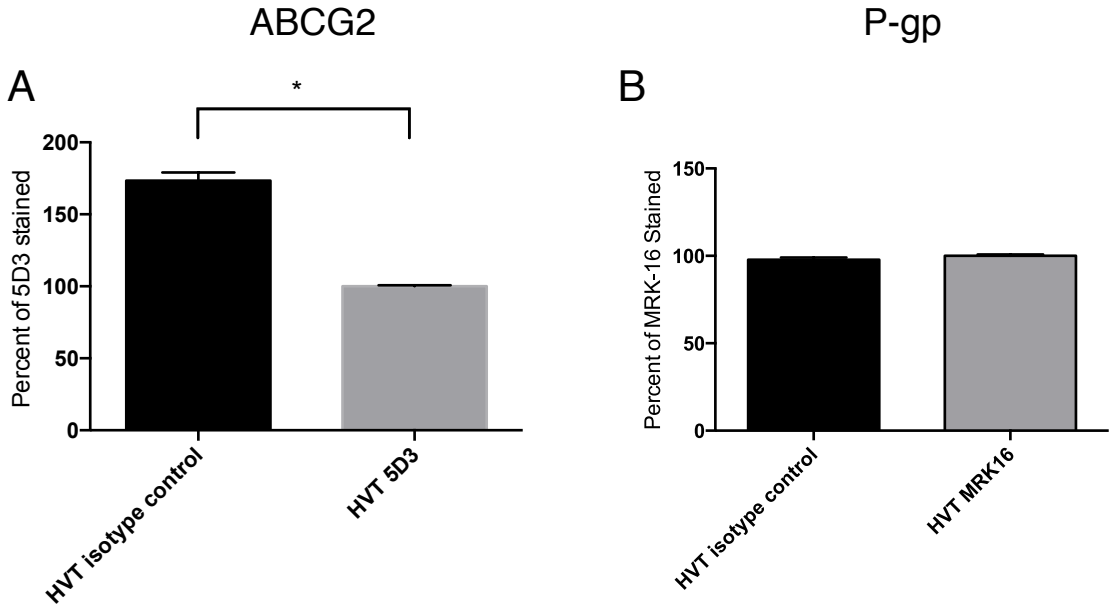


## **Supplementary Information**

### **Bioluminescent imaging of ABCG2 efflux activity at the blood-placenta barrier**

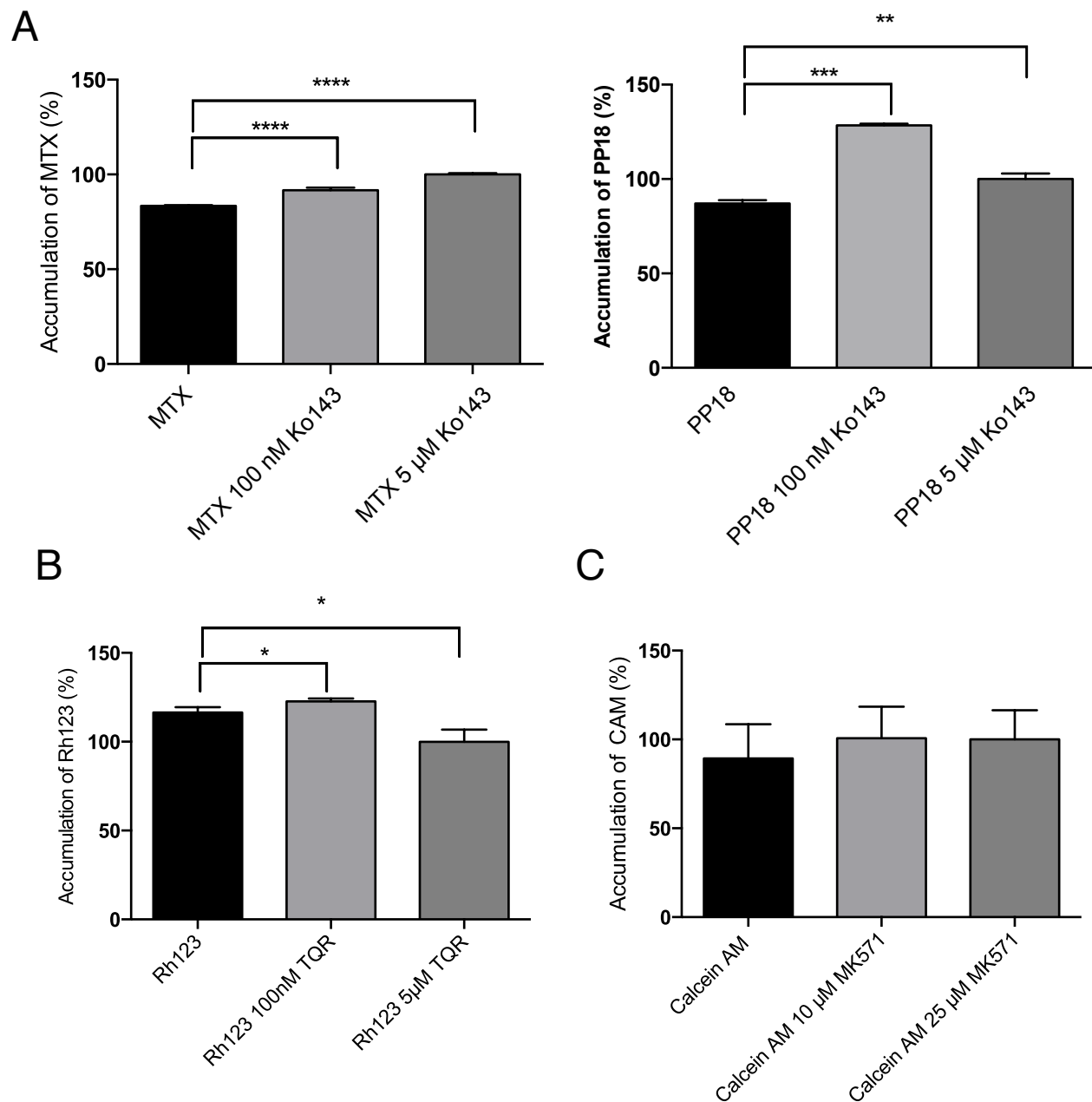
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Supplementary Figure S1



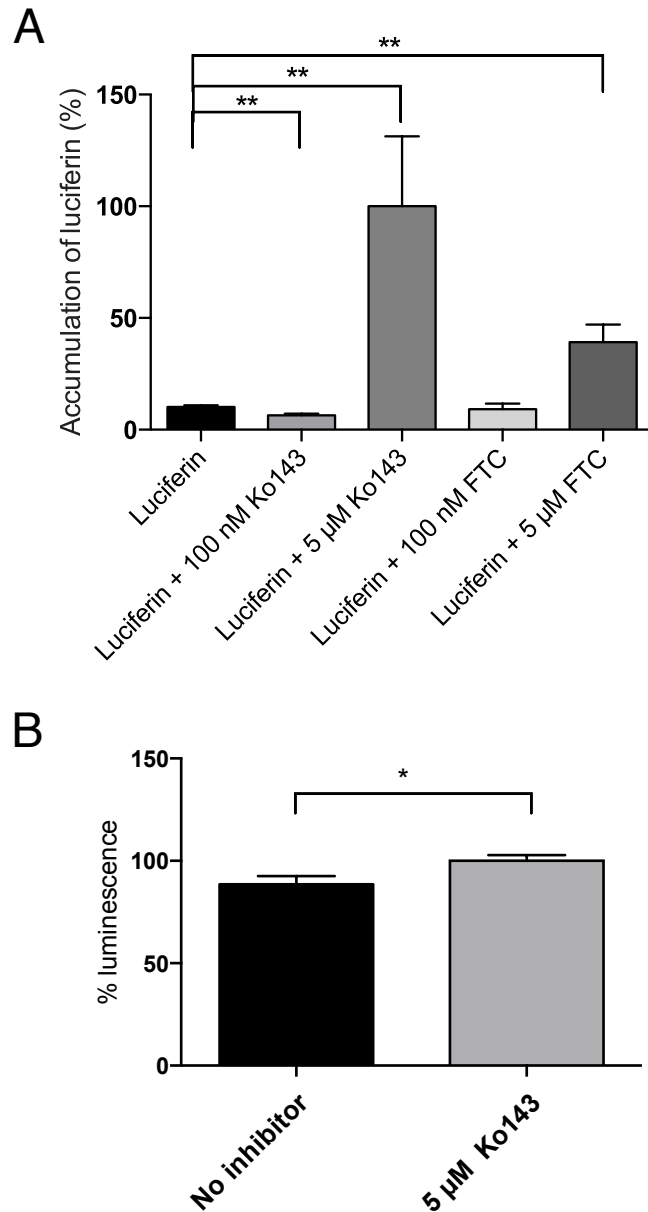
**Figure S1.** Cell surface expression of transporters by HVT cells. (A) Undetectable levels of ABCG2 expression, as determined by flow cytometry. (B) Undetectable levels of P-gp expression, as determined by flow cytometry. Data normalized to fluorescence measured in the antibody stained condition for each cell line from three experiments  $\pm$  SD (\* $p < 0.05$  by Student's t test).

Supplementary Figure S2



**Figure S2.** Accumulation of fluorescent substrates of ABCG2, P-gp, and MRP1 measured by flow cytometry in HVT cells. (A) ABCG2 substrates, mitoxantrone (10  $\mu$ M) and purpurin 18 (15  $\mu$ M) accumulation increased with Ko143 (100 nM, 5  $\mu$ M) coincubation. (B) Accumulation of P-gp substrate rhodamine 123 (2  $\mu$ M) and MRP1 substrate calcein-AM (1  $\mu$ M) accumulation did not increase with coincubation with their respective inhibitors tariquidar (100nM, 5  $\mu$ M) and MK571 (10  $\mu$ M, 25  $\mu$ M). All accumulation values are normalized to accumulation of the maximally inhibited condition. Data represent means  $\pm$  SD of three experiments (\*\* $p$  < 0.01, \*\*\* $p$  < 0.001, \*\*\*\* $p$  < 0.0001 by Student's t test).

Supplementary Figure S3



**Figure S3.** ABCG2 inhibition increases D-luciferin accumulation and bioluminescent signal in HVT cells. (A) D-luciferin (2mM) accumulation in HVT cells increases with ABCG2 inhibitors Ko143 (100nM, 5 μM) and fumitrogen C (FTC; 100 nM, 5 μM) measured by flow cytometry. (B) To measure the effect of ABCG2 on bioluminescence HVT cells were transiently transduced with a baculovirus (BacMam) containing firefly luciferase. Bioluminescence with and without 5 μM Ko143 is reported. All values are normalized to the 5 μM Ko143 condition. Data represent means ± SD of three experiments (\* $p < 0.05$ , \*\* $p < 0.01$  by Student's t test).