Cryo-EM Structures of human ABCA7 provide insights into its phospholipid translocation mechanisms

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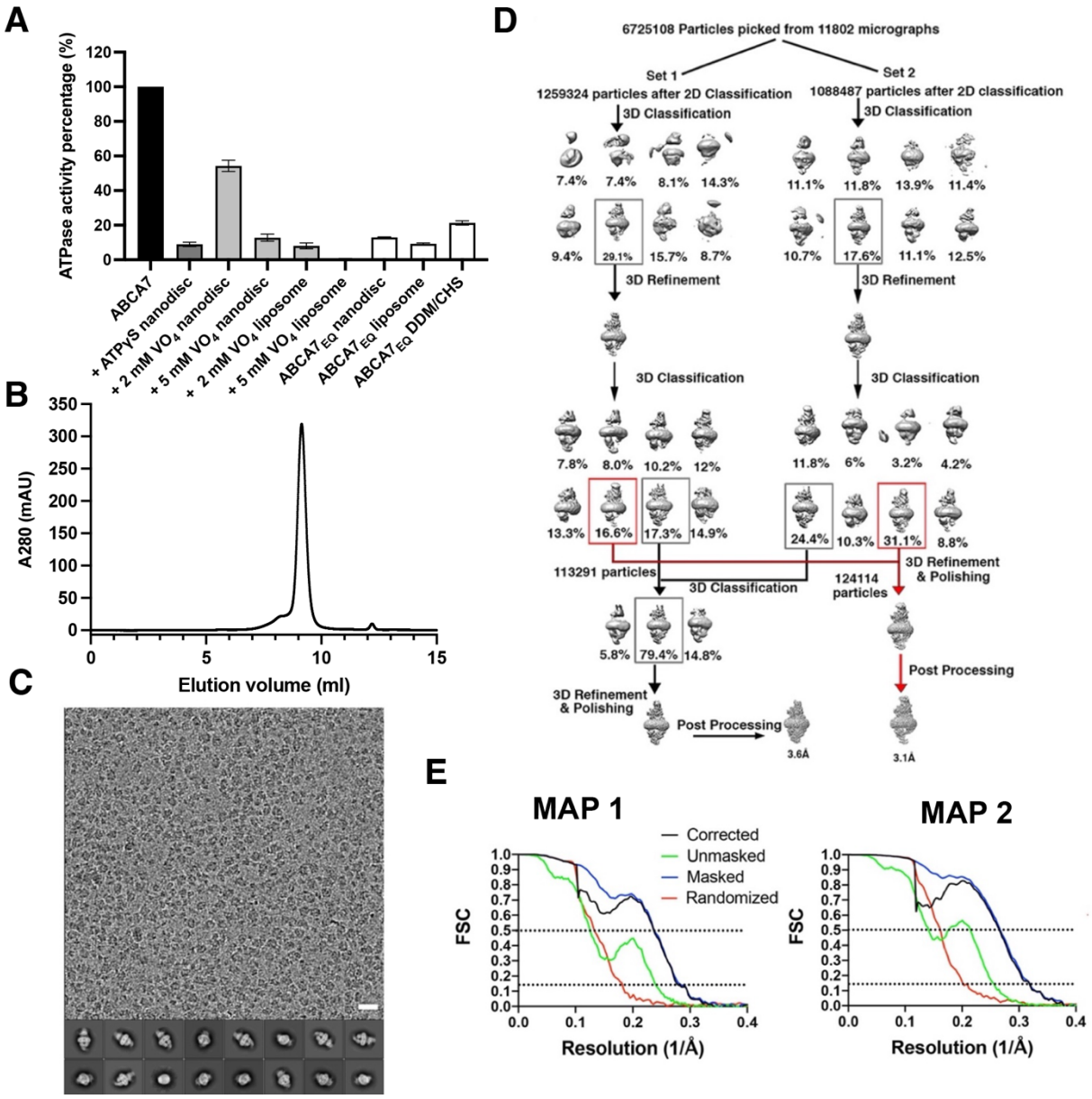
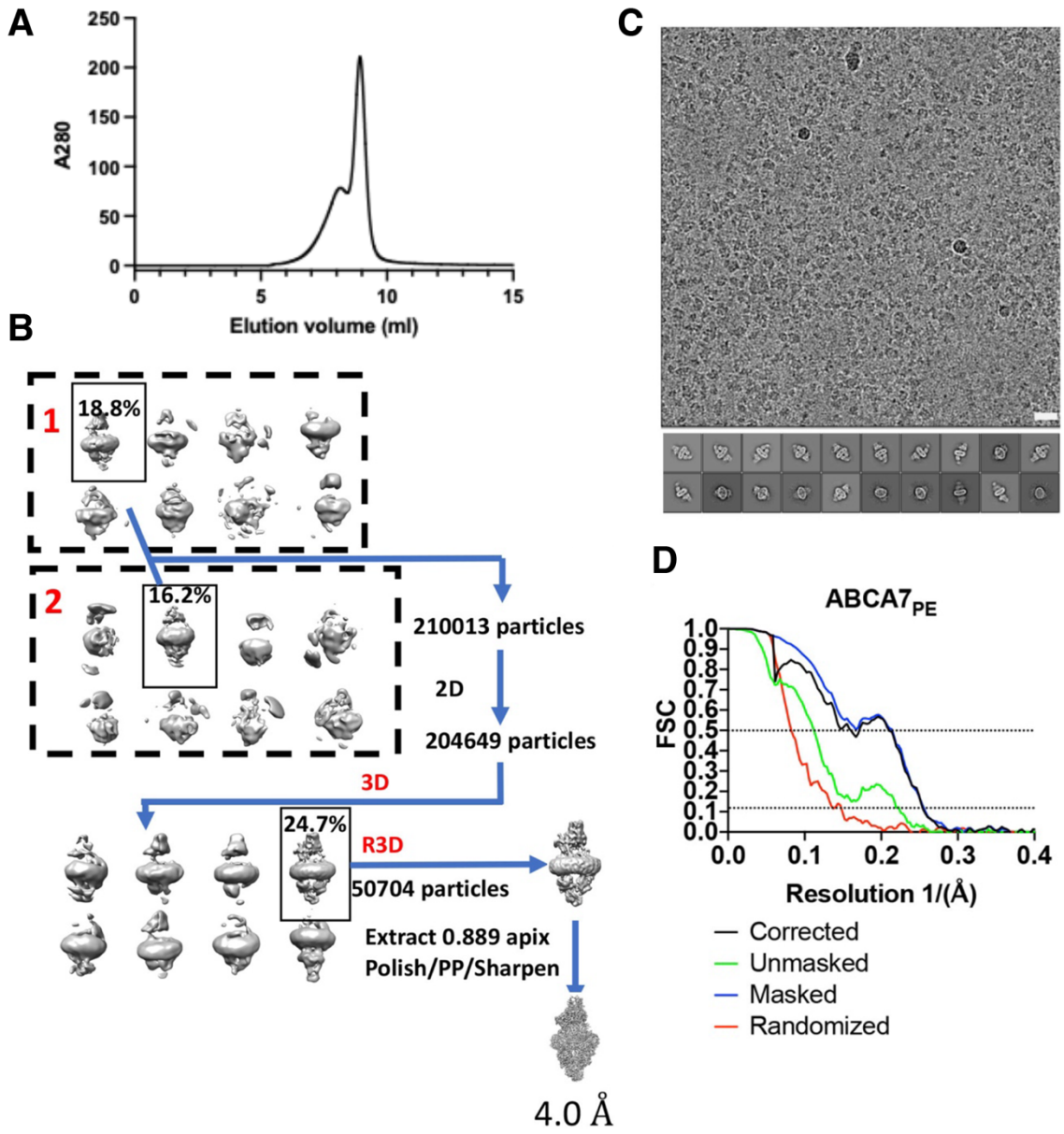
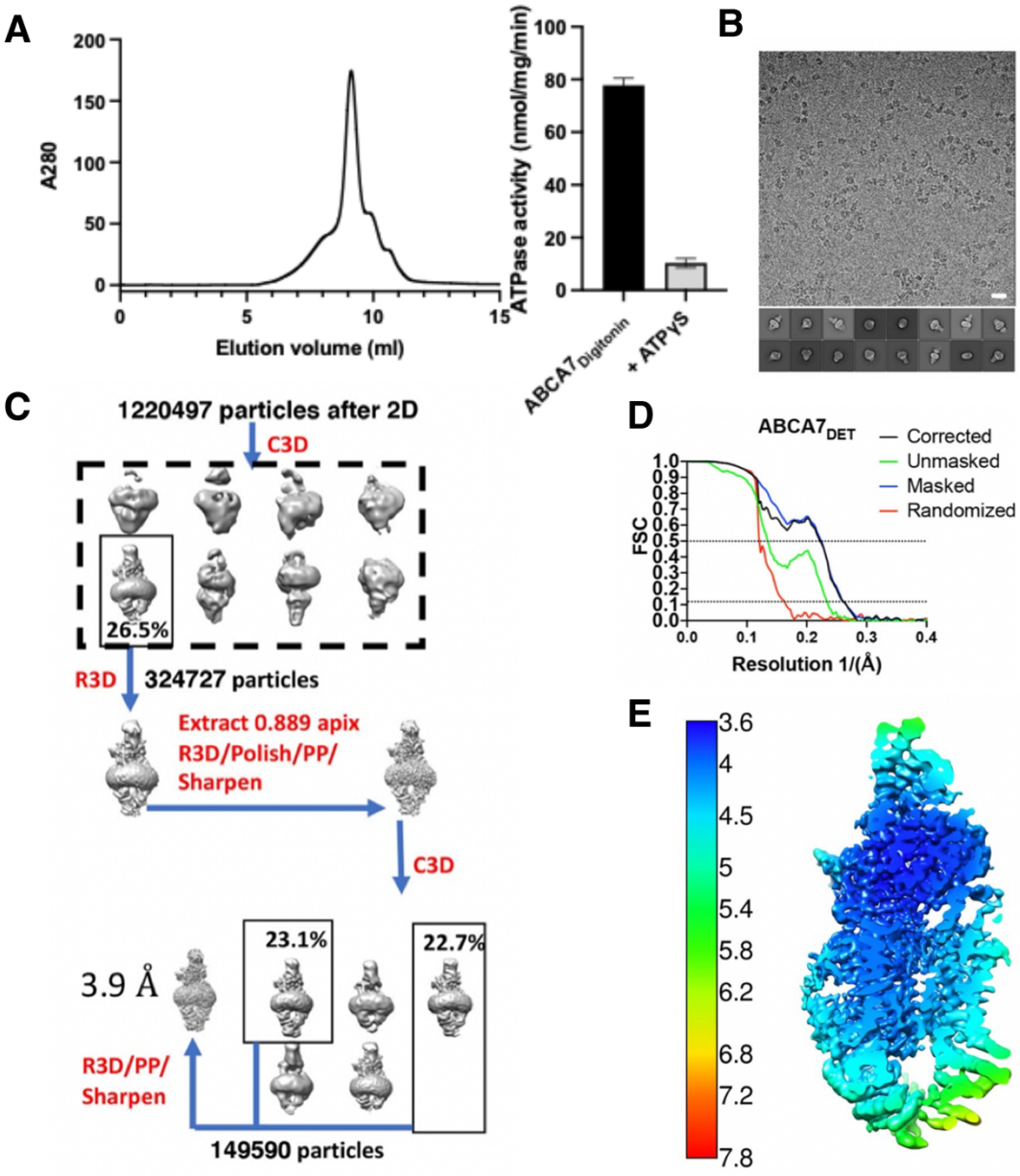


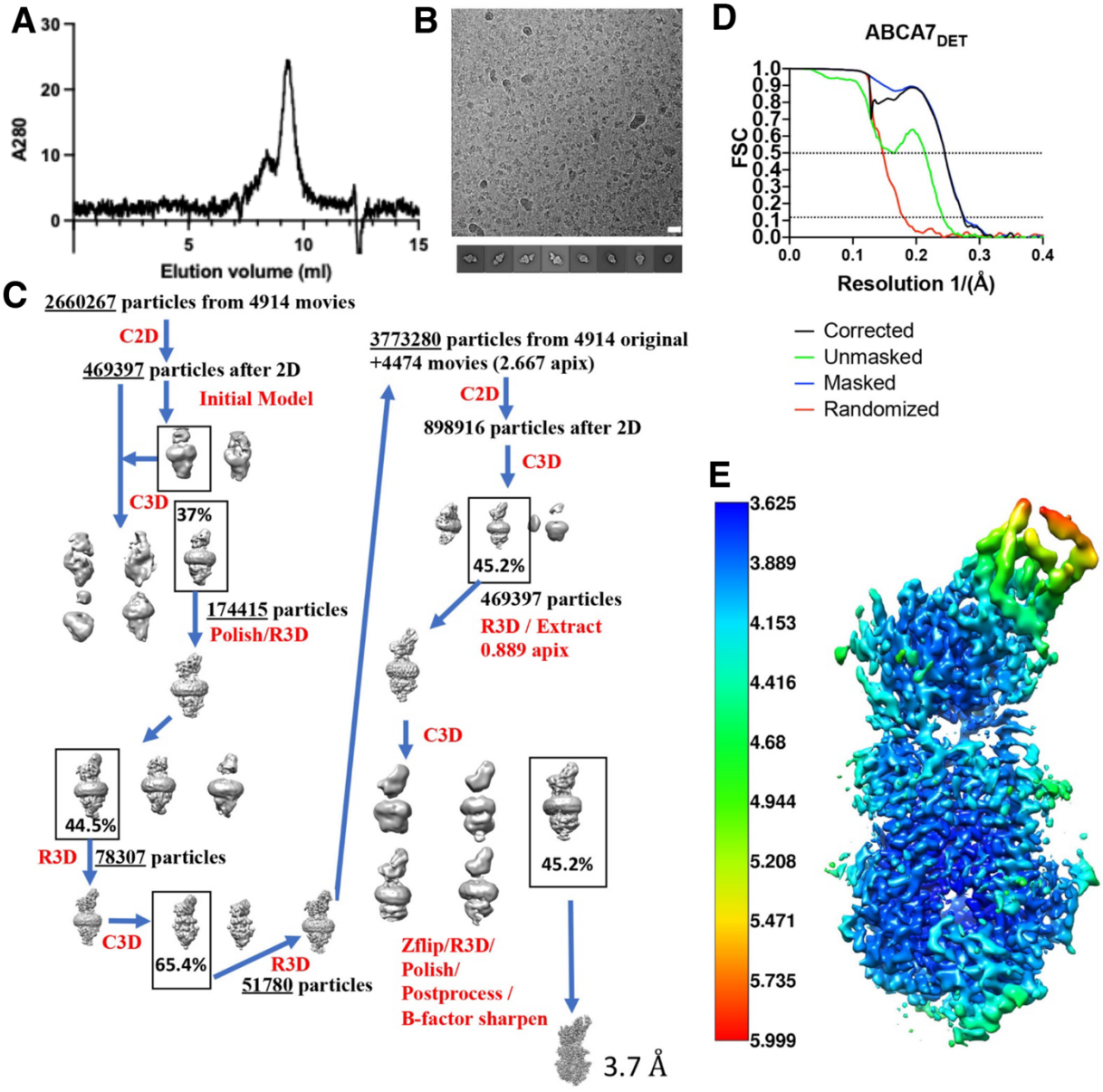
Appendix Figure S1. **ABCA7$_{BPL}$ characterization and cryo-EM data processing**. (**A)** ATPase rates for ABCA7 under various experimental conditions as percentage of wildtype ABCA7 ATPase activity in liposomes, nanodiscs, and detergent. ATPase rate comparison for nanodisc and liposome reconstituted ABCA7 with and without ATP analog adenosine-5'-o-(3-thio-triphosphate) (ATPγS) or sodium orthovanadate (VO_4) along with a hydrolysis-deficient mutant ABCA7$_{EQ}$ in nanodiscs, liposomes, and detergent. Experimental replicates (n)=3 and error bars represent standard deviation (s.d). (**B)** SEC peak of ABCA7BPL samples for cryo-EM. (**C)** Representative cryo-EM micrograph at -2.5 μm defocus. Scale bar = 20 nm. (**D)** cryo-EM processing workflow. Boxes indicate 3D classes used for further refinement for both Map 1 and Map 2 (red). **E** Fourier shell correlation (FSC) curves for Map 1 (top) and Map 2 (bottom) Dotted lines indicate position 0. 143 and 0.5 cutoff criteria for resolution estimates.



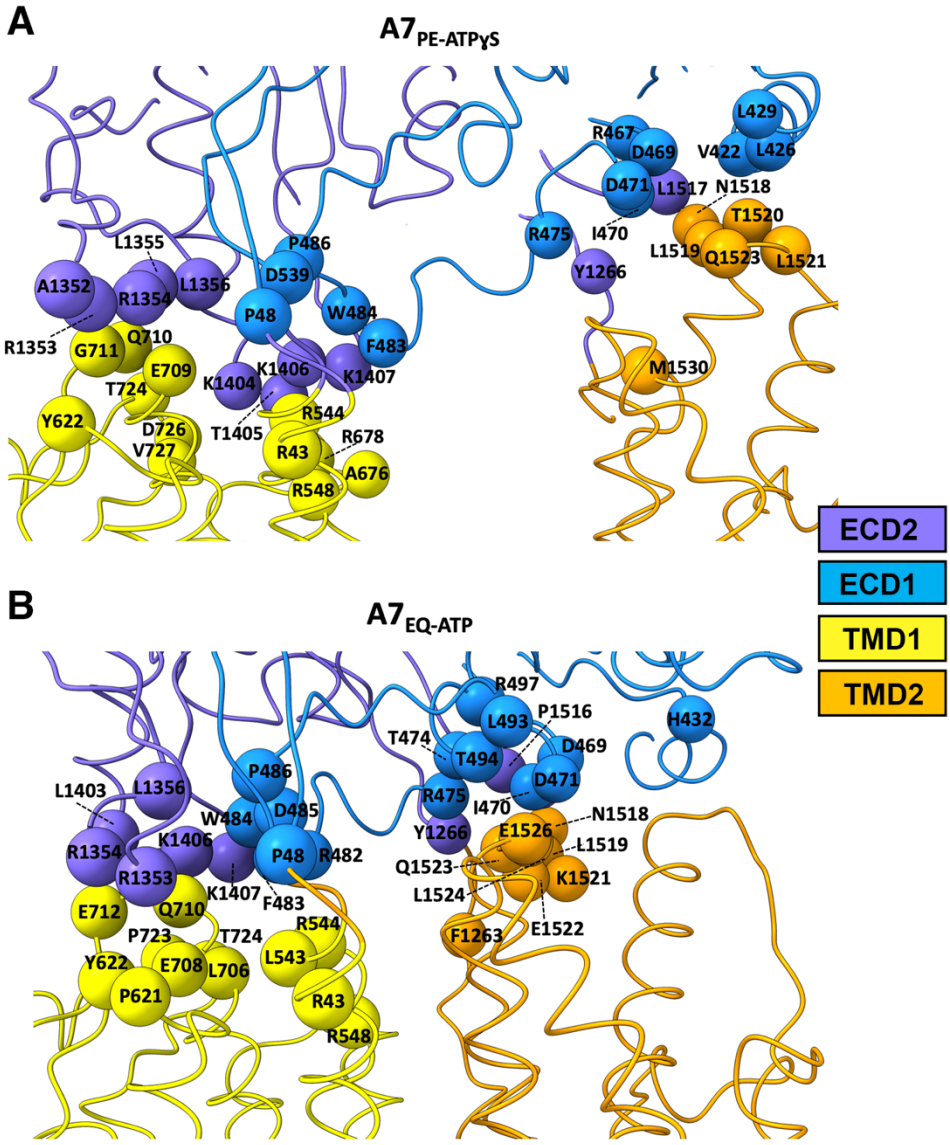
Appendix Figure S2. ABCA7$_{PE}$ Cryo-EM data processing. (A) Size exclusion chromatography micrograph of cryo-EM sample showing monodisperse ABCA7$_{PE}$ nanodisc (main peak). **(B)** Representative micrograph at -2.5 µm defocus and 2D classes. Scale bar = 20 nm. **(C)** cryo-EM processing workflow. Dashed boxes demarcate Subsets 1 and 2. Solid boxes indicate 3D classes used for further refinement. 2D = 2D Classification, 3D=3D classification, R3D = 3D refinement. **(D)** Fourier shell correlation (FSC) curves for ABCA7$_{PE}$. Dotted lines indicate position 0.143 and 0.5 cutoff criteria for resolution estimates.



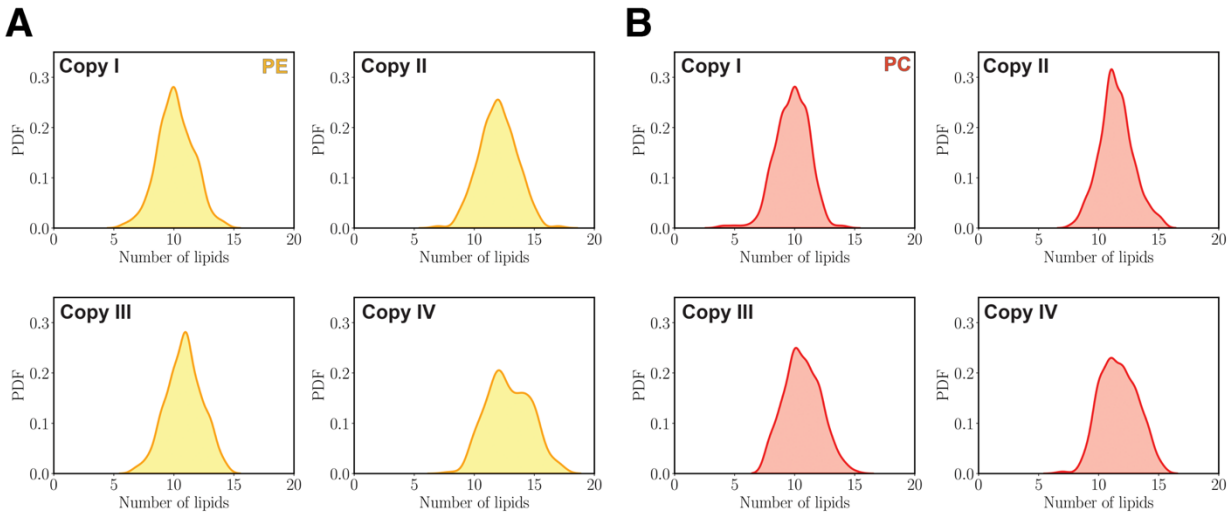
Appendix Figure S3. **ABCA7$_{DIGITONIN}$ cryo-EM processing. (A)** SEC profile of ABCA$_{7DIGITONIN}$ and its ATPase activity with and without ATPγS. **(B)** Representative micrograph at -2.5 µm defocus and 2D classes. Scale bar = 20 nm. **(C)** cryo-EM processing workflow. C2D = 2D Classification, C3D=3D classification, R3D = 3D refinement. **(D)** Local resolution colored EM map of ABCA7$_{DIGITONIN}$. **(E)** Fourier shell correlation (FSC) curves for ABCA7$_{DIGITONIN}$. Dotted lines indicate position 0.143 and 0.5 cutoff criteria for resolution estimates.



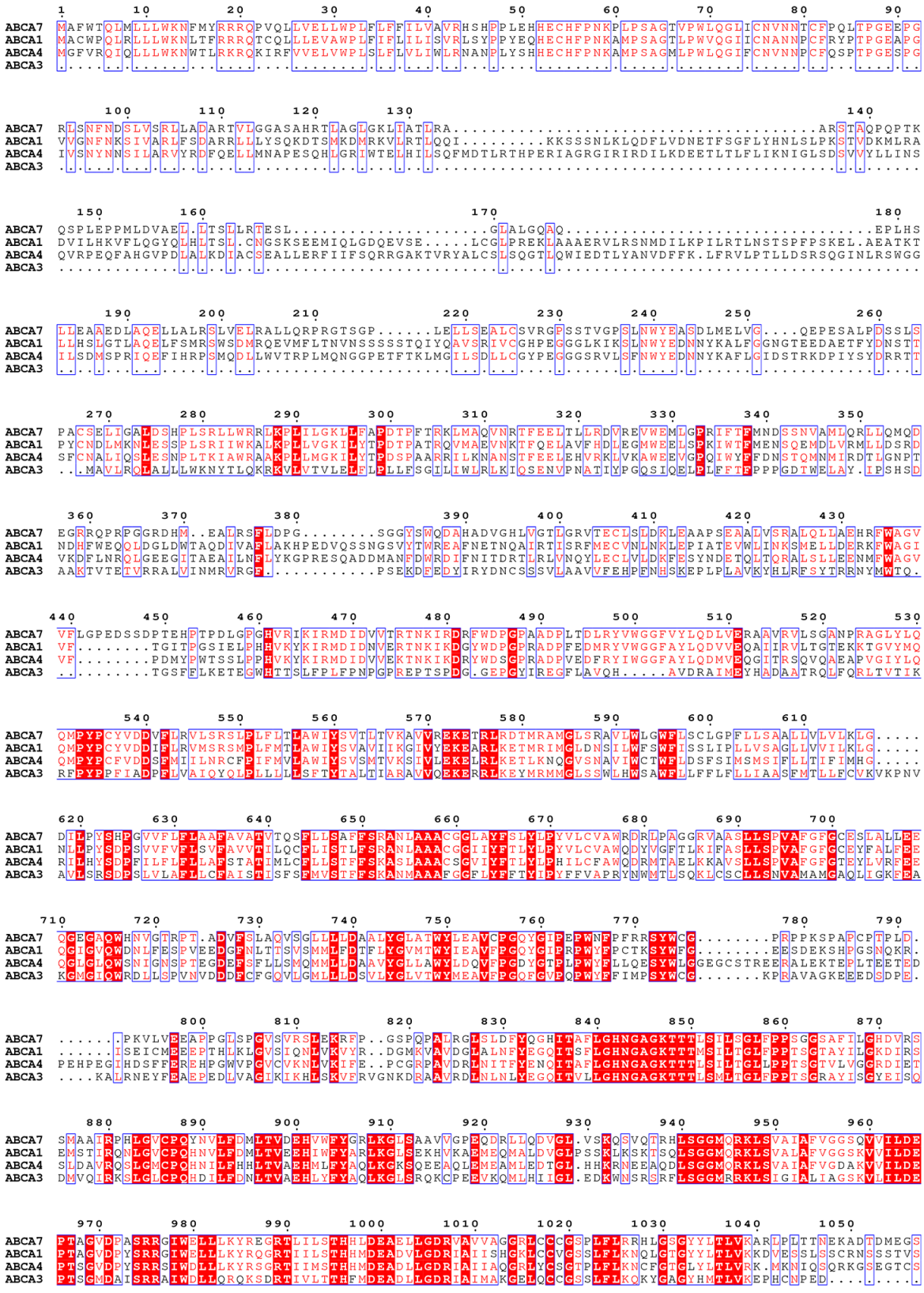
Appendix Figure S4. ABCA7$_{EQ\text{-}ATP}$ cryo-EM processing. **(A)** SEC peak for ABCA7$_{EQ\text{-}ATP}$ in nanodiscs. (**B)** Representative EM micrograph (-2.5 defocus) and rep 2D classes for ABCA7$_{EQ\text{-}ATP}$ sample. © Cryo-EM data processing pipeline. C2D = 2D Classification, C3D=3D classification, R3D = 3D refinement. **(D)** FSC curves for ABCA7$_{EQ\text{-}ATP}$. Dotted lines indicate position 0.143 and 0.5 cutoff criteria for resolution estimates. (**E)** Local resolution colored EM map.

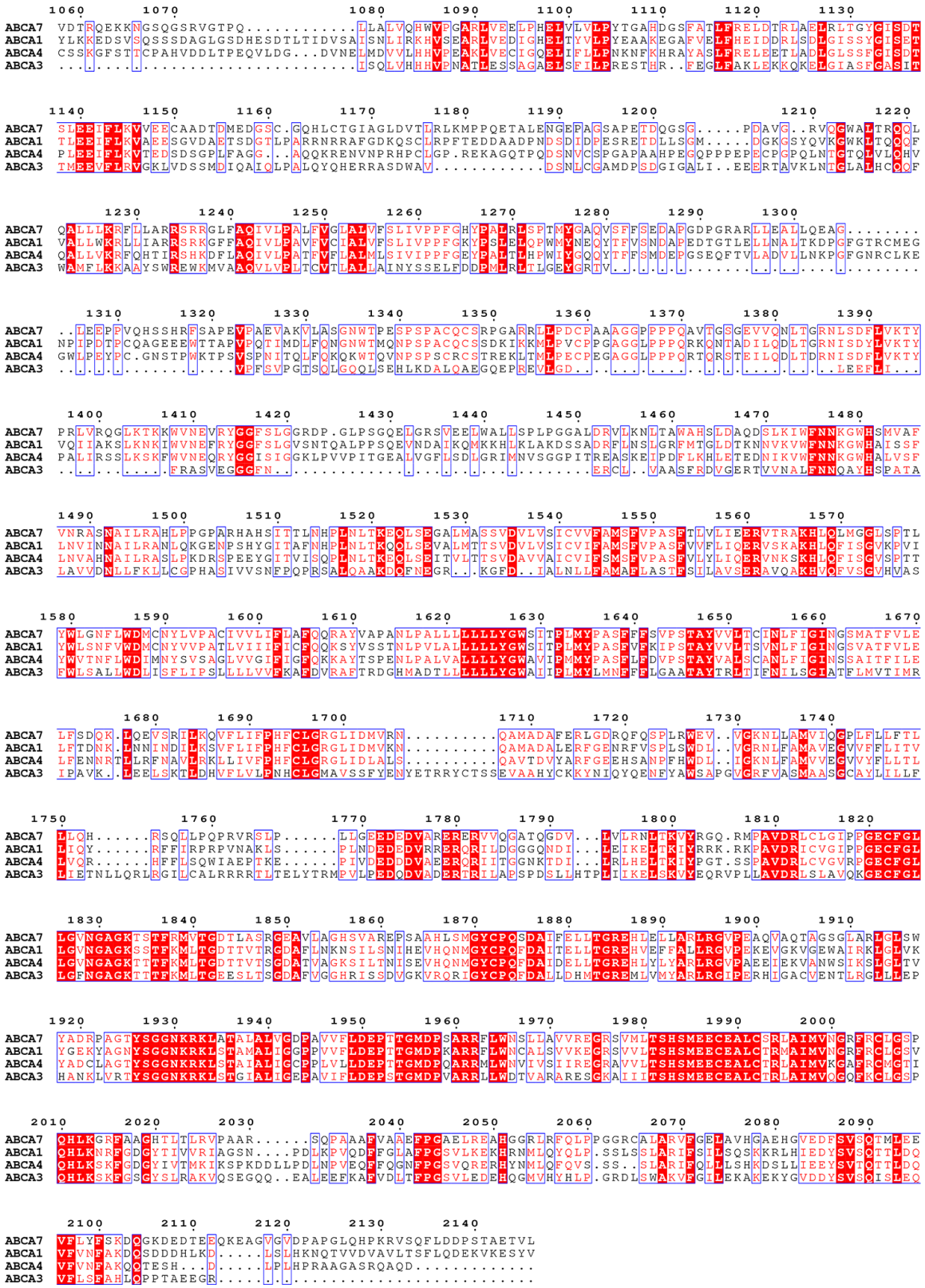


Appendix Figure S5. TMD-ECD interfaces of open and closed form ABCA7. (A) The TMD-ECD binding interfaces of ABCA7$_{PE}$ with TMD1 and TMD2 and with Cα for residues in TMD1 and TMD2 within 5Å of either ECD or vice versa shown as spheres. **(B)** The same analysis for the TMD-ECD binding interfaces of ABCA7$_{EQ\text{-}ATP}$ with closed cavity.

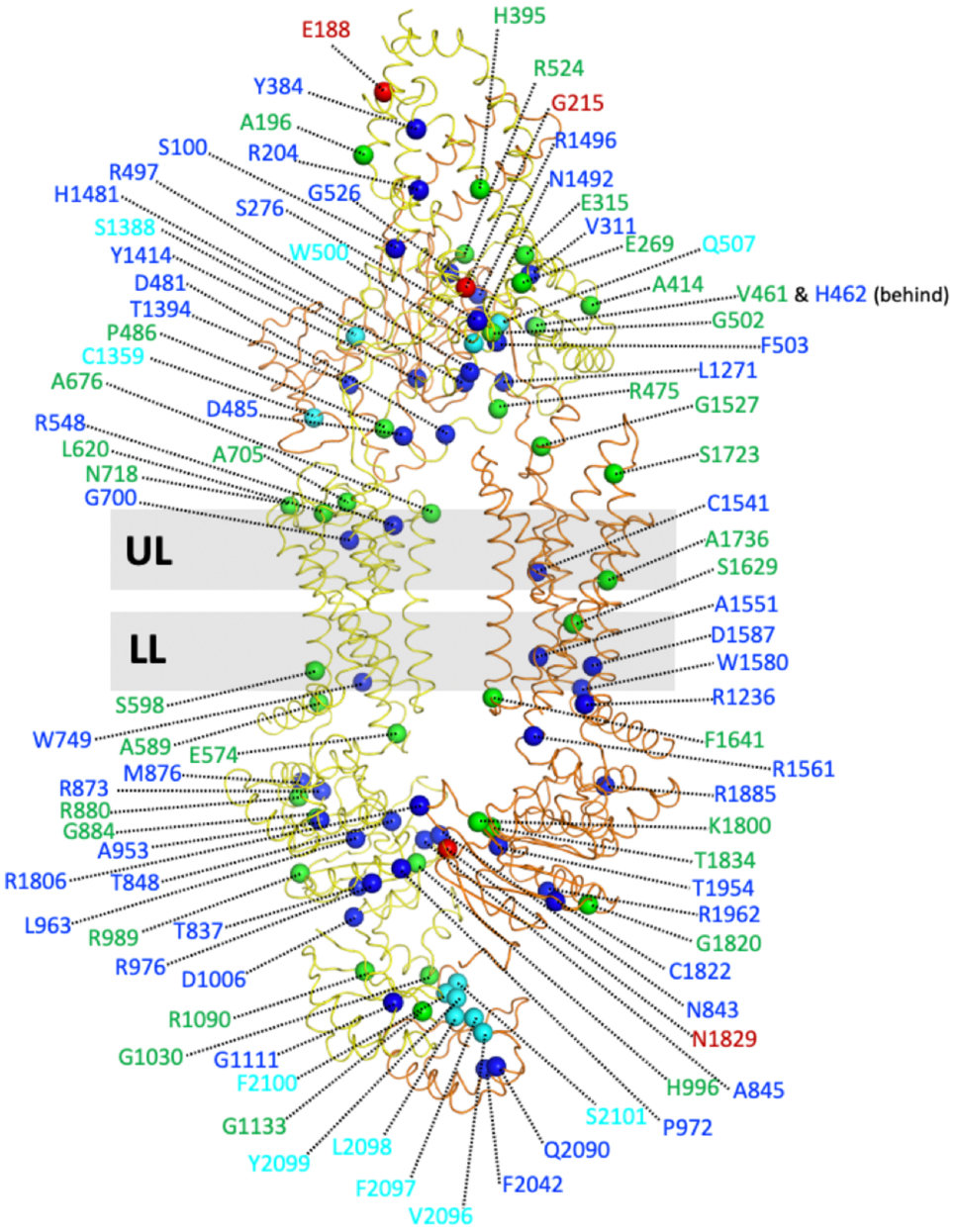


Appendix Figure S6. Histograms of lipid counts in the TMD lumen for POPE and POPC systems. (A/B) POPE and POPC lipid counts in the lumen of ABCA7 captured through the 2 μs of MD simulations. The histograms were calculated for each protein's copy separately and shown here. Y-axis of the histograms represent probability distribution function (PDF). Copy IV of the POPE system shows more lipid penetration to the lumen of TMD compared to other POPE and POPC systems.


ABCA7
ABCA1
ABCA4
ABCA3



Appendix Figure S7. Sequence alignment of human (hs) ABCA7, ABCA1, and ABCA4, and ABCA3.



Appendix Figure S8. Amino acid positions of ABCA7 with variants associated with risk of AD. Structure of ABCA7 with Cα atoms shown for amino acid with known pathogenic variants (green), protective variants (red), and ABCA7 residues conserved in ABCA1 with mutations known to disrupt the latter's binding to apoA1 and/or phospholipid translocation (blue). Cyan spheres highlight residue positions of ABCA1 mutations known to disrupt phospholipid efflux including those equivalent to the VFVNFA motif within the ABCA1 RD. Grey bars indicate the upper (extracellular) and lower (cytoplasmic) membrane bilayer leaflets (UL and LL, respectively).

	Nanodiscs	Detergent (DDM/CHS)	Liposomes
K_M	0.51	0.43	0.74
V_{Max}	37.41	162.9	167.8

Appendix Table S1. Table of K_M and V_{Max} values of ABCA7 incorporated in nanodiscs, liposomes, and detergent.

Dataset	$ABCA7_{BPL}$		$ABCA7_{PE}$	$ABCA7_{DIGITONIN}$	$ABCA7_{EQ-ATP}$
Magnification	96k		96k	96k	96k
Pixel Size (Å)	0.895		0.889	0.889	0.89
Total Dose (e/Å^2)	60		40	40	40
Defocus Range (um)	-0.8 to 2.6		-0.8 to 2.6	-0.8 to 2.6	-0.8 to 2.6
Maps	Map 1	Map 2	Map3		
EMDB ID	EMD-28041	EMD-28044	EMD-28047	EMD-28050	EMD-28451
# Particles in final Class	91381	124114	50704	149590	177230
Resolution (Å) (0.143 threshold)	3.6	3.2	4.0	3.9	3.7
Sharpening B factor	-82.9	-20	-132	-50	-134.1
Refined Coordinates	$ABCA7_{BPL}$		$ABCA7_{PE}$	$ABCA7_{DIGITONIN}$	$ABCA7_{EQ-ATP}$
PDB ID	8EDW		8EE6	8EEB	8EOP
# Residues/Non-hydrogen Atoms	1804/14311		1808/14475	1873/14673	1687/13266
Ligands	20		26		4
R.M.S deviations					
Bond Length (Å)	0.003		0.003	0.002	0.003
Bond Angles (°)	0.676		0.683	0.626	0.593
MolProbity Statistics					
MolProbity Score	1.76		1.69	1.73	1.76
Clashscore	8.80		8.30	9.05	6.82
Poor rotamers (%)	0.07		0.07	0.00	0.00
Ramachandran statistics					
Favored (%)	95.80		96.42	96.61	94.30
Allowed (%)	4.14		3.52	3.39	5.64
Outliers (%)	0.06		0.06	0.00	0.06

Appendix Table S2. Data collection and refinement statistics.