Kühnlenz et al.:

A Microfluidic Thyroid-Liver Platform to Assess Chemical Safety in Humans

Supplementary Data

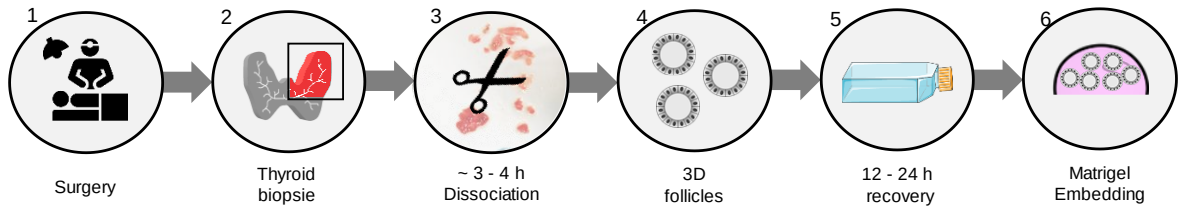

Fig. S1: Generation of the 3D thyroid model from primary tissue
(1,2) Fresh thyroid explants were obtained from clinical surgeries within less than 8 h. (3) Abnormal, adipose, and connective tissue pieces were trimmed and discarded. Prior to a 3-4 h enzymatic digestion via collagenase NB4 and dispase II, the tissue was mechanically shredded. (4,5) Isolated follicles were obtained from a 100 µm filtrate and recovered for 12-24 h in suspension culture. (6) The following day, an erythrocyte lysis was performed, follicles were counted, and 1000 follicles were embedded in growth factor-reduced Matrigel. The 3D thyroid model was then ready to use for subsequent studies like the evaluation of direct thyroid toxicities via methimazole.

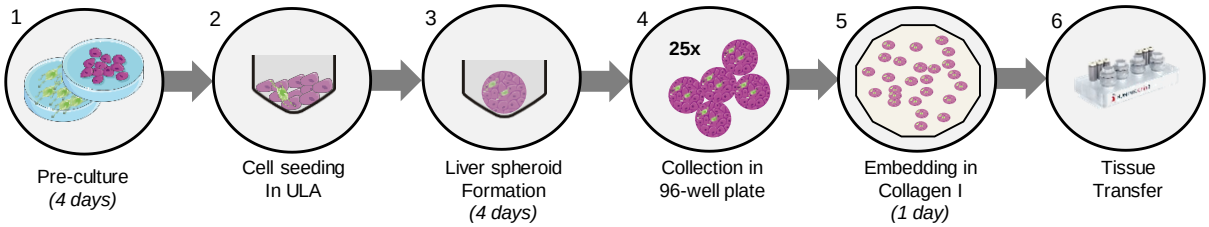

Fig. S2: Generation of the HepaRG liver spheroid model
(1) Differentiated HepaRG cells and primary human hepatic stellate cells (HSteCs) were pre-cultured in monolayers for 4 and 3 days, respectively. (2) 10,000 HepaRG cells were seeded per well together with 4,000 HSteCs in ultra-low attachment plates. (3) Self-assembly of cells to form compact 3D HepaRG liver spheroids took a total of four days. (4) 25 liver spheroids were collected per 96-well (5) and immediately embedded in collagen I, a morphology supporting extracellular matrix. (6) After 24 h recovery and finalized solidification of the collagen I, the liver model was ready to be transferred into the desired culture format, e.g., the Chip2.

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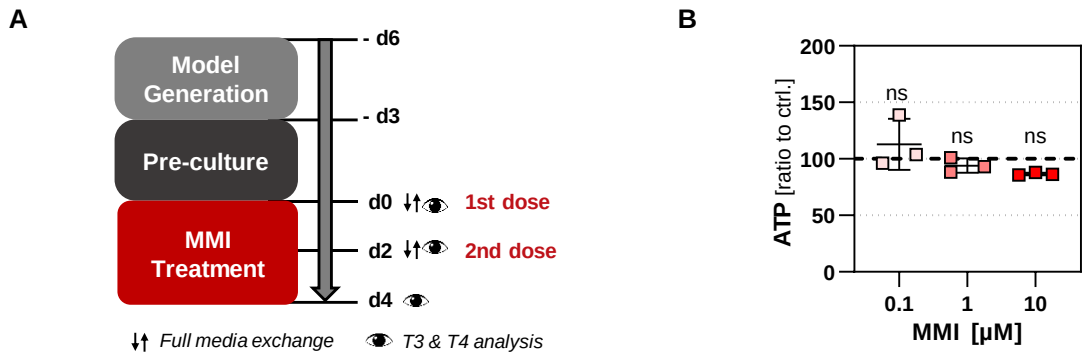

Fig. S3: Methimazole (MMI) treatment of the 3D thyroid model does not induce cytotoxicity
(A) Experimental set-up of MMI treatment. (B) Viability of thyroid follicles was assessed via luminescence measurement of intracellular ATP after a 7-day MMI exposure (one additional dose at day 4). Data depict the average ATP ratio of follicles dosed with 0.1, 1 or 10 µM MMI to untreated (DMSO solvent control) follicles and are shown as means ± SD of three independent thyroid donors (one square per donor □, Donor 9, 10 and 11). Differences between control and treatment group were evaluated by donor matching using a one-way ANOVA followed by Dunnett's multiple comparison based on log-transformed raw data. Difference was considered significant for $p < 0.05$; ns, non-significant.

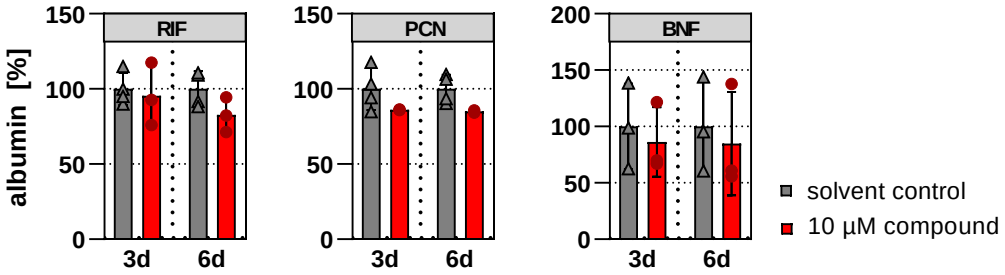

Fig. S4: Cytotoxic evaluation of 10 µM rifampicin (RIF), pregnenolone-16α-carbonitrile (PCN), and β-naphthoflavone (BNF) in HepaRG/HSteC liver spheroids
HepaRG/HSteC liver spheroids were cultured under static conditions and treated with 10 µM of the respective compound supplemented with thyroxine (T4) on culture day 5 and 8. Albumin concentrations were measured in cell culture supernatants after 3 and 6 days (d) of respective compound treatment. Bars represent the ratio to the solvent control DMSO as means ± SD (n = 3 for RIF & BNF, n = 2 for PCN). Differences were evaluated by multiple t-test ($p < 0.05$) for n = 3. None of the means were significantly different compared to each other.

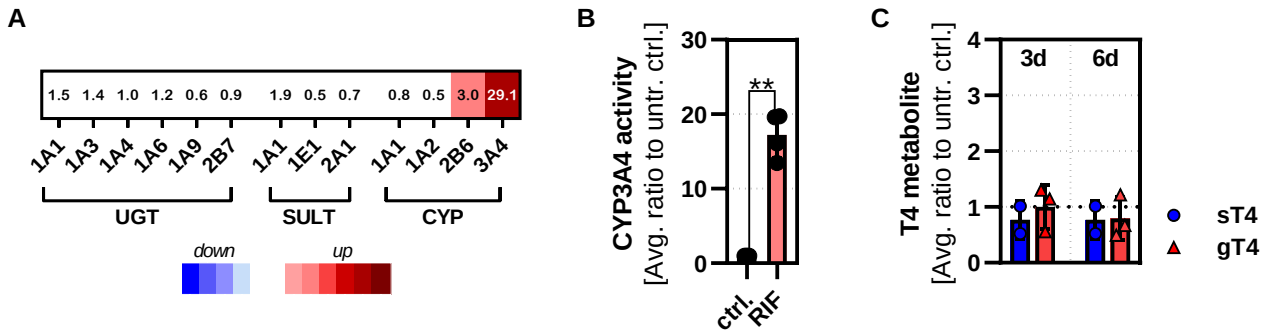

Fig. S5: 6-day rifampicin exposure in the HUMIMIC Chip2 significantly impacts CYP3A4 activity but not T4 metabolite formation in HepaRG/HSteC liver model

HepaRG/HSteC liver spheroids were cultured under dynamic conditions and treated with 10 µM rifampicin supplemented with thyroxine (T4) at culture day 5 and 8. (A) Effect of rifampicin exposure on mRNA expression of phase I and II enzymes post 6-day exposure. Numbers indicate geometric mean fold change calculated to solvent control (1% DMSO), n = 2. (CYP, cytochrome P450; SULT sulfotransferase; UGT UDP-glucuronosyltransferase) (B) Fold induction of CYP3A4 activity in response to 6-day rifampicin (RIF) treatment normalized to solvent control (ctrl.). For comparison, differences were evaluated via an unpaired t-test using Welch's correction and considered significant for $p < 0.05$. **, $p < 0.01$, n = 4. (C) gT4 and sT4 metabolite levels were quantified in culture supernatants after 3 and 6 days (d) rifampicin exposure via LC-MS analysis. Depicted are the average ratios to solvent control. Data are the means ± SD of n = 3 (gT4) and n = 2 (sT4). Differences were evaluated by two-way ANOVA followed by Sidak's multiple comparison post-hoc test ($p < 0.05$). None of the means were significantly different compared to each other.

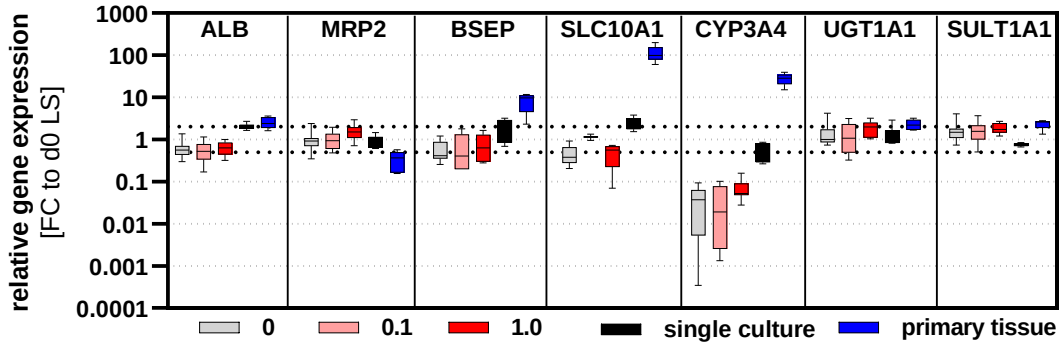

Fig. S6: Relative gene expression of 21-day co-cultured HepaRG/HSteC liver spheroids

Liver spheroids were co-cultured with the 3D thyroid model in the HUMIMIC Chip2 at different concentrations of the thyroid-stimulating hormone: 0, 0.1 and 1 mIU/mL (represented by different shades of red). Identically cultivated single liver spheroids were not treated with TSH. Fold changes (FCs) to freshly generated liver spheroids (d0) are depicted as box plots showing min to max whiskers from three independent co-cultures and two independent single cultures. Primary tissue data were derived from three independent donors. TBP (TATA box binding protein) served as a housekeeper. The two thick dotted lines indicate FCs of 0.5 and 2.0, respectively. (ALB, albumin; MRP2, multidrug resistance-associated protein 2; BSEP, bile salt export pump; SLC10A1, sodium/bile acid cotransporter 10A1; CYP3A4, cytochrome P450 3A4; UGT1A1, UDP-glucuronosyltransferase 1A1; SULT1A1, sulfotransferase 1A1).

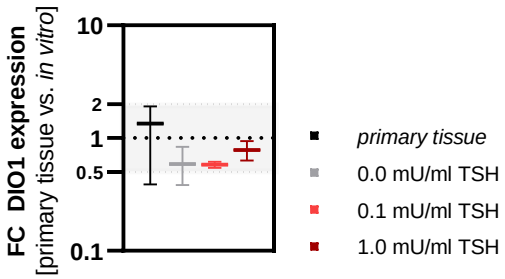

Fig. S7: *DIO1* gene was not overexpressed in thyroid follicles cultured for21-days
Thyroid follicles were dynamically cultured for 21 days with 0, 0.1 or 1.0 mIU/mL thyroid-stimulating hormone (TSH). Gene expression analysis was performed on day 21. Box plots represent the fold change (FC) ± min-max of two independent donors (n = 2) relative to primary tissue from three independent donors (Donor 7, 8 and 13; n = 3). *GAPDH* was used as a housekeeper. Grey area indicates irrelevant fold changes between 0.5 and 2. DIO1, iodothyronine deiodinase 1.

Tab. S1: Primers for qPCR using SYBR Green

Gene symbol	Name		Primer Sequence 5' → 3'
ABCB11/BSEP	ATP binding cassette subfamily B member 11	*up* *down*	GCAGACACTGGCGTTTGTTG ATGTTTGAGCGGAGGAACTGG
ABCC2/MRP2	ATP binding cassette subfamily C member 2	*up* *down*	GCATCCACAGACATCAGGTTCAC CTGCGGCTCTCATTCAGTCTTTC
ALB	Albumin	*up* *down*	TCAGCTCTGGAAGTCGATGAAAC AGTTGCTCTTTTGTTGCCTTGG
CYP3A4	Cytochrome P450 family 3 subfamily A member 4	*up* *down*	GGAAGTGGACCCAGAAACTGC TTACGGTGCCATCCCTTGAC
SLC10A1	Solute carrier family 10 member 1	*up* *down*	TCCAACTCTGTTCCACCATCC CCCCTTTGTAGGTGCCATTTC
SULT1A1	Sulfotransferase family 1A member 1	*up* *down*	ACCACAGCATCTCCCCCTTC CAGGTTTGATTCGCACACTCC
TBP	TATA-box binding protein	*up* *down*	CCTTGTGCTCACCCACCAAC TCGTCTTCCTGAATCCCTTTAGAATAG
UGT1A1	UDP glucuronosyltransferase family 1 member A1	*up* *down*	TTTTGTTGGTGGAATCAACTGC CCCAAAGAGAAAACCACAATTCC

Tab. S2: Primers for qPCR using TaqMan with FAM-MGB dye

Gene symbol	Name	Assay ID
ALB	Albumin	Hs00609411_m1
CYP1A1	Cytochrome P450 family 1 subfamily A member 1	Hs00153120_m1
CYP1A2	Cytochrome P450 family 1 subfamily A member 2	Hs00167927_m1
CYP2B6	Cytochrome P450 family 2 subfamily B member 6	Hs03044634_m1
CYP3A4	Cytochrome P450 family 3 subfamily A member 4	Hs00430021_m1
DIO1	Iodothyronine deiodinase 1	Hs00174944_m1
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Hs99999905_m1
NR1I2 (PXR)	Nuclear receptor subfamily 1 group I member 2	Hs01114267_m1
NR1I3 (CAR)	Nuclear receptor subfamily 1 group I member 3	Hs00901571_m1
SDHA	Succinate dehydrogenase complex flavoprotein subunit A	Hs00188166_m1
SLC10A1	Solute carrier family 10 member 1 (NTCP)	Hs00161820_m1
SLC16A2	Solute carrier family 16 member 2 (MCT8)	Hs00185140_m1
SULT1A1	Sulfotransferase family 1A member 1	Hs00419411_m1
SULT1B1	Sulfotransferase family 1B member 1	Hs00234898_m1
SULT1E1	Sulfotransferase family 1E member 1	Hs00960938_m1
SULT2A1	Sulfotransferase family 2A member 1	Hs00234219_m1
SERPINA7	Serpin family A member 7 (TBG)	Hs02384980_m1
THRβ	Thyroid hormone receptor B	Hs00230861_m1
TTR	Transthyretin	Hs00174914_m1
UGT1A1	UDP glucuronosyltransferase family 1 member A1	Hs02511055_s1
UGT1A3	UDP glucuronosyltransferase family 1 member A3	Hs04194492_g1
UGT1A4	UDP glucuronosyltransferase family 1 member A4	Hs01655285_s1
UGT1A6	UDP glucuronosyltransferase family 1 member A6	Hs01592477_m1
UGT1A9	UDP glucuronosyltransferase family 1 member A9	Hs02516855_sH
UGT2B7	UDP glucuronosyltransferase family 2 member B7	Hs00426592_m1

Tab. S3: TSH-dependent thyroxine (T4) secretion in the 21-day statically cultured 3D thyroid model
T4 was measured in culture supernatants of 3D thyroid models exposed to 0, 0.1, or 1 mIU/mL TSH by LC-MS/MS analysis. Three independent thyroid donors were analyzed (Donor 6, 7 and 8, d6-8). Values represent donor-specific T4 secretion levels in nM measured at the respective time points. Dotted lines (---) indicate T4 concentrations below limit of quantification (< 0.1 nM). n.d., no data were obtained.

	w/o TSH			**0.1 mIU/mL TSH**			**1 mIU/mL TSH**		
Day	d6	d7	d8	d6	d7	d8	d6	d7	d8
2	---	0.4	1.0	*n.d.*	0.8	1.7	---	1.3	1.6
5	1	0.2	0.8	*n.d.*	---	---	2.4	0.5	---
7	0.85	---	0.2	*n.d.*	---	---	2.8	0.7	---
9	4.9	---	1.2	*n.d.*	---	---	2.2	0.8	---
12	0.8	2.6	---	*n.d.*	---	---	2.6	---	---
14	0.9	---	---	*n.d.*	---	---	1.9	---	---
16	0.6	---	---	*n.d.*	---	---	2	---	---
19	---	---	---	*n.d.*	---	---	1.8	---	---
21	1.5	---	---	*n.d.*	---	---	2.2	---	---

Tab. S4: TSH-dependent thyroxine (T4) secretion in the 21-day dynamically cultured 3D thyroid model
T4 was measured in culture supernatants of 3D thyroid models exposed to 0, 0.1, or 1 mIU/mL TSH by LC-MS/MS analysis. Three independent thyroid donors were analyzed (Donor 7 (D7), 8 (D8), 13 (D13)). Values represent donor-specific T4 secretion levels in nM measured at the respective time points. Dotted lines (---) indicate T4 concentrations below limit of quantification (< 0.1 nM).

	w/o TSH			**0.1 mIU/mL TSH**			**1 mIU/mL TSH**		
Day	d7	d8	d13	d7	d8	d13	d7	d8	d13
2	---	1.55	---	---	---	---	---	---	---
5	---	1.8	---	---	---	---	1.4	0.7	---
7	---	0.35	---	---	0.25	---	1.85	1.05	0.357
9	---	---	---	---	---	---	0,3	---	---
12	---	---	---	---	---	---	---	---	---
14	---	---	---	---	---	---	0.55	0.4	---
16	---	---	---	---	---	---	---	---	0.311
19	---	---	---	---	---	0.37	---	---	---
21	---	---	---	---	---	---	---	---	0.693