Supplementary Methods

RNA ISOLATION AND QUANTITATIVE PCR

Total RNA was isolated from liver tissue that was stored in an RNAlater solution at 20 °C. RNA was isolated using the PureLink RNA Mini Kit (Cat. No. 12183025) following the protocol for purifying RNA from the liver tissue. The PureLink DNase Kit (Cat. No. 12185-010) was used in conjunction with the RNA mini kit to remove DNA from the sample. Next, the SuperScript III Platinum One-Step qRT-PCR Kit (Cat. No.11732-088) was used according to the manufacturer's instructions. The cycle conditions were set to the following: 50 °C for 15 min and 95 °C for 2 min and then 40 cycles of 95 °C for 15 s and 60 °C for 30 s. The following primers were used: *Mrp3* (Rn01452854_m1, ThermoFisher Scientific, Waltham, MA), *Mrp2* (Rn00563231_m1), *Ntcp* (Rn00566894_m1) *Oatp1* (Rn00755148_m1), *Bsep* (Rn01515444_m1), *Gapdh* (Rn01775763_g1), *Ae2* (Rn01442178_m1), *Nfκb1* (Rn01399572_m1), *Tnf-α* (Rn99999017_m1), *Il-6* (Rn01410330_m1). Expression levels were presented by relative fold changes to the reference gene (glyceraldehyde-3-phosphate dehydrogenase, *Gapdh*).

WESTERN BLOTTING ASSAY

Proteins were extracted from liver tissue that was snap frozen in liquid nitrogen. Approximately 25 mg of tissue was placed in 1 mL of RIPA buffer (Thermo Scientific, 89901) mixed with 10 µL Protease Inhibitor Cocktail (Sigma-Aldrich, P8340) and placed on ice for 15 min. The tissue was then homogenized with an electric homogenizer, centrifuged for 10 min. at 4,300 RPM and 4°C, and the supernatant was collected. Protein concentration for each sample was measured using the BCA Protein Assay Kit (Thermo Scientific). Protein levels were determined using the Simple Western machine (ProteinSimple, San Jose, CA) and following the manufacturer's instructions. Anti-Mouse Detection Module Kit and Anti-Rabbit Detection Module Kit (DM-001), 12-230 kDa WES separation Module and 8×25 Capillary Cartridges (SM-W004), and EZ Standard Pack (PS-ST01EZ-8; ProteinSimple, San Jose, CA) were also used. Equal amounts of protein from each sample were loaded into each lane and the target band intensity was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Multiplexing was used to account for lane variation. Primary antibodies used are as follows: OATP1 (bs-0607R; Bioss Antibodies), NTCP (bs-1958R; Bioss Antibodies), BSEP (bs-1954R; Bioss Antibodies), MRP2 (ab203397; Abcam), MRP3 (250760; Abbiotec),AE2 (sc-376632; Santa Cruz Biotechnology Inc.), and GAPDH (sc-376632, Santa Cruz Biotechnology Inc. for primary antibodies from a mouse host; or 2118, Cell Signaling Technology for antibodies from a rabbit host). Protein expressions were determined by area under the chemiluminescence curve corrected to GAPDH expressions using a software (Compass for SW, Version 3.1.7, ProteinSimple, San Jose, CA).

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Polymer Detection (1) – Refine Kit Leica (DS9800)
ANTIBODIES-Cat# / Program with primary incubation:

- **MRP2 – ENZO AIX-801-037) /ModF 90 minute incubation**

: Refine Kit Components

Leica Reagents:

Refine Kit-HRP (DS9800)
DAB Enhancer (AR9432)
Dewax (AR9220)
Wash Buffer (AR9590)
Epitope Retrieval H1 (AR9961) – citrate buffer
Epitope Retrieval H2 (AR9640) - EDTA

All slides were deparaffinized and antigen retrieved on the Bond Rx automated immunostaining platform. Epitope retrieval solutions citrate buffer H2(30). *Epitope Retrieval H2 at 100C applied to slides for 30 minutes with a 12 minute cool down prior to staining.

Staining Protocols – Programmed Steps are as follows with Refine Kit:

- Primary antibody incubation:
  - MRP2 1:100 diluted in Leica Diluent, 1-hour incubation
- Wash Buffer Rinse (3X2min each)
- Post Primary 8 minutes
- Wash Buffer Rinse (3x2min each)
- Polymer 8 minutes
- Wash Buffer Rinse (3x2 min each)
- Perioxidase Block 5 minutes
- Wash Buffer Rinse (3x2 min each)
- DAB Refine 10 minutes
- Di H20 wash (3x 2min each)
- DAB enhancer 5 minutes
- DiH20 Rinse (3x2 min each)
- Hematoxylin counterstain 5minutes
- Di H20 rinse (3x2 min each)
- Wash Buffer Rinse (Bluing) – 1 minute
- Di H20 rinse until unloaded from stainer
- Dehydrate, Clear and Mount with synthetic mounting media.

Polymer Detection (2) – Refine Kit Leica (DS9800)

ANTIBODIES-Cat# / Program with primary incubation:

- **MRP3 – Abbiotec (250760) /ModF-Rbt 1hour incubation**
- **OATP1 – Bioss (bs-0607R) / ModF-Rbt 15-minute incubation**

: Refine Kit Components

Leica Reagents:

Refine Kit-HRP (DS9800)
DAB Enhancer (AR9432)
Dewax (AR9220)
Wash Buffer (AR9590)
Epitope Retrieval H1 (AR9961) – citrate buffer
Epitope Retrieval H2 (AR9640) - EDTA

All slides were deparaffinized and antigen retrieved on the Bond Rx automated immunostaining platform. Epitope retrieval solutions citrate buffer H1(20). *Epitope Retrieval H1 at 100C applied to slides for 20 minutes with a 12 minute cool down prior to staining.

Staining Protocols – Programmed Steps are as follows with Refine Kit:

- Primary antibody incubation:
  - MRP3 1:250 diluted in Leica Diluent, 1-hour incubation
  - OATP1 1:400 diluted in Leica Diluent, 15-minute incubation
- Wash Buffer Rinse (3X2min each)
- Polymer 8 minutes
- Wash Buffer Rinse (3x2 min each)
- Perioxidase Block 5 minutes
- Wash Buffer Rinse (3x2 min each)
- DAB Refine 10 minutes
- Di H20 wash (3x 2min each)
- DAB enhancer 5 minutes
- DiH20 Rinse (3x2 min each)
- Hematoxylin counterstain 5minutes
- Di H20 rinse (3x2 min each)
- Wash Buffer Rinse (Bluing) – 1 minute
- Di H20 rinse until unloaded from stainer
- Dehydrate, Clear and Mount with synthetic mounting media.

LSAB Detection (Labeled Streptavidin-Biotin)
ANTIBODIES:

- **NTCP ThermoFisher (PA5-80001)**
- **BSEP LS Bio (LSC490094/135382)**

Blocking/Detection Kit/Counterstaining Products:

- DAKO Peroxidase block(S200389)
- DAKO – Protein Block (X090930-2)
- Jackson Immuno- Donkey Anti-Rabbit-Biotinylated (711-066-152)
- DAKO - DAB+ (K346811-2)
- DAKO - Mayer's Hematoxylin Solution(S330930-2)
- 0.1% Ammonium Hydroxide Solution (Bluing Reagent)
- DAKO Streptavidin-HRP (P039701-2)
- Leica Dewax Solution – (AR9222)
- Leica Bond Wash Reagent (TBST) – (AR9590)
- Leica Antibody Diluent – (AR9352)
- Leica Antigen Retrieval Citrate Buffer Solution - (H1) (AR9661)

Paraffin embedded sections, section thickness – 4um

1. All slides are deparaffinized, antigen retrieved and stained on the Leica BondRx automated staining platform.
2. Antigen Retrieval was performed using H1(10) – Retrieval solution 1 for 10 minutes at 100C w/ cool down, 12 minutes.

Programmed steps are as follows for blocking and detection:

1.) Wash Buffer rinse – 1min.
2.) Peroxidase block – 15min.
3.) Wash Buffer Rinse-TBST (2 X3min)
4.) Avidin Application 15 minutes
5.) Wash Buffer Rinse-TBST (2 X3min)
6.) Biotin Application 15 minutes
7.) Wash Buffer Rinse-TBST (2 X3min)
8.) Protein Block-30-minute application (No rinse - blot/blow off reagent)
9.) Primary antibody incubated 60min RT. (NTCP conc 1:600)
10.) Wash Buffer Rinse TBST (3 X3min)
11.) Secondary Antibody – 30-minute application (Donkey anti Rabbit-Biotin 1:500)
12.) Wash Buffer Rinse TBST(3X3min)
13.) Streptavidin-HRP 15-minute incubation (1:300)
14.) Wash Buffer Rinse-TBST (2 X3min)
15.) DAB application – 3min
16.) Wash DiH20 – (2x3min)
17.) Counterstained with hematoxylin followed by bluing reagent, dehydrate and clear and mount with coverslip.

NEGATIVE CONTROL – Omission of primary antibody.