Stem Cell Reports, Volume *17*

Supplemental Information

Ebola virus infection induces a delayed type I IFN response in bystander cells and the shutdown of key liver genes in human iPSC-derived hepatocytes

Whitney A. Scoon, Liliana Mancio-Silva, Ellen L. Suder, Carlos Villacorta-Martin, Jonathan Lindstrom-Vautrin, John G. Bernbaum, Steve Mazur, Reed F. Johnson, Judith Olejnik, Elizabeth Y. Flores, Aditya Mithal, Feiya Wang, Adam J. Hume, Joseph E. Kaserman, Sandra March-Riera, Andrew A. Wilson, Sangeeta N. Bhatia, Elke Mühlberger, and Gustavo Mostoslavsky

Supplemental information

Supplemental Methods

Vesicular Stomatitis Virus

Recombinant vesicular stomatitis virus (VSV) expressing GFP or mCherry from an additional transcription unit (VSV-GFP and VSV-mCherry; kindly provided by J. Connor, Boston University) was propagated in Vero E6. Viral titers were determined by $TCID_{50}$.

qRT-PCR Analysis

Cells were harvested for RNA analysis by using either the RNeasy Kit (Qiagen) or TRIzol Reagent (ThermoFisher) according to manufacturer's instructions. For hepatic cellular markers, the TaqMan qRT-PCR system (ThermoFisher) was used. cDNA libraries of the harvested cellular RNA were prepared using SuperScript III Reverse Transcriptase (ThermoFisher). 500 ng of the cDNA library was added to a TaqMan probe reaction master mix containing 10 µL of the TaqMan Universal PCR Master Mix 2x Buffer, 1 µL of TaqMan gene probe, and nuclease-free water to a final volume of 20 µL per reaction. For qRT-PCR of IFNβ, $1x10^6$ or $4x10^5$ iPSC-HLCs and Huh7 cells were harvested 1 day post-VSV infection by using TRIzol Reagent (ThermoFisher) according to manufacturer's instructions. The QuantiTect SYBR Green RT-PCR kit was used (Qiagen) for qRT-PCR analysis. 50 ng of purified cellular RNA was added to a SYBR Green reaction master mix containing 12.5 µL of SYBR master mix, 2.5 µL of QuantiTect gene probe, 0.25 µL of QuantiTect Reverse Transcriptase mix, and nuclease-free water to a final volume of 25 µL per reaction. For both systems, triplicate samples (n= 3 per gene per sample) were run on a StepOnePlus Real Time PCR machine (ThermoFisher). CT values were plotted using Prism GraphPad Software. Statistical significance was determined by paired t-tests with comparisons of interest plotted on each graph.

Immunofluorescence Analysis

$1x10^6$ or $4x10^5$ cells were fixed with either 4% PFA in DMEM for 10 minutes at room temperature or 10% formalin for at least 6 hours at 4°C. Cells were washed in PBS, permeabilized with a solution of acetone-methanol in a 1:1 volume ratio for 5 minutes at -20°C and incubated in blocking buffer for at least 20 minutes at room temperature. Due to cross-reactivity with the BSA in the blocking buffer, the cells were not incubated in blocking buffer when stained for albumin. Cells were incubated with primary antibody overnight at 4°C, and primary antibody names and dilutions are listed in Table S3. Cells were incubated with secondary antibody and 4′,6-diamidino-2-phenylindole (DAPI) for one hour at room temperature (dilutions listed in Table S3).

Illumina Sequencing

Duplicate samples of $1x10^6$ BU3 iPSC-HLCs were mock-infected or infected with EBOV at an MOI of 10 (n = 2 per condition per time point). Cells were harvested for RNA analysis at 1, 2, 3, and 7 dpi using TRIzol reagent (ThermoFisher). RNA was isolated according to manufacturer's instructions. 1 µg of purified cellular RNA was diluted to a concentration of 50 ng/µL in a final volume of 20 µL and shipped to Novogene on dry ice for library preparation and Illumina sequencing. The quality of the raw data was assessed using FastQC v.0.11.7. The sequence reads were aligned to a combination of the human genome reference (GRCh38) and the EBOV reference sequence (NC_002549.1) using STAR v.2.6.0c. Counts per gene were summarized using the featureCounts function from the subread package v.1.6.2. Log counts per million (lcpm) per gene were averaged across both technical duplicates for each sample. After exploratory data analysis (Glimma v. 1.11.1), contrasts for differential expression testing were done for each EBOV-infected sample compared to the mock control at each time point separately. Statistical significance of the logFC for each time point contrast was determined by a paired t-test and reported as a Benjamini-Hochberg false discovery rate adjusted p-value. Gene Set Enrichment Analysis (GSEA) was performed using all genes in the dataset, ranked on logFC, using WebGestalt to analyze Biological Processes in GO databases or the Reactome in Pathway databases with the following parameters: minimum number of

genes in a category = 15; false discovery rate (FDR) ≤ 0.01; permutations = 1000 (Liao et al., 2019). All RNA-Seq data have been deposited to Gene Expression Omnibus (GEO id GSE184640).

Fluorescence in-situ Hybridization Assay

Fluorescence in-situ hybridization (RNA-FISH) was performed using the Advanced Cell Diagnostics RNAscope v2 kit according to manufacturer's instructions (ACD Bio). $5x10^5$ BU1, BU2, and BU3 iPSC-HLCs were seeded onto 2 well glass chamber slides, and $1x10^5$ Huh7 cells were seeded onto 8 well glass chamber slides. Replicates per experiment varied by condition and are listed in the corresponding figure legends. Both cell types were infected at an MOI of 10 as described above. Cells were fixed with 10% formalin for a minimum of 6 hours. After fixation, cells were dehydrated with ethanol washes before being stored in 100% ethanol at -20ºC until staining. On the day of the assay, cells were rehydrated with ethanol. Cells were pre-treated with hydrogen peroxidase and Protease III according to the manufacturer's instructions. Cells were probed with RNAscope probes for 2 hours at 40ºC in the ACD hybridization oven. The following probes (all from ACD Bio) were used: ebolavirus Zaire VP35- C1 (undiluted), human IL6- C2 (1:50), human IFNβ- C3 (1:50), human CXCL10- C3 (1:50). After hybridization, the cells were treated with the amplification and development reagents according to manufacturer's instructions. An additional blocking and washing step was added following amplification of channels containing viral RNA probes to prevent cross-reaction due to viral gene abundance. Fluorophores used for detection (Perkin Elmer) include Opal 520 (1:3000), Opal 570 (1:3000), and Opal 690 (1:1500). Samples were visualized on a Nikon Eclipse Ti2 inverted microscope using Photometrics Prime BSI camera, and quantification of gene-expressing cells was performed in QuPath software (https://doi.org/10.1038/S41598-017-17204-50) using the cell detection feature and manual identification of positive cells.

Immortalized Cell Culture

Vero E6 (Cercopithecus aethiops kidney cell line, ATCC CRL-1586) and Huh-7 cells (human hepatocellular carcinoma cell line, kindly provided by J. Alonso, Texas Biomedical Research Institute, San Antonio, TX) were maintained in Dulbecco's Modified Eagle Media (DMEM) (Gibco), 10% fetal bovine serum (FBS), 200 nM L-glutamine in solution, and 5000 units/mL Penicillin Streptomycin solution. Cells were incubated at 37°C with 5% CO_2.

Flow Cytometry Analysis

$2.5x10^5$ D0 and D5 BU2 cells (n = 5) were stained for flow cytometry analysis of the endoderm markers cKit and CXCR4. Cells were harvested by centrifugation at 300xg at 4°C for 5 minutes. Cells were resuspended in PBS and stained with APC-conjugated cKit (BioLegend) and PE-conjugated CXCR4 (Invitrogen) primary antibodies for 30 minutes. Cells were washed with FACS buffer and filtered through a 35 µm filter into a 5 mL polystyrene FACS tube and analyzed on a Stratadigm Flow Cytometry machine or a FACS Calibur Flow Cytometry machine. Analysis of the flow plots was performed using FlowJo.

Periodic Acid-Schiff (PAS) Staining

PAS staining was performed on triplicate samples of $1x10^6$ BU2 iPSC-HLCs, $1x10^6$ Huh7 cells, and $1x10^4$ Donor A PHHs using the Periodic Acid-Schiff (PAS) kit (Sigma-Aldrich) according to manufacturer's instructions (n = 3 for each cell type). Cells were fixed with 1 mL of 4% paraformaldehyde (PFA) solution for 10 minutes at room temperature prior to PAS staining. PBS was added to the cells for imaging on an inverted microscope.

LDL-Uptake Assay

Duplicate samples of $1x10^6$ BU1 and BU2 iPSC-HLCs, $1x10^6$ Huh7 cells, and $1x10^4$ PHHs were analyzed using the LDL Uptake Assay Kit (Cell-Based) (Abcam) according to manufacturer's instructions (n = 2 for each donor and cell type). The LDL-DyLight reagent was diluted in the appropriate cell culture medium for

each cell type. The cells were incubated with the LDL reagents at 37°C for 4 hours. Cells were washed one time with 1 mL of PBS, and 2 mL of PBS was added to the cells for visualization of LDL update on an inverted microscope with a filter capable of capturing excitation and emission wavelengths of 540 and 570 nm.

CYP3A4 P450-Glo Assay

The quantification of CYP3A4 in triplicate samples of $1x10^6$ BU2 iPSCs, BU2 iPSC-HLCs, and Huh7 cells was performed using the P450-Glo Assay kit (Promega) according to manufacturer's instructions (n = 3 for each cell type). One day prior to the assay, Huh7 cells were treated with cell culture media containing 100 nM Dexamethasone. One the day of the assay, the Luciferin Glo substrate was added to the appropriate cell culture medium for each cell type. Cells were incubated with luciferin reagents at 37°C for 4 hours. Supernatant was clarified at 300xg for 5 minutes at room temperature. Clarified supernatant and detection reagent were incubated at room temperature for 20 minutes. Luminescence was measured on a LUMIStar Omega Luminometer (BMG Labtech).

VSV Bioassay

Duplicate samples of $4x10^5$ iPSC-HLCs from BU1, BU2, and BU3 and triplicate samples of Huh7 cells were infected with VSV-GFP or VSV-mCherry at an MOI of 10 (n = 2 for each iPSC-HLC donor and n = 3 for Huh7 cell samples). 1 dpi, cells were imaged for GFP expression and cellular RNA was purified for qRT-PCR analysis.

Luminex analysis

Triplicate samples of $2x10^5$ BU3 iPSC-HLCs cells were infected with EBOV at an MOI of 10 or left uninfected (n = 3 per condition). Cell supernatants (1mL per sample) were collected at the indicated time points. Samples were clarified by low-speed centrifugation and analyzed using the ProcartaPlex Human IP-

10 Simplex assay (Invitrogen). Mean fluorescence intensity was measured to calculate final concentration in picograms per milliliter using Bioplex200 and Bioplex Manager 5 software (Bio-Rad). Experiment was performed in triplicate. Statistical analysis of data was performed using unpaired t-test in GraphPad Prism.

SmartSeq2 Sequencing

$1x10^6$ BU1, BU2, and BU3 iPSC-HLCs or triplicate samples of Huh7 cells were seeded in 6-well tissue culture plates, and $1x10^4$ PHHs from Donor A and Donor B were seeded in 96-well glass-bottom plates (n = 1 per iPSC-HLC donor; n = 3 Huh7 cell samples; n = 2 per donor of PHHs). The cells were infected with EBOV at an MOI of 10 or left uninfected and lysed 1 dpi. Cellular RNA was isolated using TRIzol reagent (ThermoFisher) according to the manufacturer's instructions. 50 ng of purified RNA was used for sequencing by diluting to a final concentration of 5 ng/µL in a final volume of 10 µL. Samples were pipetted into a twin.tec® PCR 96-well plate (Eppendorf) and sent out for sequencing on dry ice. Sequencing was performed at the Broad Institute Genomics Services Core. The quality of the raw data was assessed using FastQC v.0.11.7. The sequence reads were aligned to a combination of the human genome reference (GRCh38) and the Ebola virus reference (NC_002549.1) using STAR v.2.6.0c. Counts per gene were summarized using the featureCounts function from the subread package v.1.6.2. The matrix of counts per gene per sample was then analyzed using the limma/voom normalization (limma v. 3.39.19, edgeR v.3.25.10). After exploratory data analysis (Glimma v. 1.11.1), contrasts for differential expression testing were done for each EBOV-infected sample compared to mock-infected samples (controls) at each time point separately. Functional predictions were performed using WebGestalt for gene set analysis (Liao *et al.*, 2019).

Luminescent Caspase 3 Assay

To quantify the activity of caspase 3 in Huh7 cells and HLCs, we utilized the Promega Caspase-Glo 3/7 Kit (cat# G8090). Triplicate samples of $1x10^5$ Huh7 cells or $4x10^5$ HLCs from bBU1, BU2, and BU3 were

infected with EBOV MOI 10 or mock-infected. Cells were also treated with 2 μM Staurosporine (Sigma-Aldrich). At the designated time point, cell supernatant was mixed at a 2:1 ratio with the detection reagent from the Promega kit and incubated for 30 minutes at room temperature. 200 μL of this supernatant mixture was analyzed on a LUMIStar Omega Luminometer (BMG Labtech). Luminescent values were normalized to a blank control and reported as the fold change in luminescent activity over Mock-infected controls. Results from bBU1, BU2, and BU3 samples are averaged and represented as a single HLC sample.

Macrophage Differentiation from Peripheral Mononuclear Blood Cells

MDMs were generated from apheresed peripheral blood mononuclear cells using Ficoll separation (GE Healthcare) from BU1, BU2, and BU3 donors. Whole blood was diluted to a final volume of 30 mL with PBS. 15 mL of Ficoll was added to a 50 mL conical tube, and the diluted whole blood was overlayed onto the Ficoll. Blood was centrifuged for 30 minutes at 450xg with the brake turned off of the centrifuge. The blood separated into three layers: the off-white plasma layer, a thin white buffy coat containing leukocytes, and the pelleted erythrocytes. The plasma layer was removed, and the buffy coat was isolated into a fresh 50 mL conical tube. The buffy coat was diluted to a final volume of 50 mL using 37ºC PBS. Cells were centrifuged at 350xg for 10 minutes with the centrifuge brake on. The cell pellet was then washed three times with 50 mL of warm PBS and centrifuged at 350xg for 10 minutes. After the final wash, cells were resuspended in 1 mL of warm PBS and counted. $1x10^7$ cells were plated in a T75 flask with RPMI medium for one hour at 37ºC and 5% CO_2, non-adhered cells were removed, and 10 ml RPMI medium with 10% FBS, 1% L-glutamine, 10 mM HEPES, 10ng/mL GM-CSF, and 50 U/mL penicillin with 50 mg/mL streptomycin (MDM medium) was added to the cells. Medium was changed every 2 to 3 days for a minimum of 6 days before differentiated macrophages were split into 6-well TC plates for infection experiments.

Supplementary Figures

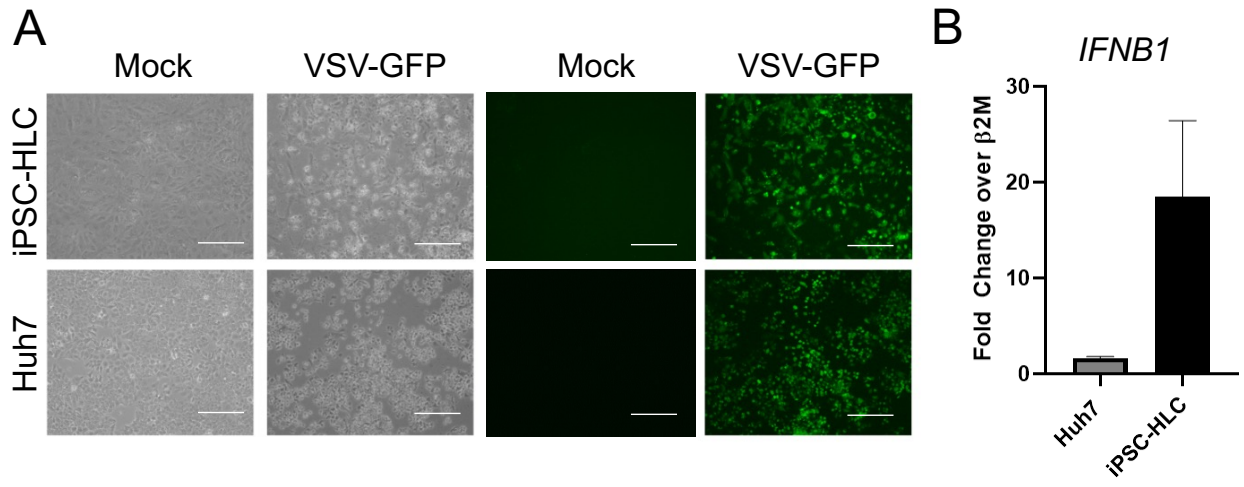


Figure S1. Induction of a type I IFN response in VSV-GFP-infected iPSC-HLCs. (A) Representative brightfield and fluorescence images of iPSC-HLCs and Huh7 cells either mock-infected or infected with VSV-GFP at an MOI of 10 at 1 dpi. Images taken at 10x magnification. Images are representative of iPSC-HLCs from three donors and three replicates of Huh7 cells. Scale bars are 20μm. (B) SYBR green qRT-PCR analysis of iPSC-HLCs and Huh7 cells infected with VSV-GFP. Expression of *IFNB1* was normalized to expression of β-2 microglobulin. Error bars represent SEM of three independent replicates of iPSC-HLCs (BU1, BU2, BU3) and three replicates of Huh7 cells.

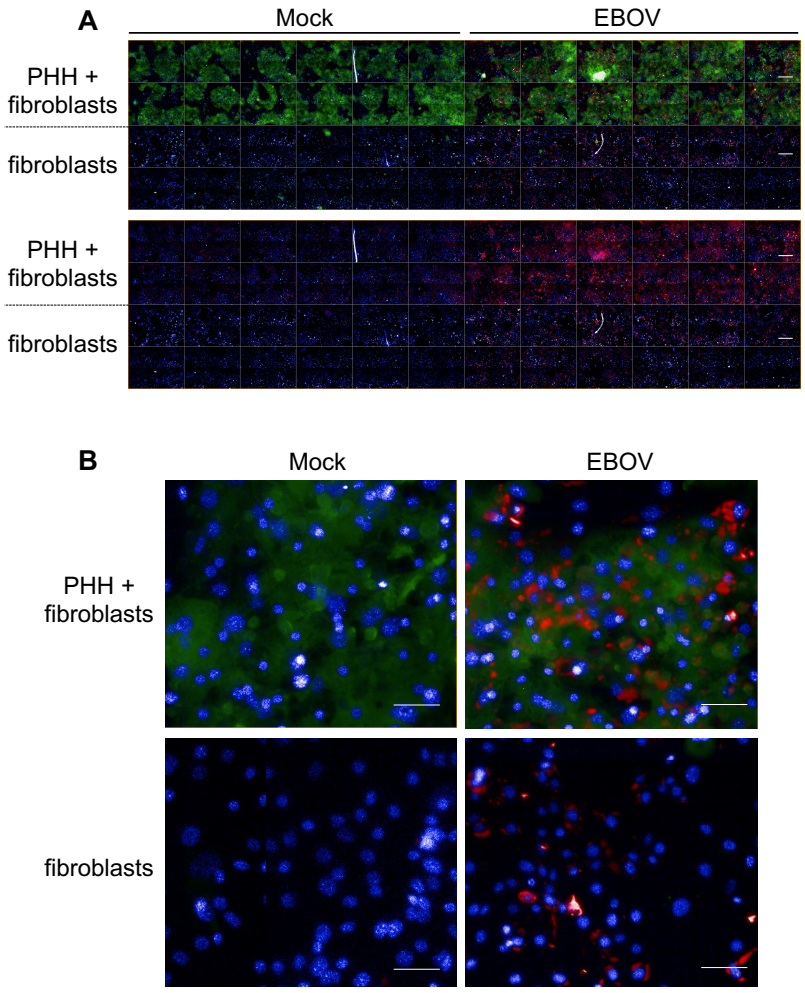


Figure S2. EBOV infection of micropatterned cocultures consisting of primary human hepatocyte islands supported by J2-3T3 murine embryonic fibroblasts. Murine fibroblasts were seeded in 96-well plates with and without primary human hepatocytes (PHH). Cells were mock-infected or infected with EBOV at an MOI of 3 and fixed at 4 dpi. Cells were immunostained with antibodies directed against the EBOV nucleoprotein (red) and human CK18 (liver cell specific; green). Cell nuclei were stained with DAPI (blue). Images captured on an Operetta at 10x magnification. (A) Representative images for donor B. Shown are images taken from three wells per condition with 4 images per well. Upper panel shows DAPI, NP, and CK18 staining, lower panel shows DAPI and NP staining only. Scale bars are 5µm. (B) Higher magnifications of the infected cells. Experiments were performed with PHHs from two donors with similar outcome. Scale bars are 20µm.

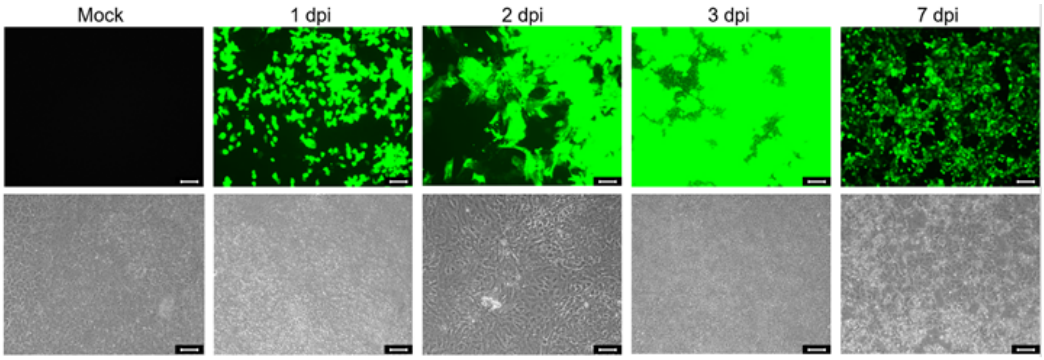


Figure S3. EBOV-infected iPSC-HLCs show CPE at late time points post infection. iPSC-HLCs were infected with EBOV-GFP at an MOI of 10 and imaged at the indicated time points. Mock-infected cells imaged at 1 dpi. Representative images from three independent experiments with similar outcome using cells from three donors (BU1-3). Scale bars are 20 µm.

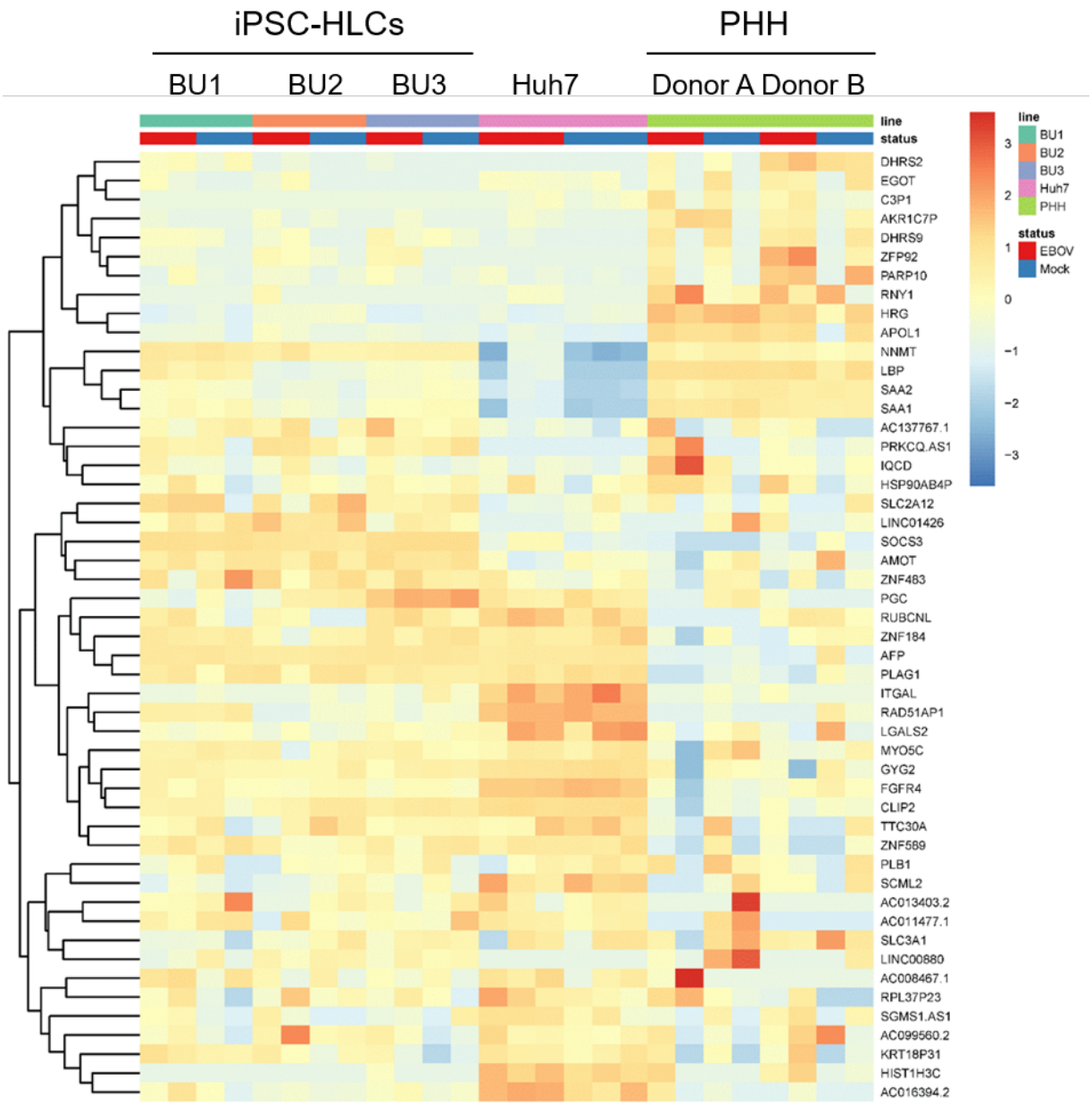


Figure S4. Hepatocytes do not respond to EBOV infection 1 dpi. Heatmap displaying the normalized relative expression of the top 50 genes ranked on absolute logFC across all EBOV-infected hepatocyte infection platforms compared to uninfected controls. No gene in this list was differentially expressed at significant levels when compared to uninfected controls (p-value ≥ 0.05). Each transcript is labeled on the right of the plot. The cell platform and infection status are color-coded on the top of the plot, and the key is located to the right of the plot. Red, EBOV; blue, Mock.

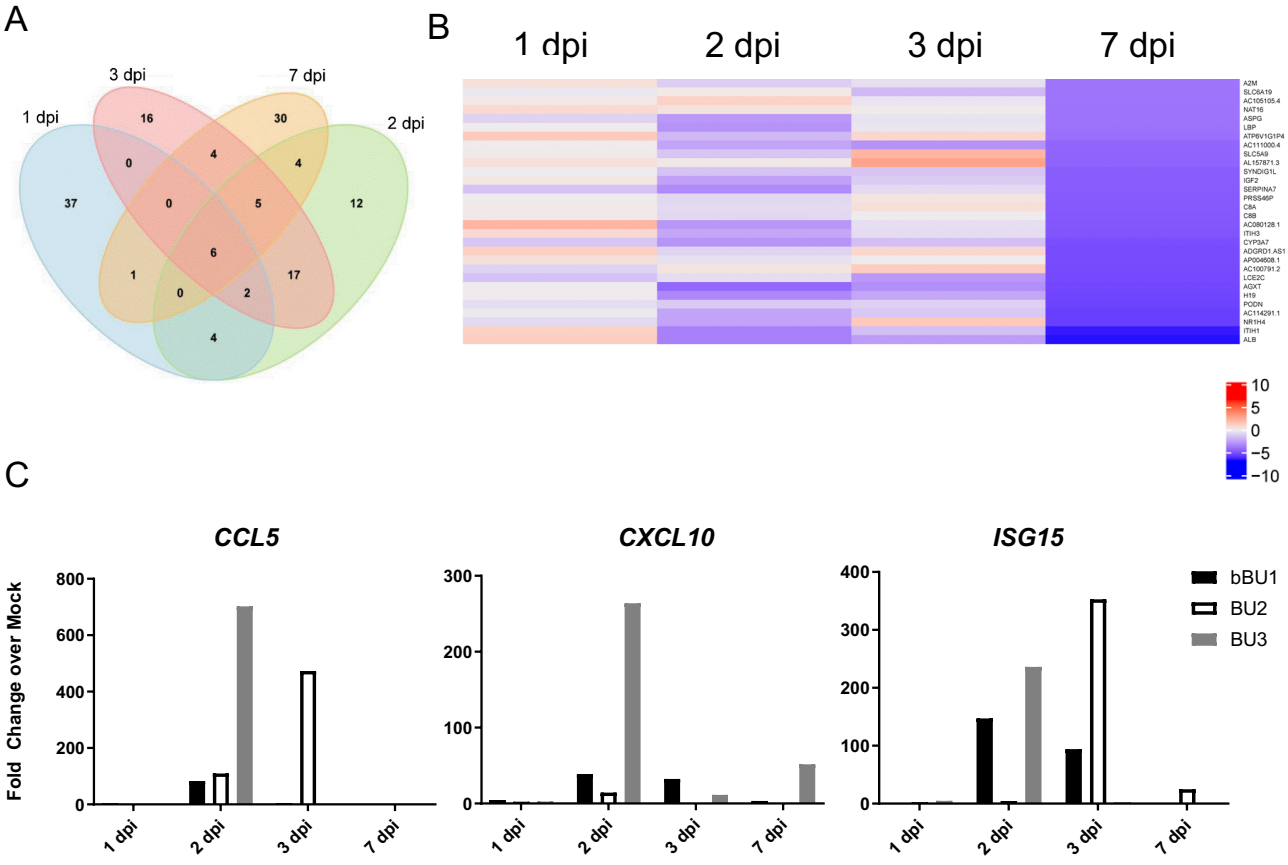


Figure S5. iPSC-HLCs respond to EBOV infection 2-7 dpi. (A) Venn diagram displaying the 50 most DEG for each time point comparison (1 dpi, 2 dpi, 3 dpi, and 7 dpi) and the number of overlapping genes between time points. (B) Heatmap displaying the logFC for the top 30 downregulated DEG at 7 dpi plotted for each time point. List of genes on the right of the heatmap. Key for the value of the logFC to the bottom left of the plot. Time point contrast labeled on the bottom of the plot. (C) qRT-PCR analysis of *CCL5*, *CXCL10* and *ISG15* in three independent iPSC-derived HLC (bBU1, BU2 and BU3) infected with EBOV at 1 dpi, 2 dpi, 3 dpi and 7 dpi.

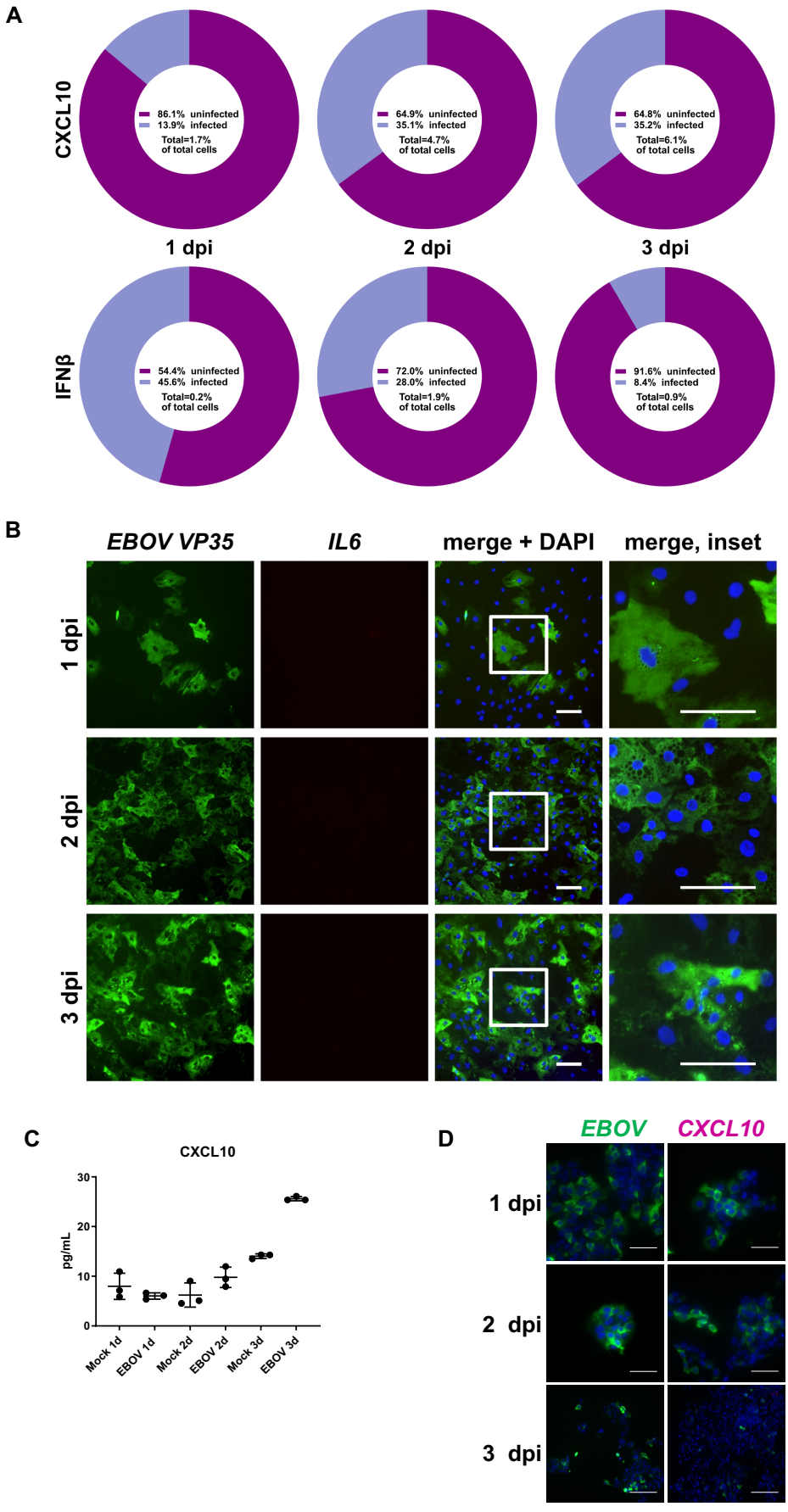
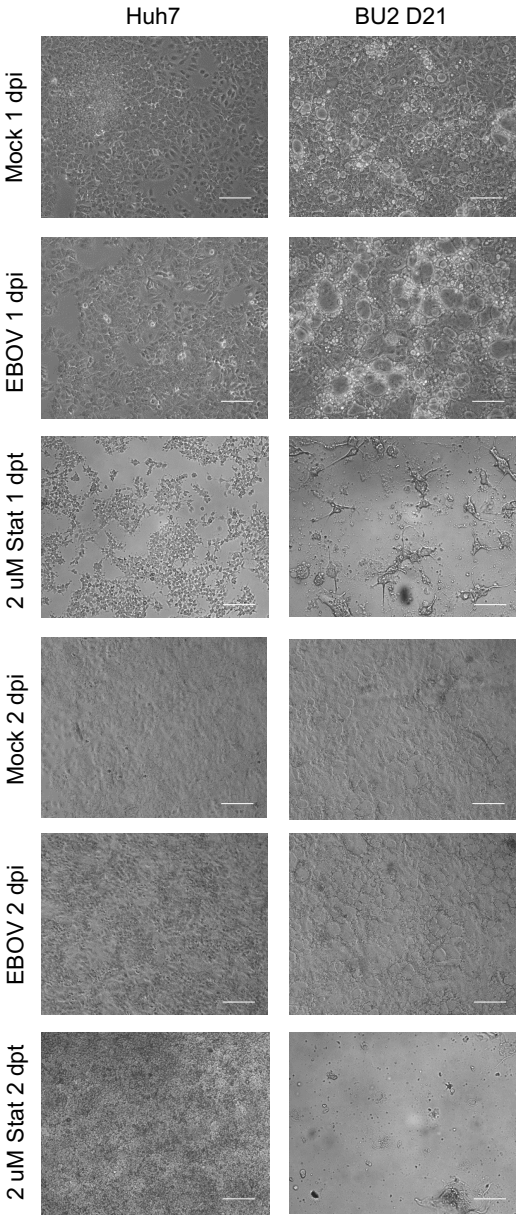

A
CXCL10
86.1% uninfected
13.9% infected
Total=1.7%
of total cells
64.9% uninfected
35.1% infected
Total=4.7%
of total cells
64.8% uninfected
35.2% infected
Total=6.1%
of total cells
1 dpi
2 dpi
3 dpi
IFNβ
54.4% uninfected
45.6% infected
Total=0.2%
of total cells
72.0% uninfected
28.0% infected
Total=1.9%
of total cells
91.6% uninfected
8.4% infected
Total=0.9%
of total cells
B
EBOV VP35
IL6
merge + DAPI
merge, inset
1 dpi
2 dpi
3 dpi
C
CXCL10
pg/mL
30
20
10
0
Mock 1d
EBOV 1d
Mock 2d
EBOV 2d
Mock 3d
EBOV 3d
D
EBOV
CXCL10
1 dpi
2 dpi
3 dpi

Figure S6. RNA FISH analysis of EBOV-infected iPSC-HLCs and Huh7 cells. (A) Ring chart displaying RNA FISH quantification data of *CXCL10*- or *IFNB1* -expressing cells shown in Figure 6. Shown is the percentage of uninfected and infected cells expressing either *CXCL10* (upper panel) or *IFNB1* (IFNβ, lower panel). (B) RNA FISH analysis of EBOV-infected iPSC-HLCs. RNA FISH probes target EBOV mRNA (green) and *IL6* mRNA (red). No red staining was detected. Scale bars are 10μm (C) Luminex analysis of BU1, BU2, and BU3 EBOV-infected and mock-infected iPSC-HLCs. Cells were infected with EBOV at an MOI 10 and cell supernatants were collected from infected and corresponding mock-infected controls 1, 2, and 3 dpi. CXCL10 protein expression was quantified from triplicate technical replicates using a Bioplex. (D) RNA FISH analysis of EBOV-infected Huh7 cells. Cells were infected at an MOI of 10 and fixed at the indicated time points. RNA FISH probes target EBOV mRNA (green) and *CXCL10* mRNA (magenta). No magenta staining was observed. Note that there is significant cell death at 2 and 3 dpi. Scale bars are 20μm.

A


Huh7
BU2 D21
Mock 1 dpi
EBOV 1 dpi
2 uM Stat 1 dpt
Mock 2 dpi
EBOV 2 dpi
2 uM Stat 2 dpt

B

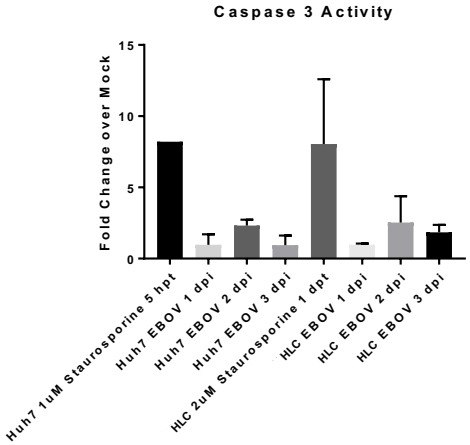

Caspase 3 Activity
Fold Change over Mock
15
10
5
0
Huh7 1uM Staurosporine 5 hpt
Huh7 EBOV 1 dpi
Huh7 EBOV 2 dpi
Huh7 EBOV 3 dpi
HLC 2uM Staurosporine 1 dpt
HLC EBOV 1 dpi
HLC EBOV 2 dpi
HLC EBOV 3 dpi

Figure S7. EBOV infection does not induce apoptosis in infected HLCs or Huh7 cells. (A) Brightfield images of Huh7 cells and BU2 HLCs either infected with EBOV MOI 10, mock infected, or treated with 2μM Staurosporine. Cells were imaged at the indicated time point. (B) Luminescence values of caspase 3 activity using the Promega Caspase-Glo 3/7 Assay kit. Triplicate samples of Huh7 cells and a single replicate each of bBU1, BU2, and BU3 HLCs were infected with EBOV MOI 10, mock infected, or treated with 2μM Staurosporine. Cell supernatants were harvested as the indicated time points and analyzed using a LUMIStar Omega Luminometer (BMG Labtech). Luminescent values were normalized to a blank control and reported as the fold change in luminescent activity over Mock-infected controls. Results from bBU1, BU2, and BU3 samples are averaged and represented as a single HLC sample.

Supplementary Tables

Gene Name	Adjusted p-value	logFC
COL3A1	9.58162E-09	2.079037109
ALB	1.10993E-06	0.791786818
GTF2A1	2.88433E-05	-0.823365329
ACTC1	8.32575E-05	0.166593898
HSPA1B	0.00012209	-1.932617071
HSD3B1	0.000145946	1.098272689
OR2I1P	0.000155119	0.483260067
COL1A2	0.000247591	1.28257751
HSPA1A	0.000373155	-2.049418845
CXCL2	0.000572529	0.671972125
AL161431.1	0.000793635	-0.541597932
EGR1	0.000793635	-0.668323271
ABCC3	0.001710955	-0.029215609
A2M	0.00178052	0.492236335
FMOD	0.001901718	0.699395715
ACTA1	0.002256423	0.144448347
TXNDC5	0.002626023	-1.819276835
MBNL3	0.002934011	0.863087837
AP000892.3	0.003258406	0.394206526
SAA1	0.003258406	0.0002776
TAGLN	0.003274745	0.424662869
AL355916.1	0.003274745	2.400345681
GDF15	0.003274745	-1.426469054
SPON2	0.003446481	0.509329633
H1FX	0.006171997	-1.404491248
ERRFI1	0.007591647	-0.595140628
CD24	0.00802999	0.930873507
MT-ATP6	0.00802999	1.065510209
POLR2A	0.00802999	-0.269318403
AP000648.3	0.008278758	-1.378343082
SRSF3	0.00844516	0.713503971
MX1	0.00844516	-1.76665914
MCAM	0.008722908	-0.504260289
TPM1	0.0091873	0.73398065

Table S1. Summary of host DEG at 1 dpi in EBOV-infected iPSC-HLCs. Transcripts were ranked) and sorted for transcripts with a False Discovery Rate adjusted p-value ≤ 0.01 when compared to the other time point and uninfected contrasts. The logFC of the transcript when compared to the uninfected iPSC-HLCs at 1 dpi is indicated in the logFC column (last column). logFC, log fold change compared to uninfected control.

Time Point	Pathway	Leading Edge Number	Enrichment Score	FDR
1 dpi	N/A			
2 dpi	Interferon alpha response	29	0.69408	0
	Interferon gamma response	51	0.59635	0
	TNFA signaling via NFkB	60	0.45735	0.0053618
	Blood vessel formation	24	-0.70587	0
	Epithelial mesenchymal transition	97	-0.44221	0.00066813
	Biosynthesis of bile acids	35	-0.48976	0.00083516
	Metabolism of xenobiotics	69	-0.47167	0.00083516
	Blood coagulation cascade	51	-0.48574	0.0011135
3 dpi	Interferon alpha response	31	0.70268	0
	Interferon gamma response	46	0.58008	0
	Oxidative phosphorylation and citric acid cycle	116	-0.58008	0
	MYC targets, variant 1	80	-0.40318	0.0015312
	Blood vessel formation	16	-0.60886	0.0015312
	Adipocyte development	77	-0.37443	0.0020416
	Reactive oxygen species pathway	30	-0.51705	0.0020416
	Epithelial mesenchymal transition	78	-0.38571	0.0024499
	Blood coagulation cascade	45	-0.40118	0.0034998
7 dpi	Metabolism of xenobiotics	47	-0.48082	0
	Blood coagulation cascade	39	-0.62257	0
	Biosynthesis of bile acids	30	-0.48532	0.0012961
	Unfolded protein response; ER stress	33	-0.45886	0.0077766
	Complement cascade	31	-0.41966	0.0089431

Table S2. Summary of Hallmark Pathway Analysis of EBOV-infected iPSC-HLCs. GSEA of Hallmark 50 Pathways for each time point of EBOV-infected iPSC-HLCs as compared to uninfected controls. Leading Edge Number represents the number of genes mapping to the hallmark pathway. Enrichment score is a measurement of the significance of the up or downregulation of the pathway based on the ranked genes that map to the particular pathway (closer to 1, more significantly upregulated; closer to -1, more significantly downregulated). Values greater than 0 are upregulated pathways, values less than 0 are downregulated pathways. FDR = false discovery rate. Parameters: minimum number of genes in a category = 15; FDR ≤ 0.01; number of permutations = 1000.

Antigen	Company (catalog number)	Species	Dilution
Albumin	Dako	Rabbit	1:100
Alexa Fluor α-mouse 594	Thermo Fisher (A32744)	Donkey	1:200

Alexa Fluor α-rabbit 488	Thermo Fisher (A48282)	Goat	1:200
Alexa Fluor α-goat 488	Thermo Fisher (A32814)	Donkey	1:200
CK18, human	Abcam (EPR1626)	Rabbit	1:100
EBOV NP	Gift from G. Olinger, USAMRIID	Mouse	1:200
EBOV VP35	Antagene, custom	Goat	1:1000
EBOV VP40	BEI	Mouse	1:200
FOXA1	Abcam (ab173287)	Rabbit	1:100
FOXA2	Abcam (ab108422)	Rabbit	1:100
HNF4α	Abcam (ab41898)	Mouse	1:50
NFκB p65 (A)	Santa Cruz (sc-8008)	Rabbit	1:200
Transferrin	ThermoFisher (MA5-29600)	Rabbit	1:50

Table S3. Antibodies used for immunofluorescence analysis. Not all antibodies in this table are commercially available at this time. In those cases, the catalog number has been omitted.

Medium Component	Final Concentration or Volume	Source
D5-D6		
Activin A	50 µg/mL	R&D Systems
BMP-4	10 ng/mL	Peprotech
FGF-2	10 ng/mL	Peprotech
VEGF	10 ng/mL	Peprotech
D7-D12		
Ascorbic Acid	50 µg/mL	Sigma Aldrich
BMP-4	50 ng/mL	Peprotech
FGF-2	10 ng/mL	Peprotech
VEGF	10 ng/mL	Peprotech
EGF	10 ng/mL	Peprotech
TGF-α	20 ng/mL	Peprotech
HGF	20 ng/mL	Peprotech
Dexamethasone	100 nM	Invitrogen
D13-D18		
FGF-2	10 ng/mL	Peprotech
VEGF	10 ng/mL	Peprotech
EGF	10 ng/mL	Peprotech

HGF	20 ng/mL	Peprotech
Dexamethasone	100 nM	Invitrogen
Oncostatin M	20 ng/mL	Peprotech
Vitamin K	6 ug/mL	Sigma Aldrich
DMSO	1%	Sigma Aldrich
γ-Secretase Inhibitor	1.5 μM	Calbiochem
D19-D30		
HGF	20 ng/mL	Peprotech
Dexamethasone	100 nM	Invitrogen
Oncostatin M	20 ng/mL	Peprotech
Vitamin K	6 μg/mL	Sigma Aldrich

Table S4. Differentiation protocol for hepatocyte-like cells from iPSCs. The concentration of each medium component and manufacturer are listed for each period of differentiation.

REFERENCES

Liao, Y., Wang, J., Jaehnig, E.J., Shi, Z., and Zhang, B. (2019). WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. Nucleic Acids Res *47*, W199-W205. 10.1093/nar/gkz401.