1. (b)



(c)



**Supplementary Figure S1.** Titration of transport substrates for development of flow cytometry assay. HEK293T cells were transiently transfected with pABCB1. Cells were labelled with the anti-ABCB1 primary 4E3 antibody as described in the methods. The red-fluorescent secondary antibody was added and the cells divided into 100 μl aliquots to which were added increasing concentrations of green-fluorescent transport substrate. After 20 minutes at 37oC the cells were recovered by gentle centrifugation, washed and resuspended in transport buffer for flow cytometry. Ten thousand cells of normal size and granularity were analysed for antibody binding to distinguish the ABCB1-expressing from the untransfected cells. The level of transport substrate uptake by the two populations was plotted against the final concentration of transport substrate added to the cell samples using Graphpad Prism version 8. The transport substrate content of the ABCB1-expressing (transfected) cells is shown in red (left Y axis) and the non-expressing (untransfected) cells in black (right Y axis) for: **(a)** Calcein-AM; **(b)** BODIPY-verapamil; **(c)** OREGON GREEN taxol bisacetate. The concentration of each drug to use for subsequent experiments was determined as the point at which the red curve begins to accumulate transport substrate indicating that transporter density at the plasma membrane is becoming saturated. This was deemed to be 500 nM for Calcein-AM, 800 nM for BODIPY-verapamil and 400 nM for OREGON GREEN-Taxol bisacetate. These concentrations also provide good discrimination between the ABCB1-expressing and non-expressing cells in the population but are minimal thus limiting any possible cytotoxic effects of verapamil or taxol derivatives during the assay.

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Q725/990A

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WT

**Supplementary Figure S2.** Confirmation of equivalent levels of ABCB1 at the plasma membrane ensured that any differences in transport activity were due to ABCB1 functionality rather than the transporter expression level. Histogram of the 4E3-positive populations showing significant overlap in red fluorescence of wild-type ABCB1 and each of the mutant transporters.