Improving Hepatic Metabolic Function with 3D iPSC-derived Human Hepatocytes

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Keywords: iPSCs, metabolism, organobodies, liver, 3D spheroid, DILI

SUPPLEMENTARY INFORMATION TABLES

Table S1 Materials used in cell culture

Material	**Supplier**	**Catalogue number**	**CAS number**
Fetal Bovine Serum, qualified, Brazil	Gibco	10270106	
DMEM, low glucose, pyruvate	Gibco	31885023	
MEM amino acids solution (50X)	ThermoFisher	15040033	
MEM non-essential amino acids solution (100X)	ThermoFisher	12519059	
L-Glycine	Merck Life Science	G7126	56-40-6
Trypsin-EDTA solution	Merck Life Science	T4049	
Geltrex ™	Gibco	A14133-02	
Gibco™ Versene Solution/ EDTA	ThermoFisher	15040033	
Penicillin-Streptomycin	Merck Life Science	P4333	
DMSO for cell culture	Merck Life Science	D8418	67-68-5
Gibco™ StemPro™ Accutase™ Cell Dissociation Reagent	ThermoFisher	11599686	
Albumin, Bovine Serum, Fraction V, Fatty Acid-Free, Nuclease- and Protease-Free	Merck Life Science	126609	
Human Recombinant Albumin	Merck Life Science	A9731	

Table S2 Materials used in VU8 media preparation in iPSCs culture

Material	**Supplier**	**Catalogue number**	**CAS number**
DMEM, high glucose	Gibco	41965-039	
Ham's F-12 Nutrient Mix	Gibco	21765-029	
L-Ascorbic acid 2-phosphate	Merck Life Science	A8960	1713265-25-8
Insulin	Gibco	A11382II	
Transferrin	Merck Life Science	T3705	11096-37-0
Sodium selenite	Merck Life Science	S5261	10102-18-8
Recombinant FGF2-G3 protein	Qkine	Qk053	
Human Recombinant TGF-beta 1	StemCell Technologies	78067.1	
GlutaMAX	Gibco	35050-038	

Table S3 Materials used in LDM media preparation and cytokines used in HLCs differentiation

Material	**Supplier**	**Catalogue number**	**CAS number**
DMEM-Low glucose	Gibco	31885	
MCDB 201 Medium	Merck Life Science	M6770	
Penicillin-Streptomycin	Merck Life Science	P4333	
L-Ascorbic acid 2-phosphate	Merck Life Science	A8960	1713265-25-8
Insulin-Transferrin-Selenium (ITS-G) (100X)	Gibco	4140-045	
Linoleic Acid-Albumin from bovine serum albumin	Merck Life Science	L9530	

2-Mercaptoethanol (50 mM)	Gibco	31350010	
Dexamethasone-Water Soluble	Merck Life Science	D-2915	50-02-2
Hydrocortisone-21-hemisuccinate sodium salt	Merck Life Science	H2270	125-04-2
Recombinant mouse Wnt3a protein	Abcam	ab81484	
Human Recombinant BMP-4	StemCell Technologies	78211	
Human Recombinant HGF	StemCell Technologies	78019.1	
Human/Mouse Recombinant Activin A	Stem cell technologies	78001.1	
Human Recombinant FGF-acidic	StemCell Technologies	78187.1	
Y-27632 dihydrochloride, Rho kinase inhibitor	Abcam	Ab120129	
Doxycycline hydrochloride	Merck Life Science	D3447	10592-13-9

Table S4 Materials used in primary human hepatocytes (PHHs) culture

Material	**Supplier**	**Catalogue number**
Collagen I Cellware plates	Corning	354408
LiverPool 10-donor Human hepatocytes 5M cells	BioIVT	X008001
OptiThaw Hepatocyte Media	Tebu bio	K8000
InVitroGRO CP medium	BioIVT	Z99029
InVitroGRO HI medium	BioIVT	Z99009

Table S5 Materials used for 3D HLCs and 3D HepG2 preparation

Material	**Supplier**	**Catalogue number**	**CAS number**
Collagen Type I, rat tail, 10 mg/ml, 1 x 5 ml	IBIDI	50204	
Corning® PuraMatrix™ Peptide Hydrogel, 5 mL	Corning	354250	
Gibco™ rhLaminin-521	ThermoFisher	A29248	
Sucrose	PanReac AppliChem	A3935	57-50-1
CellCarrier Spheroid ULA 96-well Microplates	Perkin Elmer	6055330	
TripLE Extpress	ThermoFisher	12604-013	
RevitaCell™ Supplement (100X)	Gibco	A2644501	

Table S6 Antibodies used for immunofluorescence

Antibody	**Supplier**	**Catalogue number**
CYP3A4	Abcam	AB124921
Albumin	Dako	A0001
Phosphoenolpyruvate carboxykinase (PEPCK)	Merck Life Science	SAB5701540
Hoechst 33342	ThermoFisher	11534886
Alexa 647 donkey anti-rabbit IgG	ThermoFisher	A32795

Table S7 Chemicals used to perform CYP metabolic incubations

Chemical	Supplier	Catalogue number	CAS number
Bupropion hydrochloride	TCI chemicals	B3649	31677-93-7
Phenacetin	Merck Life Science	77440	62-44-2
Rosiglitazone	TCI chemicals	R0106	122320-73-4
Diclofenac sodium salt	Merck Life Science	D6899	15307-79-6
Dextromethorphan	Merck Life Science	D2531	6700-34-1
Chlorzoxazone	Merck Life Science	C4397	95-25-0
Benzydamine hydrochloride	Merck Life Science	B5524	132-69-4
Midazolam hydrochloride	Duchefa	-	59468-44-9
Coumarin	Merck Life Science	C4261	91-64-5
7-Ethoxycoumarin	Merck Life Science	E1379	31005-02-4
Meloxicam sodium salt	Merck Life Science	M3935	71125-39-8

Table S8 Inhibitors of central carbon metabolism pathways

Materials	Supplier	Catalogue number	CAS number
Etomoxir sodium salt hydrate	Merck Life Science	E1905	828934-41-4
2-Deoxy-D-glucose	Merck Life Science	D8375	154-17-6
UK5099	Merck Life Science	PZ0160	56396-35-1
3-Nitropropionic acid	Merck Life Science	164603	504-88-1
Rotenone	Merck Life Science	R8875	83-79-4

Table S9 Solvents and materials for LC-MS sample preparation and acquisition

Chemical	Supplier	Catalogue number	CAS number
Acetonitrile LC-MS grade	Biosolve	1207802	75-05-8
MeOH LC-MS grade	Biosolve	13687801	67-56-1
Ammonium Acetate	Merck Life Science	73594	631-61-8
Ammonia solution 25%	Merck Life Science	1.05432.1011	1336-21-6

Table S10 Drug induced liver injury (DILI) drugs used for cytotoxicity assays

Compound	Supplier	Catalogue number	CAS number
Diclofenac	Merck Life Science	D6899	15307-79-6
Rosiglitazone	Merck Life Science	PHR2513	155141-29-0
Azathioprine	Merck Life Science	A4638	446-86-6
Troglitazone	Merck Life Science	T2573	97322-87-7
Nefazodone	Merck Life Science	N5536	82752-99-6
Omeprazole	Merck Life Science	PHR1059	73590-58-6
Cyclosporine A	Merck Life Science	30024	59863-13-3
Amiodarone	Merck Life Science	A8423	19774-82-4

Table S11 Materials used in lactate, resazurin, and calcein AM assays

Material	Supplier	Catalogue number	CAS number
Triethanolamine HCl	Merck Life Science	T9534	637-39-8
EDTA.disodium salt dihydrate	PanReac AppliChem	A2937-0500	6381-92-6
MgCl2.6H2O	Merck Life Science	M2670	7791-18-6
Phenazine methosulfate (PMS)	Merck Life Science	P9625	299-11-6
Iodonitrotetrazolium chloride (INT)	Merck Life Science	I8377	146-68-9
Albumin, Bovine Serum, Fraction V, Fatty Acid-Free, Nuclease- and Protease-Free	Merck Life Science	126609	9048-46-8
Sodium L-lactate	Merck Life Science	71718	867-56-1
Adenosine 5'-triphosphate disodium salt hydrate	Merck Life Science	A26209	34369-07-8
Triton X-100	Merck Life Science	T8787	9036-19-5
Beta-Nicotinamide-adenine Dinucleotide	Merck Life Science	N7004-25G	53-84-9
Lactate dehydrogenase	Merck Life Science	L2625-12.5KU	
Resazurin	Merck Life Science	R7017	62758-13-8
Calcein AM	ThermoFisher	C1430	
Sodium Hydroxide	Merck Life Science	1.06498	1310-73-2

Table S12 Putative identified metabolites in extracellular contents of 2D and 3D HLCs incubated with model drugs (benzydamine, midazolam, rosiglitazone, phenacetin, diclofenac, dextromethorphan, 7-ethoxycoumarin, bupropion, coumarin, and chlorzoxazone) analyzed by LC-MS xenobiotic metabolite analysis. This table reports the metabolite molecular formula (MF), theoretical mass $[M+H]^+$ for positive ions and $[M-H]^-$ for negative ions, retention time (RT, in minutes), and detected mass error in ppm. All identified metabolites were present in drug-incubated samples, absent in control, and absent or higher than in blank samples

Compound Name	**MF**	**Theoretical m/z $[M+H]^+$**	**Theoretical m/z $[M-H]^-$**	**RT (min)**	**Mass error in positive ion mode**	**Mass error in negative ion mode**	**2D HLCs**	**3D HLCs**
benzydamine	C19H23N3O	310.1913888	308.1768359	12.3	-1.00		x	x
3-((1H-indazol-3-yl)oxy)-*N*.*N*-demethylpropan-1-amine	C12H17N3O	220.1444386	218.1298857	6.7	0.18		x	x
benzydamine hydroxide	C19H23N3O2	326.1863034	324.1717505	10.4	-1.22		x	
benzydamine hydroxide2	C19H23N3O2	326.1863034	324.1717505	10.1	-1.22		x	
benzydamine hydroxide3	C19H23N3O2	326.1863034	324.1717505	10.9	2.46		x	
benzydamine *N*-oxide	C19H23N3O2	326.1863034	324.1717505	12.5	-0.60		x	x
benzydamine *N*-oxide hydroxide	C19H23N3O3	342.0803943	340.1666651	10.2	-1.48		x	
norbenzydamine	C18H21N3O	296.1757387	294.1611858	11.9	-1.22		x	x
midazolam	C18H13ClFN3	326.0854797	326.0854797	11.1	-2.21		x	x
dihydroxymidazolam	C18H13ClFN3O2	358.0753089	356.0607560	10.5	1.42		x	x
hydroxymidazolam 1	C18H13ClFN3O	342.0803943	340.0658414	10.4	-1.48		x	
hydroxymidazolam 2	C18H13ClFN3O	342.0803943	340.0658414	10.7	-0.89		x	
rosiglitazone	C18H19N3O3S	358.1219891	356.1074362	8.5	-1.15	1.22	x	x
N-desmethylrosiglitazone	C17H17N3O3S	344.1063390	342.0917861	8.1	-0.18	1.71	x	x
N-despyridinyl rosiglitazone	C13H16N2O3S	281.0954400	342.0917861	3.8	-2.35		x	x
para-hydroxyrosiglitazone	C18H19N3O4S	374.1169037	372.1023508	8.7	0.81		x	
para-hydroxyrosiglitazone2	C18H19N3O4S	374.1169037	372.1023508	7.7	-1.06		x	
para-hydroxyrosiglitazone3	C18H19N3O4S	374.1169037	372.1023508	7.1	-2.40		x	
O-sulfate rosiglitazone	C18H19N3O7S2	454.0737187	452.0591658	8.7		-0.08	x	
phenacetin	C10H13NO2	180.1019051	178.0873522	9.9	1.14		x	x
diclofenac	C14H11Cl2NO2	296.0239604	294.0094075	17.1	-0.13	-0.99	x	x

hydroxydiclofenac	C14H11Cl2NO3	312.0188750	310.0043221	14.2		-0.25	x	
dextromethorphan	C18H25NO	272.2008909	270.1863380	10.7	2.17		x	x
dextrorphan	C17H23NO	258.1852408	256.1706879	7.5	-1.00		x	x
methoxymorphinan	C17H23NO	258.1852408	256.1706879	10.7	-2.55		x	x
7-ethoxycoumarin	C11H10O3	191.0702706	189.0557177	13.3	1.42		x	x
7-hydroxycoumarin	C9H6O3	163.0389705	161.0244176	7.6		0.73	x	x
coumarin-7-*O*-sulfate	C9H6O6S	242.9957855	240.9812326	4.3		-1.94	x	
bupropion	C13H18ClNO	240.1149683	238.1004154	9.5	-2.63		x	x
hydroxybupropion	C13H18ClNO2	256.1098829	254.0953300	7.5		0.91	x	x
coumarin	C9H6O2	147.0440559	145.0295030	9.9	0.38		x	x
7-hydroxycoumarin	C9H6O3	163.0389705	161.0244176	7.6		0.73	x	x
coumarin-7-*O*-sulfate	C9H6O6S	242.9957855	240.9812326	4.3		-1.94	x	
M22	C10H12O4	197.0808353	195.0662824	8.8		0.42	x	
chlorzoxazone	C7H4ClNO2	170.0003325	167.9857796	11.2		1.66	x	x
hydroxychlorzoxazone	C7H4ClNO3	185.9952471	183.9806942	6.0		-4.92	x	

Table S13 Targeted list of central carbon metabolites found in 2D HLCs and 3D HLCs analyzed by HILIC-MS.

MF: molecular formula; m/z: mass over charge; RT (min): retention time in minutes; ppm: mass error in ppm; ASC: authentic standard confirmation; IS assigned: ^{13}C IS standard assignment for data normalization

Compound Name	MF	Theoretical m/z	Ion polarity	RT (min)	Mass error	ASC	^{13}C IS assigned
Aminobutyric acid	C4H9NO2	102.056	negative	9.3	-0.1	yes	Aminobutyric acid
(Iso)leucine	C6H13NO2	132.102	positive	6.7	-1.0	yes	Glutamine
Adenosine 5'-diphosphate (ADP)	C10H15N5O10P2	428.037	positive	10.1	-0.2	yes	AMP
Alanine	C3H7NO2	90.055	positive	8.9	0.1	yes	Glutamine
Adenosine 5'-monophosphate (AMP)	C10H14N5O7P	348.070	positive	9.1	0.1	yes	AMP
Arginine	C6H14N4O2	175.119	positive	15.1	0.2	yes	Arginine
Asparagine	C4H8N2O3	133.061	positive	9.1	-0.1	yes	Glutamine
Aspartate	C4H7NO4	134.045	positive	9.5	-0.3	yes	Glutamine
Adenosine 5'-triphosphate (ATP)	C10H16N5O13P3	508.003	positive	10.7	-0.3	yes	AMP
Creatine	C4H9N3O2	132.077	positive	8.7	-0.8	yes	Glutamine
Citrate	C6H8O7	191.020	negative	11.6	-1.3	yes	Glutamate
Fructose-1,6-bisphosphate (F1,6BP)	C6H14O12P2	338.989	negative	12.1	-0.5	yes	Glucose-phosphate
Fumarate	C4H4O4	115.004	negative	10.4	-2.4	yes	Glutamate
Glucose-6-phosphate (G6P)	C6H13O9P	259.022	negative	10.2	-0.1	yes	Glucose-6-phosphate
Lactate	C3H6O3	89.024	negative	7.0	0.0	yes	Glutamate
Malate	C4H6O5	133.014	negative	10.4	0.0	yes	Glutamate
N-Acetyl aspartate	C6H9NO5	174.041	negative	9.7	-3.1	no	UDP-Glucose
Pyruvate	C3H4O3	87.009	negative	9.9	-0.5	yes	Glutamate
Glutamate	C5H9NO4	148.060	positive	9.3	0.7	yes	Glutamate
Glutamine	C5H10N2O3	147.076	positive	9.0	0.4	yes	Glutamine
Glycine	C2H5NO2	76.039	positive	9.5	-0.1	yes	Glutamine
Glutathione (GSH)	C10H17N3O6S	308.091	positive	9.2	0	yes	AMP
Glutathione oxidized (GSSG)	C20H32N6O12S2	613.159	positive	10.9	1.3	yes	AMP
Histidine	C6H9N3O2	156.077	positive	9.1	-2.9	yes	Glutamine

Lysine	C6H14N2O2	147.113	positive	14.6	-0.9	yes	Glutamine
Methionine	C5H11NO2S	150.058	positive	7.2	-0.4	yes	Glutamine
Nicotinamide adenine dinucleotide (NAD^+)	C21H27N7O14P2	664.116	positive	9.0	-0.8	yes	NAD
Pantothenic acid	C9H17NO5	220.118	positive	6.5	-0.3	yes	Glutamine
Phenylalanine	C9H11NO2	166.086	positive	6.6	0.2	yes	Glutamine
Phosphocholine	C5H14NO4P	184.073	positive	9.3	0.1	no	Glutamine
Proline	C5H9NO2	116.071	positive	7.5	-1.9	yes	Glutamine
S-(5'-Adenosyl)-L-methionine iodide (SAM)	C15H22N6O5S	399.145	positive	10.1	-0.4	yes	AMP
Serine	C3H7NO3	106.050	positive	9.4	0.0	yes	Glutamine
Threonine	C4H9NO3	120.066	positive	8.9	-3.5	yes	Glutamine
Tyrosine	C9H11NO3	182.081	positive	8.1	0.5	yes	Glutamine
Uridine 5'-monophosphate (UMP)	C9H13N2O9P	325.043	positive	9.9	-0.1	yes	AMP
Valine	C5H11NO2	118.086	positive	8.1	0.0	yes	Glutamine
Succinate	C4H6O4	117.019	negative	10.0	0.1	yes	Glutamate
Glycerol-phosphate	C3H9O6P	171.006	negative	9.7	-0.6	no	Glutamate
Uridine diphosphate-glucose (UDP-Glc)	C15H24N2O17P2	565.048	negative	10.7	-1.8	yes	UDP-Glucose
Uridine diphosphate N-acetylglucosamine (UDP-GlcNAc)	C17H27N3O17P2	606.074	negative	10.1	-1.0	yes	UDP-Glucose
Uridine 5'-diphosphate (UDP)	C9H14N2O12P2	405.009	positive	10.5	2.4	no	AMP
Sedoheptulose-7-phosphate	C7H15O10P	289.033	negative	10.5	-0.4	yes	Sedoheptulose-7-phosphate

SUPPLEMENTARY INFORMATION METHODS

LC-MS xenobiotic metabolomics analysis:

The LC-MS method was previously reported (Pozo Garcia et al. 2025). All solvents used were LC-MS grade (BioSolve), and ultrapure water was obtained from a water purification system (Milli-Q EQ 7000, Merck Life Science). The LC-MS system consisted of an ultra-high performance liquid chromatograph, UHPLC (Agilent 1290 UHPLC), coupled to a high-resolution time-of-flight mass spectrometer, MS (Agilent 6230B TOF), equipped with an electrospray ionization source (ESI). A reverse phase column (Waters XBridge BEH C18 2.1 mm internal diameter x 100 mm long, 2.5 µm particle size and 130 Å pore size) with a guard column (Waters XBridge BEH C18 3.9 mm x 5 mm, 3.5 µm particle size, 130 Å pore size) was used for the chromatography at 40 °C column oven temperature and 0.2 mL/min flow rate. A linear gradient was applied: 10 to 80% of B (0.1% (v/v) formic acid in acetonitrile) in 18 min (0-1 min: 10% B; 1-19 min: 80% B; 19-21 min: 80% B; 21.5-24 min: 10% B) with a total run of 24 min (A, 0.1% (v/v) formic acid in ultrapure water). The following MS source parameters were used: nebulizer gas (nitrogen) at 20 psi, 325 and 400 °C of gas and sheath gas temperatures, with sheath gas flow of 12 L/min, using 4 kV and 3 kV of capillary voltage in positive and negative ion modes, respectively. The acquisition rate was 1 spectra/s and 1000 ms/spectrum in centroid mode. Mass calibration was done on-the-fly by infusing along the run a reference solution with masses 161.050873 and 922.009798 for positive ion mode, while 112.985587 and 1033.988109 for negative ion mode. A volume of 1 µL was injected for each sample, and samples were kept at 10 °C before analysis. Acquisition was done under the control of MassHunter Workstation version 11.0 (Agilent). Data analysis, including signal integration, was done with MassHunter 11.0 (Agilent).

LC-MS central carbon metabolome analysis:

The LC-MS method was previously reported (Pozo Garcia et al. 2026). Separation was performed using a UHPLC (Agilent 1290 UHPLC), coupled to a SCIEX ZenoTOF 7600 system with an electrospray ionization source (ESI). Hydrophilic interaction liquid chromatography (HILIC) was used with a ZIC®-pHILIC column (5 µM, polymeric, 150 x 2.1 mm) and a guard column ZIC®-pHILIC (5 µM, polymeric, 20 x 2.1 mm). For the analysis, mobile phase A (water: 10 mM ammonium acetate (NH_4Ac) and 0.04% (v/v) ammonium hydroxide) and mobile phase B (100% acetonitrile) were used at a flow rate of 0.2 mL/min. Gradient went from 90 to 25% of B in 16 min (0-0.50 min: 90% B; 0.50-16 min: 25% B; 16-21 min: 25% B; 21.10-30 min: 90% B) with a total run of 30 min. The column temperature was maintained at 35 °C. The injection volume was 3 µL, and samples were maintained at 10 °C until the analysis. Acquisition was performed in TOF-MS full scan. The nebulizer and drying gas were maintained at a pressure of 40 psi, while the curtain gas and the collision-activated dissociation (CAD) gas were at 35 and 8 psi, respectively. Source temperature was set at 400 °C. The accumulation time was 0.1 s. Capillary voltage was 5.5 kV for positive and 4.5 kV for negative ion mode; with a declustering potential of 50 and 80 V, respectively. Collision energy was set at 10 V for both polarities. The data storage was performed in profile mode. Acquisition was done under the control of SCIEX OS V.3.1.6 software. Sample order was randomized within each batch.

SUPPLEMENTARY INFORMATION FIGURES

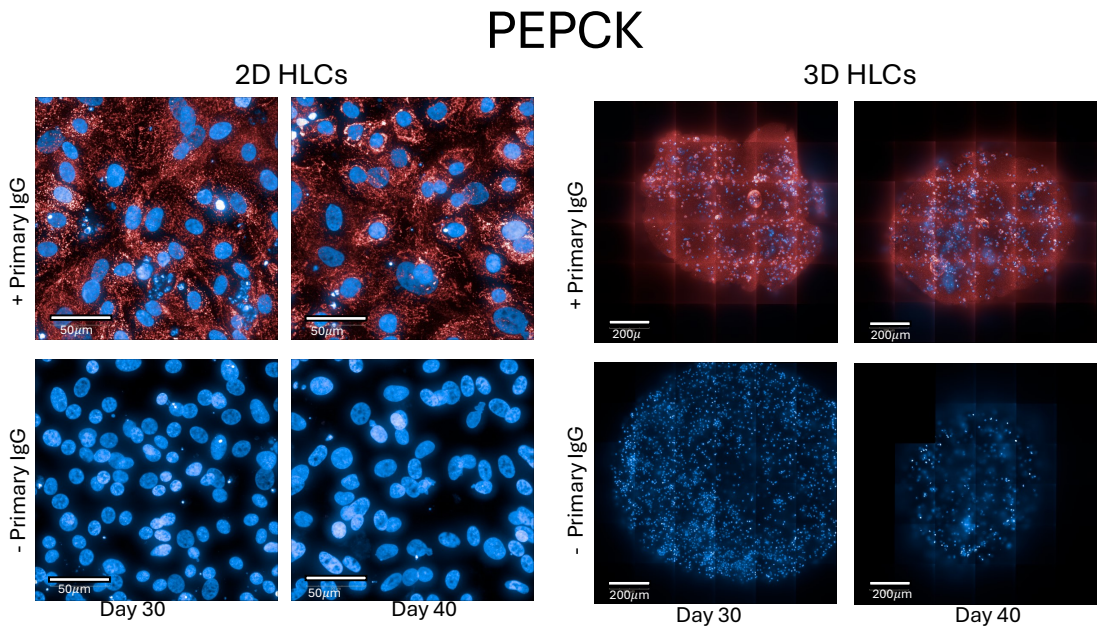

Fig. S1 Immunostaining of phosphoenolpyruvate carboxykinase (PEPCK) comparing day 30 and day 40 of differentiation in 2D (scale represents 50 µm) and 3D HLCs (scale represents 200 µm). Intensity contrast was set equal among the two time points; thus, image intensities are comparable within model. PEPCK is represented in red, and nuclear staining using Hoechst 33342 is in blue. The cells were imaged using a 63x water immersion objective with confocal imaging. 3D images are a stitched compilation of several image fields

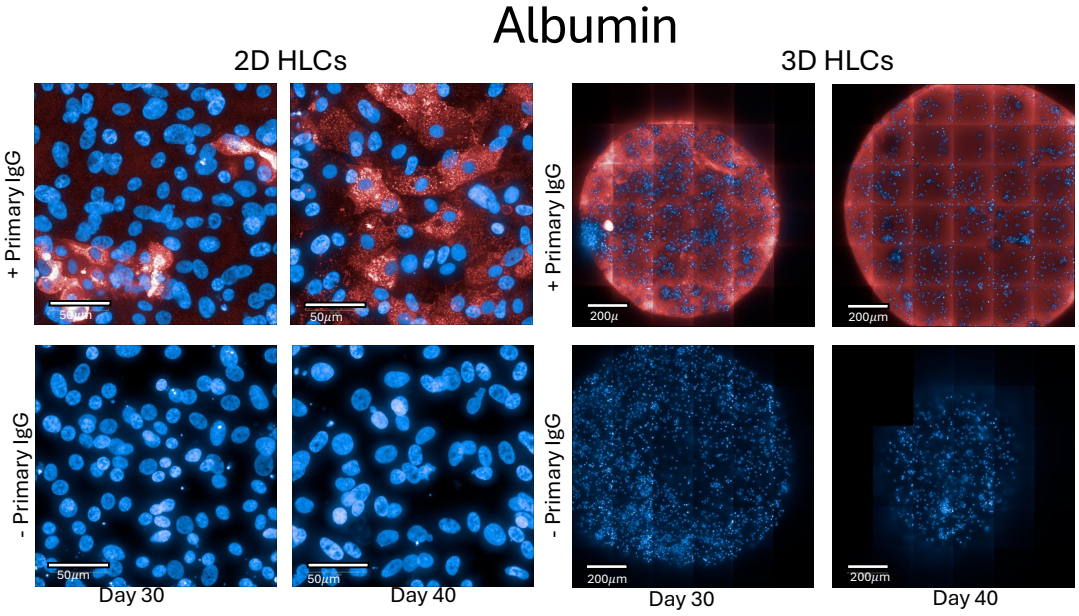

Fig. S2 Immunostaining of albumin comparing day 30 and day 40 of differentiation in 2D (scale represents 50 µm) and 3D HLCs (scale represents 200 µm). Intensity contrast was set equal among the two time points; thus, image intensities are comparable within model. Albumin is represented in red, and nuclear staining using Hoechst 33342 is in blue. The cells were imaged using a 63x water immersion objective with confocal imaging. 3D images are a stitched compilation of several image fields

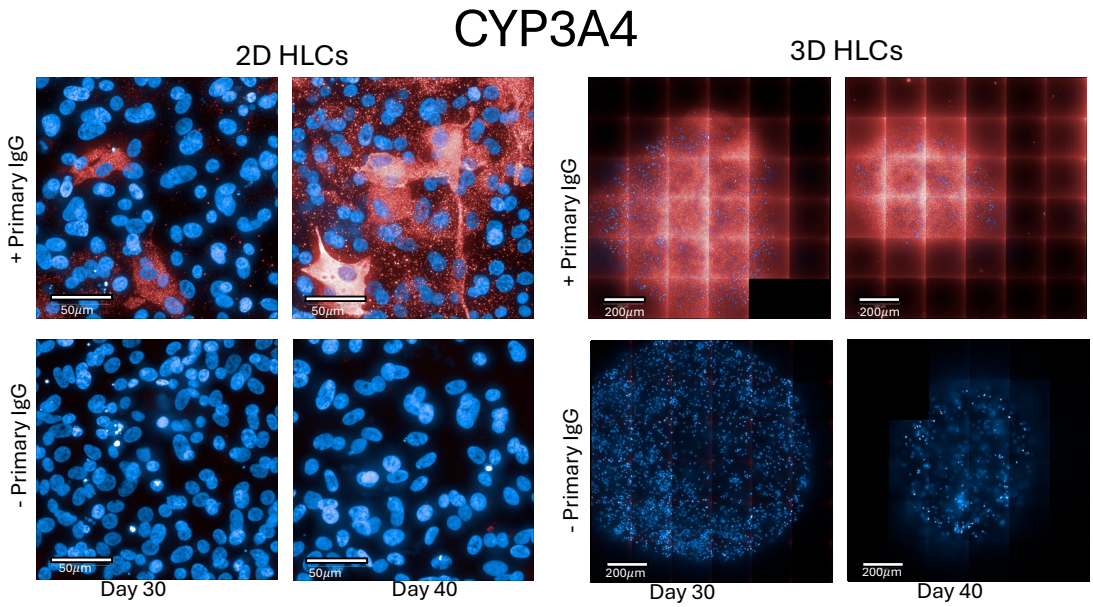

Fig. S3 Immunostaining of CYP3A4 comparing day 30 and day 40 of differentiation in 2D (scale represents 50 µm) and 3D HLCs (scale represents 200 µm). Intensity contrast was set equal among the two time points; thus, image intensities are comparable within model. CYP3A4 is represented in red, and nuclear staining using Hoechst 33342 is in blue. The cells were imaged using a 63x water immersion objective with confocal imaging. 3D images are a stitched compilation of several image fields

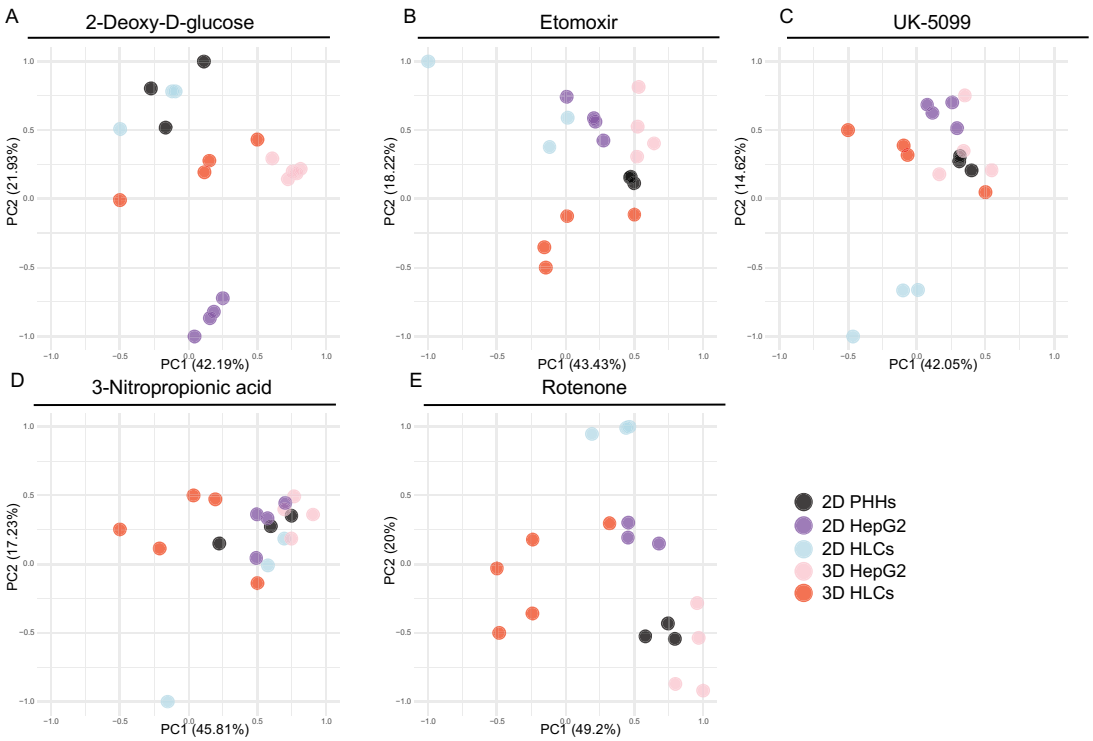

Fig. S4 3D cultures improve mitochondrial function in HepG2 and HLCs, compared to 2D. Principal component analysis (scores plot) of LC-MS central carbon metabolome of 2D PHHs (black), 2D HepG2 (purple), 2D HLCs (blue), 3D HepG2 (pink), and 3D HLCs (red), challenged with **A.** 2-Deoxy-D-glucose (10 mM), **B.** Etomoxir (30 µM), **C.** UK-5099 (10 µM), **D.** 3-Nitropropionic acid (100 µM), and **E.** Rotenone (5 nM) for 24 h. PCAs were centered and scaled using the min-max method N=3-5

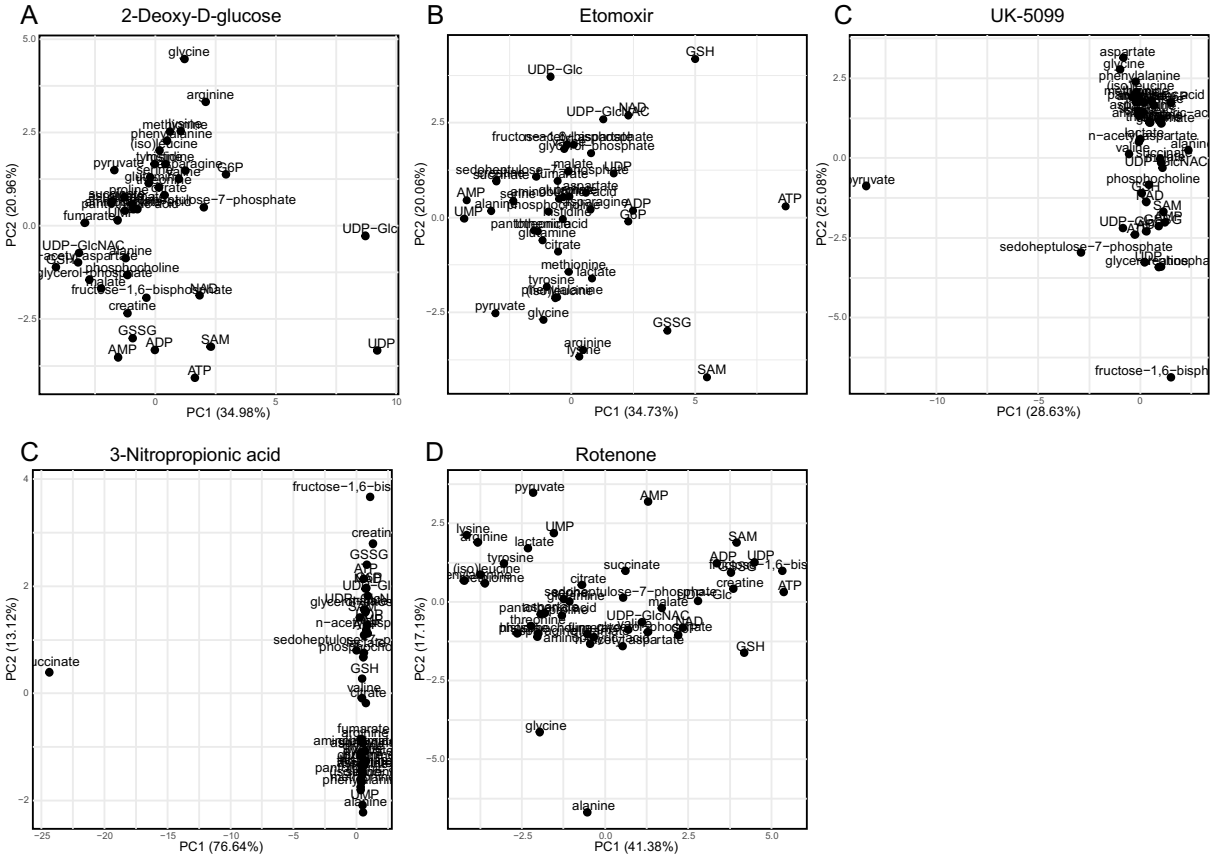

Fig. S5 3D cultures improve mitochondrial function in HepG2 and HLCs, compared to 2D. Principal component analysis (loadings plot) of LC-MS central carbon metabolome of 2D PHHs, 2D HepG2, 2D HLCs, 3D HepG2, and 3D HLCs, challenged with **A.** 2-Deoxy-D-glucose (10 mM), **B.** Etomoxir (30 µM), **C.** UK-5099 (10 µM), **D.** 3-Nitropropionic acid (100 µM), and **E.** Rotenone (5 nM) for 24 h. PCAs were centered and scaled N=3-5

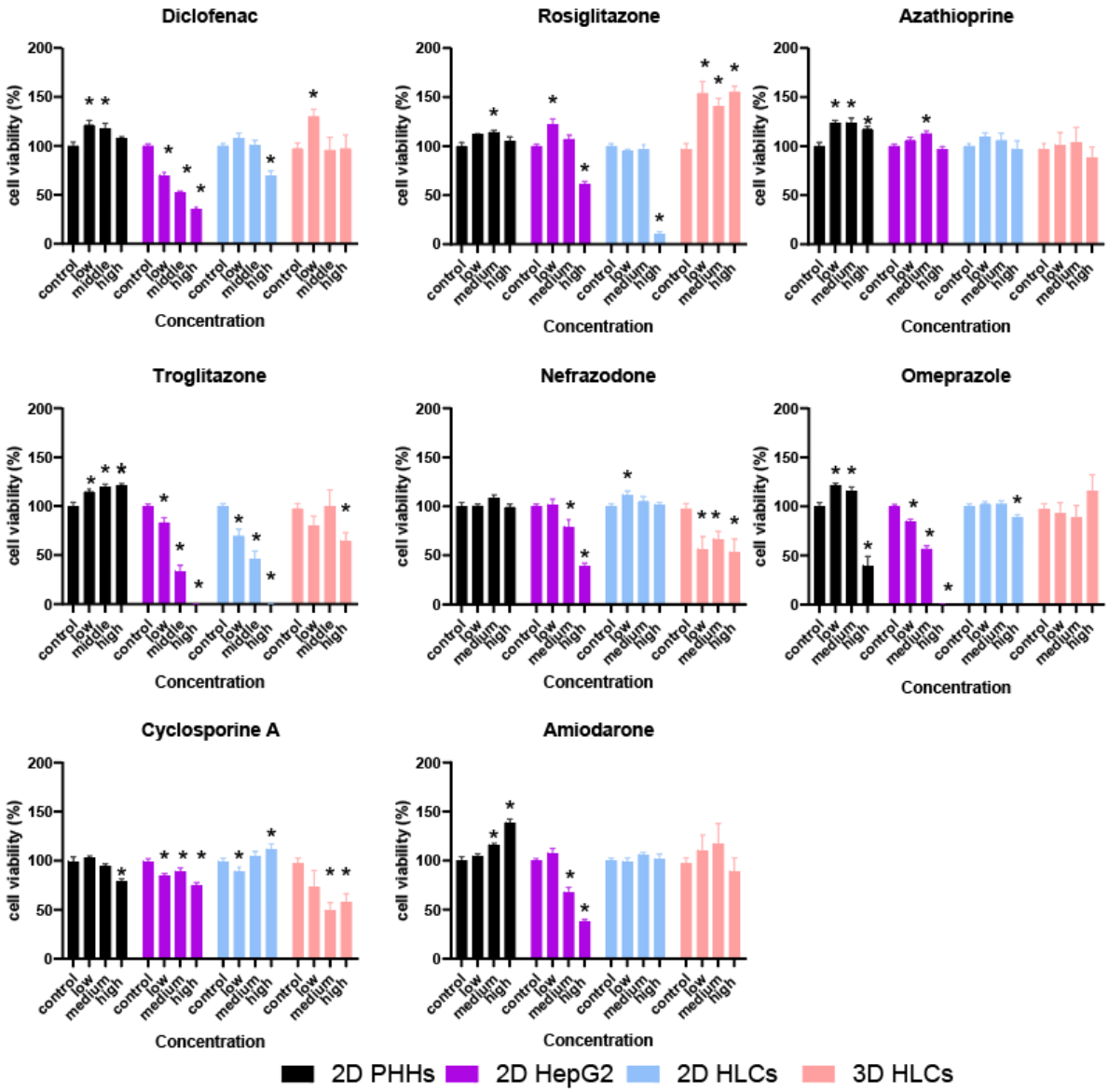

Fig. S6 Resazurin Assay. Cell viability of hepatic cell models in response to 8 known drug-induced liver injury (DILI) drugs tested in 2D PHHs (black), HepG2 (purple), 2D HLCs (blue), and 3D HLCs (red). Low, medium and high drug concentrations, used for the treatment were as follows: diclofenac (150 µM, 300 µM, 600 µM); rosiglitazone (117.5 µM, 235µM, 470 µM); azathioprine (2.5 µM, 5 µM, 10 µM); troglitazone (25 µM, 50 µM, 100 µM); nefazodone (9.5 µM, 19 µM, 38 µM); omeprazole (210 µM, 420 µM, 840 µM); cyclosporine A (5 µM, 10 µM, 20 µM); amiodarone (7 µM, 14 µM, 28 µM). Data is represented as % to control condition (0 µM). Average ± SEM (N=4-15, statistics were done using an unpaired t-test, considering significant when p value <0.05*)

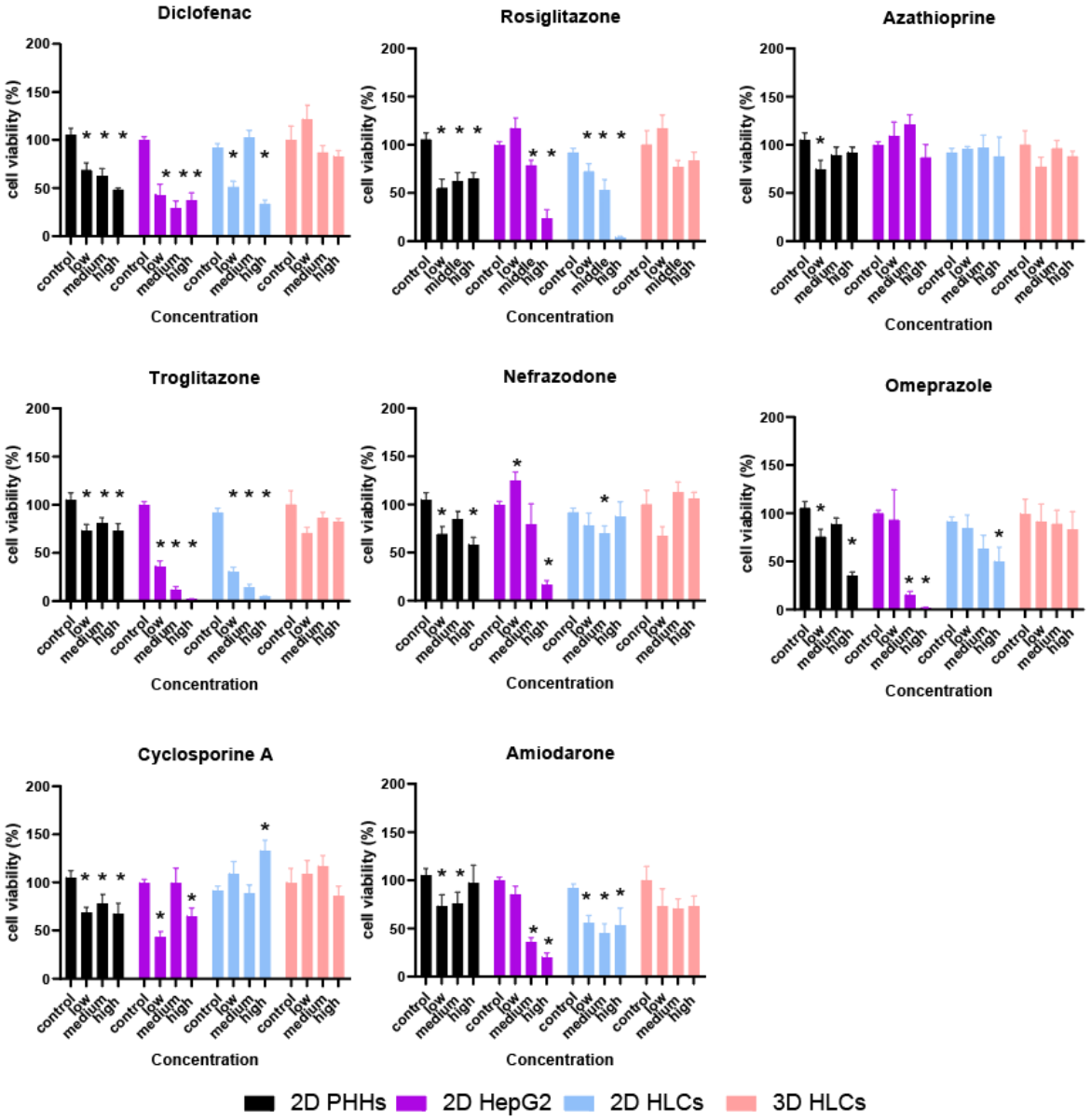

Fig. S7 Calcein AM assay. Cell viability of hepatic cell models in response to 8 known drug-induced liver injury (DILI) drugs tested in 2D PHHs (black), HepG2 (purple), 2D HLCs (blue), and 3D HLCs (red). Low, medium and high drug concentrations, used for the treatment were as follows: diclofenac (150 µM, 300 µM, 600 µM); rosiglitazone (117.5 µM, 235µM, 470 µM); azathioprine (2.5 µM, 5 µM, 10 µM); troglitazone (25 µM, 50 µM, 100 µM); nefazodone (9.5 µM, 19 µM, 38 µM); omeprazole (210 µM, 420 µM, 840 µM); cyclosporine A (5 µM, 10 µM, 20 µM); amiodarone (7 µM, 14 µM, 28 µM). Data is represented as % to control condition (0 µM). Average ± SEM (N=4-10, statistics were done using an unpaired t-test, considering significant when p value <0.05*)

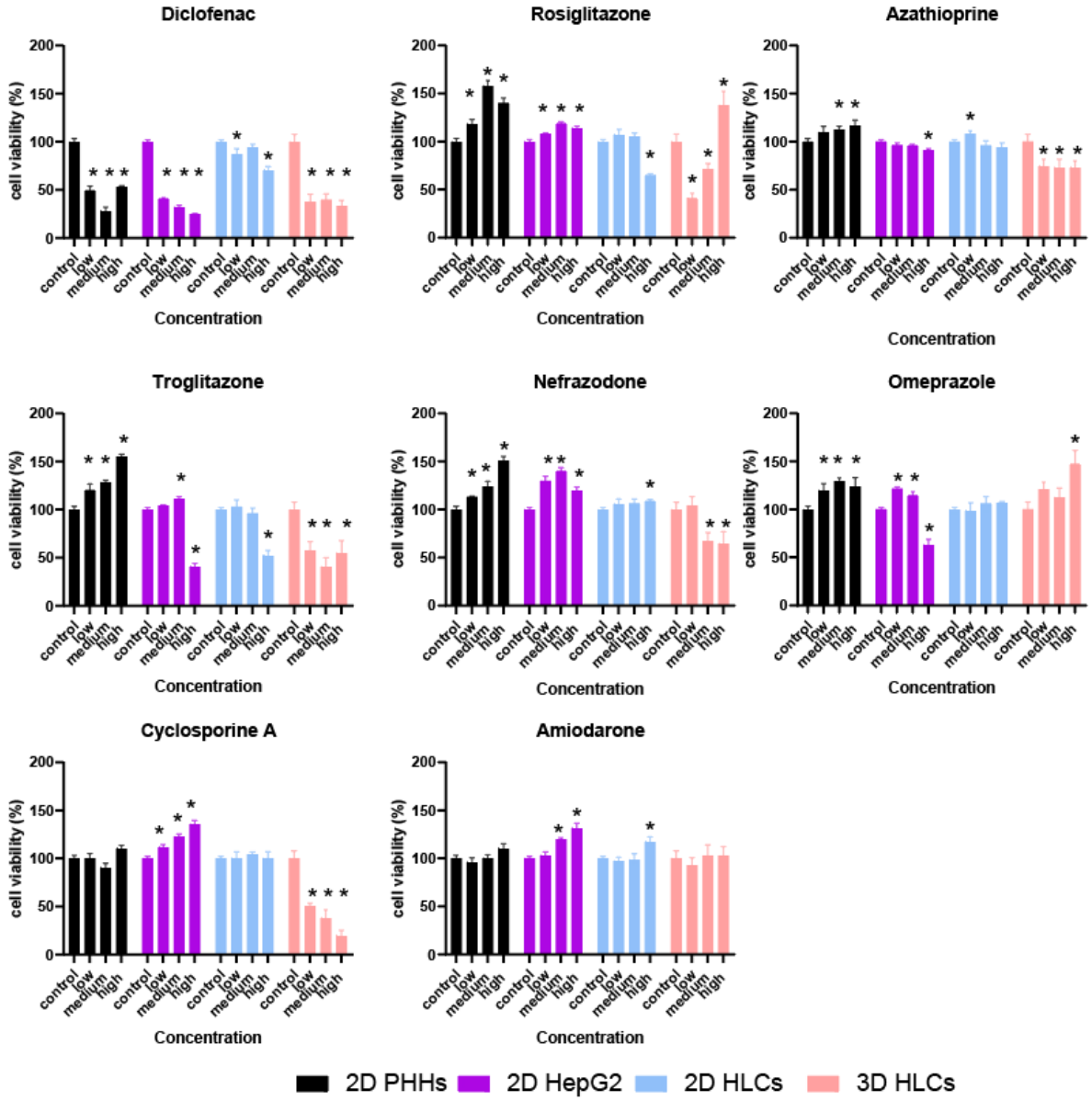

Fig. S8 Lactate assay. Cell viability of hepatic cell models in response to 8 known drug-induced liver injury (DILI) drugs tested in 2D PHHs (black), HepG2 (purple), 2D HLCs (blue), and 3D HLCs (red). Low, medium and high drug concentrations, used for the treatment were as follows: diclofenac (150 µM, 300 µM, 600 µM); rosiglitazone (117.5 µM, 235µM, 470 µM); azathioprine (2.5 µM, 5 µM, 10 µM); troglitazone (25 µM, 50 µM, 100 µM); nefazodone (9.5 µM, 19 µM, 38 µM); omeprazole (210 µM, 420 µM, 840 µM); cyclosporine A (5 µM, 10 µM, 20 µM); amiodarone (7 µM, 14 µM, 28 µM). Data is represented as % to control condition (0 µM). Average ± SEM (N=3-15, statistics were done using an unpaired t-test, considering significant when p value <0.05*)

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