Table S1.
List of the ABCC1, ABCC5 and ABCC6 sequences and their corresponding UniprotKB codes used to generate the alignment for the homology models.

Name	UniProtKB code
ABCC6_Rat	O88269
ABCC6_Mouse	Q9R1S7
ABCC6_Human	O95255
ABCC6_Chimpanzee	A0A2I3SJS2
ABCC6_Gorilla	G3S3B7
ABCC6_Bovine	G5E62
ABCC6_Dog	F1PB44
ABCC6_Rabbit	G1SYV5
ABCC6_Horse	F7B7N5
ABCC6_Gpig	H0V465
ABCC1_Human	P33527
ABCC1_Macaque	Q864R9
ABCC1_BosTauros	Q8HXQ5
ABCC1_Dog	Q6UR05
ABCC1_Rat	Q8CG09
ABCC1_Mouse	O35379
ABCC1_Gallus	Q5F364
ABCC1_Zebrafish	A0A0R4IC81
ABCC6_Zebrafish	E7F118
ABCC5_Mouse	Q9R1X5
ABCC5_Rat	Q9QYM0
ABCC5_Human	O15440
ABCC5_Zebrafish	E1B2R7

TABLE S2. Functional consequences of mutating rAbcc6 R1168 and R1220 and corresponding residues in hABCC6, hABCC1 and hABCC2

ABCC6						hABCC1			hABCC2		
rAbcc6	mutation[a]	phenotype	hABCC6	variant[b]	ref	mutation	phenotype	ref	mutation	phenotype	ref
R1168	R1168A	ATP efflux reduced ≥75%[a]	R1169	-	-	R1197E R1197K	Organic anion[c] transport reduced ≥80%	[1]	-	-	-
R1220	R1220A	ATP efflux reduced ≥90%[a]	R1221	R1221C	[2-4]	R1249A	LTC_4 transport reduced 80%; vincristine resistance abolished	[5]	R1257A	glutathione conjugate transport markedly (>50%) reduced	[6]
				R1221H	[4, 7, 8]	R1249K R1249D	Organic anion[c] transport reduced ≥80%	[1]			

[a]this paper; [b]pathogenic hABCC6 variant associated with PXE; [c] organic anions tested included estradiol glucuronide, LTC_4, estrone sulphate, methotrexate

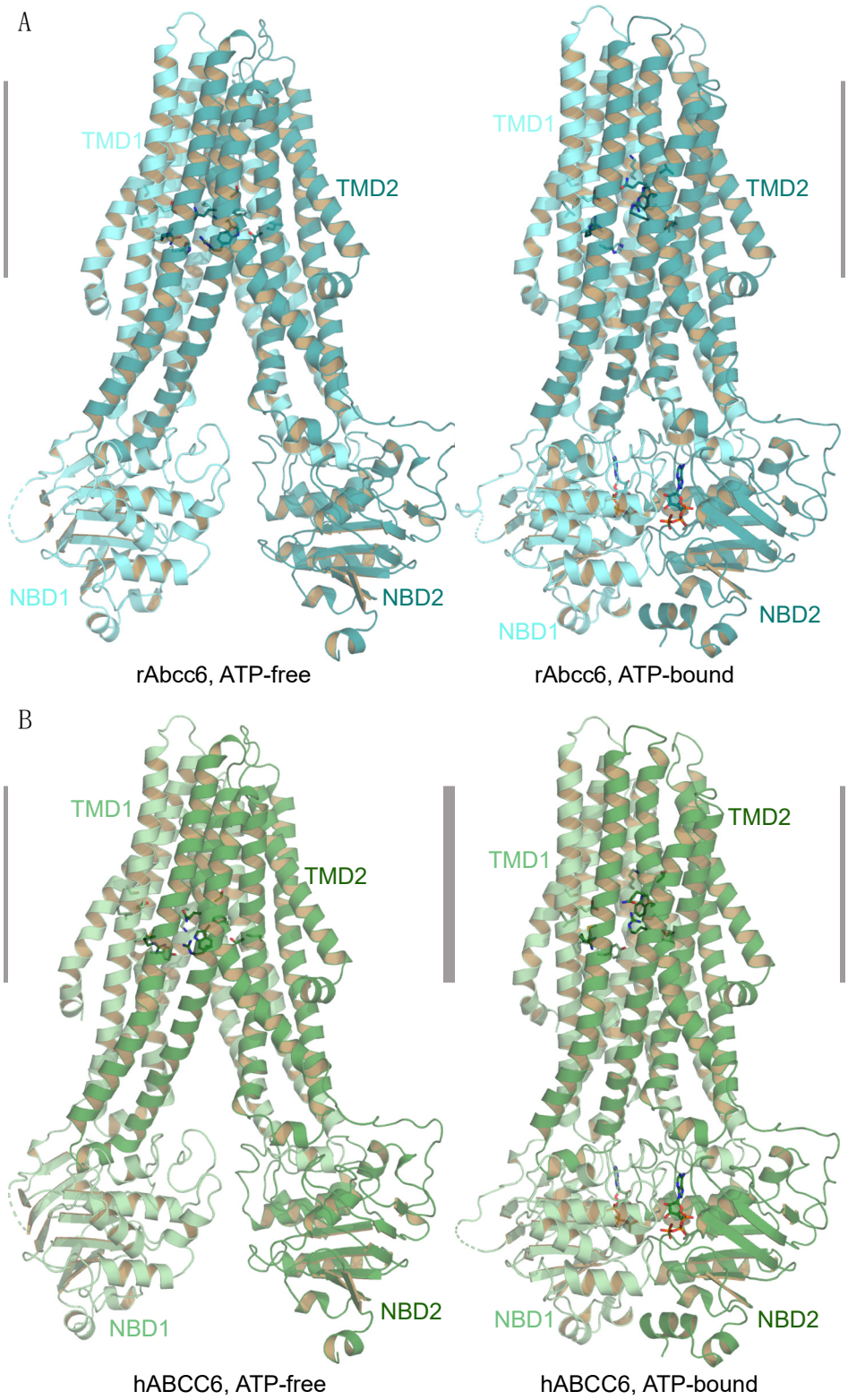

Figure S1. Structural models of rAbcc6 and hABCC6. The ABC core of ABCC6 (residues of TMD1-NBD1 and of TMD2-NBD2) is shown for (A) the rAbcc6 and (B) the hABCC6 ATP-free, inward-facing model (left panels) and ATP-bound, outward-facing conformational state of the transport cycle. The residues of the transmembrane cavity that are the subject of this study and described more in detail in the main text are shown as sticks. The ATP molecules bound to the NBDs in the outward-facing states are shown as sticks. TMD1, transmembrane domain 1; TMD2, transmembrane domain 2; NBD1, nucleotide binding domain 1; NBD2, nucleotide binding domain 2. The region of the TMDs embedded in the membrane is highlighted in gray.

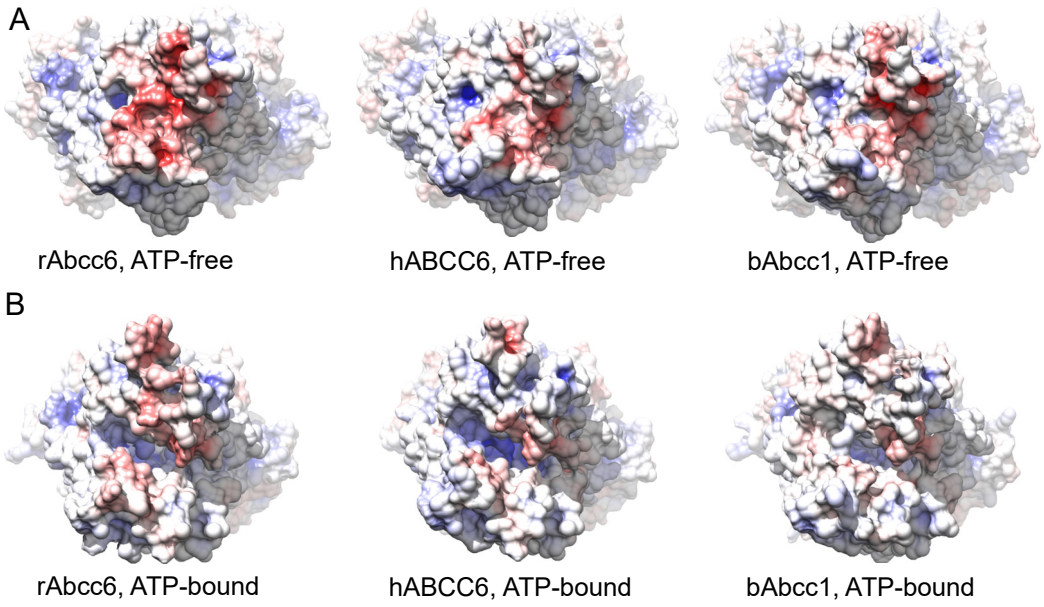

Figure S2. Electrostatic potential at the extracellular end of the TMDs for rAbcc6 hABCC6 and bAbcc1. View from the extracellular side of the electrostatic potential mapped on the molecular surface of (A) the ATP-free, inward-facing and (B) the ATP-bound, outward-facing homology models of rAbcc6 and hABCC6 and of the experimental structures of bAbcc1 [9, 10]. The isovalue was set at −10 k_BT/e for the negative potential (red) and +10 k_BT/e for the positive potential (blue).

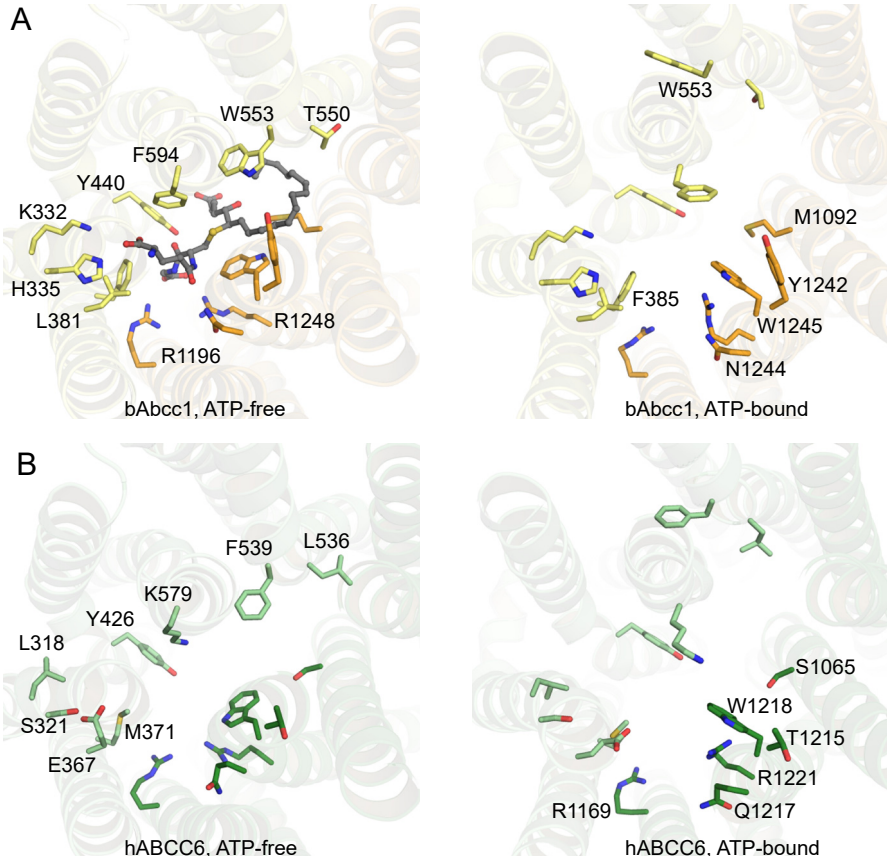

Figure S3. The LTC_4 binding cavity in bAbcc1 and hABCC6. A. Residues in the transmembrane cavity of (left) the ATP-free, inward-facing and (right) ATP-bound, outward-facing experimental structures of bAbcc1 [9, 10]. Residues of TMD1 are shown in yellow sticks, and residues of TMD2 are shown in orange. the LTC4 ligand present in the ATP-free state is shown as gray sticks. B. The corresponding residues in the hABCC6 homology models. Residues of TMD1 are shown in light green sticks, and residues of TMD2 are shown in darker green for the (left) ATP-free, inward-facing and (right) ATP-bound, outward-facing model.

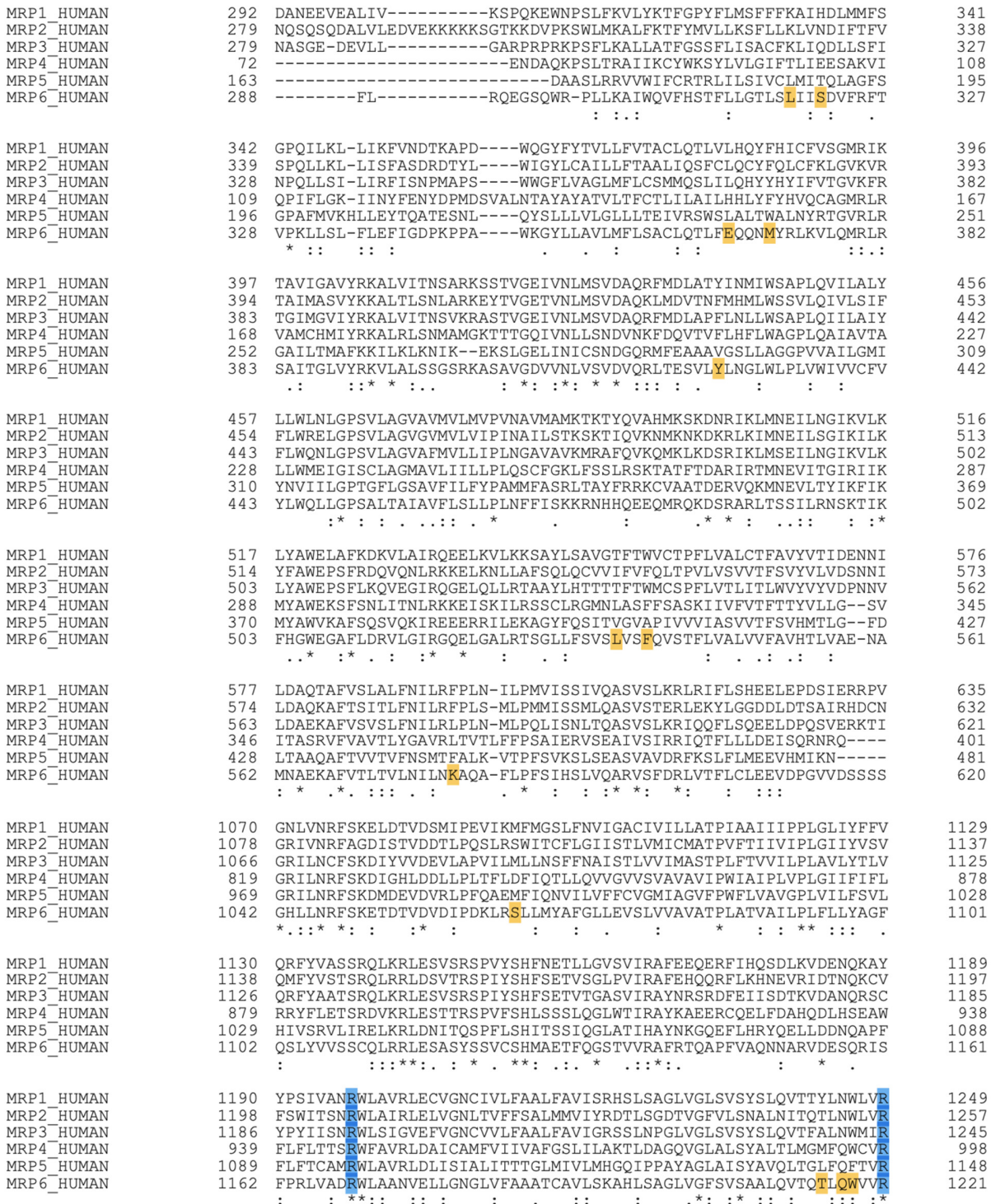

Figure S4. Alignment of ABCC1-6 amino acid sequences. Sequences of the human ABCC1 to ABCC6 proteins retrieved from UniprotKB were aligned using Clustal Omega. ABCC6 amino acid residues mutagenized are indicated by colors. Non conserved residues of ABCC6 are highlighted in yellow and residues conserved amongst ABCC1-ABCC6 are highlighted in blue.

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