Supplementary 1 - Materials and methods

Chemicals and reagents. Rifampicin [4-methylpiperazine-^{3}H] (46.6 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA, USA). Fetal bovine serum (FBS) was purchased from Greiner Bio-one (Sollingen, Germany). Hank's Balanced Salt Solution (HBSS) and GlutaMAX high glucose Dulbecco's modified Eagle's Medium (DMEM) were purchased from Life Technologies Invitrogen (Carlsbad, CA, USA). Rifampicin, bovine serum albumin fraction V (BSA), sodium butyrate, sodium hydroxide (NaOH) and HEPES were purchased from Merck (Darmstadt, Germany). BD BioCoat poly-D-lysine-coated 24-well tissue culture plates were ordered from Corning (Corning, NY, USA). Protein concentrations were determined with an assay kit from Bio-Rad Laboratories (Hercules, CA, USA).

Cell line maintenance. Human embryonic kidney 293 (HEK293) cells were maintained at 37°C with 5% CO_2 in DMEM culture medium containing 10% (*v/v*) FBS. After growing to 70% confluence, ~300,000 cells in DMEM were seeded into poly-D-lysine-coated 24-well tissue culture plates in a volume of 400 µl per well. Twenty-four hours after seeding, cells were transduced with 60 µl recombinant baculovirus to express the transporter of interest (OATP1B1 WT or variant) or the vector control (background) expressing enhanced yellow fluorescent protein (EYFP). To enhance protein expression, 140 µl DMEM containing sodium butyrate (3 mM final concentration) was added to each well. Uptake experiments were performed 3 days after transduction.

Cloning of wild type and polymorphic OATP1B1. The *SLCO1B1* transporter (wild type OATP1B1; accession number NM_006446.3) as well as OATP1B1*1b, *4 and *15 in a pENTR4 vector were ordered from GenScript (Piscataway, NJ, USA). OATP1B1*5 was kindly provided by Dr. H. Kidron (University of Helsinki, Helsinki, Finland). To produce the recombinant baculovirus, genes of interest were cloned downstream a cytomegalovirus (CMV) promoter into a baculovirus for protein expression in mammalian cells as described previously by El-Sheikh *et al*. (2007) (1).

Cellular transport assays. On the day of experiment, the culture medium was removed and cells were washed with 37°C buffer (Hank's Balanced Salt Solution with 10 mM HEPES (HBSS-HEPES), pH 7.4). Uptake was initiated by replacing the wash-buffer with fresh transport buffer (HBSS-HEPES, pH 7.4, at 37°C) supplemented with the substrate of interest. First, [^{3}H]-Estradiol 17 β-D-Glucuronide ($E_2$17βG; 18.9 nM) was used to confirm functional OATP1B1-mediated uptake for wild type, *1b, *4 and *5 (**Supplementary Figure 1**). Subsequently,time-dependent uptake of [^{3}H]-rifampicin (13.7 nM) was assessed by performing transport assays at incubation times of 30 seconds and 1, 2, and 5 minutes (**Supplementary Figure 2**). Concentration-dependent uptake was determined by performing uptake assays at a concentration range (13.7 nM [^{3}H]-rifampicin supplemented with 0.015, 0.05, 0.15, 0.5, 1.5, 3 and 5 µM rifampicin) after 1 minute of incubation at 37°C. To terminate the reaction,

the transport buffer and replaced with 400 µl ice-cold HBSS-HEPES buffer containing 0.5% BSA (*m/v*), after which the cells were washed twice with ice-cold HBSS-HEPES (pH 7.4). To measure the intracellular [^{3}H]-rifampicin concentrations, cells were solubilized in 200 µl 1M NaOH and radioactivity was measured in aliquots of the lysate after adding 4 ml Opti-fluor scintillation fluid (PerkinElmer, Waltham, MA, USA) using the Hidex automatic TDCR liquid scintillation counter (Turku, Finland). Transporter dependent uptake was calculated by subtracting the background (vector control cells containing EYFP) from the values measured in cells expressing the OATP1B1 transporter.

Membrane fraction preparation and Western blotting. The OATP1B1 expression in OATP1B1 WT, *1b, *4, *5 and *15 of the membrane fractions of HEK293 cells was detected by Western blot. Cells were cultured and transduced, in the same conditions as for the transport experiment, in T175 flasks and harvested 3 days posttransduction by centrifugation at 3000*g* for 15 minutes. The pellets were resuspended in ice-cold homogenization buffer (10 mM TRIS and 250 mM Sucrose, pH 7.4 with HEPES (TS buffer)) supplemented with protease inhibitors (100 µM phenylmethylsulfonyl fluoride, 5 µg/ml aprotinin, 5 µg/ml leupeptin, 1 µg/ml pepstatin, and 10 µM E-64) and lysed with a microfluidizer. Lysed cells were spun for 20 minutes at 4000*g* at 4°C, subsequently, the supernatant was spun for 90 minutes at 25,000*g* at 4°C. The pellets were resuspended in TS buffer and samples were homogenized by syringing 10 times with a 27G x ½ needle. Protein amount was measured using the protein determination assay kit from Bio-Rad Laboratories, measured using the Benchmark Plus plate reader (595 nm) and all samples were diluted to a concentration of 2.5 mg/ml.

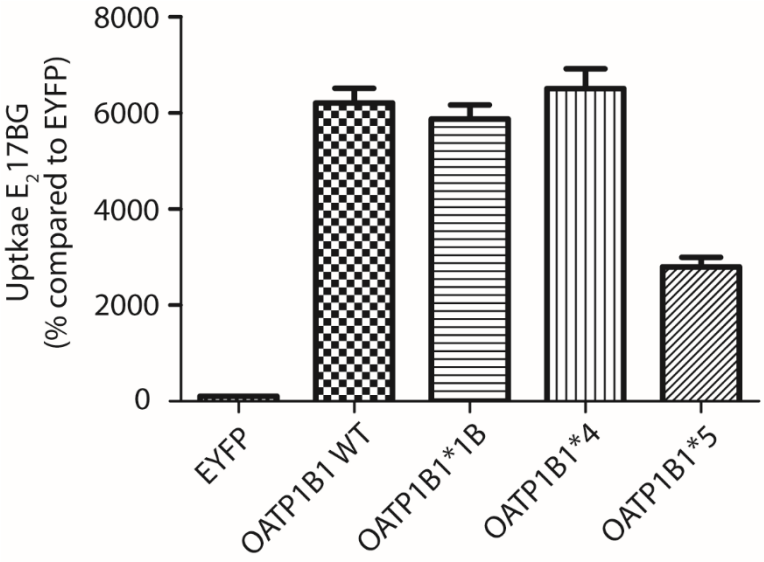
Protein samples (25 µg) were subjected to SDS-PAGE (TGX Stain-Free™ Precast Gels (Bio-Rad Laboratories), 4% to 20%) and electrophoretically transferred to a nitrocellulose membrane (Life Technologies Invitrogen). Immunoblots were probed with rabbit polyclonal anti-OATP2 antibody (diluted 1:1000) which was a kind gift of prof. dr. B. Stieger (University Hospital Zurich, Zurich, Switzerland). After incubation with AlexaFluor 680 goat anti-rabbit IgG antibody secondary antibody (diluted 1:10,000, Life Technologies Invitrogen), signal was detected with the ChemiDoc MP Imaging System (Bio-Rad Laboratories). Quantification was performed using ImageLab Software (Bio-Rad Laboratories) with global background subtraction.Total protein staining (Ponceau) confirmed equal loading across the lanes.

Statistical analysis. Intrinsic clearance was evaluated with linear regression analysis using Graphpad Prism Version 5.3 (GraphPad Software Inc, San Diego, CA, USA) per experiment (i.e. three independent experiments, by using three different virus batches each in duplicate). After normalization to the wild type intrinsic clearance and expression of OATP1B1 protein per experiment (as percentage of the WT), four one-sample t-tests were performed to compare each experimental

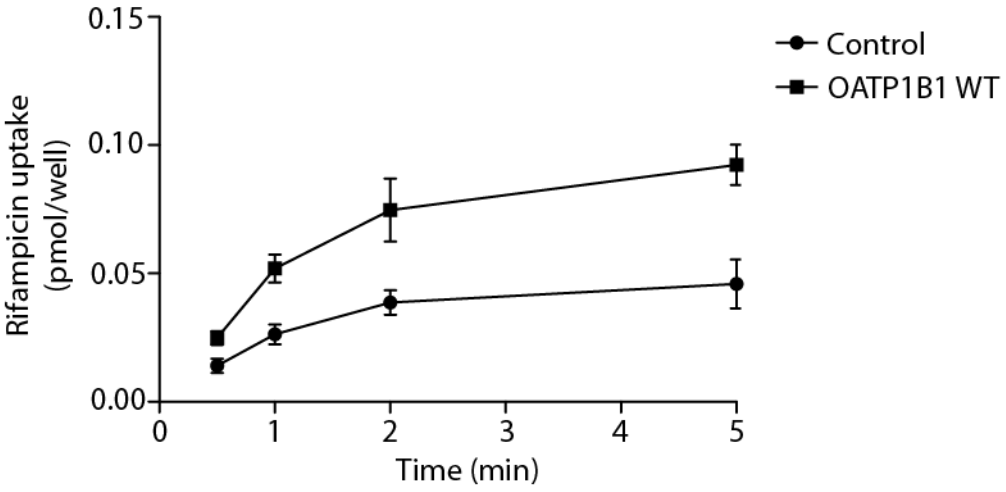
group (*1b, *4, *5 and *15) with the wild type group (100%) using SPSS version 25 (IBM Corp., Armonk, NY, USA). The $p<0.05$ was considered as statistically significant.

References

1. El-Sheikh AA, van den Heuvel JJ, Koenderink JB, Russel FG. 2007. Interaction of nonsteroidal anti-inflammatory drugs with multidrug resistance protein (MRP) 2/ABCC2- and MRP4/ABCC4-mediated methotrexate transport. J Pharmacol Exp Ther 320:229-35.



Supplementary Figure 1| Confirmation of functional OATP1B1-mediated uptake with the model substrate Estradiol 17 β-D-Glucuronide by OATP1B1 WT, *1b, *4 and *5, resulting in equal activity of *1b and *4 and a reduced activity of *5 (residual activity of 44% compared to WT). The x-axis represent the different OATP1B1 variants, the y-axis the uptake of $E_2$17βG as a percentage compared to the vector control EYFP (100%). Bars represent mean with standard error the mean of one experiment in triplicate (i.e. three independent virus batches)



Supplementary Figure 2| Time curve of [^{3}H]-rifampicin uptake in vector control (EYFP) and OATP1B1 WT expressing cells. Data were pooled from three independent experiments and are presented as presented as means ± SD. Optimal incubation time was determined to be 1 minute.