Supplementary Information

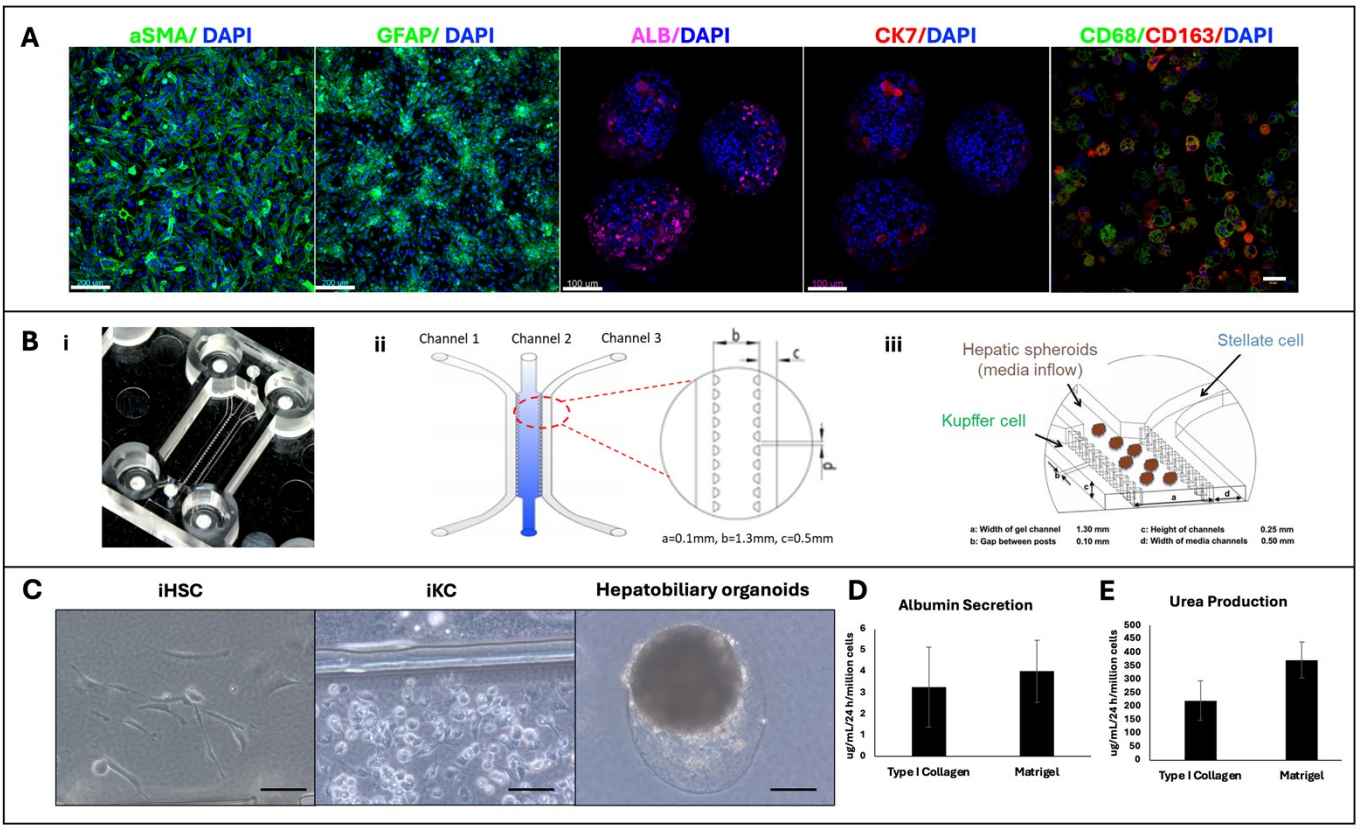
Table S1: List of antibodies and reagents

Antibody/Dye	Catalogue Number
Albumin	Bethyl Laboratories A80-129A
CK 7	Abcam ab9021
CK 19	Abcam ab76539
M30	Roche M30 CytoDEATH
Bodipy	Invitrogen D3922
DAPI	Sigma D9542
Hoecsht	Sigma 94403
SiR-actin	Spirochrome SC001 SiR-actin Kit
Fibronectin	BD Biosciences 610077
Type I Collagen	Abcam ab34710
GFAP	Abcam ab7260
αSMA	Abcam ab5694
CD68	Abcam ab955
CD163	Abcam ab182422
PDGFrβ	Abcam ab32570
CK8/18	Abcam ab17139
Ubiquitin	Invitrogen PA1-10023
LIVE/DEAD™ Viability/Cytotoxicity Kit	Invitrogen L3224
Anti-Mouse Secondary Ab	Invitrogen A-21235, A-11001, A-21123
Anti-Goat Secondary Ab	Invitrogen A-21089, A-21445
Anti-Rabbit Secondary Ab	Invitrogen A-11035, A-31573, A-11008

Table S2: Comparison of co-culture media in the reported literature:

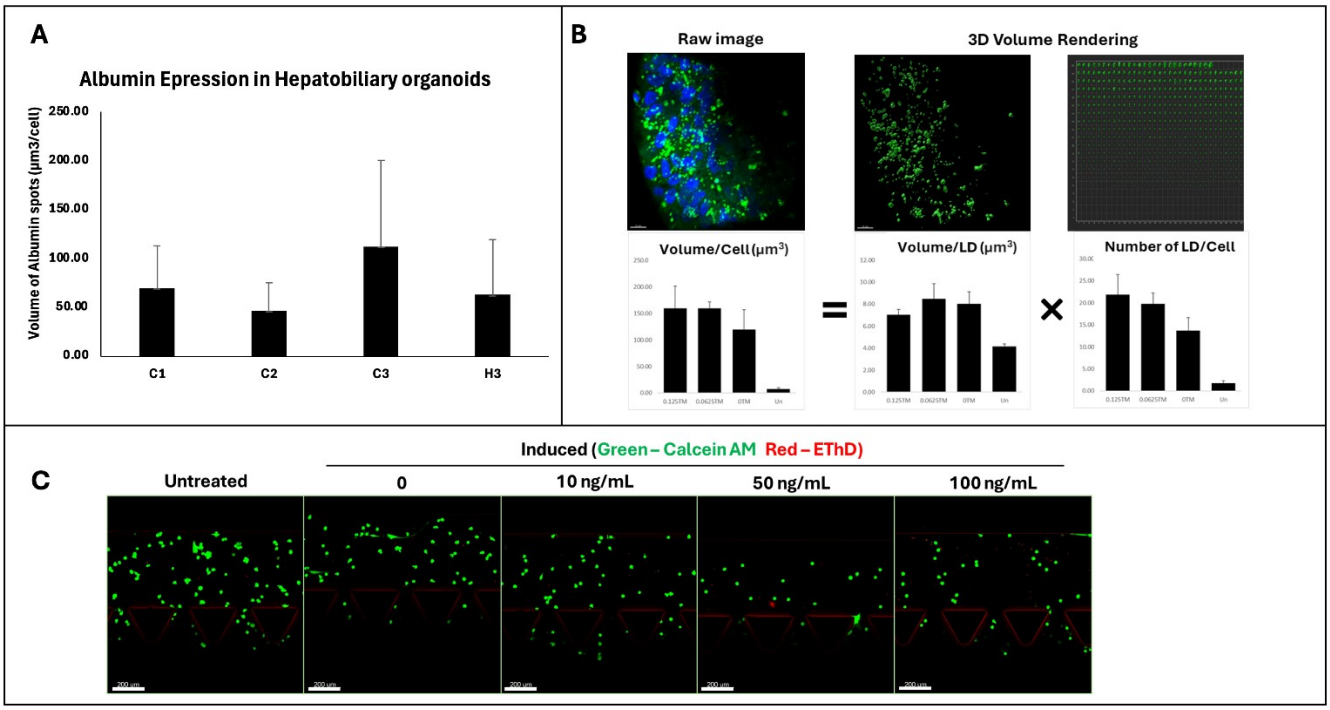
	Feaver et al., JCI Insight, 2016 (Hemoshear)[1]	**Freag et al., Hepatology Communications, 2021** [2]	**Duriez et al., Journal of Clinical and Translational Hep**[3]
Cell source	**Primary cells** Hepatocytes – isolated and cryopreserved Stellate cells – commercial sources Macrophages – isolated from peripheral blood	**Primary cells** Hepatocytes, Kupffer cells, Stellate cells and Liver sinusoidal endothelial cells – commercial source (Zen Bio)	**Primary cells** PHH - Lonza Kupffer cells, Stellate cells and Liver sinusoidal endothelial cells – commercial source (Samsara)
Co-culture media ratio	**Uniform media** • Maintenance media –DMEM/F-12 + 10% FBS, Antibiotics, 0.2% ITS, 1mM Dex at plating and 250 nM thereafter • Proprietary Hepatocytes Flow media – 690 pM Insulin; 5.6 mM Glucose • NASH – 6.9 nM Insulin; 25 mM Glucose	**Mixture** • HC plating media – William's E • KC plating media – DMEM high glucose • HSC growth media – DMEM/Ham's F12 • LSEC media – MEM α-mod **Ratio** – HC plating media: HSC growth media: KC plating media of **10:1:3**	**Uniform media** PHH, HSC, LEC – William's E media with 5% FBS, primary hepatocyte plating supplements **Co-culture medium** – DMEM (low glucose), 0.1 uM Dex and other supplements NASH media - 25mM Glucose, 5 ng/mL TNFα

Supplementary Figure 1



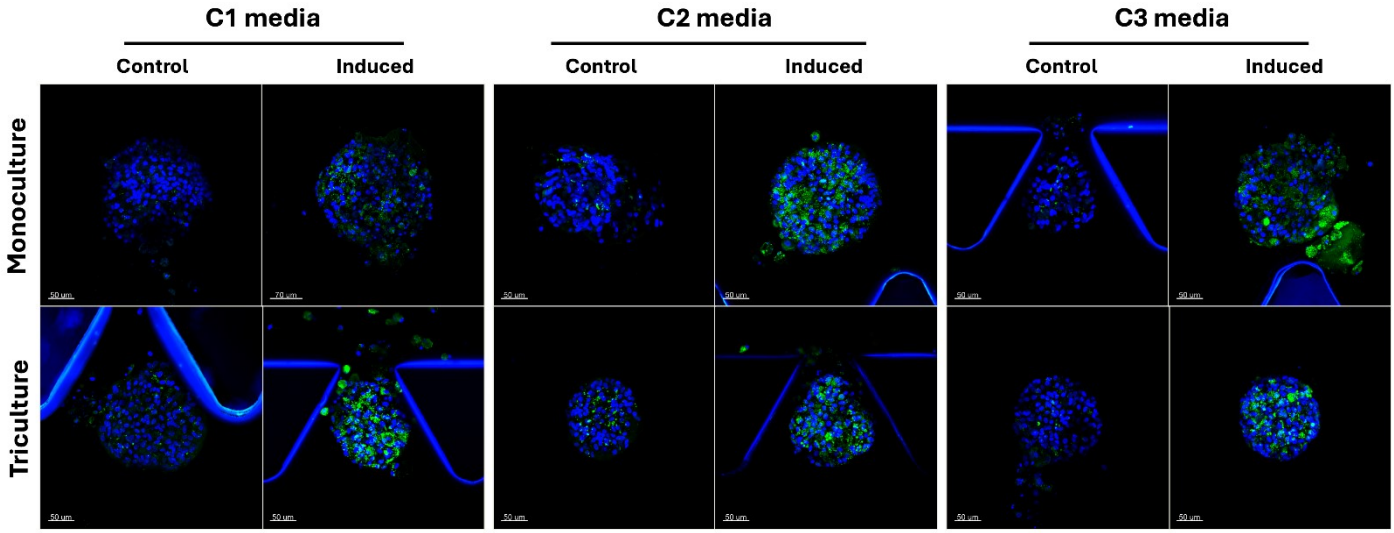
Supplementary Figure 1: Panel A shows the representative images of immunostaining of the iHSCs for aSMA and GFAP, hepatobiliary organoids for albumin and CK7, and, iKCs for CD68 and CD163. Scale bar for the iKC immunostaining is 30μm. The indicated proteins are shown in color and the nuclei is counterstained with DAPI. Panel B shows the (i) digital images, (ii) drawing and the (iii) plan for different cell types in each compartment of the idenTx 9 chip. Panel C shows the iHSCs, iKCs and the organoids 24 hours post seeding on the chip. Scale bar represent 100μm. D shows the ELISA based quantification of secreted Albumin (N=2). E shows the quantification of urea production (N=2) when the organoids were seeded in Type I Collagen and Matrigel.

Supplementary Figure 2



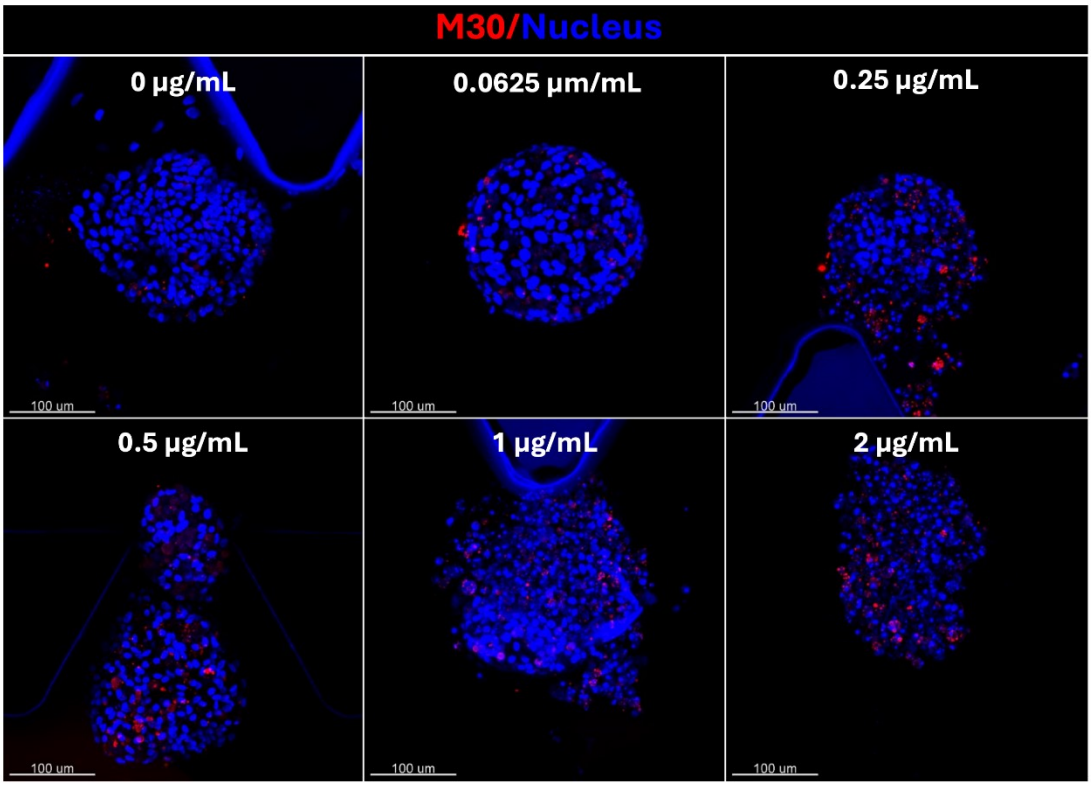
Supplementary Figure 2: Graph (A) shows the quantification of Albumin expression per cell in the organoids in three different media used for co-culture. Panel (B) shows the volume rendering and the quantification of the size and number of lipid droplets in the organoids. Scale bar represents 10 µm. Panel (C) shows the live-imaging of iKCs on the chip treated with varying levels of LPS and stained with Calcein AM and EthD. Scale bar represents 200 µm.

Supplementary Figure 3



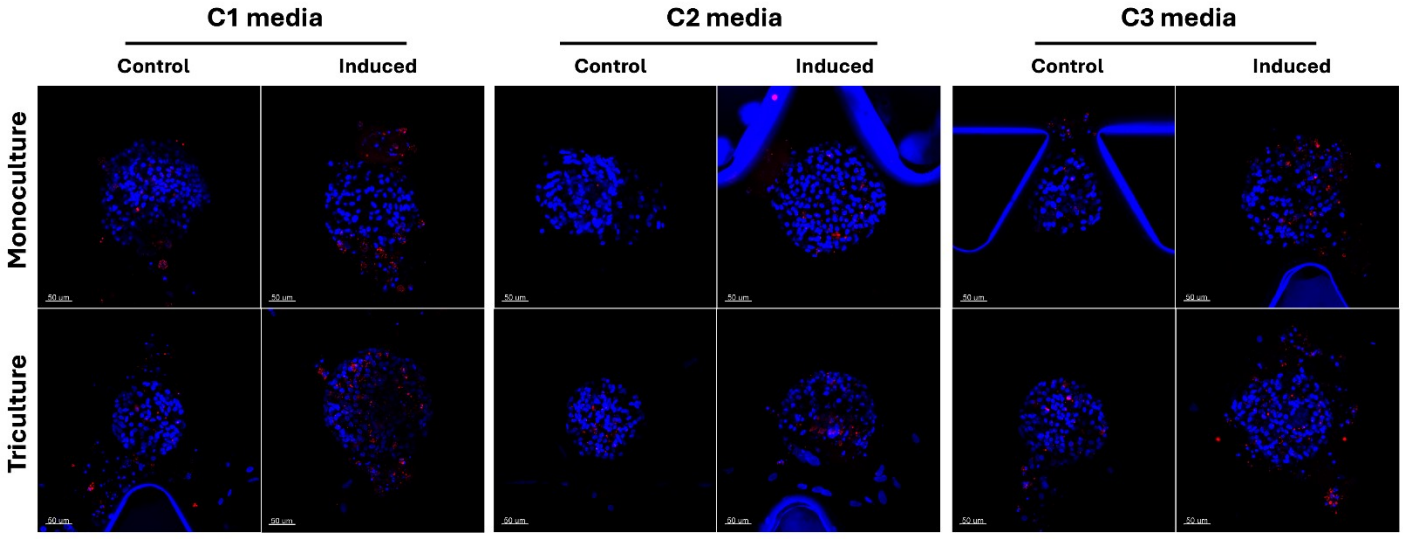
Supplementary Figure 3: Representative images of the organoids stained with BODIPY (green) for visualising lipid droplets and counterstained with DAPI (blue) for the nucleus under control and induced conditions in three different co-culture media. Imaging was performed using a confocal microscope and maximum intensity projections are shown. Scale bar represents 50 µm.

Supplementary Figure 4



Supplementary Figure 4: Representative images of the organoids immunostained with M30 (red) and counterstained with DAPI (blue) for the nucleus treated with varying amounts of Tunicamycin. Imaging was performed using a confocal microscope and maximum intensity projections are shown. Scale bar represents 100 µm.

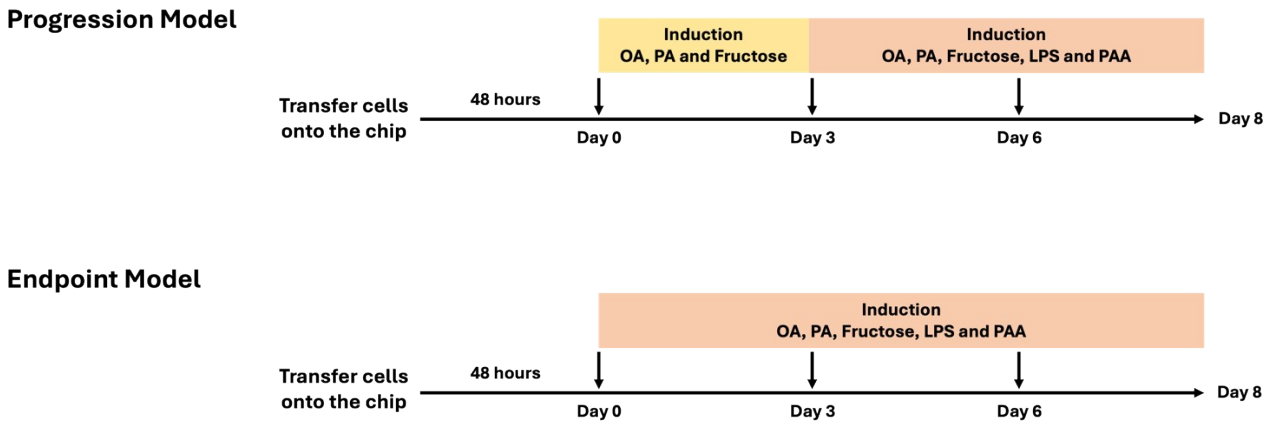
Supplementary Figure 5



Supplementary Figure 5: Representative images of the organoids immunostained with M30 (red) and counterstained with DAPI (blue) for the nucleus under control and induced conditions in three different

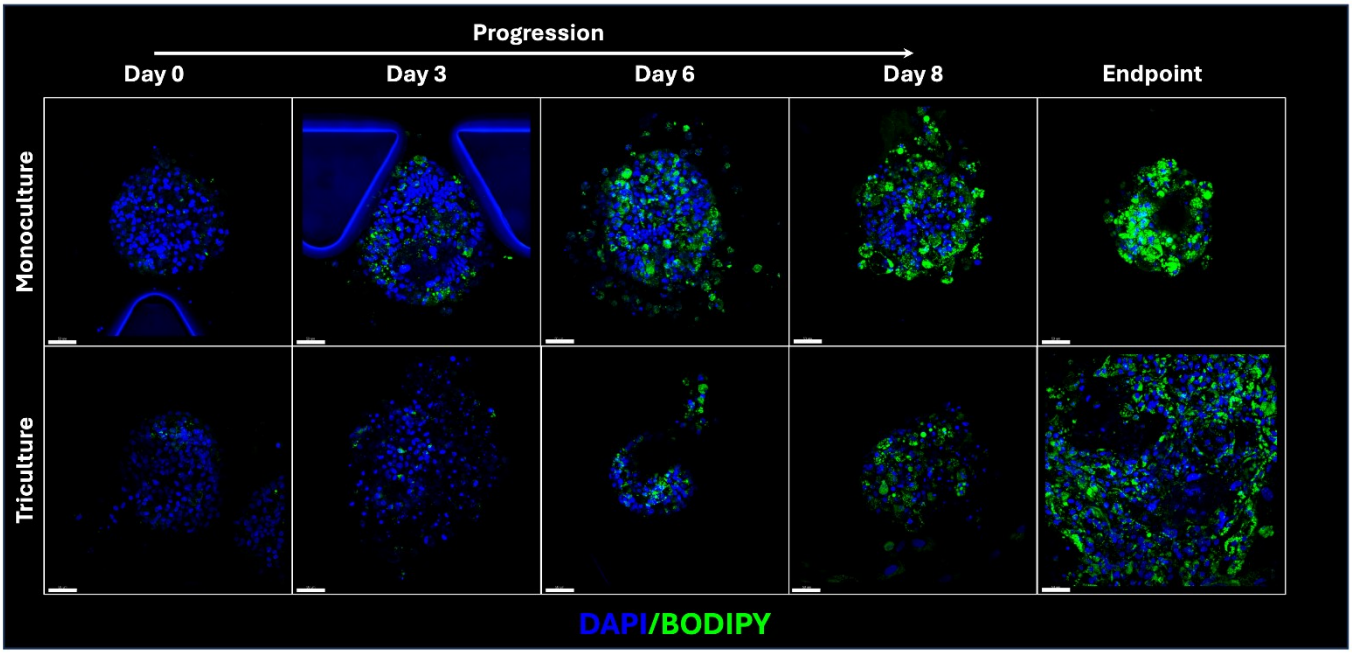
co-culture media. Imaging was performed using a confocal microscope and maximum intensity projections are shown. Scale bar represents 50 µm.

Supplementary Figure 6



Supplementary Figure 6: Induction scheme for the Progression and Endpoint models. The arrows indicate the timepoints when the induction was performed and the color bar indicate the period of exposure to the specified induction factors.

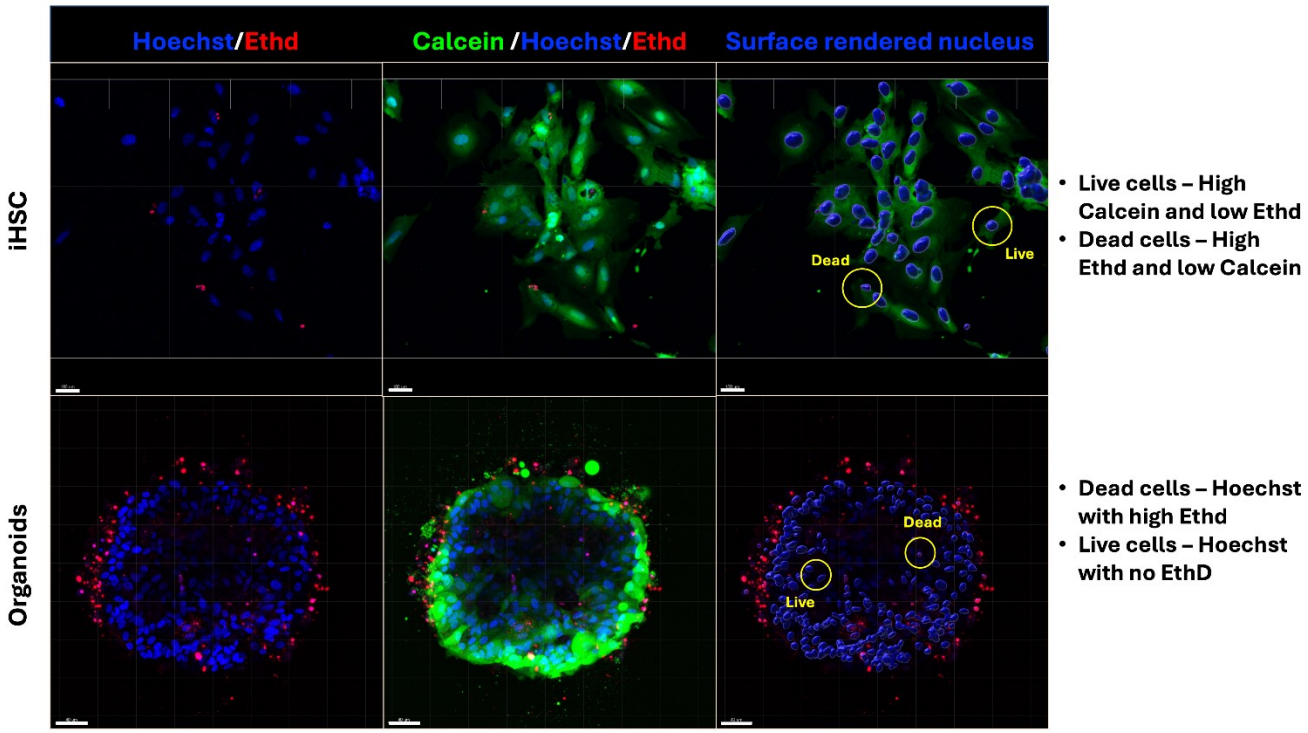
Supplementary Figure 7



Supplementary Figure 7: Representative images of the organoids stained with BODIPY (green) and counterstained with DAPI (blue) for the nucleus at Days 0, 3, 6 and 8 under the Progression model

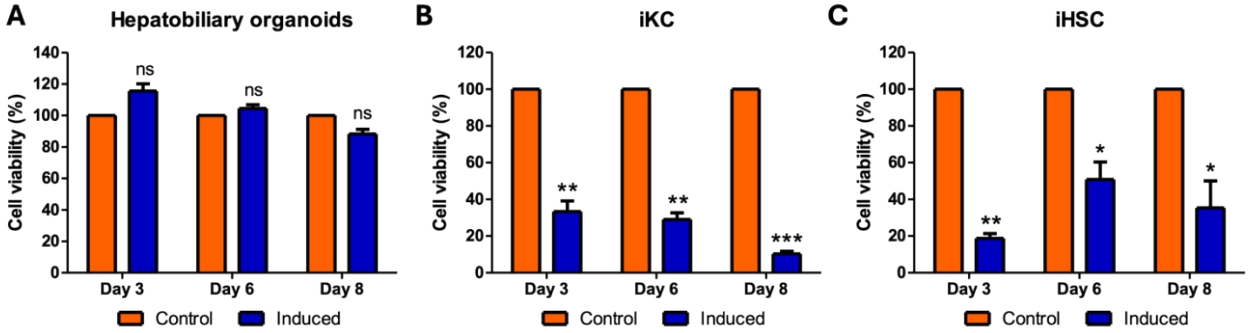
induction scheme. The far right panel shows the organoids after 8 days of induction under the Endpoint model induction scheme. Imaging was performed using a confocal microscope and maximum intensity projections are shown. Scale bar represents 50 µm.

Supplementary Figure 8



Supplementary Figure 8: Representative images of iHSCs and organoids stained with Calcein AM (green), EthD (red) and Hoechst (blue). Scale bar represents 100 µm in the top panel and 40 µm in the lower panel. Live imaging was performed using a confocal microscope with a live cell incubation centre and maximum intensity projections are shown.

Supplementary Figure 9



D

	Day 3	Day 6	Day 8
Control	100	100	100
Induced	115.52	104.43	88.28

E

	Day 3	Day 6	Day 8
Control	100.00	100.00	100.00
Induced	33.32	29.01	10.32

F

	Day 3	Day 6	Day 8
Control	100.00	100.00	100.00
Induced	18.71	50.83	35.37

G

	Organoid number	Day 3	Day 6	Day 8
Only organoids	✓	1	1	1
Only iHSC	-	0.19	0.51	0.35
Only iKC	-	0.33	0.29	0.10
iHSC+iKC	-	0.26	0.40	0.23
Organoids+iHSC+iKC	✓	0.26	0.40	0.23
Organoids+iHSC	✓	0.19	0.51	0.35
Organoids+iKC	✓	0.33	0.29	0.10

Supplementary Figure 9: Graphs showing the relative viability of hepatobiliary organoids (A), iKCS (B) and iHSCs (C) at 3, 6 and 8 days after induction in induced versus control conditions. The values are tabulated in (D), (E) and (F) for hepatobiliary organoids, iKCs and iHSCs respectively. Table (G) shows the normalising ratios for cytokines secreted by the different cell types on the chip. Error bars represent standard deviation. N = 3 experiments. Paired t-tests were performed. For each timepoint, induced (blue) was compared against control (orange). ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

References:

[1] R.E. Feaver, B.K. Cole, M.J. Lawson, S.A. Hoang, S. Marukian, B.R. Blackman, R.A. Figler, A.J. Sanyal, B.R. Wamhoff, A. Dash, Development of an in vitro human liver system for interrogating nonalcoholic steatohepatitis, JCI Insight 1 (2016) 0–18. https://doi.org/10.1172/jci.insight.90954.

[2] M.S. Freag, B. Namgung, M.E. Reyna Fernandez, E. Gherardi, S. Sengupta, H.L. Jang, Human Nonalcoholic Steatohepatitis on a Chip, Hepatol Commun 5 (2021) 217–233. https://doi.org/10.1002/hep4.1647.

[3] M. Duriez, A. Jacquet, L. Hoet, S. Roche, M.D. Bock, C. Rocher, G. Haussy, X. Vigé, Z. Bocskei, T. Slavnic, V. Martin, J.C. Guillemot, M. Didier, A. Kannt, C. Orsini, V. Mikol, A.C. Le Fèvre, A 3D human liver model of nonalcoholic steatohepatitis, J Clin Transl Hepatol 8 (2020) 359–370. https://doi.org/10.14218/JCTH.2020.00015.