Localization of Xenobiotic Transporters Expressed at the Human Blood-Testis Barrier Supplemental Material

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Supplementary Methods

CHO Cell Cultures

Untransfected Flp-In CHO cells were grown in F12K (Sigma-Aldrich, St. Louis, MO, USA, Catalog #N3520) supplemented with 10% fetal bovine serum, 1% penicillin–streptomycin, and 100 µg/mL zeocin in a 37°C humidified 5% CO_2 incubator. CHO cell lines stably expressing a human transporter were grown in the identical medium described above, except with 100 µg/mL hygromycin B instead of zeocin. All cells were washed with standard PBS during routine maintenance.

Immunocytofluorescence Staining

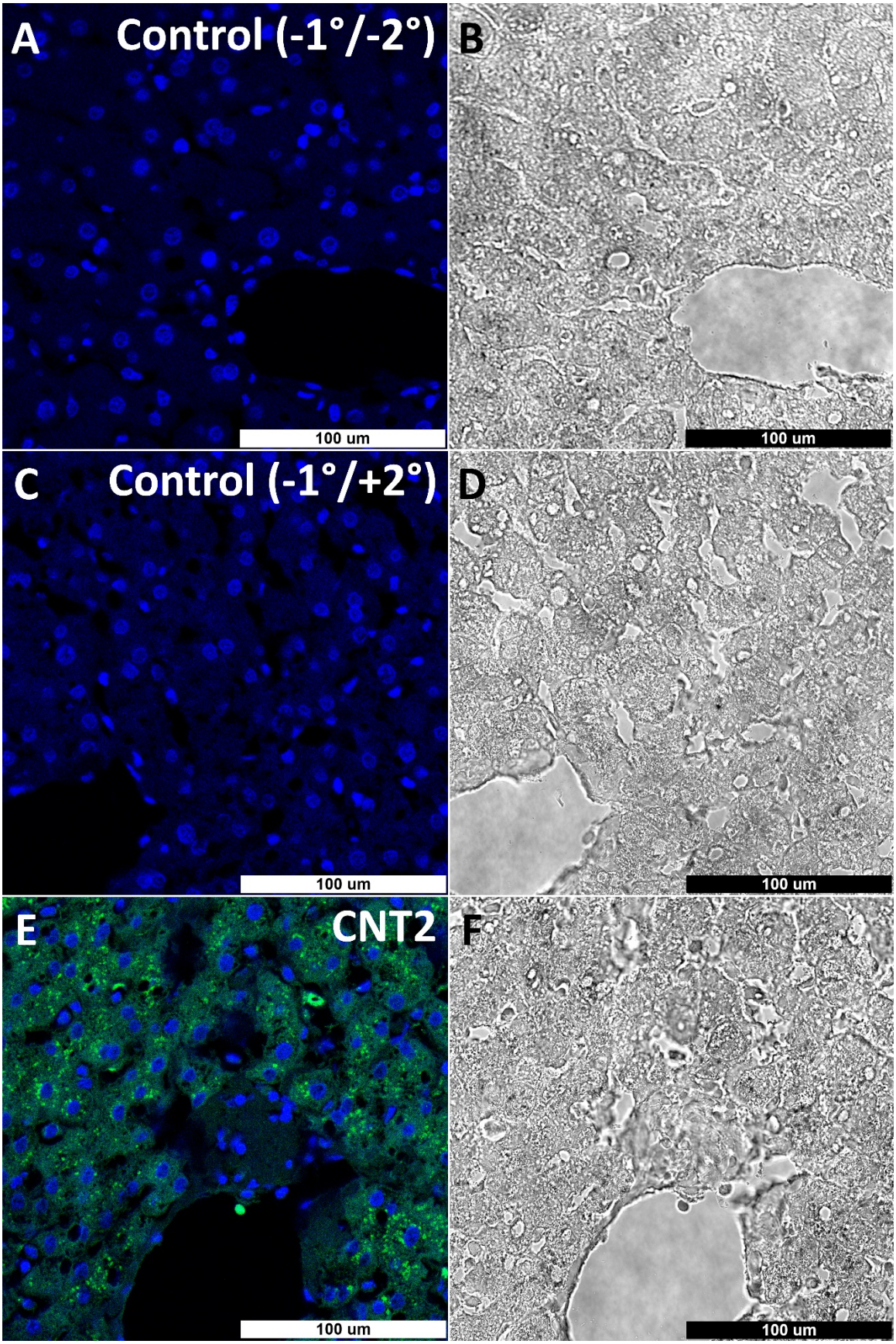
Flp-In CHO, CHO-OAT3, CHO-MATE2-K, and CHO-OATP6A1 cells were grown on round glass coverslips to 80-90% confluence before fixation with 100% ice-cold methanol for 20 min. Non-specific epitopes in each sample were blocked using a solution of 5% goat serum in PBS containing 0.1% Tween 20 (PBS-T) for 30 min. Following epitope blocking, the coverslips were washed with PBS-T three times and the samples were probed with a V5 Tag Monoclonal Antibody (1:1000, Invitrogen, Waltham, MA, USA, Catalog #R960) or a DYKDDDDK (Flag) Tag Antibody (1:10, Developmental Studies Hybridoma Bank, Iowa City, IA, USA, Catalog #12C6c) diluted in PBS-T with 2% goat serum for 1 hr at room temperature. The coverslips were washed three times with PBS-T before probing with Alexa Fluor 488 Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (1:1000, Invitrogen, Carlsbad, CA, USA, Catalog #A-11001) or Alexa Fluor 488 Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (1:1000, Invitrogen, Carlsbad, CA, USA, Catalog #A-11008) in PBS-T with 2% goat serum for 1 hr

at room temperature. Coverslips were washed three times with PBS-T, then rinsed with water before counterstaining the nuclei using 1.5 µg/mL DAPI for 5 min. Following nuclei staining, the coverslips were washed three times with PBS and rinsed once with water before mounting onto glass slides using ProLong™ Diamond Antifade Mountant (Invitrogen, Carlsbad, CA, Catalog #P36970).

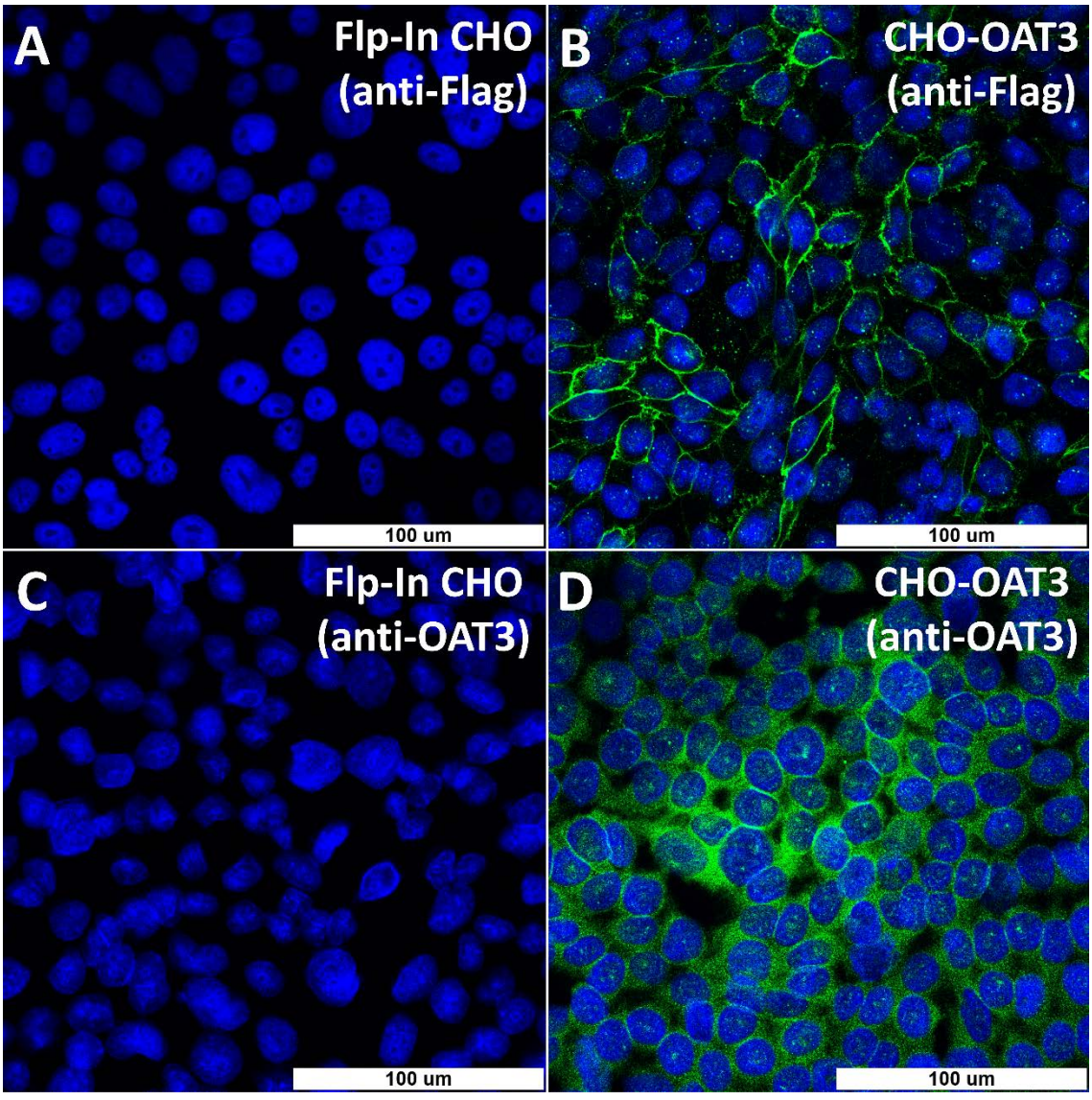
Slides were imaged using a Leica SP5-II confocal microscope (Leica Camera AG, Wetzlar, Germany) with a HC PL APO 40x/1.25 GLYC CORR CS2 objective (Leica Camera AG, Wetzlar, Germany). The image representing the green channel for Alexa Fluor 488-stained proteins and the image representing the blue channel for DAPI-stained nuclei were superimposed to generate the final merged image for each figure. The Flp-In CHO cells did not exhibit a fluorescent signal when probed with the V5, Flag, or transporter antibodies. Images were cropped from the original image to illustrate clearer staining patterns.

Supplementary Figures

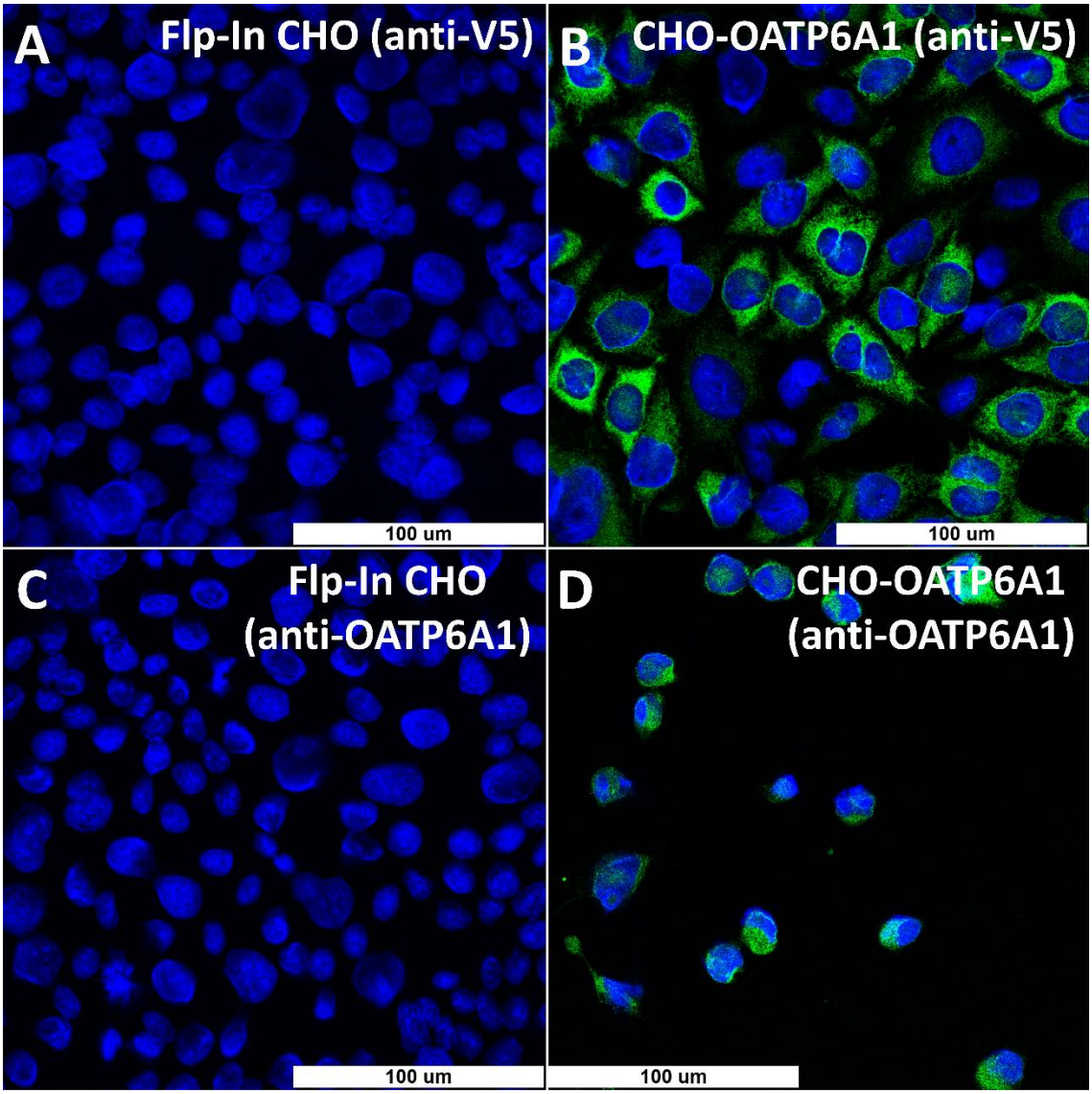
Supplementary Figure 1: Immunohistofluorescence analysis for CNT1 in formalin-fixed paraffin-embedded human liver tissue. Representative control images for all the following immunohistofluorescence experiments that were negative for a fluorescent signal when probed with (A) no primary or secondary antibodies or (C) only a secondary antibody. (E) Intense punctate staining in green for CNT1 was primarily observed in the cytosol of hepatocytes. Nuclei of all cells were counterstained with DAPI in blue. (B, D, F) Bright-field images of each tissue sample illustrates intact tissue structure. Images were captured at 40× magnification with a laser confocal microscope.


A
Control (-1°/-2°)
100 um
B
100 um
C
Control (-1°/+2°)
100 um
D
100 um
E
CNT2
100 um
F
100 um

Supplementary Figure 2: Immunocytofluorescence analysis for Flag-tagged human OAT3 in CHO cells. Flp-In CHO or CHO-OAT3 cells were probed with an (A, B) anti-Flag or (C, D) anti-OAT3 antibody to validate antibody specificity. Intense positive staining for (B) V5 was observed at the plasma membrane whereas (D) OAT3 was observed throughout the cytosol of the CHO-OAT3 cell line but no staining was observed in (A, C) Flp-In CHO control cells, indicating greater non-specificity of the OAT3 antibody for membrane-bound proteins. Nuclei of all cells were counterstained with DAPI in blue. Images were captured at 40× magnification with a laser confocal microscope.



Supplementary Figure 3: Immunocytofluorescence analysis for V5-tagged human OATP6A1 in CHO cells. Flp-In CHO or CHO-OATP6A1 cells were probed with an (A, B) anti-V5 or (C, D) anti-OATP6A1 antibody to validate antibody specificity. Intense positive staining for V5 and OATP6A1 was observed throughout the cytosol of the (B, D) CHO-OATP6A1 cell line but not in the (A, C) Flp-In CHO control cells, indicating antibody specificity for OATP6A1. Nuclei of all cells were counterstained with DAPI in blue. Images were captured at 40× magnification with a laser confocal microscope.



Supplementary Figure 4: Immunocytofluorescence analysis for V5-tagged human MATE2-K in CHO cells. Flp-In CHO or CHO-MATE2-K cells were probed with an (A, B) anti-V5 or (C, D) anti-MATE2 antibody to validate antibody specificity. Intense positive staining for V5 and MATE2 was observed throughout the cytosol of the (B, D) CHO-MATE2-K cell line but not in the (A, C) Flp-In CHO control cells, indicating antibody specificity for MATE2. Nuclei of all cells were counterstained with DAPI in blue. Images were captured at 40× magnification with a laser confocal microscope.