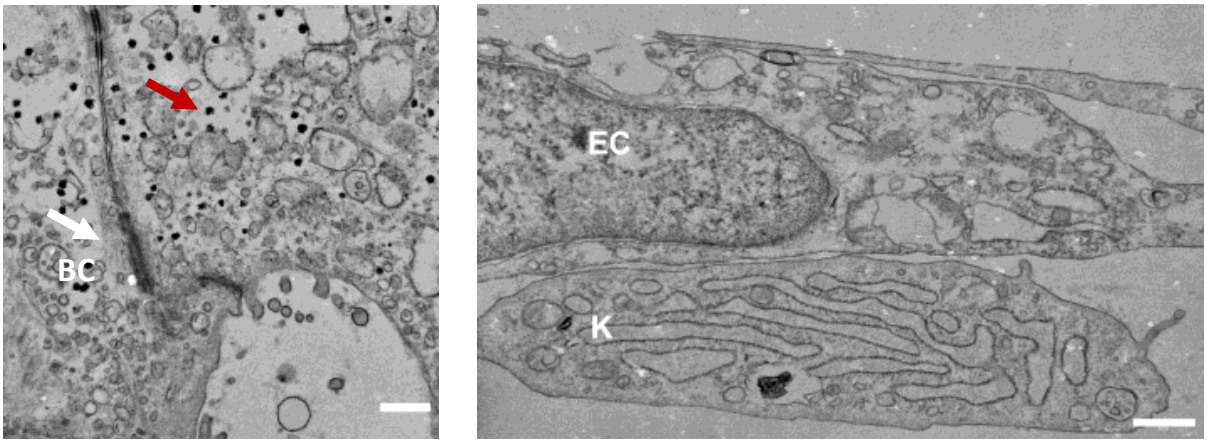
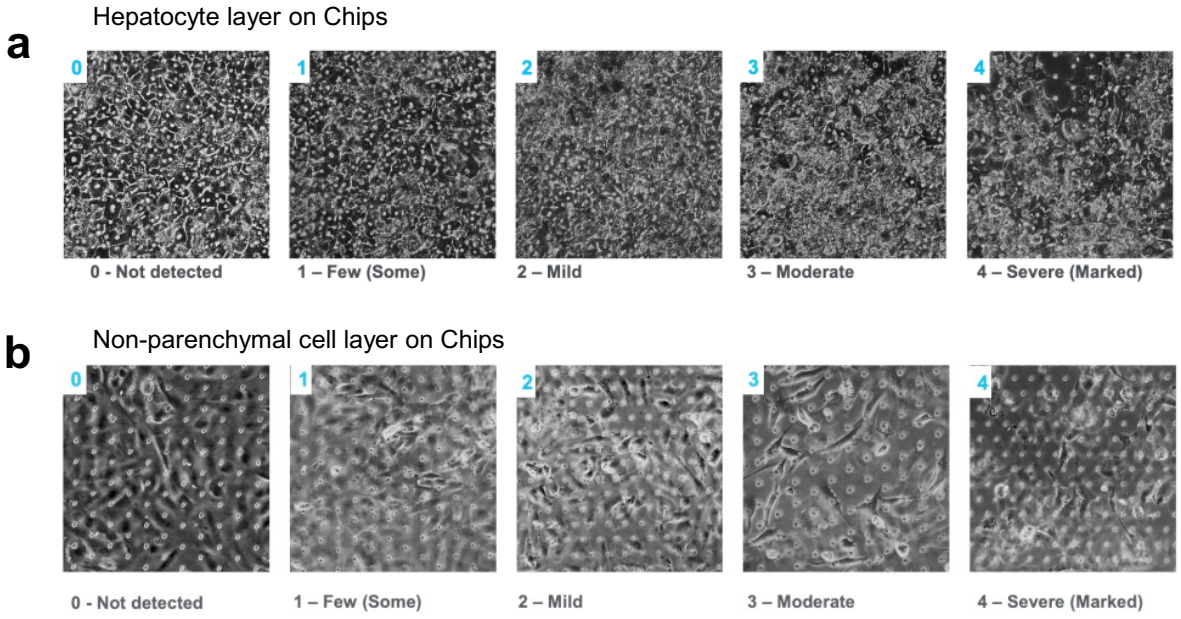
Qualifying a human Liver-Chip for predictive toxicology: Performance assessment and economic implications

Ewart et al.

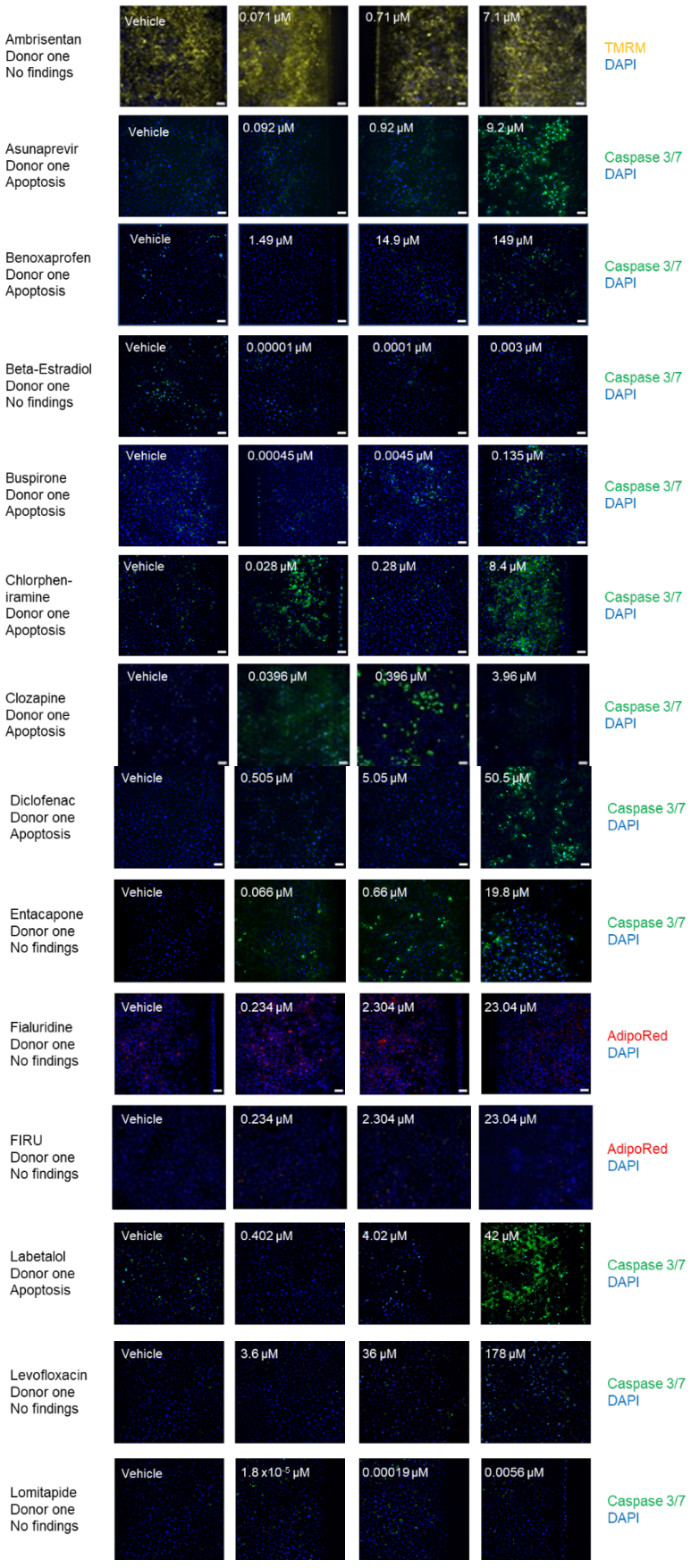
Supplementary Material

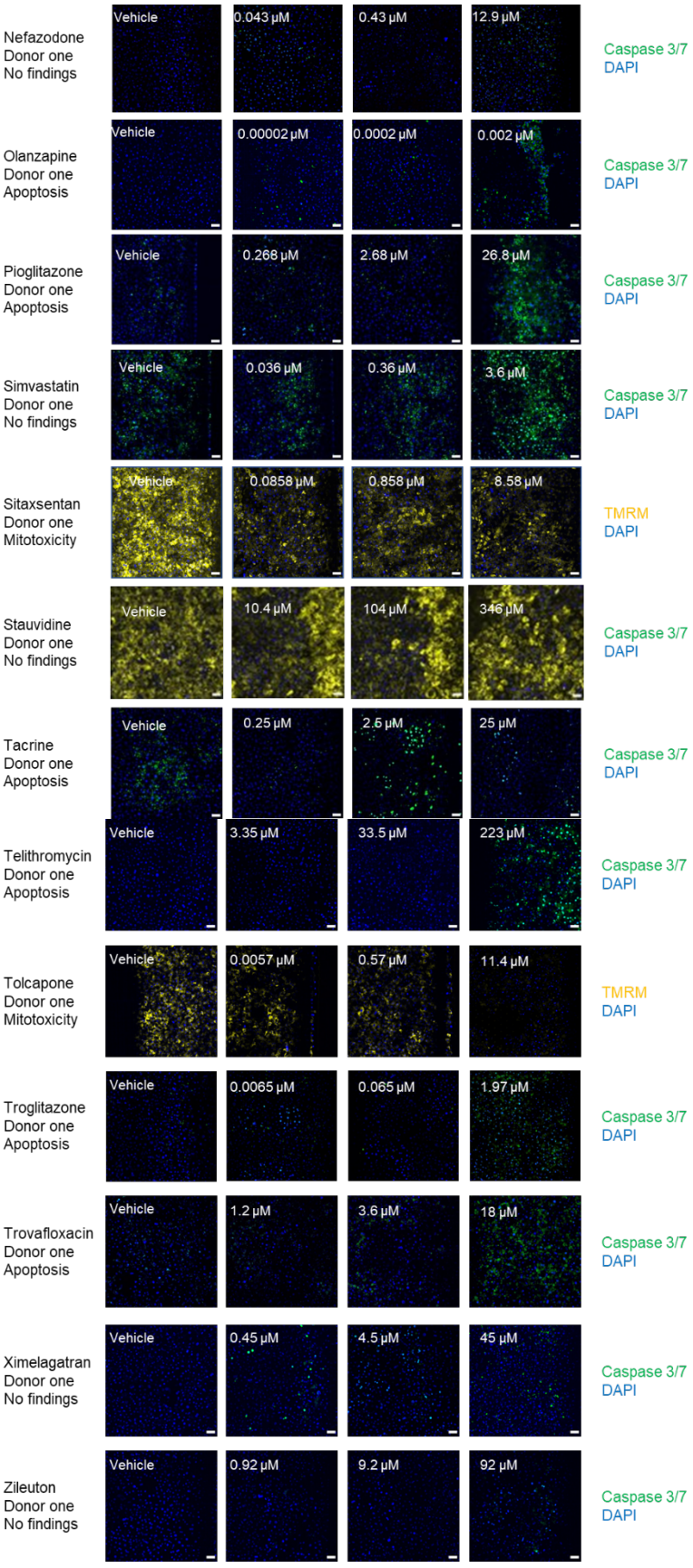


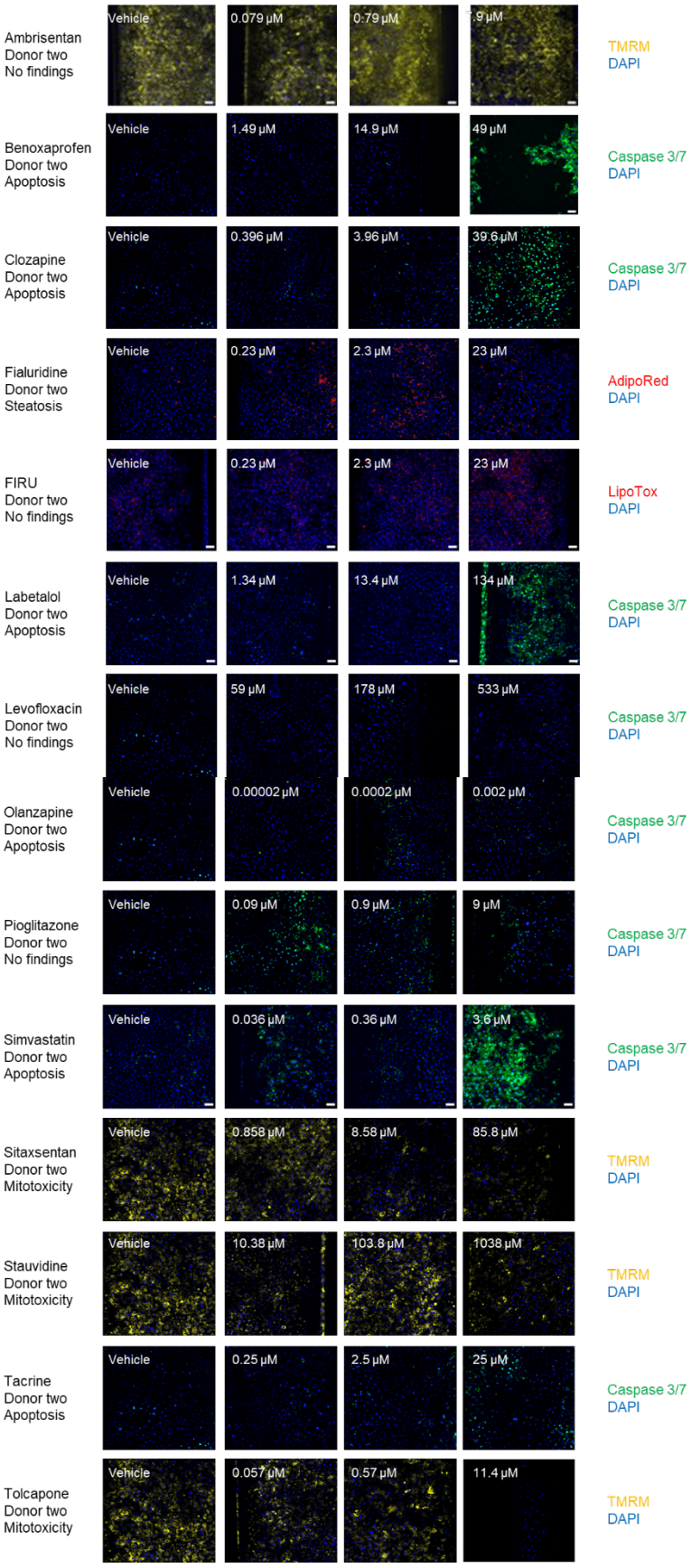
Supplementary Figure S1. Representative transmission electron microscopy images showing a well-formed bile canaliculus (bc) between neighboring hepatocytes (left) and cell-cell contact formation between a Kupffer (K) cell and liver sinusoidal endothelial cell (right) (bar, 0.5 μm).

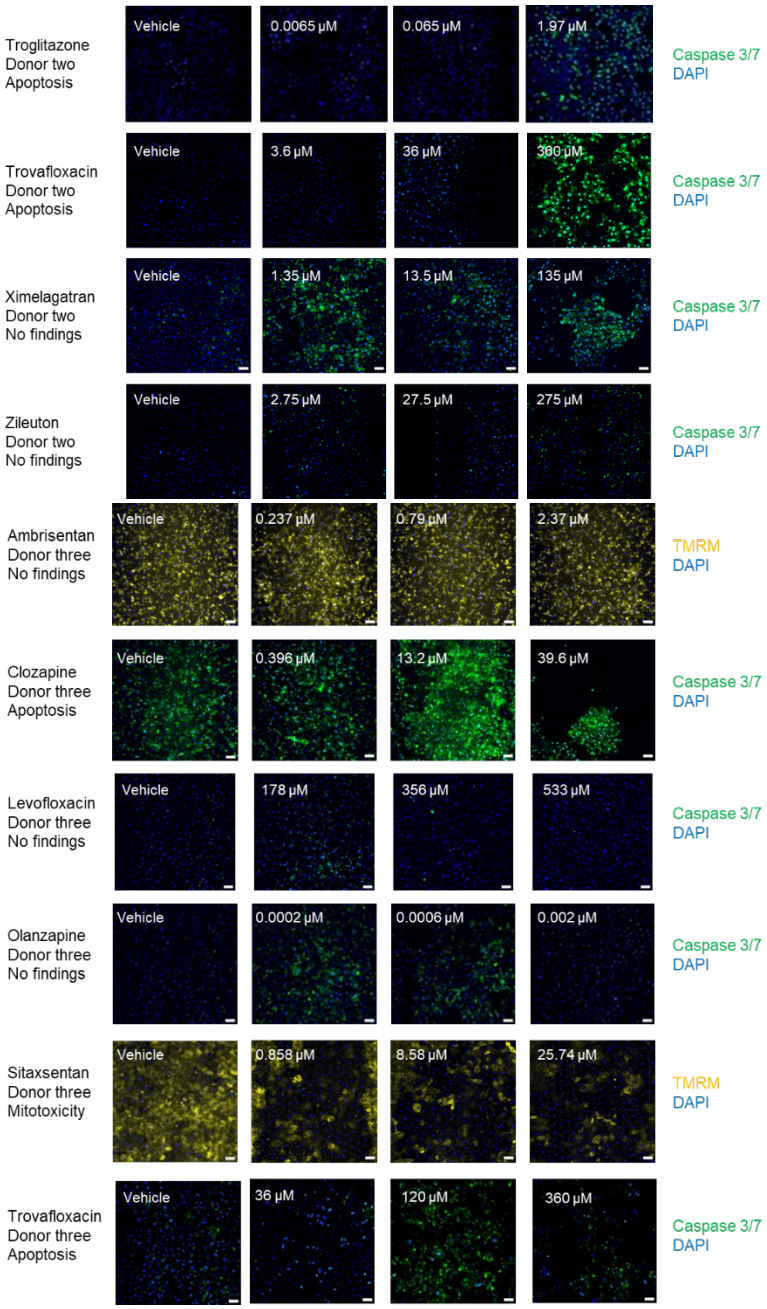


Supplementary Figure S2. a) Representative brightfield images to depict the cellular morphology score in the top channel of the Liver-Chip which contains hepatocytes. A score of 0 represents no hepatotoxicity detected, which is defined by 95-100% healthy hepatocyte morphology, hexagonal shape containing binucleated cells, clear cell cytoplasm, distinctive cell junctions and less than 5% dead cells. A score of 1 represents at least 85% healthy hepatocyte morphology, a hexagonal shape containing binucleated cells, clear cell cytoplasm, distinctive cell junctions but < 15% are dead cells. A score of 2 represents mild hepatotoxicity with > 70% monolayer of hepatocytes visible, evidence that cells have begun to lose their distinct cell junctions, many cells contain a granulated cytoplasm but < 30% are dead cells. A score of 3 represents moderate hepatotoxicity with severe granulation of cytoplasm and most of the cells have lost their junctions. Approximately 50% of the cells are considered dead. A score of 4 represents severe hepatotoxicity with agglomeration of cell debris and > 50% of the cells are considered dead. The pores on the membrane become visible as there is no longer a cellular monolayer. b) Representative brightfield images to depict the cellular morphology score in the bottom channel of the Liver-Chip which contains non-parenchymal cells. A score of 0 represents no cytotoxicity detected, with an intact monolayer and <1% of the cells are dead. A score of 1 represents at least 90% of the monolayer is present and there are <10% dead cells. A score of 2 represents mild cytotoxicity with > 80% of the monolayer present and < 20% are dead cells. A score of 3 represents moderate cytotoxicity with > 50% of the monolayer present and < 50% are dead cells. A score of 4 represents severe cytotoxicity with > 50% of the cells are considered dead.


Ambrisentan
Donor one
No findings
Vehicle
0.071 µM
0.71 µM
7.1 µM
TMRM
DAPI
Asunaprevir
Donor one
Apoptosis
Vehicle
0.092 µM
0.92 µM
9.2 µM
Caspase 3/7
DAPI
Benoxaprofen
Donor one
Apoptosis
Vehicle
1.49 µM
14.9 µM
149 µM
Caspase 3/7
DAPI
Beta-Estradiol
Donor one
No findings
Vehicle
0.00001 µM
0.0001 µM
0.003 µM
Caspase 3/7
DAPI
Buspirone
Donor one
Apoptosis
Vehicle
0.00045 µM
0.0045 µM
0.135 µM
Caspase 3/7
DAPI
Chlorphen-
iramine
Donor one
Apoptosis
Vehicle
0.028 µM
0.28 µM
8.4 µM
Caspase 3/7
DAPI
Clozapine
Donor one
Apoptosis
Vehicle
0.0396 µM
0.396 µM
3.96 µM
Caspase 3/7
DAPI
Diclofenac
Donor one
Apoptosis
Vehicle
0.505 µM
5.05 µM
50.5 µM
Caspase 3/7
DAPI
Entacapone
Donor one
No findings
Vehicle
0.066 µM
0.66 µM
19.8 µM
Caspase 3/7
DAPI
Fialuridine
Donor one
No findings
Vehicle
0.234 µM
2.304 µM
23.04 µM
AdipoRed
DAPI
FIRU
Donor one
No findings
Vehicle
0.234 µM
2.304 µM
23.04 µM
AdipoRed
DAPI
Labetalol
Donor one
Apoptosis
Vehicle
0.402 µM
4.02 µM
42 µM
Caspase 3/7
DAPI
Levofloxacin
Donor one
No findings
Vehicle
3.6 µM
36 µM
178 µM
Caspase 3/7
DAPI
Lomitapide
Donor one
No findings
Vehicle
1.8 x10-5 µM
0.00019 µM
0.0056 µM
Caspase 3/7
DAPI


Nefazodone
Donor one
No findings
Vehicle
0.043 µM
0.43 µM
12.9 µM
Caspase 3/7
DAPI
Olanzapine
Donor one
Apoptosis
Vehicle
0.00002 µM
0.0002 µM
0.002 µM
Caspase 3/7
DAPI
Pioglitazone
Donor one
Apoptosis
Vehicle
0.268 µM
2.68 µM
26.8 µM
Caspase 3/7
DAPI
Simvastatin
Donor one
No findings
Vehicle
0.036 µM
0.36 µM
3.6 µM
Caspase 3/7
DAPI
Sitaxsentan
Donor one
Mitotoxicity
Vehicle
0.0858 µM
0.858 µM
8.58 µM
TMRM
DAPI
Stauvidine
Donor one
No findings
Vehicle
10.4 µM
104 µM
346 µM
Caspase 3/7
DAPI
Tacrine
Donor one
Apoptosis
Vehicle
0.25 µM
2.5 µM
25 µM
Caspase 3/7
DAPI
Telithromycin
Donor one
Apoptosis
Vehicle
3.35 µM
33.5 µM
223 µM
Caspase 3/7
DAPI
Tolcapone
Donor one
Mitotoxicity
Vehicle
0.0057 µM
0.57 µM
11.4 µM
TMRM
DAPI
Troglitazone
Donor one
Apoptosis
Vehicle
0.0065 µM
0.065 µM
1.97 µM
Caspase 3/7
DAPI
Trovafloxacin
Donor one
Apoptosis
Vehicle
1.2 µM
3.6 µM
18 µM
Caspase 3/7
DAPI
Ximelagatran
Donor one
No findings
Vehicle
0.45 µM
4.5 µM
45 µM
Caspase 3/7
DAPI
Zileuton
Donor one
No findings
Vehicle
0.92 µM
9.2 µM
92 µM
Caspase 3/7
DAPI


Ambrisentan
Donor two
No findings
Vehicle
0.079 µM
0.79 µM
7.9 µM
TMRM
DAPI
Benoxaprofen
Donor two
Apoptosis
Vehicle
1.49 µM
14.9 µM
49 µM
Caspase 3/7
DAPI
Clozapine
Donor two
Apoptosis
Vehicle
0.396 µM
3.96 µM
39.6 µM
Caspase 3/7
DAPI
Fialuridine
Donor two
Steatosis
Vehicle
0.23 µM
2.3 µM
23 µM
AdipoRed
DAPI
FIRU
Donor two
No findings
Vehicle
0.23 µM
2.3 µM
23 µM
LipoTox
DAPI
Labetalol
Donor two
Apoptosis
Vehicle
1.34 µM
13.4 µM
134 µM
Caspase 3/7
DAPI
Levofloxacin
Donor two
No findings
Vehicle
59 µM
178 µM
533 µM
Caspase 3/7
DAPI
Olanzapine
Donor two
Apoptosis
Vehicle
0.00002 µM
0.0002 µM
0.002 µM
Caspase 3/7
DAPI
Pioglitazone
Donor two
No findings
Vehicle
0.09 µM
0.9 µM
9 µM
Caspase 3/7
DAPI
Simvastatin
Donor two
Apoptosis
Vehicle
0.036 µM
0.36 µM
3.6 µM
Caspase 3/7
DAPI
Sitaxsentan
Donor two
Mitotoxicity
Vehicle
0.858 µM
8.58 µM
85.8 µM
TMRM
DAPI
Stauvidine
Donor two
Mitotoxicity
Vehicle
10.38 µM
103.8 µM
1038 µM
TMRM
DAPI
Tacrine
Donor two
Apoptosis
Vehicle
0.25 µM
2.5 µM
25 µM
Caspase 3/7
DAPI
Tolcapone
Donor two
Mitotoxicity
Vehicle
0.057 µM
0.57 µM
11.4 µM
TMRM
DAPI



Supplementary Figure S3. Representative immunofluorescent images from day 7 post-vehicle or drug administration of the hepatocyte cell layer in the top channel of the chip. Each drug is shown with its free drug concentration and corresponding vehicle image that was used for thresholding across each donor the drug was tested in. The data support the immunofluorescent findings statement in Table 2 and 3.

Criteria/ Cell Type	Hepatocyte Donor one	Hepatocyte Donor two	Hepatocyte Donor three	Liver Sinusoidal Endothelial Cells	Kupffer Cell	Stellate Cell
Donor gender	Male	Male	Female	Male	Female	Male
Donor age	53 years	26 years	77 years	Unknown	25 years	21-25 years
Race	Caucasian	Caucasian	African-American	Unknown	Caucasian	Unknown
BMI	18.1	22.2	28.5	Unknown	19.7	20-25
Viability	90%	93%	96%	93%	70%	91%
Vendor	Thermo	Thermo	Thermo	Cell Systems	Lonza	iXCells
Phenotypic Markers	MRP2 positive Vimentin negative	MRP2 positive Vimentin negative	MRP2 positive	Stabilin positive Vimentin positive	CD68 positive, CD11b positive, Alpha-SMA negative	Vimentin positive, AdipoRed positive, Alpha-SMA positive

Supplementary Table S1. Details of the cell sources and their defining characteristics used in the investigation.

Drug	Human Cmax Total	Expected Fraction Unbound in Plasma	Multiplier xCmax	Chip Dosing Concentration (uM) Total	Chip Dosing Concentration (uM) Free	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Ambrisentan	0.79µM[26]	0.01 [60]	0.1	0.0024	0.00079					
			0.3	0.0071	0.00237					
			1	0.0235	0.0079					
			3	0.0705	0.0237					
			10	0.2351	0.079					
			30	0.7054	0.237					
			100	2.351	0.79					
			300	7.054	2.37					
			1000	23.51	7.9					
Asunaprevir	0.7644µM[51]	0.012 [61]	3	0.073	0.0275184					
			10	0.243	0.091728					
			30	0.728	0.275184					
			100	2.428	0.91728					
			300	7.284	2.75184					
			1000	24.280	9.1728					
Benoxaprofen***	149.1µM[52]	0.01 [62]	0.1	0.444	0.1491					
			0.3	1.331	0.4473					
			1	4.438	1.491					
			3	13.31	4.473					
			10	44.38	14.91					
			30	133.13	44.73					
			100	443.75	149.1					
Beta-Estradiol	0.0006µM[53]	0.016 [61]	0.1	0	0.00000096					
			0.3	0	0.00000288					
			1	0	0.0000096					
			3	0.0001	0.0000288					
			10	0.0002	0.000096					
			30	0.0007	0.000288					
			100	0.0022	0.00096					
			300	0.0066	0.00288					
Buspirone	0.009µM[54]	0.05 [62]	0.1	6.25869E-05	0.000045					
			0.3	0.00019	0.000135					
			1	0.00063	0.00045					
			3	0.00188	0.00135					
			10	0.00626	0.0045					
			30	0.01878	0.0135					
			100	0.06259	0.045					
			300	0.18776	0.135					
Chlorpheniramine maleate	0.04µM[54]	0.7 [61]	0.1	0.0028	0.0028					
			0.3	0.0085	0.0084					
			1	0.0283	0.028					
			3	0.0849	0.084					
			10	0.2831	0.28					
			30	0.8493	0.84					
			100	2.8311	2.8					
			300	8.4934	8.4					
Clozapine	2.4µM[54]	0.055 [61]	0.1	0.0178	0.0132					
			0.3	0.0535	0.0396					
			1	0.1784	0.132					
			3	0.5351	0.396					
			10	1.7838	1.32					
			30	5.3514	3.96					
			100	17.838	13.2					
			300	53.514	39.6					
Diclofenac sodium	10.1µM[54]	0.005 [61]	3	0.7545	0.1515					
			10	2.5149	0.505					
			30	7.5448	1.515					
			100	25.149	5.05					
			300	75.448	15.15					
			1000	251.49	50.5					

Drug	Human Cmax Total	Expected Fraction Unbound in Plasma	Multiplier xCmax	Chip Dosing Concentration (uM) Total	Chip Dosing Concentration (uM) Free	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Entacapone	3.3µM[55]	0.02 [61]	0.1	0.0131	0.0066					
			0.3	0.0392	0.0198					
			1	0.1307	0.066					
			3	0.3921	0.198					
			10	1.3069	0.66					
			30	3.9208	1.98					
			100	13.069	6.6					
			300	39.208	19.8					
Fialuridine	0.64µM[27]	0.36 [62]	0.1	0.0239	0.02304					
			0.3	0.0716	0.06912					
			1	0.2385	0.2304					
			3	0.7155	0.6912					
			10	2.3851	2.304					
			30	7.1553	6.912					
			100	23.851	23.04					
			300	71.553	69.12					
FIRU (5-iodo-1-92-fluoro-2-deoxyribofuranosyl)uracil)*	*0.64µM[27]	*0.36 [62]	0.1	0.0239	0.02304					
			0.3	0.0716	0.06912					
			1	0.2385	0.2304					
			3	0.7155	0.6912					
			10	2.3851	2.304					
			30	7.1553	6.912					
			100	23.851	23.04					
			300	71.553	69.12					
Labetalol***	2.68µM[27]	0.5 [61]	0.1	0.1367	0.134					
			0.3	0.4102	0.402					
			1	1.3673	1.34					
			3	4.1020	4.02					
			10	13.673	13.4					
			30	41.020	40.2					
			100	136.73	134					
Levofloxacin***	15.8µM[33]	0.75 [61]	0.1	1.1934	1.185					
			0.3	3.5801	3.555					
			1	11.934	11.85					
			3	35.801	35.55					
			5	59.668	59.25					
			10	119.34	118.5					
			15	179.00	177.75					
			30	358.01	355.5					
			45	537.01	533.25					
Lomitapide	0.0017µM[56]	0.002 [61]	0.9	3.41906E-05	3.11374E-06					
			2.6	9.87728E-05	8.99524E-06					
			5.3	0.0002	1.83365E-05					
			15.8	0.0006	5.46634E-05					
			55.3	0.0021	0.000191322					
			163.2	0.0062	0.000564624					
			544.9	0.0207	0.001885195					
			1634.7	0.0621	0.005655585					
Nefazodone hydrochloride	4.3µM[54]	0.01 [62]	0.1	0.0128	0.0043					
			0.3	0.0384	0.0129					
			1	0.1280	0.043					
			3	0.3839	0.129					
			10	1.2798	0.43					
			30	3.8393	1.29					
			100	12.798	4.3					
			300	38.393	12.9					
Olanzapine	0.00009µM[57]	0.07 [63]	0.1	7.97468E-07	0.00000063					
			0.3	2.39241E-06	0.00000189					
			1	7.97468E-06	0.0000063					
			3	2.39241E-05	0.0000189					
			10	7.97468E-05	0.000063					
			30	0.00024	0.000189					
			100	0.00080	0.00063					
			300	0.00239	0.00189					

Drug	Human Cmax Total	Expected Fraction Unbound in Plasma	Multiplier xCmax	Chip Dosing Concentration (uM) Total	Chip Dosing Concentration (uM) Free	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Pioglitazone	3µM[33]	0.01 [62]	0.1	0.0089	0.003					
			0.3	0.0268	0.009					
			1	0.0893	0.03					
			3	0.2679	0.09					
			10	0.8929	0.3					
			30	2.6786	0.9					
			100	8.9286	3					
			300	26.786	9					
Simvastatin	0.02µM[26]	0.06 [63]	0.1	0.0002	0.00012					
			0.3	0.0005	0.00036					
			1	0.0016	0.0012					
			3	0.0047	0.0036					
			10	0.0158	0.012					
			30	0.0473	0.036					
			100	0.1577	0.12					
			300	0.4731	0.36					
			1000	1.5769	1.2					
			3000	4.7306	3.6					
Sitax(s)entan sodium salt	28.6µM[58]	0.01 [64]	0.1	0.0851	0.0286					
			0.3	0.2554	0.0858					
			1	0.8512	0.286					
			3	2.5536	0.858					
			10	8.5119	2.86					
			30	25.536	8.58					
			90	76.607	25.74					
			100	85.119	28.6					
			300	255.36	85.8					
Stavudine	3.46µM[33]	1 [61]	0.1	0.346	0.346					
			0.3	1.038	1.038					
			1	3.460	3.46					
			3	10.38	10.38					
			10	34.60	34.6					
			30	103.8	103.8					
			60	207.6	207.6					
			100	346	346					
			300	1038	1038					
Tacrine	0.1µM[33]	0.25 [61]	0.1	0.0027	0.0025					
			0.3	0.0080	0.0075					
			1	0.0265	0.025					
			3	0.0795	0.075					
			10	0.2651	0.25					
			30	0.7953	0.75					
			100	2.6511	2.5					
			300	7.9533	7.5					
			1000	26.511	25					
Telithromycin	2.79µM[59]	0.4 [61]	0.00004	0.0001	0.00012					
			0.00013	0.0004	0.00036					
			0.00044	0.0012	0.0012					
			0.00133	0.0037	0.0036					
			0.00443	0.0124	0.012					
			0.01329	0.0371	0.036					
			0.04429	0.1236	0.12					
			0.13289	0.3708	0.36					
			1	1.1307	1.116					
			3	3.3921	3.348					
			10	11.307	11.16					
			30	33.921	33.48					
			100	113.07	111.6					
			200	226.14	223.2					

Drug	Human Cmax Total	Expected Fraction Unbound in Plasma	Multiplier xCmax	Chip Dosing Concentration (uM) Total	Chip Dosing Concentration (uM) Free	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Tolcapone***	47.6µM[54]	0.0012 [61]	0.1	0.10074	0.005712					
			0.3	0.30222	0.017136					
			1	1.00741	0.05712					
			3	3.02222	0.17136					
			10	10.0741	0.5712					
			30	30.2222	1.7136					
			100	100.741	5.712					
			200	201.481	11.424					
Troglitazone	6.08µM[33]	0.0011 [65]	0.1	0.01259	0.000657036					
			0.29	0.03778	0.001971107					
			0.98	0.12592	0.006570357					
			2.95	0.37775	0.019711072					
			9.82	1.25917	0.065703574					
			29.47	3.77751	0.197110722					
			98.2	12.5917	0.65703574					
			294.72	37.7751	1.971107219					
Trovafloxacin mesylate	5µM[33]	0.24 [61]	0.1	0.12793	0.12					
			0.3	0.38380	0.36					
			1	1.27932	1.2					
			3	3.83795	3.6					
			10	12.7932	12					
			15	19.1898	18					
			30	38.3795	36					
			100	127.932	120					
			300	383.795	360					
Ximelagatran	0.45µM[53]	1	0.1	0.045	0.045					
			0.3	0.135	0.135					
			1	0.45	0.45					
			3	1.35	1.35					
			10	4.5	4.5					
			30	13.5	13.5					
			100	45	45					
			300	135	135					
Zileuton	13.1µM[33]	0.07 [62]	0.1	0.1161	0.0917					
			0.3	0.3482	0.2751					
			1	1.1608	0.917					
			3	3.4823	2.751					
			10	11.608	9.17					
			30	34.823	27.51					
			100	116.08	91.7					
			300	348.23	275.1					

*FIRU values assumed same as Fialuridine

**A calculation error for Troglitazone led to slightly misaligned dosing concentrations such that the Multiplier xC_{max} values aren't exact multiples of C_{max}

***Highest concentration limited by solubility

Supplementary Table S2. Drug information and dosing concentrations used in the investigation. Cycle-specific concentrations have been added for further clarity.

Incremental DILI sensitivity	Tox FNR vs. Baseline	Clinical dev. success rate	Portfolio IRR	Portfolio eNPV ($m)	NPV of chip costs ($m)	IRR on chip investment	Portfolio NPV / NPV of base R&D costs	Steady state uplift in pre-tax profit (%)	Annualized industry-wide pre-tax profit uplift ($m)
0.0%	100.0%	11.5%	11.0%	-3.5	-3.5	-3.5%	-0.2%	-0.1%	(207)
7.5%	99.0%	11.5%	11.0%	1.1	-3.6	14.4%	0.1%	0.0%	61
14.9%	98.0%	11.6%	11.0%	5.7	-3.6	24.7%	0.3%	0.2%	331
22.4%	97.0%	11.6%	11.1%	10.3	-3.6	30.4%	0.5%	0.3%	602
29.8%	96.0%	11.7%	11.1%	15.0	-3.6	33.8%	0.8%	0.5%	876
37.3%	95.0%	11.7%	11.2%	19.8	-3.6	36.0%	1.0%	0.7%	1,151
44.7%	94.0%	11.7%	11.2%	24.5	-3.6	37.6%	1.3%	0.8%	1,428
52.2%	93.0%	11.8%	11.2%	29.3	-3.6	38.7%	1.6%	1.0%	1,707
61.5%	91.8%	11.8%	11.3%	35.3	-3.6	39.9%	1.9%	1.2%	2,058
67.1%	91.0%	11.9%	11.3%	39.0	-3.7	40.4%	2.1%	1.3%	2,270
74.5%	90.0%	11.9%	11.4%	43.9	-3.7	41.0%	2.3%	1.5%	2,555
82.0%	89.0%	11.9%	11.4%	48.8	-3.7	41.5%	2.6%	1.6%	2,841
87.0%	**88.3%**	**12.0%**	**11.4%**	**52.1**	**-3.7**	**41.8%**	**2.8%**	**1.8%**	**3,034**
96.0%	87.1%	12.0%	11.5%	58.1	-3.7	42.3%	3.1%	2.0%	3,384

Supplementary Table S3. Portfolio value and industry profits increase with reductions in the false negative rate (FNR) of the preclinical toxicology assessment, which cause fewer toxic drugs to enter the clinic. The leftmost column tabulates the proportional improvement in DILI detection versus the base case, and the next column shows the improvement in the toxicology FNR relative to the model's base case. Since DILI is around 13% or tox failure in development, near total DILI sensitivity can reduce the FNR to around 87% of its base case value. Subsequent columns then show the clinical development success rate from entry into Phase I to launch, the internal rate of return (IRR) of the R&D portfolio, the NPV of the portfolio discounted to the time of drug launch, the capitalized cost of Liver-Chips used in assessing the portfolio (discounted to the time of launch), and the marginal IRR on the Liver-Chip investment. The remaining columns capture the value uplift due to FNR improvement as a percentage uplift of the portfolio's NPV relative to the baseline NPV of R&D, percentage uplift of steady state pre-tax profits, and the estimated increase in annual pre-tax profits for the small-molecule drug development industry. The row highlighted in dark gray relates to the improvement in FNR that may result from incorporating the Liver-Chip into DILI prediction workflows in accordance with the 87% sensitivity estimated by the present study. The rows highlighted in light gray correspond to the 95% confidence interval around this point estimate. The economic model behind these calculations is provided in full in the Supplementary Materials.