Sensitivity analysis on transport inhibition of arbitrary substrates

In order to determine whether the inhibition of verapamil toward cABCB1 is applicable to an arbitrary substrate with varying K_m values, sensitivity analysis was carried out. The transport velocity of a substrate in the presence of an inhibitor is as shown in Eq. 1. In addition, IC_{50} could be described as Eq. 2 (i.e., rearrangement of Cheng-Prusoff equation), and assuming that active transport is predominant (i.e., $V_0/V_{max} = 0$), Eq. 1 could be rearranged as Eq 3 and 4:

$$V = V_{max} - (V_{max} - V_0) \cdot \left[\frac{[I]}{[I] + IC_{50}}\right] \quad (1)$$

$$IC_{50} = K_i \cdot \left(1 + \frac{[S]}{K_m}\right) \quad (2)$$

$$V = V_{max} - (V_{max} - V_0) \cdot \frac{[I]}{[I] + K_i\left(1 + \frac{[S]}{K_m}\right)} \quad (3)$$

$$\frac{V}{V_{max}} = 1 - \frac{[I]}{[I] + K_i\left(1 + \frac{[S]}{K_m}\right)} \quad (4)$$

where K_i, K_m and [S] represent the inhibition coefficient of the inhibitor, the Michaelis-Menten constant and concentration of the substrate, respectively. By using Berkeley Madonna™ software (version 8.3.18; University of California, Berkeley, CA, USA), % inhibition of the transport was then simulated using the equation below (Eq. 5):

$$\%\ inhibition = 100 \times \left(1 - \frac{V}{V_{max}}\right) \quad (5)$$

Inhibition of olaparib efflux using other ABCG2 inhibitors

To further determine hABCG2-mediated transport of olaparib, transcellular transport of olaparib was also studied in the presence of hABCG2 inhibitors other than Ko143 (i.e., elacridar or fumitremorgin C). The transcellular transport of 10 μM olaparib was determined in verapamil-treated or shRNA-transfected MDCKII/WT and MDCKII/hABCG2 cells, and 1 μM Ko143, 10 μM elacridar or 10 μM fumitremorgin C was added for inhibition of the transport. The transport was studied for a period of 120 min at 37˚C, and concentrations of olaparib were determined by chromatographic quantification at the end of the incubation.

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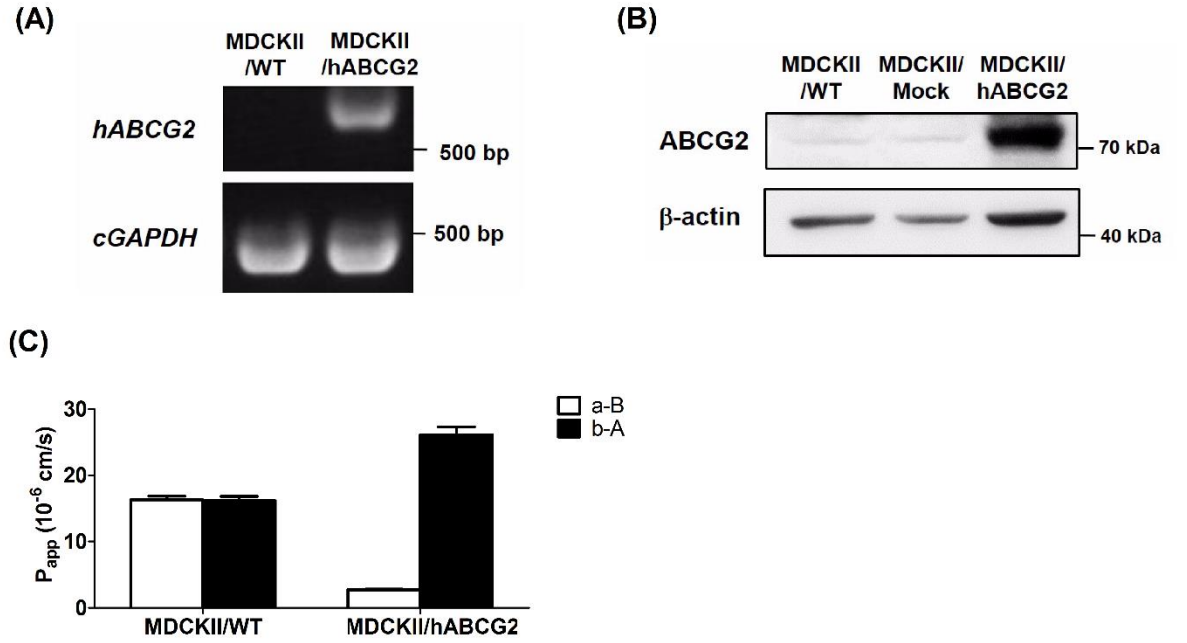

Figure S1. (A) Agarose gel electrophoresis of PCR products using the total RNA extract from MDCKII/WT and MDCKII/hABCG2 cells. PCR products were generated by *hABCG2* (product size: 577 bp) and *cGAPDH* (product size: 497 bp) primer, respectively. (B) Western blot analysis from cell lysates (60 μg) of MDCKII/WT, MDCKII/Mock and MDCKII/hABCG2, probed with anti-ABCG2 and anti-β-actin antibodies, respectively. (C) The functional expression of hABCG2 in MDCKII/hABCG2, compared to MDCKII/WT cells, as evaluated by bi-directional transport of [^{3}H]-prazosin. The apparent permeability coefficients of the compound are shown in apical to basolateral (a-B) and basolateral to apical (b-A) directions. Data are presented as mean ± S.D. of triplicate runs.

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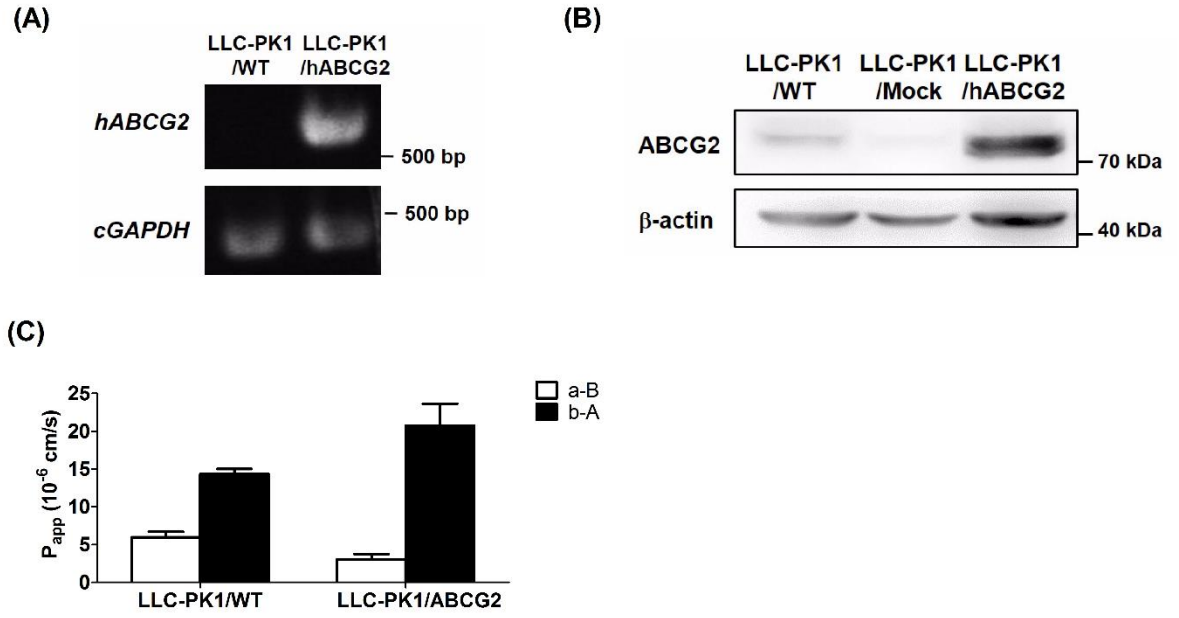

Figure S2. (A) Agarose gel electrophoresis of PCR products using the total RNA extract from LLC-PK1/WT and LLC-PK1/hABCG2 cells. PCR products were generated by *hABCG2* (product size: 577 bp) and *porcine GAPDH* (product size: 396 bp) primer, respectively. (B) Western blot analysis from cell lysates (60 μg) of LLC-PK1/WT, LLC-PK1/Mock, and LLC-PK1/hABCG2, probed with anti-ABCG2 and anti-β-actin antibodies, respectively. (C) The functional expression of hABCG2 in LLC-PK1/hABCG2, compared to LLC-PK1/WT cells, as evaluated by bi-directional transport of [^{3}H]-prazosin. The apparent permeability coefficients of the compound are shown in apical to basolateral (a-B) and basolateral to apical (b-A) directions. Data are presented as mean ± S.D. of triplicate runs.

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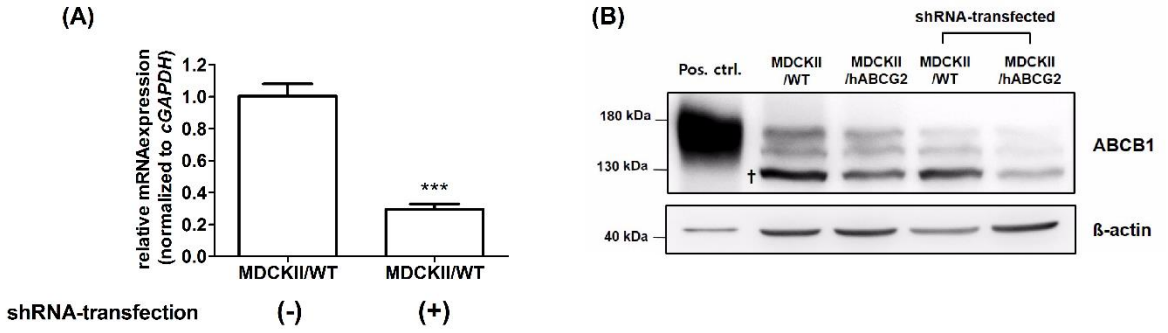

Figure S3. (A) Expression levels of *cABCB1* mRNA in shRNA-transfected and non-transfected MDCKII cell lines were evaluated by qPCR. Data are presented as mean ± S.D. of triplicate runs. Asterisks indicate statistical difference from the control (i.e., without transfection) by the unpaired t test (***$P < 0.005$). (B) Expression levels of cABCB1 protein in shRNA-transfected and non-transfected MDCKII cell lines were evaluated by Western blot. The protein amount of sample in the Western blot analysis was 200 μg for ABCB1 and 60 μg for β-actin. In parallel, 10 μg (i.e., for the detection of ABCB1) or 3 μg (i.e., for the detection of β-actin) protein was loaded for positive control (i.e., NCI/ADR-RES). Dagger in panel B indicates potential interaction of the antibody with ABCB4 (Schinkel et al., 1991; Scheffer et al., 2000).

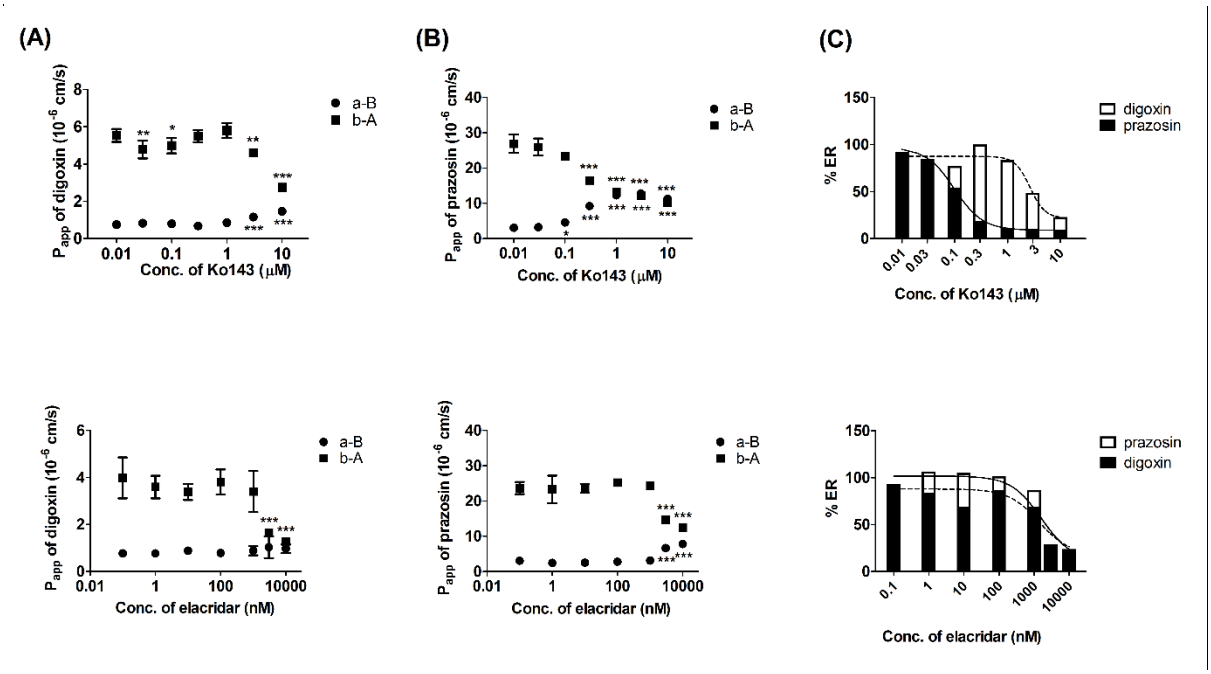

Figure S4. The inhibition profiles of Ko143 in various concentrations against the transport of digoxin in MDCKII/WT cells (A) and prazosin in MDCKII/hABCG2 cells (B) are shown in apical to basolateral (a-B) and basolateral to apical (b-A) direction. The percent of control ER (%ER) was shown together with the best-fit values obtained from the nonlinear regression analysis based on Eq. 1 (C). Similarly, the inhibition profiles of elacridar against the transport of digoxin in MDCKII/WT cells (D) and prazosin in MDCKII/hABCG2 cells (F) are shown together with the %ER values (G). Asterisks indicate statistical differences (*P < 0.05, **P < 0.01; ***P < 0.001) from the control group (i.e., without inhibitor) by one-way ANOVA, followed by the Tukey's *post hoc* test. Data are presented as mean ± S.D. of triplicate runs.

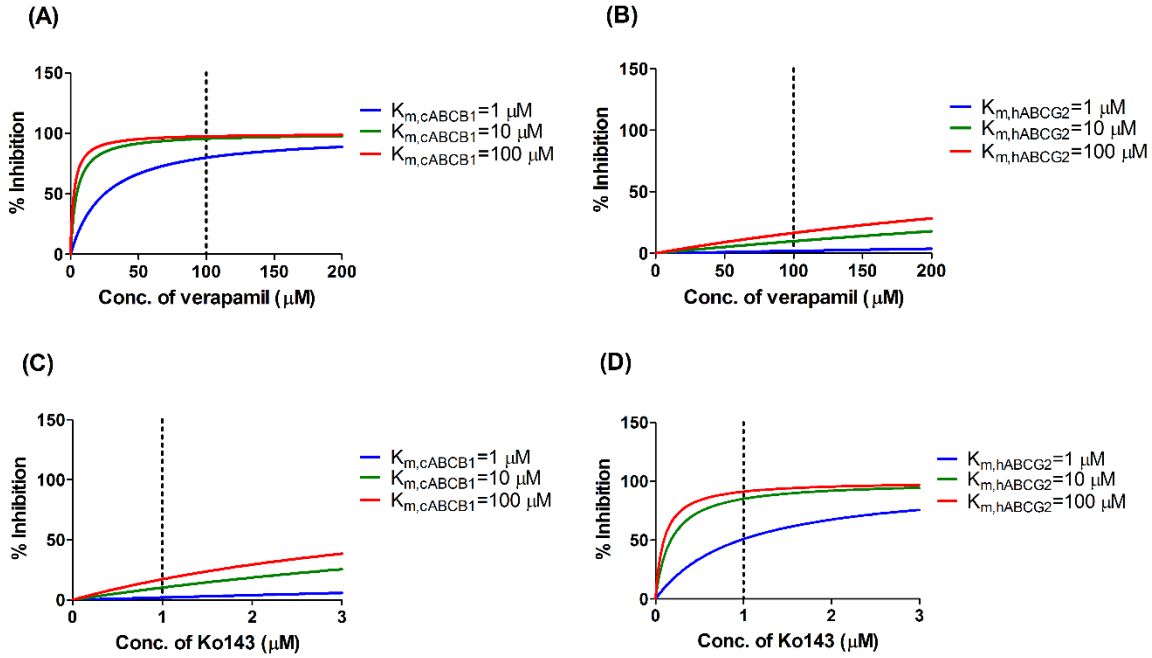

Figure S5. Sensitivity analysis of transport inhibition by 100 μM verapamil towards cABCB1 (A) and hABCG2 (B), and sensitivity analysis of transport inhibition by 1 μM Ko143 towards cABCB1 (C) and hABCG2 (D) assuming arbitrary substrate having a K_m value of 1~100 μM towards cABCB1 and hABCG2, respectively.

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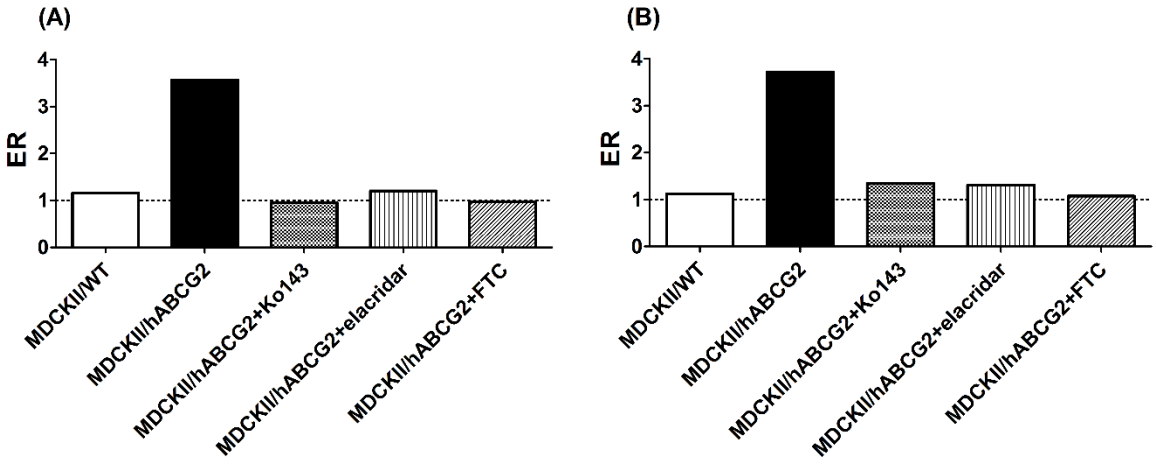

Figure S6. Bi-directional transport of olaparib in the presence of verapamil treatment (A), or in the presence of shRNA-transfected (B) conditions for MDCKII/WT and MDCKII/hABCG2 cells. When necessary, Ko143 (1 μM), elacridar (10 μM), or fumitremorgin C (FTC; 10 μM) was added to the incubation mixture.