

## Supplemental Materials

### Title:

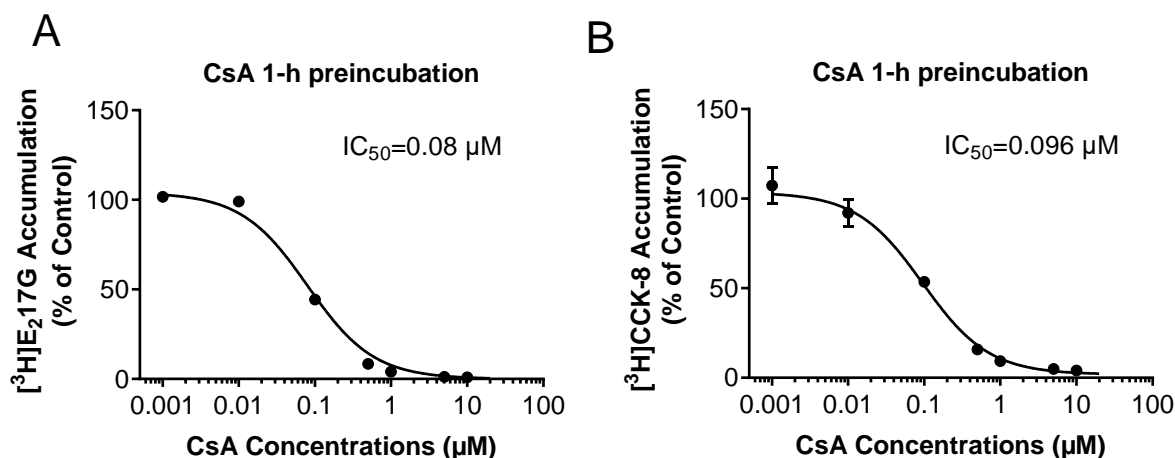
Differential preincubation effects of nicardipine on OATP1B1- and OATP1B3-mediated transport in the presence and absence of protein: implications in assessing OATP1B1- and OATP1B3-mediated drug-drug interactions

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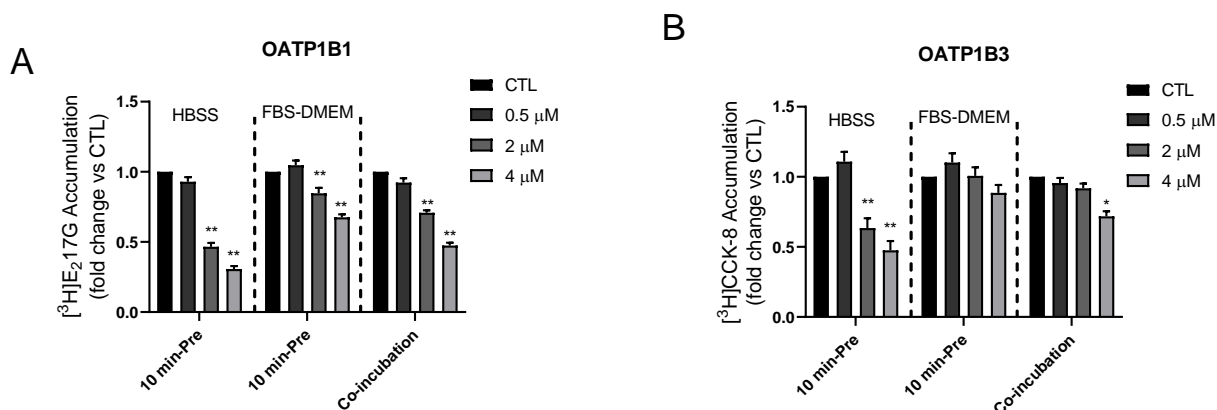
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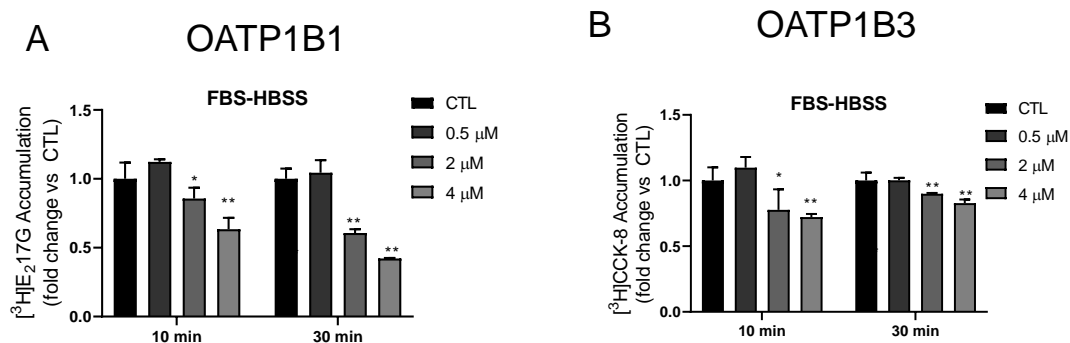
**Year:** 2023



**Figure S1. Preincubation  $\text{IC}_{50}$  values of CsA against OATP1B1 and OATP1B3.** HEK293-FLAG-OATP1B1 and HEK293-FLAG-OATP1B3 cells were preincubation with CsA at indicated concentrations for 1 h in FBS-DMEM culture medium followed by washing. OATP1B1-mediated  $[^3\text{H}]\text{E}_217\beta\text{G}$  accumulation (1 μM, 2 min) (A) and OATP1B3-mediated  $[^3\text{H}]\text{CCK-8}$  (1 μM, 3 min) accumulation (B) were determined in the absence of CsA in protein-free HBSS (10 mM HEPES, pH 7.4). Data represents mean  $\pm$  SD (n=1 in triplicate). Error bars in (A) are smaller than the symbol and appear invisible. The  $\text{IC}_{50}$  values were determined by fitting dose response curves to the data via nonlinear regression analysis same as described in the Material and Methods. Solid lines represent the fitted curves.



**Figure S2. Comparison of effects of 10-min pre-incubation and co-incubation with nicardipine.** The same 10 min-preincubation data in Fig. 1 and the same co-incubation data in Fig. 2 and Fig. 3 were replotted here for comparison purpose. Data represent model-estimated fold changes and associated SE vs. vehicle control treatment for both pre-incubation (N=3 for both OATP1B1 and OATP1B3) and co-incubation data (N=6), all in triplicate. Preincubation solution (HBSS or FBS-DMEM) are indicated. Linear mixed effects models were fit to the data as described in the “Materials and Methods”. \* and \*\* indicates statistically significant differences vs. CTL with Bonferroni-adjusted p values <0.05 and 0.01, respectively.



**Figure S3. Effects of preincubation with nicardipine in FBS-containing HBSS on OATP1B1- and OATP1B3-mediated transport.** (A) OATP1B1-mediated accumulation of [<sup>3</sup>H]E217βG (1 μM, 2 min) and (B) OATP1B3-mediated accumulation of [<sup>3</sup>H]CCK-8 (1 μM, 3 min) after 10 or 30 min preincubation with vehicle control (CTL) or nicardipine (0.5-4 μM). After preincubation and washing, substrate transport was determined in the absence of protein in HBSS (10 mM HEPES, pH 7.4) buffer. Data represent mean ± SD (n=1 in triplicate). \* and \*\* indicates statistically significant differences vs. CTL by one way analysis of variance (ANOVA) with Bonferroni-adjusted p values <0.05 and 0.01, respectively.