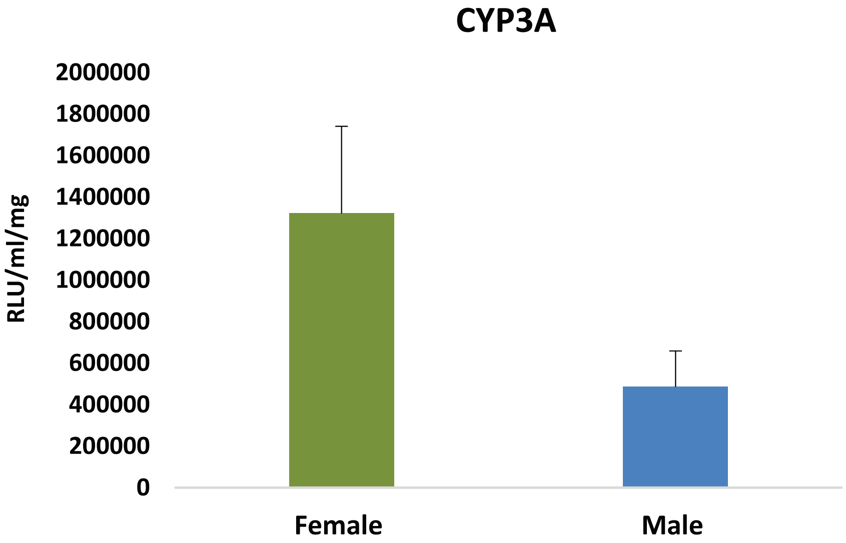
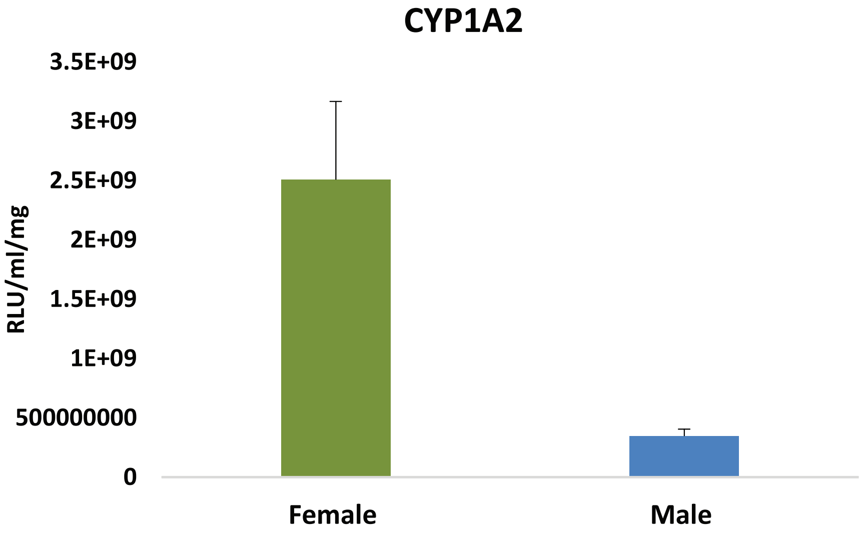
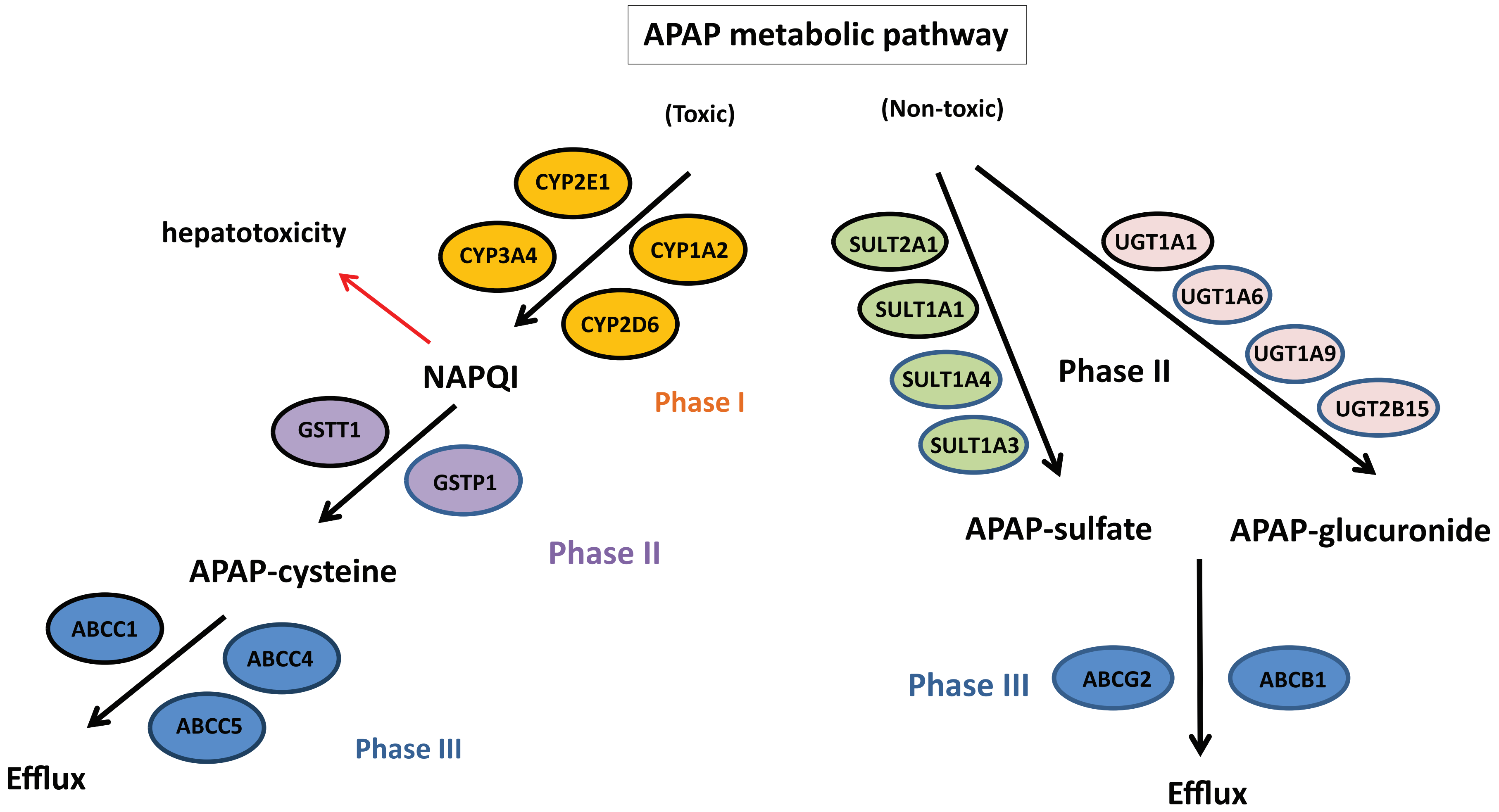




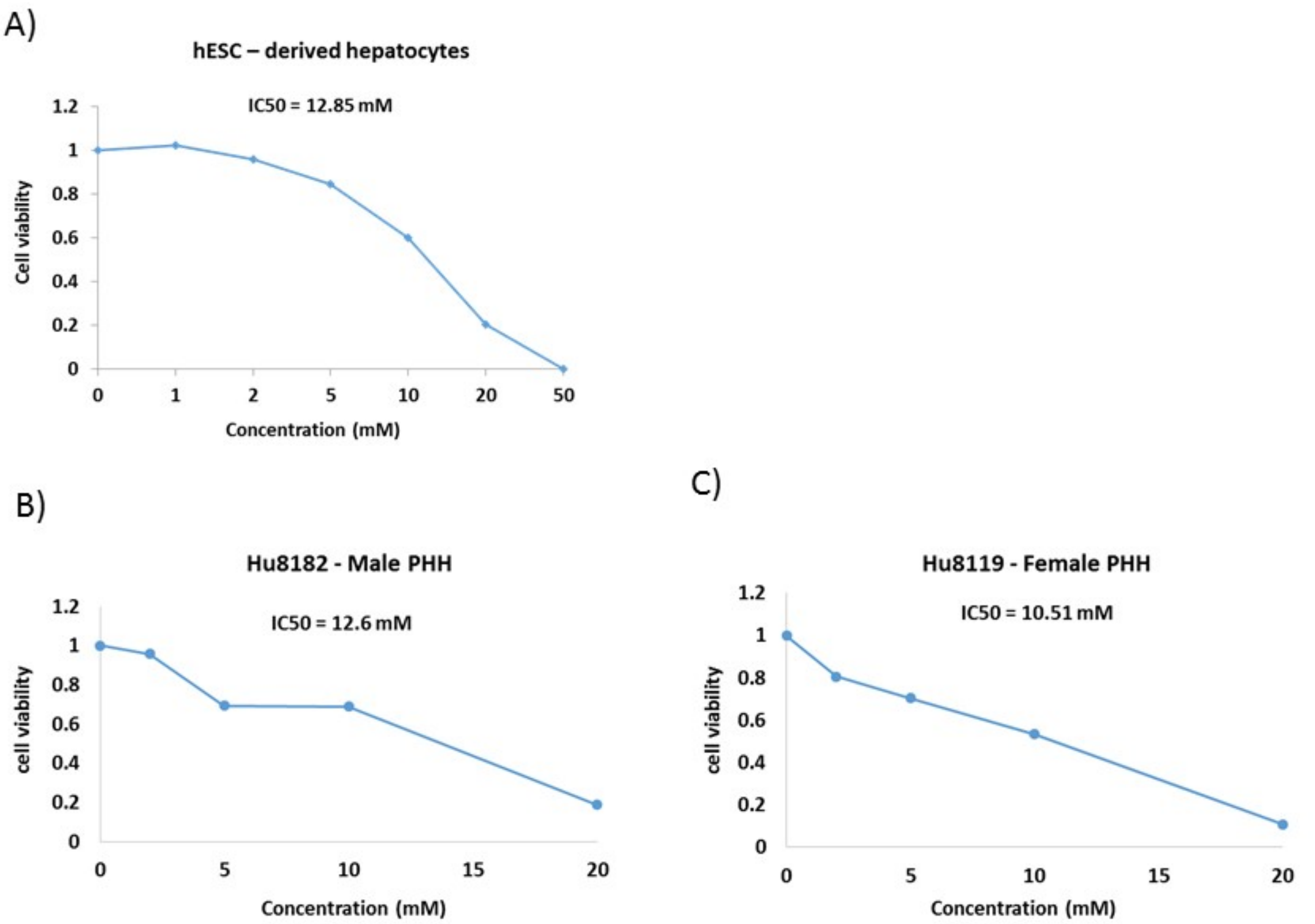
Supplementary Figure 1

Figure S1: Metabolic activity of female and male primary human hepatocytes. Cyp3A and Cyp1A2 metabolism were measured in both lines 48 hours post-replating using the Promega pGlo system^{TM}. Experiments were performed in triplicate and measured on a luminometer. The units of activity quoted are relative light units per millilitre of supernatant per milligram of protein (RLU/mg/ml).

Figure S2: Paracetamol (APAP) metabolism (based on Pharmacogenomics Knowledge Base PharmGKB; www.pharmgkb.org). At normal doses APAP is metabolised by two major families of phase II enzymes: Sulfotransferases (SULTs) and UDP-glucuronosylltransferases (UGTs). This leads to the production of two metabolites: APAP-sulfate and APAP-glucuronide, which are further effluxed from the cell by one of the ABC transporters. At higher doses than recommended, paracetamol is metabolised by Cytochrome P450 enzymes. This leads to the formation of a reactive metabolite, N-acetyl-p-benzoquinoneimine (NAPQI), which is further transformed to a non-toxic APAP- cysteine by Glutathione –S- transferases (GSTT1,GSTP1). The metabolite is effluxed from the cell by one of the phase III transporters.

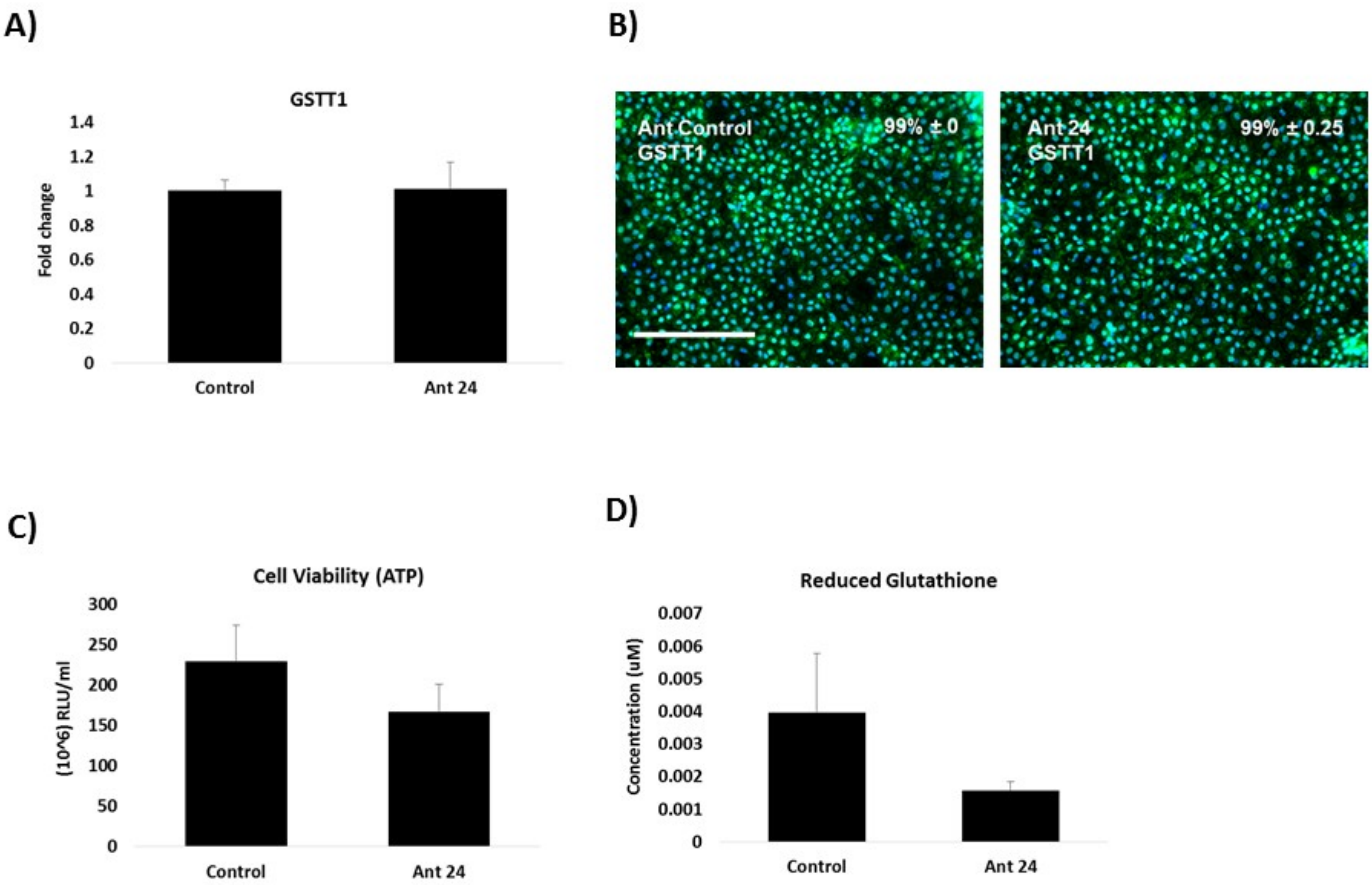


Supplementary Figure 2



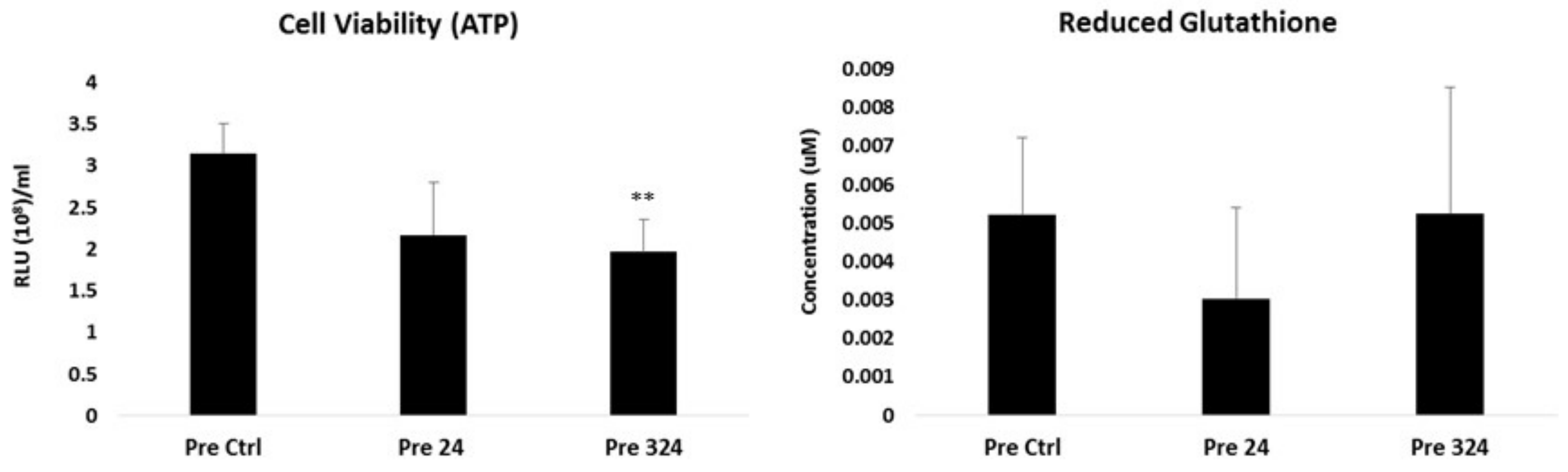
Supplementary Figure 3

Figure S3: Paracetamol *in vitro* toxicity in stem-cell derived hepatocytes (hESC-heps) and primary human hepatocytes (PHH). hESC-heps (at day 17) and PHH (24 hours post- replating) were induced with different concentrations of paracetamol (0-50 mM) for 24 hours. The CellTitre viability assay (Promega) was used to measure the ATP levels. The IC50 was calculated from the function f(x)= ax + b.



Supplementary Figure 4

Figure S4: At day 17, hESC-heps were transfected with the antagomir of the corresponding microRNA at 50 nM concentration for 24 hours. Transfection with the antagomir of microRNA 24-3p did not have any effect either on gene (A) or protein (B) expression of GSTT1. At day 18, the transfected hESC-heps were exposed to paracetamol concentration that causes 50% of the cell death (IC50 = 12.85 mM) for another 24 hours. At day 19, the cell viability was measured using CellTitre Assay (Promega) and Glutathione Depletion Assay (Promega). The Ant-24 decreased cell viability (C) and reduced glutathione (D) after exposure to paracetamol comparing to the scrambled control. The percentage of positive cells is provided in the top right of each panel. This was calculated from four random fields of view and is quoted as ± standard error. The scale bar represents: 100 um. Abbreviations: Ant Control; Scrambled Antagomir Control; Ant 324; Antagomir 324-5p.



Supplementary Figure 5

Figure S5: At day 17, hESC-derived hepatocytes were transfected with precursors at 50nM concentration for 24 hours. At day 18, cells were exposed to paracetamol (APAP) concentration that causes 50% of the cell death (IC50 = 12.85 mM) for 24 hours. At day 19, the cell viability and glutathione reduction were measured using CellTiter – Glo® Luminescent Cell Viability Assay (Promega) and GSH/GSSG – Glo™ Assay (Promega) respectively. Abbreviations: Pre; precursor. Pre ctrl/Pre 24/Pre 324, precursor scrambled control/ precursor of miR -24/ precursor of miR -324-5p. Levels of significance are quoted and measured by Student's *t*-test. Significance levels are denoted by one asterisks to indicate $p < 0.05$.

Supplementary Table 1

RT^2 PROFILER PCR ARRAY - Human Drug Metabolism Phase I (QIAGEN; PAHS-002Z)						
Gene Symbol	**AVG ΔCt**		**2^ - ΔCt**		**Fold Change**	**Fold Up – or Down-Regulation**
	Group1 (HLC)	**Control Group (PHH)**	**Group1 (HLC)**	**Control Group (PHH)**	**Group 1/ Control**	**Group 1/ Control**
CYP1A1	0.38	7.02	0.768483	0.007705	99.74	99.74
ADH4	4.08	6.10	0.059302	0.014562	4.07	4.07
ADH5	1.01	2.39	0.495439	0.190431	2.60	2.60
EPHX1	1.64	1.52	0.321538	0.347956	0.92	-1.08
ADH6	4.78	3.34	0.036387	0.098713	0.37	-2.71
ALDH1A1	0.45	-0.45	0.729565	1.363349	0.54	-1.87
CYPB5R	0.65	-0.38	0.636322	1.303635	0.49	-2.05
CYP2B6	7.46	6.75	0.005694	0.009309	0.61	-1.63
CYP3A5	2.01	-1.18	0.248204	2.272074	0.11	-9.15
CYP2J2	6.06	1.98	0.015040	0.252693	0.06	-16.80
CYP2C8	6.76	0.63	0.009224	0.647110	0.01	-70.16
CYP2C9	7.46	-0.49	0.005694	1.402808	0.00	-246.36
CYP3A4	7.46	1.34	0.005694	0.394672	0.01	-69.31
CYP2C19	6.91	2.15	0.008323	0.225670	0.04	-27.11
CYP2D6	7.09	4.56	0.007318	0.042426	0.17	-5.80
CES1	4.46	-1.36	0.045546	2.559360	0.02	-56.19
CES2	4.07	-0.66	0.059552	1.579842	0.04	-26.53
ADH1B	6.89	4.26	0.008442	0.052291	0.16	-6.19
ADH1C	7.46	4.16	0.005694	0.056056	0.10	-9.84

Supplementary Table 1

RT^2 PROFILER PCR ARRAY - Human Drug Metabolism Phase II (QIAGEN; PAHS-069Z)						
Gene Symbol	AVG ΔCt		2^ - ΔCt		Fold Change	Fold Up – or Down-Regulation
	Group1 (HLC)	Control Group (PHH)	Group1 (HLC)	Control Group (PHH)	Group 1/ Control	Group 1/ Control
GSTP1	-0.20	4.85	1.148277	0.034646	33.14	33.14
GSTA1	-0.42	2.08	1.341208	0.236264	5.68	5.68
GSTA4	3.37	6.86	0.096681	0.008588	11.26	11.26
GSTA3	6.20	8.55	0.013622	0.002677	5.09	5.09
SULT1E1	3.85	9.74	0.069583	0.001166	59.66	59.66
SULT1A2	4.37	7.98	0.048511	0.003948	12.29	12.29
SULT1C2	8.20	12.73	0.003411	0.000147	23.14	23.14
SULT1C3	8.20	12.73	0.003411	0.000147	23.14	23.14
SULT1C4	6.63	8.40	0.010075	0.002967	3.40	3.40
NAT 1	9.80	11.43	0.001118	0.000362	3.09	3.09
GSTO1	0.04	0.46	0.971041	0.724547	1.34	1.34
GSTO2	8.00	7.95	0.003893	0.004040	0.96	-1.04
GSTT1	3.47	3.39	0.089944	0.095282	0.94	-1.06
GSTK1	2.45	2.52	0.182812	0.174788	1.05	1.05
GSTM4	6.98	7.30	0.007905	0.006338	1.25	1.25
UGT2B28	2.98	2.15	0.126827	0.225954	0.56	-1.78
UGT3A1	7.78	6.94	0.004559	0.008118	0.56	-1.78
SULT1A1	3.08	3.96	0.117951	0.064148	1.84	1.84
SULT2A1	4.55	3.61	0.042611	0.081891	0.52	-1.92
COMT	2.01	2.76	0.249051	0.147120	1.69	1.69
UGT1A1	6.58	4.83	0.010486	0.035145	0.30	-3.35
UGT1A4	10.39	3.28	0.000746	0.102607	0.01	-137.61
UGT2B4	8.36	3.41	0.003054	0.093826	0.03	-30.73
UGT2B7	3.56	0.83	0.084852	0.564152	0.15	-6.65
UGT2B10	8.65	5.59	0.002489	0.020726	0.12	-8.33
UGT2A3	10.59	4.69	0.000649	0.038640	0.02	-59.49
UGT2B17	10.85	4.95	0.000543	0.032289	0.02	-59.46
NAT2	7.58	5.64	0.005230	0.020051	0.26	-3.83
SULT1B1	8.60	5.59	0.002579	0.020766	0.12	-8.05

Supplementary Table 1

RT^2 PROFILER PCR ARRAY - Human Drug Transporters Phase III (QIAGEN; PAHS-070Z)						
Gene Symbol	AVG ΔCt		2^ - ΔCt		Fold Change	Fold Up – or Down-Regulation
	Group1 (HLC)	Control Group (PHH)	Group1 (HLC)	Control Group (PHH)	Group 1/ Control	Group 1/ Control
ABCC1	6.89	8.73	0.008423	0.002350	3.58	3.58
ABCC4	4.89	9.01	0.033689	0.001934	17.42	17.42
ABCC5	5.93	8.98	0.016410	0.001982	8.28	8.28
ABCG2	4.52	9.39	0.043502	0.001491	29.17	29.17
SLCO1A2	7.29	11.41	0.006390	0.000368	17.38	17.38
SLCO2A1	6.64	12.26	0.009994	0.000204	49.01	49.01
SLC19A3	4.75	7.85	0.037173	0.004328	8.59	8.59
SLC22A7	8.55	11.07	0.002659	0.000466	5.70	5.70
SLC22A9	6.43	8.37	0.011561	0.003030	3.82	3.82
ABCC3	5.53	3.98	0.021683	0.063471	0.34	-2.93
ABCB11	8.58	9.07	0.002616	0.001859	1.41	1.41
ABCG8	8.74	7.25	0.002338	0.006587	0.35	-2.82
SLC19A1	9.35	10.40	0.001529	0.000738	2.07	2.07
SLC19A2	4.95	6.46	0.032350	0.011345	2.85	2.85
SLC22A3	6.28	6.05	0.012855	0.015105	0.85	-1.18
ABCB1	8.16	5.09	0.003490	0.029260	0.12	-8.38
ABCB4	8.73	6.51	0.002351	0.011003	0.21	-4.68
ABCC2	6.42	2.84	0.011709	0.139434	0.08	-11.91
SLCO1B1	10.59	6.34	0.000648	0.012367	0.05	-19.09
SLCO1B3	10.88	7.76	0.000532	0.004626	0.12	-8.69
SLC22A1	11.12	7.00	0.000448	0.007829	0.06	-17.46

Table S1: Fold-Change (2^(- Delta Delta Ct)) is the normalized gene expression (2^(- Delta Ct)) in the Test Sample divided the normalized gene expression (2^(- Delta Ct)) in the Control Sample. Fold-Regulation represents fold-change results in a biologically meaningful way. Fold-change values greater than one indicate a positive- or an up-regulation, and the fold-regulation is equal to the fold-change. Fold-change values less than one indicate a negative or down-regulation, and the fold-regulation is the negative inverse of the fold-change. Up-regulated genes (fold differences larger than a 3 fold threshold) are indicated in red, down-regulated genes (fold differences narrower than 3 fold treshold) are indicated in green. Genes similarly expressed for the Group 1 and Control (fold differences between -3 to +3 boundary) are indicated in black. Abbreviations: HLC, hepatocyte-like-cells; PHH, primary human hepatocytes.

Table S2: The list of 220 microRNAs commonly expressed in stem cell – derived hepatocytes (day 18) and primary human hepatocytes (PHH). The RNA samples (4 replicates of PHH and 4 experimental samples of hESC-derived hepatocytes) were analysed on the Agilent miRNA platform (using Agilent's SurePrint G3 Human v16 microRNA 8x60K microarray slides; miRBase version 16.0.

Supplementary Table 3

A)

mRNA/microRNA	Predicted consequential pairing of target region (top) and microRNA (bottom)

Position 639-645 of SULT2A1 3' UTR

hsa-miR-324-5p

```
5' ...AGGGUUCAAAGCCCA--GGGAUGCA...
                ||||   |||||||
3'      UGUGGUUACGGGAUCCCCUACGC
```

mRNA/microRNA	Predicted consequential pairing of target region (top) and microRNA (bottom)

Position 266-272 of GSTT1 3' UTR

hsa-miR-24

```
5'   ...AAACCUGGGCUCAGCCUGAGCCU...
           ||||        |||||||
3'    GACAAGGACGACUU---GACUCGGU
```

B)

Gene/ microRNA	Seed Match	Context + score percentile
SULT2A1/ hsa-miR- 324-5p	8mer	96
GSTT1/ has-miR-24-3p	7mer-m8	84

C)

microRNA/ Gene (symbol)	Accession Number (miRBase)	
hsa-miR-324-5p	MI0000813 / MIMAT0000761	
hsa-miR-24-3p	MI0000080 / MIMAT0000080	
	UniGene	**GeneBank**
SULT2A1	Hs.515835	NM_003167
GSTT1	Hs.268573	NM_000853

Table S3: Predicted microRNA/mRNA binding site by TargetScanHuman5.2 (www.targetscan.com). (A) Human microRNA hsa-miR-324-5p has been predicted to bind its target gene SULT2A1 at the 3' untranslated region at position between 639-645 nt. Human microRNA has-miR-24-3p has been predicted to bind its target gene GSTT1 at the 3' untranslated region at position between 266-272 nt. (B) The table represents type of seed match and context score for each of the microRNA/mRNA binding. (C) The table represents the miRBase accession number of each of the selected microRNAs and UniGene and GeneBank of selected genes of interest. Abbreviations: SULT2A1, sulfotransferase 2A1; GSTT1, glutathione-s-transferase theta 1.

Supplementary Table 4

Name	**Host**	**Dilution**	**Source**
Primary Antibody			
SULT2A1	Rabbit polyclonal	1:250	Abcam
GSTT1	Rabbit polyclonal	1:500	Abcam
ABCG2	Mouse monoclonal	1:100	Abcam
ABCC1	Mouse monoclonal	1:50	Abcam
Secondary Antibody			
Alexa Fluor 568	Goat	1:400	Invitrogen
Alexa Fluor 488	Rabbit	1:400	Invitrogen
Name	**Ref Number (primers)**		**Source**
Genes			
SULT2A1	Hs00234219_m1 (Taqman)		Applied Biosystems
GSTT1	Hs00184475_m1 (Taqman)		Applied Biosystems

Table S4: The table demonstrates qPCR primers, and primary/secondary antibodies used in this study.

Supplementary Table 5

A)

STUDY NO.	AGE	ALIVE OR DEAD	ALT (IU/L)	CREATININE (umol/L)	BILIRUBIN (umol/L)	INR RATIO (ratio)
1	29	DEAD	8696	241	64	11.5
8	38	ALIVE	5699	272	77	8.7
58	33	ALIVE	9424	216	48	3

B)

THE NORMAL RANGES	
NAME	**RANGE (UNIT)**
ALT	10-50 (IU/L)
CREATININE	30-120 (umol/L)
BILIRUBIN	3-17 (umol/L)
INR	0.8-1.2 (RATIO)

Table S5: Anonymised patient details. (A) The table represents the plasma contents of each of the patients used in this study. (B) The table represents normal ranges for particular sera and international normalized ratio (INR). ALT and BILI are liver function tests. Creatinine is a measure of renal function, which is often compromised in liver disease INR is International Normalized Ratio of the prothrombin time, a coagulation function test.