Supplemental Materials

Title

Functional proliferating human hepatocytes: *in vitro* hepatocyte model for drug metabolism, excretion and toxicity

Authors

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Supplemental data

Supplemental Table 1. Demographics, supplier and metabolic activity of primary human hepatocytes used for metabolism, disposition and toxicity.

No.	Donor	Supplier	Race	Age	Sex	Thaw viability	CYP isoform (pmol/min/million cells)		
							1A2	2B6	3A4
1	MRW[1]	1	C	11mos	M	85%	15.5	4.62	14.0
2	QIE[1]	1	C	7mos	M	86%	12.5	7.19	11.8
3	LHum15101[2]	2	C	33	M	92%	25.0	8.33	6.67
4	LHuf17905A[2]	2	C	58	F	94%	31.6	6.67	6.67

C: Caucasian; [1)] BioreclamationIVT GmbH (Frankfurt/Main, Germany/Baltimore, MD, USA). Cells were incubated in suspension either for 4 hours at 37°C with the following test substrates: phenacetin (15μM), midazolam (15μM), bupropion (250μM).[2)] XenoTech (Lenexa, KS, USA). Cells were incubated in suspension either for 1 hour at 37°C with the following test substrates: phenacetin (200μM), midazolam (50μM), bupropion (100μM). Metabolite rate of formation was measured in CYP1A2 acetaminophen, CYP2B6 hydroxybupropion, and CYP3A4 1-hydromidazolam.

Supplemental Table 2. HPLC/MS-MS conditions and parameters for CYP450 enzyme activity assays.

CYP450 isoform	Probe Substrate	HPLC column	Flow rate (mL/min)	Q1/Q3 (m/z)	Mode	CE
1A2	Phenacetin	1	0.25	180/110.1	Positive	25
2B6	Bupropion	1	0.25	235.2/86.15	Positive	24
2C9	Diclofenac acid	1	0.25	294.1/250.1	Negative	11
2C19	s-Mephenytoin	1	0.25	219.2/133.9	Positive	21
2D6	Dextromethorphan	1	0.25	271.8/171	Positive	40
2E1	Chlorzoxazone	1	0.25	167.9/132.0	Negative	20
3A4	Testosterone	1	0.25	289.1/97.05	Positive	20

HPLC column: 1 INERTSIL ODS-4 C18 (2.1×100 mm), GL Sciences, Japan

Supplemental Table 3. Primer sequences used for RT-qPCR

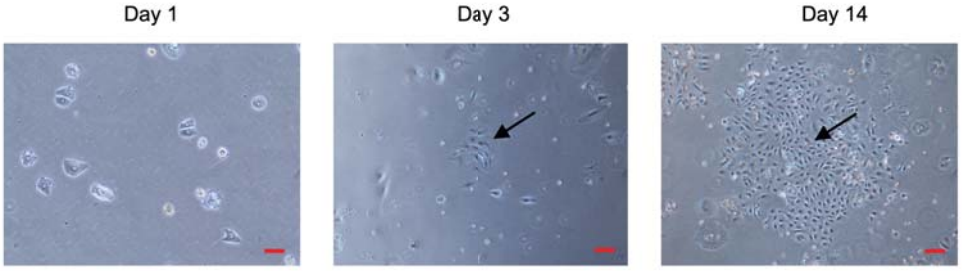
Gene	Sequences (Forward, 5' to 3')	Sequences (Reverse, 5' to 3')
CK7	GACATCTTTGAGGCCCAGATT	CTTGAAGTCCTCCACCACATC
SOX17	CTGGTGATGGTTGCACAATTC	CGCCCTTCACCTTCATGT
HNF4A	TCCAACCCAACCTCATCCTCCTTCTT	TCCTCTCCACTCCAAGTTCCTGTT
CEBPA	GATAACCTTGTGCCTTGGAAATG	GAGGCAGGAAACCTCCAAATA
ALB	GTGAAACACAAGCCCAAGGCAACA	TCCTCGGCAAAGCAGGTCTC
AAT	AGGGCCTGAAGCTAGTGGATAAGT	TCTGTTTCTTGGCCTCTTCGGTGT
CYP1A2	ATGCCCTCAACACCTTCTC	CTCCTGCAACCTGCTGAT
CYP2B6	TTGGATGTGTAGAGGACAGAGA	ATACACAGCAAGGCTACAGC
CYP3A4	GTGACCAAATCAGTGTGAGGAGGTAGA	AGGAGGAGTTAATGGTGCTAACTGG
BSEP	TACACAGAGGCGGGTCTATAA	CTGGTCTTCAGTCCTTCTGTTC
MRP2	CCACAAGCCCAGAATAAGGTAG	ACTGACAATTGGTAGGTGAAAGT
NTCP	GTGGCAATCAAGAGTGGTGTC	ACTGGTCCTGGTTCTCATTCC
OATP1B1	TTGGAGGTGTTTTGACTGCTT	ACAAGTGGATAAGGTCGATGTTG
TBP	TTGCTGAGAAGAGTGTGCTGGAGATG	CGTAAGGTGGCAGGCTGTTGTT

Supplemental Table 4. *In vitro* toxicity test TC_{50} values in PHH, ProliHH-P and ProliHH-M for 12 compounds.

Compounds	TC_{50} values (μM)			
	PHH	ProliHH-P	ProliHH-M	literature
Lithocholic acid	153.9	108.7	58.76	
Cyclosporin A	17.87	6.14	63.35	
Troglitazone	7.03	4.41	108.8	
Tamoxifen	3.03	24.94	9.05	
Chlorpromazine	34.83	15.48	30.06	
Amiodarone	11.44	10.83	18.25	
Imipramine	44.43	425.9	770.1	
Chenodeoxycholic Acid	754.7	526.1	500.1	
Ibuprofen	3717	3486	3836	
Valproic acid	>5000 [1]	>5000	>5000	(Albrecht et al., 2019)
Isoniazid	>5000 [1]	>5000	>5000	(Albrecht et al., 2019)
Rifampicin	255 [1]	878.8	488.8	(Albrecht et al., 2019)

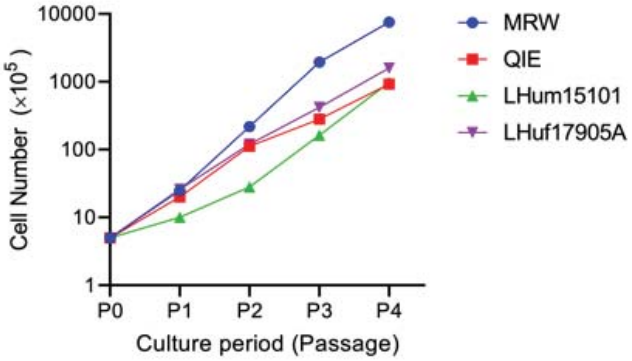
Supplemental Figure 1: Morphology of ProliHH at indicated days in HM culture.

Phase microscopy shows colonies of ProliHH generated from primary hepatocytes in day 1, day 3, and day 14. ProliHH were derived from donor QIE. Scale bar, 100 μm.



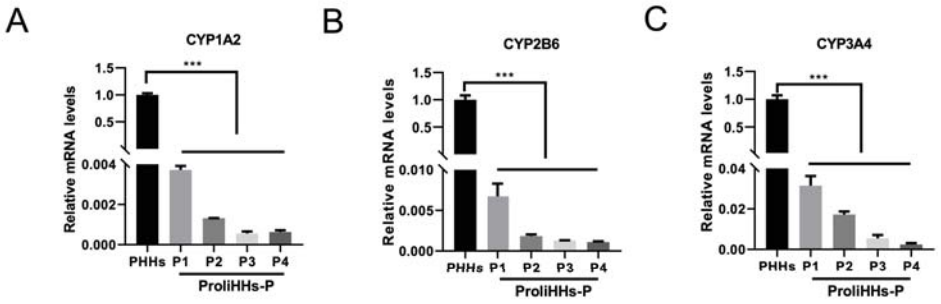
Supplemental Figure 2: ProliHH were proliferative in HM culture.

Growth curves of cultured ProliHH were analyzed at indicated passages.



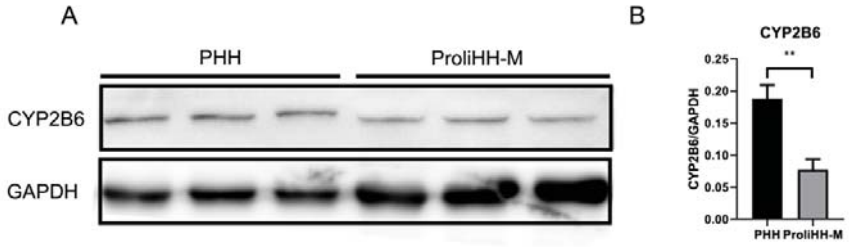
Supplemental Figure 3: Impact of culture time on CYP450 mRNA expression in ProliHH.

Comparison of CYP450 mRNA expression determined at PHH (freshly thawed PHH source) to P4 (week four) of ProliHH-P (donor QIE) by qPCR. (A) CYP1A2 (B) CYP2B6 (C) CYP3A4. Data were normalized to PHH. All error bars indicate ± SD.

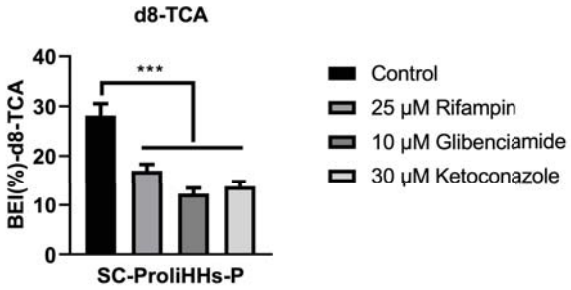


Supplemental Figure 4: Protein expression level of CYP2B6 enzyme in ProliHH-M.

Protein expression level of CYP2B6 enzyme in ProliHH-M was analyzed by Western blotting compared with the PHH. (A) The Western blotting results of CYP2B6 expression in ProliHH-M and PHH (B) The statistical analysis for (A).



Supplemental Figure 5: Effect of cholestatic drugs on d8-TCA efflux in sandwich cultured ProliHH. SC-ProliHH-P were treated with 5 µM d8-TCA alone or in combination with BSEP inhibitors for 15 min. BSEP inhibitors include 25 µM rifampicin, 10 µM glibenclamide and 30 µM ketoconazole. ProliHH were generated from donor LHum15101.



References

Albrecht W, Kappenberg F, Brecklinghaus T, Stoeber R, Marchan R, Zhang M, Ebbert K, Kirschner H, Grinberg M, and Leist M (2019) Prediction of human drug-induced liver injury (DILI) in relation to oral doses and blood concentrations. Archives of toxicology 93:1609-1637.