

Supplemental Table 1. List of studies used in Figures with links to the MPS-Db.

Figure	Study Name in the MPS-Database	URLs
1 and 2	<p>Liver_Mimetas 2-lane_Exp.7_PHH (TF HU8373) pre-differentiated in spheroid plate</p> <p>Liver_Mimetas 2-lane_Exp.8_iHep ± NPCs with Midazolam</p> <p>Liver_Mimetas 2-lane_Exp.9_PHH (TF HU8373) ± NPCs with Midazolam</p>	<p>https://mps.csb.pitt.edu/assays/assaystudy/698/ https://mps.csb.pitt.edu/assays/assaystudy/681/ https://mps.csb.pitt.edu/assays/assaystudy/696/</p>
3	<p>Exp.1; Liver_Mimetas 2-lane_Exp.1_iHep and NPCs with Troglitazone</p> <p>Exp.2; Liver_Mimetas 2-lane_Exp.11_iHep and NPCs with Trovafloxacin ± LPS</p> <p>Exp.3; Liver_Mimetas 2-lane_Exp.3_iHep and NPCs (Transporters)</p> <p>Exp.4; Liver_Mimetas 2-lane_Exp.4_iHep and NPCs with Five Chemical Cocktail_2nd</p> <p>Exp.5; Liver_Mimetas 2-lane_Exp.6_iHep ± NPCs with Five Chemical Cocktail</p> <p>Exp.6; Liver_Mimetas 2-lane_Exp.12_iHep ± NPCs with Midazolam_2nd</p>	<p>https://mps.csb.pitt.edu/assays/assaystudy/566/ https://mps.csb.pitt.edu/assays/assaystudy/699/ https://mps.csb.pitt.edu/assays/assaystudy/582/ https://mps.csb.pitt.edu/assays/assaystudy/588/ https://mps.csb.pitt.edu/assays/assaystudy/680/ https://mps.csb.pitt.edu/assays/assaystudy/727/</p>
4	<p>Liver_Mimetas 2-lane_Exp.4_iHep and NPCs with Five Chemical Cocktail_2nd</p> <p>Liver_Mimetas 2-lane_Exp.8_iHep ± NPCs with Midazolam</p>	<p>https://mps.csb.pitt.edu/assays/assaystudy/588/ https://mps.csb.pitt.edu/assays/assaystudy/681/</p>
5	Liver_Mimetas 2-lane_Exp.1_iHep and NPCs with Troglitazone	https://mps.csb.pitt.edu/assays/assaystudy/566/
6	Liver_Mimetas 2-lane_Exp.13_iHep and NPCs with Trovafloxacin ± LPS_2nd	https://mps.csb.pitt.edu/assays/assaystudy/759/

Supplemental Table 2. Loading success rate of iHeps/collagen I and HMEC1/THP1.

iHep all		Exp.0 (Mps- Db Exp. 8)	Exp.1 (Mps- Db Exp.1)	Exp.2 (Mps- Db Exp. 11)	Exp.3 (Mps- Db Exp. 3)	Exp.4 (Mps- Db Exp. 4)	Exp.5 (Mps- Db Exp. 6)	Exp.6 (Mps- Db Exp. 12)	Total	Success rate
2.5 uL iHeps/collagen I	Total No. of chips	88	73	88	96	88	92	76	601	95.5%
	Successful No. of chips	84	71	88	95	88	84	64	574	
2.5 uL HMEC1/THP1	Total No. of chips	44	73	88	96	88	44	40	473	96.8%
	Successful No. of chips	44	70	86	90	88	42	38	458	

iHep+NPCs		Exp.0 (Mps- Db Exp. 8)	Exp.1 (Mps- Db Exp.1)	Exp.2 (Mps- Db Exp. 11)	Exp.3 (Mps- Db Exp. 3)	Exp.4 (Mps- Db Exp. 4)	Exp.5 (Mps- Db Exp. 6)	Exp.6 (Mps- Db Exp. 12)	Total	Success rate
2.5 uL iHeps/collagen I	Total No. of chips	48	73	88	96	88	40	40	473	97.9%
	Successful No. of chips	44	71	88	95	88	39	38	463	
2.5 uL HMEC1/THP1	Total No. of chips	48	73	88	96	88	40	40	473	97.0%
	Successful No. of chips	48	70	86	90	88	39	38	459	

Supplemental Table 3. Trend and variability of each phenotype in OrganoPlate® 2-lane 96 plates seeded with iHeps and non-parenchymal cells.

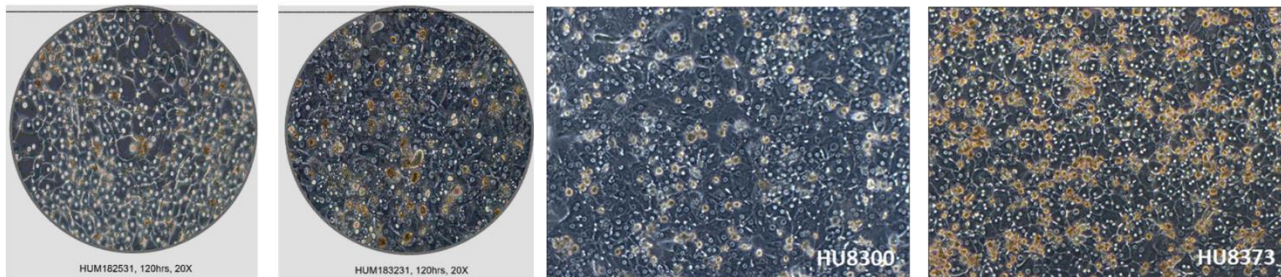
Endpoints	No. of chips	Mean	Min.	Max.	95%CI
% Live cells					
Day 10	18	80.3	64.6	92.1	74.5-86.2
Day 15	16	78.5	64.2	87.5	74.5-82.4
Day 17	19	73.8	49.6	89.6	66.8-80.7
LDH (mU/mL/day)					
Day 1-3	29	5.8	1.5	12.8	3.8-7.8
Day 4-6	30	3.4	0.8	9.2	1.9-4.8
Day 7-9	27	2.2	0.5	7.3	1.0-3.5
Day 10-13	27	2.4	0.4	4.7	1.5-3.3
Day 14-17	19	9.7	2.3	28.6	5.0-14.3
Albumin (µg/day/1M Hep.)					
Day 1-3	29	4.4	1.3	12.3	2.5-6.3
Day 4-6	30	16.6	3.2	53.1	7.6-25.7
Day 7-9	31	18.5	3.8	53.1	9.5-27.5
Day 10-13	31	18.9	3.5	55.6	10.3-27.5
Day 14-17	19	14.2	2.3	47.8	5.1-23.3
Urea (µg/day/1M Hep.)					
Day 1-3	22	11.1	0.1	30.3	5.5-16.6
Day 4-6	30	17.2	1.8	47.3	8.8-25.6
Day 7-9	31	22.2	3.0	100.7	7.5-36.8
Day 10-13	31	36.4	4.9	141.3	16.6-52.3
Day 14-17	19	48.2	7.3	170.4	16.5-80.0
CYP3A4 (pmol/min/1M Hep.)					
Day 4-6	19	0.19	0.11	0.31	0.15-0.22
Day 7-9	27	0.28	0.09	0.88	0.15-0.41
Day 10-13	25	0.35	0.16	0.73	0.27-0.43
Day 14-17	19	0.37	0.08	0.64	0.28-0.46

Supplemental Table 4. Coefficient of variation and its trend of each phenotype in OrganoPlate® 2-lane 96 plates seeded with iHeps and non-parenchymal cells.

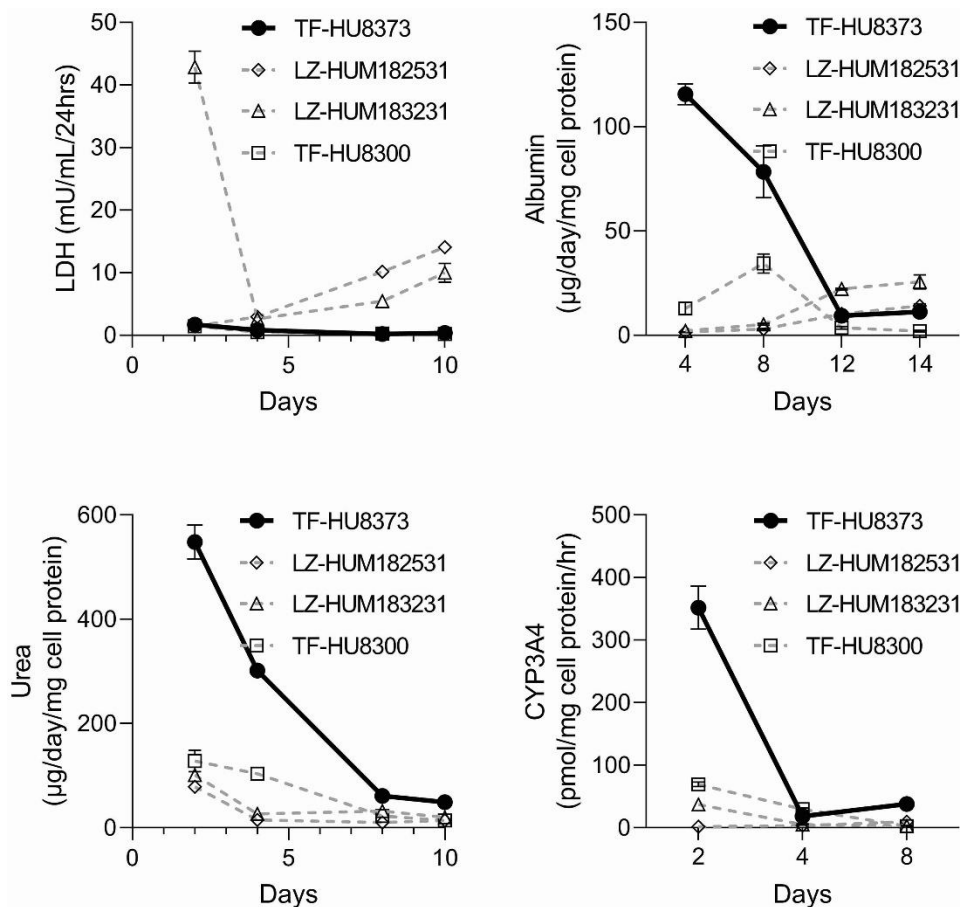
Endpoints	Exp.1 (Mps-Db Exp.1)	Exp.2 (Mps-Db Exp. 11)	Exp.3 (Mps-Db Exp. 3)	Exp.4 (Mps-Db Exp. 4)	Exp.5 (Mps-Db Exp. 6)	Exp.6 (Mps-Db Exp. 12)
% Live cells						
Day 10	8%	12%	-	-	-	-
Day 15	-	-	7%	-	-	-
Day 17	-	-	-	6%	12%	4%
LDH						
Day 1-3	39%	20%	27%	26%	14%	25%
Day 4-6	32%	9%	31%	42%	16%	10%
Day 7-9	34%	19%	46%	18%	17%	26%
Day 10-13	28%	8%	41%	11%	17%	22%
Day 14-17			17%	66%	26%	9%
Albumin						
Day 1-3	33%	13%	25%	39%	35%	50%
Day 4-6	34%	2%	21%	70%	22%	29%
Day 7-9	56%	34%	23%	44%	24%	52%
Day 10-13	46%	29%	22%	32%	26%	47%
Day 14-17			12%	37%	18%	38%
Urea						
Day 1-3	66%	13%	95%	20%	15%	49%
Day 4-6	25%	46%	62%	47%	20%	40%
Day 7-9	20%	27%	60%	47%	20%	43%
Day 10-13	25%	25%	43%	40%	19%	47%
Day 14-17			51%	28%	15%	43%
CYP3A4						
Day 4-6	-	-	-	21%	25%	23%
Day 7-9	32%	32%	-	16%	21%	26%
Day 10-13	36%	15%	-	24%	29%	12%
Day 14-17	-	-	-	23%	26%	35%

Supplemental Figure 1. Basic characteristics (top) and plated morphology (bottom) of 4 lots of primary human hepatocytes from different suppliers (Lonza and ThermoFisher) that were considered for these experiments. Lot HU8373 was selected for subsequent studies.

Supplier	Lot #	Avail. Qty	Sex	Race	Age (yrs)	BMI	Alcohol Use	Tobacco Use	Drug Use	ALT	AST	Viability (%)	Verified for 96-well plating	Induction Fold mRNA CYP3A4	Induction Fold CYP3A4
Lonza	HUM182531	782	F	Caucasian	51	25.7	Social	No	No	20	22	80	Yes	76.8	122
Lonza	HUM183231	668	F	Caucasian	50	28.8	Social	No	No	29	31	84	Yes	111.7	9
ThermoFisher	HU8300	227	M	Caucasian	31	21	Yes	Yes	Yes	-	-	94	Yes	24.6	21.1
ThermoFisher	HU8373	813	F	Caucasian	26	18.6	Yes	Yes	Yes	-	-	93	Yes	33.14	34.6



Supplemental Figure 2. Comparison of basic function (LDH, albumin, urea, and CYP3A4 activity) of in traditional 2D cultures of 4 lots of primary human hepatocytes from different suppliers (Lonza and ThermoFisher) that were considered for these experiments. Data are plotted as mean \pm standard deviation (n = 3-4). Each lot is marked as shown in the insets.



Supplemental Figure 3. Comparison of hepatotoxicity screening utility between OrganoPlate® 2-lane 96 plate seeded with iHeps and non-parenchymal cells and traditional 2D culture models, as reported in (Bircsak et al., 2021).

