Chemicals

The bile acids TCDCA, GCDCA, TCA, GCA, TαMCA and TβMCA and the synthetic FXR-agonist GW4064 were purchased from Sigma. For *in vivo* studies, human recombinant FGF19 was kindly provided by Dr. Eric Zhu. Plasma immunoreactive human FGF19 was measured using a commercial ELISA kit (R&D systems DF1900, UK). The cytokine bead assay was performed according to manufacturer's manual (BD Bioscience no.552364, the Netherlands).

Mouse studies

Male *Slc10a1* knockout mice (C57Bl6/J background) were housed and bred in the Academic Medical Center, Amsterdam (1). Control wild-type mice were purchased from Envigo, the Netherlands. Male *Slco1a/1b* knockouts mice (lacking *Slco1b2* and *Slco1a1* to *Slco1a5*) and *Slco1a/1b* knockouts reconstituted with liver-specific expression of human OATP1B1 (FVB/N background) and wild-type littermates were housed and bred in the Netherlands Cancer Institute, Amsterdam (2) or obtained from Taconic, Denmark. A single-dose of myrcludex B (intravenously, 5 μg/g BW) was administered in acute taurocholate (TCA) clearance studies. Male wild-type mice were subjected to common bile duct ligation (BDL) and cholecystectomy, with a single analgesic dose of buprenorphine (30 μg/kg BW subcutaneously), as described previously (3). Treatment with myrcludex B (5-day subcutaneously, 2.5 μg/g BW) was performed in male (OATP1B1-humanized) *Slco1a/1b*-knockouts and in BDL-mice. For the T cell transfer colitis model, female Balb/C and C.B-17 SCID animals were obtained from Envigo (Boxmeer, The Netherlands) and used between the age of 8 and 20 weeks. The T cell transfer model has been described previously (4). Briefly, CD4+CD45RBhigh cells are isolated from the spleen of wild-type animals,

and adoptively transferred into C.B-17 SCID animals (2.5x10E5 cells, i.p.). Control animals did not receive a transfer. Onset of disease (colitis) was confirmed by endoscopy three weeks after transfer. To investigate regulation of hepatic bile acid uptake transporters, male wild-type and FGF15 knockout mice (3-4 months of age, described previously in (5)) were injected intraperitoneally with recombinant hFGF19 (1 mg/kg) or saline at 3 am. Given the even distribution of FGF19, plasma concentrations should reach 1 µg/ml. Between 8 and 9 am, mice were killed by isofluorane overdosage and tissues were collected. *Db/db* (#000642, on BKS background) and DIO male mice (#380050 on C57BL6/J background) were purchased from Jackson Laboratory. For studies in *db/db* mice, 11 week-old mice received a single intravenous dose of $3x10^{11}$ *v.g.* of AAV carrying either FGF19 or a control gene green fluorescent protein. *Db/db m*ice were euthanized 24 weeks post AAV administration. Diet-induced obese (DIO) mice were fed a high fat diet (Research Diets #D12492i, 60% kcal from fat) beginning at 6 weeks of age and throughout the study. Mice received a single tail vein injection of $1x10^{11}$ *v.g.* AAV carrying FGF19 or a control gene green fluorescent protein when 18-week old. DIO mice were euthanized 52 weeks post AAV administration.
The study design and animal care and handling were approved by the Institutional Animal Care and Use Committee of the University of Amsterdam and the University of Texas Southwestern Medical Center of Dallas.

Gall bladder cannulation and bile acid kinetics in vivo

To investigate TCA transport in wild-type and *Slco1a/1b* knockout mice, the gall bladder was cannulated and bile was collected after distal ligation of the common bile duct, as described in (6). Myrcludex B (5 µg/g BW) was injected intravenously at the start of the cannulations to block NTCP-mediated TCA uptake. Bile output was calculated as the product of the bile flow and the bile concentration during the indicated collection period. After the 30 minutes depletion-phase, a single submicellar bolus of 50 µmol TCA/kg mouse (including a trace amount of tritium-labelled TCA) was administered intravenously in 100 µL 0.9% NaCl per 20 g mouse. Blood was collected directly prior to TCA bolus injection to measure baseline plasma bile acid levels. Blood (~30 µL) and bile samples were collected at the indicated time points after TCA administration. Radioactivity in plasma and bile was measured by liquid scintillation counting.

Human study

The Clinical Research Unit of the Department of Clinical Pharmacology and Pharmacoepidemiology at the Heidelberg University Hospital (EN ISO standard 9001) conducted a clinical study, which followed the guideline of Good Clinical Practice, the ethical principles expressed in the Declaration of Helsinki, and all legal requirements for clinical studies in Germany. The responsible Ethics Committee of the Medical Faculty of Heidelberg University and the competent national authority approved the study (BfArM, Bonn, Germany, EudraCT: 2014-003289-26). Each participant provided written informed consent before any study-related procedure was conducted. In a single center open-label phase I study, drug-drug interaction between myrcludex B and tenofovir was assessed. Twelve healthy volunteers were given 245 mg tenofovir disoproxil fumarate orally (Viread®, Gilead Sciences, Martinsried, Germany) for 11 days. On day 7, 10 mg myrcludex B was added for additional 6 days to reach steady-state for both drugs (2 consecutive subcutaneous injections daily with 5 mg each; Bachem, Bubendorf, Switzerland) *(A. Blank et al. -*

manuscript in preparation). Study procedures ensured controlled conditions for blood sampling regarding fasting state at baseline and food intake at controlled times. Samples analyzed for FGF19 were taken at baseline (0 h) and 3 h after myrcludex B intake, 2.75 h after breakfast. FGF19 day profiles included sampling at 0 h, just before myrcludex B application, and 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 h thereafter. Food intake occurred right after 0 h, at 3.25 h and 8.25 h thereafter.

DNA constructs

The plasmid encoding FRET-based bile acid sensor NucleoBAS (Addgene #62861; mutant N354K, I372V for increased dynamic range) was generated previously (7). The mouse *Ntcp*-containing cDNA3.1 plasmid was generated in our lab. The Receiver-M07 plasmid encoding mouse *Oatp1b2* was commercially obtained (Tebu-bio, the Netherlands). Mouse *Oatp1a1* and rat *Oatp1a4* were cloned in pSport6 and pExpress1, respectively (kind gifts of Prof. dr. Hagenbuch).

Cell culture and transfection

U2OS cells (human bone osteosarcoma epithelial cells) were cultured in DMEM supplemented with 2 mM L-glutamine, 100 U/L penicillin/streptomycin and 10% FCS. Cells were transiently (co-)transfected with equal amount of plasmids complexed with polyethylenimine (PEI 1 mg/ml; Polyscience) at a ratio of 1:5 (µg DNA: µL PEI). After 8-16 h, cells were seeded in equal density in 24-wells plate (Greiner, uptake assay) or in 8-well chambered coverglass slide (Nunc Labtek II, FRET assay). Sodium butyrate (10 mM) was added to culture medium to boost membrane protein overexpression. HPCT-1e3 cells, a fusion cell line between primary rat hepatocytes and Fao Reuber hepatoma cells H35 (kind gift of Prof. dr. Honscha, Leipzig) (8), were cultured in DMEM supplemented with 2 mM L-glutamine, 10 ng/ml insulin, 10 ng/ml inosine, 1,5 μM dexamethasone and 10% FCS. T84 cells (ATCC) were cultured in DMEM/F12 supplemented with 2 mM L-glutamine, 100 U/L penicillin/streptomycin and 10% FCS (cells were in passages 25-35). After seeding onto Transwell polycarbonate membrane inserts (Costar; pore size 0.4 μm, 1.12 cm^2), monolayers were grown at full confluence for 2-3 weeks, and medium was replaced every 2 to 3 days. Cell monolayer integrity was verified by measuring transepithelial electrical resistance (>350 Ω/cm^2) at the end of incubation with mentioned compounds. Murine bile acids (ratio TCA 77%, TMCAs 20%, TCDCA 3%) and human bile acids (ratio GCDCA 47%, GCA 47%, TCDCA 6%) were used to mimic plasma composition upon myrcludex B treatment.

Organoid culture

Intestinal epithelial organoids were obtained from the small intestines of C57Bl6/J mice. Harvest and expansion of intestinal organoid culture was performed as described previously (9). In short, organoids were cultured in drops of Matrigel (BD) and medium was refreshed every two to three days. Organoid medium contained advanced DMEM/F12 (Invitrogen) with 1% Penicillin/Streptomycin, 1% HEPES buffer and 1% Glutamax, 50 ng/ml EGF, 20% Noggin conditioned medium, 10% R-spondin conditioned medium, 1X B-27 supplement, 1X N-2 supplement (all from Invitrogen) and 1.25 mM n-Acetyl Cysteine (Sigma-Aldrich). Organoids were split by pipetting through a narrowed pipet. Six days after splitting, organoids were stimulated for 24 hours with the indicated stimulus.

Bile acid transport assay

Transfected U2OS cells were pre-washed with sodium-containing uptake buffer (136 mM NaCl, 5 mM KCl, 1.1 mM KH_2PO_4, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 10 mM D-Glucose, 10 mM HEPES, pH 7.4) at room temperature. To determine bile acid transport, cells were incubated at 37 °C for 2 minutes with uptake buffer containing 20 µM taurocholic acid (TCA) of which a trace amount (0.1 µM) was tritium-labelled ($[^3H]$TCA, Perkin Elmer, Groningen, the Netherlands). Cells were washed four times with ice-cold PBS buffer and lysed with milliQ water containing 0.05% (w/v) SDS. Radioactivity in the lysates was measured by liquid scintillation counting. Data are presented of two independent experiments, each condition performed in triplicate.

Live cell bile acid dynamics by confocal microscopy

Uptake of 40 µM TCDCA by NTCP and OATP-isoforms was measured using the nuclear FRET sensor for bile acids NucleoBAS on a Leica SP8-X SMD confocal microscope as described earlier (7) (10). To fully activate NucleoBAS, each experiment ended with addition of 5 µM GW4064.

Biotinylation and Western blot

Transfected U2OS cells were washed twice with ice-cold PBS containing 1.0 mM $MgCl_2$ and 0.5 mM $CaCl_2$ (PBS-CM), and incubated for 30 min with Sulfo-NHS-ss-biotin (0.5 mg/ml in ice-cold PBS-CM; Thermo Scientific). Non-bound biotin was quenched by washing twice with PBS-CM containing 0.1% BSA and a final wash with PBS-CM. Cells were lysed for 5 min on ice in lysis buffer (20 mM Tris-HCl pH 7.4, 135 mM NaCl, 5 mM EDTA, 1.0% (v/v) NP-40, 10% (w/v) sucrose, 1 mM PMSF, 1 µg/mL Leupeptin, 10 µg/mL Aprotinin, 1 µg/mL Pepstatin A). Biotinylated proteins were precipitated with high-affinity Neutravidin beads (Thermo Scientific) for 2 h at 4

°C by end-over-end rotations. Beads were washed three times with lysis buffer. Crude mouse liver membranes were isolated from ~50 mg of frozen liver, which was pestle homogenized on ice in hypotonic lysis buffer (10 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 7.4) supplemented with protease inhibitor cocktail. Liver homogenates were ultracentrifugated at 200,000 x g for 45 minutes, and pellets were resuspended in RIPA buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% (v/v) NP-40, 0.5% (w/v) Na-deoxycholate, 0.1% (w/v) SDS). Both biotinylated and crude liver membrane proteins were eluted with SDS-containing sample buffer for 15 min at room temperature, and separated on a 8% SDS-polyacrylamide electrophoresis gel (50 µg protein/lane). Proteins were transferred by wet-blotting to PVDF-membrane and probed with anti-mouse OATP1B2 (1:750; prof. dr. R. Kim), anti-rat OATP1A1 (1:1000; prof. dr. B. Stieger) or anti-mouse/rat OATP1A4 (1:1000; prof. dr. B. Stieger, and 1:250; Santa Cruz sc-18436). Immune complexes were detected with a horseradish peroxidase-conjugated secondary antibody (Biorad), visualized using enhanced chemiluminescence detection reagent (Lumi-light, Roche) and detected using ImageQuant LAS 4000 (GE Healthcare).

Co-immunofluorescence staining

Small cubes of fresh frozen liver tissue were embedded in OCT (Agar Scientific), and 7 µm cryosections were fixed in 100% acetone for 10 minutes. Slides were blocked with 5% (v/v) normal goat serum in PBS + 0.05% (v/v) tween-20 (blocking buffer) for 1 hour, followed by incubation with rabbit anti-OATP1A1 (1:50; prof. dr. B. Stieger) or goat anti-OATP1A4 (1:50; Santa Cruz sc-18436) in blocking buffer for 1 hour. After washings, the slides were incubated another hour with mouse anti-glutamine synthetase (1:1000; BD Biosciences no. 610517). After washings, slides were incubated with secondary antibodies goat anti-rabbit / rabbit anti-goat Alexa 594 IgG (red; directed to OATPs) or goat anti-mouse Alexa 488 IgG (green; directed to GS). Images were captured with a Leica SP8-X confocal microscope.

RNA isolation and qRT-PCR

Total RNA was isolated from approximately 50 mg of liver with TRI Reagent (Sigma). For gene expression experiments in organoids, mRNA isolation was performed using the Bioline ISOLATE II RNA Mini kit (BIO-52073, Bioline) according to manufacturer's instructions. RNA integrity was assessed spectrophotometrically at 260 nm using a Nanodrop 1000 (Thermo Scientific, Wilmington, US). Five-hundred nanograms of total RNA were treated with DNAse (Promega, Madison, US) and first-strand cDNA was synthesized with Oligo-dT$_{12-18}$ and Superscript II reverse transcriptase (Invitrogen). Quantitative real-time PCR (qRT-PCR) was carried out in a Roche Lightcycler 480 II instrument using SensiFAST SYBR No-ROX kit (Bioline, UK) and was analysed using LinRegPCR 12.5 software. Expression levels in each sample were normalized for the geometrical mean of two reference genes, as summarized in Supplementary table 1.1-1.3.

Plasma biochemistry and urine creatinine

Plasma biomarkers for liver injury (ALT, AST) and cholestatic parameters (bilirubin, ALP) were determined by routine clinical biochemistry testing on a Roche Cobas c502/702 analyzer (Roche Diagnostics, US). Urine creatinine was measured by UPLC-MS/MS in multiple reaction monitoring mode.

Quantification of bile acids and C4

Feces were collected (24 h) from individually housed mice (to avoid coprophagy), lyophilized and weighed. The dried stools were homogenized and bile acids were extracted in 50% (v/v) t-butanol (Merck). Total bile acid concentrations in feces, urine and plasma were quantified with the Total Bile Acid Assay kit (Diazyme Laboratories, Poway, US) by measuring absorbance at 410 nm using a NOVOstar analyzer (BMG-Labtech, Offenburg, Germany). Concentrations of different bile acid species in plasma were determined by reverse-phase HPLC. For HPLC, plasma was deproteinized by adding dropwise 5 volumes of acetonitrile (Biosolve, Valkenswaard, the Netherlands), followed by vortex-mixing, centrifugation at 12,000 x g for 10 min and vacuum-drying of the supernatant. Plasma bile acids were solubilized in 25% methanol. 100 µl was applied to a Hypersil C18 (3 µm, 15 cm HPLC column, Thermo Scientific, the Netherlands). Starting eluent consisted of 6.8 mM ammonium formate (pH 3.9), followed by several steps of linear gradients to different concentrations of acetonitrile (Biosolve, the Netherlands). Detection was performed using a Dionex Corona Veo RS Charged Aerosol Detector (Thermo Scientific, the Netherlands). Quantification of the different bile acid species was performed using calibration curves for all bile acid species. The bile acid precursor 7α-hydroxy-4-cholesten-3-one (C4) was measured in plasma by high-performance LC/MS, as described in (11).

Species	Gene name	Sense and antisense	Product length	Used in
Mouse	*Shp*	5'-GGCACGATCCTCTTCAACCC-3'	287-bp	Liver, ileum
		5'-GGCACCAGACTCCATTCCAC-3'		
Mouse	*Fgf15*	5'-ATACGGGCTGATTCGCTACT-3'	123-bp	Ileum, organoid
		5'-GGCTTGGCCTGGATGAAGAT-3'		
Mouse	*Slc10a2*	5'-CCATGGGGTATCTTCGTGGG-3'	278-bp	Kidney
		5'-GTTCCCGAGTCAACCCACAT-3'		
Mouse	*Slc51a*	5'-GGCATCTATGACCCAGGAGA-3'	151-bp	Ileum, kidney
		5'-TGGATCCCATGTTCTGTTCA-3'		
Mouse	*Fabp6*	5'-GAGACGTGATTGAAAGGGGA-3'	294-bp	Ileum, organoid
		5'-TTACGCGCTCATAGGTCACA-3'		
Mouse	*Cyp7a1*	5'-CTGGGGGATTGCTGTGGTAG-3'	315-bp	Liver
		5'-CTGTGTCCAAATGCCTTCGC-3'		
Mouse	*Slc10a1*	5'-TGGCTACCTCCTCCCTGATG-3'	380-bp	Liver
		5'-GCCAGGTTGTGTAGGAGGAT-3'		
Mouse	*Slco1a1*	5'-TGAGAAAGACAGCAGTAGGACTTT-3'	162-bp	Liver
		5'-GTGATTTGGCTAGGTATGCAC-3'		
Mouse	*Slco1a4*	5'-TACATGTCAGCTTGCCTCGC-3'	140-bp	Liver
		5'-GCACACTCAGGACCCTTGTC-3'		
Mouse	*Slco1b2*	5'-GGGTGAATGCCCAAGAGACA-3'	282-bp	Liver
		5'-TATAGCCTGCATGCTCCACG-3'		
Mouse	*Rplp0*	5'-CCAGCGAGGCCACACTGCTG-3'	169-bp	Liver, kidney
		5'-ACACTGGCCACGTTGCGGAC-3'		
Mouse	*Tbp*	5'-GGAGAATCATGGACCAGAACA-3'	89-bp	Liver
		5'-GATGGGAATTCCAGGAGTCA-3'		
Mouse	*Ppib*	5'-TCGGAGCGCAATATGAAGGT-3'	65-bp	Ileum
		5'-AAAAGGAAGACGACGGAGCC-3'		
Mouse	*Hprt*	5'-TTGCTCGAGATGTCATGAAGGA-3'	91-bp	Ileum, kidney, organoid
		5'-AGCAGGTCAGCAAAGAACTTATAG-3'		

Supp. table 1.1: Mouse oligonucleotide primers used for qRT-PCR analysis (each used at a final concentration of 5 µM). Genes of interest are involved in hepatic BA transport (*Slc10a1, Slco1a1, Slco1a4, Slco1b2*) and synthesis (*Cyp7a1*), intestinal and renal BA homeostasis (*Shp, Fgf15, Slc10a2, Ibabp, Slc51a*). Reference genes (*Rplp0, Tbp, Ppib and Hprt*) are also shown.

Species	Gene name	Sense and antisense	Product length	Used in
Rat	*Rplp0*	5'-GAGGTGCTGGACATCACAGA-3'	100-bp	HPCT-1e3
		5'-CAACAGTCGGTAGCCAATC-3'		
Rat	*Hprt*	5'-GCGAAAGTGGAAAAGCCAAGT-3'	76-bp	HPCT-1e3
		5'-GCCACATCAACAGGACTCTTGTAG-3'		
Rat	*Slco1b2*	5'-ACTACAAGTCAGCGGCTTCA-3'	198-bp	HPCT-1e3
		5'-GGGTTCATTTTGGCGATTCCA-3'		
Rat	*Slco1a1*	5'-CTTACAGCCATACCTGGGTACAT-3'	376-bp	HPCT-1e3
		5'-GCTCGCTTTCCTTCTCTCCG-3'		
Rat	*Slco1a4 (1a2)*	5'-CTGCACACTTAGCATTCTGGC-3'	126-bp	HPCT-1e3
		5'-GTTGGTGCTGAACCCCTTCAT-3'		
Rat	*Slc10a1*	5'-ATCAAGCCTCCAAAGGACCAAA-3'	137-bp	HPCT-1e3
		5'-AGGCCATTAGGGGAAGGACC-3'		

Supp. table 1.2: Rat oligonucleotide primers used for qRT-PCR analysis of HPCT-1e3 hepatoma cell line (each used at a final concentration of 5 μM). Genes of interest are involved in BA uptake in hepatocytes. Reference genes are also shown.

Species	Gene name	Sense and antisense	Product length	Used in
Human	*RPLP0*	5'-TCATCAACGGTACAAACGA-3'	78-bp	T84
		5'-GCCTTGACCTTTTCAGCAAG-3'		
Human	*HPRT*	5'-TGACCTTGATTTATTTTGCATACC-3'	102-bp	T84
		5'-CGAGCAAGACGTTCAGTCCT-3'		
Human	*FABP6*	5'-TCACTTGGTCCCAGCACTA-3'	182-bp	T84
		5'-CTTGTCACCCACGATCTCT-3'		
Human	*SLC51*	5’-AGATAACGCTGACCCTGGTG-3’	196-bp	T84
		5’-AATTTGGCTCCCATGTTCTG-3’		
Human	*FGF19*	5’-TTTCTCATCACTTCCCCAGG-3’	222-bp	T84
		5’-AGGCTTCCCCTACTCCTGAA-3’		

Supp. table 1.3: Human oligonucleotide primers used for qRT-PCR analysis of T84 colon cell line (each used at a final concentration of 5 μM). Genes of interest are involved in BA transport and homeostasis. Reference genes are also shown.

	Unit	Oatp-KO + vehicle	Oatp-KO + myrcludex B	OATP1B1-humanized + vehicle	OATP1B1-humanized + myrcludex B
Total Bilirubin	**µM**	34.0 ± 1.0	35.8 ± 3.6	5.7 ± 0.9 *	5.0 ± 0.8 #
Conjugated Bilirubin	**µM**	29.8 ± 1.7	33.8 ± 3.2	4.3 ± 0.6 *	4.3 ± 0.6 #
AST	**U/L**	108.8 ± 15.2	193.9 ± 99.8	119.4 ± 27.7	146.1 ± 35.9
ALT	**U/L**	52.6 ± 6.6	94.4 ± 19.6	65.4 ± 15.9	83.5 ± 23.3
ALP	**U/L**	79.9 ± 2.5	87.2 ± 4.4	81.2 ± 6.5	82.9 ± 4.7

Supp. table 2: Biochemical indicators in plasma of 2-month-old male *Slco1a/1b* knockout mice and OATP1B1-humanized *Slco1a/1b* knockout mice. Mice were treated for 5 days with myrcludex B (2.5 µg/g mouse s.c.) or vehicle. AST: aspartate aminotransferase. ALT: alanine aminotransferase. ALP: alkaline phosphatase. Asterisk indicates significant change for *Slco1a/1b*-KO mice, and hash indicates significant change for OATP1B1-humanized *Slco1a/1b*-KO mice ($p<0.05$, Kruskal Wallis; n=6/group).

Supp. figure 1: (A) Plasma total BA concentrations (μM) in *Slco1a/1b* knockout and OATP1B1-humanized *Slco1a/1b* knockout mice after myrcludex B or vehicle s.c. treatment for 5 days. Plasma was analyzed 3 hours or 24 hours after treatment. (B) Relative mRNA expression of renal BA transporters for the same mouse cohort. Data is calculated using the geometric mean of reference genes *Rplp0* and *Hprt* and normalized to vehicle-treated *Slco1a/1b* knockout mice. Asterisk or hash symbols indicate significant increase ($p<0.05$; Mann-Whitney U; 6 male mice/group) compared to vehicle controls.

Supp. figure 2: Liver hematoxylin-eosin histology comparing adult male *Slco1a/1b* knockout and OATP1B1-humanized *Slco1a/1b* knockout mice after myrcludex B or vehicle treatment. Section thickness is 7 μm. Portal vein (pv) and central vein (cv) are indicated. Scale bar is 100 μm.

Supp. figure 3: (A) Total RNA was isolated from mouse livers and gene expression was measured using qRT-PCR. Relative hepatic mRNA expression of *Ntcp* and *Oatp1a1* was determined in a sterile inflammation (T cell transfer). Data is calculated using the reference gene *Rplp0* and normalized to healthy mice. Asterisk indicates significant decrease ($p<0.05$; 4 female mice/group) compared to healthy mice. (B) Mouse plasma cytokine concentrations were determined by cytometric bead assay measured on FACS apparatus (BD LSRFortessa). Cytokines concentrations (IL-6, IL-10, MCP-1, TNFα, IL-12p70, IFNγ) were calculated (^{10}log scale) in wild-type, *Slco1a/1b* knockouts and OATP1B1-humanized *Slco1a/1b* knockouts after myrcludex B or vehicle, *Slc10a1* knockouts and wild-type mice injected with LPS (20 μg/kg BW, as a positive control for the assay). Data is presented from 5-6 mice/group. B.D. is below detection limit (~10 ng/mL).

Supp. figure 4: (A) Plasma concentrations of total conjugated and unconjugated bile acids after 5-days BDL, as quantified using HPLC. Asterisk indicates significant change ($p<0.05$; Mann-Whitney U; 6 male mice/group) upon myrcludex B treatment. (B) Plasma total bile acid concentrations in humans before and after steady-state (6 day) myrcludex B treatment. (C) Human plasma FGF19 levels before (open dots) and after myrcludex B (closed dots) treatment during daytime. Blood samples were drawn at 9:45 AM (after fasting) and at 9 consecutive time points. FGF19 levels were measured by ELISA in 4 individuals/group.

Supp. figure 5: (A) Lactate dehydrogenase (LDH) release in medium of polarized, filter-grown intestinal cells. Murine (M) bile acids and human (H) bile acids were used at increasing amounts (200 and 1000 μM) for 24 hours. Data is normalized to LDH activity in control (DMSO) medium, set at 1.0. (B) Gross morphology of mouse organoid culture, incubated with indicated bile acid-mixtures. Scale bar: 50 μm. (C) *Fgf15* and *Ibabp* mRNA expression measured using qRT-PCR in mouse organoid culture after 24 hour incubation with mouse and human bile acids mixture. Data is calculated using the reference gene *Hprt* and normalized to vehicle-treated controls. Asterisks indicate significant increase ($p<0.05$; 3 replicates/group, measured in duplo) compared to vehicle controls.

Supp. Figure 6: (A) Relative hepatic mRNA expression of *Ntcp* and *Oatp*-isoforms was determined 6 hours (acutely) after a single injection of hFGF19 (1 mg/kg i.p.) or vehicle in male *Fgf15* knockout mice, using the housekeeping gene *Rplp0* as a reference. Data is normalized to vehicle-treated *Fgf15* knockout mice. Asterisk symbols indicate significant ($p<0.05$; 5 fasted male mice/group). (B) Relative hepatic mRNA expression of *Ntcp* and *Oatp*-isoforms after 52-weeks of AAV-induced FGF19 overexpression in diet-induced obese (DIO) male mice, using the housekeeping gene *Rplp0* as a reference. Data is normalized to AAV-GFP treatment. Asterisk indicates significant change ($p<0.05$; 6-10 fasted male mice/group).

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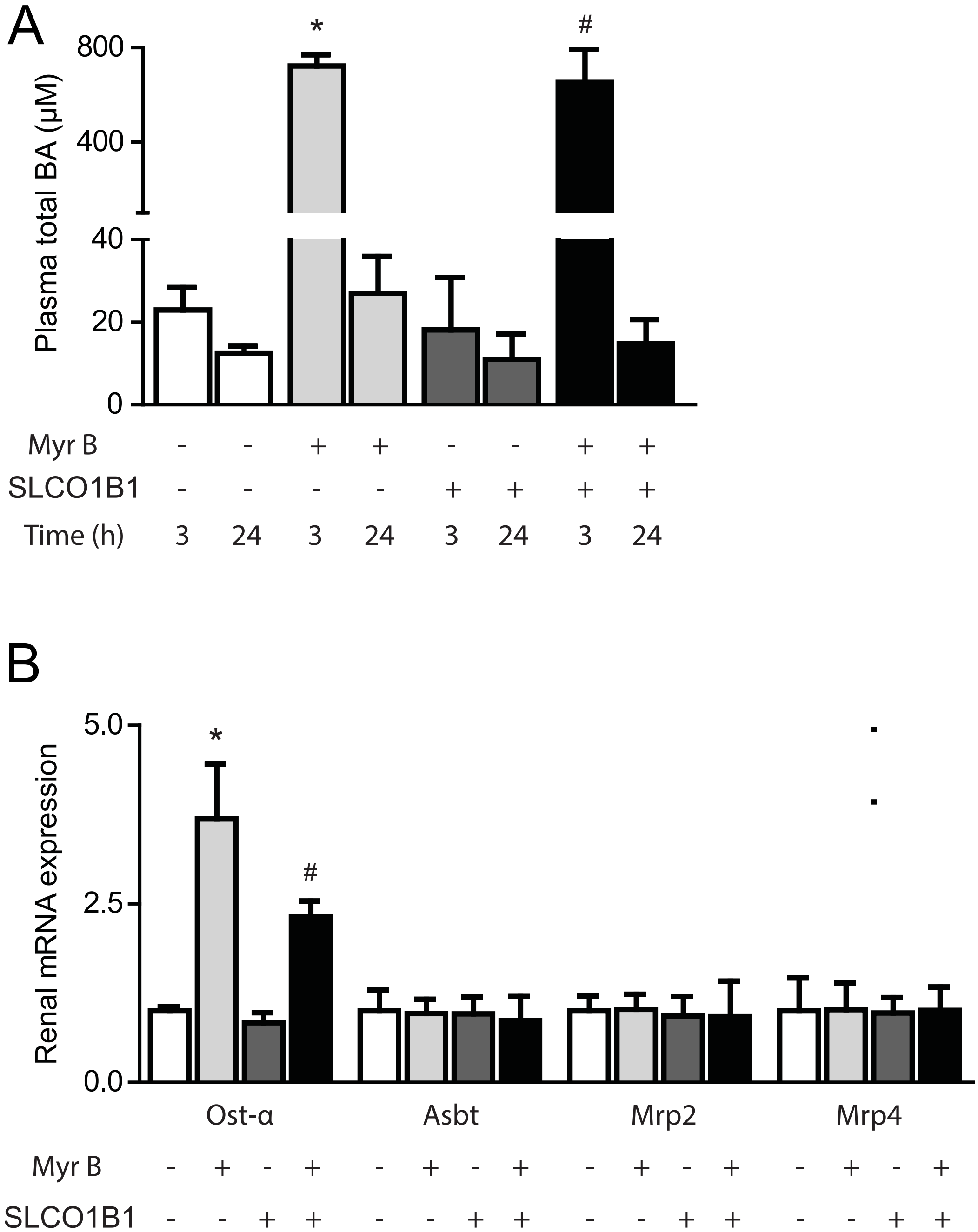
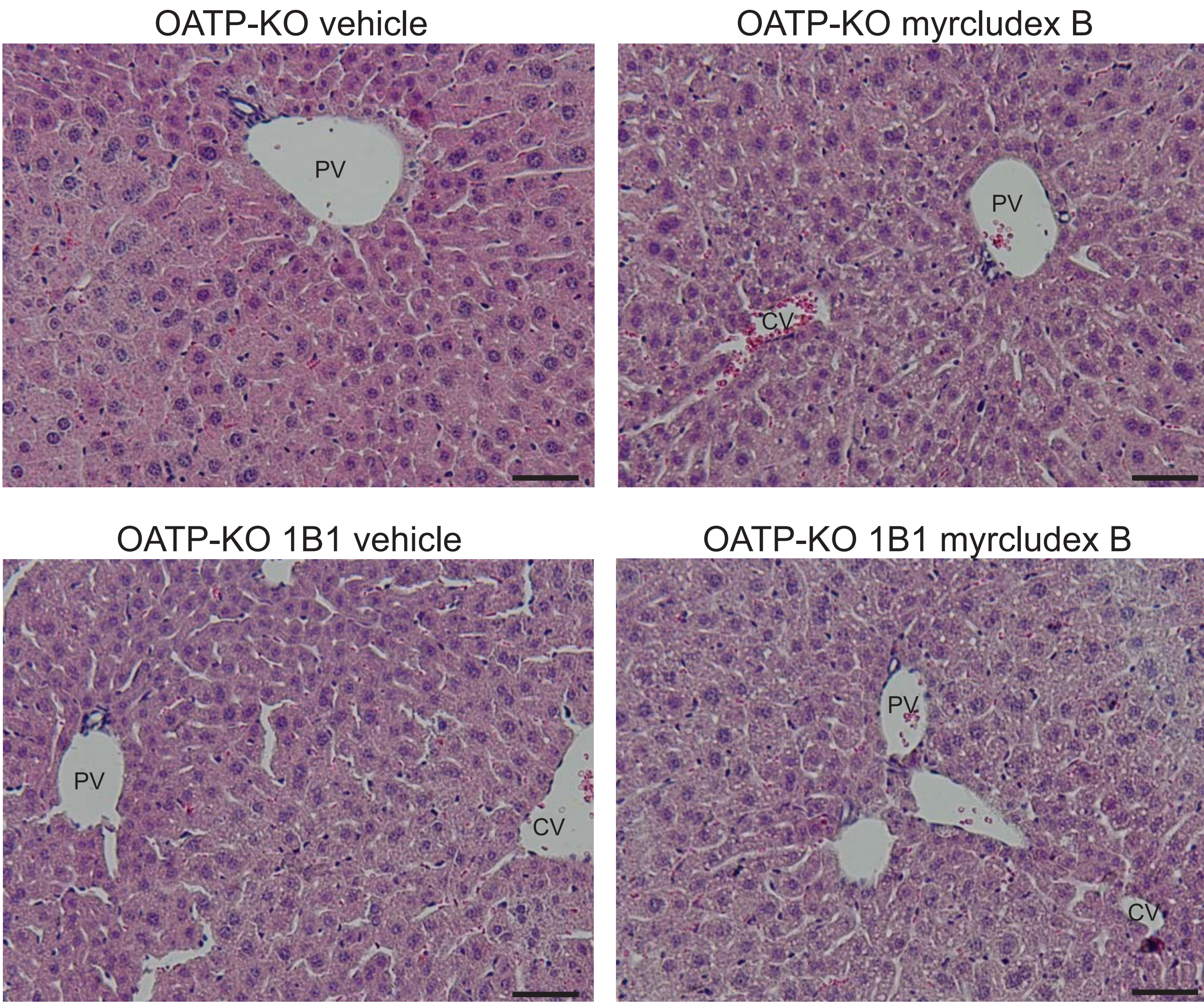
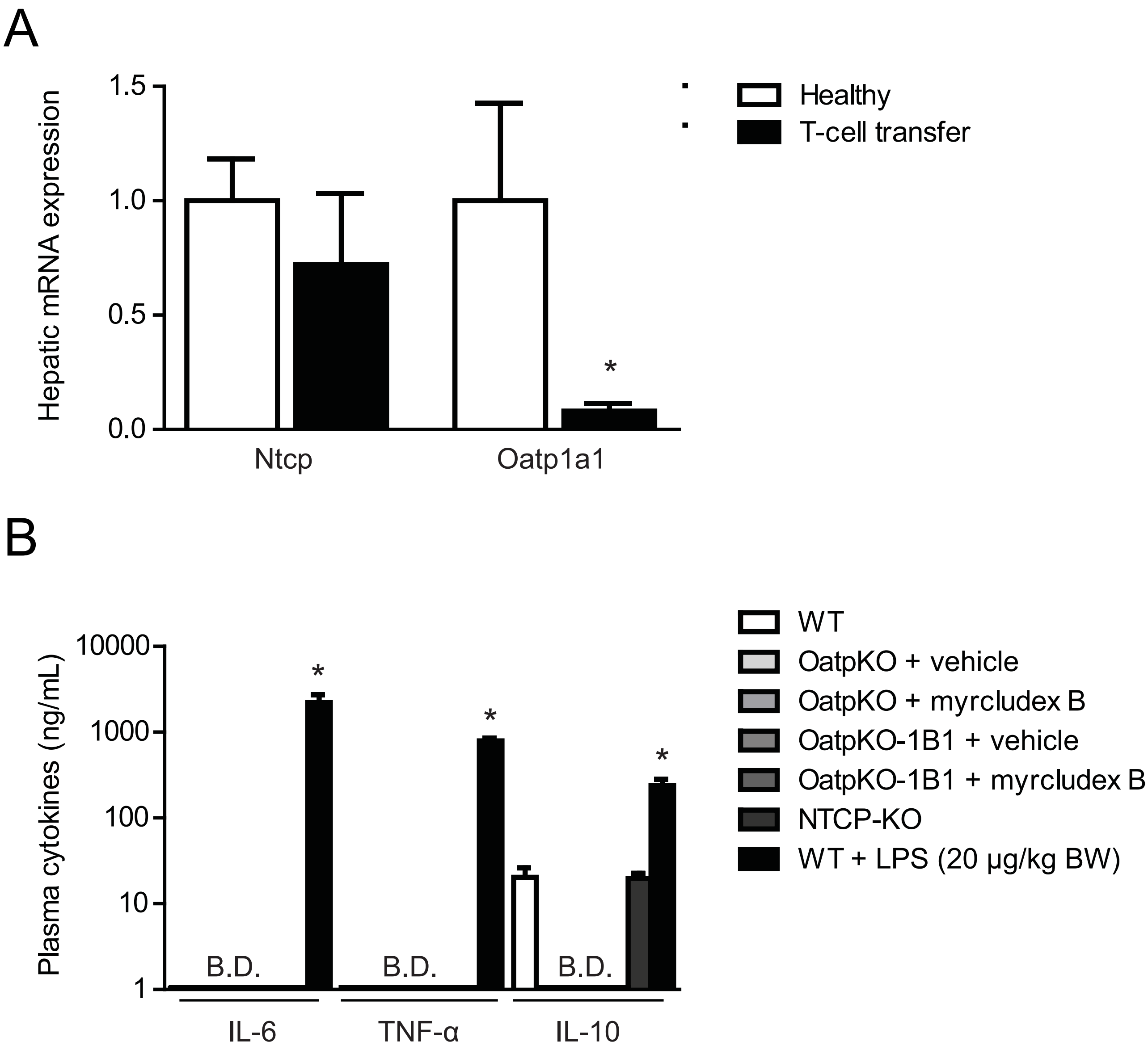
Supp. figure 1



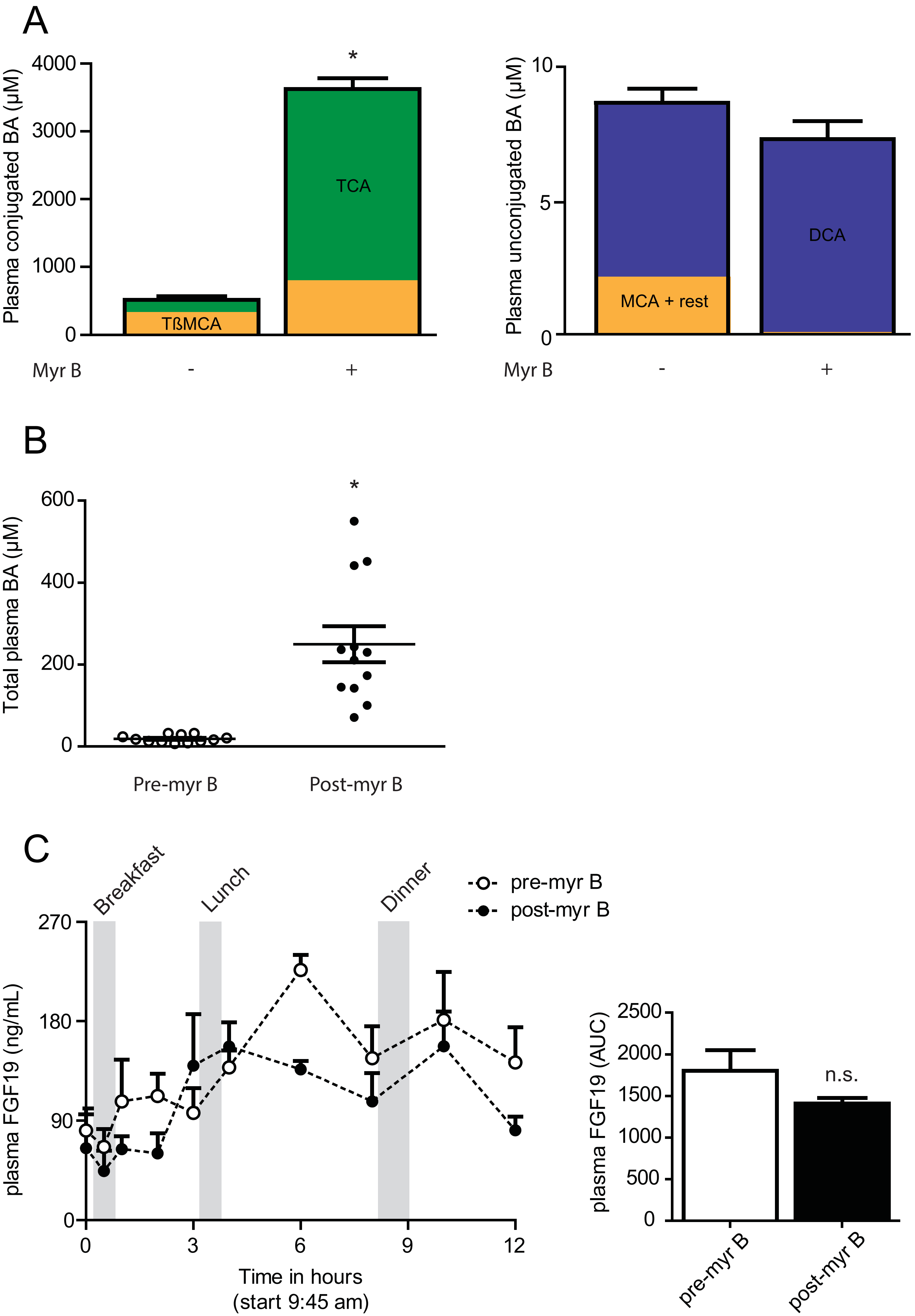
Supp. figure 2



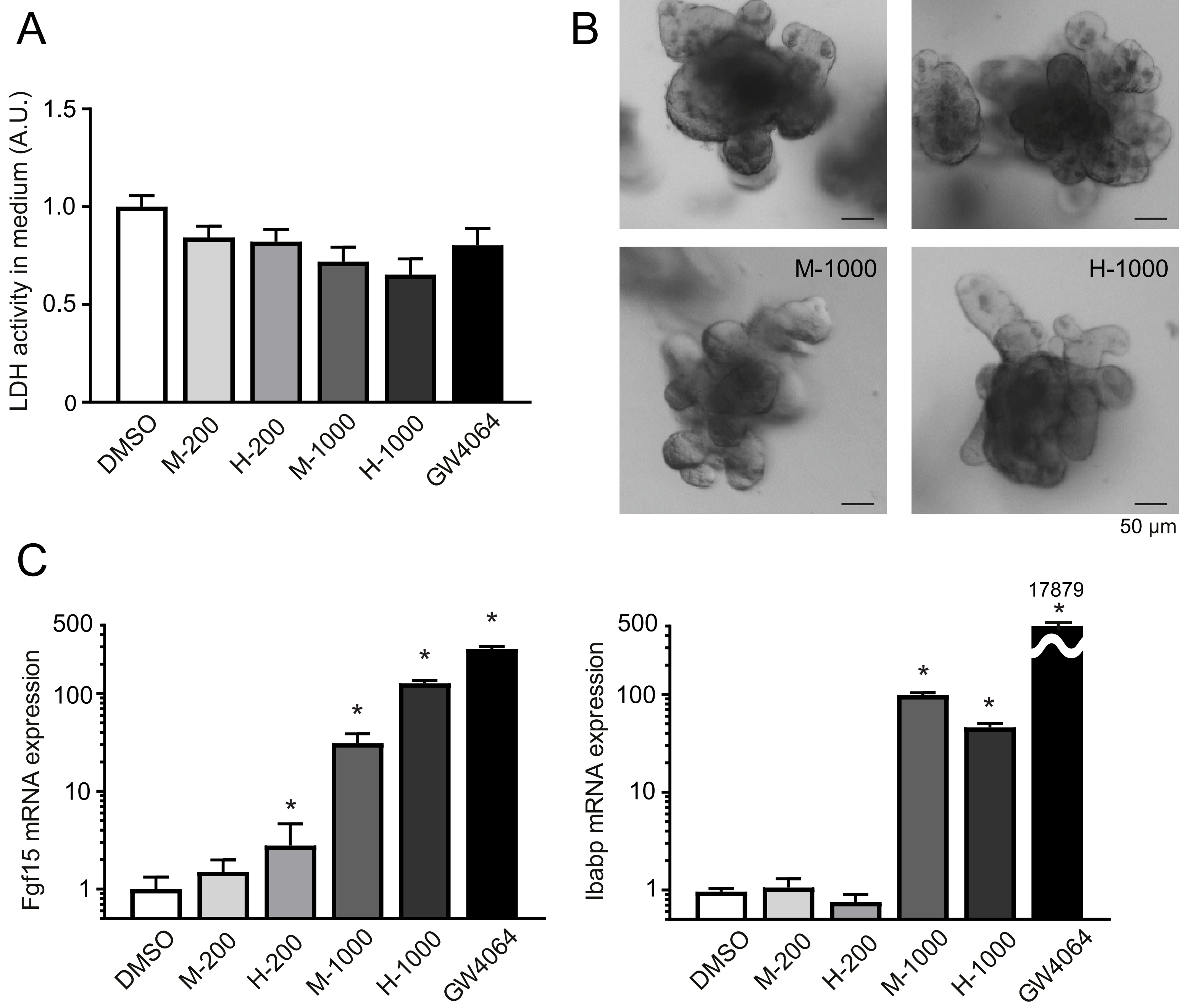
Supp. figure 3



Supp. figure 4



Supp. figure 5



Supp. figure 6