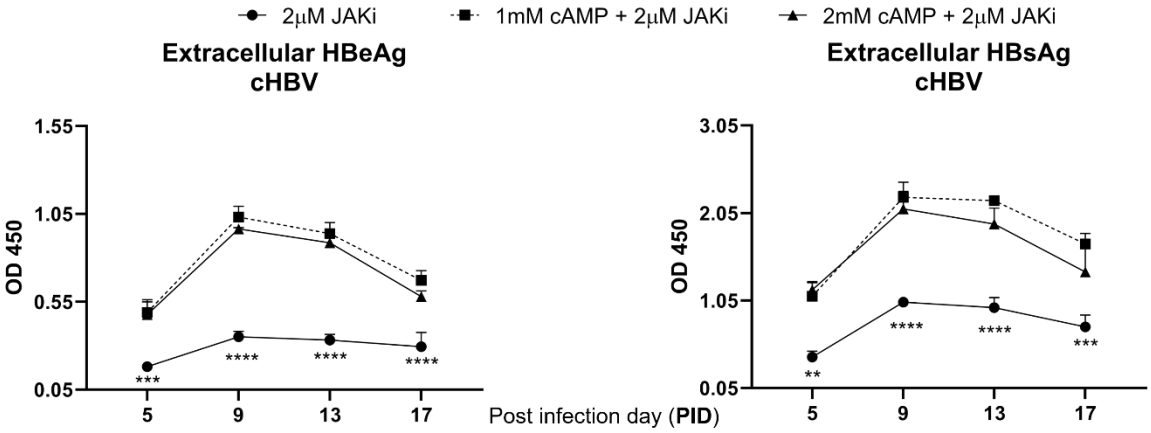

Fig. S6. Long-term infection of iMPCCs with cell culture-derived HBV (cHBV). Kinetics of extracellular (A) HBeAg and (B) HBsAg secretions in the supernatants from infected iMPCCs (donor 2 iHeps). OD 450 is the optical density at 450 nm from the ELISA. Mock-treated iMPCC showed values of 0.05 (start of the y-axis) for the HBeAg and HBsAg ELISAs. Statistical significance is displayed for secretion levels relative to the 2 mM cAMP- and 2 μM JAKi-treated group. **p<0.01, ***p<0.001, ****p<0.0001.

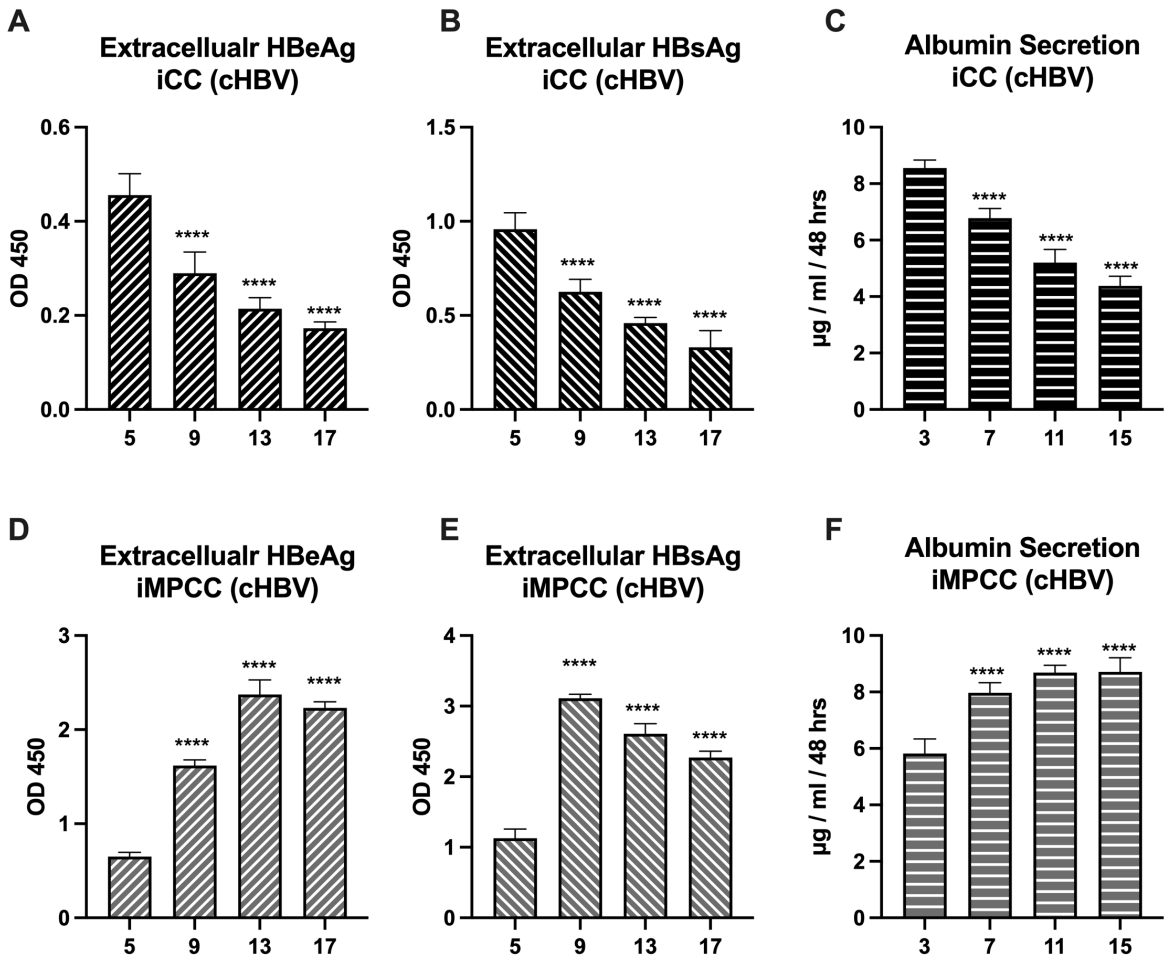

Fig. S7. Infection of iCC and iMPCC with cHBV. Comparison of (A) HBeAg, (B) HBsAg, and (C) albumin secretions in infected iCCs created using donor 3 iHeps. Comparison of (D) HBeAg, (E) HBsAg, and (F) albumin secretions in infected iMPCCs created using the same donor. Statistical significance is displayed relative to the initial time-point displayed for each graph. ****$p<0.0001$.

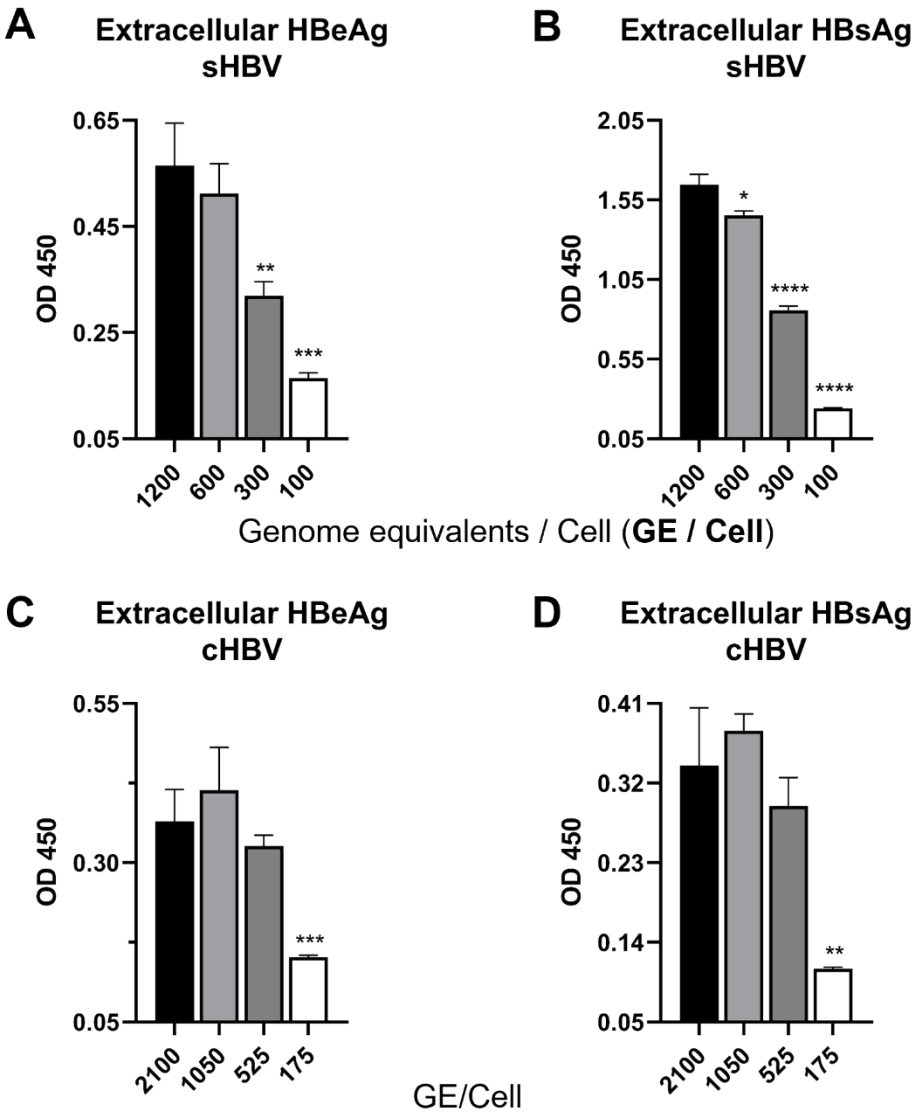

Fig. S8. Infection of iMPCCs with increasing multiplicities of infection (MOIs) of patient serum-derived HBV (sHBV, genotype C) and cHBV (genotype D). Extracellular (A) HBeAg and (B) HBsAg secretions in supernatants from iMPCCs (donor 1 iHeps) infected with different MOIs of sHBV. HBeAg was assessed PID 7, while HBsAg was assessed PID 9. OD 450 is the optical density at 450 nm from the ELISA. Mock-treated iMPCCs showed values of 0.05 (start of the y-axis) for the HBeAg and HBsAg ELISAs. Statistical significance is displayed relative to the highest MOI. (C, D) Similar data as for panels A and B except that iMPCCs were infected with cHBV. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

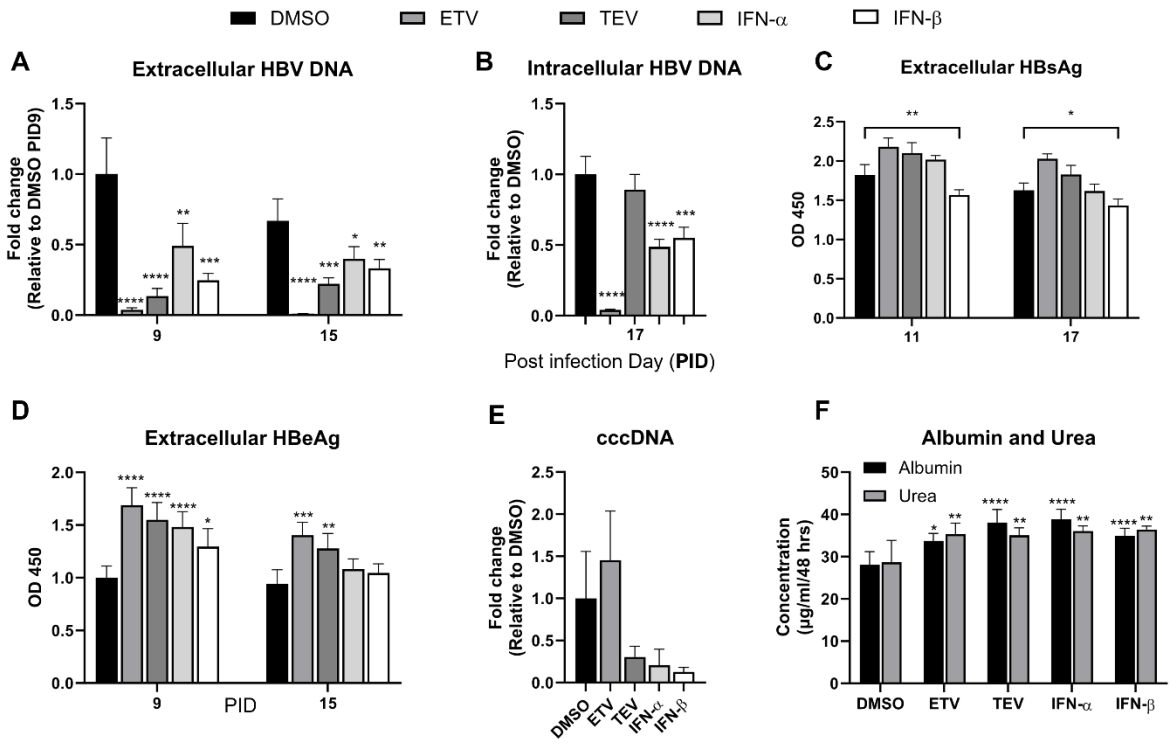

Fig. S9. Assessment of hepatotoxicity and drug efficacy in HBV-infected iMPCCs. The timeline of drug treatment of infected iMPCCs (donor 2 iHeps) is provided in panel A of Figure 8 in the main manuscript. Relative levels of (A) extracellular and (B) intracellular HBV DNA in drug-treated versus vehicle control-treated infected iMPCCs. iMPCC were treated on PID 3 with entecavir (2 µM), tenofovir (4 µM), interferon (IFN)-α (1000 U/mL), and IFN-β (40 U/mL) continuously for 14 days with fresh drug added with medium exchanges every 2 days. Statistical significance shown is relative to the DMSO-treated control infected cultures. (C) Extracellular HBsAg and (D) HBeAg secretions in drug and DMSO-treated infected iMPCCs. OD 450 is the optical density at 450 nm from the ELISA. Mock-treated cultures showed values of 0.05 for HBeAg and HBsAg ELISAs. Statistical significance shown is relative to the DMSO-treated control cultures. (E) Quantification of intracellular cccDNA from infected iMPCCs (PID 17). (F) Albumin and urea secretions in infected iMPCCs treated with drugs or DMSO-alone (PID 17). Statistical significance shown is relative to the DMSO-treated control cultures. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

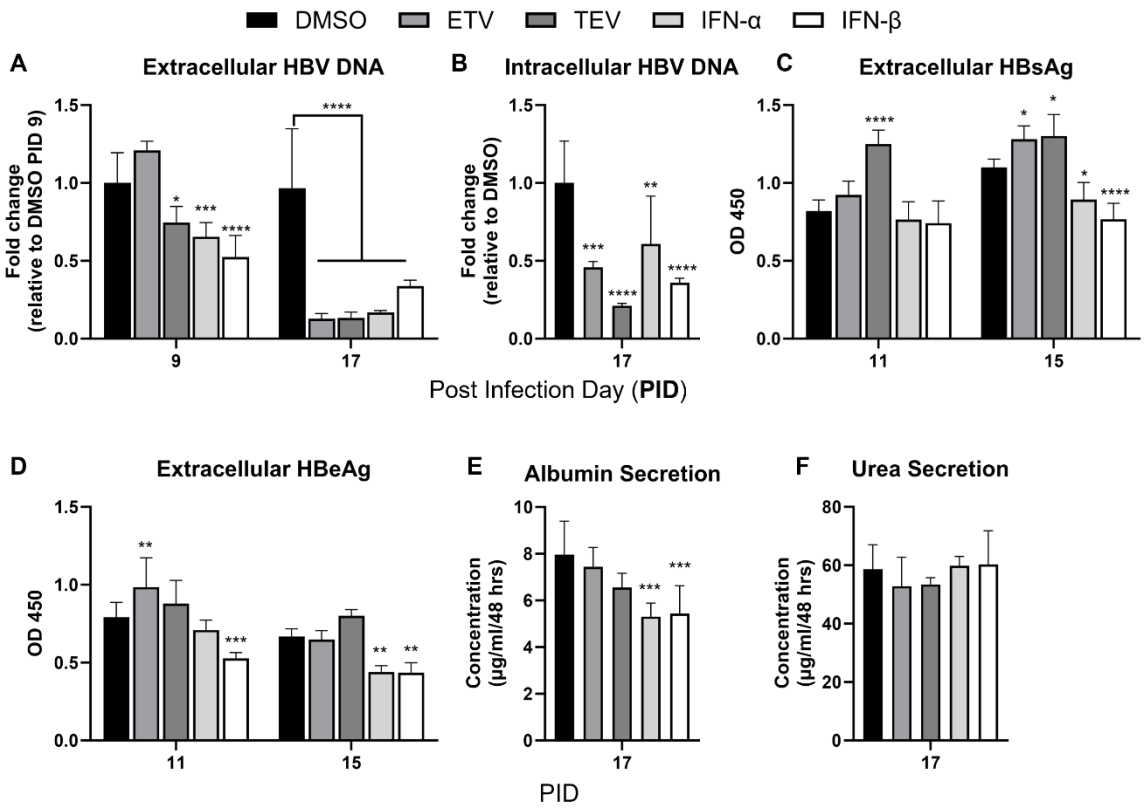

Fig. S10. Assessment of hepatotoxicity and drug efficacy in HBV-infected PHH-MPCCs (donor HUM4145). Relative levels of (A) extracellular and (B) intracellular HBV DNA in drug-treated versus vehicle control-treated infected PHH-MPCCs. PHH-MPCCs were treated on PID 3 with entecavir (2 µM), tenofovir (4 µM), interferon (IFN)-α (1000 U/mL) and IFN-β (40 U/mL) continuously for 14 days with fresh drug added with medium exchanges every 2 days. Statistical significance shown is relative to the DMSO-treated control infected cultures. (C) Extracellular HBsAg and (D) HBeAg secretions in drug and DMSO-treated infected PHH-MPCCs. OD 450 is the optical density at 450 nm from the ELISA. Mock-treated cultures showed values of 0.05 for HBeAg and HBsAg ELISAs. Statistical significance shown is relative to the DMSO-treated control cultures. Albumin (E) and urea (F) secretions in infected PHH-MPCCs treated with drugs or DMSO-alone (PID 17). Statistical significance shown is relative to the DMSO-treated control infected cultures. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

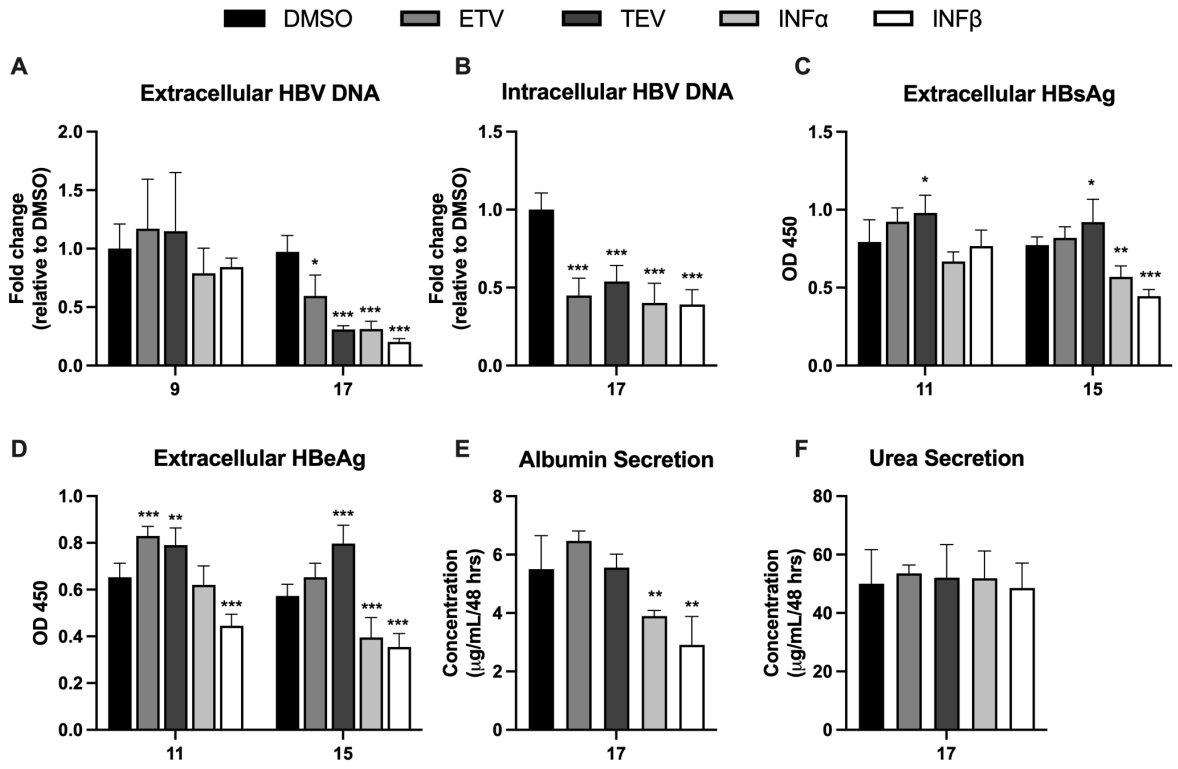

Fig. S11. Assessment of hepatotoxicity and drug efficacy in HBV-infected PHH-MPCCs (donor HUM4192). Relative levels of (A) extracellular and (B) intracellular HBV DNA in drug-treated versus vehicle control-treated infected PHH-MPCCs. PHH-MPCCs were treated on PID 3 with entecavir (2 μM), tenofovir (4 μM), interferon (IFN)-α (1000 U/mL) and IFN-β (40 U/mL) continuously for 14 days with fresh drug added with medium exchanges every 2 days. Statistical significance shown is relative to the DMSO-treated control infected cultures. (C) Extracellular HBsAg and (D) HBeAg secretions in drug and DMSO-treated infected PHH-MPCCs. OD 450 is the optical density at 450 nm from the ELISA. Mock-treated cultures showed values of 0.05 for HBeAg and HBsAg ELISAs. Statistical significance shown is relative to the DMSO-treated control cultures. Albumin (E) and urea (F) secretions in infected PHH-MPCCs treated with drugs or DMSO-alone (PID 17). Statistical significance shown is relative to the DMSO-treated control infected cultures. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Table S1. List of Primers

Gene	Species	Primers
AFP	Human	Fw: CTGCAGCCAAAGTGAAGAGGGAAG Rv: GTAGGTGCATACAGGAAGGGATGC
ALB	Human	Fw: GCT GCC ATG GAG ATC TGC TTG AAT Rv: GCA AGT CAG CAG GCA TCT CAT CAT
APOBEC3B	Human	Fw: GACCCTTTGGTCCTTCGAC Rv: GCACAGCCCCAGGAGAAG
ASGR1	Human	Fw: TGA AGA ACC TGG GAA TCA GAC CCT Rv: TTC TGA GCT GAT GGT GGT CAC TCT
CREB	Human	Fw: TAG TTT GAC GCG GTG TGT TAC GTG Rv: GTT ACA GCT GCA TCT CCA CTC TGC
CYP3A4	Human	Fw: TTT ATG ATG GTC AAC AGC CTG TGC Rv: CTG GTG AAG GTT GGA GAC AGC AAT
CYP3A7	Human	Fw: GGCTATGAAACCACGAGCAGTGTT Rv: AGCACAGTATCATAGGTGGGTGGT
GAPDH	Human	Fw: GGA AGG TGA AGG TCG GAG TCA A Rv: CTT GAC GGT GCC ATG GAA TTT GC
HNF4α	Human	Fw: CCA GTT CAT CAA GCT CTT CGG CAT Rv: TAT GTT CCT GCA TCA GGT GAG GGT
HNF1α	Human	Fw: CCATCCTCAAAGAGCTGGAGAACC Rv: CAGGTAGGACTTGACCATCTTCGC
MT-CO3	Human	Fw: CCC CAC AAA CCC CAT TAC TAA ACC CA Rv: TTT CAT CAT GCG GAG ATG TTG GAT GG
NTCP	Human	Fw: TCC TCA AAT CCA AAC GGC CAC AAT Rv: GAG GTG GCA ATC AAG AGT GGT GTC
PPARα	Human	Fw: GTG GCT GCT ATC ATT TGC TGT GGA Rv: AGA GAA AGA TAT CGT CCG GGT GGT
PRNP	Human	Fw: TGC TGG GAA GTG CCA TGA G Rv: CGG TGC ATG TTT TCA CGA TAG TA
RXRα	Human	Fw: TCG CAG ACA TGG ACA CCA AAC ATT Rv: CTC AGG GTG CTG ATG GGA GAA T

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