*Supporting documents*

# Solute Carrier Organic Anion Transporter Family Member 3A1 is a Bile Acid Efflux Transporter in Cholestasis

**Short Title: Function of OATP3A1 in bile acid efflux**

Qiong Pan[1]*, Xiaoxun Zhang[1]*, Liangjun Zhang[1]*, Ying Cheng[1], Nan Zhao[1], Fengju Li[1], Xueqian Zhou[1], Sheng Chen[2], Jianwei Li[3], Senlin Xu[4], [5]Dingde Huang, Yue Chen[6], Lihua Li[7], Huaizhi Wang[3], Wensheng Chen[1], Shi-ying Cai[8], James L. Boyer[8] and Jin Chai[1#]

[1]Cholestatic Liver Diseases Center and Department of Gastroenterology, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

[2]Department of Pediatrics, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

[3]Institute of Hepatobiliary Surgery, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

[4]Department of Pathology, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

[5]Department of Nuclear Medicine, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

[6]Department of Nuclear Medicine, the Affiliated Hospital of Southwest Medical University, Luzhou 646000, China

[7]Department of Cell Biology, Jinzhou Medical University, Liaoning 121001, China

[8]Department of Internal Medicine and Liver Center, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, USA

*These authors contributed equally to this study.

**Contact Information:**

#Jin Chai, M.D., Ph.D., Director of Cholestatic Liver Diseases Center, Department of Gastroenterology, Southwest Hospital, Third Military Medical University, Chongqing, 400038, China. Tel: 86-23-68765191; Fax: 86-23-65410853; E-mail: jin.chai@cldcsw.org. Chai' research team website: www.cldcsw.org.

## Suppl. Materials and Methods

### *Chemicals and recombinant cytokines*

Bile acids, including chenodeoxycholic acid (CDCA), taurochenodeoxycholate acid (TCDCA), glycochenodexycholate acid (GCDCA), cholic acid (CA), taurocholic acid (TCA), glycocholate acid (GCA), deoxycholic acid (DCA), taurodeoxycholate acid (TDCA) and tauroursodeoxycholate acid (TUDCA), and inhibitors (PD98059 and BAY 11-7082) were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). 7alpha-hydroxy-4-cholesten-3-one (or 7-alpha-C4) standard samples for LC-MS/MS analysis were purchased from Toronto Research Chemicals Inc (North York, Canada). Tritiated [$^{3}$H]-taurocholate and [$^{3}$H]-prostaglandin E2 (1 mCi/mL) were purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA, USA). Thyroxine (T4) labeling with [$^{125}$I] was performed by the Nuclear Medicine at the Southwest Hospital affiliated to the Third Military Medical University (Chongqing, China). Human recombinant FGF19, tumor necrosis factor (TNF) α,

interleukin (IL)-6, IL-8 and interferon-γ-inducible protein (IP)-10 were purchased from Peprotech (Rocky Hill, NJ, USA), with endotoxin levels less than < 0.1 ng/μg of protein (< 1 EU/μg) as provided by the manufacturer. These cytokines were dissolved in PBS containing 0.1% bovine serum albumin (BSA), aliquoted, and stored at -80°C. The endotoxin levels of BSA (Equitech-Bio, Kerrville, Texas) were also < 1 EU/μg, according to the manufacturer's information.

***Generation and verification of Slco3a1 knockout mice***

The *Slco3a1*-knockout mouse model was developed by Shanghai Model Organisms Center Inc. (Shanghai, China). In brief, Cas9 mRNA was transcribed *in vitro* with mMESSAGE mMACHINE T7 Ultra Kit (Ambion, TX, USA) according to the manufacturer's instructions. Two sgRNAs targeting exon2 of the *Slco3a1* gene were designed using the online designer (http://crispr.mit.edu/). The target sequences of two sgRNAs were 5'-GGAGGTTGAACCTGCGTTCCAGG-3' and 5'-GGGCAC CGACCGCGCCTCATCGG-3'. The sgRNAs were transcribed *in vitro* using the MEGAshortscript Kit (ThermoFisher, USA) and subsequently purified using MEGAclearTM Kit (Ambion, Life Technologies). Cas9 mRNA and sgRNAs were co-injected into zygotes of C57BL/6J mouse by microinjection (Suppl. Fig.1A). F0 mice were genotyped by PCR, using primer pairs: forward: 5'-CCTTTTAATAG CGTATTGCCC-3'; reverse: 5'-AACTCTCCACCAGCTCTT GGT-3'. The genotype of positive F0 mice were confirmed by sequencing. The positive F0 mice with Oatp3a1 protein frame shift were crossed with C57BL/6J mice to obtain F1

heterozygous *Slco3a1*-knockout mice. The genotype of F1 mice was identified by PCR and confirmed by sequencing as F0 generation mice. F1 mice with a 127bp deletion in the exon2 were intercrossed to obtain the homozygous *Slco3a1*-knockout mice. This deletion in exon2 resulted in a frameshift mutation of *Slco3a1* and inactivated the *Slco3a1* gene. The generation of homozygous *Oap3a1* knockout mice was identified by PCR using the primer set P1 (5'-CTAGCAGGGCTACAGT GCTTACAA-3') / P2 (5'- CCCATTGGTGGCACAGAC ATCG - 3') with a 970bp PCR product (Suppl. Fig.1B). Finally, *Slco3a1* KO mice were further confirmed using western blotting and immunofluorescent analysis (Suppl. Fig.1C&D). *Slco3a1*-KO mice appear normal.

***LC-MS/MS analysis of bile acids and 7-alpha-C4 in mouse liver tissues extracts***

HPLC/MS were carried out on a UPLC-LTQ/Orbitrap (Waters Corp., Milford, MA, USA). In the positive ESI mode, cholestatic mouse liver extract from 1% CA-fed WT mice (n=8) or *Slco3a1*-KO mice (n=7), were loaded on Waters BEH C8 column (100 mm × 2.1 mm, 1.7 μm). Water and acetonitrile with 0.1% acetic acid were used as eluent A and eluent B, respectively. The gradient started with 10% B for 1.0 min, then linearly changed to 40% B over 4.0 min, and to 100% B over the next 12 min, maintaining for 5 min. Then, eluent was changed back to 10% B over 2.9 min. In the negative ESI mode, the reaction sample was loaded on an ACQUITY UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 μm). Water and 95% acetic acid with 6.5mM $NH_4HCO_3$ were used as eluent A and eluent B in HPLC, respectively. The gradient

started with 100% eluent A for 1.0 min, changing to 40% eluent B over the next 13.0 min and then to 100% eluent B over an additional 13.0 min. The composition was held constant at 100% eluent B for a further 5 min, after which the solvent composition was changed back to 5% eluent B over 2.9 min. For MS conditions, electrospray ionization mode was applied by a TurboIonSpray inlet operating at 350°C in positive and negative ESI modes in separate experiments. TurboIonSpray Voltage was set at 4500V, curtain gas at 45 psi, auxiliary gases at 8 psi, and capillary voltage at 49V (using the positive detection mode and scanning between 50 and 900 m/z for MS analysis). In addition, levels of hepatic 7-alpha-C4 (7alpha-hydroxy-4-cholesten-3-one, 7-alpha-C4) in mice were determined by Shanghai Omicspro Biotech Company (Shanghai, China) as described previously [17, Suppl. Ref1].

***Plasmid construction, transfection, and generation of stably transfected cell lines***

Human hepatoma PLC/PRF/5 cells (ATCC, Manassas, VA) were kindly provided by Prof. Cheng Qian (Southwest Cancer Center, Southwest Hospital, Third Military Medical University, Chongqing, China) [Suppl. Ref.2]. MDCK cell lines were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The eGFP-*OATP3A1* and DsRed2-*ASBT* (apical sodium-dependent bile acid transporter) plasmids were generated by GENCHEM (Shanghai, China). The pcDNA3.1-*SP1* vector was used previously [Suppl. Ref.3]. The pcDNA3.1-*NFκBp65* and *p50* vectors were kindly provided by Dr. Xinshou Ouyang (Yale University

School of Medicine). G418 (750 μg/mL) or puromycin (2 μg/mL) were used to select stably transfected PLC/PRF/5 and MDCK cells.

***SLCO3A1* promoter luciferase reporter assays**

The pGL3-basic vector containing the human *SLCO3A1* gene proximal promoter (-5000 to +25) and its truncated forms (-3689, -3478, -2578, -643, -427, and -183 to +25) were produced using their primer pairs (Table.S3). The key motifs of potential transcription factor response elements in the pGL3-3478/+25 and pGL3-427/+25 constructs were mutated, generating the pGL3-*SLCO3A1*-3478MUT and pGL3-*SLCO3A1*-427MUT constructs (Suppl.Fig.7A and Table.S3). The promoter luciferase reporter constructs (100 ng) were transiently transfected into PLC5/PRF/5 cells along with *SP1* (100 ng) or *NFκB p65/p50* (50 ng/50 ng) and phRL-CMV (1.5 ng) as the transfection control using Fugene HD transfection reagent (Promega Corp, WI, USA). Following transfection (24 hr), the cells were treated with FGF19 (100 ng/mL) for 12 h. The treated cells were lysed using 1X passive lysis buffer and the luciferase activity was measured using the Dual Luciferase Assay kit (Promega Corp, WI, USA).

***OATP3A1-farnesoid X receptor (FXR/NR1H4)-ileal bile acid-binding protein (I-BABP) luciferase reporter assay for bile salt transport, bile acid uptake, and trans-cellular transport of [$^3$H] taurocholate assays***

To investigate the functional role of OATP3A1 in hepatocytes, we initially selected four stably transfected PLC/PRF/5 (or MDCK) cell lines (-*CTR*, -*ASBT*, -*OATP3A1*,

and -*ASBT* plus *OATP3A1*) in which the protein level of *ASBT* or *OATP3A1* in single-plasmid cells was similar to the protein level in *ASBT* and *OATP3A1* in double-plasmid cells (Suppl. Figs.2A&B and data not shown). The phRL-CMV, pCMX-human *FXR* (farnesoid X receptor), pCMX-human *RXRα* (retinoid X receptor alpha), and pGL3-human *I-BABP* (ileal bile acid-binding protein) constructs were used for the OATP3A1-FXR-*I-BABP* luciferase reporter assay for bile salt transport in the above four stably transfected PLC/PRF/5 cell lines as described previously [19]. For the bile acid uptake assay, these four stably transfected cell lines were maintained in a T-75 cell culture flask. After the cell density reached ~90%, cells were treated with the unconjugated bile acid CDCA (100 μM) and the conjugated bile acids GCA or TCA (100 μM) for 12 hr. The cells were quickly washed with cold PBS three times, and collected for ultrasonication. The cell lysates were used to determine bile acid concentration. The obtained data were normalized to the relative sample protein concentrations that were determined by a BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA). In addition, uptake or trans-cellular transport of [$^{3}$H]-taurocholate (TCA), [$^{3}$H]-prostaglandin E2 (PGE2), or [$^{125}$I] labeling thyroxine (T4) in the stably transfected MDCK or PLC/PRF/5 cell lines (-*CTR*, -*ASBT*, -*OATP3A1* and -*ASBT* plus *OATP3A1*) were performed as reported previously [3, 4, 20]. The measurements of radioactive substrates in these cell lines were performed by the Department of Nuclear Medicine at the Southwest Hospital affiliated to the Third Military Medical University (Chongqing, China) and the Affiliated Hospital of Southwest Medical University (Luzhou, China). Although human OATP3A1 has two isoforms

(*OATP3A1*_v1 and v2), both exerted similar functions (Suppl.Fig.3E). Therefore, the long variant *OATP3A1*_v2 expression construct, named *OATP3A1*, was chosen in this study.

***RNA extraction, reverse transcription, and quantitative real-time polymerase chain reaction***

Total RNA was extracted from tissues or cultured cells using TRIzol® reagent (Invitrogen; San Diego, CA), and then purified using RNeasy® MinElute Cleanup Kit (Cat#74204, Qiagen Co., Hilden, Germany). The cDNA was prepared using AffinityScript Multi Temperature cDNA Synthesis Kit (Cat#200436, Agilent Technologies, Palo Alto, CA, USA). Real-time quantitative polymerase chain reaction (qPCR) was performed in a Bio-Rad CFX96 real-time system machine (Bio-Rad, Hercules, CA) using a LightCycler® 480 Probes Master (Roche Diagnostics, IN, USA) or a SYBR® premix Ex Taq™ II kit (Cat#RR820A, Takara Biotechnology, Tokyo, Japan) [22, Suppl. Ref4]. The TaqMan probes (Life Technologies Co., CA, USA) and SYBR primers used in this study are listed in Table S4. Based on the manufacturer's information, the commercially available OATP3A1 primer/probe (Cat#: Hs00203184_m1) had the best coverage for human *SLCO3A1* transcript variants, including splice variants 1 and 2. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as reference gene for normalizing data.

***Western blot analysis***

Total liver tissues and cells lysates were prepared as previously described [22, Suppl. Ref4]. Cell surface protein biotinylation and nuclear extraction were performed using the commercial kits from Thermo Scientific (Waltham, MA, USA) according to the manufacturer's instructions [22, Suppl.Ref4]. Protein samples were resolved using SDS-PAGE and transferred to PVDF membranes (0.22 μm). The sources of primary antibodies and their dilutions are listed in Table S5. To ensure high specificity of the OATP3A1 antibody used in this study, we chose an affinity purified rabbit polyclonal antibody from Sigma-Aldrich (Cat#: SAB2102229), mainly because it was raised against a region without sharing any identity to that of other OATPs family members (Suppl.Fig.11). The specificity was further determined by the western blotting and immunofluorescent analysis in the 1% CA-fed *Slco3a1*-KO mice (Suppl.Fig.1C&D). In addition, the OATP3A1 antibody from Sigma-Aldrich was raised against amino acids 359-410 of human OATP3A1 origin, a region shared by both isoform 1 and 2, and therefore has the capability to determine human OATP3A1 isoforms 1 and 2.

***Chromatin immunoprecipitation assays***

Chromatin immunoprecipitation (ChIP) assays were performed using a commercial ChIP Assay Kit (Millipore, Bedford, MA) according to the manufacturer's instructions. Soluble chromatins were prepared from cultured PLC/PRF/5 cells and isolated hepatocytes of human liver tissues. The chromatins were immunoprecipitated using antibodies against SP1 and NFκB p65 (Table S5). The primer sequences and the sizes of the amplicon are listed in Table S6. These assays were performed as

previously described [Suppl. Ref.5].

### *Immunofluorescence and immunohistochemistry analysis*

Immunofluorescence (IF) and immunohistochemistry (IHC) were performed as previously described [22, Suppl.Ref.4]. Primary antibody dilution is described in Table S5.

### *Examination of serum FGF19 levels in patients with obstructive cholestasis*

Serum samples from control patients (n=21) and from obstructive cholestatic patients (n=22) were determined using a FGF19 ELISA Kit (R&D systems, Minneapolis, MN) according to the manufacturer's instructions.

## Suppl. References

[1] Camilleri M, Nadeau A, Tremaine WJ, et al. Measurement of serum 7alpha-hydroxy-4-cholesten-3-one (or 7alphaC4), a surrogate test for bile acid malabsorption in health, ileal disease and irritable bowel syndrome using liquid chromatography-tandem mass spectrometry. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 2009, 21(7):734-e743.

[2]. Liu L, Liu C, Zhang Q, et al. SIRT1-mediated transcriptional regulation of SOX2 is important for self-renewal of liver cancer stem cells. Hepatology 2016; 64(3):814-27

[3]. Chen W, Cai SY, Xu S, et al. Nuclear receptors RXRalpha:RARalpha are

repressors for human MRP3 expression. Am J Physiol Gastrointest Liver Physiol 2007, 292(5):G1221-7.

[4]. Chai J, Cai SY, Liu XC, et al. Canalicular membrane MRP2/ABCC2 internalization is determined by Ezrin Thr567 phosphorylation in human obstructive cholestasis. J. Hepatol 2015; 63 :1440–1448

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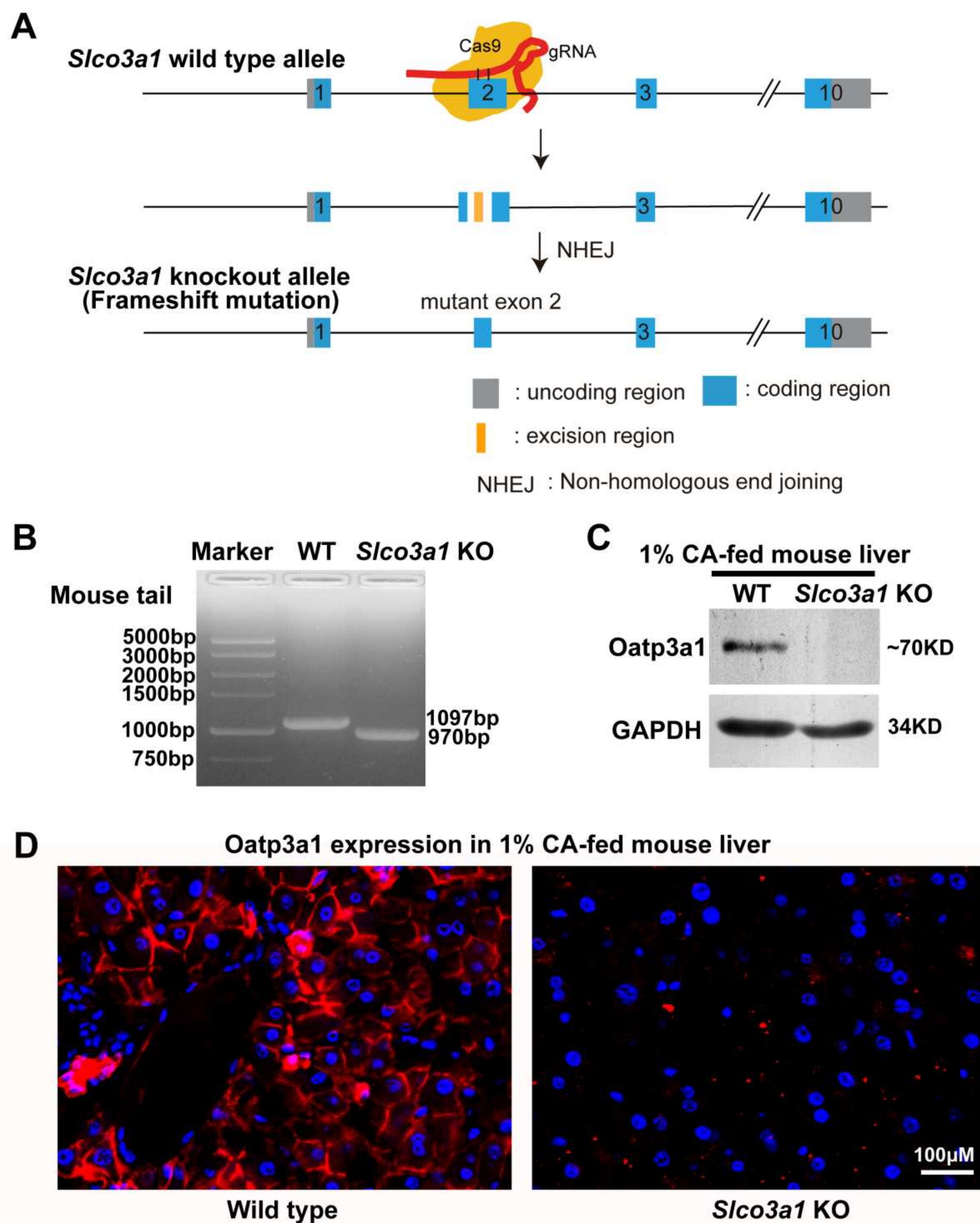
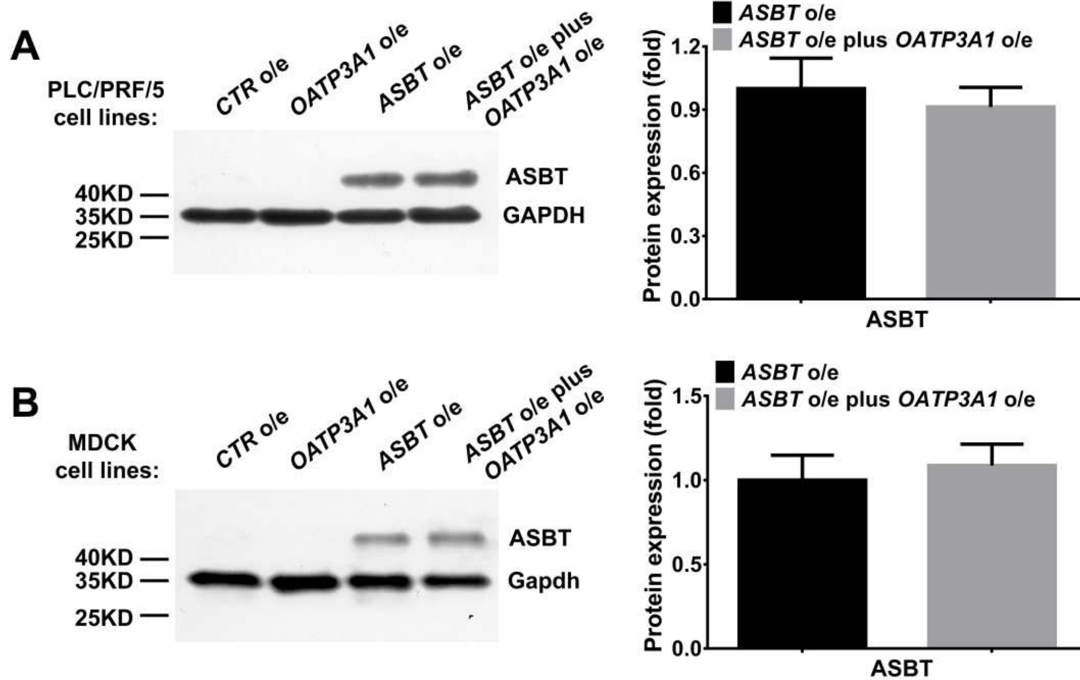
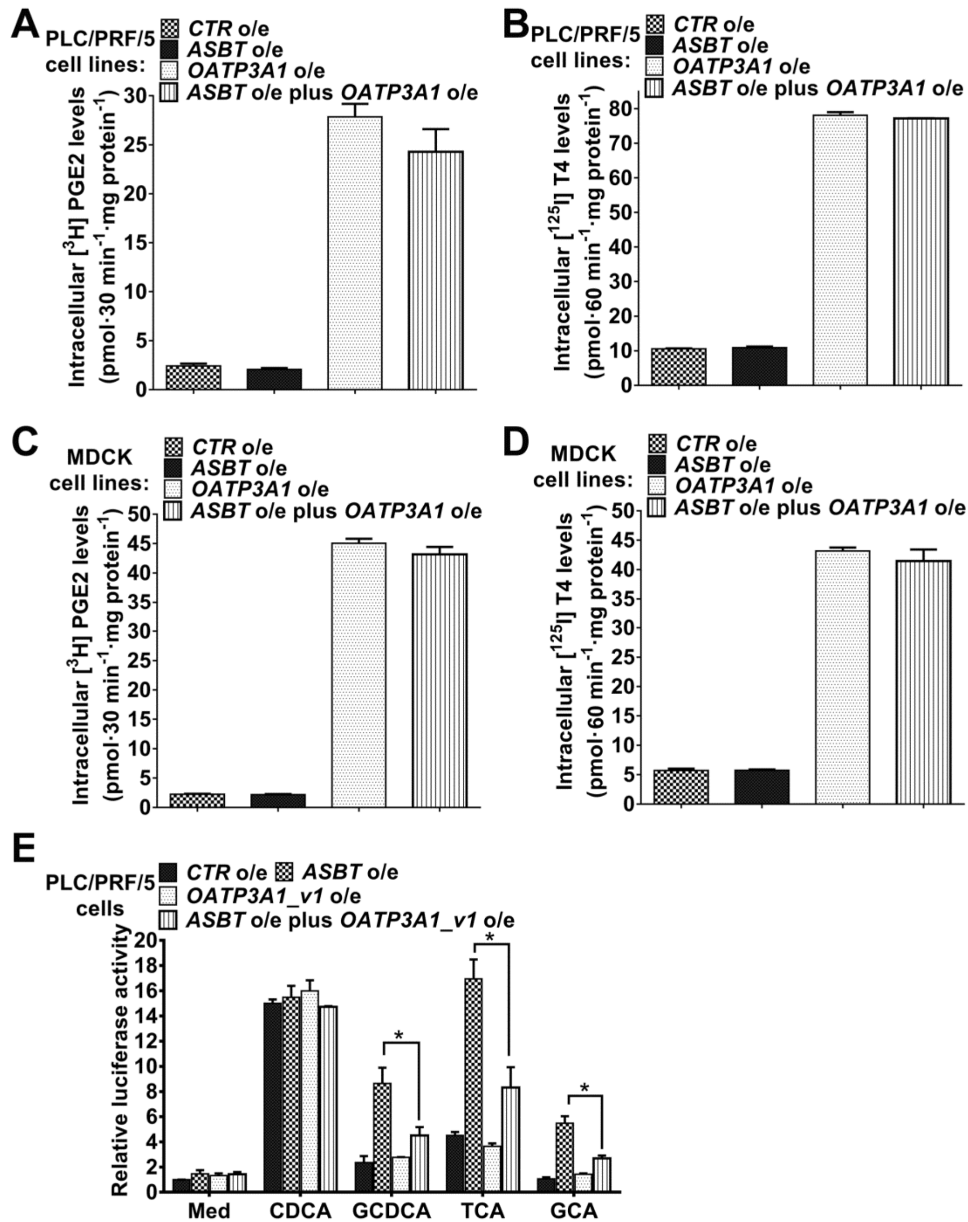
## Suppl. Figures and Figure legends:



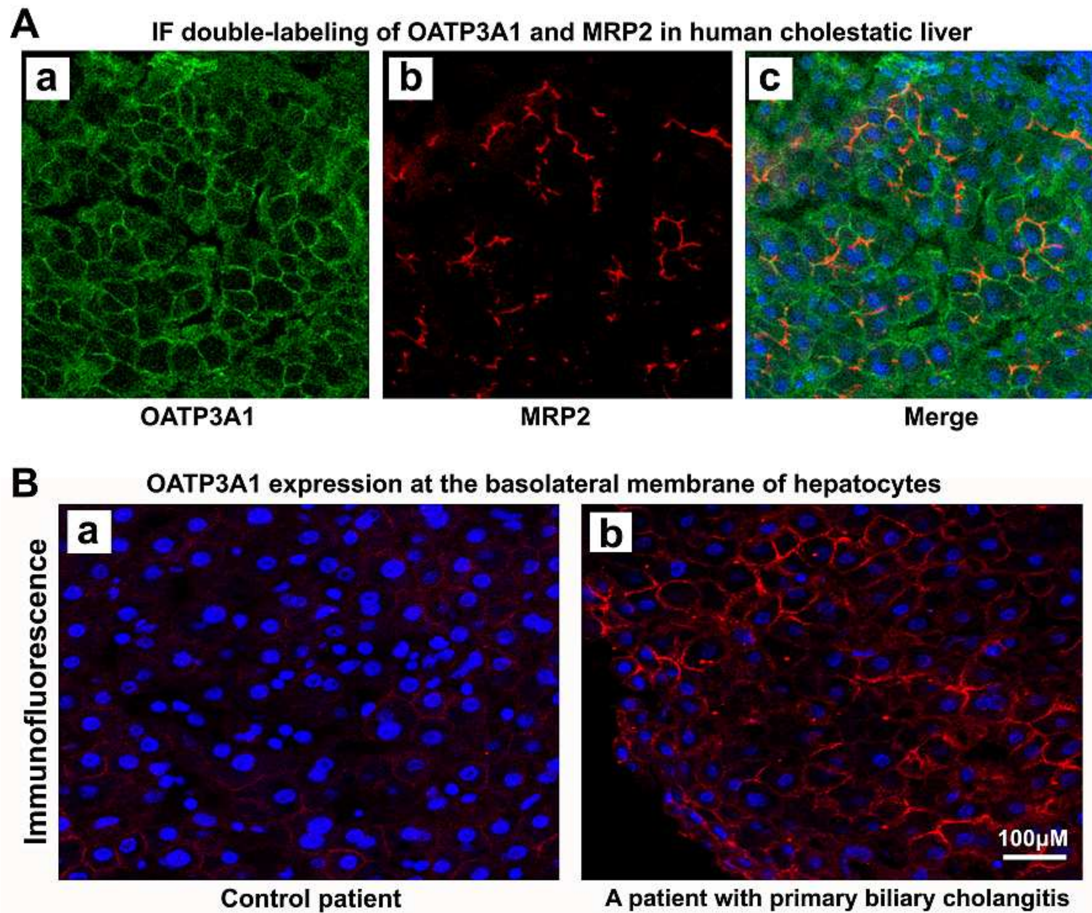

**Suppl. Figure 1.** Generation and characterization of *Slco3a1*-knockout (KO) mice. (A) Schematic diagram of *Slco3a1*-knockout mice. (B) Genotyping of *Slco3a1*-KO mice. PCR was performed using genomic DNA extracted from mouse tails. (C) & (D) Western blot and IF labeling confirmed the ablation of Oatp3a1 protein in *Slco3a1*-KO mice.



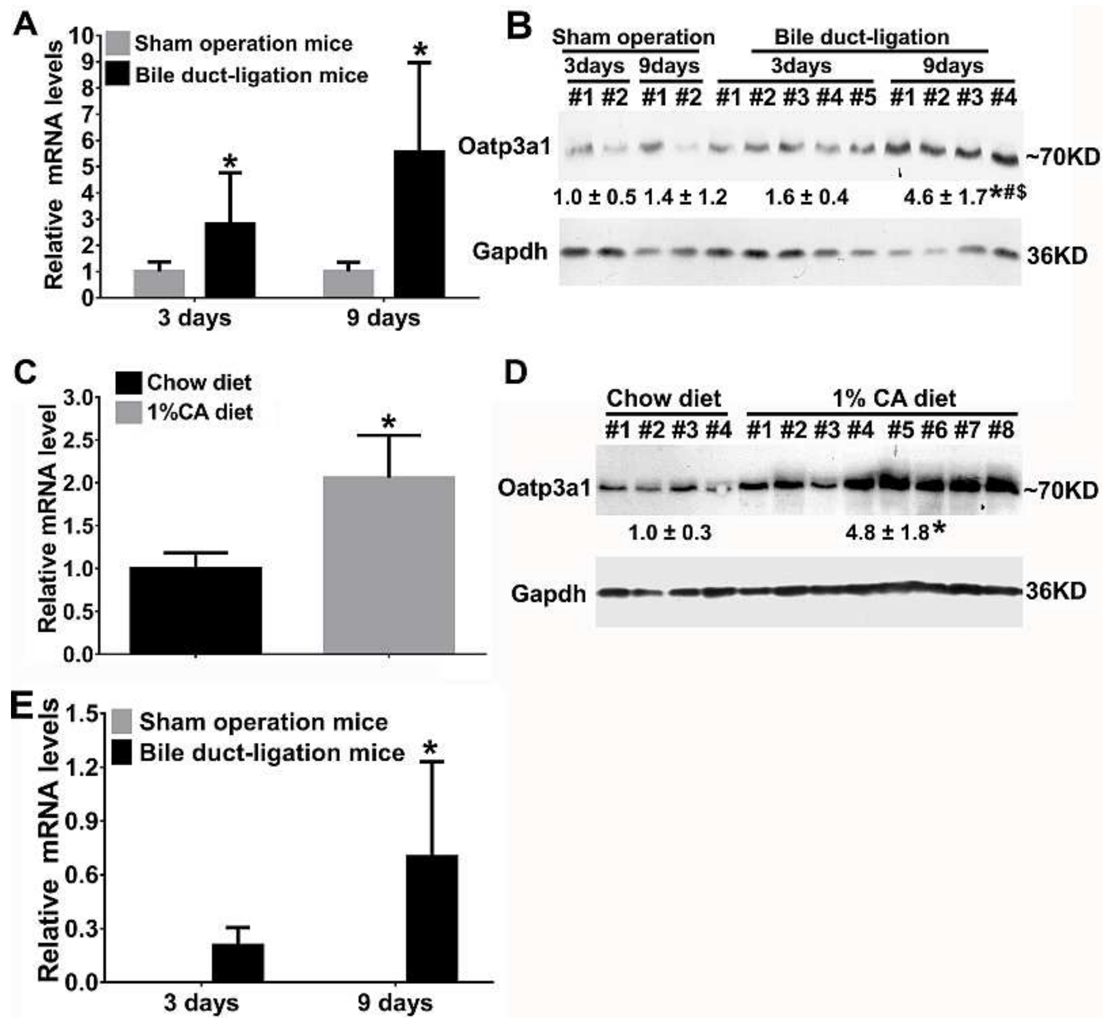

**Suppl. Figure 2.** Selection of four cell lines with stably transfected PLC/PRF/5 (or MDCK) cell lines (-*CTR*, -*ASBT*, -*OATP3A1*, and -*ASBT* plus *OATP3A1*). (A) The ASBT protein levels in PLC/PRF/5-*ASBT* cell lines were similar to those in the PLC/PRF/5-*ASBT* plus *OATP3A1* cell lines. (B) The protein ASBT levels in single-plasmid MDCK-*ASBT* cell lines were similar to those in the double-plasmid MDKC-*ASBT* plus *OATP3A1* cell lines. Values were presented as mean ± SD (n=3).




**Suppl. Figure 3.** (A) - (D) The control uptake experiments using two substrates, [$^3$H]-PGE2 and [$^{125}$I]-T4 in four stably transfected PLC/PRF/5 (or MDCK) cell lines (-*CTR*, -*ASBT*, -*OATP3A1*, and -*ASBT* plus *OATP3A1*). Values were presented as mean ± SD (n=3); (E) Similar to *OATP3A1*_v2 (namely *OATP3A1* in the manuscript, Fig.2D), *OATP3A1*_v1 can also eliminate bile acids in hepatocytes. Therefore, the long variant *OATP3A1*_v2 expression construct, namely *OATP3A1*, was used in this study. Values were presented as mean ± SD (n=3).

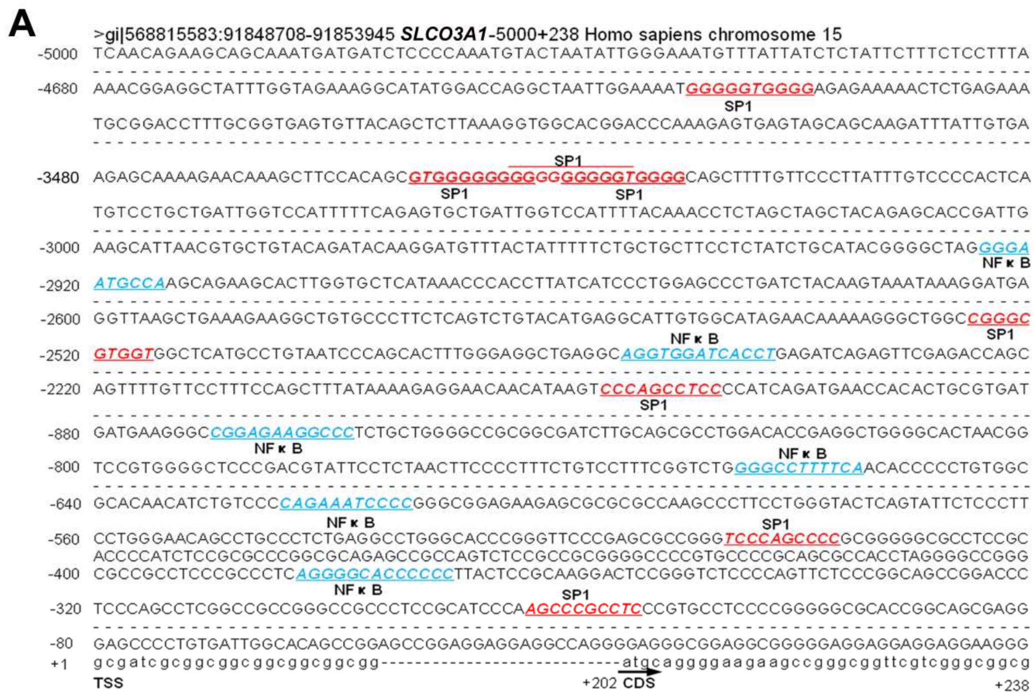



**Suppl. Figure 4.** (A) Immunofluorescence (IF) double-labeling of OATP3A1 (green) and MRP2 (red) in the human cholestatic liver. a, OATP3A1; b, MRP2; c, Merge. (B) IF labeling of OATP3A1 protein (red) in the liver of a non-cholestatic control patient (a) and a patient with primary biliary cholangitis (PBC) (b). The nuclei were stained with 4′-6-Diamidino-2 -phenylindole (DAPI) (blue).

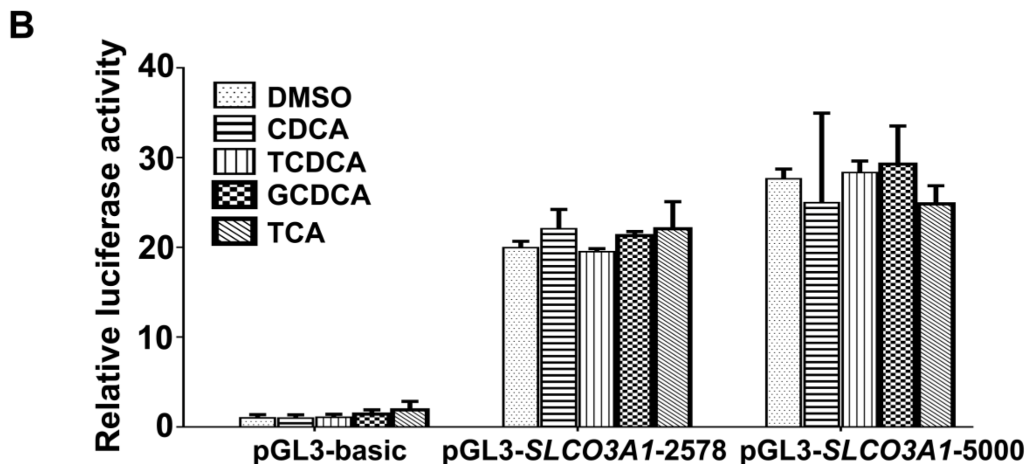



**Suppl. Figure 5.** Oatp3a1 expression was significantly increased in the liver of cholestatic mice. Levels of hepatic Oatp3a1 mRNA (A) and protein (B) expression in BDL mice (relative to control group, n=5 for each sham operation group, n=8-9 for each bile duct-ligation (BDL) group). *$P < 0.05$ vs. sham 3-day group; #$P < 0.05$ vs. sham 9-day group; $\$P < 0.05$ vs. BDL 3-day group; (C) & (D) Levels of hepatic Oatp3a1 mRNA and protein expression in 1% CA fed mice. *$P < 0.05$ vs. chow diet group; (E) Hepatic Fgf15 mRNA expression in BDL 3-day and 9-day mice (n=8-9 for each group). Fgf15 mRNA was undetectable in the sham liver (n=5 for each group). *$P < 0.05$ vs. BDL 3-day group.

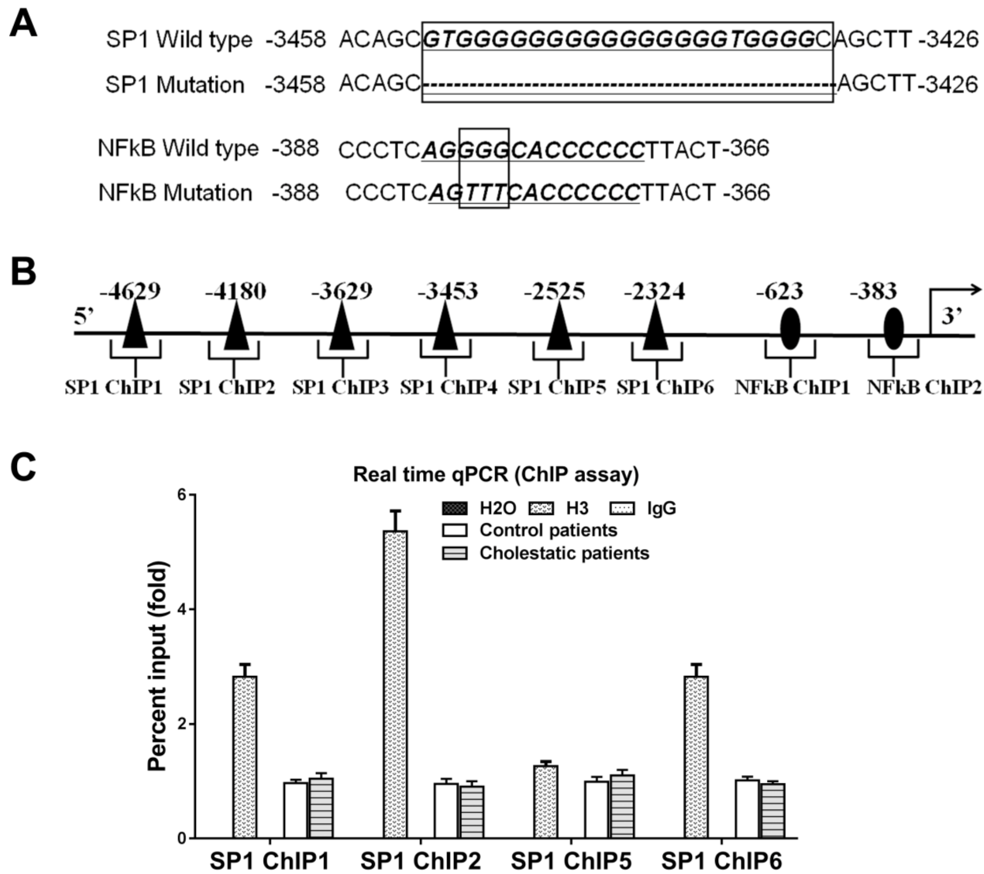
**A**



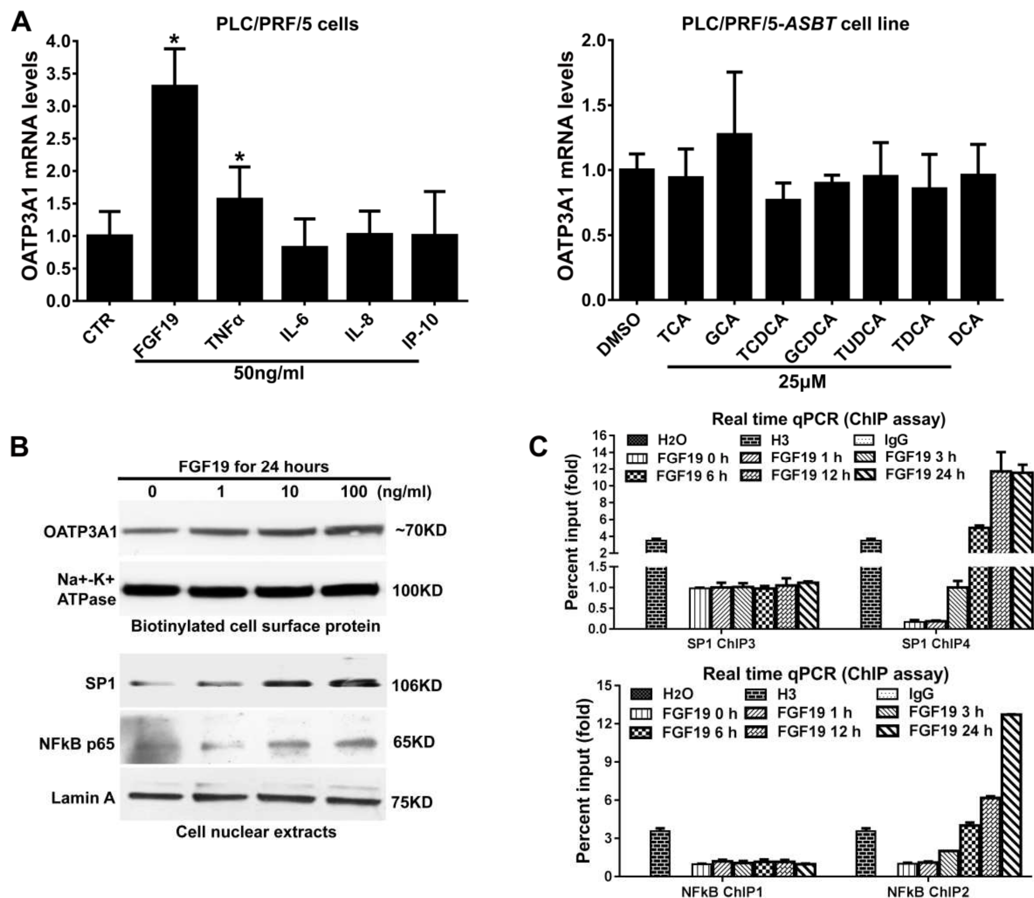

**B**




**Suppl. Figure 6.** (A) Putative SP1 and NFκB p65 response elements rather than FXR/RXR response elements were identified in the promoter region of the human *SLCO3A1* gene; (B) The bile acids-*FXR/RXR*-*SLCO3A1* promoter luciferase reporter assay demonstrated that bile acids did not affect the activity of the *SLCO3A1* promoter in PLC/PRF/5-*ASBT* cell lines, further supporting that FXR/RXR response elements was not detected in the promoter region of the human *SLCO3A1* gene.

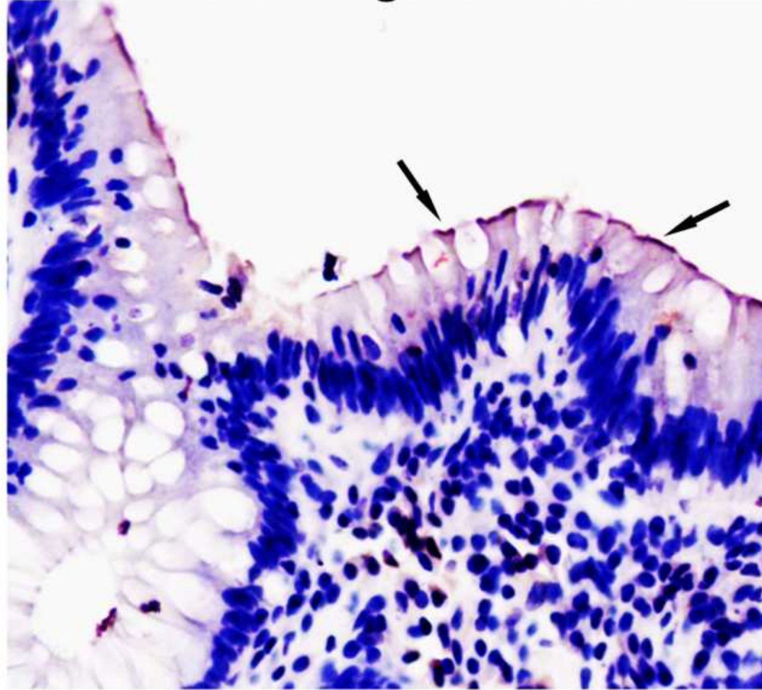



**Suppl. Figure 7.** (A) Mutated key motifs of SP1 and NFκB potential response elements in pGL3-3478/+25 and pGL3-427/+25 constructs (pGL3- *SLCO3A1* -3478 MUT and pGL3- *SLCO3A1* -427MUT constructs). (B) A schematic representation of the human *SLCO3A1* promoter. (C) ChIP assays (real time qPCR) analyzed the binding activities of SP1 to the *SLCO3A1* promoter (SP1 ChIP1-2 and ChIP5-6) in human livers (control livers, n=12; cholestatic livers, n=15).

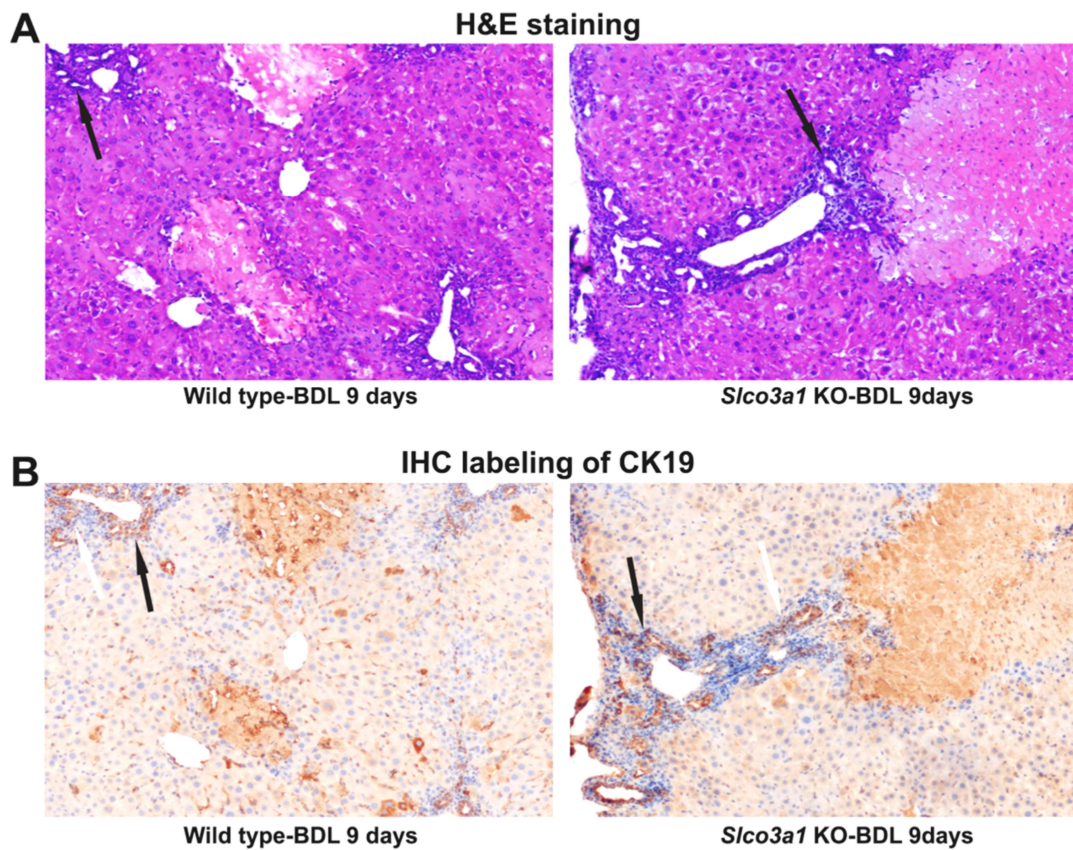



**Suppl. Figure 8.** TaqMan qPCR, western-blot, and ChIP assays were performed in PLC/PRF/5 cells. (A) The mRNA expression of OATP3A1 was significantly induced in PLC5/PRF/5 cells treated with FGF19 (50 ng/mL) and TNFα (50 ng/mL). However, the expression of OATP3A1 at the mRNA level was unaltered by IL-6, IL-8 and IP-10 treatment. Furthermore, the mRNA expression of OATP3A1 in the stably transfected cell line PLC/PRF/5-*ASBT* was not changed by conjugated bile acids taurocholic acid (TCA), glycocholate acid (GCA), taurochenodeoxycholate acid (TCDCA), glycochenodexycholate acid (GCDCA), tauroursodeoxycholate acid (TUDCA), taurodeoxycholate acid (TDCA) and deoxycholic acid (DCA), which were markedly increased in the serum of cholestatic patients. (B) FGF19 induced cell surface expression of OATP3A1 protein and nuclear

expression of SP1 and NFκB p65 protein in a dose-dependent manner in PLC/PRF/5 cells. (C) ChIP analysis (real time qPCR) demonstrated that FGF19 increased binding activities of SP1 and NFκB p65 to the *SLCO3A1* promoter in a time-dependent manner in PLC/PRF/5 cells (SP1 ChIP4 and NFκB ChIP2).




**Suppl. Figure 9.** IHC analysis showed that OATP3A1 protein expression was localized at the apical membrane of human normal colon (arrows).




**Suppl. Figure 10.** H&E staining (A) and IHC labeling of CK19 (B) demonstrated that bile duct injury, proliferation and inflammation in *Slco3a1*-KO BDL 9-day group were worse compared to the WT BDL 9-day group (arrows).

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                       360       370       380       390       400       410       420
                 ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Human  SLCO3A1 322 ------LEPDSSASCFQQLRVIPKVTKHLLSNPVFTCIILAAC MEIAVVAGFAAFLGKYL EQQFNLTTSS 386
       SLCO1B3 310 -------LTNQGKNVTKNVTGFFQSLKSILTNPLYVIFLLLTLLQVSSFI GSFTYVFKYMEQQYGQSASH 373
       SLCO2B1 339 KKQDGLVQIAPNLTVIQFIKVFPRVLLQTLRHPIFLLVVLSQVCLSSMA AGMATFLPKFLERQFSITASY 408
       SLCO1A2 293 -------VKKEKYGITK---DFLPFMKSLSCNPIYMLFILVSVIQFNAFVNMIS FMPKYLEQQYGISSSD 353
       SLCO1B1 310 -------LTNQGKNITKNVTGFFQSFKSILTNPLYVMFVLLTLLQVSSYI GAFTYVFKYVEQQYGQPSSK 373
Mouse  Slco3a1 322 ------LEPDSSASCFQQLRVIPKVTKHLLSNPVFTCIVLAAC MEIAVVAGFAAFLGKYLEQQFNLTTS S 386
       Slco2a1 306 -------------LMDFIKRFPRIFLRLLMNPLFMLVVLSQCTFSSVI AGLSTFLNKFLEKQYDASAAY 362
       Slco1a4 293 -------IKEENRGITK---DFFLFMKSLSCNPIYMIFILISVIQVNAFINSFT FMPKYLEQQYGKSTAE 353
       Slco1a5 293 -------AKEENRGITK---DFLPFMKSLSCNPIYMLLILTSVLQINAFINMFT FLPKYLEQQYGKSTSE 353
       Slco1a1 293 -------AKKENLGITK---DFLPFMKSLCCNPIYMLFSLTSVLQINGF ASTFTFLPKYLEQQYGKSTSE 353
       Slco1a6 293 -------AKEENQGIIK---EFFLMMKNLFCNPIYMLCVLTSVLQVNG VANIVIYKPKYLEHHFGISTAK 353
       Slco2b1 328 KKQAGLAQIAPDLTLVQFVKVFPRVLLRNLRHPIFLLVVLSQVCTSSM VAGMATFLPKFLERQFSITASF 397

                       430       440       450       460       470       480       490
                 ....|....|....|....|....|....|....|....|....|....|....|....|....|
Human  SLCO3A1 387 ANQLLGMTAIPCACLGIFLGGLL VKKLSLSALGAIRMAMLVNLVSTACYVSFLFLGCDTGPVAGVTVP Y- 455
       SLCO1B3 374 ANFLLGIITIPTVATGMFLGGFIIKKFKLSLVGIAKFSFLTSMISFLFQLLYFPLICESKSVAGLTLTY D 443
       SLCO2B1 409 ANLLIGCLSFPSVIVGIVVGGVLVKRLHLGPVGCGALCLLGMLLCLFFSLPLFFIGCSSHQIAGITHQT S 478
       SLCO1A2 354 AIFLMGIYNLPPICIGYIIGGLIMKKFKITVKQAAHIGCWLSLLEYLLYFLSFLMTCENSSVVGINTSY E 423
       SLCO1B1 374 ANILLGVITIPIFASGMFLGGYIIKKFKLNTVGIAKFSCFTAVMSLSFYLLYFFILCENKSVAGLTMTY D 443
Mouse  Slco3a1 387 ANQLLGMTAIPCACLGIFLGGLL VKKLSLSALGAIRMAMLVNLVSTACYVSFLFLGCDTGPVAGVTVR Y- 455
       Slco2a1 363 ANLLIGAVNLPAAALGMLFGGILMKRFVFPLQTIPRVAATIMTISIILCAPLFFMGCSTPAVAEVYPPS T 432
       Slco1a4 354 IVFLMGLYMLPPICLGYLIGGLIMKKFKITVKKAAYIGFWLSLTEYLLSFVSYIMTCDNFPVAGLTTSY E 423
       Slco1a5 354 VVLLIGVCNLPPICIGYLLIGFIMKKFRITVKKAAYMAFCLSLFEYLLSYFHFMISCDNFQVAGLTTSY E 423
       Slco1a1 354 AVFLIGVYSLPPVCLGYLISGFIMKKFKITVKKAAYIAFGLSLSEYFIFLCNYLLTCDNFPVAGLTTSY K 423
       Slco1a6 354 AVFLIGLYTTPSVSAGYLISGFIMKKLKITLKKAAIIALCLFMSECLLSLCNFMLTCDTTPIAGLTTSY E 423
       Slco2b1 398 ANMLLGCLTIPLVIVGIMMGGVLVKRLHLSPVQCSALCLLGSLLCLLFSVPLFFIGCSTHQIAGI TQDLG 467
```

**Suppl. Figure 11.** Specificity analysis of the OATP3A1 (SLCO3A1) antibody used in this study. The OATP3A1 antibody was purchased from Sigma-Aldrich that was raised against a region (red) where it does not share any identity to that in other OATPs. The specificity was further determined by the western blotting and immunofluorescent analysis in the 1% CA-fed *Slco3a1*-KO mice (Suppl.Figs.1C&D).

## Suppl. Table.1-6

**Table S1. Clinical Features of Patients**

| Clinical Features | Control patients | Obstructive Cholestatic patients |
|---|---|---|
| Total samples (Male/Female) | 21 (13/8) | 22 (14/8) |
| Age (years) | 53±10 | 57±10 |
| ALT (IU/L) | 37.0±34.1 | 156.7±133.0* |
| AST (IU/L) | 31.3±15.2 | 124.9±85.5* |
| ALP (IU/L) | 88.7±37.3 | 423.2±211.4* |
| GGT (IU/L) | 45.9±50.8 | 620.4±446.0* |
| TBA (μmol/L) | 13.9±43.8 | 86.3±93.8† |
| TBIL (μmol/L) | 20.7±32.8 | 201.3±105.4* |
| DBIL (μmol/L) | 7.4±19.0 | 106.5±56.3* |
| IBIL (μmol/L) | 13.3±14.7 | 90.3±56.7* |

Values are means ± SD.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; TBA, total bile salts; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin.

*$P < 0.001$.

†$P < 0.01$ versus controls.

**Table S2A. Survival rates and serum biochemistry in BDL mice for 3 days**

| | Sham operation 3 d | | Bile duct-ligation (BDL) 3 d | |
|---|---|---|---|---|
| | **WT** | ***Slco3a1*-KO** | **WT** | ***Slco3a1*-KO** |
| Survival/total mice (%) | 5/ 5 (100%) | 3 / 3 (100%) | 8 / 8 (100%) | 4 / 4 (100%) |
| Serum ALT (IU/L) | 28.22±16.02 | 17.64±3.22 | 435.04±229.04*,¶ | 424.76±137.54*,¶ |
| Serum AST (IU/L) | 99.62±23.74 | 120.96±10.92 | 685.19±338.72*,¶ | 608.30±164.08*,¶ |
| Serum ALP (IU/L) | 99.40±25.51 | 114.8±55.92 | 478.44±181.92*,¶ | 418.6±206.58*,¶ |
| Serum TBA (μmol/L) | 5.35±3.58 | 1.43±0.56 | 389.76±219.30*,¶ | 291.37±140.95*,¶ |
| Serum TBIL (μmol/L) | 1.57±1.43 | 4.28±6.02 | 178.64±66.89*,¶ | 123.9±18.31*,¶ |
| Serum DBIL (μmol/L) | 1.23±1.15 | 1.96±3.14 | 119.22±68.66*,¶ | 69.02±26.82*,¶ |

Values are means ± SD. *$P$ < 0.05 VS Sham WT mice; ¶$P$ < 0.05 VS Sham *Slco3a1*-KO mice; §$P$ < 0.05 VS BDL WT mice.

**Abbreviations:** BDL, bile duct ligation; KO, Knock out; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TBA, total bile salts; TBIL, total bilirubin; DBIL, direct bilirubin.

**Table S2B. Survival rates and serum biochemistry in BDL mice for 9 days**

| | Sham operation 9 d | | Bile duct-ligation (BDL) 9 d | |
|---|---|---|---|---|
| | **WT** | ***Slco3a1*-KO** | **WT** | ***Slco3a1*-KO** |
| Survival/total mice (%) | 5 / 5 (100%) | Not detected | 9 / 9 (100%) | 6 / 9 (67%) |
| Serum ALT (IU/L) | 23.13±2.83 | Not detected | 208.71±102.59* | 364.59±120.344*,§ |
| Serum AST (IU/L) | 97.89±14.78 | Not detected | 340.37±140.31* | 606.90±293.89*,§ |
| Serum ALP (IU/L) | 57.12±12.12 | Not detected | 386.46±113.62* | 437.724±170.27* |
| Serum TBA (μmol/L) | 4.37±1.93 | Not detected | 250.12±116.62* | 366.1±213.892* |
| Serum TBIL (μmol/L) | 1.85±2.13 | Not detected | 145.01±70.7* | 188.66±47.99* |
| Serum DBIL (μmol/L) | 1.04±1.29 | Not detected | 72.44±41.55* | 123.31±35.95*,§ |

Values are means ± SD. *$P < 0.05$ VS Sham WT mice; §$P < 0.05$ VS 1% BDL WT mice.

**Abbreviations:** BDL, bile duct ligation; KO, Knock out; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TBA, total bile salts; TBIL, total bilirubin; DBIL, direct bilirubin.

**Table.S3 The primers were used in *SLCO3A1* promoters production**

| pGL3-*SLCO3A1*-Luc | Primer pairs | Products (bp) |
|---|---|---|
| pGL3-*SLCO3A1* -Luc(-5000/+25) | F: 5'- CGACGCGT TCAACAGAAGCAGCAAATG -3'<br>R: 5'-GAAGATCT CCGCCGCCGCCGCCGCCGCGATC-3' | 5025 bp |
| pGL3-*SLCO3A1* -Luc(-3689/+25) | F: 5'- CGACGCGT GCTGGGGCTGAGGGGGTGAG -3'<br>R: 5'-GAAGATCT CCGCCGCCGCCGCCGCCGCGATC-3 | 3714 bp |
| pGL3-*SLCO3A1* -Luc(-3478/+25) | F: 5'- CGACGCGT GCCCCGCAGCGCCACCTAGG -3'<br>R: 5'-GAAGATCT CCGCCGCCGCCGCCGCCGCGATC-3 | 3503 bp |
| pGL3-*SLCO3A1* -Luc(-2578/+25) | F: 5'- CGACGCGT AGAGCACAACATCTGTCCCC -3'<br>R: 5'-GAAGATCT CCGCCGCCGCCGCCGCCGCGATC-3 | 2603 bp |
| pGL3-*SLCO3A1* -Luc(-643/+25) | F: 5'- CGACGCGT TGCCCTTCTCAGTCTGTACATG -3'<br>R: 5'-GAAGATCT CCGCCGCCGCCGCCGCCGCGATC-3 | 668bp |
| pGL3-*SLCO3A1* -Luc(-427/+25) | F: 5'- CGACGCGT AGCAAAAGAACAAAGCTTCC -3'<br>R: 5'-GAAGATCT CCGCCGCCGCCGCCGCCGCGATC-3 | 452 bp |
| pGL3-*SLCO3A1* -Luc(-183/+25) | F: 5'- CGACGCGT GGAGGGGGCACTGCAGTTC -3'<br>R: 5'-GAAGATCT CCGCCGCCGCCGCCGCCGCGATC-3 | 208 bp |

The underlined bases in the primers are Mlu I and Bgl II adapters.

**The primer sequences were used in mutation PCR of *SLCO3A1* promoters**

| Mutants | Primer pairs |
|---|---|
| pGL3-*SLCO3A1*<br>-3478/+25 MUT | F:5'-AGCAAAAGAACAAAGCTTCCACAGCAGCTTTTGTTC-3'<br>R: 5'-GGACAAATAAGGGAACAAAAGCTGCTGTGGAAGC-3' |
| pGL3-*SLCO3A1*<br>-427/+25 MUT | F: 5'-GCCTCCCGCCCTCAGTTTCACCCCCCTTACT-3'<br>R: 5'-AGTAAGGGGGGTGAAACTGAGGGCGGGAGGC-3' |

**Table S4. Real time qPCR probes (TaqMan) and primers (SYBR)**

| Gene | Sequences (5'→3') | Species/Source |
|---|---|---|
| *GAPDH* | Proprietary to ABI | Human/Hs02758991_g1 |
| *OATP3A1* (*SLCO3A1*)* | Proprietary to ABI | Human/ Hs00203184_m1* |
| *CYP7A1* | Proprietary to ABI | Human/ Hs00167982_m1 |
| *NFκB1* | Proprietary to ABI | Human/Hs00765730_m1 |
| *SP1* | Proprietary to ABI | Human/ Hs00916521_m1 |
| *FGF19* | Proprietary to ABI | Human/ Hs00192780_m1 |
| *FGFR4* | Forward: 5'- CCGCTATAACTACCTGCTA -3' | Human/Primers (SYBR) |
| | Reverse: 5'- TTGATGACGATGTGCTTC-3' | NM_002011.4 |
| *βKlotho*(*KLB*) | Forward: 5'- ATCCTGTCAGCACTTATTCT -3' | Human/Primers (SYBR) |
| | Reverse: 5'- TCCATCCTTCTTCCAACTC -3' | NM_175737.3 |
| *Mrp2 (Abcc2)* | Proprietary to ABI | Mouse/Mm00496899_m1 |
| *Mrp3 (Abcc3)* | Proprietary to ABI | Mouse/Mm00551550_m1 |
| *Mrp4 (Abcc4)* | Proprietary to ABI | Mouse/Mm01226381_m1 |
| *Ostα (Slc51a)* | Proprietary to ABI | Mouse/Mm00521530_m1 |
| *Ostβ (Slc51b)* | Proprietary to ABI | Mouse/Mm01175040_m1 |
| *Asbt (Slc10a2)* | Proprietary to ABI | Mouse/Mm00488258_m1 |
| *Mdr1a (Abcb1a)* | Proprietary to ABI | Mouse/Mm00440761_m1 |
| *Mdr2 (Abcb4)* | Proprietary to ABI | Mouse/Mm00435630_m1 |
| *Cyp7a1* | Proprietary to ABI | Mouse/Mm00484150_m1 |
| *Cyp8b1* | Proprietary to ABI | Mouse/Mm00501637_s1 |
| *Shp (Nr0b2)* | Proprietary to ABI | Mouse/Mm00442278_m1 |

| *Fgf15* | Proprietary to ABI | Mouse/Mm00433278_m1 |
|---|---|---|
| Gapdh | Forward: 5'- acagcaacagggtggtggac-3' | Rat/Primers (SYBR) |
| | Reverse: 5'- tttgagggtgcagcgaactt-3' | NM_017008.4 |
| *Oatp3a1*(*Sclo3a1*) | Forward: 5'-acaagcaacctggacatcact-3' | Rat/Primers (SYBR) |
| | Reverse: 5'- gaagaagagtaaggcaccacaga-3' | NM_177481.1 |

***Note:** This commercially available OATP3A1 primer/probe has the best coverage for human SLCO3A1 transcript variants, including splice variants 1 and 2.

**Table S5. Antibodies used in western blot, Chromatin co-immunoprecipitation, and immunofluorescence**

| Protein | Host | Company / Catalog | Antibody dilution |
|---|---|---|---|
| GAPDH | Rabbit | Proteintech, Chicago, IL/10494-1-AP | WB 1:3000 |
| Na+/K+ATPase | Rabbit | Abcam, Cambridge, MA/ ab76020 | WB 1:10,000 |
| OATP3A1(SLCO3A1)* | Rabbit | Sigma-Aldrich, St Louis, MO/ SAB1304633 | WB 1:1000; IF 1:50; IHC 1:50 |
| OATP3A1(SLCO3A1) | Goat | Santa Cruz, Dallas, CA/ sc-66566 | WB 1:1000, |
| MRP2 (ABCC2) | Mouse | Abcam, Cambridge, MA/ab3373 | WB 1:1600; IF:1:100 |
| MRP3 (ABCC3) | Mouse | Abcam, Cambridge, MA/ab3375 | WB 1:1600 |
| MRP4 (ABCC4) | Rat | Abcam, Cambridge, MA/ab15602 | WB 1:1600 |
| OSTα (SLC51A) | Rabbit | Santa Cruz, Dallas, CA/sc-100078 | WB 1:2000 |
| OSTβ (SLC51B) | Rabbit | Sigma-Aldrich, St Louis, MO/HPA008533 | WB 1:500 |
| ASBT (SLC10A2) | Rabbit | Proteintech, Chicago, IL/25245-1-AP | WB 1:1000 |
| SP1(H-225) | Rabbit | Santa Cruz, Dallas, CA/ sc-14027 | WB 1:1600; ChIP 2μg per sample |
| SHP (NR0B2) | Rabbit | Abcam, Cambridge, MA/ab96605 | WB 1:1000 |
| CYP7A1 | Rabbit | Santa Cruz, Dallas, CA/sc-25536 | WB 1:1000 |
| Phoshpo-ERK1/2 | Rabbit | Cell Signaling, Beverly, MA/#4370 | WB 1:2500 |
| ERK1/2 | Rabbit | Cell Signaling, Beverly, MA/#9102 | WB 1:2500 |
| NFκB p65 (L8F6) | Mouse | Cell Signaling, Beverly, MA/#6956 | WB 1:1000 |
| NFκB p65 (ChIP-Grad) | Rabbit | Abcam, Cambridge, MA/ ab7970 | WB 1:1000; ChIP 2μg per sample |
| Phoshpo-NFκB p65 (pS529) | Rabbit | Abcam, Cambridge, MA/ ab109458 | WB 1:2000 |
| Lamin A | Rabbit | Abcam, Cambridge, MA/ ab26300 | WB 1:1000 |
| FGF19 | Rabbit | Abcam, Cambridge, MA/ ab225942 | WB 1:2000 |
| FGFR4 | Rabbit | Proteintech, Chicago, IL/10098-1-AP | WB 1:2000 |
| βKlotho (KLB) | Mouse | USCN/MAH756Hu21 | WB 1:2000 |

| CK19 | Rabbit | Abcam, Cambridge, MA/ab52625 | IHC 1:50 |
|---|---|---|---|

***Note:** The OATP3A1 antibody used through this study was an affinity purified rabbit polyclonal antibody (Sigma-Aldrich) raised against amino acids 359-410 of human OATP3A1 origin, a region shared by both isoform 1 and 2. The antibody has capability determine human OATP3A1 isoforms 1 and 2.

**Table. S6 PCR primers for ChIP assays (Real time qPCR and semi-quantity PCR)**

| ChIP | Primer pairs | Products (bp) |
|---|---|---|
| SP1 ChIP1(-4629) | Forward: 5'- TAGTAAGTAGCAAACGGA -3'<br>Reverse: 5'- ATTTGGCTTGAAACTAAT -3' | 138 bp |
| SP1 ChIP2(-4180) | Forward: 5'- CCCACGACCATATAGTTAACCC -3'<br>Reverse: 5'- ATAAACTGGCTTTTGGTTCGTA -3' | 147 bp |
| SP1 ChIP3(-3629) | Forward: 5'- TTTAAGTTGTCGGCTTTC -3'<br>Reverse: 5'- TAACACTCACCGCAAAGG -3' | 212 bp |
| SP1 ChIP4(-3453) | Forward: 5'- CCCAAAGAGTGAGTAGCA -3'<br>Reverse: 5'- GTAAAAACGCACCAATCG -3' | 206 bp |
| SP1 ChIP5(-2525) | Forward: 5'- GGCATTGTGGCATAGAAC -3'<br>Reverse: 5'- TAGCTGGAATTACAGACG -3' | 196 bp |
| SP1 ChIP6(-2324) | Forward: 5'-CGTCTGTAATTCCAGCTACTCCA -3'<br>Reverse: 5'- GGAGTTTCGCTCTTGTTGCC -3' | 124 bp |
| NFκB ChIP1(-623) | Forward: 5'- AAGCCTCAAGCAGCTCTGAC -3'<br>Reverse: 5'- TCAGAGGGCAGGCTGTTCCCA -3' | 148 bp |
| NFκB ChIP2(-383) | Forward: 5'- GCGCAGAGCCGCCAGTCTCC -3'<br>Reverse: 5'- GACCCGGAGTCCTTGCGGAGT -3' | 115 bp |
| ChIP for positive control (GAPDH) | Forward: 5'-TACTAGCGGTTTTACGGGCG-3'<br>Reverse: 5'-TCGAACAGGAGGAGCAGAGAGCGA-3' | 166 bp |