**Supplemental data 1**

*Chemicals and reagents*

MTX (100 mg/ml Emthexate PF) was from Pharmachemie (Haarlem, The Netherlands), [$^3$H]FEX was custom-made by GE Healthcare/Amersham Biosciences (UK), FEX, rifampicin, and ascorbate were from Sigma (St. Louis, USA), methoxyflurane (Metofane) from Medical Developments Australia (Springvale, Australia) and heparin (5,000 IE/ml) from Leo Pharma BV (Breda, The Netherlands). The Oatp antibodies M-13 (sc-18436) and K-17 (sc-47270) were from Santa Cruz Biotechnology, Inc (Santa Cruz, USA). $M_2$III-5 and $M_4$I-80 were kind gifts of Dr. G.L. Scheffer (Free University Hospital, Amsterdam, The Netherlands), $M_3$-18 was a kind gift of the group of Dr. P. Borst in our institute. BXP-53 was described before (*Proc Natl Acad Sci* **99**:15649-15654 (2002)).

*RNA isolation, cDNA synthesis and RT-PCR*

DNA isolation from mouse liver, kidney, and small intestine and subsequent cDNA synthesis and RT-PCR were performed as described (*Mol Pharmacol* **73**:1029-1036 (2008)). Specific primers (QIAGEN, Hilden, Germany) were used to detect expression levels of the following mouse genes: *Slco1a1, Slco1a4-6, Slco1b2, Slco2b1, Slc10a1, Slc22a1-3, Slc22a6-10, Slc22a12, Abcc2-4, Abcb1a, Abcb1b, Abcg2, Aox1, Aox3,* and *Ugt1a1*.

*Western blot analysis*

Isolation of crude membrane fractions from liver, kidney and small intestine, and Western blotting were performed as described previously (*Biochem Biophys Res Commun* **220**:848-852 (1996)). Equal protein loading was confirmed by Ponceau S staining of the membranes after transfer. Oatp1a4 and Oatp1b2 were detected with respectively OATP2 (M-13; sc-18436) (dilution 1:200) and OATP4 antibodies (K-17; sc-47270) (dilution 1:200). For the detection of Abcc2, Abcc3, Abcc4 and Abcg2 the primary antibodies $M_2$III-5 (dilution 1:1000), $M_3$-18 (dilution 1:25), $M_4$I-80 (dilution 1:400) and BXP-53 (dilution 1:1000) were used, respectively.

*Clinical-chemical analysis of plasma and urine and hematological analysis of blood*

Standard clinical-chemical analyses on EDTA plasma were performed on a Roche Hitachi 917 analyzer to determine levels of total and conjugated bilirubin, alkaline phosphatase, aspartate aminotransaminase, alanine aminotransaminase, γ-glutamyl transferase, lactate dehydrogenase, creatinine, urea, $Na^+$, $K^+$, $Ca^{2+}$, total protein, albumin, uric acid, cholesterol and triglyceride. Standard clinical-chemical analyses were also performed on urine (collected using Ruco Type M/1 stainless steel metabolic cages [Valkenswaard, The Netherlands]) to determine the levels of

creatinine, glucose, $Na^+$, $K^+$, $Ca^{2+}$, urea, uric acid and total protein. Hemoglobin, hematocrit, mean corpuscular volume, red and white blood cells, and platelets were analyzed in peripheral EDTA blood on a Beckman Coulter Ac·T Diff analyzer (Beckman Coulter, Fullerton, CA).

*Histological analysis*

Tissues were fixed in 4% phosphate-buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin according to standard procedures.

*Construction of targeting vectors for generation of Slco1a/1b$^{-/-}$ mice.* The mouse *Slco1a* and *-1b* genes were mapped using data from the ENSEMBL database (www.ensembl.org). Total RPCI-21 female (129S6/SvEvTac) mouse PAC library-derived DNA was used as a template for the construction of suitable gene targeting vectors. For both the centromeric and telomeric side of the *Slco1a/1b* cluster, targeting vectors were constructed allowing insertion of *loxP* sequences at the cluster flanks (i.e. *Slco1b2* and *Slco1a5*). For the telomeric side of the cluster, a targeting vector was made in which a 2.2-kb fragment containing exon 3 and 4 of *Slco1a5* (according to ENSEMBL release 52, corresponding to the two exons after the exon containing the start codon) was replaced by a 2-kb *Pgk*-neomycin cassette in reverse transcriptional orientation with *LoxP* sequences. PCR primers used for generation of the *Slco1a5* targeting construct were 5'-GCCTCTATCACATTGCCCTGTG-3' (forward) and 5'-GCTAGTGTATGCAATGGTGTC-3' (reverse). A 10.2-kb ApaI/SacI (located in vector polylinker) linearized DNA fragment was used for electroporation into ES cells. For the centromeric side of the *Slco1a/1b* cluster, a targeting vector was made in which a ~5.9-kb fragment containing exon 4 of *Slco1b2* (according to ENSEMBL release 52, corresponding to the first exon after the exon containing the start codon) was replaced by a ~5-kb *Pgk*-hygromycin cassette with *LoxP* sequences and a *Pgk*-thymidine kinase cassette, both in transcriptional orientation. PCR primers used for generation of the *Slco1b2* targeting construct were 5'-CAACCACTCTGGAAATCGGTCTG-3' (forward) and 5'-GGCTTCCACAGGATTGGCATAC-3' (reverse). A 12.9-kb PmeI/SfiI (located in vector polylinker) linearized DNA fragment was used for electroporation into targeted ES cells.

*Electroporation and selection for Slco-recombinant ES cells.* 129/Ola-derived E14 embryonic stem (ES) cells were cultured as described before (*Mol Cell Biol* **21**:5471-5477 (2001)). Electroporation with linearized DNA of *Slco1b2* and *Slco1a5* targeting vectors, or with circular DNA encoding Cre recombinase/puromycin selection genes, and subsequent selection were performed as described previously (*Mol Cell Biol* **21**:5471-5477 (2001)).

*PCR and Southern blot analysis of Slco-recombinant ES cells.* Correct homologous recombination of the *Slco1a5* targeting construct was confirmed by Southern blot analysis. Hybridization of XbaI-digested genomic DNA with the 3' *Slco1a5* probe (**Figure 1A**) yielded a wild-type band of 12.0 kb and a targeted band of 4.9 kb, and hybridization with the 5' *Slco1a5* probe (**Figure 1A**) resulted in a wild-type band of 12.0 kb and a targeted band of 5 kb. Absence of additional *Slco1a5* targeting construct integrations was confirmed with a neomycin-specific probe. An ES cell clone with correctly inserted *loxP*-neomycin into *Slco1a5*, and with the correct karyotype was electroporated with the *loxP*-hygromycin *Slco1b2* targeting construct. Correct homologous recombination of the *Slco1b2* targeting construct was confirmed by hybridization with the 3' *Slco1b2* probe (**Figure 1A**) yielding a wild-type band of 8.9 kb and a targeted band of 8.3 kb, and hybridization with the 5' *Slco1b2* probe (**Figure 1A**) resulted in a wild-type band of 10.9 kb and a targeted band of 9.3 kb. Absence of additional *Slco1b2* targeting construct integrations was confirmed with a hygromycin-specific probe. Clones with correctly integrated *loxP* sites at both flanks of the cluster were cotransfected with *Pgk-Cre* recombinase and *Pgk-puromycin* expression plasmids in order to excise the cluster. After selection with puromycin, surviving ES cell clones were screened for correct Cre recombinase-mediated excision of the *Slco1a/1b* cluster by PCR, using primers at the *Slco1b2* flank (5'-GCATTCTTGGCACTTGCC-3') and at the *Slco1a*5 flank (5'-CAGCACTTGTCCTGCAGG-3').

*Generation of Slco1a/1b$^{-/-}$ mice.* Chimeric mice were generated by micro-injection of 2 independently targeted ES clones with the *Slco1a/1b* cluster deleted (and with correct karyotype) into blastocysts derived from C57BL/6J mice. Subsequently, these blastocysts were implanted into the oviducts of pseudopregnant F1 fosters (C57BL/6J), which carried them to term. Cross-breeding of resulting *Slco1a/1b*$^{-/-}$ mice yielded 2 independent *Slco1a/1b*$^{-/-}$ mouse lines.

*PCR analysis of Slco1a/1b$^{-/-}$ mice. Slco1a/1b$^{-/-}$* founder lines were detected by PCR. DNA was extracted from ear snips or tail tips of mice. Forward 5'-GCATTCTTGGCACTTGCC-3' (F1) and reverse 5'-CAGCACTTGTCCTGCAGG-3' (R1) specific primers were used for the detection of the *Slco1a/1b*-deleted cluster allele, which resulted in a 388-bp band (**Figure 1A**). For the detection of the wild-type cluster allele, forward 5'-GCATAATGCAGGACATGAGG-3' (F2) and reverse 5'-CAGCACTTGTCCTGCAGG-3' (R1) specific primers were used, yielding a 543-bp band (**Figure 1A**).

*Southern blot analysis of Slco1a/1b$^{-/-}$ mice. Slco1a** and *Slco1b2* probes to demonstrate absence of *Slco1a/1b* genes in *Slco1a/1b*$^{-/-}$ mice by Southern blot analysis were generated by PCR from wild-type liver cDNA. Primers used for this PCR reaction were 5'-CCTCATTTCCTCATGGGC-

3' (forward) and 5'-GGATGCTGGTCAGGATATTC-3' (reverse) for *Slco1a** and 5'-CCTGACTGGTTTTCTATGG-3' (forward) and 5'-CTATGTGAGAGTCCACTGGG-3' (reverse) for the *Slco1b2* probe. Sequences of these probes were verified and revealed sequence identity of the *Slco1a** probe with *Slco1a* genes (and not with *Slco1b2*, *-1c1* or *-2b1*) and of the *Slco1b2* probe with *Slco1b2* (and not with *Slco1a*, *-1c1* or *-2b1*). Genomic DNA from tail tips of *Slco1a/1b*$^{-/-}$ founders was digested with Asp718 and probed with either *Slco1a** or *Slco1b2* probe.

*Detection of BMG, BDG, and UCB in plasma and urine.* The protocol was adapted from the method described by Spivak and Carey (*Biochem J* **225**:787-805 (1985)). In brief, urine and plasma samples were deproteinized with 2 volumes of MeOH after the addition of KOH (final concentration 5 µM). Following centrifugation for 2 minutes at 14000 rpm, the supernatant was injected. 100 µl was applied to a Pursuit C18, 5 µm, 10 cm HPLC column (Varian, Middelburg, The Netherlands). Starting eluent consisted of 50% MeOH/ 50% ammonium acetate (1%, pH 4.5), followed by a linear gradient to 100% MeOH in 20 minutes. BDG, BMG and UCB eluted from the column at retention times of 8, 12 and 22 min, respectively. Detection of bilirubin was performed at 450 nm. Quantification of BDG, BMG and UCB was done by using a calibration curve of UCB.

*Determination of total bile acid pool.* To determine the total bile acid pool, the protocol described by Pawlikowska et al (*Hum Mol Genet* **13**:881-892 (2004)) was slightly adapted. In brief, wild-type and *Slco1a/1b*$^{-/-}$ mice were i.v. injected with 0.5 µCi [$^{3}$H]taurocholate and after 5 hours bile was collected for 20 min by gall bladder cannulation. Total radioactivity was analyzed by liquid scintillation counting and total bile acids as described (*J Inherit Metab Dis* **22**:307-310 (1999)). The obtained specific radioactivity was used to calculate the total bile acid pool size from the total radioactivity that was injected.

**Supplemental data 2**

Overview of ΔCt values of the RT-PCR analysis to investigate expression of several endogenous uptake and efflux transporters in liver, kidney and small intestine of male wild-type and *Slco1a/1b*$^{-/-}$ mice (n = 3; each sample was assayed in duplicate). Part of these data are also shown in Figure 1C. Analysis of the results was done by comparative Ct method. Quantification of the target cDNAs in all samples was normalized against the endogenous control β-actin ($Ct_{target} - Ct_{\beta\text{-}actin} = \Delta Ct$). Accordingly, the lower the value, the higher the expression level.

**Liver**

| | **Wild-type** | ***Slco1a/1b*$^{-/-}$** |
|---|---|---|
| ***Slco1a1* (Oatp1a1)** | 0.76 ± 0.08 | 18.9 ± 2.65 [C] |
| ***Slco1a4* (Oatp1a4)** | 3.13 ± 0.06 | 18.0 ± 2.79 [C] |
| ***Slco1a5* (Oatp1a5)** | 15.8 ± 1.39 | 15.8 ± 0.90 |
| ***Slco1a6* (Oatp1a6)** | 16.9 ± 1.17 | 17.0 ± 0.99 |
| ***Slco1b2* (Oatp1b2)** | -0.09 ± 0.08 | 19.8 ± 2.24 [C] |
| ***Slco2b1* (Oatp2b1)** | 4.46 ± 0.19 | 4.39 ± 0.50 |
| ***Slc22a1* (Oct1)** | 3.48 ± 0.82 | 3.82 ± 0.42 |
| ***Slc22a3* (Oct3)** | 13.1 ± 1.25 | 13.6 ± 0.29 |
| ***Slc22a7* (Oat2)** | 12.5 ± 0.84 | 13.9 ± 1.29 |
| ***Slc10a1* (Ntcp)** | 0.99 ± 0.19 | 0.80 ± 0.52 |
| ***Abcb1a* (Mdr1a)** | 10.3 ± 0.17 | 11.6 ± 0.53 [A] |
| ***Abcb1b* (Mdr1b)** | 10.3 ± 0.69 | 9.58 ± 1.17 |
| ***Abcb11* (Bsep)** | 2.06 ± 0.24 | 2.52 ± 0.84 |
| ***Abcc2* (Mrp2)** | 3.26 ± 0.40 | 3.33 ± 0.74 |
| ***Abcc3* (Mrp3)** | 7.21 ± 0.72 | 7.17 ± 0.52 |
| ***Abcc4* (Mrp4)** | 10.9 ± 0.37 | 11.1 ± 0.70 |
| ***Abcg2* (Bcrp1)** | 5.83 ± 0.50 | 5.56 ± 0.78 |
| ***Aox1*** | 6.67 ± 0.72 | 6.18 ± 0.26 |
| ***Aox3*** | 2.17 ± 0.64 | 1.29 ± 0.68 |
| ***Ugt1a1*** | 1.49 ± 0.34 | 1.39 ± 0.53 |

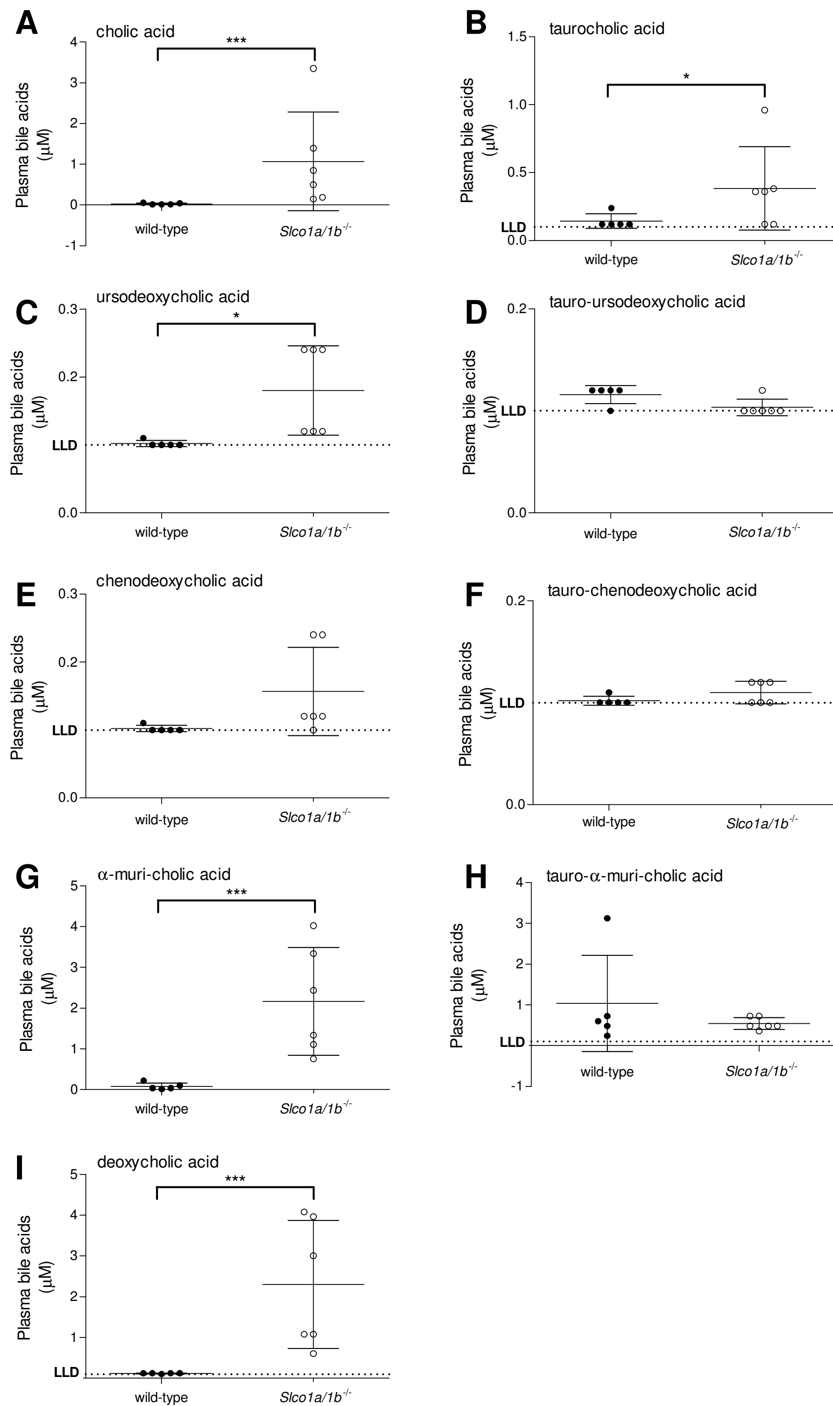
**Kidney**

| | **Wild-type** | ***Slco1a/1b$^{-/-}$*** |
|---|---|---|
| ***Slco1a1*** **(Oatp1a1)** | 3.26 ± 0.19 | 17.4 ± 1.97 $^{C}$ |
| ***Slco1a4*** **(Oatp1a4)** | 10.7 ± 0.31 | 22.9 ± 3.69 $^{B}$ |
| ***Slco1a5*** **(Oatp1a5)** | 20.0 ± 0.38 | 21.7 ± 1.25 |
| ***Slco1a6*** **(Oatp1a6)** | 2.10 ± 0.00 | 18.6 ± 0.87 $^{C}$ |
| ***Slco1b2*** **(Oatp1b2)** | 14.8 ± 0.77 | 25.5 ± 2.59 $^{B}$ |
| ***Slco2b1*** **(Oatp2b1)** | 7.02 ± 0.22 | 7.12 ± 0.33 |
| ***Slc22a1*** **(Oct1)** | 3.00 ± 0.35 | 2.86 ± 0.27 |
| ***Slc22a2*** **(Oct2)** | 4.10 ± 0.56 | 3.45 ± 0.38 |
| ***Slc22a3*** **(Oct3)** | 14.4 ± 0.41 | 14.2 ± 0.64 |
| ***Slc22a6*** **(Oat1)** | 0.94 ± 0.29 | 1.02 ± 0.18 |
| ***Slc22a7*** **(Oat2)** | 9.10 ± 0.27 | 8.85 ± 0.86 |
| ***Slc22a8*** **(Oat3)** | 2.90 ± 0.40 | 2.65 ± 0.09 |
| ***Slc22a9*** **(Oat5)** | 6.05 ± 0.26 | 5.30 ± 0.42 |
| ***Slc22a12*** **(Urat-1)** | 1.44 ± 0.33 | 1.17 ± 0.12 |
| ***Abcb1a*** **(Mdr1a)** | 9.88 ± 0.38 | 9.97 ± 0.33 |
| ***Abcb1b*** **(Mdr1b)** | 8.21 ± 0.42 | 6.51 ± 1.28 |
| ***Abcc2*** **(Mrp2)** | 4.38 ± 0.06 | 4.17 ± 0.12 |
| ***Abcc3*** **(Mrp3)** | 11.9 ± 0.44 | 12.2 ± 0.52 |
| ***Abcc4*** **(Mrp4)** | 6.67 ± 0.46 | 6.88 ± 0.38 |
| ***Abcg2*** **(Bcrp1)** | 3.14 ± 0.19 | 3.01 ± 0.26 |

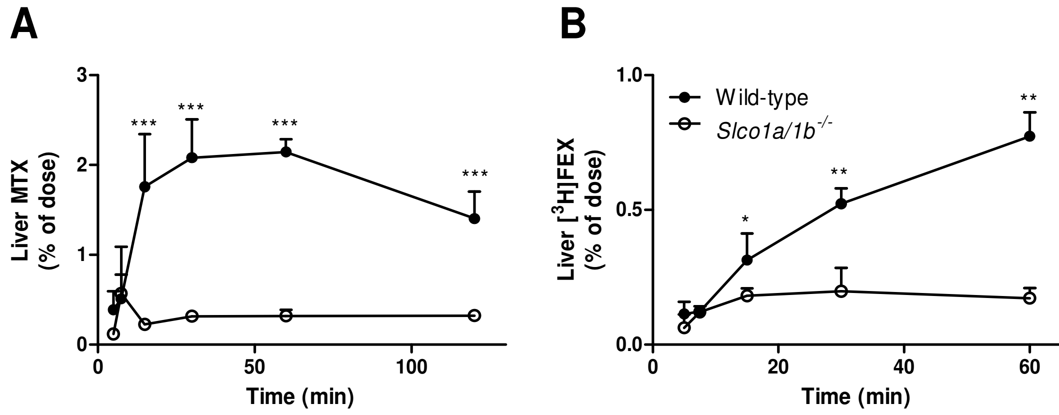
**Small intestine**

| | **Wild-type** | ***Slco1a/1b$^{-/-}$*** |
|---|---|---|
| ***Slco1a1*** **(Oatp1a1)** | 27.7 ± 0.3 | 28.0 ± 0.26 |
| ***Slco1a4*** **(Oatp1a4)** | 10.6 ± 1.56 | 21.9 ± 1.70 $^{B}$ |
| ***Slco1a5*** **(Oatp1a5)** | 20.0 ± 1.47 | 21.2 ± 1.70 |
| ***Slco1a6*** **(Oatp1a6)** | 18.8 ± 1.94 | 25.6 ± 1.26 $^{B}$ |
| ***Slco1b2*** **(Oatp1b2)** | 25.5 ± 1.73 | 24.7 ± 1.57 |
| ***Slco2b1*** **(Oatp2b1)** | 9.47 ± 0.30 | 9.09 ± 0.21 |
| ***Slc10a2*** **(Asbt)** | 13.9 ± 1.45 | 13.4 ± 0.98 |
| ***Slc22a1*** **(Oct1)** | 9.15 ± 0.51 | 8.76 ± 0.67 |
| ***Slc22a3*** **(Oct3)** | 15.4 ± 0.49 | 14.9 ± 0.57 |
| ***Abcb1a*** **(Mdr1a)** | 7.92 ± 0.09 | 8.02 ± 0.21 |
| ***Abcb1b*** **(Mdr1b)** | 14.4 ± 0.35 | 13.9 ± 0.54 |
| ***Abcc2*** **(Mrp2)** | 7.50 ± 0.44 | 7.30 ± 0.13 |
| ***Abcc3*** **(Mrp3)** | 11.7 ± 0.41 | 11.4 ± 0.32 |
| ***Abcc4*** **(Mrp4)** | 12.4 ± 0.27 | 12.4 ± 0.26 |
| ***Abcg2*** **(Bcrp1)** | 7.60 ± 0.28 | 7.34 ± 0.20 |
| ***Ostα*** | 8.14 ± 0.74 | 8.45 ± 1.02 |
| ***Ostβ*** | 7.63 ± 0.18 | 7.42 ± 0.79 |

Note: All data are presented as means ± S.D. (n = 3, male).
$^{A}P < 0.05$; $^{B}P < 0.01$; $^{C}P < 0.001$ when compared with wild-type.




**Supplemental data 3.** Levels of individual bile acids in plasma of male wild-type and *Slco1a/1b*$^{-/-}$ mice (**A-I**). Data are presented as means ± S.D. (n = 5-7; $^{*}$, $P < 0.05$; $^{**}$, $P < 0.01$; $^{***}$, $P < 0.001$ when compared with wild-type). The logarithm of the data was determined prior to assess the statistical significance of the data by two-sided unpaired Student's *t* test. LLD, lower limit of detection. Detection limit was 0.1 μM for all bile acids, except for cholic acid and α-muri-cholic acid, for which it was 0.01 μM.




**Supplemental data 4.** Liver accumulation (% of dose) after oral dosing of MTX (10 mg/kg) (**A**) and [$^3$H]FEX (1 mg/kg) (**B**) to wild-type and *Slco1a/1b*$^{-/-}$ mice. All data are presented as means ± S.D. (n = 4-8; $^{*}$, $P < 0.05$; $^{**}$, $P < 0.01$; $^{***}$, $P < 0.001$ when compared with wild-type).

**Supplemental data 5**

Plasma AUCs of MTX and [$^3$H]FEX in the portal vein ($AUC_{PV}$) and systemic circulation ($AUC_{SC}$) after oral administration of, respectively, MTX (10 mg/kg) and FEX (1 mg/kg).

| | **MTX** | | **[$^3$H]FEX** | |
|---|---|---|---|---|
| | $AUC_{PV}$ | $AUC_{SC}$ | $AUC_{PV}$ | $AUC_{SC}$ |
| (A) Wild-type | 49.6 ± 3.1 | 14.4 ± 0.9 | 2.28 ± 0.21 | 0.41 ± 0.02 |
| (B) *Slco1a/1b*$^{-/-}$ | 94.1 ± 4.6$^B$ | 54.5 ± 2.9$^A$ | 3.32 ± 0.21$^A$ | 1.90 ± 0.22$^B$ |
| Difference (B) – (A) | ~44.5 | ~40.1 | ~1.0 | ~1.5 |

Note: AUCs are represented as min · µg/ml. All values represent means ± S.E. (n = 4-8).
$^A P < 0.05$; $^B P < 0.01$ when compared with wild-type.