**Supplementary Material**

**Supplementary Methods**

*Cultivation and generation of Fly Stocks*

Flies were cultivated in vials with standard fly yeast-cornmeal-molasses food (0.8% agar, 1.8% dried yeast, 1.0% soybean meal, 2.2% molasses, 8.0% malt extract, 8.0% corn meal, 0.63% propionic acid) at 25˚C.

To generate the transgenic stocks carrying either UAS-hOCT1, UAS-hOCT2, UAS-hOATP1B1, UAS-hOATP2B1, UAS-hOATP1B3 or UAS-hABCB1 the respective constructs were produced applying the Takara In-Fusion method (Takara Bio Europe SAS, Saint-Germain-en-Laye, France). In principle, the coding sequence of the human gene was amplified with the KOD-Taq polymerase (Merck Millipore, Darmstadt, Germany) and inserted into the pUAST-*att*B fly transformation plasmid. Using these constructs, transgenic flies were generated by the BestGene Inc. company (Chino Hills, CA, USA). Following standard fly crossing schemes, the insertion UAS-hOCT1 was recombined with the insertion UAS-GFP; the insertions UAS-hOATP1B1 and UAS-hOATP1B3 were recombined with the insertion UAS-CD8-RFP; the insertion UAS-hOATP2B1 was recombined with the insertion UAS-RFPnls; the insertion UAS-hOCT1 was recombined with the insertion UAS-hABCB1.

*Preparation of Amp-LB Medium*

To prepare Amp-LB medium, 10 g NaCl, 5 g yeast extract, and 10 g tryptone were dissolved in 950 mL deionized water. The medium was adjusted to pH 7.0 with 3 M NaOH and filled up with additional water to a total of 1 L, autoclaved for 2 hours. After cooling down to 55°C, 1 mL ampicillin solution (50 mg/mL in water) was added to the medium and the medium was stored at room temperature.

*Preparation of Amp-LB Agar Plates*

After preparing 1 L Amp-LB medium, 15 g agar was added and autoclaved. It was cooled down to 55°C and 1 mL ampicillin was added to the medium. The mixed solution was transferred onto Petri dishes on the clean bench. After waiting until the agar became hard, the dish was inverted, packed, and stored at 4°C.

*Quantitative PCR*

The SYBR Green Kit (Roche, Basel, Switzerland) was used for qPCR experiments. 2 μL of mixed primers were added to 13 μL of Master SYBR Green solution as reaction mixture solution. cDNA was diluted to 125 ng/μL. Then, 5 μL cDNA was added to the reaction mixture solution as a sample for qPCR. The RpS20 gene of the fly was used as a reference gene. The qPCR program of the Light Cycler Nano machine was as follows:

|  |  |  |  |
| --- | --- | --- | --- |
| Program | Temperature | Time | Cycles |
| Hold | 95°C | 10 min | 1 |
| Amplification | 95°C - 60°C - 72°C | 20s – 20s – 20s | 45 |
| Melting | 65°C - 95°C | 60s - 1s | 1 |

Primers are given in Supplementary Table S4.

*Immunofluorescence Staining of Embryos*

Collection and Dechorionation of Embryos

3 - 5 days old female and male flies were used to set up the cage with an attached apple juice plate with a small amount of fresh yeast. Apple juice plates were changed every day. After 3 days, flies had adapted to life in a cage and could produce enough eggs. To collect enough embryos of stages 15 - 16, the new apple juice plate was changed before collection after about 16 h.

To dechorionate embryos, they were gently transferred to a wire mesh basket from the apple juice plate by a soft short brush. Embryos were rinsed in tap water to remove yeast. Eggs were soaked in 3% sodium hypochlorite solution for about 3 min at room temperature. After dechorionation, embryos become transparent and floated on the surface of the solution. Embryos were rinsed in tap water several times. These embryos may easily dehydrate, so they should be fixed immediately.

Formaldehyde-Based Fixation Procedures

For preparation of the fixative buffer, 750 µL heptane and 750 µL 3.7% formaldehyde were mixed in a 1.5 mL Eppendorf tube. The solution was shaken vigorously for about 30 s. Embryos were gently transferred to this fixative buffer with a paint brush. Embryos were incubated at room temperature for 30 min with gently shaking. Embryos localized between the formaldehyde and the heptane phase. The formaldehyde solution in the lower layer was removed and 750 µL of methanol was added. The mixture solution was vigorously shaken for 15 s. Embryos stayed at in the methanol phase and sank to the bottom of the Eppendorf tube. Heptane was removed from the upper solution and 750 µL of methanol was added. Embryos were washed using methanol three times to remove heptane thoroughly. Embryos can be stored in methanol at −20°C for months.

Heat-Methanol Fixation

20 mL salt solution (0.4% NaCl, 0.003% Triton X-100) was added into a 100 mL beaker and heated to boiling in the microwave. A wire mesh basket with embryos was put to the boiling solution and incubated for 10 s. Then, embryos were quickly put into cold salt solution for 10 s. Embryos were transferred to mixture solution with 750 µL of methanol and 750 µL of heptane. This solution was quickly shaken for 15 s. Embryos fell to the bottom of the Eppendorf tube. The mixture solution was removed, and the embryos were washed using methanol three times.

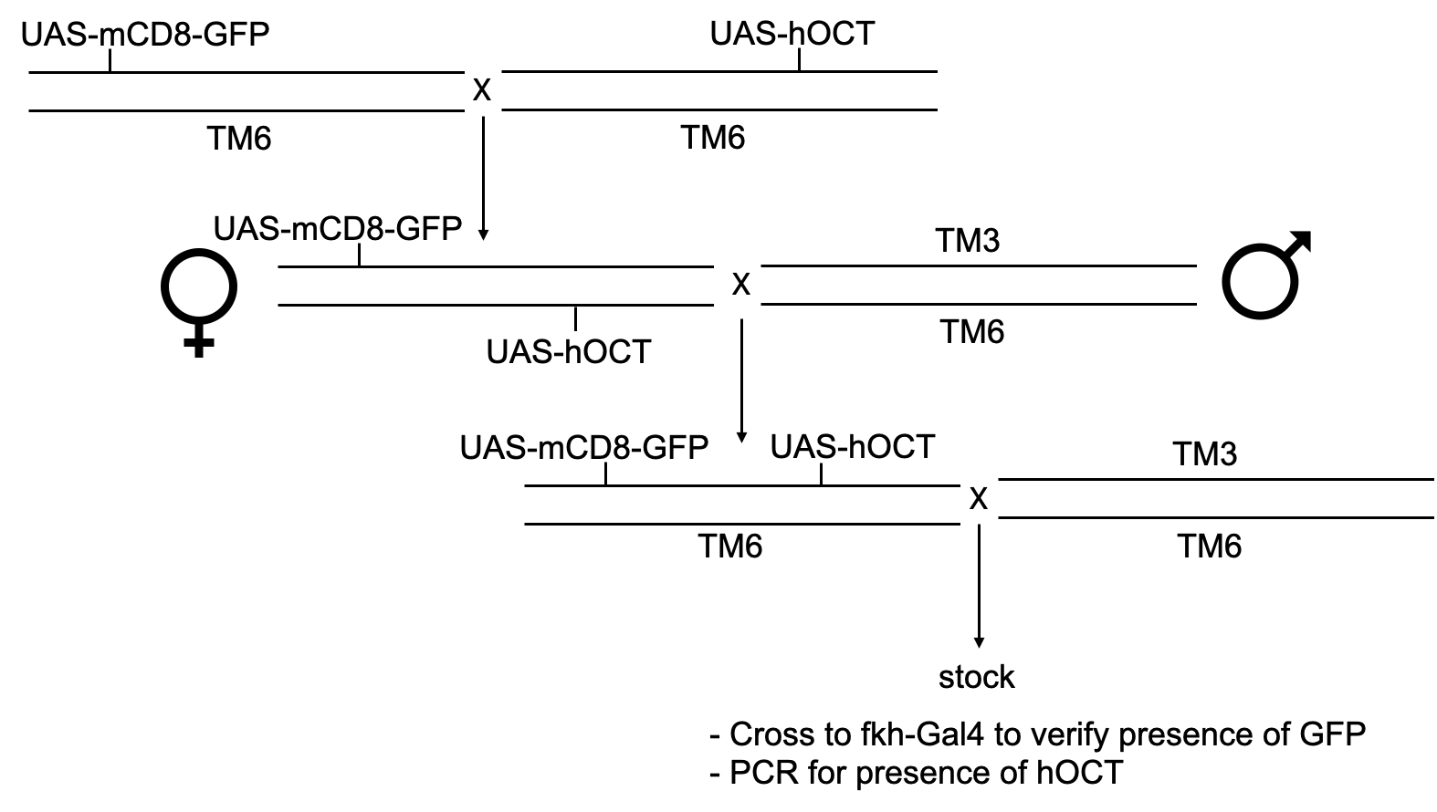
Staining

After fixation, embryos were washed with 1 mL PBST for 10 min at room temperature, wash was repeated three times. Then 1000 μL PBST containing 5% BSA was used to block unspecific antibody binding sites for 60 min at room temperature. The primary antibody was diluted to a suitable concentration by blocking buffer. Embryos were incubated in the primary antibody overnight at 4°C. On the next day, the primary antibody solution was removed, and embryos were washed with 1mL PBST for 10 min at room temperature, this was repeated three times. The secondary antibody was prepared using the blocking buffer. 500 μL of the secondary antibody solution was added to the embryos and incubated for 60 min at room temperature with shaking. Embryos were washed again using PBST for 10 min at room temperature, this was repeated three times. Mowiol was dropped on the slide. Then embryos were transferred into the Mowiol of the slide by a trimmed yellow pipet. After embryos were mixed with Mowiol, a coverslip was put onto the sample. Antibodies used are given in Supplementary Table S5.

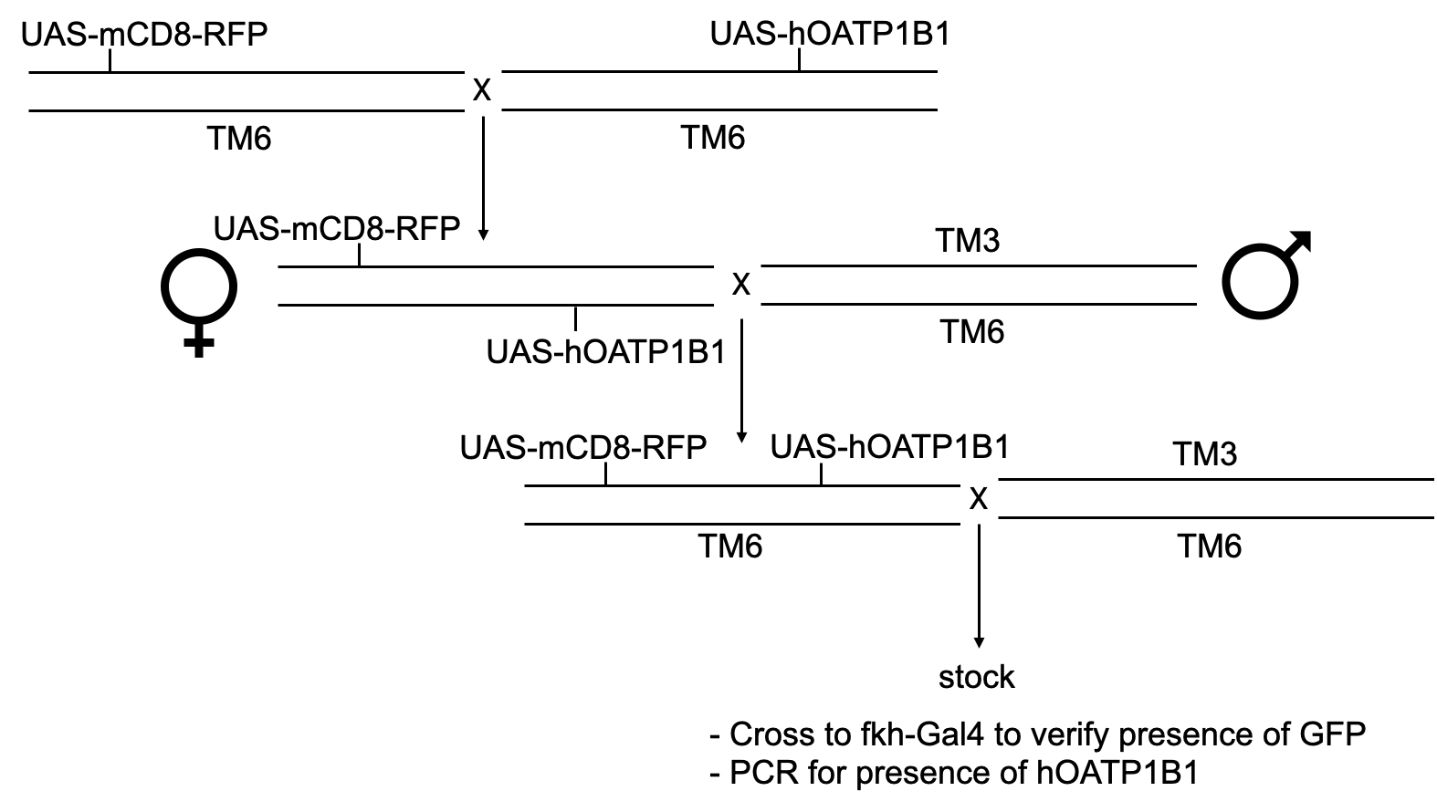
**Supplementary Fig. 1**

**Schemes of the fly crossing experiments**

**A Scheme of the procedure of the recombination to couple the UAS-mCD8-GFP with UAS-hOCTs**



**B Scheme of the procedure of the recombination between UAS-CD8-RFP and UAS-hOATP1B1**



The same procedure was used to generate UAS-hOATP1B3, UAS-CD8-RFP stock and UAS-hOATP2B1, UAS-CD8-RFP.

**Supplementary Fig. 2**

**Representative images of ASP+ uptake by hOCT1*-*Ref and the negative control**

The uptake of ASP+ was detected by confocal microscopy 4h after injection only in the embryos expressing hOCT1-Ref (arrow) but not in the negative control.



**Supplementary Fig. 3**

**Time-dependent uptake of Et+ in cells expressing hOCT1 and hOCT2 and variants thereof**

Et+ fluorescence was measured after incubation of cells with 5 µM EtBr at 37 °C for different time points (A-C). (D) Quantification of hOCT1- or hOCT2-mediated or Et+ uptake after 15 min. Et+ fluorescence values were subtracted from the values of accumulation into vector-transfected cells and are expressed as % of uptake by hOCT1-Ref or hOCT2-A270. Data are from 3 independent experiments with 6 measurements performed in each experiment. Error bars indicate SE.

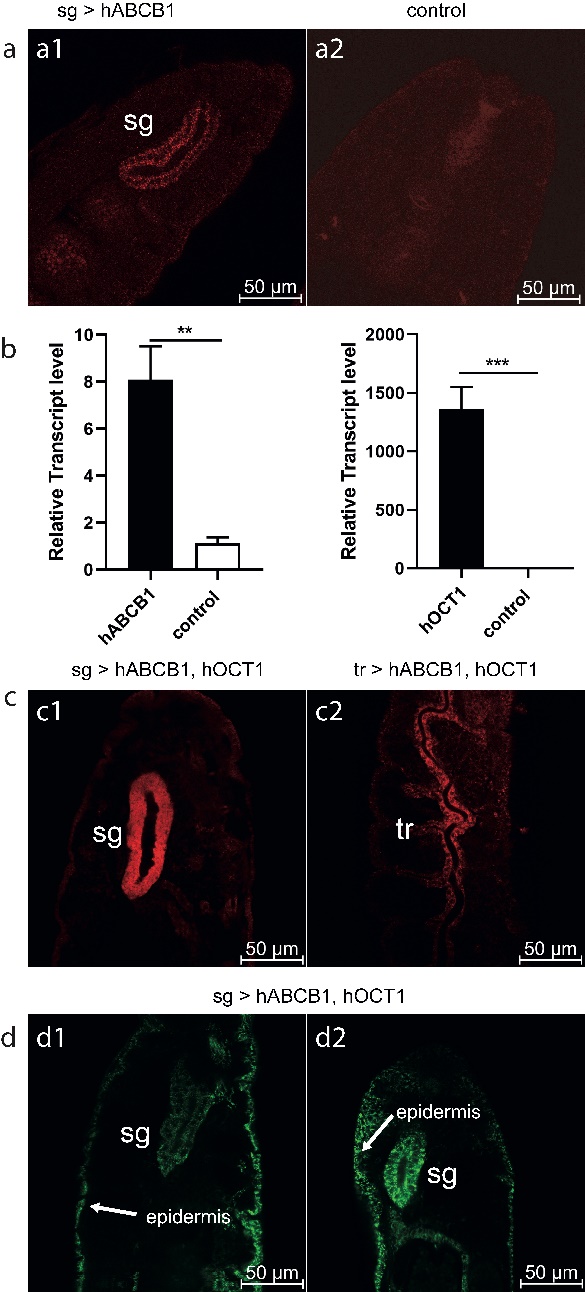
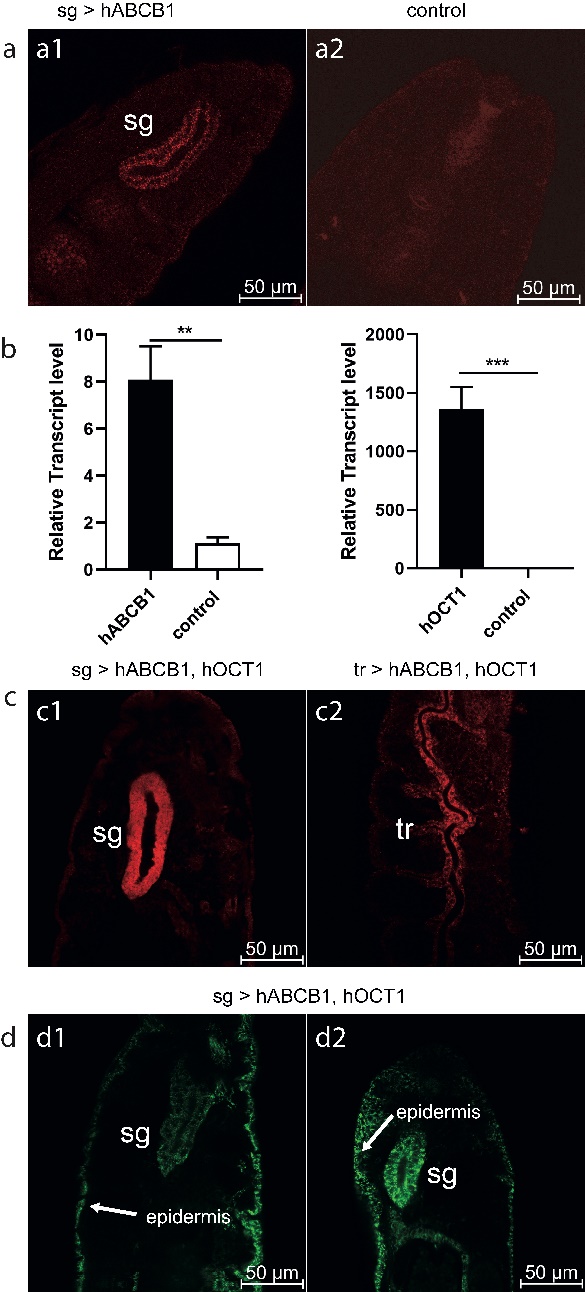
RFU, relative fluorescence unit



**Supplementary Fig. 4**

**Et+ and rhodamine 123 accumulation in salivary gland lumen by hOCT1-Ref and hABCB1**

(A) After formaldehyde fixation treatment and detection with the anti-hABCB1 (Anti-ABCB1, # HPA002199, Sigma) antibody, a signal was observed in the salivary glands of respective embryos (*fkh*-Gal4; UAS-hABCB1; A1). The staining of wild-type Dijon embryos is shown as the negative control (A2). Scale bar = 50 µm. (B) The expression of hABCB1 in the humanized hABCB1 and hOCT1 embryos (*fkh*-Gal4, UAS-hABCB1, UAS-hOCT1) was significantly higher than in wild-type Dijon embryos (B, \*\* p < 0.01). Embryos expressing hABCB1 and hOCT1 had a much higher transcription level of hOCT1 compared to wild-type Dijon embryos (B, \*\*\* p < 0.001). Data shown are mean ±SEM and represent two independent experiments. (C) Four hours after injection, the Et+ signal was not observed in the salivary gland lumen of embryos co-expressing hOCT1 and hABCB1 (*fkh*-Gal4, UAS-hOCT1, UAS-hABCB1) (C1). Similarly, there was no signal detected in the tracheal lumen of embryos expressing both transporters (*btl*-Gal4, UAS-hOCT1, UAS-ABCB1, C2). Scale bar = 50 µm. (D) Four hours after injection, the rhodamine 123 signal was observed in the salivary gland cells; the lumen was, however, clear (*fkh*-Gal4, UAS-hOCT1, UAS-hABCB1, D1, D2). Scale bar = 50 µm.



C

A

A

B2

B1

D2

D1

D

C1

C2

B

A2

A1

**Supplementary Fig. 5**

**Gene transcription levels in humanized embryos measured by qPCR**

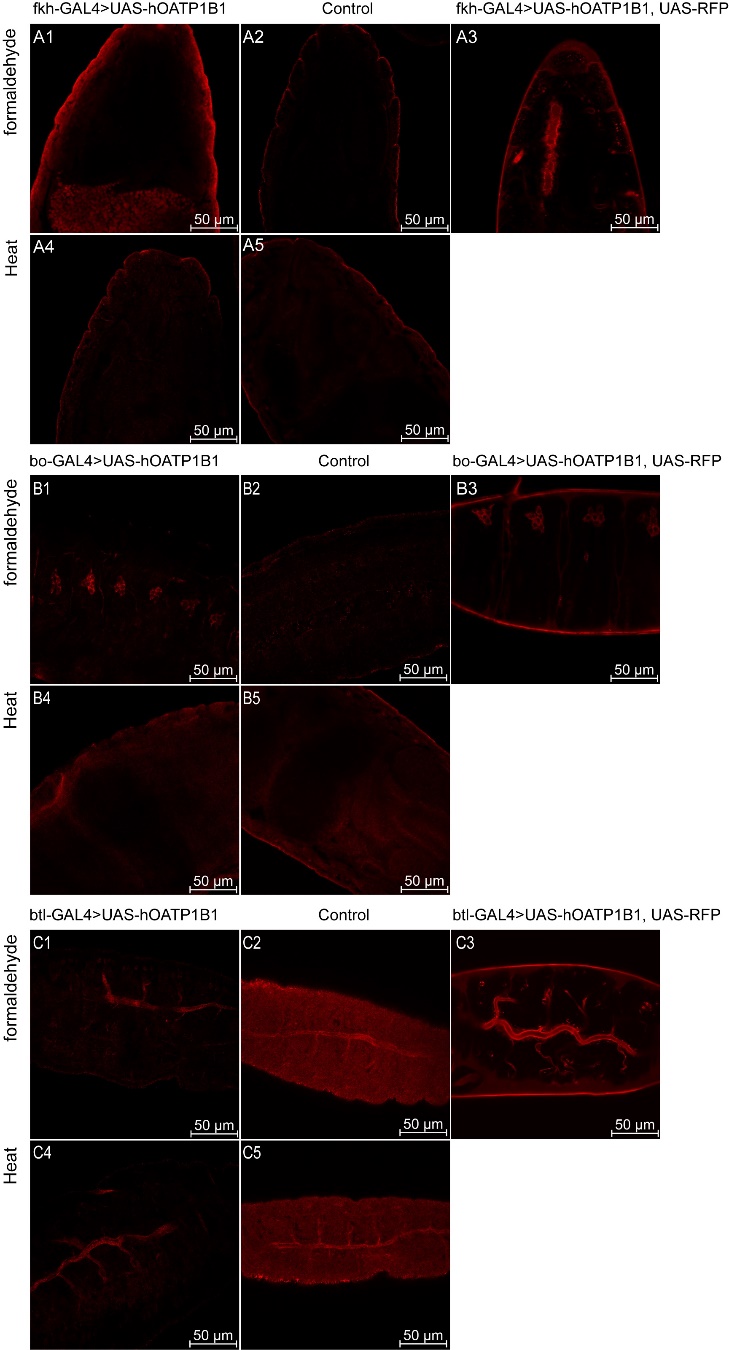
Compared to the wild-type Dijon embryos, expression of *hOATP1B1*, *hOATP2B1*, and *hOATP1B3* genes in respective embryos (*fkh*-Gal4, UAS-hOATP1B1*, fkh*-Gal4, UAS-hOATP2B1*, fkh*-Gal4, UAS-hOATP1B3) was significantly higher (\* p < 0.05, \*\* p < 0.01) than in control embryos. Data shown are mean ±SEM and represent two independent experiments.



**Supplementary Fig. 6**

**Localization of hOATP1B1 protein in *D. melanogaster* embryos**

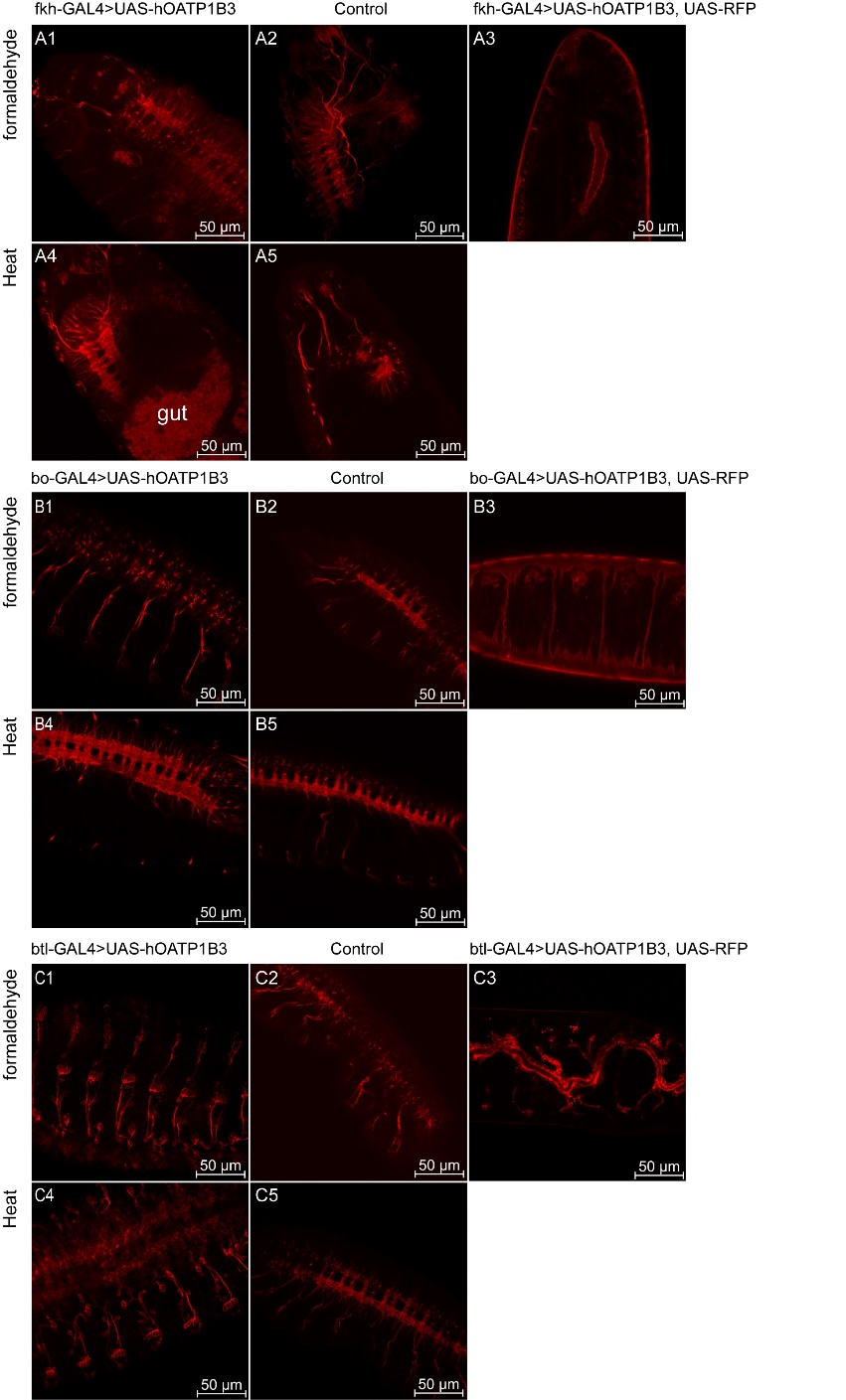
After formaldehyde and heat fixation, hOATP1B1 was not detected by antibody staining in the salivary glands of stage 16 embryos that expressed hOATP1B1 under the control of a salivary gland-specific Gal4 driver (*fkh*-Gal4; UAS-hOATP1B1; A1, A4). The staining of wild-type Dijon embryos is shown as the negative control (A2, A5). After formaldehyde fixation and staining with the antihOATP1B1 antibody, a signal was observed in the oenocytes of respective embryos (*bo*-Gal4; UAS-hOATP1B1; B1). No signal was detected after heat fixation (B4). Wild-type Dijon embryos were used as a negative control for hOATP1B1 antibody staining (B2, B5). The hOATP1B1 protein was localized in the tracheae of *btl*-Gal4; UAS-hOATP1B1 and wild-type Dijon embryos after formaldehyde or heat fixation (C1, C4, C2, C5). Targeted expression of RFP in the salivary glands, oenocytes, and tracheae allowed identifying this tissue by fluorescence microscopy (A3, B3, C3). The salivary glands, oenocytes, and tracheae are indicated by a white arrow. Scale bar = 50 μm.

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**Supplementary Fig. 7**

**The hOATP1B3 (OATP1B3, HPA004943, Sigma) antibody did not show localization of the protein in the *D. melanogaster* embryos.**

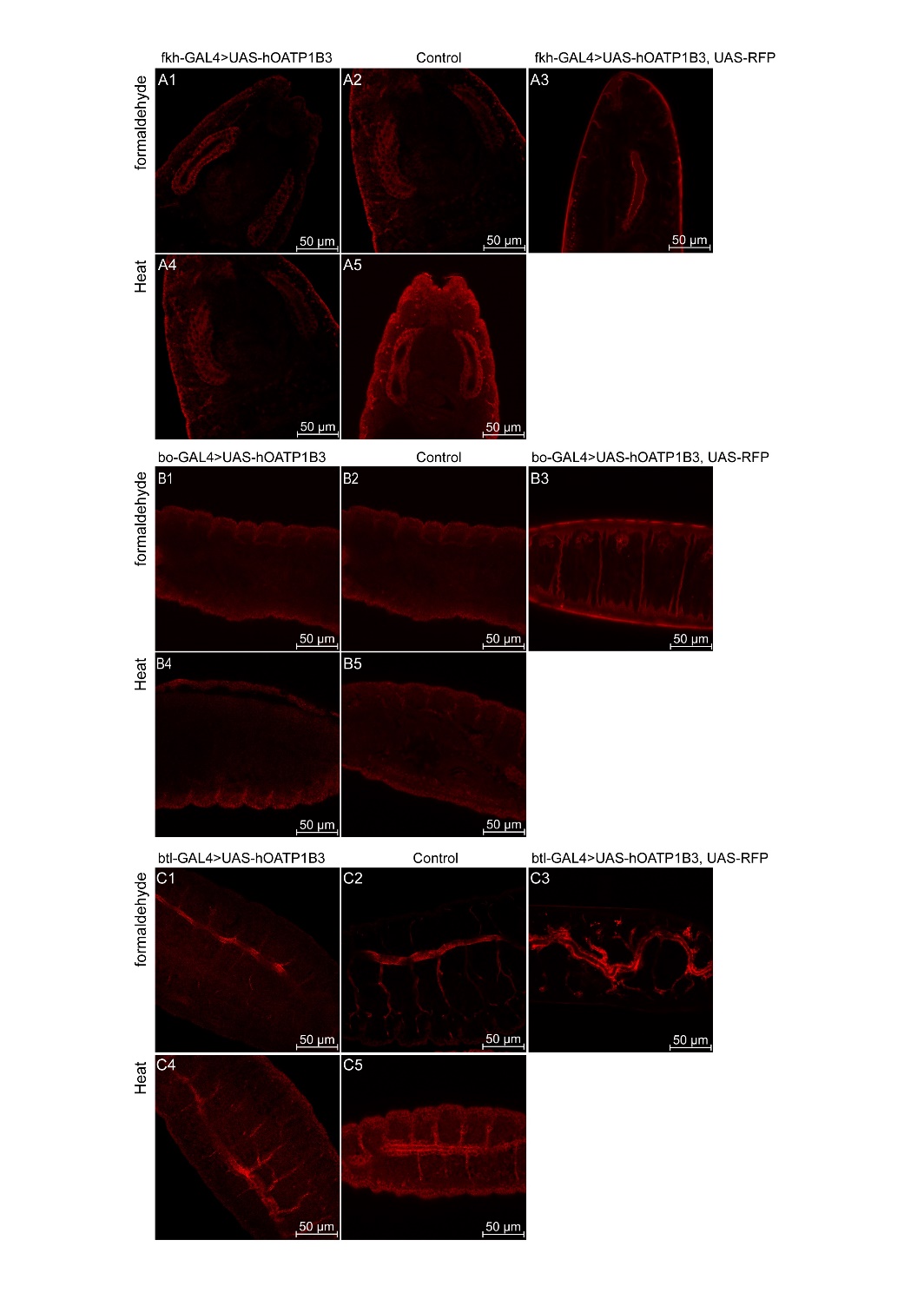
The hOATP1B3 was detected by antibody staining in the neuronal system that expresses hOATP1B3 under the control of a salivary gland-specific Gal4 driver (*fkh*-Gal4; UAS-hOATP1B3; A1, A4), oenocytes-specific Gal4 driver (*bo*-Gal4; UAS-hOATP1B3; B1, B4), and tracheae-specific Gal4 driver (*btl*-Gal4; UAS-hOATP1B3; C1, C4), and fixed with formaldehyde or by heat fixation. The staining of wild-type Dijon embryos was also observed signal in the neuron system (A2, A5, B2, B5, C2, C5). Targeted expression of RFP in the salivary glands, oenocytes, and tracheae allowed identifying this tissue by fluorescence microscopy (A3, B3, C3). The salivary glands, oenocytes, and tracheae are indicated by white arrows. Scale bar = 50 μm. Please note that the controls in Suppl. Figs. 7 and 8 A3, B3 and C3 are identical.

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**Supplementary Fig. 8**

**Immunodetection of hOATP1B3 (SKT antiserum, OATP1B3) did not show localization of the protein in the *D. melanogaster* embryos.**

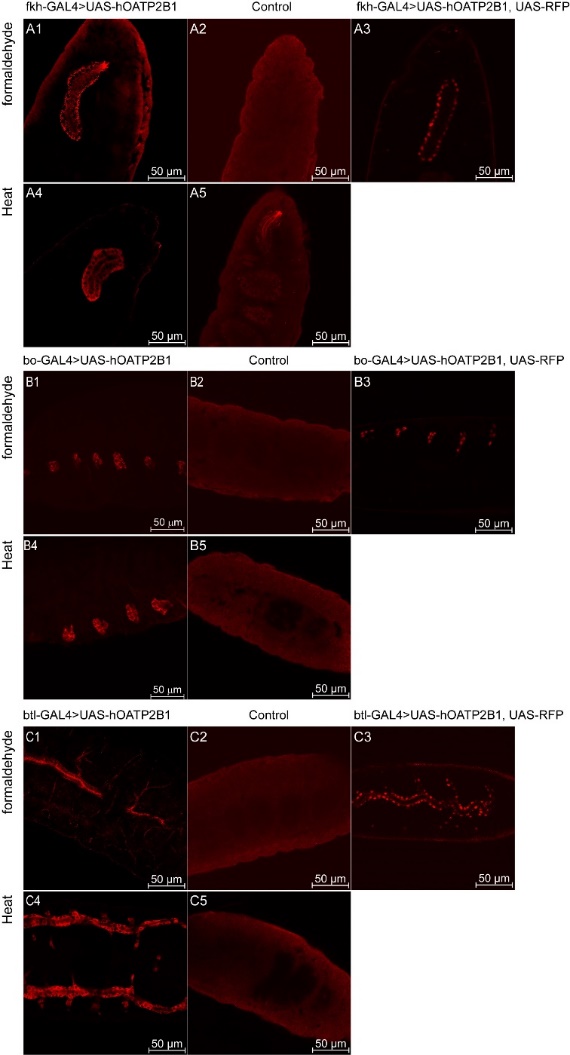
After formaldehyde or heat treatment fixation and staining with the anti-hOATP1B3 antibody, a signal was observed in the salivary glands and tracheae of respective embryos (*fkh*-Gal4; UAS-hOATP1B3; A1, A4; *btl*-Gal4; UAS-hOATP1B3; C1, C4), but the signal was also detected in wild-type Dijon embryos (A2, A5, C2, C5). No signal was detected after anti-hOATP1B3 staining in the oenocytes of embryos in hOATP1B3-flies (*bo*-Gal4; UAS-hOATP1B3; B1, B4) or in wild-type Dijon embryos (B2, B5) fixed with formaldehyde or by heat treatment. Targeted expression of RFP allowed identifying these tissues by fluorescence microscopy (A3, B3, C3). The salivary glands, oenocytes, and tracheae are indicated by white arrows. Scale bar = 50 μm. Please note that the controls in Suppl. Figs. 7 and 8 A3, B3 and C3 are identical.

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**Supplementary Fig. 9**

**Localization of hOATP2B1 protein in the *D. melanogaster* embryo**

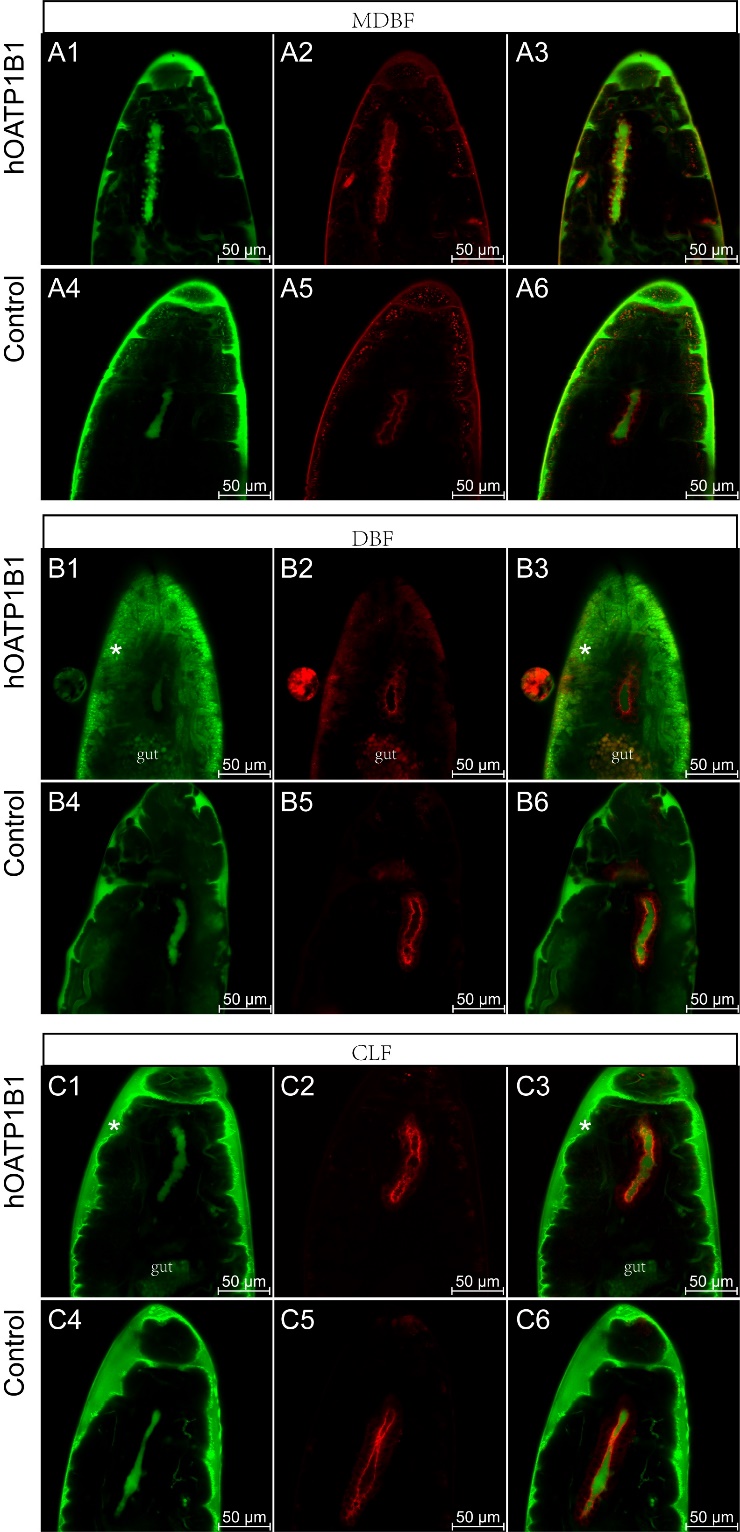
After formaldehyde or heat treatment fixation and staining with the anti-hOATP2B1 antibody, a signal was observed in the salivary gland of respective embryos (*fkh*-Gal4; UAS-hOATP2B1; A1, A4). But the signal was only detected in wild-type Dijon embryos by heat treatment (A2, A5). hOATP2B1 was detected by antibody staining in the oenocytes and tracheae that express hOATP2B1 under the control of an oenocytes-specific Gal4 driver (*bo*-Gal4; UAS-hOATP2B1; B1, B4) and tracheae-specific Gal4 driver (*btl*-Gal4; UAS-hOATP2B1; C1, C4) and fixed with formaldehyde or by heat fixation. The staining of wild-type Dijon embryos is shown as the negative control (B2, B5, C2, C5). Targeted expression of RFP in the salivary glands, oenocytes, and tracheae allowed identifying this tissue by fluorescence microscopy (A3, B3, C3). The salivary glands, oenocytes, and tracheae are indicated by white arrows. Scale bar = 50 μm.



**Supplementary Fig. 10**

**Fluorescent hOATP substrates penetrated into the lumen of salivary glands independently of hOATP1B1.**

Four hours after injection, the MDBF signal (A1), the DBF signal (B1), and the CLF signal (C1) were observed in the salivary gland lumen (*fkh*-Gal4; UAS-CD8-RFP). The salivary glands of embryos were marked by RFP co-expression (A2, A5, B2, B5, C2, C5). In negative control embryos (*fkh*-Gal4; UAS-CD8-RFP) injected with MDBF (A4), DBF (B4), or CLF (C4), a signal was detected in the salivary gland lumen. A3, A6, B3, B6, C3, and C6 show mergers of the GFP and RFP channels. Asterisks mark the space between eggshells and embryos. Scale bar = 50 µm. Please note that the controls in Suppl. Figs. 10, 11 and 12 are identical.

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**Supplementary Fig. 11**

**Fluorescent hOATP substrates were accumulated in the lumen of salivary glands independently of hOATP2B1.**

Four hours after injection, the MDBF (A1), the DBF (B1), and the CLF signals (C1) were observed in the salivary gland lumen (*fkh*-Gal4; UAS-CD8-RFPnls). The salivary glands of embryos were visualized through RFPnls co-expression by microscopy (A2, A5, B2, B5, C2, C5). In negative control embryos (*fkh*-Gal4; UAS-CD8-RFPnls) injected with MDBF (A4), DBF (B4), and CLF (C4), a signal was accumulated in the salivary gland lumen. A3, A6, B3, B6, C3, and C6 show mergers of the GFP and RFP channels. Asterisks mark the space between the eggshell and the embryo. Scale bar = 50 µm. Please note that the controls in Suppl. Figs. 10, 11 and 12 are identical.

A collage of images of a lung

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**Supplementary Fig. 12**

**Fluorescent hOATP substrates were taken up into the lumen of salivary glands independently of hOATP1B3.**

Four hours after injection, the salivary gland lumen of these embryos visualized through RFP co-expression (A2, A5, B2, B5, C2, C5) was observed by confocal microscopy. MDBF (A1), DBF (B1), and CLF signals (C1) were detected not only in the salivary gland lumen of these embryos but also in control embryos (*fkh*-Gal4; UAS-CD8-RFP) (A4), (B4), (C4). A3, A6, B3, B6, C3, and C6 show mergers of the GFP and RFP channels. Asterisks mark the space between eggshells and embryos. Scale bar = 50 µm. Please note that the controls in Suppl. Figs. 10, 11 and 12 are identical.

A collage of images of a human lung

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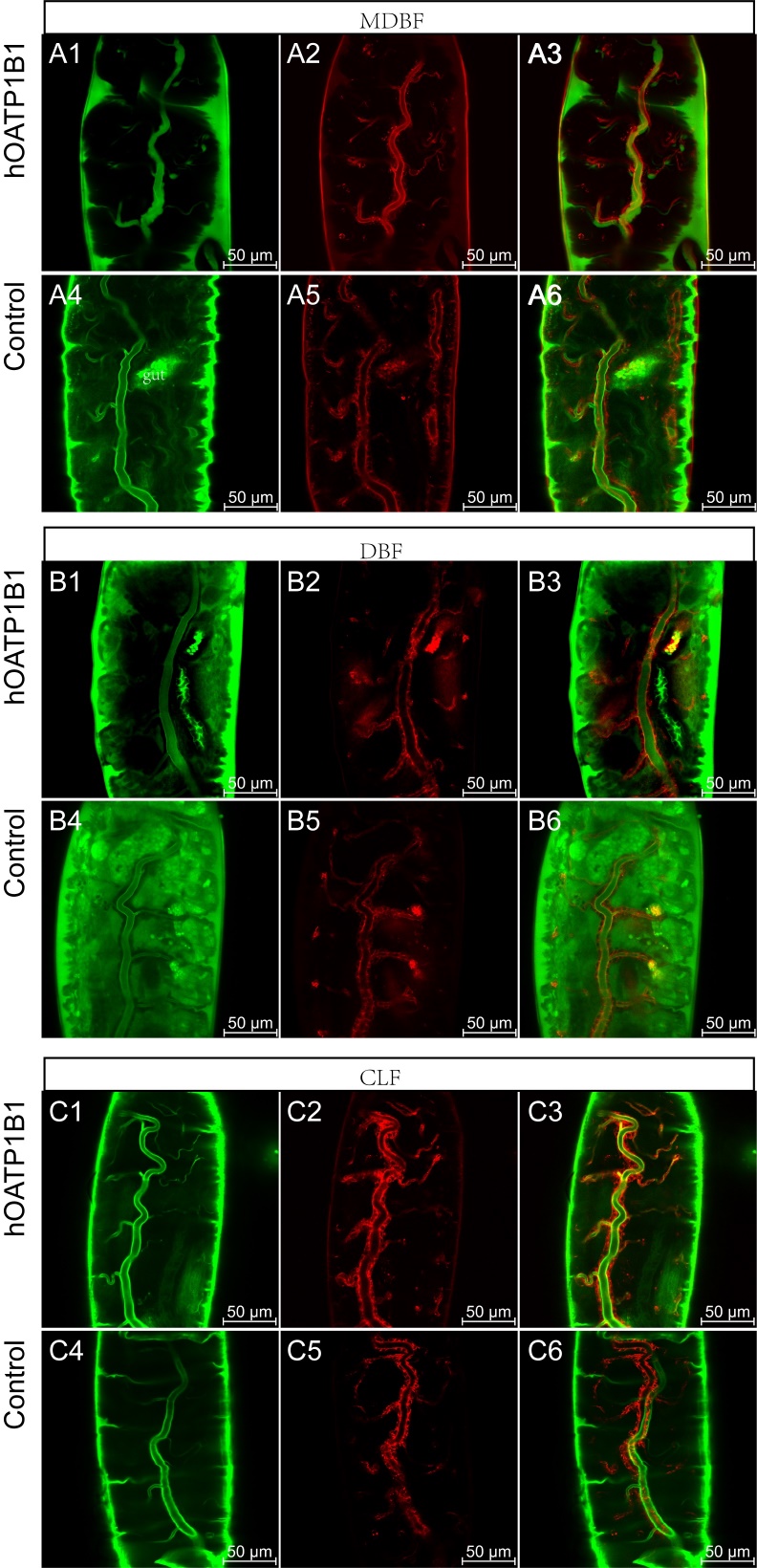
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**Supplementary Fig. 13**

**Fluorescent hOATP substrates accumulated in the lumen of the tracheae independently of hOATP1B1.**

Four hours after injection MDBF (A1), DBF (B1), and CLF signals (C1) were visualized not only in the tracheae of these embryos but also in control embryos (*btl*-Gal4; UAS-CD8-RFP) (A4), (B4), (C4). The tracheae of these embryos co-expressed RFP (A2, A5, B2, B5, C2, C5). A3, A6, B3, B6, C3, and C6 show merged images of the GFP and RFP channels. Asterisks mark the space between eggshells and embryos. Scale bar = 50 µm. Please note that the controls in Suppl. Figs. 13, 14 and 15 are identical.

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**Supplementary Fig. 14**

**Fluorescent hOATP substrates were accumulated in the tracheae independently of hOATP2B1.**

Four hours after injection, the tracheae of stage 15-16 embryos were visualized through RFPnls co-expression (A2, A5, B2, B5, C2, C5) by confocal microscopy. The MDBF (A1), DBF (B1), and CLF (C1) signals were observed in the tracheae of embryos expressing hOATP2B1 in their tracheae (*btl*-Gal4; UAS-hOATP2B1; UAS-CD8-RFPnls). Again, the MDBF (A4), DBF (B4), and CLF signal (C4) were also detected in the tracheae of control embryos (*btl*-Gal4; UAS-CD8-RFPnls). A3, A6, B3, B6, C3, and C6 show merged images of the GFP and RFPnls signals. Asterisks mark the space between eggshells and embryos. Scale bar = 50 µm. Please note that the controls in Suppl. Figs. 13, 14 and 15 are identical.

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**Supplementary Fig. 15**

**Fluorescent hOATP substrates were taken up into the lumen of tracheae independently of hOATP1B3.**

Four hours after injection, the tracheae were visualized through RFP co-expression (A2, A5, B2, B5, C2, C5) and the MDBF signal was observed in the tracheae (A1). Also, in negative control embryos (*btl*-Gal4; UAS-CD8-RFP) injected with MDBF, a signal was detected in the tracheae (btl-Gal4; UAS-CD8-RFP; A4). Similarly, a DBF signal (B1) and a CLF signal (C1) were detected in the tracheae of embryos expressing hOATP1B3 in their tracheae (*btl*-Gal4; UAS-hOATP1B3; UAS-CD8-RFP). Again, the DBF signal (B4) and the CLF signal (C4) were also detected in the tracheae of control embryos (*btl*-Gal4; UAS-CD8-RFP). A3, A6, B3, B6, C3, and C6 show merged images of the GFP and RFP signals. Asterisks mark the space between eggshells and embryos. Scale bar = 50 µm. Please note that the controls in Suppl. Figs. 13, 14 and 15 are identical.

A collage of images of a human body

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**Supplementary Fig. 16**

**Fluorescent hOATP substrates did not penetrate oenocytes expressing hOATP1B1.**

Four hours after injection MDBF (A1), DBF (B1), or CLF (C1), no signal was detected in respective embryos (*bo*-Gal4; UAS-hOATP1B1; UAS-CD8-RFP). Similarly, there was no signal detected in the oenocytes of negative control embryos (*bo*-Gal4; UAS-CD8-RFP) after injection of MDBF (A4), DBF (B4), or CLF (C4). The oenocytes (arrows) of stage 15-16 embryos were visualized through RFPnls co-expression (A2, A5, B2, B5, C2, C5). A3, A6, B3, B6, C3, and C6 show merged images of the GFP and RFP signals. Asterisks mark the space between eggshells and embryos. Scale bar = 50 µm. Please note that the controls in Suppl. Figs. 16, 17 and 18 are identical.

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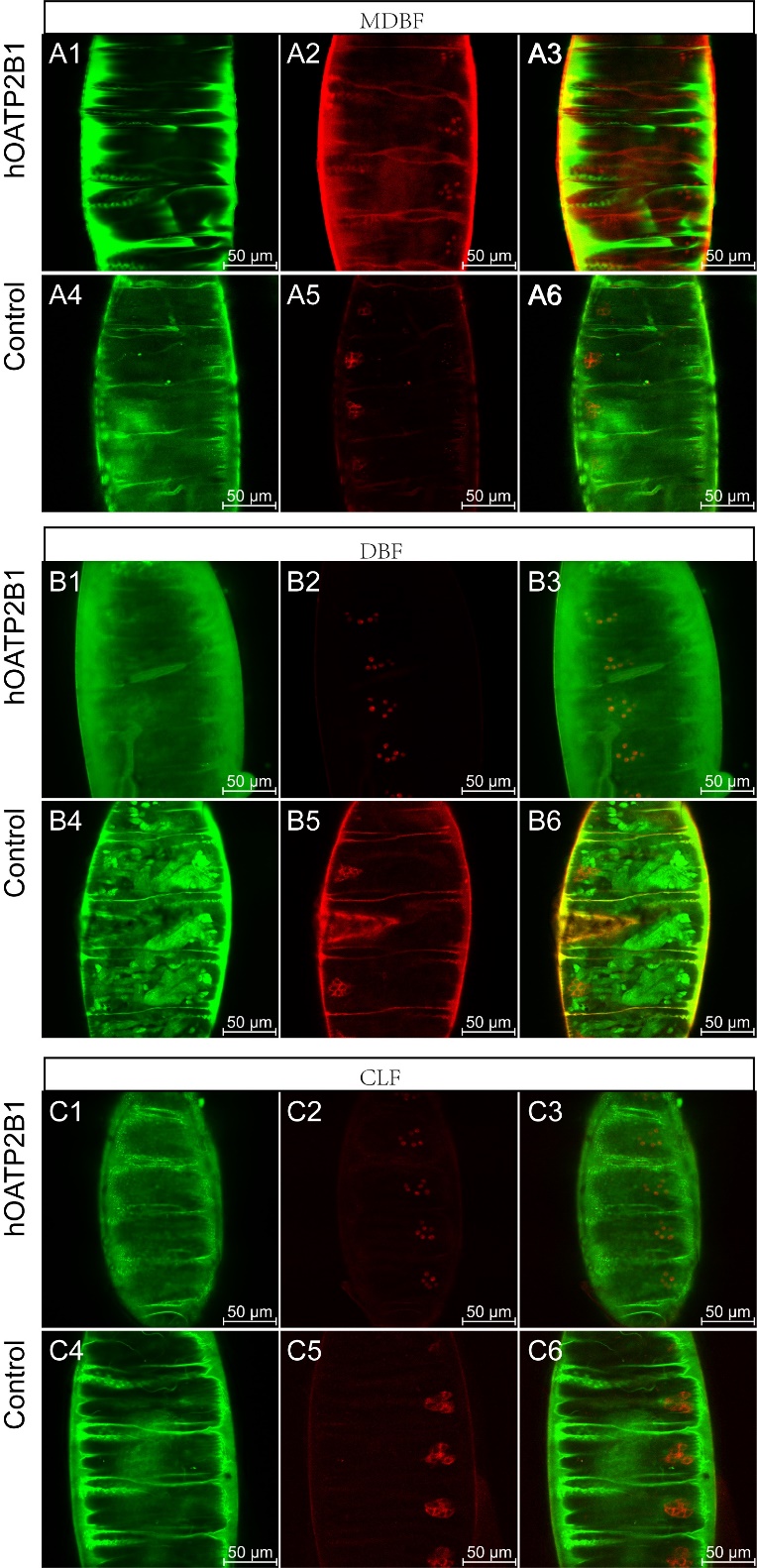
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**Supplementary Fig. 17**

**Fluorescent hOATP substrates were not accumulated in oenocytes expressing hOATP2B1.**

Four hours after injection into respective embryos (*bo*-Gal4; UAS-hOATP2B1; UAS-CD8-RFPnls), oenocytes (arrows) co-expressing RFPnls were observed (A2, A5, B2, B5, C2, C5). Neither MDBF (A1), nor DBF (B1), nor CLF (C1) was detected in the oenocytes of these embryos. Similarly, the MDBF (A4), DBF (B4), and CLF (C4) signals were not detected in the oenocytes of negative control embryos (*bo*-Gal4; UAS-CD8-RFPnls). A3, A6, B3, B6, C3, and C6 show merged images of the GFP and RFPnls signals. Asterisks mark the space between eggshells and embryos. Scale bar = 50 µm. Please note that the controls in Suppl. Figs. 16, 17 and 18 are identical.



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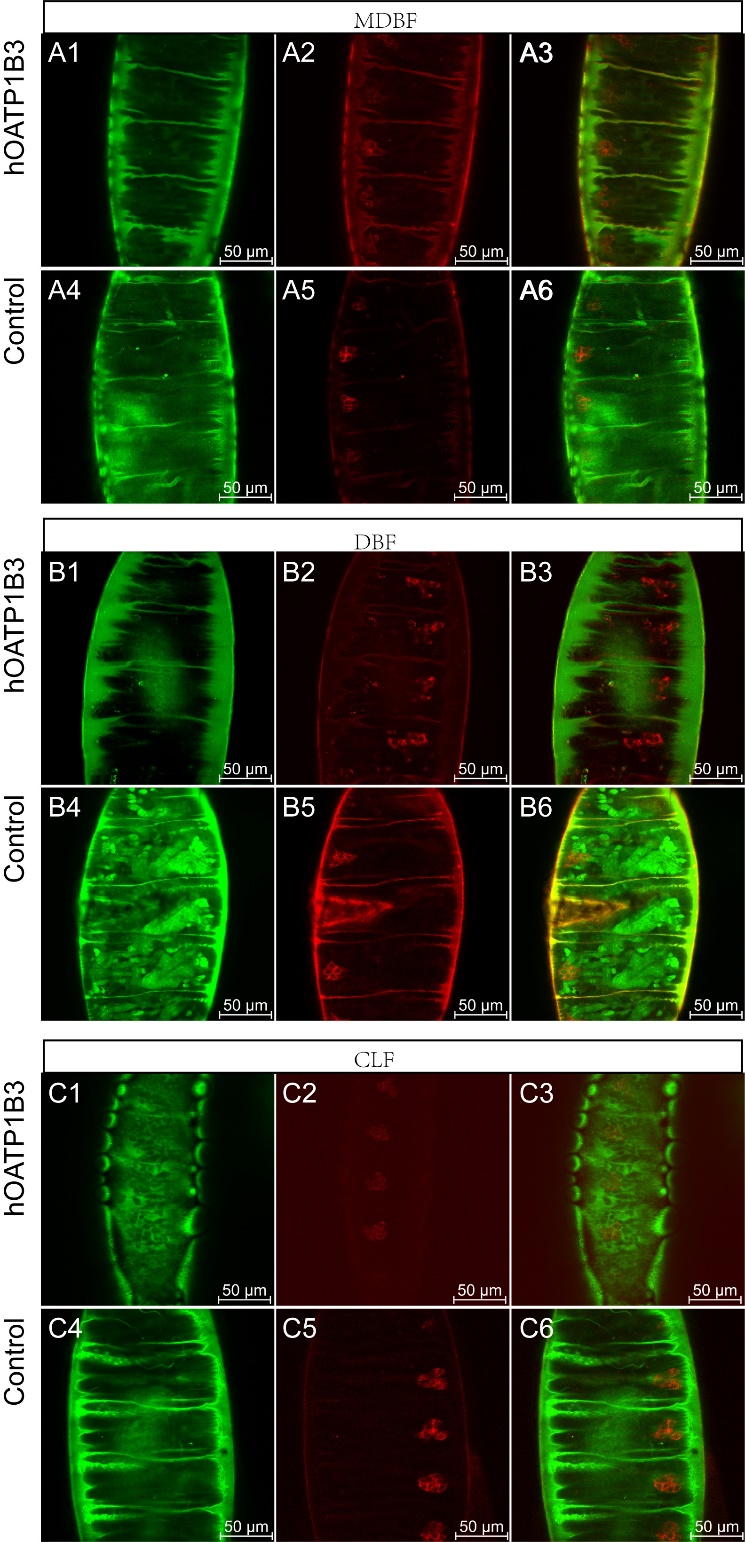
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**Supplementary Fig. 18**

**Fluorescent hOATP substrates were not taken up into the oenocytes by hOATP1B3.**

Four hours after injection into respective embryos (*bo*-Gal4; UAS-hOATP1B3; UAS-CD8-RFP), neither MDBF (A1), nor DBF (B1), nor CLF (C1) was detected in the oenocytes of these embryos. Likewise, in negative control embryos (*bo*-Gal4; UAS-CD8-RFP) no signal was observed in oenocytes after injection of MDBF (A4), DBF (B4), or CLF (C4). The oenocytes (arrows) were visualized through RFP co-expression (A2, A5, B2, B5, C2, C5). A3, A6, B3, B6, C3, and C6 show merged images of the GFP and RFP signals. Asterisks mark the space between eggshells and embryos. Scale bar = 50 µm. Please note that the controls in Suppl. Figs. 16, 17 and 18 are identical.



**Supplementary Table 1**

**Mutagenesis primers for generation of hOCT1 and hOCT2 variants**

|  |  |
| --- | --- |
| **Primer name** | **Primer sequence** |
| SLC22A1\_C61-for | 5’-tgagctgagccagtgctgtggctggag |
| SLC22A1\_C61-rev | 5’-CTCCAGCCACAGCACTGGCTCAGCTCA |
| SLC22A1\_F160-for | 5’-gaatgcgggcttcttctttggctctctcggt |
| SLC22A1\_F160-rev | 5’-ACCGAGAGAGCCAAAGAAGAAGCCCGCATTC |
| SLC22A1\_S401-for | 5’-cattgaccgcgtgagccgcatctaccc |
| SLC22A1\_S401-rev | 5’-GGGTAGATGCGGCTCACGCGGTCAATG |
| SLC22A1\_V408-for | 5’-gcatctaccccatggccgtgtcaaatttgttggcg |
| SLC22A1\_V408-rev | 5’-CGCCAACAAATTTGACACGGCCATGGGGTAGATGC |
| SLC22A1\_del420-for | 5’-ggcagcctgcctcgtcatttttatctcacctga |
| SLC22A1\_del420-rev | 5’-TCAGGTGAGATAAAAATGACGAGGCAGGCTGCC |
| SLC22A1\_R465-for | 5’-cattcgtcaggaacctcagagtgatggtgtgttcc |
| SLC22A1\_R465-rev | 5’-GGAACACACCATCACTCTGAGGTTCCTGACGAATG |
| SLC22A2\_S270-for | 5’-ggtggttgcagttcacagtttctctgcccaac |
| SLC22A2\_S270-rev | 5’-GTTGGGCAGAGAAACTGTGAACTGCAACCACC |

**Supplementary Table 2**

**A Primers for In-Fusion cloning**

|  |  |
| --- | --- |
| **Primer name** | **Primer sequence** |
| hOCT1-Rec-for | 5’-AGGGAATTGGGAATTCATGCCCACCGTGGATGAC |
| hOCT1-Rec-rev | 5’-ATCTGTTAACGAATTCTCAGGTGCCCGAGGGTTC |
| hOCT2-Rec-for | 5’-AGGGAATTGGGAATTCATGCCCACCACCGTGGAC |
| hOCT2-Rec-rev | 5’-ATCTGTTAACGAATTCTTAGTTCAATGGAATGTCTAG |
| Seq T-attB F | 5’-CAAGTAAATCAACTGCAACTACTG |
| Seq T-attB R | 5’-CACACCACAGAAGTAAGGTTCCTTC |
| hOATP1B1-Rec-for | 5’-TGAATAGGGAATTGGGAATTATGGACCAAAATCAACATTTGAA |
| hOATP1B1-Rec-rev | 5’-CGCAGATCTGTTAACGAATTCTACAGGAACAGGTGGTGGC |
| hOATP1B3-Rec-for | 5’-TGAATAGGGAATTGGGAATTATGGACCAACATCAACATTTGA |
| hOATP1B3-Rec-rev | 5’-CGCAGATCTGTTAACGAATTCTACAGGAACAGGTGGTGGC |
| hOATP2B1-Rec-for | 5’-TGAATAGGGAATTGGGAATTGCAGTCATGGGACCCAGG |
| hOATP2B1-Rec-rev | 5’-CGCAGATCTGTTAACGAATTCTACAGGAACAGGTGGTGGC |
| RFP (hOATP1B1)-for | 5’-ACATTGTTAAATGGTGCGCTCCTCCAAG |
| RFP (hOATP1B1)-rev | 5’-AGCGCACCATTTAACAATGTGTTTCACTATCTGCC |
| RFP (hOATP1B3)-for | 5’-TGCCAACTAAATGGTGCGCTCCTCCAAG |
| RFP (hOATP1B3)-rev | 5’-AGCGCACCATTTAGTTGGCAGCAGCATTGT |
| RFP (hOATP2B1)-for | 5’-CCGAGTGTGAATGGTGCGCTCCTCCAAG |
| RFP (hOATP2B1)-rev | 5’-AGCGCACCATTCACACTCGGGAATCCTCTGG |
| hABCB1-Rec-for | 5’-AGGGAATTGGGAATTATGGATCTTGAAGGGGACCGC |
| hABCB1-Rec-rev | 5’-ATCTGTTAACGAATTTCACTGGCGCTTTGTTCCAGC |

**B Primers for validation of transgenes in the recombined lines**

|  |  |
| --- | --- |
| **Primer name** | **Primer sequence** |
| OCT1\_for301 | 5’-AGCTGTGTAGACCCCCTGG |
| OCT1\_for701 | 5’-AACGGTGGCGATCATGTAC |
| OCT1\_for1049 | 5’-GATGTACCTGTGGTTCACGG |
| OCT1\_rev | 5’-AGCAAAGCCCAAAGAAAACA |
| OCT2\_rev2 | 5’-CTGTGTCTCCTGGGAACTGT |
| OCT1\_for368 | 5’-GGAGTCCTGCAGAGGAACTG |
| OCT2\_for699 | 5’-CCGTAAGCTCTGCCTCCTAAC |
| OCT2\_for1060 | 5’-AATGCTGAAGCCATGAGATC |
| OCT2\_for1421 | 5’-CAGCCTGTCTGTCTGGCCTCAG |
| hOATP1B1 | 5’-GCTCTGATTGATACAACG |
| hOATP2B1 | 5’-GAAAACCTGGCTGTTGTCCA |
| hOATP1B3 | 5’-TCCTAAGGACTCTCGTTGGGT |
| hABCB1-1 | 5’-CAGGGTTCTTCATGAATCTGG |
| hABCB1-2 | 5’-GGCCATCAGTCCTGTTCTTGG |
| hABCB1-3 | 5’-TAATGCTGACGTCATCGCTGG |
| hABCB1-4 | 5’-ATAAATGGAGGCCTGCAACC |
| hABCB1-5 | 5’-CCCATCATTGCAATAGCAGG |
| hABCB1-6 | 5’-GGCAAGTCAGTTCATTTGCTCC |
| hABCB1-7 | 5’-AGCGACTGAATGTTCAGTGG |

**Supplementary Table 3**

**List of the fly lines and their sources**

|  |  |
| --- | --- |
| **Strain** | **Source** |
| w1118, Wild-type | Bloomington Drosophila stock center, Bloomington, IN, USA |
| R24, Balancer stock for chromosome 3 | In house stock |
| UAS-mCD8-GFP | Parisa Kakanj (University of Cologne) |
| UAS-CD8-RFP | Bloomington Drosophila stock center |
| UAS-mCherry nls | Bloomington Drosophila stock center |
| UAS hOCT1 ref | BestGene Inc (Chino Hills, CA, USA) |
| UAS hOCT1 C61 | BestGene Inc |
| UAS hOCT1 F160 | BestGene Inc |
| UAS hOCT1 S401 | BestGene Inc |
| UAS hOCT1 V408 | BestGene Inc |
| UAS hOCT1 del420 | BestGene Inc |
| UAS hOCT1 R465 | BestGene Inc |
| UAS hOCT2 A270 | BestGene Inc |
| UAS hOCT2 S270 | BestGene Inc |
| UAS hOCT1 R61C; UASmCD8-GFP | Made in house |
| UAS hOCT1 F160; UASmCD8-GFP | Made in house |
| UAS hOCT1 S401 | Made in house |
| UAS hOCT1 V408 | Made in house |
| UAS hOCT1 del420 | Made in house |
| UAS hOCT1 R465 | Made in house |
| UAS hOCT2 A270 | Made in house |
| UAS hOCT2 S270 | Made in house |
| UAS-hABCB1 | BestGene Inc |
| UAS-hOATP1B1 | BestGene Inc |
| UAS-hOATP1B3 | BestGene Inc |
| UAS-hOATP2B1 | BestGene Inc |
| UAS-hOATP1B1; UAS-CD8-RFP | Made in house |
| UAS-hOATP2B1; UAS-mCherry nls | Made in house |
| UAS-hOATP1B3; UAS-CD8-RFP | Made in house |
| UAS-hOATP1B1-RFP | Made in house |
| UAS-hOATP2B1-RFP | Made in house |
| UAS-hOATP1B3-RFP | Made in house |
| fkh-Gal4 | Marta Llimargas (IBMB, Barcelona, Spain) |
| btl-Gal4 | Bloomington Drosophila stock center |
| bo-Gal4 | Bloomington Drosophila stock center |

**Supplementary Table 4**

**Primers for qPCR**

|  |  |
| --- | --- |
| **Primer name** | **Primer sequence** |
| q-hOCT1-for | 5’-CTCTGCCTCGTCATGATTTTT |
| q-hOCT1-rev | 5’-ACGAATGTGGGGTACAGCTC |
| q-hOCT2-for | 5’-TGGCCATTTCCCCAACCTAT |
| q-hOCT2-rev | 5’-AGGAGCCCAACTGTATAGGC |
| q-hOATP1B1-for | 5’-ACTGATTCTCGATGGGTTGG |
| q-hOATP1B1-rev | 5’-TATTTGGAGTTTGGGGCAAG |
| q-hOATP1B3-for | 5’-ATATGCTTCGTGGCATAGGG |
| q-hOATP1B3-rev | 5’-AAAGCCAATGACTGGACCAA |
| q-hOATP2B1-for | 5’-GAAAACCTGGCTGTTGTCCA |
| q-hOATP2B1-rev | 5’-CCATTGGACGGCTTTAACCC |
| q-hABCB1-for | 5’-CAAGAAGCCCTGGACAAAGC |
| q-hABCB1-rev | 5’-ATGCTCCTTGACTCTGCCAT |
| q-RpS20-Dmel-for | 5’-TTCGCATCACCACCCGTAAGAC |
| q-RpS20-Dmel-rev | 5’-TTGTGGATTCTCATCTGGAAGCG |

**Supplementary Table 5**

**Antibodies used for staining of OATP1B1, OATP1B3, OATP2B1 and ABCB1**

|  |  |  |  |
| --- | --- | --- | --- |
| **Antigen** | **Host species** | **Company** | **Dilution** |
| hOATP1B1 | rabbit | Thermo Fisher Scientific (MA3-934) | 1:500 |
| hOATP2B1 | rabbit | Abcam (ab222094) | 1:500 |
| hOATP1B3 | rabbit | Sigma-Aldrich (HPA004943) | 1:500 |
| hOATP1B3 | rabbit | Peptide Specialty Laboratories, Heidelberg, antiserum SKT described in König et al., 2000 | 1:500 |
| hABCB1 | rabbit | Sigma-Aldrich (HPA002199) | 1:100 |
| Rabbit IgG | goat | Invitrogen (A-11011) Alexa Fluor 568 conjugated | 1:500 |

J. König, Y. Cui, A.T. Nies, D. Keppler, Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide, J. Biol. Chem. 275 (2000) 23161-8. https://doi.org/10.1074/jbc.M001448200

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**Supplementary Table 6**

**Allele frequencies, other annotations and plasma membrane localizations of common hOCT1 and hOCT2 variants investigated in this study**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Genetic  variant** | **Nucleotide change**  **(Ref > Alt)** | **Amino acid change** | **Alt allele frequency Europeans [1]** | **In vitro localization** | |
| **HEK293 cells** | **Fruit fly salivary gland cells** |
| SLC22A1 | rs12208357 | C > T | p.R61>C | 0.063 | Reduced PM expression [1] | Similar PM expression as hOCT1-Ref |
| rs683369 | G > C | p.L160>F | 0.785 | ND [1] | Similar PM expression as hOCT1-Ref |
| rs34130495 | G > A | p.G401>S | 0.021 | Reduced PM expression [1] | Reduced PM expression |
| rs628031 | A > G | p.M408>V | 0.586 | ND [1] | Similar PM expression as hOCT1-Ref |
| rs202220802 | ATG >  in frame deletion | p.M420>del | 0.184 | Similar PM expression as hOCT1-Ref [1] | Similar PM expression as hOCT1-Ref |
| rs34059508 | G > A | p.G465>R | 0.02 | Reduced PM expression [1] | Reduced PM expression |
| SLC22A2 | rs316019 | T > G | p.S270>A | 0.89 | Similar PM expression of hOCT2-S270 and hOCT2-A270 [2] | Similar PM expression of hOCT2-S270 and hOCT2-A270 |

Ref, reference allele; Alt, alternate allele; PM, plasma membrane; ND, not determined

[1] S.W. Yee, D.J. Brackman, E.A. Ennis, Y. Sugiyama, L.K. Kamdem, R. Blanchard, A. Galetin, L. Zhang, K.M. Giacomini, Influence of transporter polymorphisms on drug disposition and response: a perspective from the International Transporter Consortium, Clin.Pharmacol.Ther. 104 (2018) 803–817. https://doi.org/10.1002/cpt.1098.

[2] O. Zolk, T.F. Solbach, J. König, M.F. Fromm, Functional characterization of the human organic cation transporter 2 variant p.270Ala>Ser, Drug Metab.Dispos. 37 (2009) 1312–1318. https://doi.org/10.1124/dmd.108.023762