

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 $\geq 75\%$ ^a	R1169	-	-	R1197E R1197K	Organic anion ^c transport reduced $\geq 80\%$	[1]	-	-	-
R1220	R1220A	ATP efflux reduced $\geq 90\%$ ^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 $\geq 80\%$	[1]			

^athis paper; ^bpathogenic hABCC6 variant associated with PXE; ^c organic anions tested included estradiol glucuronide, LTC₄, estrone sulphate, methotrexate

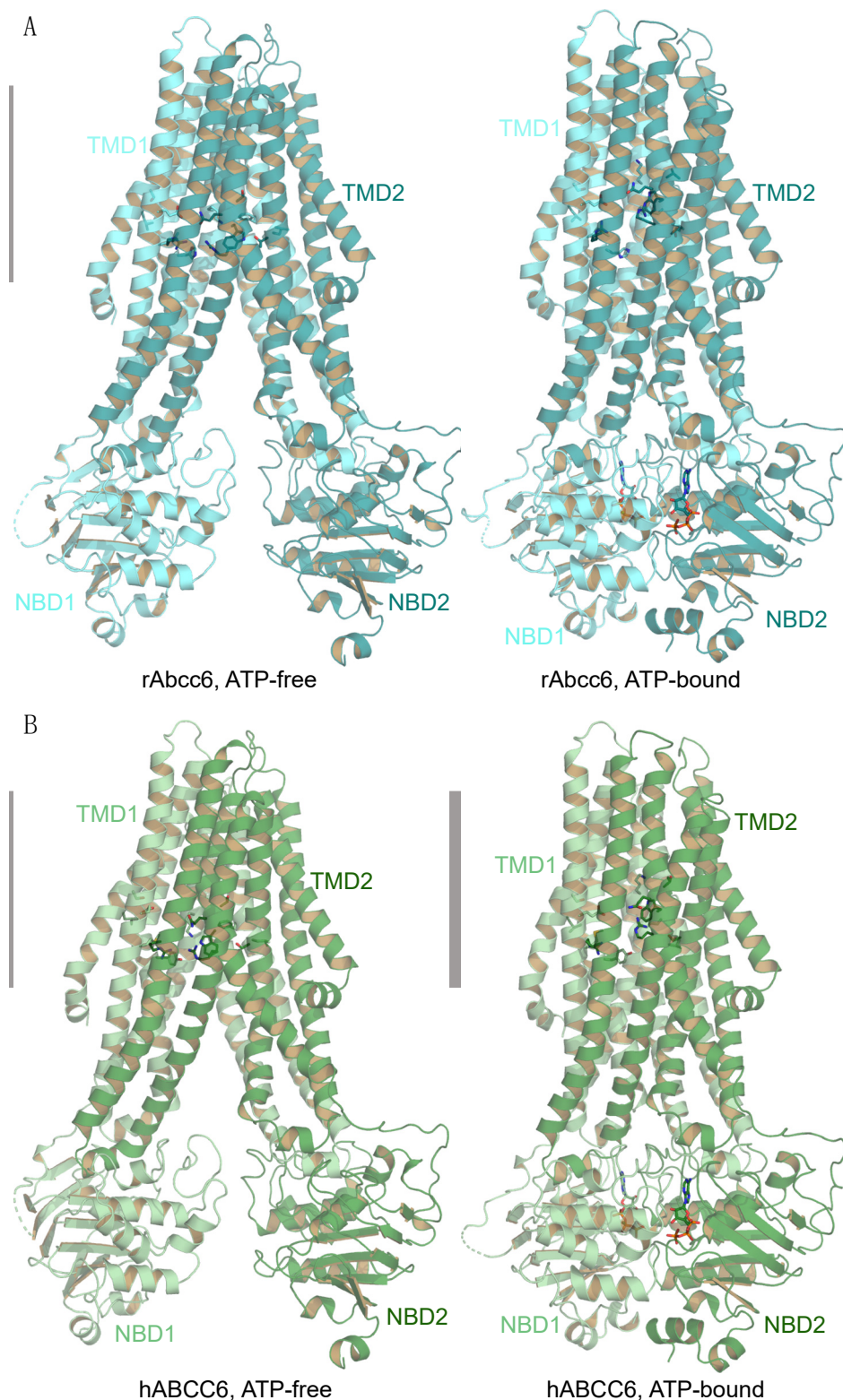


Figure S1. Structural models of rAbcc6 and hABCC6. The ABC core of ABCC6 (residues of TMD1-NBD1 and of TMD2-NBD2) is shown for (A) the rAbcc6 and (B) the hABCC6 ATP-free, inward-facing model (left panels) and ATP-bound, outward-facing conformational state of the transport cycle. The residues of the transmembrane 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.

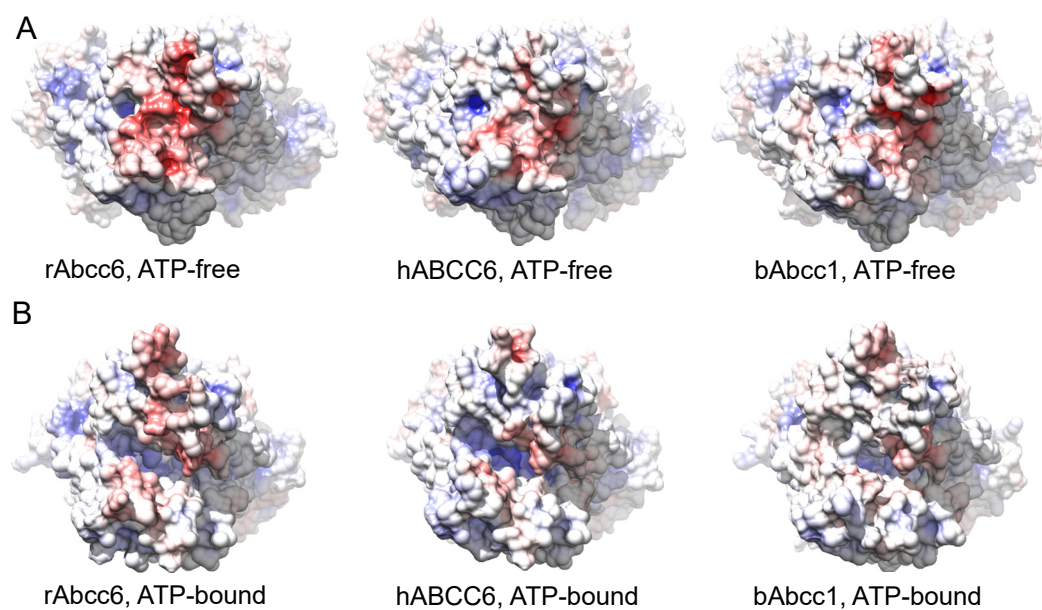


Figure S2. Electrostatic potential at the extracellular end of the TMDs for rAbcc6 hABCC6 and bAbcc1. View from the extracellular side of the electrostatic potential mapped on the molecular surface of (A) the ATP-free, inward-facing and (B) the ATP-bound, outward-facing homology models of rAbcc6 and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

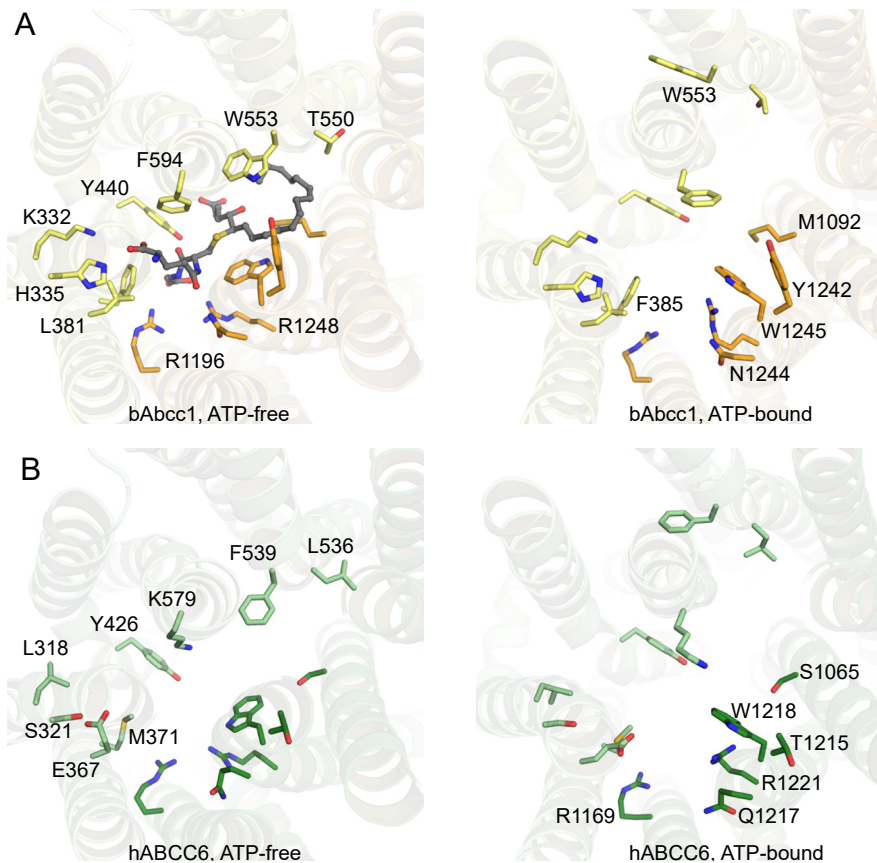


Figure S3. The LTC₄ binding cavity in bAbcc1 and hABCC6. A. Residues in the transmembrane cavity of (left) the ATP-free, inward-facing and (right) ATP-bound, outward-facing experimental structures of bAbcc1 [9, 10]. Residues of TMD1 are shown in yellow sticks, and residues of TMD2 are shown in orange. 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.

MRP1_HUMAN	292	DANEVEEALIV-----KSPQKEWNP	SLFKVLYKTFGPGYFLMSFFFKAIHDLMMFS	391
MRP2_HUMAN	279	NQSQSQDALVLEDDVEKKKKSGTKKDV	PKSWLMKALFKTFYFVLLKSFLKLKLVNDIFTFV	338
MRP3_HUMAN	279	NASGE-DEVLL-----GARPRPRKPS	FLKALLATFGSSFLISACFKLIQDLLSFI	327
MRP4_HUMAN	72	-----ENDAQKPSLTRA	IKCYWKSFLVGLGFIETLIESAKVI	108
MRP5_HUMAN	163	-----DAASLRVVVMI	FCFTRLLILSIVCLMITQLAGFS	195
MRP6_HUMAN	288	-----FL-----RQEGSQWR	-PLLKAIWQVFHSTFLLGLTSLIISDVFRFT	327
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MRP1_HUMAN	342	GPQILKL-LIKFVNDTKAPD----	WQGYFYTVLLFVTAACLQTLVLHQQYFHICFVSGMRIK	396
MRP2_HUMAN	339	SPQLLKL-LISFASDRDTYL----	WIGYLCAILLFTAALIQSFCLQCYFQLCFKLGKVVR	393
MRP3_HUMAN	328	NPQLLSI-LIRFISNPMAPS-----	WWGFLVAGLMFLFCSMQSLILQHYHYHIFVTGVKFR	382
MRP4_HUMAN	109	QPIFLGK-IINYENYDPMDSVALNTAY	AYATVLTFTCLLILHLHYFYHVQCAGMRLR	167
MRP5_HUMAN	196	GPAFMVKHLLLEYTQATESNL----	QYSLLLVLGLLLTEIVRSWSLALTWALNYRTGVRLR	25
MRP6_HUMAN	328	VPKLLSL-FLEFIGDPKPPA----	WKGYLLAVLMLFSLACLQTLFEEQNMYRLKVLQMRLR	382
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MRP1_HUMAN	397	TAVIGAVYRKALVITNSARKSSTVGEI	VNLMVSVDARFMDLATYINMIWSAPLQVILALY	456
MRP2_HUMAN	394	TAIMASVYKKALTLNSLNARKEYTVGE	TVNLMVSVDADQKLMVDVTFNMHMLWSSSVLQIVLSIF	453
MRP3_HUMAN	383	TGIMGVIRYKALVITNSVKRASVTGEI	VNLMVSVDARFMDLAPFLNLLWSAPLQIILAIY	442
MRP4_HUMAN	163	VAMCHMIYRKALRSLNMAMGKTTGGV	INLLSNDVNKDTQGVTVFLHFWAGPLQAIQAVTA	227
MRP5_HUMAN	252	GAILTMFAKKILKLKNIK--EKS	LGELINICSDNGQRMFEAAAVGSSLAGGPVVAAILGMI	309
MRP6_HUMAN	383	SAITGLVYRKVALSSGSRKASAVGDV	VNLSVDVQRLTESVLILNGLWLPLVWIVVCFV	442
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MRP1_HUMAN	457	LLWNLNGPSVLAGVAVMLVMPVNAV	MAMKTKTYQVAHMKSKDNRIKLMNEILNGIKVLK	516
MRP2_HUMAN	454	FLWRELGPSVLAVGVVMVLVIPINAIL	STKSKTIQVNMKNKDKRLKIMNEILSGIKILK	513
MRP3_HUMAN	443	FLWQNLGPSVLAVGAVFMVLLIPLNG	AVAVKMRAFQVKQMKLKDSRIKLMSEILNGIKVLK	502
MRP4_HUMAN	228	LLWMEIGISCLGMAVLIILLPLQSC	FGKLFSSLSRKTATFTDARIRTMNEVITGIRI	287
MRP5_HUMAN	310	YNVILGPPTGLGSAVILFYFPA	MMFASRLTAYFRKRCVAATDERVQKLMNEVLT	369
MRP6_HUMAN	443	YLWQLLGPSALTAIAVFISLLPLN	FFITSKRNHHQEEQMRQKDSARLTSSILRNSKTIK	502
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MRP1_HUMAN	517	LYAWELAFKDKVLAIHQEELKVLK	KSAYLSAVGTFTVCTPFLVALCTFAVYVITIDENNI	576
MRP2_HUMAN	514	YFAWEPFRDQVQNLRRKLEKNLLA	FSQLQCVFVFFQTLPLVLSVVTFSVLVVDSDNNI	573
MRP3_HUMAN	503	LYAWEPSFLKQVEGIRQGEQLQLLR	TAAYLHTTTTTFTWMCSPFLVTLITLWVYVYVDPNNV	562
MRP4_HUMAN	288	MYAWEKSFNLIINLRKKEISKILRS	SCLRGMNLASFFSASKIIVFVTFTTYVLLG--SV	345
MRP5_HUMAN	370	MYAWWKAFSQSVQKIREERRILE	KAGYFQGITVGVAPIVVVIASVVTFSVHMTLG--FD	427
MRP6_HUMAN	503	FHWEGEAFLDRLVLGIRGQELGAR	LRTSGLLFSVSLVSEFQVSTFLVALVVFVAVHTLVAE-NA	561
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MRP1_HUMAN	577	LDAQTAFAVSLALFNILRFPLN-IL	PMVISSIVQASVSLKRLRIFLSHEELEPDSIERRPV	635
MRP2_HUMAN	574	LDAQKAFTSITLFNILRFPLS-ML	PMMISSMLQASVSTERLEKYLGDDLDTSAIRHDCN	632
MRP3_HUMAN	563	LDAEKAFAVSVSLFNILRLPLN-ML	PQLISNLTQASVSLRKIQQLFSQEELDPQSVERKTI	621
MRP4_HUMAN	346	ITASRVFVAVTLYGAVRLTVTLFF	PSPAIERVSEAIIVSIRRTQTFLLLDETSQRNRQ----	401
MRP5_HUMAN	428	LTAQAQFATVTVTFNSMTFALK-V	TPFSVKLSLSEASAVDRKFSFLMEVEHMIKN-----	481
MRP6_HUMAN	562	MNAEKAFTVLTVLNLIINLKAQA-	FLPFSIHSLVQARVSDRLVTLFLEEVDGPDGVSSSS	620
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MRP1_HUMAN	1070	GNLVNRFSEKELDTVDSMIPEVIK	FMGSLFNIVIGACIVILLATPIAAIIIPPLGLIYFFV	1129
MRP2_HUMAN	1078	GRIVNRFAGDISTVDDTLQPSLR	SWITCFGLGIISTLVMICAMPTVETIIVIPGLIIYVS	1137
MRP3_HUMAN	1066	GRILNCFSKDIYVDEVLAIPVIL	MLNSFFNAISTLVIMASTPLTFTVVLPLAVLYTLV	1125
MRP4_HUMAN	819	GRILNRFSDKIGHLDDLLPLTFL	DFIQTLTLLQVVGVSVAVAVIPWIAIPLVPLGIIFIFL	878
MRP5_HUMAN	969	GRILNRFSDKMDDEVDRLFPQ	AEFMFIQNVILVFFFCVGMIAGVFPWFLVAVGVLPLIFL	1028
MRP6_HUMAN	1042	GHLNRFSEKETDTVDVDPDKLR	SLLMYAFGLLEVLVAVATPLATVAIPLVLIFYAGF	1101
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MRP1_HUMAN	1130	QRFYVASSRQLKRLESVSRSPY	SHFNETLLGVSVIRAFEEQERFIHQSDLKVDENQKAY	1189
MRP2_HUMAN	1138	QMFYVSTSRQLRRLDSVTRSPI	YSHFSETVSGLPVIRAFEHQQRFLKHNEVIDRTNQKCV	1197
MRP3_HUMAN	1126	RRFYAATSRQLKRLESVSRSPY	SHFSETVTGASVIRAYNRSDRDEIISDTKVDANQRSC	1185
MRP4_HUMAN	879	RRYFLETSLRQVDRKLESTRSP	VSFLSHSSSLQGLWTRIRAYKAEERCQELFPAHQDLHSEAW	938
MRP5_HUMAN	1029	HIVSRVLIQELKRLDNIITQSP	FLSHITSSIQGLATHAYNKGQEFHLHRYQELLDNDQAPF	1088
MRP6_HUMAN	1102	QSLYVVSQCRLRLESASYSVC	SHMAETFCQSTTVVRAFRTQAPFVAQNNAVVDQSQRIS	1161
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MRP1_HUMAN	1190	YPSIVANRWLAVRLECVGNCIV	LFAALFAVISRHSLSAGLVGLSVSYSLQVTTYLNWLVR	1249
MRP2_HUMAN	1198	FSWITSNRWLAIRLELVGNLT	VFFSALMMVIYRDTLSGDTVGFVLSNALNITQTLNWLVR	1257
MRP3_HUMAN	1186	YPYIISNRWLSIGVEFVGCN	CVLFAALFAVIGRSSLNPLVGLSVSYSLQVTFALNMWIR	1245
MRP4_HUMAN	939	FLFLTTSRWFAVRDLDAICAM	FVIIIVAFGSLDILAKTLDAGQGLALSYALTLMGMFQWCVR	99

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