Karwelat et al.:

A Rodent Thyroid-Liver Chip to Capture Thyroid Toxicity on Organ Function Level

Supplementary Data

Tab. S1: Utilized Taqman probes for gene expression analysis

Gene symbol	Name	Assay ID
Oatp1	Organic anion transporting polypeptide 1	Rn00755148_m1
Alb	Albumin	Rn01413928_m1
Bsep	Bile salt export pump	Rn00582179_m1
Cyp1a1	Cytochrome P450, family 1a, polypeptide 1	Rn00487218_m1
Cyp1a2	Cytochrome P450, family 1a, polypeptide 2	Rn00561082_m1
Cyp2b1	Cytochrome P450, family 2b, polypeptide 1	Rn01457880_m1
Cyp2b2	Cytochrome P450, family 2b, polypeptide 2	Rn02786833_m1
Cyp2b3	Cytochrome P450, family 2b, polypeptide 3	Rn01476084_m1
Cyp3a1	Cytochrome P450, family 3a, polypeptide 1	Rn01640761_gH
Dio1	Deiodinase type I	Rn00572183_m1
Dio3	Deiodinase type II	Rn00568002_s1
Duox1	Dual oxidase 1	Rn00596688_m1
Duox2	Dual oxidase 2	Rn00666512_m1
Duoxa2	Dual oxidase maturation factor 2	Rn01512829_g1
Mct8	Monocarboxylate carrier 8	Rn00596041_m1
Nis	Sodium iodine symporter	Rn00583900_m1
Nkx-2.1	NK2 homeobox 1	Rn01512482_m1
Oatp2	Organic anion transporting polypeptide 2	Rn00756233_m1
Pax8	Paired box 8	Rn00579743_m1
Pds	Pendrin	Rn01469208_m1
Ppia	Peptidylprolyl isomerase A	Rn00690933_m1
Sult1a1	Sulfotransferase, family 1a, polypeptide 1	Rn01510633_m1
Sult1b1	Sulfotransferase, family 1b, polypeptide 1	Rn00673872_m1
Sult2a6	Sulfotransferase, family 2a, polypeptide 6	Rn04219376_m1
Tg	Thyroglobulin	Rn00667257_g1
Tpo	Thyroid peroxidase	Rn00571159_m1
Tshr	Thyroid stimulating hormone receptor	Rn00563612_m1
Ugt1a1	UDP-glucuronyltransferase family 1a, polypeptide 1	Rn00754947_m1
Ugt1a6	UDP-glucuronyltransferase family 1a, polypeptide 6	Rn00756113_mH

doi:10.14573/altex.2108262s

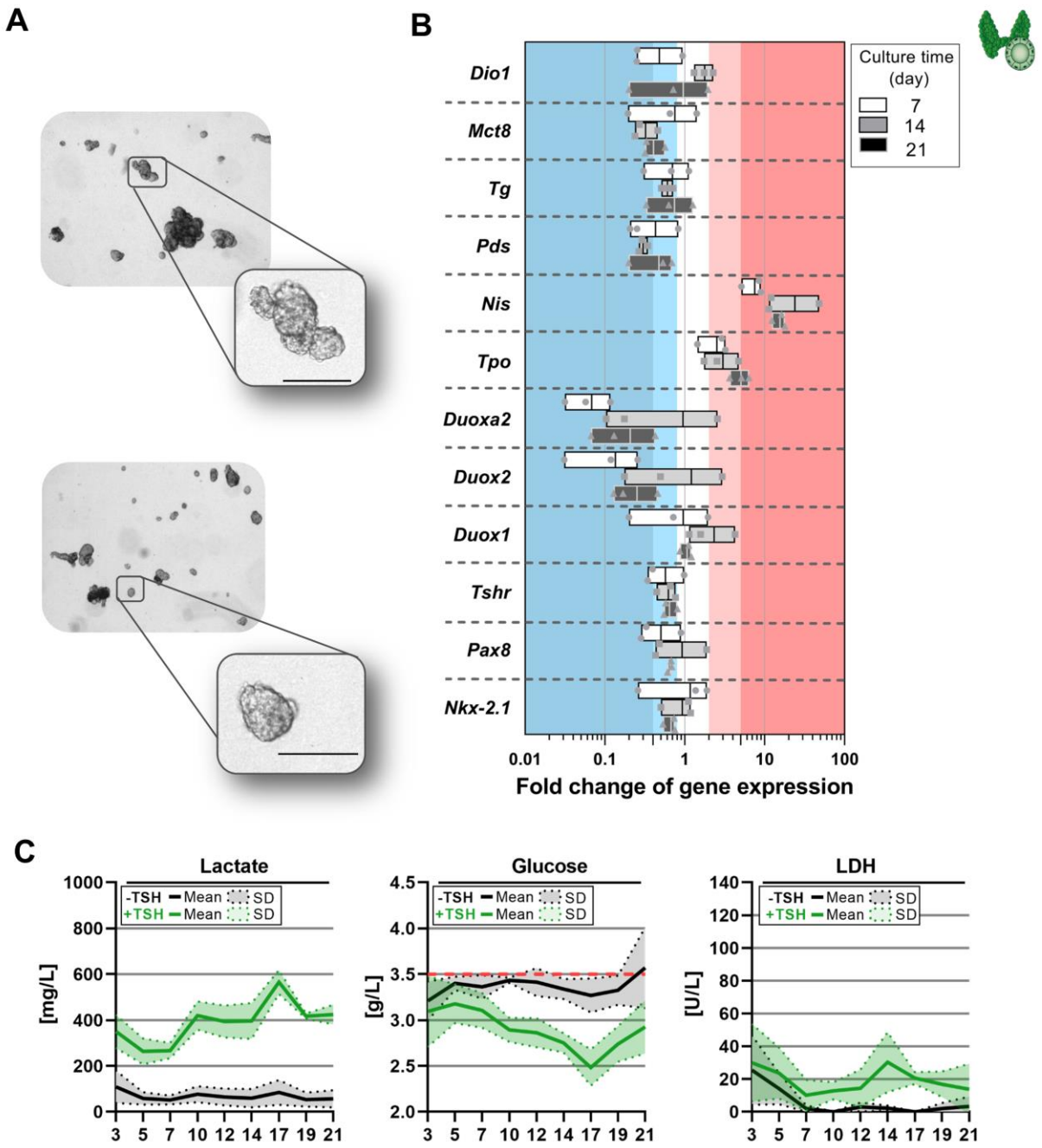


Fig. S1: Morphology, gene expression profile, and energy metabolism of TSH-stimulated and unstimulated thyroid follicles
(A) Representative bright-field images of isolated thyroid follicles embedded in collagen after 14 days in culture. The scale bar is 50 µM. (B) Gene expression analysis of target genes involved in hormone biosynthesis after 7 (white bar), 14 (grey bar), and 21 (black bar) days of culture. All relevant genes were expressed. Upregulation was evident for *TPO* and *NIS*. Bars depict min to the max with the line at the mean of fold-change of gene expression relative to unstimulated thyroid follicles. Data represent 3 independent experiments with cells from 1 animal per experiment (single data points). (C) Lactate (mg/L), glucose (g/L), and lactate dehydrogenase (LDH) (U/L) were monitored in culture medium over 21 days. The red dotted line indicates glucose level in fresh medium (cell-free). Data presented as mean ± SD of 3 independent experiments with cells from 1 animal (AE) per experiment and two technical replicates per experiment (n = 3). Supplementary data to Fig. 2.

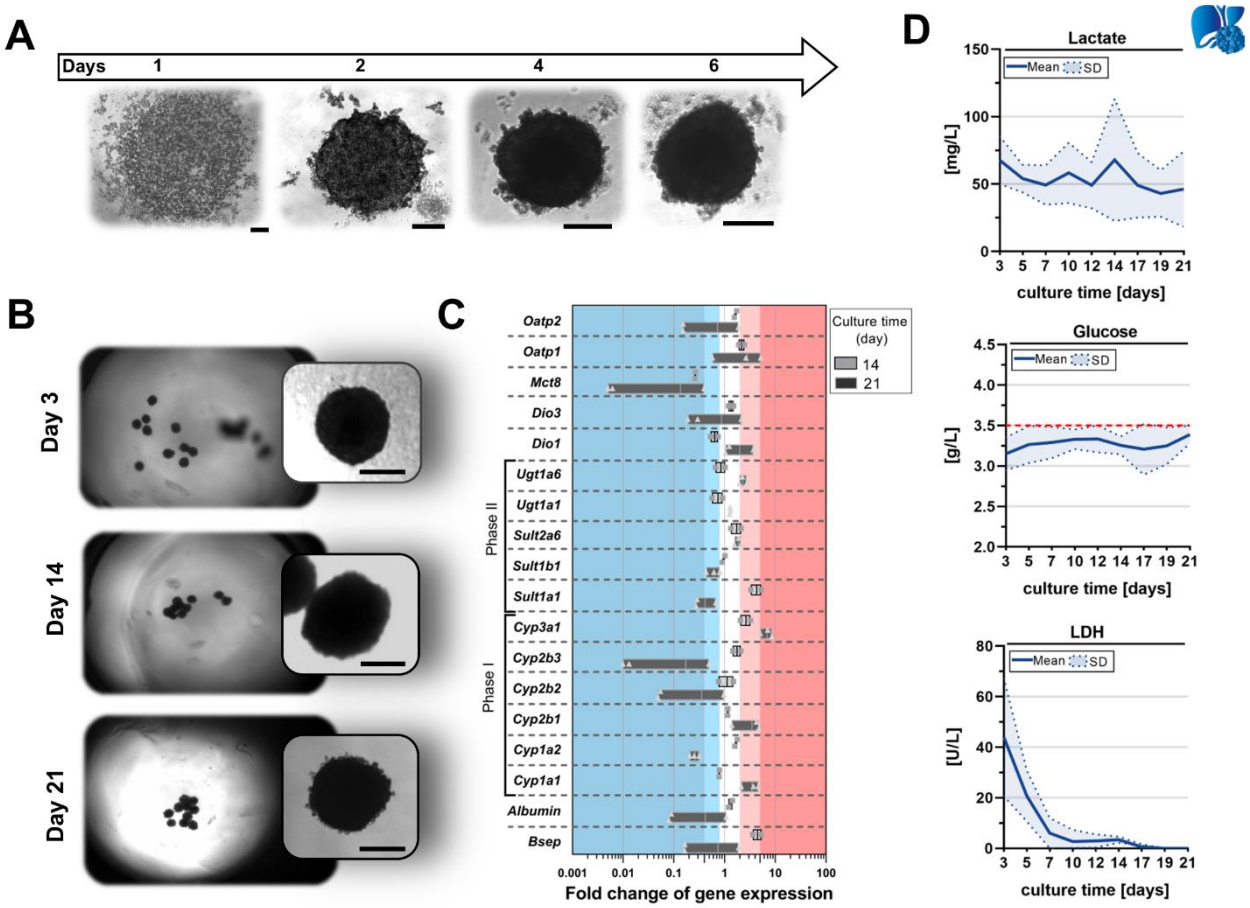


Fig. S2: Morphology, energy metabolism, and gene expression profile of self-assembled liver spheroids
(A) Primary rat hepatocytes and non-parenchymal cells were plated in ultra-low-attachment plates to enable self-assembly and spheroid formation. Representative bright-field images taken on days 1, 2, 4, and 6 evince a successful self-assembly. The scale bar is 200 µm. (B) Representative bright-field images were taken on days 3, 14, and 21 of embedded liver spheroids cultured in the Chip2. The scale bar is 200 µm. (C) Gene expression analysis of liver-specific target genes after 14 (grey bar) and 21 (black bar) days of culture. All relevant genes were expressed. Bars depict min to the max with the line at the mean of fold-change of gene expression relative to day 1 of culture (spheroid formation). Data presented of 2-3 independent experiments (2 experiments for day 14, 3 experiments for day 21) with cells from 1 animal per experiment (single data points). (D) Lactate (mg/L), glucose (g/L), and lactate dehydrogenase (LDH) (U/L) were monitored over the culture period of 21 days. Red dotted line indicates glucose level in fresh medium (cell-free). Supplementary data to Fig. 3.

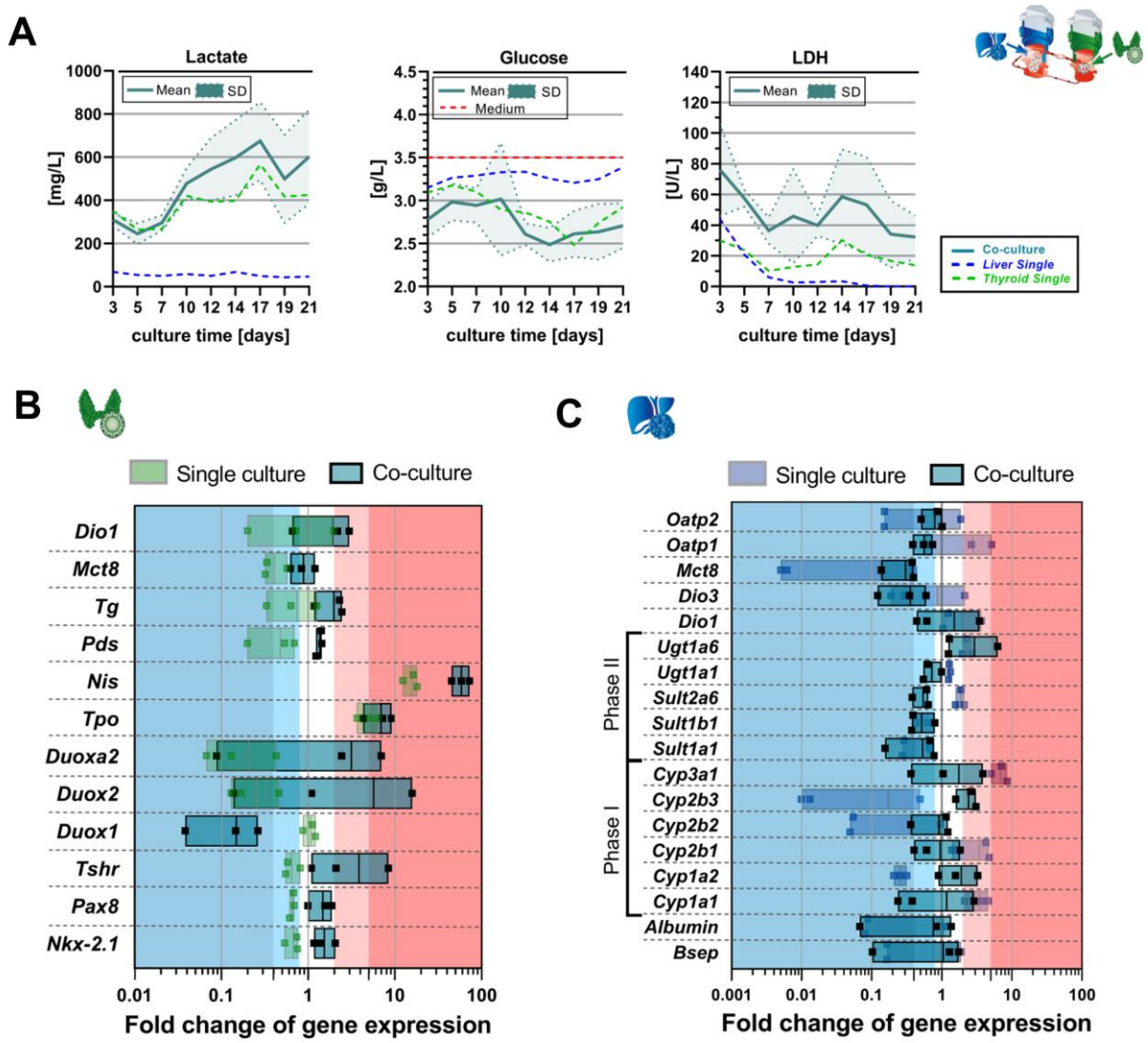
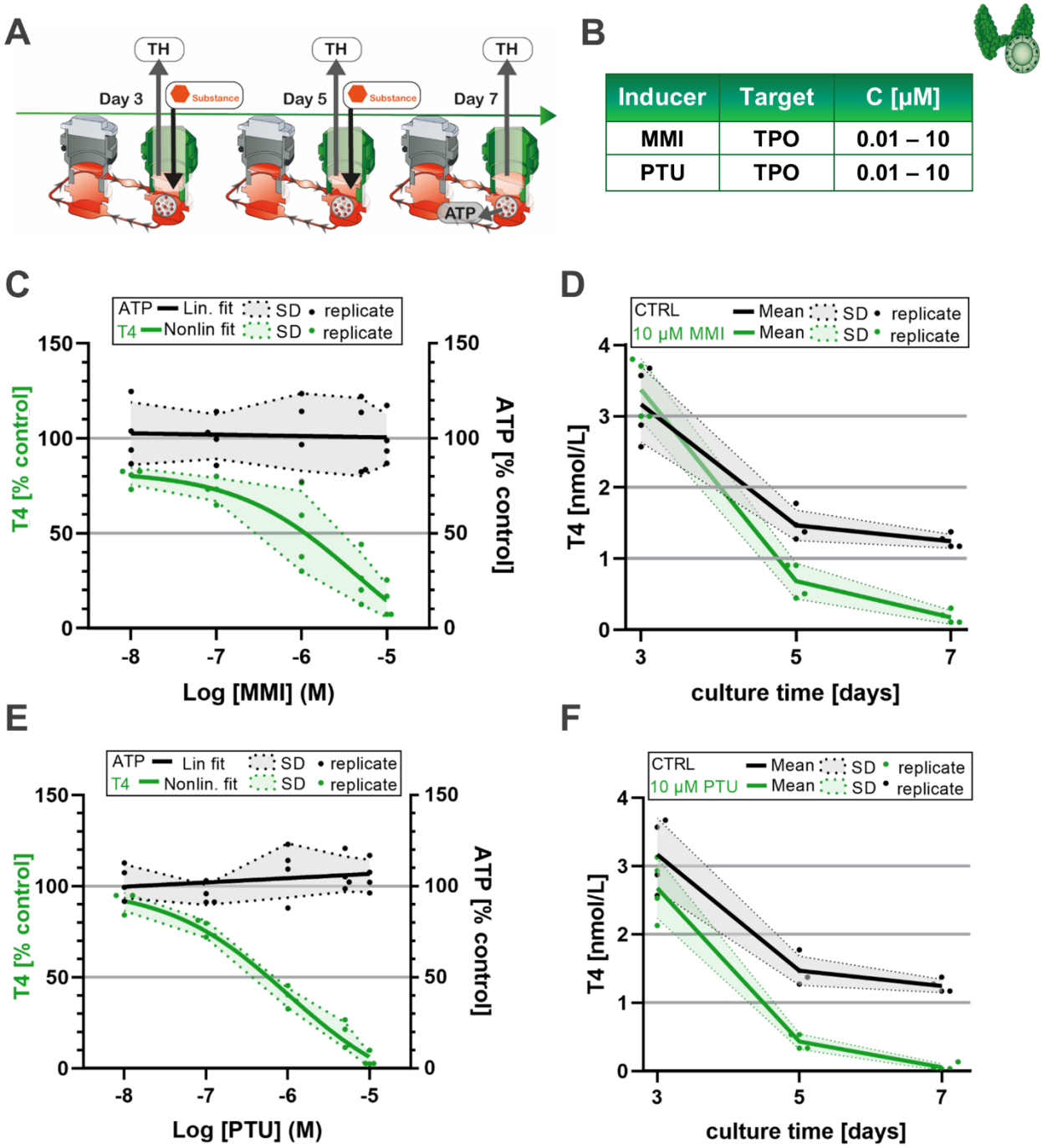


Fig. S3: Energy metabolism and gene expression profile of co-cultured liver and thyroid models
(A) Lactate concentrations (mg/L), glucose concentrations (g/L), and lactate dehydrogenase activity (LDH) (U/L) were monitored over the culture period of 21 days. The red dotted line indicates medium glucose levels (cell-free). Dashed green and blue lines indicate mean values of thyroid and liver single culture, respectively. Data presented as mean ± SD of 3 independent experiments with two technical replicates per experiment (n = 3). (B) Gene expression analysis of transcripts involved in thyroid hormone biosynthesis after 21 days of single (light green) or co-culture (turquoise). In co-cultures, all transcripts (except *Duox1*) trended towards a greater expression compared to single cultures, presumably indicating a supportive effect of the co-culture. Bars depict min to the max with the line at the mean of fold-change of gene expression relative to unstimulated follicles. Data presented from 3 independent experiments with cells from 1 animal per experiment (single data points) (n = 3). (C) Gene expression analysis of liver-specific transcripts after 21 days of single (light blue) or co-culture (turquoise). Overall, in co-culture most analyzed liver-specific transcripts show a more robust expression. Bars depict min to the max with the line at the mean of fold-change of gene expression relative to day 1 of culture. Data presented from 3 independent experiments with cells from 1 animal per experiment (single data points) (n = 3). Supplementary data to Fig. 4.



Inducer	Target	C [µM]
MMI	TPO	0.01 – 10
PTU	TPO	0.01 – 10

Fig. S4: Effects of MMI and TPO inhibitor exposure on thyroxine levels of follicles cultured under microfluidic conditions
(A) Schematic representation of TPO inhibitor treatment regimen. After 3-day culture in the Chip2, the thyroid culture model was treated every 2 days for 4 days. Culture supernatants were analyzed for T4 on days 3, 5, and 7. ATP levels were measured after 4 days of treatment (day 7 in culture). (B) Chemicals with enzyme inhibitory mechanisms were selected as a reference set of compounds disrupting thyroid hormone secretion (methimazole (MMI) and 6-propyl-2-thiouracil (PTU)). Specifications for chemical name, target enzyme, and utilized concentrations are noted. (C) Thyroxine (green) and ATP (black) levels in % relative to vehicle control (DMSO) after 4 days of treatment with MMI (0.01 µM, 0.1 µM, 1 µM, 5 µM, and 10 µM). Non-linear fitted regression lines ([agonist] vs. response-variable slope (four parameters)) were calculated. (D) Thyroxine concentration over time after treatment with 10 µM MMI (green) or vehicle control (DMSO, black). (E) Thyroxine (green) and ATP (black) levels in % relative to vehicle control (DMSO) after 4 days of treatment with PTU (0.01 µM, 0.1 µM, 1 µM, 5 µM, and 10 µM). Non-linear fitted regression lines ([agonist] vs. response-variable slope (four parameters)) were calculated. (F) Thyroxine concentration over time of treatment with 10 µM PTU (green) or vehicle control (DMSO, black). Presented as pooled data from 2 independent experiments (2 technical replicates/experiment) as mean ± SD (n=2). Liver cells were isolated from 1 animal per experiment (AE) and thyroid cells from 4 animals (AS) per experiment. Supplementary data to Fig. 5.

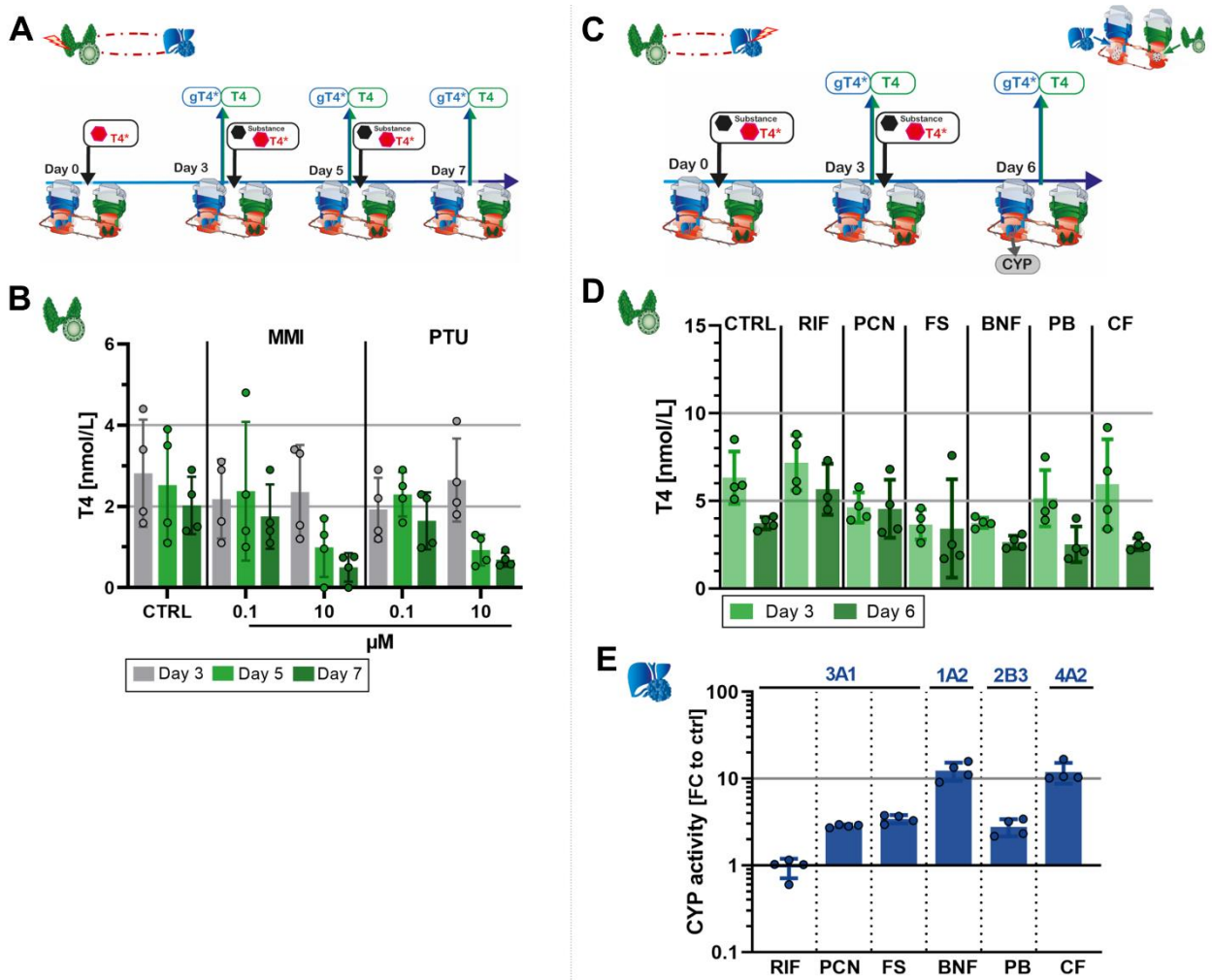


Fig. S5: Co-culture exposed to TPO inhibitors or biotransformation inducers
(A) Schematic representation of TPO inhibitor treatment regimen of liver-thyroid co-culture. After 3 days in culture, the thyroid culture model was treated every 2 days (culture day 5) for 4 days (culture day 7) with either 0.1 µM or 10 µM of methimazole (MMI) and 6-propyl-2-thiouracil (PTU). Culture supernatants were analyzed for T4 on days 3, 5, and 7. ATP levels were measured after 4 days of treatment (day 7 in culture). The medium was supplemented with 1 µM labeled T4. (B) T4 concentrations after 3 and 6 days of treatment with MMI or PTU (CTRL = solvent control; DMSO). (C) Schematic representation of liver spheroid treatment regimen in co-culture. Liver spheroids were treated every 3 days for 6 days with 10 µM rifampicin (RIF); 10 µM pregnenolone 16-α-carbonitrile (PCN), 10 µM fipronil sulfone (FS), 10 µM beta-naphthoflavone (BNF), 1 mM phenobarbital (PB) or 1 mM clofibrate (CF). The medium was supplemented with 1 µM labeled L-thyroxine. Culture supernatants were analyzed for gT4 formation after 3 and 6 days of treatment. CYP450 activity was measured after 6 days of treatment. (D) Corresponding T4 levels after 3 and 6 days of treatment (CTRL = solvent control; DMSO). (E) Fold-induction of CYP450 isozymes after 6 days of treatment with inducers (3A1: RIF, PCN, FS; 1A2: BNF; 2B3: PB; 4A2: CF). Presented as pooled data from 2 independent experiments (2 technical replicates/experiment) as mean ± SD. Supplementary data to Fig. 7.