SUPPLEMENTAL INFORMATION

Mouse Engraftment and HCV Infection of Human Stem Cell-Derived Hepatocytes

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Supplemental Table 1: Genes Implicated in HCV Life Cycle (34, 35) and Induced During Hepatic Differentiation of Pluripotent Stem Cells (36).

Symbol	**Alternate symbol**	**Gene name**	**Overexpressed during**	**Role in HCV cycle**	**Ref.**
FOXA2	*HNF3B*	Forkhead box A2	Definitive endoderm to hepatocytes maturation	Unknown	-
SMAD6		SMAD family member 6	Definitive endoderm to hepatocyte maturation	Unknown	-
HIST1H2BK		Histone cluster 1, H2bk	Definitive endoderm to hepatocyte maturation	Unknown	-
APOB		Apolipoprotein B	Hepatic specification and hepatocyte maturation	Assembly/ Maturation	31, 33
MTTP	*MTP*	Microsomal triglyceride transfer protein	Hepatic specification and hepatocyte maturation	Assembly/ Maturation	31, 33
FER1L3	*MYOF*	Myoferlin	Hepatic specification and hepatocyte maturation	Unknown	-
TWIST1		Twist homolog 1	Hepatic specification and hepatocyte maturation	Unknown	-
CLDN1		Claudin 1	Hepatocyte maturation	Viral entry	65

Supplemental Table 2: Clinical Isolates Used for in Vitro and in Vivo Infection of HLCs.

To be noted, 21 sera from patients chronically infected with HCV, with CHC, routinely followed at the NIH Clinical Center, were first screened for in vitro infectivity of PHHs. Patients were not under treatment at the time the sera were collected. Out of these 21 sera, 12 were infectious in vitro for PHHs, as demonstrated with visualization of HDFR relocalization events (Fang Zhang, unpublished observations). The most infectious serum for each genotype was then used for inoculation of HLCs in the same conditions.

Sera	**HCV RNA**	**HDFR in PHHs**	**HDFR in HLCs**
Genotype	Copies/mL of serum	% of cells with relocalization 3 days pi	
Genotype 1a	14,800,000	0.1%	0.04%
Genotype 1b	40,900,000	0.2%	0.02%
Genotype 2	10,500,000	0.3%	0.01%
Genotype 3	16,500,000	0.1%	0.02%
Genotype 4	13,400,000	0.4%	0.02%
Ctrl non-inf		0%	0%

Supplemental Table 3: Antibodies Used in our Analyses.

Antigen	Distributor	Cat#	Host	Dilution
AAT	Dako	A0012	Rabbit	1:50
AAT (in situ staining)	Cell Marque	223A-15	Rabbit	1:1000
AFP	Sigma Aldrich	A8452	Mouse	1:330
AFP (in situ staining)	Cell Marque	203A-15	Rabbit	1:100
ALB	Cedarlane	CL2513A	Mouse	1:330
ALB (in situ staining)	Bethyl Laboratories	A80-229A	Goat	1:500
Mouse ALB	Bethyl	A90-234F	Goat	1:100
CD81	BD Pharmingen	555675	Mouse	1:200
CLDN1	Life Technologies	18-7362	Rabbit	1:200
CK7	Dako	M7018	Mouse	1:50
CK18	Dako	M7010	Mouse	1:50
CYP2D6	Sigma Aldrich	AV41675	Rabbit	1:200
CYP3A4	Abcam	ab135813	Rabbit	1:50
Glutamine synthetase	Abcam	ab16802	Rabbit	1:1000
HCV Core	Thermo Scientific	C7-50	Mouse	1:200
HCV NS5A	Abcam	ab20773	Goat	1:200
Hep Par-1	Dako	M7158	Mouse	1:50
HNF3B	Santa Cruz Biotechnology	sc-271103	Mouse	1:100
HNF4A	Santa Cruz Biotechnology	sc-6556	Goat	1:100
KI67	Abcam	ab15580	Rabbit	1:100
OCLN	Life Technologies	404700	Mouse	1:200
OCT4	Santa Cruz Biotechnology	sc-9081	Rabbit	1:400
SCARB1	Novus Biologicals	NB400-104	Mouse	1:200
SOX17	R&D Systems	AF1924	Goat	1:100
TRA-1-81	Stemgent	09-0069	Mouse	1:1000

Supplemental Table 4: Taqman RT-qPCR Assays (from IDT and Applied Biosystems)

Name	Gene Symbol	Ref Seq #	IDT Assay ID
AFP	*AFP*	NM_001134	Hs.PT.42.3900012
Albumin	*ALB*	NM_000477	Hs.PT.42.3975397
ApoB100	*APOB*	NM_000384.2	Hs.PT.49a.19389676
ApoE	*APOE*	NM_000041.2	Hs.PT.49a.21148243
ASGR1	*ASGR1*	NM_001671	Hs.PT.42.2279045
CD81	*CD81*	NM_004356	Hs.PT.47.3786606
CLDN1	*CLDN1*	NM_021101	Hs.PT.47.3130958
CYP3A4	*CYP3A4*	NM_017460	Hs.PT.4919228102.g
CYP3A7	*CYP3A7*	NM_000765	Hs.PT.42.663713
CYP7A1	*CYP7A1*	NM_000780	Hs.PT.42.2603300
FOXA2	*FOXA2*	NM_021784	Hs.PT.49a.3462639
GAPDH	*GAPDH*	NM_014364	Hs.PT.42.474589
HIST1H2BK	*HIST1H2BK*	NM_080593	HS.PT.49A.20210756
HNF4A	*HNF4A*	NM_178849	Hs.PT.53a.24630422
Myoferlin	*MYOF*	NM_133337	Hs.PT.49a.1682436
NANOG	*NANOG*	NM_024865	Hs.PT.49.243900
OCLN	*OCLN*	NM_002538	Hs.PT.47.19052532.g
OCT4	*POU5F1*	NM_203289	Hs.PT.49.20925921.g
SCARB1	*SCARB1*	NM_005505	Hs.PT.47.14853299
SMAD6	*SMAD6*	NM_005585	Hs.PT.49a.19248369.g
SOX17	*SOX17*	NM_022454	Hs.PT.42.543418.g
SRSF7	*SRSF7*	NM_019616	Hs.PT.42.4042596.g
TWIST1	*TWIST1*	NM_000474	HS.PT.49A.18940950

Name	Gene Symbol	Ref Seq #	Applied Biosystems Part Number
IL28B	*IFNL3*	NM_172139.2	Hs99999041_m1
IP10	*CXCL10*	NM_001565.3	Hs01911452_s1
ISG15	*ISG15*	NM_005101.3	Hs01921425_s1
RSAD2	*RSAD2*	NM_080657.4	Hs00369813_m1

Supplemental Table 5: Details about engraftment experiments

Engraftment	Mouse ID	Age at time of engraftment	Gender	SC type	Protocol of differentiation into HLCs			Serum hALB titer (µg/mL)		Sacrificed	Experiment
					Definitive Endoderm	Hepatic Specification	Maturation	at day 10 pe	at day 100 pe		
#1	M1-1	5 months	F	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	430	1	Day 100 pe	Ctrl non inf
	M1-2	5 months	M	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	1560	334	Day 100 pe	Gt1a HCV (low titer)
	M1-3	5 months	F	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	2250	290	Day 100 pe	Gt1a HCV (low titer)
	M1-4	5 months	M	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	1770	123	Day 100 pe	Gt1a HCV (low titer)
#2	M2-1	7 months	M	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	430	6415	Day 100 pe	Gt1a HCV (low titer)
	M2-2	7 months	F	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	490	520	Day 100 pe	Gt1a HCV (low titer)
	M2-3	7 months	F	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	2000	1416	Day 100 pe	Gt1a HCV (low titer)
#3	101	5 months	M	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	1710	180	Day 100 pe	Gt3a HCV
	102	5 months	F	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	1750	10	Day 100 pe	Gt3a HCV
	103	5 months	M	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	620	3800	Day 100 pe	Gt3a HCV
	104	5 months	M	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	*Died during engraftment*			
	105	5 months	F	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	1100	2340	Day 100 pe	Ctrl non inf
#4	106	6 months	M	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	1795	*Died on day 70*		Gt1b HCV
	107	6 months	M	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	463	428	Day 100 pe	Gt1b HCV
	108	6 months	F	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	85	610	Day 100 pe	Gt1b HCV
	109	6 months	F	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	842	710	Day 100 pe	Gt1b HCV
	110	6 months	F	HSF-6 ESC	Basma et al.	HGF + DMSO	DEX only	50	160	Day 100 pe	Ctrl non inf
#5	M3-1	7 months	M	iPSC	Basma et al.	HGF + DMSO	DEX only	450	nd	Day 100 pe	Gt1a HCV (high titer)
	M3-2	7 months	M	iPSC	Basma et al.	HGF + DMSO	DEX only	1179	nd	Day 100 pe	Gt1a HCV (high titer)
	M3-3	7 months	F	iPSC	Basma et al.	HGF + DMSO	DEX only	6	nd	Day 100 pe	Gt1a HCV (high titer)
	M3-4	7 months	F	iPSC	Basma et al.	HGF + DMSO	DEX only	3900	nd	Day 100 pe	Gt1a HCV (high titer)
#6	M201	8 months	F	iPSC	STEMDiff Kit	HGF + DMSO	DEX only	415	nd	Day 14 pe	
	M202	8 months	F	iPSC	STEMDiff Kit	HGF + DMSO	DEX only	701	nd	Day 14 pe	
	M203	8 months	F	iPSC	STEMDiff Kit	HGF + DMSO	DEX only	459	nd	Day 14 pe	Kinetics 14 days
	M204	7 months	M	iPSC	STEMDiff Kit	HGF + DMSO	DEX only	408	nd	Day 14 pe	
	M205	7 months	M	iPSC	STEMDiff Kit	HGF + DMSO	DEX only	243	nd	Day 14 pe	

Supplemental Experimental Procedures:

hiPSC Induction

Human iPSCs were generated using the Human STEMCCA Cre-excisable constitutive polycistronic lentiviruses, expressing the transcription factors OCT4, KLF4, SOX2 and c-MYC (28). STEMCCA lentiviruses were produced in 293T cells transduced with the pHAGE2-hSTEMCCA-loxp, RC-CMV Rev1b and HDM-Hgpm2 plasmids using Fugene 6 (Promega) and cultured on Poly L Lysine. 24, 48 and 72 hour post-transduction, the supernatants were collected, filtered on 0.22 μM nylon mesh and stored at -80^{0}C. Produced lentiviruses were titrated by ELISA for p24, following the manufacturer's instructions (BD Bioscience Clontech Laboratories).

Control primary human fibroblasts (PHF) were obtained from ATCC. Patient fibroblasts were isolated from punch skin biopsies. Briefly, skin biopsies are first subjected to an enzymatic digestion of the dermis, floating on a 5% dispase solution (Life Technologies), with only the dermis in contact with the enzyme, overnight at 4^{0}C. The epidermis is then mechanically separated from the dermis, and treated 5 minutes at room temperature with trypsin EDTA (Life Technologies). Epidermis cells are then separated by pipetting up and down in culture medium and pellet by centrifugation (1000 rpm 5 minutes). Isolated fibroblasts are then cultured on rat-tail, type I collagen (Life Technologies) in PHF medium composed of DMEM, 10% FCS, 1% NEAA and PS (Life Technologies).

Early passaged PHF at 50% confluence were incubated overnight with STEMCCA lentiviruses at a M.O.I of 1, in presence of 10 μg/mL of polybrene (Sigma Aldrich), in 1 well of a 6w plate.

The day after, the medium is changed for 2.5 mL of PHF medium (MEM, 10% FCS, NEAA, sodium pyruvate, from Life Technologies). Six days later, the cells are passed in one D100 petri dish coated the day before with 3.10^6 irradiated mouse embryonic fibroblasts (MEF CF-1 IRR from Globalstem) on 0.1% Gelatin (Sigma Aldrich). The cells are then cultured for the next 3-4 weeks in hESC medium, composed of DMEM-F12, 10% KOSR, 5% Glutamine, 1% NEAA, 1% PS (all from Life Technologies), 0.1 mM beta mercaptoethanol (Sigma Aldrich), with 10 µg/mL of basic-FGF (R&D Systems). After 3 weeks, SC-like dense colonies are visible and start growing. Cells were stained for TRA-1-81 using the Stemgent Stain Alive DyLight 488 mouse anti-human TRA-1-81, diluted 1/1000 in hESC medium, incubated for 30 min at 37^0C (Stemgent). After intensive washes, TRA-1-81 positive colonies were manually picked and expanded on MEF cells (Globalstem), and then adapted to feeder free condition, on BD Growth factor reduced Matrigel (BD Biosciences) in mTeSR1 medium (Stemcell Technologies). Stem cell phenotype of the selected iPSC colonies was confirmed by immunofluorescence assay for human OCT4 (Santa Cruz Biotechnology) (See Supplementary table 3 for working dilution), by alkaline phosphatase assay (Vector Laboratories), and analyzed by RTqPCR for SC pluripotency makers (See supplemental table 4 for reactions references).

Generated human iPSC and control HSF-6, H1 or H9 ESC (WiCell) were routinely maintained in culture on BD Growth Factor reduced Matrigel (BD Biosciences) in mTeSR1 medium (Stemcell Technologies).

Hepatic Differentiation

Hepatic differentiation of human pluripotent stem cells was performed following a three-step protocol adapted from Basma et al (9). First, human pluripotent stem cells at 60-70%

confluence are subjected to a 3 days treatment with 100 ng/mL Activin A and 100 ng/mL bFGF (R&D Systems), in the presence of increasing level of FCS (0% on day 1, 0.2% on day 2, and 2% on day 3) to induce definitive endoderm (DE). In case of limited number of cells at day 3 (for example due to high cytotoxicity after the first day of AA treatment), DE cells could be maintained 2 more days in medium containing 100 ng/ml AA and bFGF, and 2% FCS, to allow growth of enough cells for subsequent differentiation. The efficiency of induction of DE is then assessed by immunofluorescence for SOX17 (R&D Systems) and FOXA2 (Santa Cruz Biotechnology) (See Supplemental Table 3 for antibodies references and working dilution). Only cell preparations with above 75% of SOX17 positive cells were used for further differentiation. To be noted, very consistent and reproducible definitive endoderm could also generated using the STEMdiff Definitive Endoderm Kit (Stemcell Biotechnologies) following the manufacturer's instructions.

Confluent definitive endoderm cells are then passed 1 to 3 in presence of Rock Inhibitor on growth factors reduced Matrigel (BD Biosciences) and cultured for 8 days in differentiation medium (DMEM F12, 10% KOSR, with 1% NEAA and 1% Glutamine) containing 100 ng/mL of HGF (Peprotech) and 1% DMSO (Sigma Aldrich), to promote hepatic specification. The percentage of alpha-fetoprotein (AFP)-positive cells was then assessed by immunofluorescence (Sigma-Aldrich). Finally, the hepatoblasts are matured in DMSO-free differentiation medium with 10^{-7}M of Dexamethasone for 3 days. The percentage of albumin-positive cells was used to validate the efficiency of differentiation. Hepatocyte-like cells (HLCs) were then maintained for up to 1 week, in Hepatocyte Culture Medium (L15 medium, 8.4% FCS + 1% glutamine + 10%

tryptose phosphate, all from Life Technologies) containing 1 μM insulin, 10 μM hydrocortisone and 10^{-7} M of dexamethasone (all from Sigma Aldrich).

Immunofluorescence Assay

Expression of differentiation markers was assessed throughout the differentiation process by immunofluorescence assay. We also assessed the expression of the 4 HCV entry factors CD81, SCARB1, Claudin-1 (CLDN1), occludin (OCLN) in human PSCs and differentiated cells. After one wash with PBS, cells were fixed with 4% PFA 30 minutes at RT, permeabilized with PBS 0.5% Triton for 15 min at RT, and saturated with PBS 3% BSA for 15 min at RT. Primary antibodies are diluted in PBS 1% BSA 0.1% Triton, and incubated overnight at 4^0C (See Supplementary table 3 for working dilution). After several washes with PBS 0.1% Triton, preparations were incubated with anti-mouse, anti-goat (both from Life Technologies) or anti-rabbit (Abcam) IgG secondary antibodies, conjugated with ALEXA 488, 555 or 647, diluted 1/1000 in PBS 1% BSA 0.1% Triton, for 30 minutes at RT. Cells were then counterstained with DAPI, diluted 1/10,000 in PBS, for 1 minute at RT, and read on an inverted fluorescence microscope (EvosFL from AMG).

FACS analysis

Pluripotent Stem Cells and Definitive Endoderm Cells were re-suspended using Accutase (Life Technologies). Hepatoblasts and HLCs were re-suspended using 0.25% Trypsin and 2.21mM EDTA in HBSS (Cellgro) for 10 minutes at 37 degrees C. Cells were washed twice in Incubation Buffer (PBS, 1% FBS, 0.1% Sodium Azide, 2mM EDTA), by centrifuging at 200 g for 4 minutes, and fixed for 30 minutes at RT in PFA 4%. Cells were then permeabilized using PBS

0.5% Triton X100 for 10 minutes, and saturated using PBS 3% FBS for 15 minutes, at RT. Cells were incubated overnight at 4 degrees with the combination of primary antibodies diluted in incubation buffer (See Supplementary table 3 for working dilution). The day after, cells are washed twice in incubation buffer, and incubated one hour at RT in dark with secondary antibodies diluted 1/1000 in incubation buffer. After 2 washes, cells are kept in PFA 2% and run through a BD LSR II Flow Cytometer (BD Biosciences).

RT-qPCR

Total mRNA was isolated from cultured cells at the different steps of differentiation using the RNeasy minikit (QIAGEN), following the manufacturer's instructions. Isolated mRNA was quantified using a Nanodrop, stored at -80 degrees c, and a total amount of 10ng was used per reaction. Taqman primers were purchased from IDT or from Applied Biosystems (See Supplementary table 4 for reactions reference) and one step RTqPCR was performed using the Thermo Scientific Verso 1-step QRT-PCR Low ROX Kit (Thermo Scientific), according to the manufacturer's instructions. Results were then expressed as relative expression, as described by Livak et al (66).

ELISA

Secretion of human alpha-fetoprotein (AFP) (Calbiotech), albumin (Bethyl Laboratories) and apolipoprotein (Apo)-B100 (Mabtech) in the culture supernatant was assessed by ELISA, according to the manufacturers instructions. 96-wells ELISA plates pre-coated with purified antibodies are incubated overnight at 4 degrees C with pure or diluted supernatants. The day after, plates are washed and incubated 1 hour at 37 degrees C with HRP conjugated antibodies. After washes, the HRP activity is assessed using OPD and read at 492nm.

To analyze the cellular response to HCV infection, we assessed by ELISA the production of human IFNA, IFNB (PBL Interferon Source), CXCL10 and IL-28 (RayBiotech) in the supernatant of infected cells and non-infected cells, following the manufacturers instructions.

Functional Assays on Differentiated Cells

Low Density Lipoprotein (LDL) uptake was assessed by incubation of the differentiated hepatocytes with Alexa 488-conjugated acetylated LDL (Life Technologies) at a final concentration of 20 μg/mL in PHH medium, for 4h at 37^0C. After extensive washes, positive cells are visualized using an inverted fluorescence microscope. Glycogen storage was assessed by Periodic Acid Schiff (PAS) staining. Cells fixed 30 minutes in PFA 4%, were washed with PBS, permeabilized with PBS 0.1% triton, and treated with Periodic Acid solution and then Schiff solution, as described by the manufacturer (Sigma Aldrich). Cells were counterstained with hematoxyline. Lipid storage was analyzed by Oil Red O (ORO) Staining. Differentiated cells are fixed for 30 min at RT in PFA 4%, and then incubated at RT 25 minutes in a solution of Oil Red O (Sigma Aldrich). Finally cells were intensively washed with PBS.

Indocyanin Green (ICG) (Sigma Aldrich) was diluted in H2O at 10 mg/mL, and then diluted 1/10 in Hepatocyte Culture medium. Cells were incubated for 1 hour at 37^0C in the medium containing ICG, washed 3 times with PBS, and maintained in Hepatocyte Medium. ICG staining was observed at that time and 6 hours later, to observe the liver-specific internalization and secretion of ICG. Cells stained with PAS, ORO or ICG were visualized using a bright field and contrast phase microscopy (EvosFL, AMG).

In Vitro HCV Infection of HLCs

HCVcc of genotype 2a (isolate JFH1) were produced in Huh7.5.1 (provided by F. Chisari, Scripps Research Institute), as described previously (19). HLCs at day 14 of differentiation were incubated overnight at 37^0C, with JFH1-HCVcc, at a Multiplicity of Infection (MOI) of 0.5. For inoculation with clinical isolates, patient sera of different genotypes (Gt1a, 1b, 2, 3, 4) were previously screened for high infectivity on PHHs by HDFR assay (Fang Zhang et al., unpublished observations) (Supplemental table 2). HLCs were incubated overnight with 100 μL of the infectious HCV(+) serum. The day after, the cells were washed extensively with PBS and maintained for up to 6 days in Hepatocyte Culture medium. On the same day, viral inhibition assay was performed by keeping the inoculated cells in medium containing 100 μM of 2'-C-methylcytidine (2MC)(US Biological), a potent inhibitor of the HCV RNA dependent RNA polymerase.

Intracellular replication of the viral genome was assessed by quantitative RTqPCR of HCV RNA at different times post-infection (pi). Total cellular RNA was isolated with the RNeasy Minikit (QIAGEN). HCV RNA was then quantified using a Taqman RTqPCR technique as previously described (21, 63, 64) using the Verso 1-Step QRT-PCR Low ROX Kit (Thermo Scientific) on an Applied Biosystems AB ViiA7 machine (Life Technologies). HCV RNA was isolated from culture supernatant using the Viral RNA Minikit (QIAGEN), and then quantified by RTqPCR as described previously. HCV Core antigen was quantified in the cell culture supernatant by ELISA using the QuickTiter HCV Core Antigen ELISA Kit (Cell Biolabs) following the manufacturer's instructions. Infectivity of the progeny virions was assessed by inoculation of highly permissive Huh7.5.1 with serial dilutions of supernatant from infected HLCs. 3 days later, the cells were fixed in PFA 4% and stained for HCV core protein (C7-50

clone, Thermo Scientific) and HCV core positive foci were visualized using an inverted fluorescent microscope (EvosFL, AMG).

Visualization of Infected HLCs

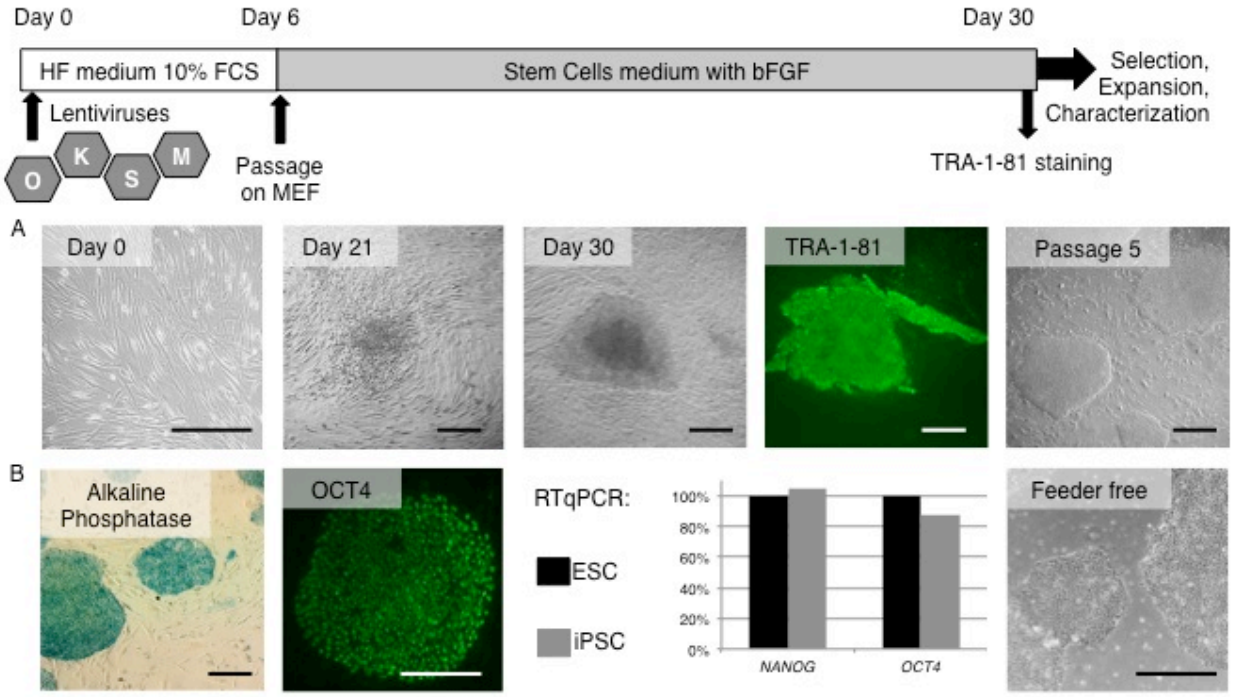
Infected HLCs were visualized using Hepatitis C Dependent Fluorescent Relocalization (HDFR) (37). This strategy is based on the observation that the HCV nuclease NS3/4A specifically and efficiently cleaves the Interferon-beta promoter stimulator 1 protein (IPS-1) (67, 68). Briefly, HLCs were transduced with a lentiviral vector, expressing the C-terminal region of IPS-1 (including the transmembrane domain and the NS3-4A cleavage site) fused to EGFP (EGFP-IPS), or fused to RFP with the SV40 nuclear localization sequence (RFP-NLS-IPS), in presence of 4 μg/mL of Polybrene. The day after, the efficiency of transduction could be assessed by live immunofluorescence, the reporter being expressed on the surface of the mitochondria. Upon infection, HCV NS3/4A cleaves the vector, leading to relocalization of the fluorescence from the mitochondria membrane to the entire cell for the EGFP-IPS vector, or to the nucleus only for the RFP-NLS-IPS vector. The cells were examined the following days for relocalization events, indicating HCV infection.

In situ immunostaining

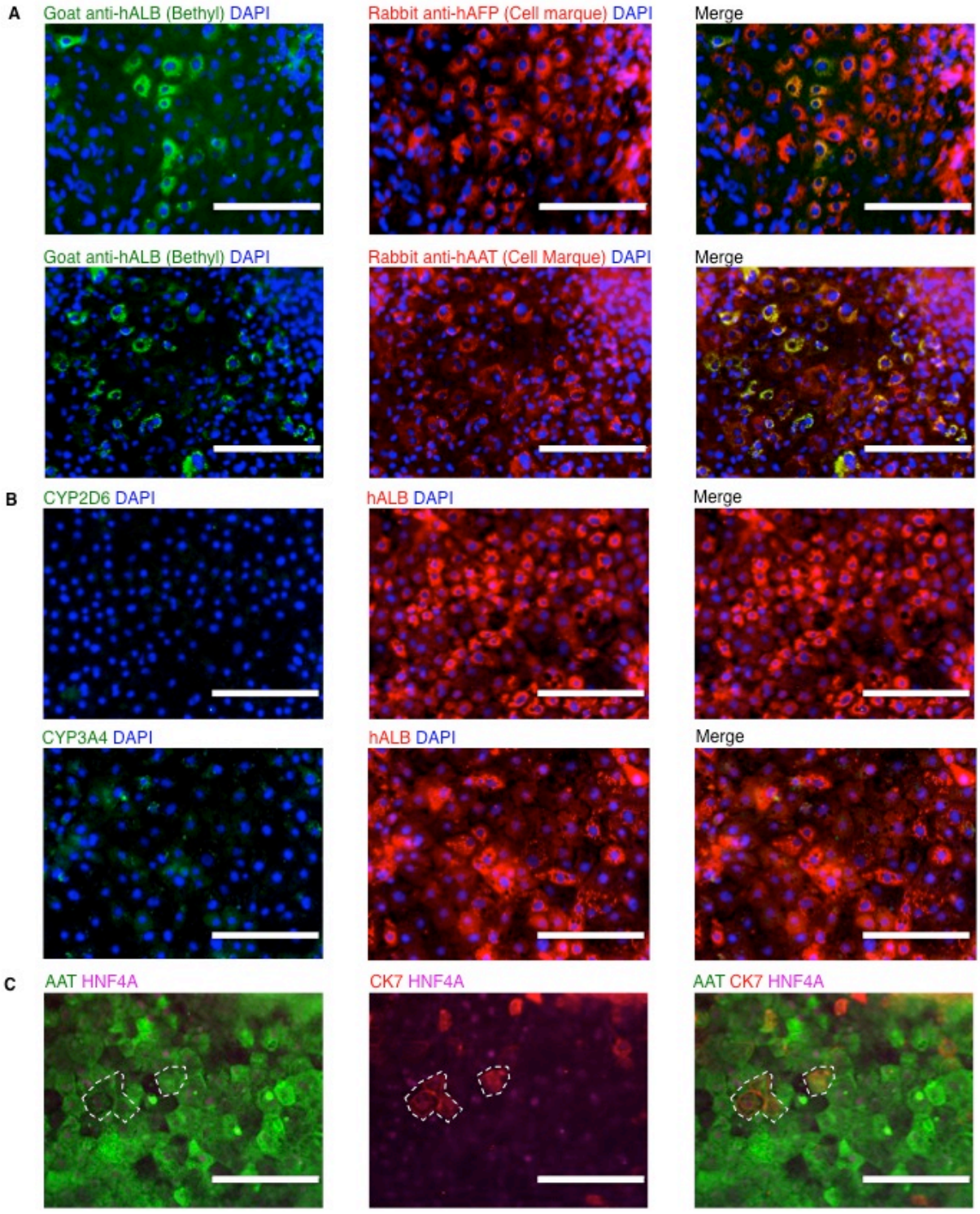
Paraffin-embedded tissues were cut using a Cryostat into sections of 5 μm. Detection of human Hep Par-1 (Clone OCH1E5) and cytokeratin CK18 (Clone DC-10) was assessed by immunohistochemistry using Background Buster (Innovex Biosciences), Citrate-based Antigen Unmasking Solution (Vector) and the Dako Animal Research Kit (ARK) Peroxidase, following the manufacturers instructions. Sections were counterstained with Hematoxyline. In situ

expression of human albumin, AFP, AAT, Ki67 and mouse/human Glutamine Synthetase, CYP2D6 and 3A4 was assessed by immunofluorescence. De-parafinized tissues were treated with Background Buster and Vector Citrate-based Antigen Unmasking Solution, then incubated overnight with primary antibodies (See supplemental table 3 for reference and working dilutions), then one hour with secondary antibodies. Stained sections were then observed using a confocal laser-scanning microscope (Axio Observer.Z1, Carl Zeiss).

Supplemental Figures

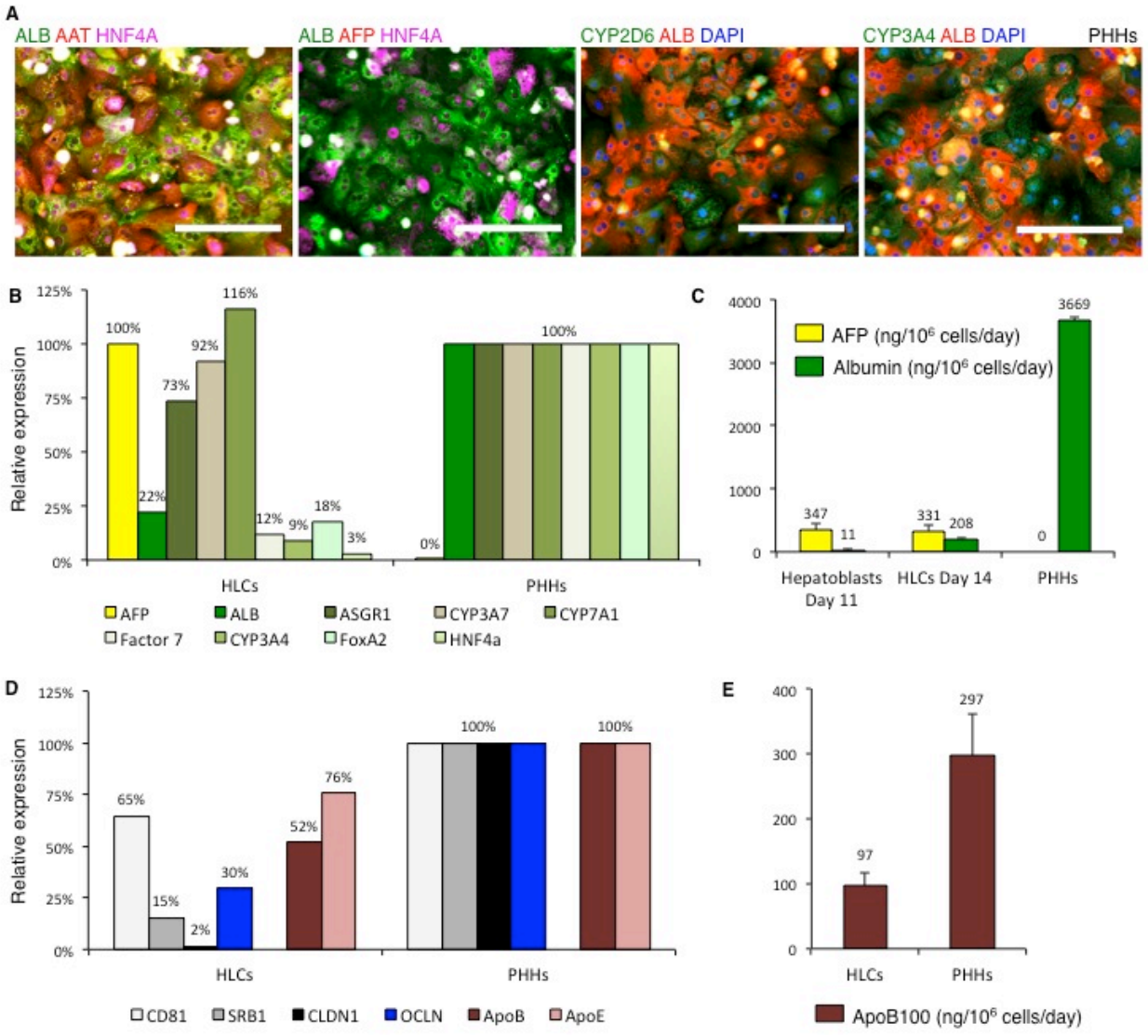


Supplemental Figure 1. Generation and Characterization of Human Induced Pluripotent Stem Cells (hiPSC). (A) Primary skin fibroblasts are cultured in HF medium containing 10% FCS. At day 0, primary fibroblasts were transduced with STEMCCA lentiviruses expressing the reprograming factors OCT4, KLF2, SOX2 and c-MYC. 6 days later, cells were passed on irradiated murine embryonic fibroblasts (MEF) and maintained in culture in stem cells medium containing 100 ng/mL of bFGF. At day 21 post-transduction, growing colonies start to emerge and exhibit typical hESC-like morphology by day 30 post-transduction. At that stage, live cells were stained for TRA-1-81, and positive colonies were picked up and cultured on MEF feeder cells. (B) SC-like colonies were also positive for alkaline phosphatase activity and express OCT4. The hiPSC express *NANOG* and *OCT4* at a level similar to hESC. The hiPSC cell lines were then adapted to feeder-free culture condition for subsequent hepatic differentiation. (Scale bars: 100 μm)



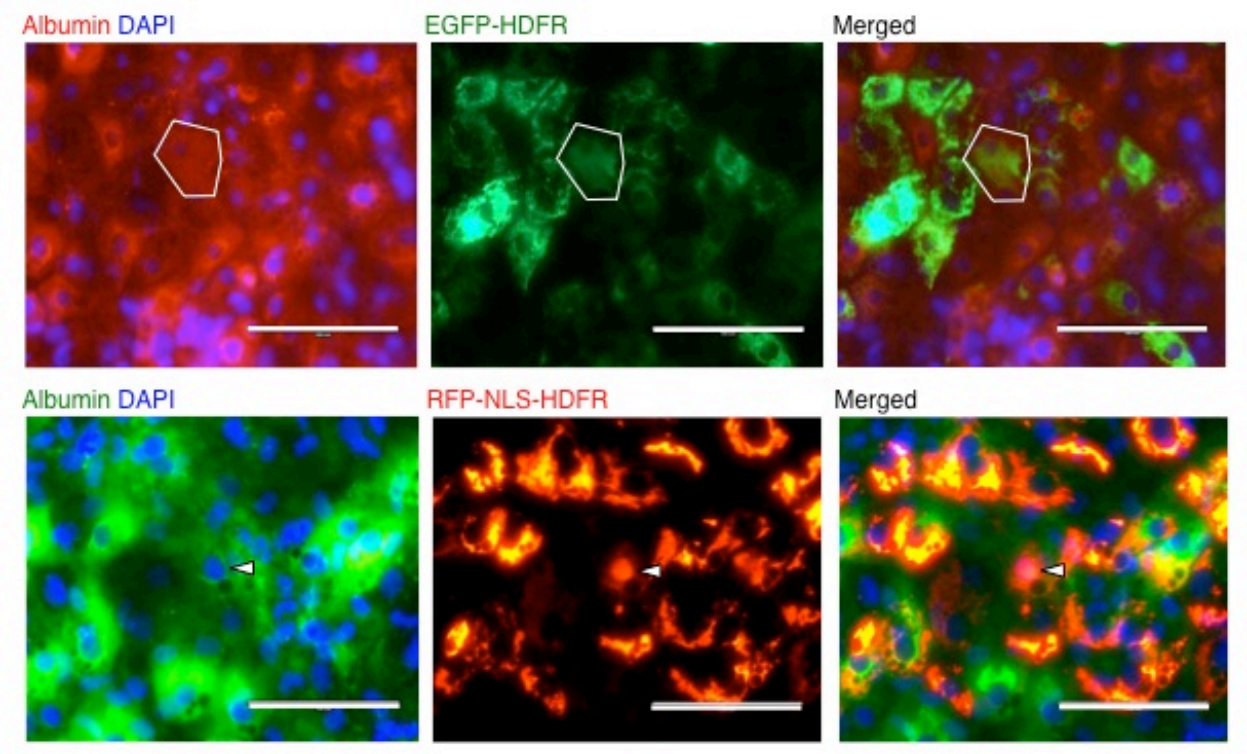
Supplemental Figure 2. In vitro characterization of HLCs at day 14 of differentiation. (A) Validation by IFA on HLCs of the non-mouse antibodies used for in situ immunostaining of hALB, hAFP and hAAT on section of liver of engrafted mice (Figure 6) **(B)** At the end of the in

vitro hepatic differentiation, cells are mainly negative or weakly stained for CYP450 isoforms 2D6 and 3A4. **(C)** Cells positive for cholangiocyte marker CK7 and hepatic marker AAT, suggesting the presence of hepatic biprogenitor cells in the HLCs population. (Scale bar: 200 μm).

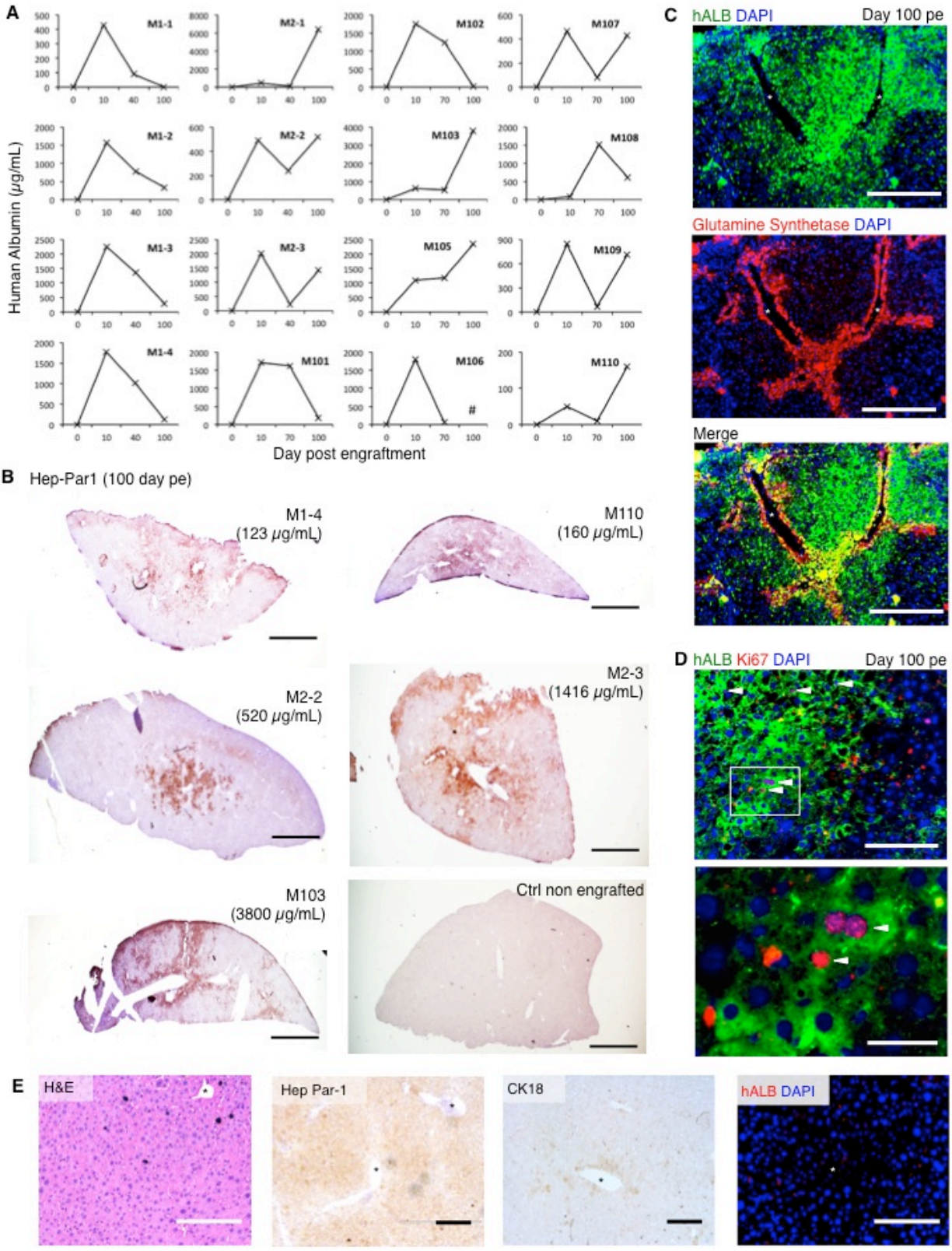


Supplemental Figure 3. In vitro comparison of HLCs with primary human adults hepatocytes (PHHs). (A) Immunostaining of PHHs for hepatic markers ALB, AAT, AFP, HNF4A, and CYP450 isoforms 2D6 and 3A4. (Scale bar: 200µm) **(B)** Relative expression of hepatic markers assessed by RTqPCR (labels denote relative expression to mean PHHs expression, except for AFP, labeled as relative expression to mean HLCs expression) and **(C)** secretion of hepatic proteins AFP and ALB in the supernatants of culture assessed by ELISA (labels indicate mean concentration). **(D)** Relative expression of genes associated with HCV life

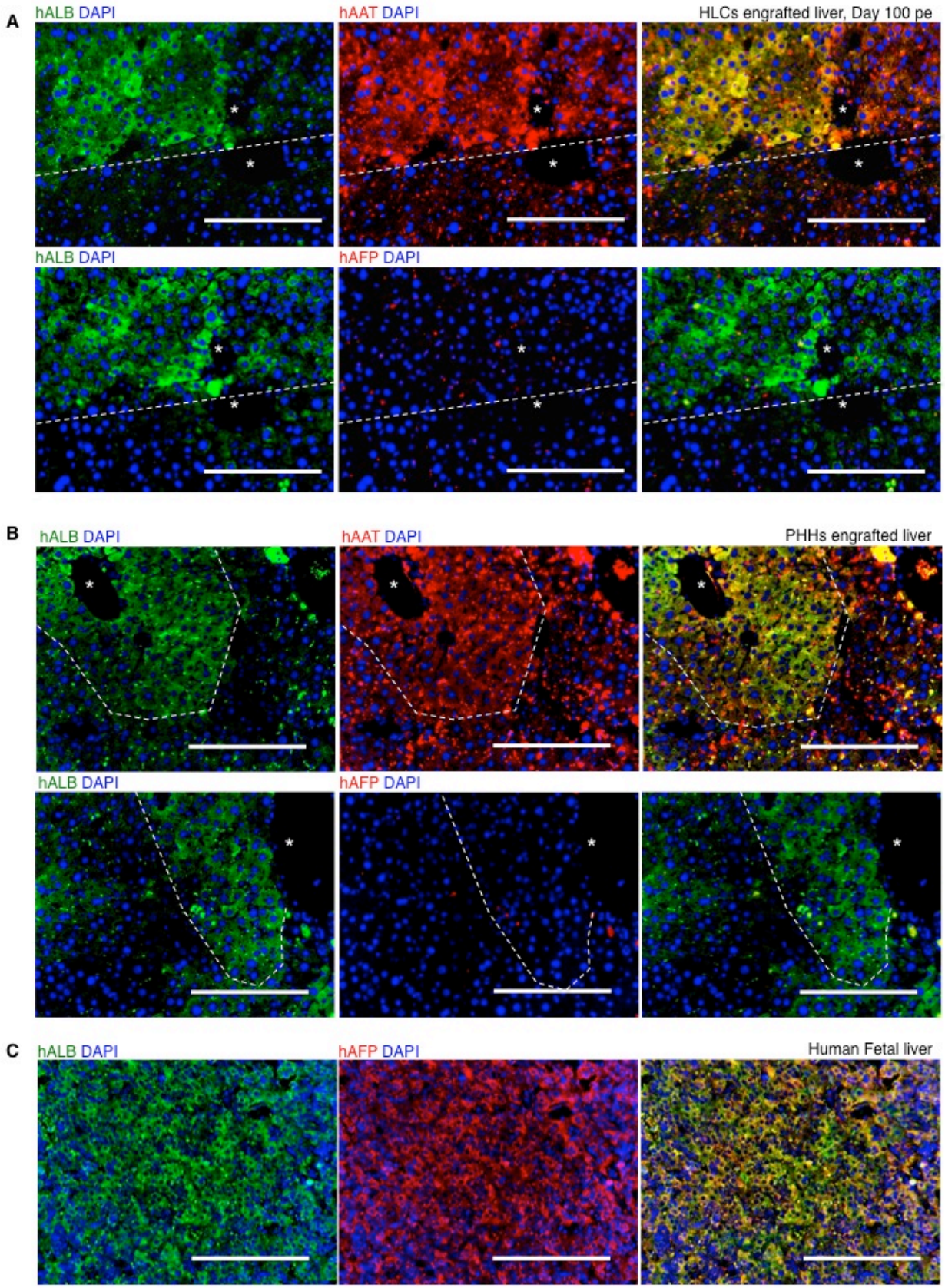
cycle assessed by RTqPCR (numbers denote percentages of mean PHHs expression) and **(E)** secretion of ApoB100 in the culture supernatants by HLCs compared to PHHs, assessed by ELISA (numbers indicate mean concentrations).



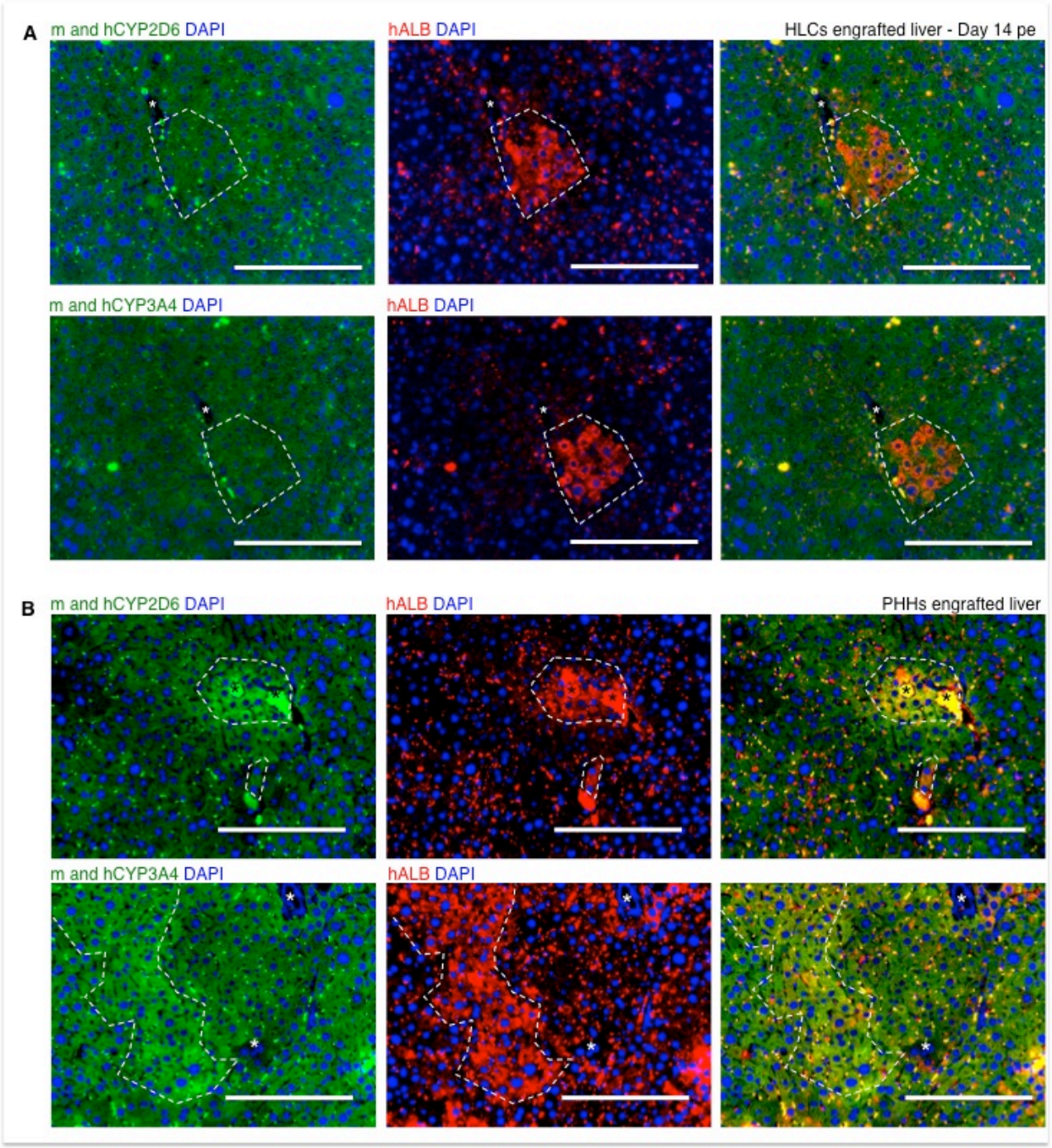
Supplemental Figure 4. HDFR infection visualization and immunostaining for human albumin. Differentiated cells at day 14 of differentiation were transduced to express the IPS-NLS-RFP vector. One day later, cells were inoculated with JFH1-HCVcc. Three days post-infection, cells were fixed, stained for human Albumin and counterstained with DAPI and observed for relocalization events, confirming infection of the hAlb positive differentiated cells only (Scale bar: 100 μm)


A
Human Albumin (μg/mL)
M1-1
M2-1
M102
M107
M1-2
M2-2
M103
M108
M1-3
M2-3
M105
M109
M1-4
M101
M106
M110
#
Day post engraftment
B
Hep-Par1 (100 day pe)
M1-4
(123 μg/mL)
M110
(160 μg/mL)
M2-2
(520 μg/mL)
M2-3
(1416 μg/mL)
M103
(3800 μg/mL)
Ctrl non engrafted
C
hALB DAPI
Day 100 pe
Glutamine Synthetase DAPI
Merge
D
hALB Ki67 DAPI
Day 100 pe
E
H&E
Hep Par-1
CK18
hALB DAPI

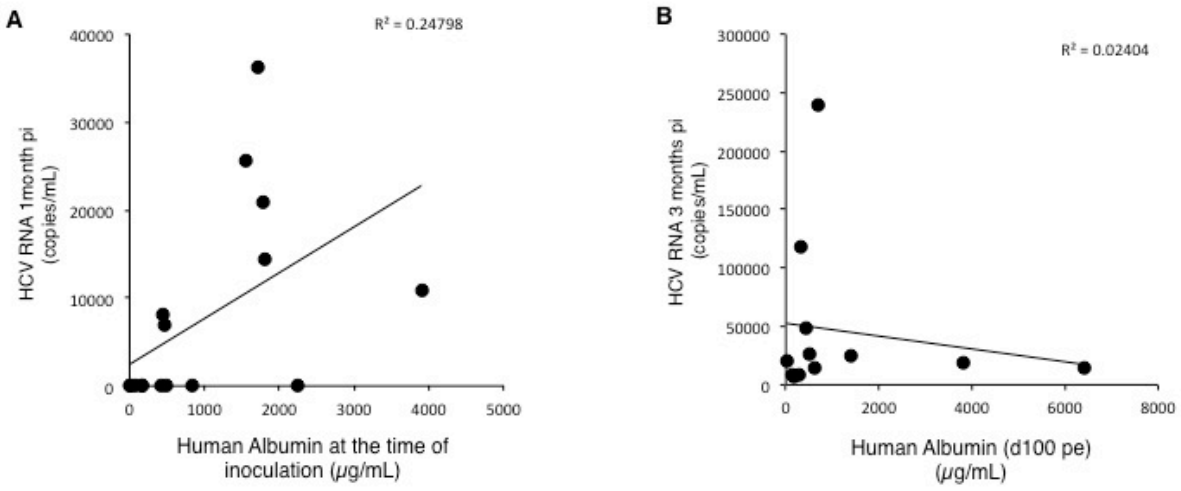
Supplemental Figure 5. Long-term engraftment of HLCs in the liver of MUP-uPA/SCID/Bg mice. (A) Individual profile of secretion of hALB in the serum of engrafted mice. # indicates death of the mouse M106. (B) Pictures of entire section of engrafted liver stained for Hep-Par1, 100 day pe (Scale bar: 2 mm) **(C)** Co-immunostaining for Glutamine Synthetase, marker of central veins, and hALB, showing engraftment around central vein (Scale bar: 200 μm). **(D)** IFA with antibodies anti-human Hep Par-1 and human proliferation marker Ki67 100 days pe confirming the in situ proliferation of human cells in the murine liver parenchyma (Scale bar: 200 μm; Higher magnification: Scale bar: 50 μm). **(E)** Control H&E staining and human specific immunostaining on liver section of non-engrafted mice (Scale bars: 200 μm).


A
hALB DAPI
hAAT DAPI
HLCs engrafted liver, Day 100 pe
hALB DAPI
hAFP DAPI
B
hALB DAPI
hAAT DAPI
PHHs engrafted liver
hALB DAPI
hAFP DAPI
C
hALB DAPI
hAFP DAPI
Human Fetal liver

Supplemental Figure 6. In situ detection of hALB, hAAT and hAFP in **(A)** engrafted HLCs engrafted compared to **(B)** engrafted PHHs in the liver of MUP-uPA/SCID/Bg mice. **(C)** Control immunostaining for hAFP on sections of human fetal liver (AFP Ctrl FTL-1, Cell Marque). (Scale bars: 200 μm)



Supplemental Figure 7. Detection of CYP450 isoforms 2D6 and 3A4 in **(A)** engrafted hALB-positive HLCs at day 14 pe, compared to **(B)** engrafted PHHs in the liver of MUP-uPA/SCID/Bg mice (Scale bars: 200 µm).



Supplemental Figure 8. No correlation between hALB and HCV RNA in the serum of engrafted MUP-uPA/SCID/Bg mice infected with HCV of different genotypes. (A) No significant correlation between hALB concentration at the time of HCV inoculation (day 10pe) and HCV RNA titer 1 month later. Data do not include mice inoculated with very high titer Gt1b. **(B)** No correlation between hALB concentration and HCV RNA titer at the time of sacrifice (Day 100 pe / 3 months pi).

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