

Supplementary Materials for

Molecular insights into the human ABCB6 transporter

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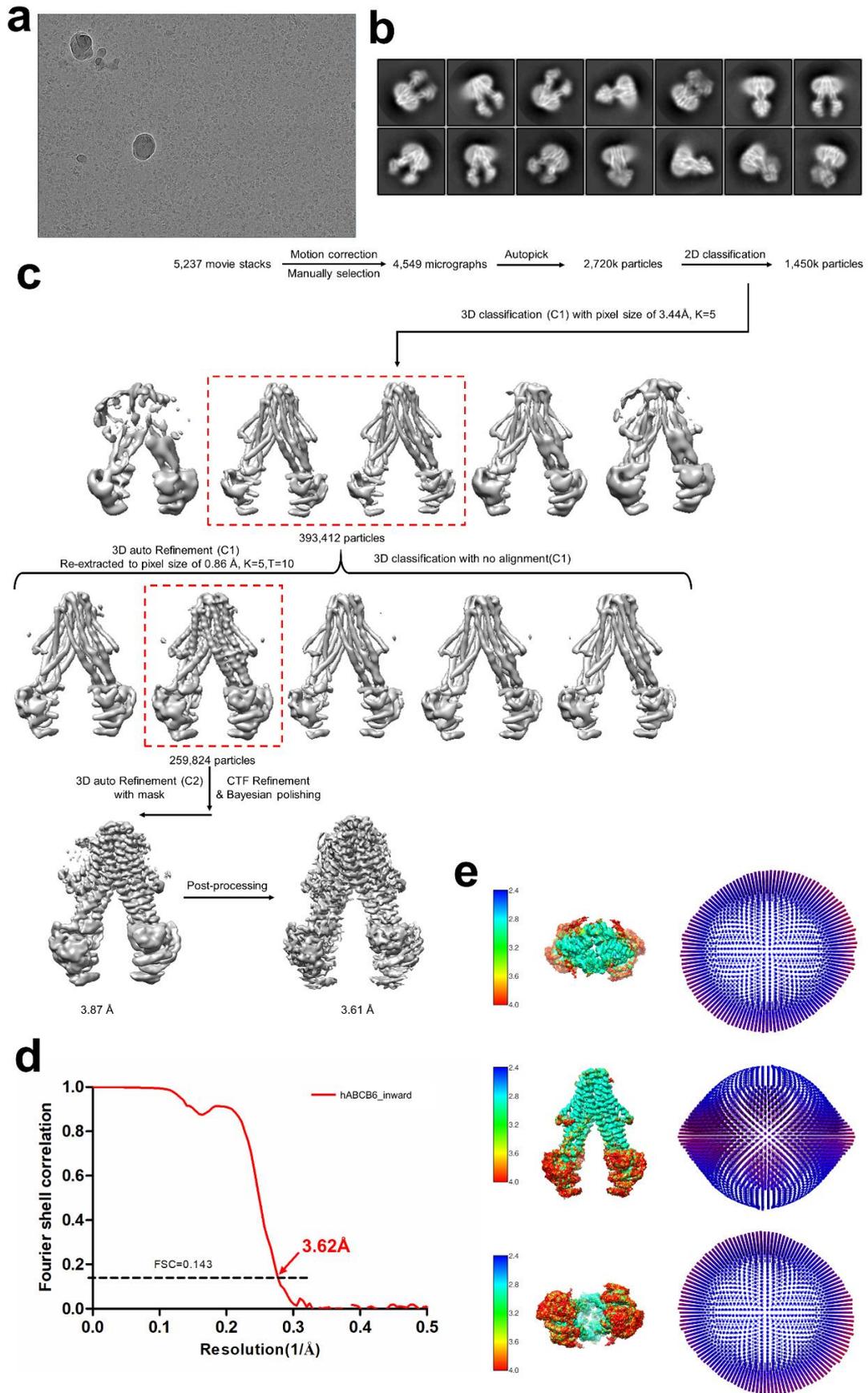
#To whom correspondence should be addressed.

This file includes:

Supplementary Figs. S1 to S11

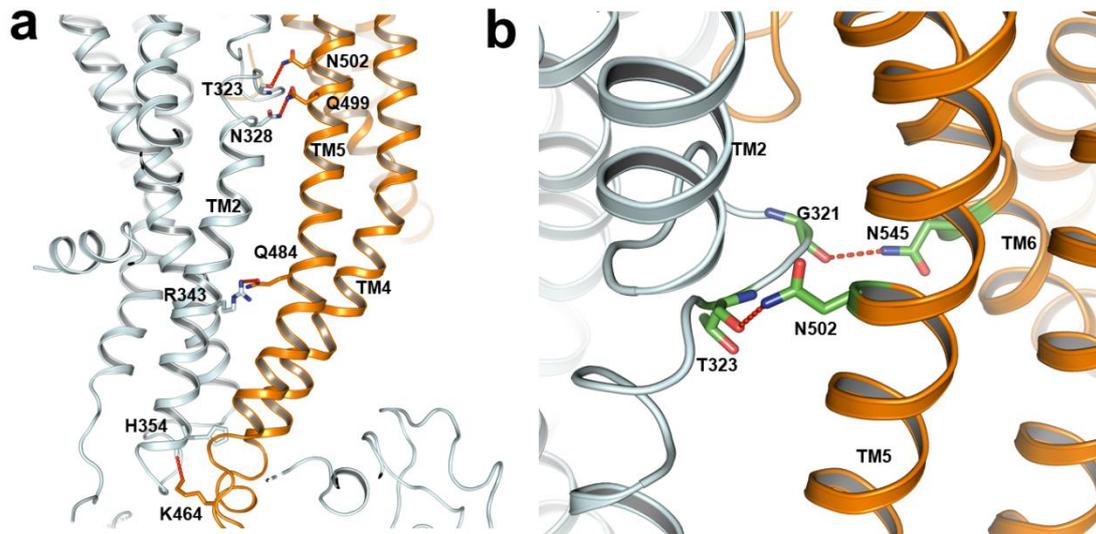
Supplementary Table S1

Supplementary Figures and Figure legends



Supplementary Fig. S1 Reconstruction and structure determination of the apo-state hABCB6.

- a**, Representative cryo-EM micrograph of apo-state hABCB6 in nanodisc.
- b**, 2D class averages of the apo-state hABCB6 sample in nanodisc.
- c**, The workflow of 2D/3D reconstruction with apo-state hABCB6 cryo-EM data. In brief, 1,450 k particles were kept after 2D classification, and subjected to three rounds of 3D classification. A final dataset containing 260 k particles were used for high-resolution refinement (see methods for more details).
- d**, Gold-standard Fourier Shell correlation (FSC) curve of apo state hABCB6 after 3D refinement. The resolution estimation was based on the criterion of FSC 0.143 cutoff.
- e**, Angular distribution of the apo-state hABCB6 final reconstruction.



Supplementary Fig. S2 The interactions within two hABCB6 monomers.

a, The interaction between TM2 and TM5 from two neighboring monomers.

b, The interaction between the “plug” and TM6 from two neighboring monomers.

Structure of hABC6

a

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1      10      20      30      40      50      60      70
ABC66  MVTVGNVCEAEGPVGPAWMDGLSPCFEFTLVPSTRMALGTLALV...LALPCRRR...ERPAGADSLSWGAGPRIS
ABC67  .....MA.....LAMHSWRWAAAAA...
ABC61  .....
HMT1   MV..LRYNS...P.....RLN.ILELVLLVYGVFFSIGSLNLLQKRKATSDPYRRKRFNGKEPIGIIISW.WILGIAL.
ATM1   .....

80     90     100    110    120    130    140    150
ABC66  PYVLQLLLATLQAALPLAGLAGRVGTARGAPLPSYLLLASVLESAGACGLWLLVVERSQARQLAMGIWIKFPH.SPGL
ABC67  AFEKRR..HSAAILIRPLVSVSGSGP.....QWPHQLGA
ABC61  .....
HMT1   TYVVDI.....SNLVIYALRV.....PNWWPCKTTVVCLILFLFWIIVLISCADSKALPKNADSIKAYRLS.V
ATM1   .....MLLLPRCPVIGRIV.....RSKFS...GL

160    170    180    190    200
ABC66  TLLWTVAFAAENLALVSWNSPQWWARADLGLG.....VQFSLWLVRYVVSGLVFLGLWAP
ABC67  LGTARAYQIPESLKS.....ITWQRLLGKG.....SQGLDA.....AKALQVWPL
ABC61  .....
HMT1   LYVVAIDIVFETIFIVYSPHPNETFQGLVLADHVARLVLCVFATAIYLYTYYRRRHITHDPLDFEERQTEESN..VNEINAI
ATM1   IRNHS...PV...I.....FTVSKLS.....TQREPLLF.....NSAVNLWNO

210    220    230    240    250    260    270    280
ABC66  GLRPQSYTL...QVHEEDQ.DVERSQVRSAAQOSTWRDFGRKLRLLDSCYLVWFRGSPAQLVVLITGLGMLGLERALNVLV
ABC67  IEKRTCWHGHAGGGLHT.D.....PKE.GLKDVDTRKIIRKMLSYVWFKDRPDLRARVAISLGLGGAKAMNIVV
ABC61  ...MDLEGRNGGAKKKNFKLNKSEKDKKEKPTVSVFSM...FRYSNWLKLYMVGVT...LAIITHGAGLPLM
HMT1   SQNPFSTVQL..GVSASTSNF.GTLKSL.SKKPSDKSWAEYFRSFTLLPYLVWFTKDYRLQFOIFICIVLFLGKRAVILA
ATM1   AQKDITHK.KSVEQFSS.AP.KV.KTQVKKTS.KAPTLESELKILKDLFRYIWFKGNKVRIRVILALGLLISAKILNVQV

290    300    310    320    330    340
ABC66  FIFVRRNVNLLF.....T...EKAPWNSLAW.....T...VTSYVFL.KFLQGGTGTGFVSNLRTFLWIRVQCFETS
ABC67  PFMFKYAVDSL...N...QM..SGMMLNLSAPNTVATMATAVLIGYGVSRAGAAEFNEVRNAVFKVAANSI
ABC61  MLVFCGEMTDIFANAGNLEDLMSNITNRSNDINDTGFMMLEEDMTRY...AYYYSIGAGVLVAAYIQVSWCKLAAGRQI
HMT1   PRLQGLVITKLL.....TKHSEKIPWSDVIL.....FVIYRFLQGNMVGIGSLRSFLWVVFVSYAY
ATM1   FFFFKQIIDSX.....N...IA..WD.....DPTVALPAAIGLITLICYGVARFGSVLFGELRNVAFAKVAANAI

350    360    370    380    390    400    410
ABC66  RRVELLIFSHLHESLRMHLGRRTGELVLRADRGTSSTVGLLSYLVFNVIPTLADTIIGIIFYSMFFNAWFGLLVVF...
ABC67  RRFAKNVFLHLHNLDLGFLHSRQTGALSKAIDRGTRGTSFVLSALVFNLLPIMFEVMLVSGVLYYKCGAOFALVTL...
ABC61  HKIRKQFFHAIMRQEGVDFVHDVGEINLRLTDDVSKINNEGIG.....DKIGMFFQSMATEFTGIVGFTR
HMT1   RAISTKALRHVNLISYDFHLNKRAGEVLTALTKGSLSL.NTFAEQVVFQIGVLLDLGAMVYVFFIKFDIYFTLIVL...
ATM1   RTVSLQTFQHLMKLDLGHLSRQTGGLTRAMDKGTGKLSQVLTAMVVFHIIPTISFETISVVCGLITLYQFGASFAITF...

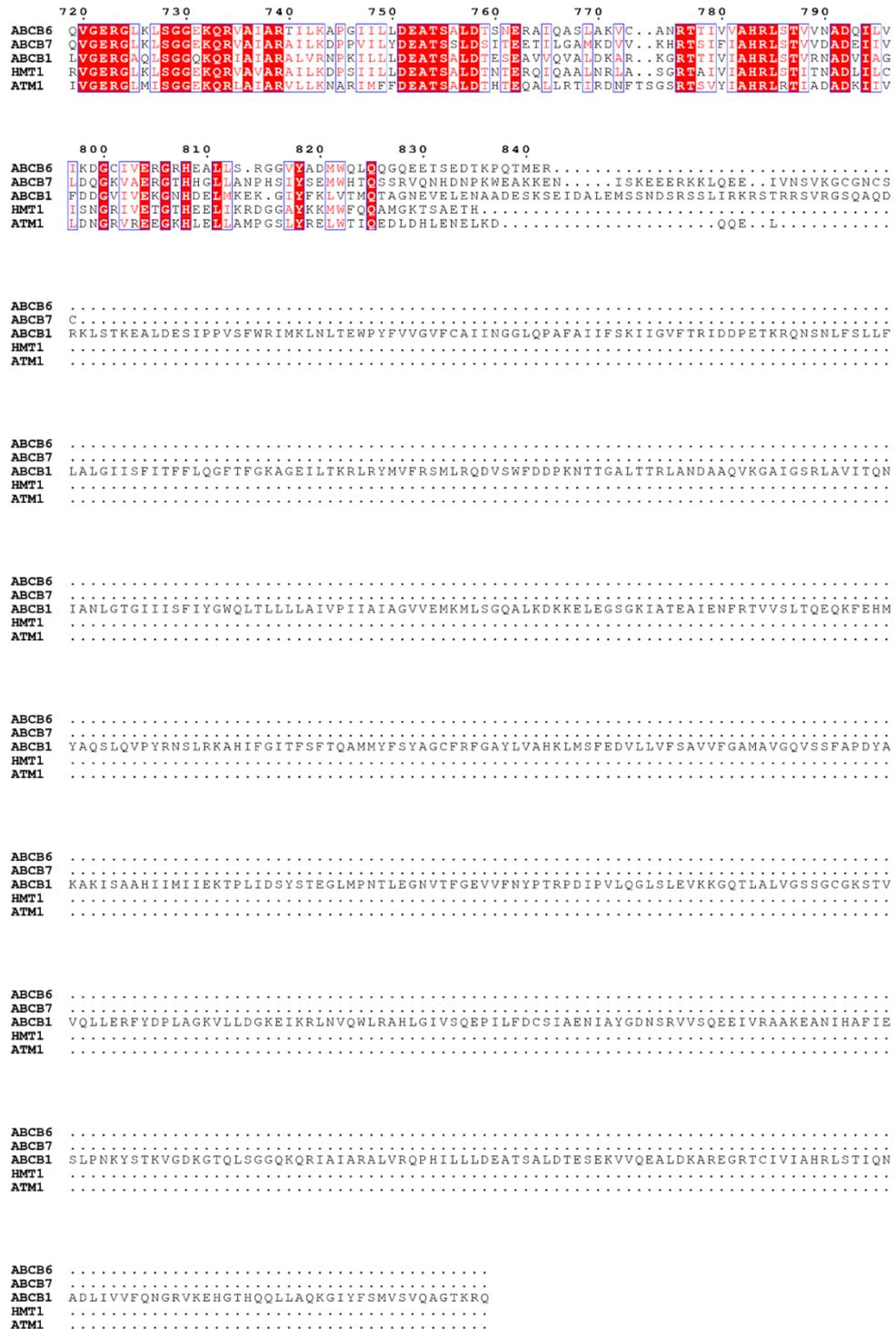
420    430    440    450    460    470    480
ABC66  ....LCMSLYLTLTIVVTPHRT...KFRRAMNTQENATRAAVVSLLNFEVRYYNNAEISVEVERREAITIKVQGLEWFK
ABC67  ...GTLTYTAFIVAVTRRR...RFRLEMNKADDNAGNAADISLLNRYETVKYFNNERYEAQSYDGLLKTIVETASLK
ABC61  GWKLLTVLAIASPLVGLSAAVWAKILSSFTDKELLAYAKAGAVAEVLAARIVTIAFGGQKKELESNKNLLEAKRIGIK
HMT1   ....IMTLCYCYVTVKITSWR...EARRKMVNSWRESYAVQNDAIMNPFVKNFEDADDFENERGHAVDYLKQERIK
ATM1   ....STMLLYSIFTKITART...HERDANKADNKAASVALSLLNFEAVKYEENNKYLVADKNGSLMNRDSQIK

490    500    510    520    530    540    550    560
ABC66  SSSASLVLLNQTLQNLVIGLGLAGSLLCAYFVTEQKLOVGDY...VLFGTYTIQLIYMBLNWFGLTYRMIQNFIDMEN
ABC67  STSTLAMLNFGQSAIFSVGLTAIMVLAQQGIVAGTLTVGDLD...VMVNGLLFQLSPLNLFGLTVYRETROALIDMNT
ABC61  KAITANISIGAAFLLIYASYALAFWYGTTLVLSGEYSIQVLTVFFSVLIGAFSVQASPS...IEAFANARGAAYE
HMT1   VLFSLNFINVQGGIIFFSLIAACLSAYRVTFGNFTVGD...VILLTYMIQLQQLNFFGLYRSLQNSIIDTER
ATM1   VSOQLAFLNSSGQLIFTTALTAMMYMGSTGVIGNLTVGD...VLIINQLVFLQSVLHNFGLSYYRDLKQSLIDMET

570    580    590    600    610    620    630
ABC66  MFDLLEETEVEKDLPGAGPDRF...QKGRIDENVHFSWADGR...ETLQDVSTVMPGQTAVVGSVSCAACKSTLRLVFR
ABC67  LFTLLKVDVDTQFKKVMASPLQI.TPQTATVADNDVHVFIEQQ...KVLGGISFEPFAKKAIVVGSVSCAACKSTVRLVFR
ABC61  LFKLIDNKPSSDSYSKS..GKPPDNIKGNLBRNVHFSWPSKKEVKLLKGLNLKYOQSGOTVAVVGSVSCAACKSTVQLMQR
HMT1   LLEIFEEKPTVVEKPNAPDLKV...TQCKVHESHVSYFADPRK...PVLSDINFAVQPKVIAVGSVSCAACKSTMRLVFR
ATM1   LFKLRKNEVKLKAER...PLMLPENVPYDITDENVTFGHPDR...KILRNASETIPAGWKTAVVGSVSCAACKSTLKLVFR

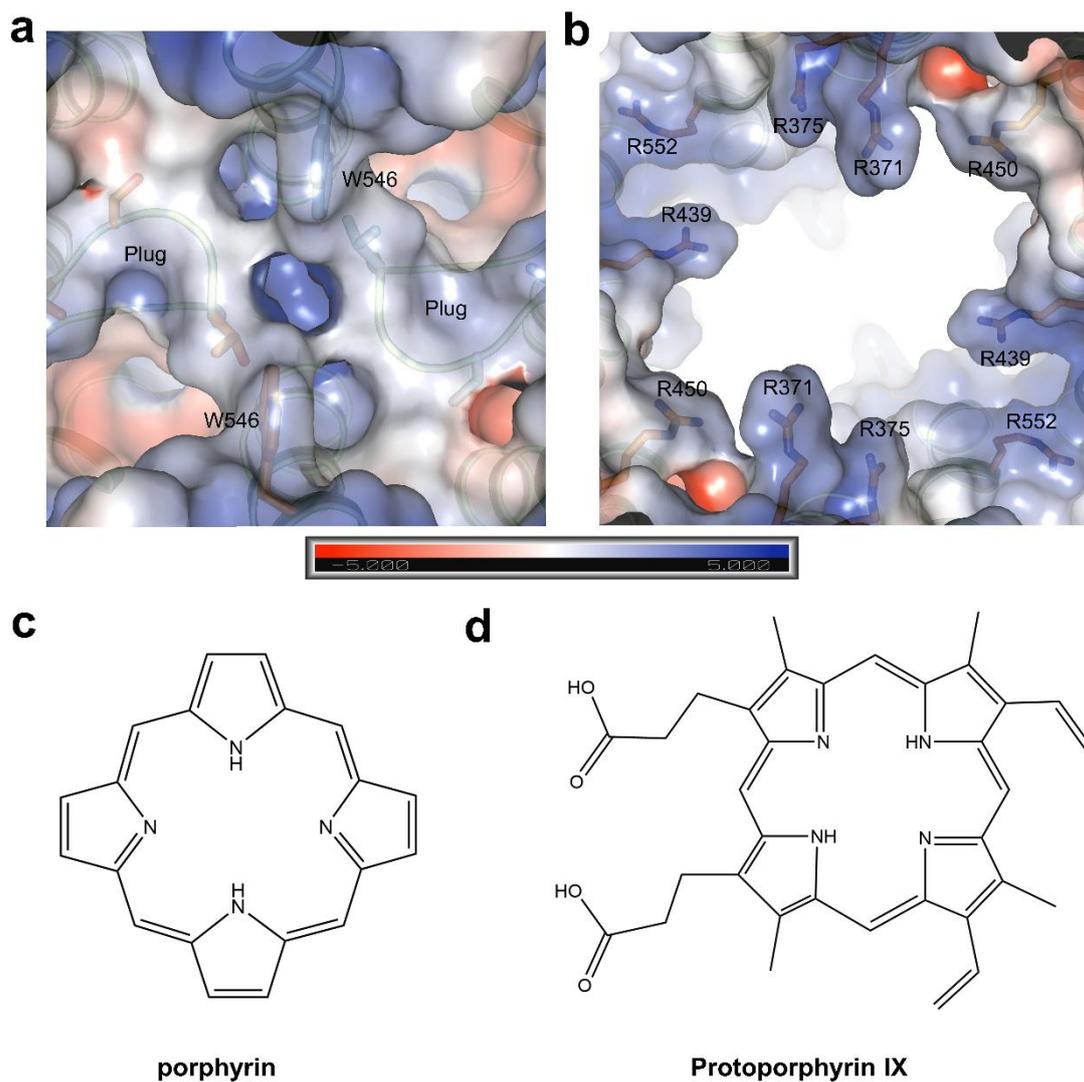
640    650    660    670    680    690    700    710
ABC66  FYDLSGSCIRTDGQDITSQVTLQASLRSHTGVVVEDTVLNDTADNIRYGRVTAAGNDEVEAAAQAAGTHDAMAFPEGYRIT
ABC67  FYEPEKQSGISYLAQNIQDVSLESLRRAVGVVVEDAVLPHNTTYYNLYGNISASPEVYAVAKLAGLHDATLRMPHGFDIT
ABC61  LYDPEGEMVSVGDQDIRTINVRFLREIIGVVVQEPVLFATTAENIRYGRVNTMDEIEKAVKEANAYDITMKLPHKFDIT
HMT1   FFFVNSGSGIRITDDQDIRNVTLSLRSSTGVVVEDSTLNDTTLNIRYKAKPSATNDEIYAAAQAAGTHDITLQEPDGYNS
ATM1   FYDPEGSCIRLNGRDIKEYDIDALRKVIGVVVEDTPVLFNDTTEWENKFKGRIDATDEVITVVEKAQLAPITIKKLPQGFDD
    
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b



Supplementary Fig. S3 Sequence alignment of ABC transporters.

a,b, Sequence alignment of human ABCB1, ABCB6, ABCB7 and yeast HMT1, ATM1 using ESPrpt3.

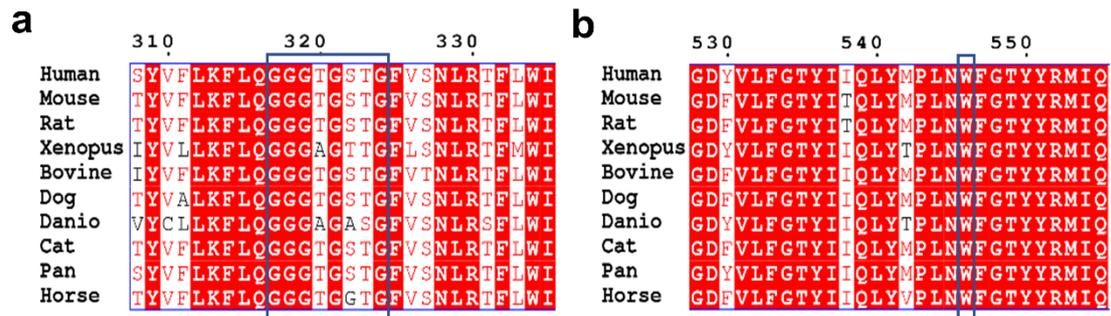


Supplementary Fig. S4 The H-P pocket of hABCB6.

a,b, The electrostatic properties of the interior surface of the translocation pathway. hydrophobic region (a) and positive region (b) were shown separately. Scale: red, negative (-5 kT/e); blue, positive (+5 kT/e). Two “plugs” and W546 are shown in (a). Side chains of representative arginine are shown in (b).

c, The structure of porphyrin.

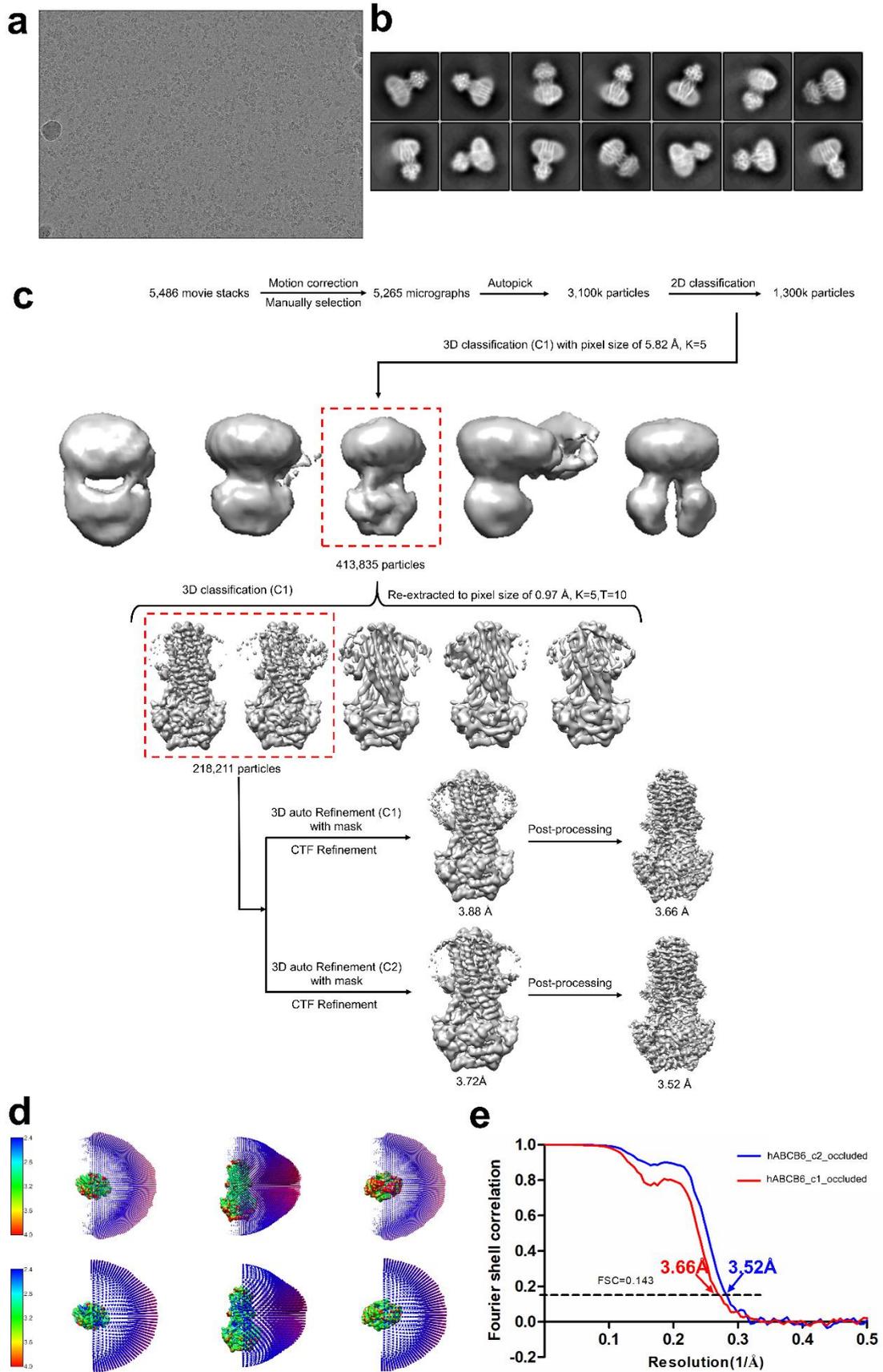
d, The structure of protoporphyrin IX.



Supplementary Fig. S5 Sequence alignment of the “plug” and W546.

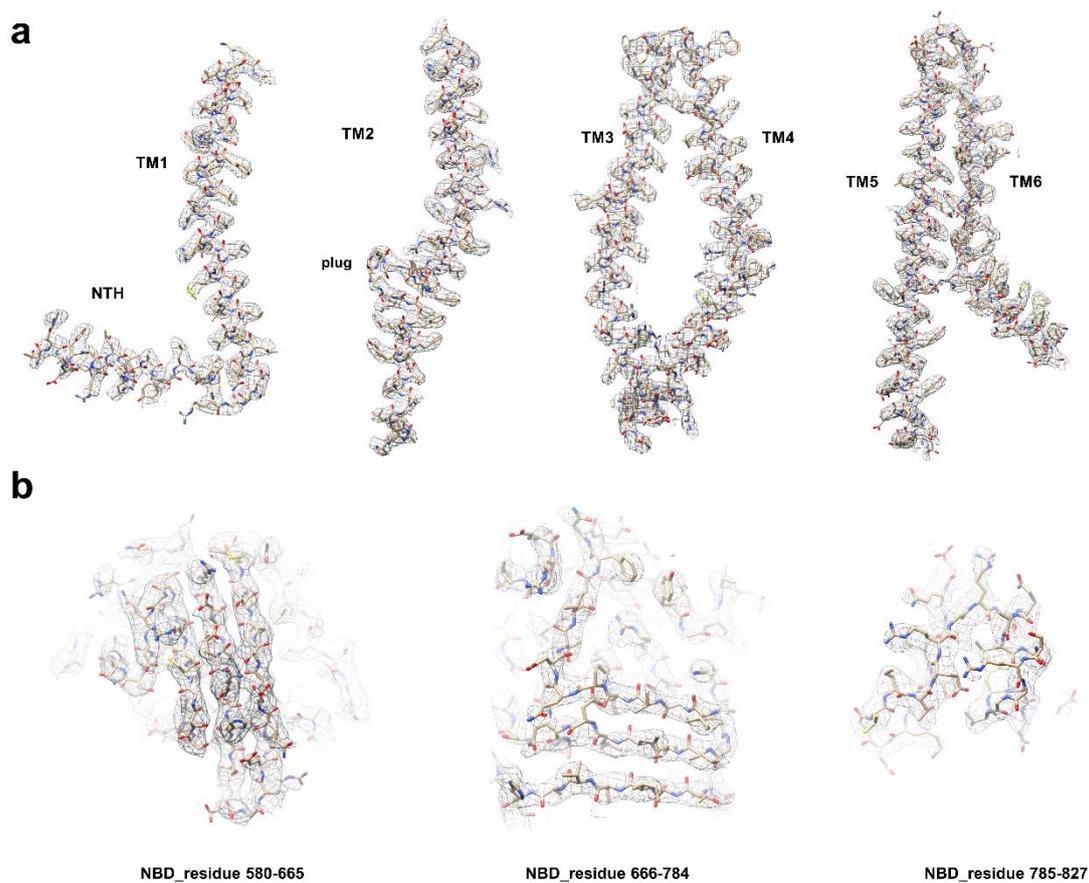
a, Sequence alignment of the “plug” in ABCB6 among different species.

b, Sequence alignment of W546 in ABCB6 among different species.



Supplementary Fig. S6 Reconstruction and structure determination of the nucleotide-bound hABCB6.

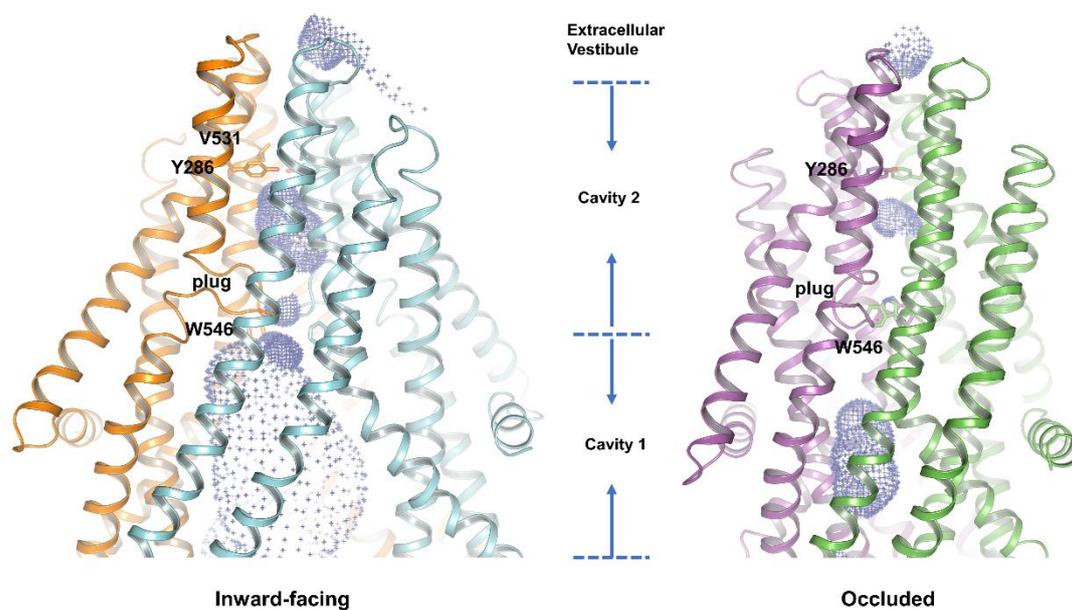
- a**, Representative cryo-EM micrograph of nucleotide-bound state hABCB6 in nanodisc.
- b**, 2D class averages of the nucleotide-bound state hABCB6 sample in nanodisc.
- c**, The workflow of 2D/3D reconstruction with nucleotide-bound state hABCB6 cryo-EM data. In brief, 1,300 k particles were kept after 2D classification, and subjected to three rounds of 3D classification. A final dataset containing 218 k particles were used for high-resolution refinement (see methods for more details).
- d**, Angular distribution of the nucleotide-bound state hABCB6 final reconstruction.
- e**, Gold-standard Fourier Shell correlation (FSC) curve of nucleotide-bound state hABCB6 after 3D refinement. The resolution estimation was based on the criterion of FSC 0.143 cutoff.



Supplementary Fig. S7 Representative cryo-EM densities of hABC6.

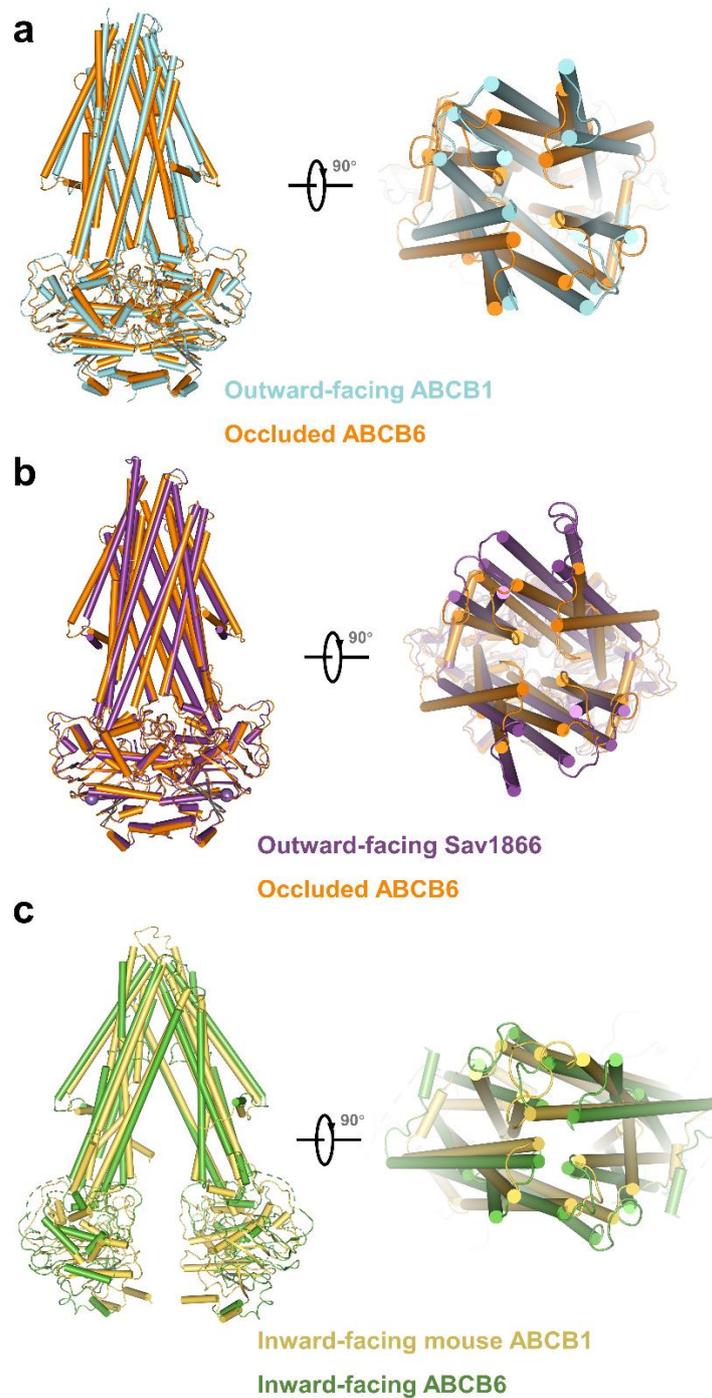
a, Density maps of representative transmembrane regions of nucleotide-bound hABC6. Stick style atomic models (gold) were fitted into the cryo-EM density maps (gray mesh). The density maps were contoured at 10.0σ .

b, Density maps of the representative nucleotide-binding region of nucleotide-bound hABC6, similar to the panel (a).



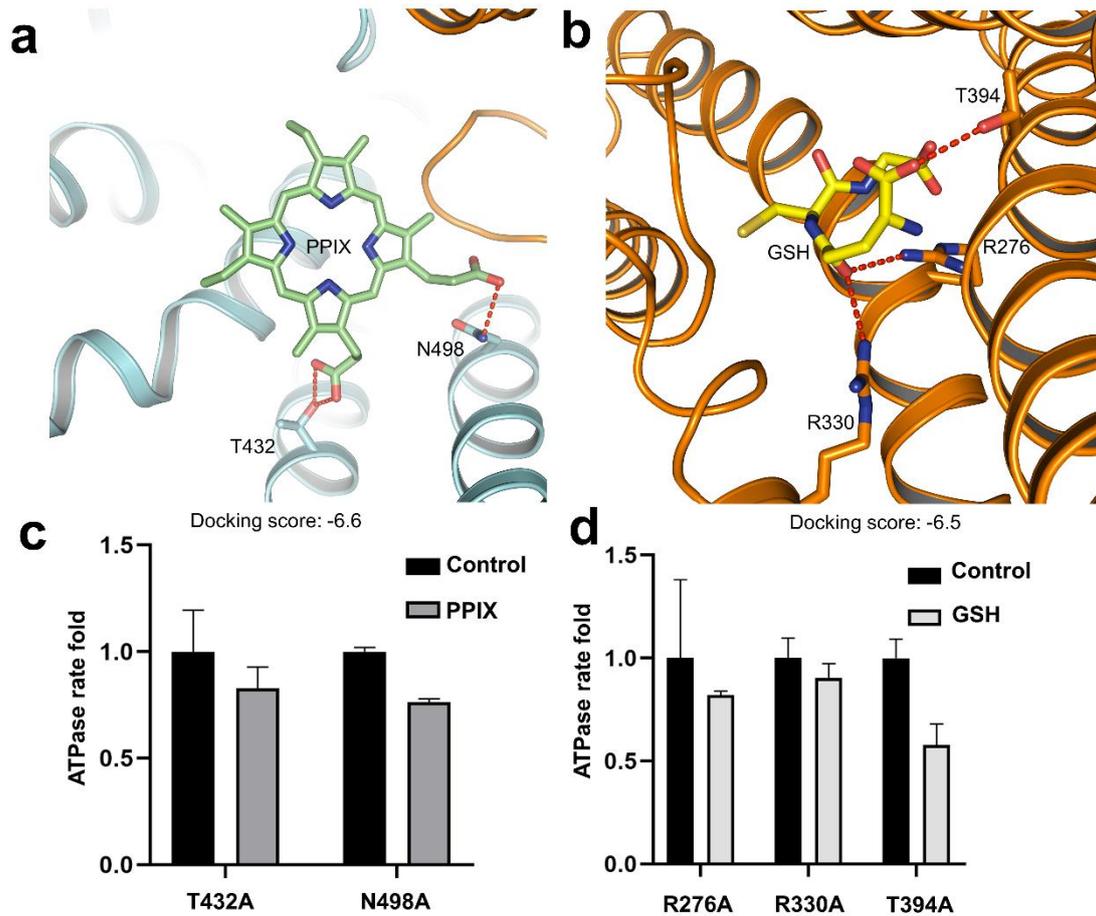
Supplementary Fig. S8 The translocation pathway in hABC6.

The substrate translocation pathway of hABC6 in two conformations calculated by HOLE software. The pockets are shown as meshed surface and two cavities are divided by the plug or W546 in different conformations.



Supplementary Fig. S9 Comparisons of hABCB6 with other homologous proteins.

- a, Comparison of occluded hABCB6 with outward-facing ABCB1 (6C0V).
- b, Comparison of occluded hABCB6 with outward-facing Sav1866 (2HYD).
- c, Comparison of inward-facing conformation hABCB6 with mouse ABCB1 (4M1M).



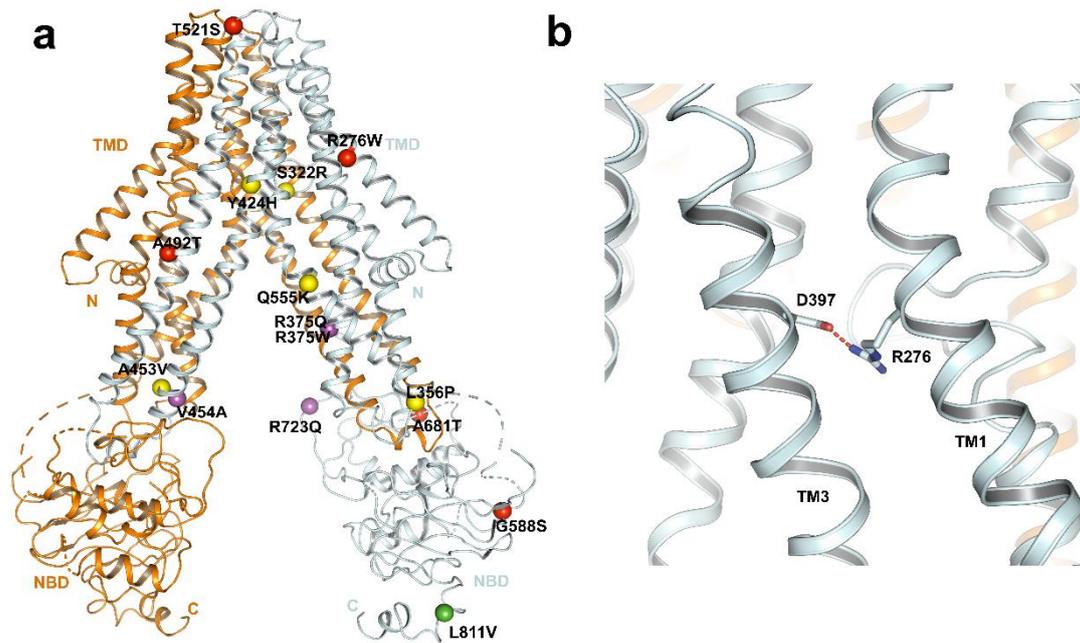
Supplementary Fig. S10 Molecular docking of PPIX and GSH in hABCB6

a, The hABCB6-PPIX dock model and the interactions between PPIX and hABCB6.

b, The hABCB6-GSH dock model and the interactions between GSH and hABCB6.

c, The ATPase rate assay of PPIX-related mutants: T432A and N498A.

d, The ATPase rate assay of GSH-related mutants: R276A, R330A, and T394A.



Supplementary Fig. S11 Structural interpretation of the pathogenic mutations.

a, The pathogenic mutations of hABCB6. Mutations related to different diseases are marked with different colors. Red: Porphyria. Yellow: dyschromatosis universalis hereditaria (DUH). Magenta: familial pseudohyperkalemia (FP). Green: ocular coloboma.

b, The interaction between R276 and D397.

Supplementary Table S1 | Cryo-EM data collection, refinement and validation statistics

	ABC6	ABC6[EQ]
Data collection and processing		
Magnification	130,000	81,000
Voltage (kV)	300	300
Electron exposure (e ⁻ /Å ²)	50	50
Defocus range (μm)	-1.5 ~ -2.5	-1.5 ~ -2.5
Pixel size (Å)	0.86	0.97
Software	RELION-3	RELION-3
Symmetry imposed	C2	C2
Initial particle images (no.)	1450,000	1300,000
Final particles images (no.)	259,000	218,000
Map resolution (Å)	3.62	3.52
FSC threshold	0.143	0.143
Local map resolution range (Å)	2.4-4.0	2.4-4.0
Refinement		
Software	PHENIX 1.14	PHENIX 1.14
Initial model used (PDB code)		
Model resolution (Å)	3.7	3.6
FSC threshold	0.5	0.5
Map sharpening <i>B</i> factor	-162.9	-198.7
Model composition		
Non-hydrogen atoms	8828	9394
Protein residues	782	1184
Ligand	0	0
B factors (Å ²)		
Protein	55.36	45.08
Ligand	0	0
R.m.s deviations		
Bond length (Å)	0.005	0.004
Bond angles (°)	1.199	0.839
Validation		
MolProbity score	2.55	1.52
Clashscore	5.69	4.66
Poor rotamers (%)	7.73	0.40
Ramachandran plot		
Favored (%)	89.28	95.92
Allowed (%)	9.56	4.08
Disallowed (%)	0.18	0.00