

Supplementary material

Drug transporter expression and activity in cryopreserved human hepatocytes isolated from chimeric TK-NOG mice with humanized livers

Table S1. Transporter mRNA expression in one hepatocyte human population (HUM4282) and in the five HUM4282-deriving batches of HepaSH cells

Transporter	mRNA transporter expression [#]		
	Human hepatocyte population (HUM4282)	HUM4282-deriving HepaSH cells (n=5)	
	Expression mean (a.u.)	Expression mean (a.u.)	Expression range (minimum-maximum) (a.u.)
NTCP	705.6	496.6	372.0-621.3
OATP1B1	2264.2	1255	723.1-1621
OATP1B3	449.1	1247	425.2-1711
OATP2B1	199.9	269.8	123.4-311.5
OAT2	931.4	1641	894.4-3068
OCT1	2596	619.9	458.4-888.0
OST α	99.4	3.8	1.9-6.0
OST β	3.9	79.3	69.9-89.9
MDR1	32.5	494.8	332.1-753.9
BSEP	3.5	21.2	13.6-45.0
MRP2	372.3	499.2	413.0-603.2
MRP3	315.7	269.8	187.1-348.2
MRP4	3.6	19.5	12.0-27.3
BCRP	43.3	12.5	8.3-15.9

a.u., arbitrary unit. [#]Transporter mRNA expression was determined in suspended thawed human hepatocytes and HepaSH cells by RT-qPCR assays.

Table S2. Variability of drug transporter mRNA expression in suspended thawed batches of HepaSH cells (n=5) deriving from the same human hepatocyte population (HUM4282), in independent primary human hepatocyte populations (n=9-13) and in human livers (n=226)

Transporter		Coefficient of variation for mRNA expression (%)		
Family	Type	HepaSH cells deriving from the HUM4282 hepatocyte population	Human hepatocytes	Human livers
SLC	NTCP	20.2	81.0	72.9
	OATP1B1	28.4	58.6	65.5
	OATP1B3	42.0	78.5	72.2
	OATP2B1	14.9	70.7	57.1
	OAT2	51.9	64.3	56.0
	OCT1	27.9	72.5	67.2
	OST α	54.2	130.3	91.7
	OST β	12.7	87.1	159.1
ABC	MDR1	34.1	84.9	81.4
	BSEP	63.0	162.7	64.3
	MRP2	16.2	54.2	54.9
	MRP3	23.1	65.5	47.6
	MRP4	34.6	97.9	50.0
	BCRP	23.8	74.2	74.1
All transporters		31.9 \pm 4.2 [#]	84.5 \pm 7.9 ^{#, ****}	72.4 \pm 7.4 ^{#, ****}

[#]defined as mean \pm SEM of coefficients of variation for transporters (n=14); ****, p<0.0001 when compared to HepaSH cells.

Table S3. Evaluation of contaminating mouse cells in cultured HepaSH cells

Time of culture	Mouse contaminating cells (%) [#]
24 h	0.06 \pm 0.01
120 h	0.02 \pm 0.02

[#]correspond to mouse H-2Kd/H-2Dd-positive and human albumin-negative cells; data are the means \pm SEM of values from four independent HepaSH cultures.

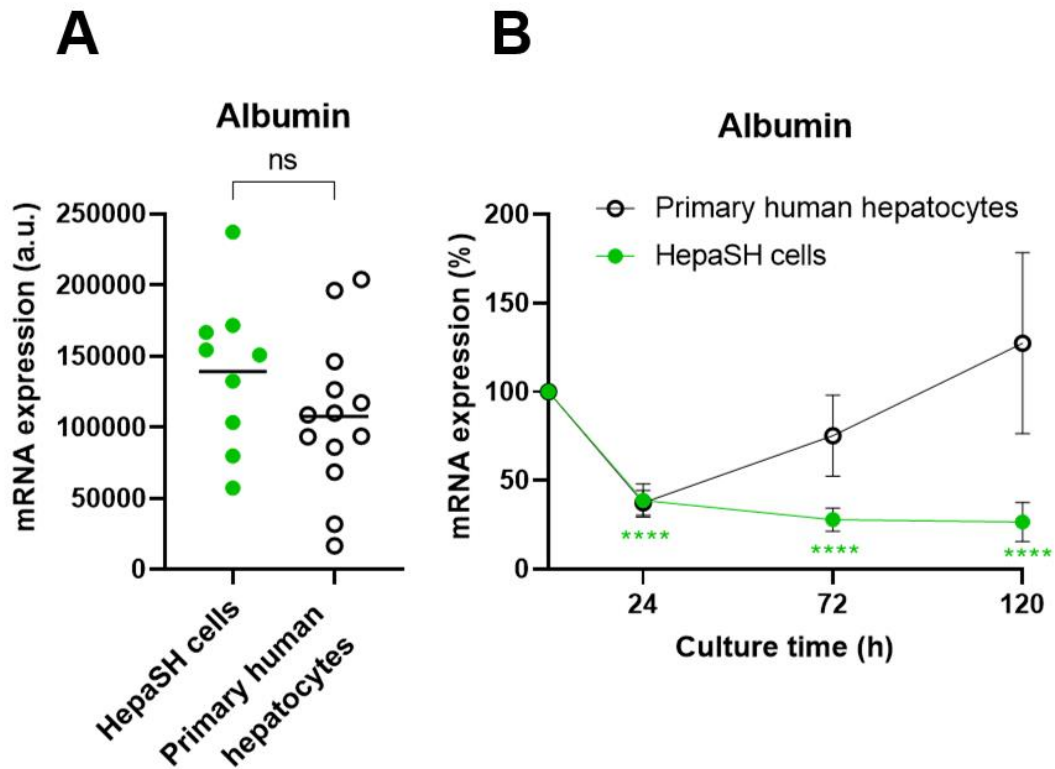


Fig. S1. Albumin mRNA levels in (A) suspended and (B) cultured HepaSH cells and primary human hepatocytes. Cryopreserved HepaSH cells and primary human hepatocytes were thawed and put in (A) suspension or (B) cultured for 24 h-120 h, before RNA extraction and analysis by RT-qPCR for determining albumin mRNA expression. Data are expressed as (A) arbitrary units (a. u.) or (B) percentages of albumin mRNA expression found in thawed cells before plating. (A) The black bar on the graph represents the expression mean of 9 batches (HepaSH cells) or 13 batches (primary human hepatocytes); ns, not statistically significant. (B) data are the means \pm SEM of 6 batches (HepaSH cells) or 10 batches (primary human hepatocytes); ****, $p < 0.0001$ when compared to thawed cells before plating.

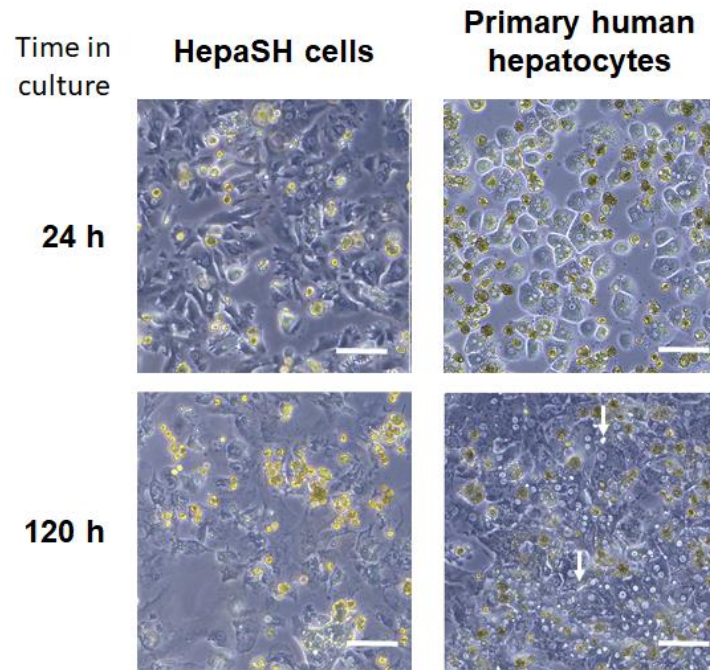


Fig. S2. Phase contrast imaging of cultured HepaSH cells and primary human hepatocytes. White bar, 50 μm . White arrows indicate refractive bile canaliculi-like structures.

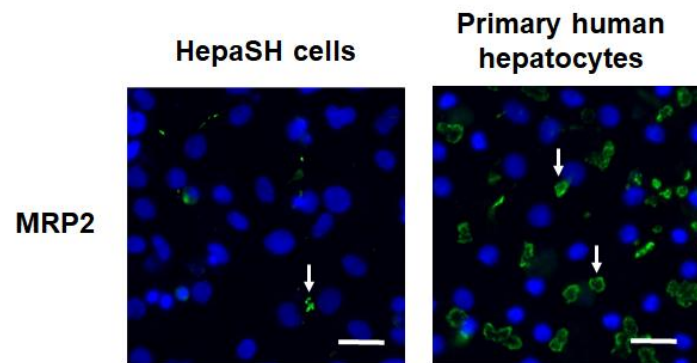


Fig. S3. Expression of MRP2 protein in 72 h-cultured HepaSH cells and primary human hepatocytes. MRP2 immunolocalization was performed as described in Materials and Methods. White arrows indicate MRP2-related green fluorescence of bile canaliculi-like structures; blue fluorescence corresponds to 4,6-diamidino-2-phenylindole-stained nuclei. White bar, 15 μm .

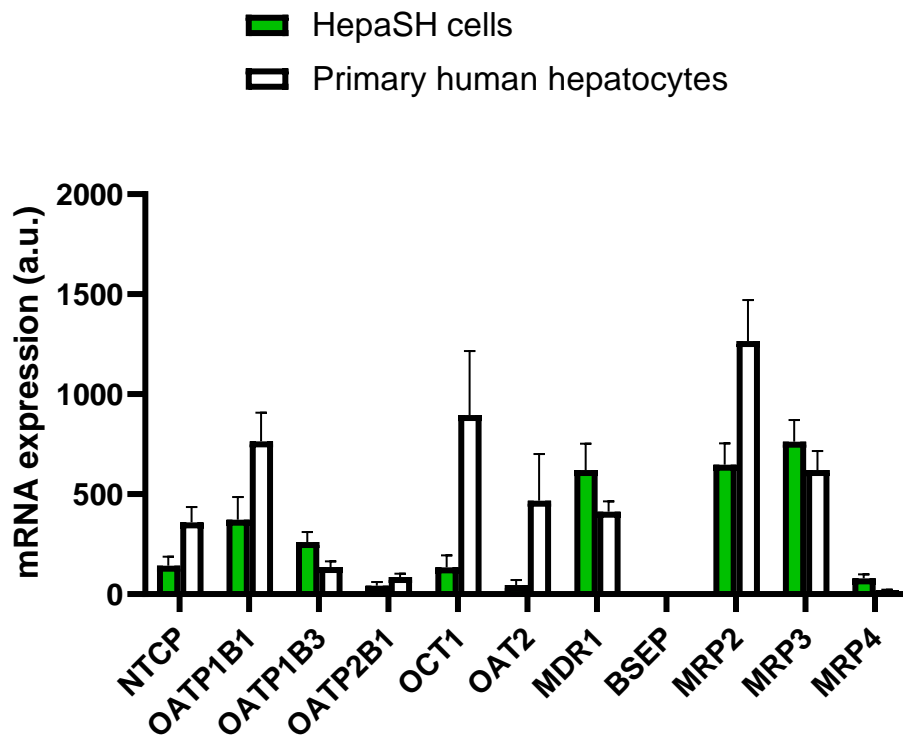


Fig. S4. Transporter mRNA levels in 120 h-cultured HepaSH cells and primary human hepatocytes. Cryopreserved HepaSH cells and primary human hepatocytes were thawed and put in culture for 120 h. Transporter mRNA expression was next determined by RT-qPCR assays. Data are expressed as arbitrary units (a. u.) and are the means \pm SEM of 3-5 batches (HepaSH cells) or 8-10 batches (primary human hepatocytes).

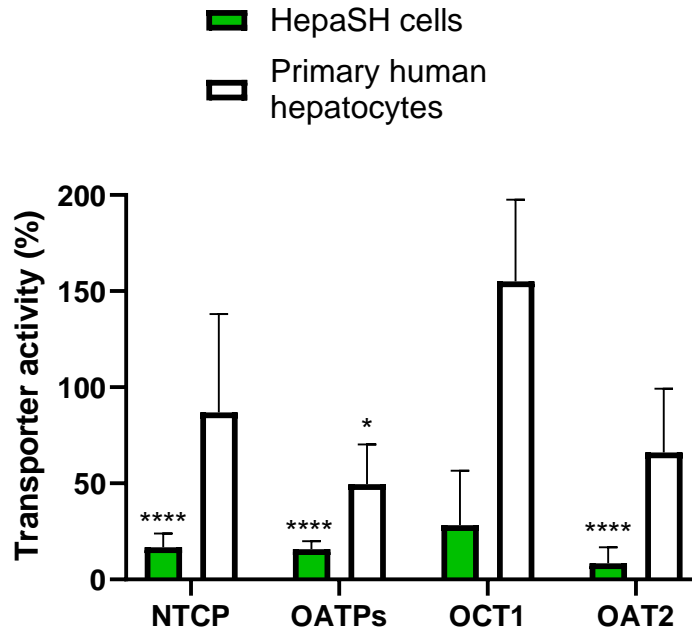


Fig. S5. SLC transport activities in 120 h-cultured HepaSH cells and primary human hepatocytes. Cryopreserved HepaSH cells and primary human hepatocytes were thawed and put in culture for 120 h. Measurement of SLC transporter activities was then performed using reference substrates, as described in the Materials and Methods. Data are expressed as percentages of transporter activities found in 24 h-cultured cells, arbitrarily set at 100% and are the means \pm SEM of 3-6 (HepaSH cells) or 6-8 (primary human hepatocytes) independent assays, each one being performed with one batch of HepaSH cells or human hepatocytes. *, $p < 0.05$ and ****, $p < 0.0001$ when compared to SLC activities in 24 h-cultured cells.