Supplementary information

Methods and Materials

Protein expression and purification

The full-length human *ABCB11* gene was synthesized after codon optimization for the mammalian cell expression system by General Biosystems Company. Both the wild-type protein and N-terminal truncation ΔN44 construct were subcloned into a pCAG vector with an N-terminal FLAG tag (DYKDDDDK).

For protein expression, the HEK 293F cells were cultured in SMM 293T-I medium (Sino Biological Inc.) at 37°C with 5% CO_2. Cells were transfected when the density reached ~2.5 × 10^6 cells per mL. For transfection, ~1.5 mg plasmids were mixed with 4 mg linear polyethylenimines (PEIs) (Polysciences, Inc) in 45 mL fresh medium for 20 min, followed by a 30-min static incubation. The transfected cells were grown at 37°C for 48 h before harvesting. Cell pellets were resuspended in the lysis buffer containing 50 mM Tris-HCl pH 8.0, 200 mM NaCl, 10% glycerol (w/v) after centrifugation at 1,500 *g* for 10 min. The suspension was frozen in liquid nitrogen and stored at -80°C for further use.

For protein purification, thawed suspension was incubated in the lysis buffer with additional 1% (w/v) lauryl maltose neopentyl glycol (LMNG, Anatrace), 0.2% (w/v) cholesteryl hemisuccinate (CHS, Sigma) at 8°C for 2 h for membrane solubilization and protein extraction. After ultracentrifugation at 45,000 rpm for 45 min (Beckman Type 70 Ti), the supernatant was incubated with the anti-FLAG M2 affinity gel (Sigma) on ice for 40 min. Then the resin was loaded onto the column and washed three times, each with 10 ml of wash buffer containing 50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.06% digitonin (w/v, Apollo Scientific). Protein was eluted with 6 mL of wash buffer plus 200 μg/ml FLAG peptide. The eluent was concentrated by a 30-kDa cut-off Centricon (Millipore) and then applied to size-exclusion chromatography (Superdex 200 Increase 10/300, GE Healthcare) in wash buffer plus 2 mM DTT (Sangon Biotech). Peak fractions were pooled and frozen in liquid nitrogen or concentrated for EM analysis.

ATPase activity assays

ATPase activities of wild-type ABCB11 and mutants were measured using the ATPase Colorimetric Assay Kit (Innova Biosciences) in 96-well plates at $OD_{630\ nm}$.

For ATPase activity assay preparation, the fresh purified proteins were collected from the peak of the Gel filtration. A final concentration of 0.2 μM protein was added to the reaction buffer containing 20 mM HEPES-KOH, pH 7.0, 50 mM KCl, 2 mM ATP and 2 mM $MgCl_2$ to 100 μL as one reaction sample. Reactions were performed at 37°C for 60 min and then the amount of released Pi was quantitatively measured. For each reaction, the final 40 μM compounds (glycocholic, Sigma; taurocholic, Sigma; tauroursodesoxycholic, Meilunbio) were added. In addition, 1% DMSO was added to dissolve rifampicin (Sigma) and glibenclamide (Macklin) for inhibitor assays, and DMSO was also added for the corresponding control and taurocholic group.

Cryo-EM sample preparation and data collection

The purified proteins were concentrated to 6.0 mg/mL and applied to glow discharged Quantifoil R1.2/1.3 grids 300-mesh Au Holey Carbon Grids. An aliquot of 4 μL of the specimen was added to the grid with the blot time 4 s and plunged into liquid ethane by using Vitrobot Mark IV (FEI) at 8°C and 100% humidity.

6959 movie micorgraphs comprising 40 frames were collected in super resolution mode with K3 camera at a nominal magnification of 22,500 × with a defocus range from -2.0 to -1.5 μm. Using Serial EM[1], a total dose of 60 electrons/$Å^2$ was used for collection. The electron dose rate is 20 $e^-/Å^2/s$.

Image processing

The movie frames were motion corrected and dose weighted using MotionCor2[2], binned 2-fold to yield a pixel size of 1.06 Å. The contrast transfer function estimation was performed using CTFFIND4[3]. A total of 3,008,703 particles were auto-picked and extracted at a 2-fold

binned pixel size of 2.12 Å in RELION3.1[4]. Then particles were subjected to 2-D classification and good classes were selected for 3-D classification. 2,655,850 particles were used for 3-D classification into 4 classes. Then, particles from the best class were refined and re-extracted, yielding a 4.5 Å map. After multi-rounds of 3-D skip alignment classification, followed by CTF refinement and Bayesian Polishing in RELION3.1[4], 224,741 particles produce a 3.5 Å map. Map resolution was estimated with the gold-standard Fourier shell correlation 0.143 criterion[5]. Local resolutions were estimated using Resmap[6] with RELION3.1[4].

Model building and refinement

The final sharpened map with a B-factor of -130 Å^2 was used for model building in Coot[7]. A poly-Ala model was first built in the model with the map by PHENIX[8]. The model building was guided by the bulky residues such as phenylalanine, tyrosine and tryptophan. And the predicted model by SWISS-MODEL[9] was considered to facilitate model building. The final model contains residues 1-31, 44-695 and 736-1315. Structure refinement was performed by PHENIX in real space with secondary structure and geometry restraints. UCSF Chimera[10] and PyMOL (https://pymol.org) were used for preparing the figures.

Data availability

All relevant data are available from the authors and/or included in the manuscript or Supplementary Information. Atomic coordinates and EM density maps of the human ABCB11 in this paper (PDB: 6LR0; EMDB: EMD-0956) have been deposited in the Protein Data Bank and the Electron Microscopy Data Bank, respectively.

References

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Supplementary information, Fig. S1

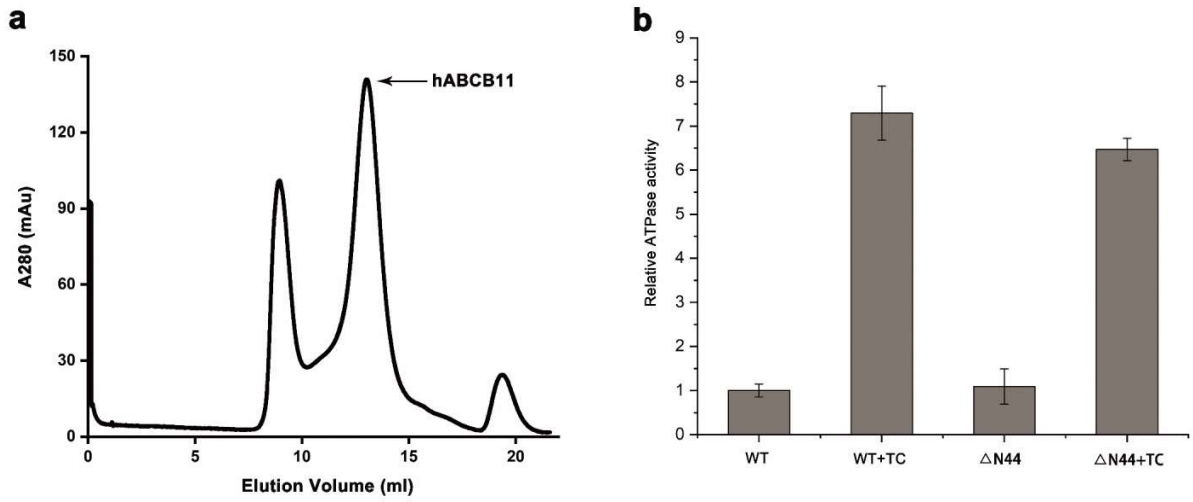

Fig. S1 Biochemical analysis of human ABCB11. **a** Gel filtration profile of purified human ABCB11 solubilized in digitonin. Peak fractions were collected for further structure determination. **b**, ATPase activity assays of wild-type ABCB11 and N-terminal truncated mutant (△N44). Data are presented as the means ± S.D. from three independent assays.

Supplementary information, Fig. S2

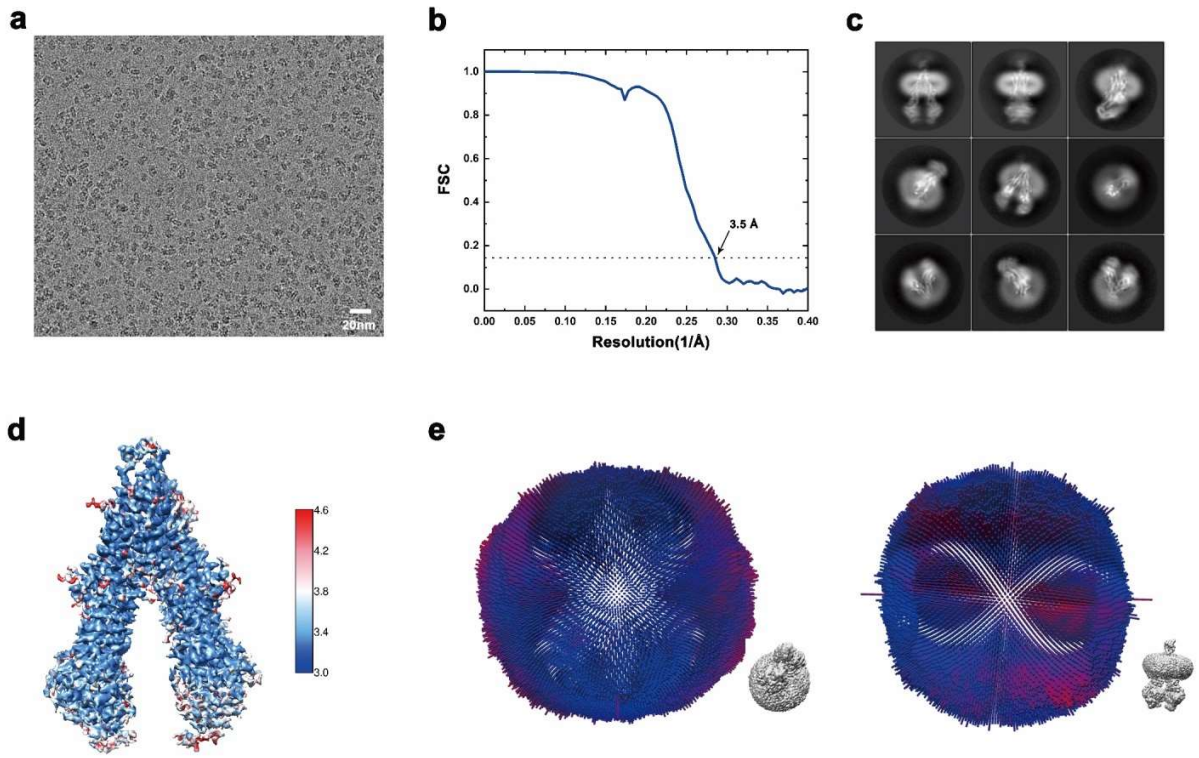

Fig. S2 Cryo-EM analysis of the human ABCB11. **a** Representative cryo-EM image of ABCB11 after motion correction and contrast transfer function correction. **b** The gold-standard Fourier shell correlation curve for the overall map. **c** Representative 2-D class averages of ABCB11. **d** Density map colored by local resolution estimation using Resmap. **e** Euler angle distribution of the classified particles used for the final 3-D refinement of the overall map.

Supplementary information, Fig. S3

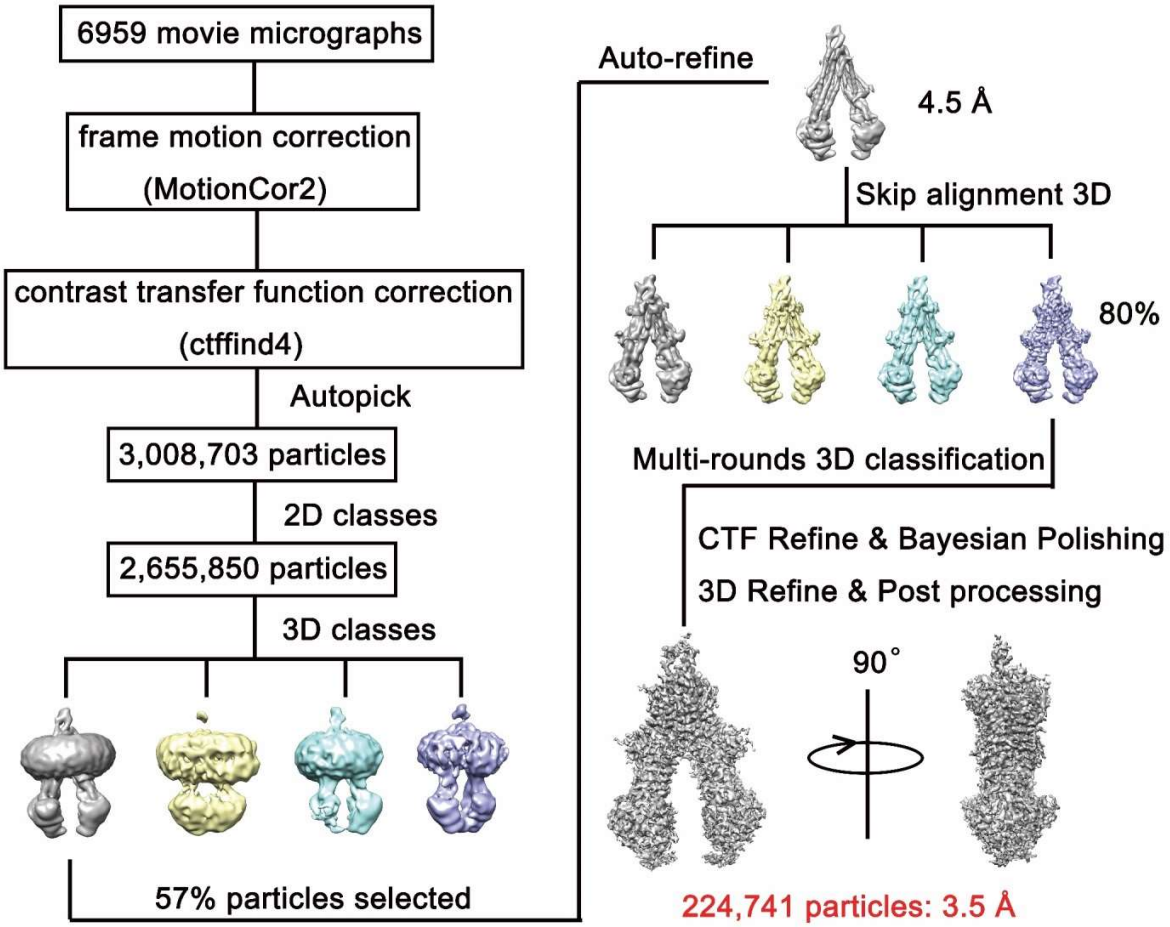

Fig. S3 Flowchart for cryo-EM data processing of the human ABCB11.

Supplementary information, Fig. S4

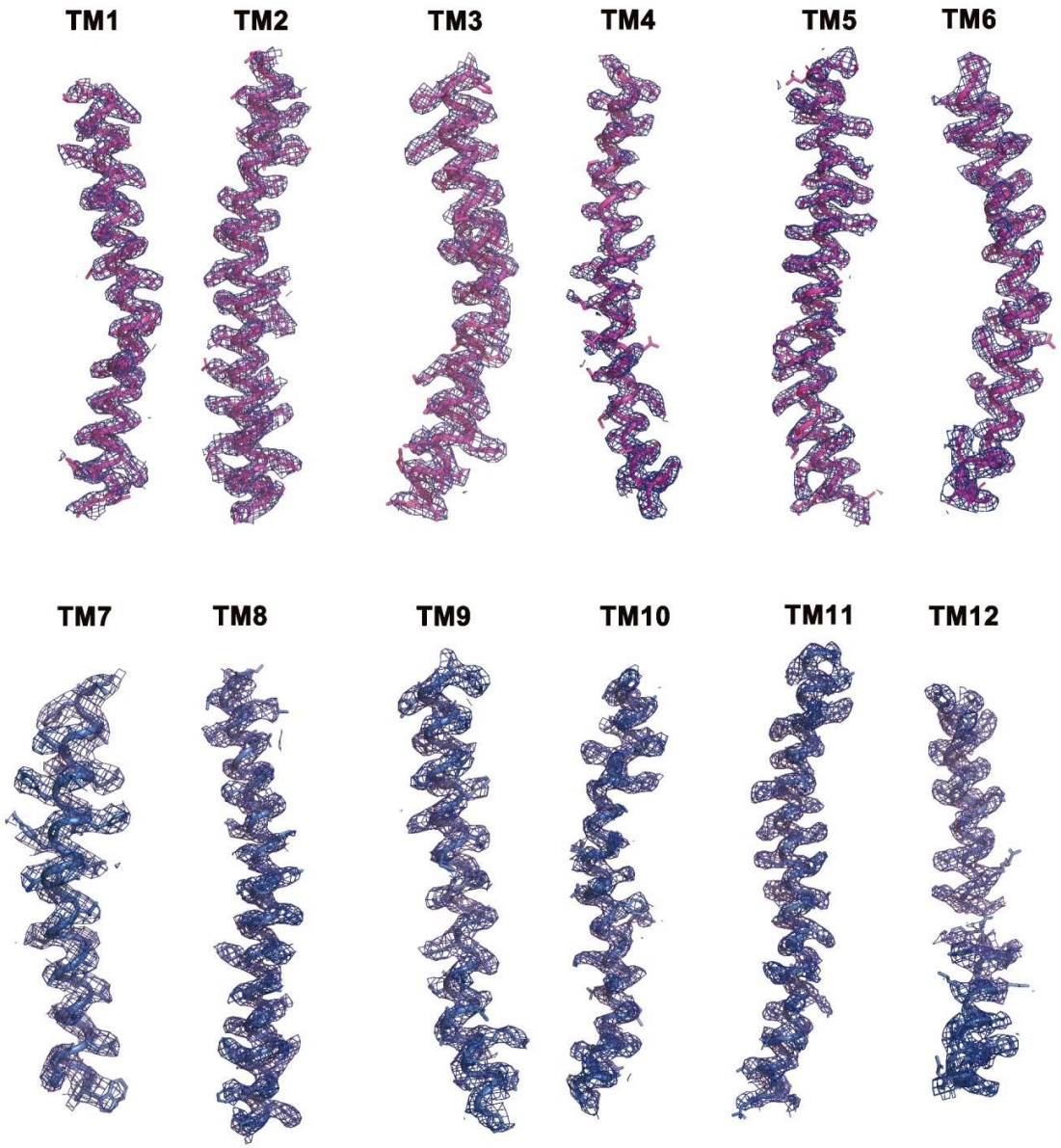

Fig. S4 EM densities of representative segments of human ABCB11. Contour level is set at σ=5 and density is carved at a distance of 2 Å for TM1~TM12.

Supplementary Table S1

Summary of cryo-EM data

	ABCB11 (EMD-0956) (PDB-6LR0)
Data collection and processing	
Magnification	22,500
Voltage (kV)	300
Electron exposure (e^{-}/Å^2)	60
Defocus range (μm)	-1.5 to -2.0
Pixel size (Å)	1.06
Symmetry imposed	C1
Initial particle images (no.)	3,008,703
Final particle images (no.)	224,741
Map resolution (Å)	3.5
FSC threshold	0.143
Map resolution range (Å)	2.12-999
Refinement	
Initial model used (PDB code)	Ab initio model
Model resolution (Å)	3.5
FSC threshold	0.143
Model resolution (Å)	3.5
Map sharpening *B* factor (Å^2)	-130.571
Model composition	
Non-hydrogen atoms	9523

Protein residues	1232
Ligands	0
R.m.s. deviations	
Bond lengths (Å)	0.009
Bond angles (°)	1.233
Validation	
MolProbity score	1.95
Clashscore	5.41
Poor rotamers (%)	1.27
Ramachandran plot	
Favored (%)	88.83
Allowed (%)	11.09
Disallowed (%)	0.08