1 **Capturing the interplay of membrane lipids and structural transitions in human ABCA7**

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14 **Abstract**

15 Phospholipid extrusion by ABC subfamily A (ABCA) exporters is central to cellular physiology,

16 although the specifics of the underlying substrate interactions and transport mechanisms remain

17 poorly resolved at the molecular level. Here we report cryo-EM structures of lipid-embedded

18 human ABCA7 in an open state and a nucleotide-bound, closed state at resolutions between 3.6-

19 4.0 Å. The former reveals an ordered patch of bilayer lipids traversing the transmembrane domain

20 (TMD), while the latter reveals a lipid-free, closed TMD with a small extracellular opening. These

21 structures offer a structural framework for both substrate entry and exit from the ABCA7 TMD

22 and highlight conserved rigid-body motions that underlie the associated conformational

23   transitions. Combined with functional analysis and molecular dynamics (MD) simulations, our

24   data also shed light on lipid partitioning into the ABCA7 TMD and localized membrane

25   perturbations that underlie ABCA7 function and have broader implications for other ABCA family

26   transporters.

27

28   **Introduction**

29   ABCA family exporters mediate efflux of phospholipids and sterols from cells,

30   contributing to membrane homeostasis, bilayer structure and asymmetry, and the formation of

31   serum lipoproteins, among other key physiological processes[1]. Their dysfunction therefore

32   underlies several human diseases[2-4]. The molecular details governing the ABCA exporter substrate

33   transport cycle are not fully resolved. To fill this knowledge gap, here we present the structural

34   and functional analysis of human ABCA7, whose dysfunction has been strongly linked to

35   Alzheimer's Disease (AD)[5-12], in a lipid environment. Deficient ABCA7 activity leads to

36   alterations in both brain lipid profiles and fatty acid and phospholipid biosynthetic pathways[13],

37   impaired memory, and reduced immune responses[14,15]. Both *in vitro* lipid flipping[16] and lipid

38   extrusion to apolipoproteins by cells over-expressing ABCA7[17] have been demonstrated, although

39   the correlation between the two processes, if any, remains unclear. To date, no direct structural

40   information exists for ABCA7. Understanding the molecular details of the ABCA7 transport cycle

41   and how its dysfunction alters inflammatory and immune responses, lipid homeostasis, and

42   phagocytosis, which all contribute to AD progression[18-22], may therefore pave the way for novel

43   therapeutics for AD.

44   ABCA7 encodes a 2146 amino acid membrane transporter found in many tissues and

45   blood, hippocampal neurons, macrophages, and microglia[23,24]. Like the phospholipid and sterol

46　　exporter ABCA1 and retinal importer ABCA4, with which it shares 54% and 59% sequence

47　　similarity, respectively, ABCA7 comprises two halves assembled as a full transporter. Each half

48　　consists of a TMD, with the first two transmembrane helices (TMs) of each separated by a large

49　　extracellular domain (ECD), and a nucleotide binding domain (NBD) attached to a cytoplasmic

50　　regulatory domain (RD). To visualize its ATP-dependent conformational cycle in a lipid

51　　environment, we resolved the structures of human ABCA7 in multiple conformations in lipid and

52　　detergent environments using cryo-EM and probed its lipid interactions using ATPase assays and

53　　MD simulations. Our data allow us to directly visualize lipid partitioning into the TMDs and the

54　　associated conformational changes in ABCA7 that provide insights into its mechanisms of

55　　substrate entry and export that likely hold true for other members of the ABCA family.

56　　**Results**

57　　**Dependence of ABCA7 ATPase activity on its lipid environment**

58　　　　Human ABCA7 expressed in a tetracycline inducible stable HEK293 cell line was

59　　reconstituted in liposomes and nanodiscs comprising 80% brain polar lipids (BPL) and 20%

60　　cholesterol (Chol) and its ATPase activity was measured (Figure 1A, Figure S1A). Although ATP

61　　hydrolysis was slowest in nanodiscs, it followed Michaelis-Menten kinetics similar to ABCA7 in

62　　detergent or liposomes comprising the same lipid/cholesterol composition and Michaelis constant

63　　(K_M) values for all three were in the 0.5-0.8 mM range. ATPase rates for a hydrolysis-deficient

64　　mutant carrying E965Q and E1951Q substitutions (ABCA7$_{EQ}$) were drastically reduced compared

65　　to wildtype in both nanodiscs and detergents, demonstrating that the observed activity was specific

66　　(Figure S1A).

67　　　　To analyze the effect of different lipid headgroups on ATPase activity, we compared

68　　ATPase rates of ABCA7 in BPL/Chol nanodiscs to those in which BPL was replaced by either

69   brain polar phosphatidylethanolamine (PE), phosphatidylserine (PS) or phosphatidylcholine (PC)

70   as well 100% BPL nanodiscs. As seen in Figure 1B, ATPase rates were highest in PE and PS

71   nanodiscs followed by 100% BPL nanodiscs lacking cholesterol and, lastly, PC nanodiscs that

72   showed a marginal increase. These results largely match previous findings[16], although differences

73   exist in extent of ATPase rate stimulation that are likely due to the divergent lipid formulations

74   used for reconstitution. Overall, they demonstrate that lipids modulate ABCA7 activity in a species

75   dependent manner and that cholesterol has an inhibitory effect on ATPase activity of ABCA7.

76   **Cryo-EM structures of ABCA7 in nanodiscs reveal an asymmetric, open TMD**

77   To visualize ABCA7 in a lipid environment, we determined its cryo-EM structures in

78   BPL/Chol nanodiscs (ABCA7$_{BPL}$) at 3.6 Å resolution (Figure 1C, Figure S1B-E). Despite the

79   addition of the non-hydrolysable ATP analog adenosine-5′-o-(3-thio-triphosphate) (ATPγS), the

80   transporter adopted an open conformation with separated NBDs and a wide open TMD pathway.

81   We observed density features consistent with a patch of ordered lipids from both bilayer leaflets

82   traversing the width of TMD as expanded upon below. A second, higher-resolution 3D class

83   displayed more complete density for the ECD tunnel region but comparatively lower quality of

84   EM density for TMD2-NBD2 and the entire RD, indicating greater conformational disorder, which

85   was therefore not analyzed further (Figure S1D-E). Analysis of TMD-ECD interfaces in the

86   ABCA7$_{BPL}$ structures revealed more extensive contacts between the ECD and TMD1 (buried

87   surface area (BSA) of 790 Å^2) compared to TMD2 (330 Å^2 BSA). Both TMD1 and TMD2 made

88   contacts with the opposite ECD subunits, leading to a domain swapped arrangement. The TMD1-

89   ECD2 interface (BSA 565 Å^2) was significantly larger than that of TMD1-ECD1 (BSA 223 Å^2)

90   and both interfaces comprised an extensive network of polar and electrostatic interactions. The RD

4

91   adopted a domain-swapped arrangement similar to that in ABCA4 structures[25], with RD1 and RD2

92   associated with the opposite NBDs. To see whether structural changes could rationalize the

93   enhanced ATPase activity observed in $ABCA7_{PE}$ compared to $ABCA7_{BPL}$, we also determined the

94   cryo-EM structure of the former to 4.0 Å resolution (Figure 1D, Figure S2). Interestingly, both

95   structures were nearly identical to each other (r.m.s.d 0.31Å), although only a single high

96   resolution 3D class was resolved for $ABCA7_{PE}$ (Figure S2). Compared to $ABCA7_{BPL}$, density for

97   TMD lipids was more homogenous , despite its comparatively lower resolution, as described

98   below, indicating overall decreased conformational heterogeneity. For both $ABCA7_{BPL}$ and

99     $ABCA7_{BPE}$, greater positional disorder was observed for TMD2-NBD2 compared to TMD1 and

100   NBD1 as indicated by relatively weaker EM density quality (Figure S3).

The ABCA7 TMD lumen is accessible to an ordered file of bilayer lipids

102   Our $ABCA7_{BPL/PE}$ structures are distinguished from available structures of ABCA1 and

103   ABCA4 (all resolved in a detergent environment) by the wider open TMD lumen that is almost

104   completely occupied by lipids. The observed lipids are continuous with the surrounding membrane

105   except at the cytoplasmic leaflet near residues L655 and T1646 from TM5 and TM11, respectively

106   (Figure 1E-F). EM density for the modeled lipids is recognizable by gaps between the extracellular

107   and cytoplasmic leaflets and the two acyl chains of a single file of phospholipids in the two lipid

108   leaflets. Towards the extracellular end, ECD residues R475, K478, R482, K1407 and TMD1

109   residues R544 and R548 form a cluster of positively charged side chains oriented towards the

110   luminal lipids (Figure 1G). Overall, the luminal lipids are more closely associated with TMD1,

111   with residues from TM2, TM5, and TM11 within 5 Å of the modeled acyl chains compared to only

112   residues from TM11 from TMD2.

113   To compare the architecture of ABCA7 in the presence or absence of lipids, we also

114   determined its structure in the detergent digitonin (Figure 2A-B, Figure S4A-E). This

115   ABCA7$_{DIGITONIN}$ structure revealed a similar overall conformation to that seen in structures of

116   ABCA1[26] and ABCA4[25,27,28] (Figure S4F) with a more complete map for the ECD compared to

117   ABCA7$_{BPL/PE}$. As expected, no EM density for TMD lipids was visible, and only isolated density

118   features attributable to detergent molecules associated with TMD1 were observed, although only

119   at a contour level below where much of the surrounding detergent micelle was visible (Figure

120   2A,C). This is in contrast to ABCA7$_{BPL/PE}$ structures where TMD lipid density was much stronger

121   than that of the bulk nanodisc (Figure 2D-E), further validating our assignment of bilayer lipids.

122   Compared to structures in nanodiscs, the TMDs in this ABCA7$_{DIGITONIN}$ structure were arranged

123   more symmetrically with respect to each other with a narrower lumen. Interestingly, EM density

124   for TMD2 was still weaker than that of TMD1, in contrast to structures of ABCA4[25,27,28] and

125   ABCA1[26] (Figure S4D) indicating that a more mobile TMD2-NBD2 pair may be a unique feature

126   of ABCA7.

Closed TMD lumen and exit pocket for lipid extrusion in ATP bound ABCA7

128   To gain insight into possible mechanisms of lipid extrusion from the ABCA7 TMD, we

129   used the hydrolysis-deficient ABCA7$_{EQ}$ mutant reconstituted in BPL/Chol nanodiscs and

130   determined its structure in a closed, ATP-bound state at 3.7 Å resolution (Figure 3A and Figure

131   S5). This ABCA7$_{EQ-ATP}$ structure revealed closely interacting TMD-NBD pairs and a largely

132   occluded TMD lumen (Figure 3B). However, at the extracellular end of the TMD lumen, we

133   observed a small central opening to the extracellular space/ECD that may represent an 'exit pocket'

134   (Figure 3C,D) akin to that seen in the yeast pleiotropic drug resistance transporter Pdr5[29] that could

135   likely accommodate two acyl chains, (Figure 3D). TMD closure involves formation of a 4-TM

136   bundle comprising TMs 2, 5, 8, and 11 that occludes the cytoplasmic bilayer leaflet. The exit

137   pocket is comprised largely of hydrophobic residues, except for R548, which is part of a cluster of

138   positively charged residues including R475, K478, R482 and R678 identified in our ABCA7$_{BPL/PE}$

139   structures that may aid in directing a negatively charged phospholipid headgroup towards the ECD.

140   Side chain density for this group of basic residues was poor compared to that in ABCA7$_{BPL/BPE}$

141   structures, indicating greater disorder. TMD closure is accompanied by a rearrangement of both

142   TMD-ECD interfaces compared to the open state structures (Figure S6). Compared to the

143   ABCA7$_{BPL}$ structure, both TMD1 and TMD2 interfaces with ECD1 increased from BSAs of 190

144    $Å^2$ to 331 $Å^2$ and from 76 $Å^2$ to 285 $Å^2$, respectively. Conversely, the BSAs of TMD1 and TMD2

145   with ECD2 decreased from 543 $Å^2$ to 275 $Å^2$ and from 317 $Å^2$ to 192 $Å^2$, respectively. As

146   expected, the ABCA7$_{EQ-ATP}$ structure shows a canonical NBD sandwich dimer with bound ATP

147   (Figure 3E). In contrast to the ATP bound ABCA4 structure, RD density was too weak for accurate

148   placement. However, contoured at lower thresholds, the available density features were more

149   compatible with a rigid body shift rather than a conformational rearrangement of RD, with RD2

150   appearing to maintain contact with NBD1, but RD1 disengaged from NBD2.

151   **Rigid-body motions of the TMDs define ABCA7 conformational transitions**

152   Despite significant overall conformational differences, the individual TMDs in

153   ABCA7$_{BPL/PE,}$ ABCA7$_{DIGITONIN}$, and ABCA7$_{EQ-ATP}$ remain largely unaltered (Figure 4A).

154   Moreover, the TMD-NBD pairs move as rigid body units from the fully open ABCA7$_{BPL}$ structure

155   to that of ABCA7$_{DIGITONIN}$, which we assert is akin to an intermediate open state between the fully

156   open ABCA7$_{BPL/PE}$ state and the closed ABCA7$_{EQ-ATP}$ states. The transition from open to

157   intermediate open involves a rigid-body rotation of 9° and translation of 2 Å for TMD2-NBD2.

158   The transition to the closed state involves a further 6° rotation and 15 Å translation of TMD2-

159   NBD2 (Figure 4B-C), albeit with a greater alteration in the NBDs owing to a movement of their

160   recA like domains upon ATP binding and dimerization. Rigid body movements were also observed

161   for the entire RD in all three structures. The RD of ABCA7$_{DIGITONIN}$ maintained molecular

162   interactions with both NBD1 (RD2) and NBD2 (RD1), whereas the RD of ABCA7$_{BPL/PE}$ separated

163   from NBD2 while maintaining contact with NBD1. Finally, the ECD base region transitioned

164   through a rigid body rotation of 34° and translation of 6.3 Å going from the open to closed states,

165   whereas the tunnel and lid regions displayed greater heterogeneity.

166   To establish whether the concerted TMD-NBD rigid body motions outlined above may

167   extend to other ABC transporters of the Type-V ABC transporter/Type II ABC exporter fold[30], we

168   extended our analysis of individual TMD-NBD pairs to members of the G family for which the

169   first structures of open and closed states were available, namely ABCG2 and ABCG5/G8 (Figure

170   S7A)[31,32]. As shown in Figure S7B, the TMD-NBD pairs from both the apo and ATP-bound closed

171   states of ABCG2, as well as from both halves of ABCG5/G8, all shared a similar overall

172   architecture. Despite the divergent topologies of ABCG and ABCA transporters, with the former

173   are arranged in an NBD-TMD configuration compared to TMD-NBD for the latter, the individual

174   TMD-NBD pairs from ABCA7 shared very strong structural similarities with those of ABCG2,

175   further establishing the role of rigid body movements of the TMDs to affect large scale

176   conformational changes in these transporters.

177   **Lipid partitioning in the ABCA7 TMD captured with MD simulations**

178   To gain additional molecular insight into the TMD-mediated lipid partitioning, membrane

179   perturbation, and lipid extrusion from ABCA7, we performed multi-microsecond MD simulations

180   of the open conformation of ABCA7$_{PE}$ after embedding into two distinct lipid bilayers, one

181   containing PE/Chol (4:1) and the other PC/Chol (4:1) (Figure 5, Figure S8). Each simulation was

182   performed for 2 µs using a system including four copies of the protein (Figure 5A), resulting in an

183   aggregate 8 µs sampling of lipid-protein interactions for each lipid composition. Interestingly,

184   during the simulations, we observed PE and PC phospholipids penetrating the TMD cavity in both

185   the cytoplasmic and extracellular leaflets in all four protein replicas (Figure 5A, insets). We

186   quantified the number of phospholipids within the TMD cavity by counting the lipid headgroups

187   (Figure S8C), revealing that on average, ABCA7 accommodated a larger number of PE lipids

188   compared to PC (Figure 5B). To further quantify ABCA7-mediated deformation of the lipid

189   bilayer bridging the TMDs, the average heights of phospholipid headgroups were calculated with

190   respect to the midplane of the bilayer. We captured both a tendency for lipids to move towards the

191   cytoplasmic gate and the formation of a dome-shaped phospholipid arrangement within the TMD

192   lumen (Figure 5C-E, Figure S8A-B). Interestingly, our MD simulations showed an accumulation

193   of phospholipid headgroups in the vicinity of residues R475, K478, R482, R544, and R548, with

194   R482 and R548 displaying the most frequent lipid contacts (Figure S8D-E), corroborating our

195   structural observations. Analysis of the cytoplasmic leaflet highlighted a continuous distribution

196   of phospholipids with the surrounding membrane, except near TM5 and TM11 around residues

197   L655 and T1646 (Figure 5D-E), which correlated well with the bilayer-like density in the

198   ABCA7$_{BPL}$ and ABCA7$_{PE}$ cryo-EM maps (Figure 1C-D) that we modeled with acyl chains.

199   **Discussion**

200   A mechanistic model derived from our results is shown in Figure 6. We establish that the

201   TMD lumen of ABCA7 is accessible to bilayer lipids in the open state (ABCA7$_{BPL/PE}$), providing

202   a basis for substrate entry from both the extracellular and cytoplasmic leaflets. These ordered lipids

203   are akin to those observed in the TMD cavity of AcrB, where they have been suggested to be

204   important for functional integrity[33]. We identify a network of positively charged residues at the

205   extracellular periphery of TMD1 and the base of the ECD directed towards the TMD luminal

206   lipids. Our MD simulations support a role for these residues in interacting with phospholipid

207   headgroups, consistent with our structural data. In ABCA7, R475 mutations have been identified

208   in AD[34] patients, and mutation of R638 in ABCA1 (equivalent to R548 in ABCA7) is associated

209   with reduced serum HDL[35] (Figures S9 and S10). While further studies are needed to pinpoint the

210   specific roles, if any of these residues, in ABCA7 function, they remain largely conserved in

211   ABCA1, ABCA4, and ABCA7. The upward protrusion of both bilayer leaflets within the TMD

212   lumen suggested by MD simulations may, upon TMD closure, aid in sequestering phospholipids

213   in the identified exit pocket to be primed for extrusion towards the ECD. Membrane deformations

214   similar to those highlighted here have also been observed in the signal peptidase complex[36], and

215   further evidence the influence membrane proteins have on local membrane bilayer structure.

216   ABCA7 could thus play a role in changing its membrane lipid environment by flipping

217   cytoplasmic leaflet lipids to the extracellular leaflet and also extruding them back out into the bulk

218   bilayer environment and/or to apolipoproteins. The reported defects in the phagocytotic activity of

219   cells that express dysfunctional ABCA7[37,38] could be related to ABCA7's influence on the

220   asymmetric distribution of bilayer lipids[39], as enrichment of PS, shown to be flipped by ABCA7[16],

221   at the extracellular surface is linked to phagocytosis and phagocytosis-associated proteins within

222   the membrane[40-42].

223   Despite significant progress in our mechanistic understanding of ABCA transporter

224   function, several questions remain open. First, it is unclear to what extent ABCA7 interacts with

225   apolipoproteins in a physiologically relevant manner and, more broadly, the structural basis for

226   apolipoprotein interactions with ABCA7 or ABCA1 is unknown. Second, while our structures

227   provide a potential basis for lipid entry into and extrusion from the ABCA7 TMD, the mechanism

228   whereby lipids get flipped, remains unresolved. Third, while our data point to a potential

229   preference for PE over PC for TMD partitioning, the exact mechanism whereby ABCA1 or

230   ABCA7 achieve lipid specificity are unknown. The observed enhancement in conformational

231   homogeneity, quality of lipid density, and ATPase activity of our $ABCA7_{PE}$ sample may be, in

232   part, due to the smaller PE headgroup, which has been shown to aid in folding and stabilization of

233   membrane proteins[43]. Finally, it is unknown how the opposite direction of substrate transport for

234   ABCA4 is achieved considering similarities in ATP bound closed and open state structures of

235   ABCA4 and ABCA7. Overall, our data will help devise better *in vitro* and *in silico* models to

236   answer these questions, which will further aid in dissecting the unique roles these proteins play in

237   cellular physiology.

238   **METHODS**

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240   **Protein Purification**

241   We utilized the Flp-In TREX system (Thermo Fisher Scientific) for tetracycline inducible

242   expression of human ABCA7. In short, a codon optimized synthetic gene construct

243   (GeneArt/Thermo Fisher Scientific) of isoform 1 of ABCA7 (Uniprot ID Q8IZY2-1), harboring a

244   C-terminal eYFP-Rho1D4 tag[43] with a 3C/precision protease site between the protein and

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245   purification tags, was cloned into a PCDNA5.1 FRT/TO vector between BamHI and NotI

246   restriction sites and a stable cell line was generated as per manufacturer's protocol (Flp-In™ T-

247   Rex™ Core Kit, Thermo Fisher Scientific). The resulting HEK293 based stable cells were grown

248   and maintained in adherent cell culture in Dulbecco's Modified Eagle Medium (DMEM, Thermo

249   Fisher Scientific) supplemented with 9% Fetal Bovine Serum (FBS, Gibco) and a

250   penicillin/streptomycin mixture (Thermo Fisher Scientific) at 37°C with 5% carbon dioxide (CO_2)

251   under humidified conditions. For protein production, cells were induced with 0.6 μg ml^{-1}

252   tetracycline at a confluency of 80% in fresh DMEM supplemented with 2% FBS under otherwise

253   identical conditions for an additional 72 hours before being washed with Phosphate Buffered

254   Saline (PBS), harvested, and flash frozen in liquid nitrogen.

255   For purification, thawed cells were resuspended in a lysis buffer (Buffer L) comprising 25

256   mM Hepes pH 7.5, 150 mM sodium chloride (NaCl), 20% glycerol, 1 cOmplete EDTA free

257   protease inhibitor tablet (Roche) per 50 ml Buffer L, 800 μM phenylmethylsulfonyl fluoride

258   (PMSF) and 20 μg ml^{-1} soybean trypsin inhibitor (both Sigma), and mechanically cracked using

259   a dounce homogenizer before addition of a 0.5%/0.1% w:v mixture of dodecyl maltoside (DDM)

260   and cholesteryl hemisuccinate (CHS) (both Anatrace). Protein extraction was allowed to proceed

261   for 90 minutes at 4°C with gentle agitation, after which, the suspension was centrifuged at 48,000

262   r.c.f for 30 minutes and the supernatant applied to rho-1D4 antibody (University of British

263   Columbia) coupled Sepharose resin (Cytiva). Binding was allowed to proceed for 3 hours before

264   the unbound fraction was discarded and beads rinsed with 4 x 10 bed volumes (BVs) of wash

265   buffer (25 mM Hepes pH 7.5, 150 mM NaCl, 20% glycerol, 0.02%/0.004% w:v DDM/CHS).

266   Protein was eluted by incubation with 3 BVs elution buffer (wash buffer supplemented with either

267   3C protease (1:10 w:w 3C:ABCA7) or 0.5 mg ml^{-1} 1D4 peptide (GenScript)) for 2-18 hours.

268   The EQ variant of ABCA7 contained two site mutations, E965Q and E1951Q. The E965Q

269   site was created within the ABCA7 construct using site directed mutagenesis by PCR with the

270   primers: A7eq1for 5'-GGTCATCCTGGATCAACCTACAGCAGGCGTGG-3' and A7eq1rev

271   5'-GCCTGCTGTAGGTTGATCCAGGATGACCACC-3'. E1951Q was generated using a

272   synthesized dsDNA block and the enzymes NheI and BsiWI. $ABCA7_{EQ}$ with C-terminal eYFP-

273   Rho1D4 tag was then transferred to a pCAG vector using KpnI and NotI restriction sites. The

274   HEK293T cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Scientific)

275   supplemented with 9% FBS (Gibco), penicillin/streptomycin mixture (Scientific) and antimycotic

276   (Gibco) at 37°C and 5% CO_2 under humidified conditions. A mixture of 37.5 μg of $ABCA7_{EQ}$

277   plasmid and 75 μg of polyethyleneimine (PEI, Sigma) was incubated for 15 min at room

278   temperature before being applied to 15 ml plates of HEK293T cells at a confluency of 60 - 80%

279   to initiate transfection and expression. The cells were further cultured for 72 hours before being

280   washed with PBS, harvested and flash frozen in liquid nitrogen. $ABCA7_{EQ}$ was purified in the

281   same approach of ABCA7; and eluted by incubation with 3 BVs elution buffer with either 3C

282   protease for ATPase assay or with 0.25 mg ml^{-1} of 1D4 peptide in an additional 2 mM ATP and

283   10 mM $MgCl_2$ for nanodisc reconstitution.

284   Membrane scaffold protein D1 (MSP1D1, addgene) and apoA1 were purified using

285   established protocols for MSP[44] with the following modifications: A synthetic construct of apoA1

286   bearing a 3C protease cleavable N-terminal deca-histidine tag (GeneArt/Thermo Fisher Scientific)

287   was cloned into a pET28a vector (Addgene) and transformed in *E. coli* BL21 DE3 cells (New

288   England Biolabs). One-liter cultures of Terrific Broth (TB) supplemented with 50 ug ml^{-1}

289   kanamycin were grown from 10 ml overnight cultures from single colonies grown in LB. Cells

290   were grown to an OD600 of 0.8 in a shaking incubator at 37 °C and induced with 1 mM isopropyl

291 β-d-1-thiogalactopyranoside (IPTG). Protein expression was allowed to proceed at 20°C for 12

292 hours. Cells were centrifuged at 12,000 r.c.f, and pellets were flash frozen in liquid nitrogen and

293 stored at -80°C until required. Frozen pellets were resuspended in 8 ml/ gram cell pellet

294 resuspension buffer comprising 25 mM Hepes pH 7.5, 150 mM NaCl and 1 mM

295 phenylmethylsulfonyl fluoride (PMSF) and sonicated. The suspension was spun down at 16,000

296 r.c.f at 4 °C for 30 min and the supernatant was applied 5 ml Ni-NTA resin (Qiagen)/ L culture

297 medium. After discarding the flowthrough, the resin was washed with 25 mM Hepes pH 7.5,

298 150 mM NaCl, 1 mM PMSF and 20 mM imidazole until a pre-established baseline A280 reading

299 was achieved. ApoA1 was eluted in 4 BVs of 25 mM Hepes pH 7.5, 150 mM NaCl, 1 mM PMSF

300 and 200 mM imidazole, concentrated using a 10 kDa molecular weight cutoff (MWCO) Amicon

301 filter (Millipore-Sigma) and desalted using a PD10 column (Cytiva) into 25 mM Hepes pH 7.5,

302 150 mM NaCl. The concentration of apoA1 was adjusted to 1 mg ml^{-1} for flash freezing in liquid

303 nitrogen and storage at -80°C.

304 **ABCA7 and ABCA7$_{EQ}$ nanodisc and proteoliposome preparation**

305 For nanodisc reconstitution, peptide eluted or 3C cleaved ABCA7 was mixed with

306 MSP1D1 and a mixture of BPL (brain polar lipid extract from Avanti) and cholesterol (80:20 w:w)

307 with 0.5%/0.1% DDM:CHS using a 1:10:350 (ABCA7:MSPD1:lipid mix) molar ratio in nanodisc

308 buffer (25 mM Hepes pH 7.5, 150 mM NaCl) that contained up to 4% glycerol for 30 minutes at

309 room temperature (RT). Nanodisc reconstitution was induced by removing detergent with 0.8 mg

310 ml^{-1} pre-washed Biobeads SM-2 (Bio-Rad) for 2 hours with gentle agitation at RT. For different

311 phospholipid compositions, BPL was replaced with brain PE, PS, or PS (all from Avanti Polar

312 Lipids). For structural studies, nanodisc-reconstituted ABCA7 bearing the eYFP-Rho1D4 tag was

313 bound to rho-1D4 resin for an additional 2 hours, washed with 4 BV of nanodisc buffer, and eluted

314 with 3C protease for 2 hours at 4°C. The eluted ABCA7 nanodiscs were concentrated using a

315 100,000 MWCO kDa Amicon filter and further purified by size exclusion chromatography using

316 a G4000swxl column (TOSOH biosciences) equilibrated with nanodisc buffer at 4°C. Figure S1A

317 shows a SEC chromatogram for pure ABCA7 placed into BPL/Ch nanodiscs, while a SEC

318 chromatogram for ABCA7 in PE/Ch nanodiscs is in Figure S2A. Generally, three fractions were

319 pooled from the main resultant peak.

320 ABCA7$_{EQ}$ was reconstituted in nanodiscs for cryo-EM preparation using the same

321 approach of ABCA7; except that an additional 2 mM ATP and 10 mM MgCl$_2$ were present until

322 the end of the purification procedure prior to grid preparation. A SEC chromatogram for ABCA7$_{EQ}$

323 in BPL/Ch nanodiscs is shown in Figure S5A, where the trace is affected by the additional ATP

324 added during the run.

325 ABCA7 proteoliposomes were generated by mixing detergent purified ABCA7 with

326 liposomes at a protein: liposome ratio of 1:10 w:w. Liposomes were prepared by extruding a 20

327 mg ml^{-1}, 80:20 w:w BPL/Ch lipid mixture 11 times using a previously described protocol [45].

328 Briefly, detergent purified ABCA7 and liposomes were added to 0.14% and 0.3% Triton X100

329 (Sigma), respectively, then incubated for 30 minutes at RT. These two samples were mixed and

330 incubated for 60 minutes. Detergent was removed by adding 40 mg fresh Biobeads SM2 (Bio-

331 Rad) per ml reaction mixture during five successive incubation steps, 30 minutes at RT, 60 minutes

332 at 4°C, overnight at 4°C, and two periods of 60 minutes at 4°C with gentle agitation. The

333 suspension was centrifuged at 80,000 rpm for 20 minutes in an ultracentrifuge. The supernatant

334 was removed, and the liposomal pellet was washed once with reconstitution buffer containing 150

335 mM NaCl, 25 mM Hepes pH 7.5. The ABCA7-liposome suspension was then centrifuged to

336   remove the supernatant, and the proteoliposomes were resuspended at a final concentration of

337   0.5 - 1 mg ml^{-1} for ATPase assays.

ABCA7$_{DIGITONIN}$ preparation

339   For the digitonin solubilized ABCA7 purification, ABCA7 was extracted from the cell in

340   a lysis buffer (Buffer L) using the same approach above, and the supernatant was applied to rho-

341   1D4 resin for a 3-hour binding period. Then, the resin was rinsed with the 4 x 10 BVs of wash

342   buffer containing 25 mM Hepes pH 7.5, 150 mM NaCl, 20% glycerol (v/v), and 0.06% digitonin

343   (w/v). Protein was eluted by incubation with 3 BVs elution buffer, which was wash buffer

344   supplemented with 3C protease (1:10 w:w 3C:ABCA7). Interestingly we obtained better particle

345   distribution and ice quality with addition of a 1:2.5 molar excess of apoA1, prepared in house, to

346   3C cleaved ABCA7 prior to grid preparation. The mixture was concentrated by a 100,000 MWCO

347   kDa Amicon filter (Millipore) and further purified by size exclusion chromatography using a

348   G4000swxl column (TOSHOH biosciences) equilibrated with a buffer containing 25 mM Hepes

349   pH 7.5, 150 mM NaCl, 0.035% digitonin (w/v), as shown in Figure S4A. Peak fractions were

350   pooled and concentrated for cryo-EM grid preparation.

ATPase assays

352   ATPase assays were based on a molybdate based colorimetric assay[46]. Protein

353   concentrations used were in the range of 0.05-0.1 mg ml^{-1}. Assays were started by the addition of

354   either 2mM ATP, except for experiments in Figure 1B where 6.25mM ATP was used, in the

355   presence of 10 mM magnesium chloride (MgCl$_2$), incubated for 30 minutes at 37°C, then stopped

356   by addition of 6% SDS. The assay was also performed in the presence of ABCA7 inhibitors as

357   additives, such as 5 mM ATPγS or sodium orthovanadate. For ATP K$_M$ measurements, a range of

358   ATP concentrations was used. Statistical analysis was done using GraphPad Prism 9. ATPase rates

16

359   were measured using simple linear regression, and the K_M of detergent, liposome, and nanodisc

360   reconstituted ABCA7 were determined from the fit to the Michaelis-Menten equation of the

361   corresponding rates. ABCA7 concentrations were measured using gel densitometry analyzed in

362   ImageStudio Lite (LI-COR Biosciences) based on detergent purified ABCA7 standards with

363   known concentrations determined by A280 measurements. All reaction components were mixed

364   with ABCA7 in detergent or reconstituted in nanodiscs and liposome in the absence of ATP,

365   incubated for 10 minutes at 37 °C prior to addition of ATP to start the reaction.

Cryo-electron microscopy grid preparation

367   For ABCA7 reconstituted in nanodisc prepared in 80:20 w:w BPL/cholesterol and

368   PE/cholesterol, SEC purified protein was mixed with 5 mM ATPγS (TOCRIS) and 5 mM $MgCl_2$

369   for 20 minutes at room temperature and concentrated to 0.5 - 1.0 mg ml^{-1}. Peak fractions from SEC

370   already containing 2 mM ATP (Sigma) and 10 mM $MgCl_2$ were pooled and concentrated to 0.5 -

371   1 mg ml^{-1} for nanodisc reconstituted ABCA7$_{EQ-ATP}$. For ABCA7 in digitonin, peak fractions were

372   pooled and concentrated between 2 to 5 mg ml^{-1}. 4 μl samples were applied to glow discharged

373   Quantifoil R1.2/1.3 grids (Electron Microscopy Sciences, Hatfield, PA, USA) using a Vitrobot

374   Mark IV (Thermo Fisher Scientific) with a 4s blotting time and 0 blotting force under >90%

375   humidity at 4°C, then plunge frozen into liquid ethane. For the nanodisc reconstituted ABCA7$_{EQ-}$

376    $_{ATP}$ and ABCA7 in digitonin, two sample droplets were applied to glow discharged grids to obtain

377   more particles per hole.

Cryo-electron microscopy data collection and processing

379   Grids were clipped as per manufacturer guidelines and cryo-EM data was collected using

380   a Titan Krios electron microscope operating at 300kV and equipped with a Falcon 3EC direct

381   electron detector (Thermo Fisher Scientific.). Automated data collection was carried out using

17

382   EPU 2.8.0.1256REL software package (Thermo Fisher Scientific) over multiple sessions in

383   counting mode at a nominal magnification of 96,000x, corresponding to a calibrated pixel size of

384   0.895 Å for nanodisc reconstituted ABCA7$_{BPL}$. Image stacks comprising 60 frames were collected

385   at a defocus range of -0.6 to -2.6 μm and estimated dose rate of 1 electron/Å^2/frame and further

386   processed in Relion-3.1 (beta). Motion correction was done using Motioncor2 (Relion

387   implementation)[47] and contrast transfer function (CTF) correction was performed using Gctf 1.06

388   [48]. A summary of the overall data processing scheme and the quality was presented in Figure S1C-

389   E. In brief, 11802 micrographs were used for template free picking of 6725108 particles, followed

390   by particle extraction at a 3x binned pixel size of 2.685 Å/pix. The dataset was processed in two

391   batches. After 2-3 rounds of 2D classification 1259324 particles from Set 1 and 1088487 particles

392   from Set 2 were selected for independent 3D classification steps (number of classes (K)=8 for

393   both). The structure of human ABCA1 (EMDB6724) was used as a 3D reference for an initial 3D

394   classification of a subset of the total data to yield an initial sub-nanometer resolution map of

395   ABCA7 that was used as a 3D reference for the full datasets. After 1 round of 3D classification,

396   both sets of data yielded a similar ensemble of classes. A total of 113291 particles from similar

397   looking classes (black boxes) were subjected to an additional round of classification (K=3), ~80%

398   of which fell into a high-resolution class that yielded a 3.6 Å map after refinement and particle

399   polishing steps. Similarly, 124114 particles from a second set of two similar classes (red boxes in

400   Figure S1D) were selected for subsequent refinement, particle polishing, and post processing to

401   yield a 3.1 Å map. All resolution estimates were based on the gold standard 0.143 cutoff criterion

402   [49]. Datasets of ABCA7$_{PE}$, ABCA7$_{EQ-ATP,}$ and ABCA7$_{DIGITONIN}$ were collected at a nominal

403   magnification of 96,000x corresponding to a calibrated pixel size 0.889 Å, and image stacks

404   containing 40 frames were collected a defocus range of -0.8 to -2.6 μm with an estimated dose rate

405   of 1 electron/$Å^2$/frame and further processed using the same software versions as for the

406   ABCA7$_{BPL}$ dataset unless otherwise indicated.

407   For ABCA7$_{PE}$, a total of 2849251 particles were picked from 9218 ctf corrected (Gctf)

408   micrographs in Relion ver. 3.1 in two batches at a 3-fold binned pixel size of 2.667 Å/pixel (Figure

409   S2B-D). Of these, 783655 particles and 413330 particles were selected from Batch 1 and Batch

410   2, respectively and independently subjected to 3D classification (K=8) using a low pass filtered

411   (60 Å) map of our ABCA7$_{BPL}$ structure as a reference. A single highest resolution class from each

412   was selected and their particles combined and subjected to an additional round of 2D classification.

413   2024649 particles were subjected to another round of 3D classification (K=8). A single, highest

414   resolution class containing 50704 particles was refined to 5.4 Å. Particles were re-extracted using

415   refined coordinates and unbinned (pixel size 0.889 Å/pixel) and subjected to a round of 3D

416   refinement, Bayesian Polishing, and postprocessing/B-factor sharpening to yield a final map 4.0

417   Å resolution and its local resolution filtered variant calculated using Relion's own algorithm. Local

418   resolution maps are shown for ABCA7$_{BPL}$ (Map1 & Map2) and ABCA7$_{PE}$ in Figure S3.

419   For the ABCA7$_{DIGITONIN}$ dataset (Figure S4B-E), a total of 7437149 particles were picked

420   from 16213 ctf corrected and motion corrected micrographs and extracted at a 3-fold binned pixel

421   size of 2.667 Å/ pixel. After 2D classification, 1220497 particles were subjected to 3D

422   classification (K=8) using a low pass filtered (60 Å) map of our ABCA7$_{BPL}$ structure as a reference.

423   A single class comprising 324727 particles was refined to 5.4 Å. The refined coordinates were

424   then used to re-extract unbinned particles (0.889 Å/pixel), subjected to 3D refinement, and

425   Bayesian polishing. Further 3D classification (K=5) was performed and two similar classes

426   containing 149590 particles were picked for 3D refinement and postprocessing/B-factor

427   Sharpening to yield a 3.9 Å map.

428   For the ABCA7$_{EQ-ATP}$ dataset, an initial set of 2660267 particles were picked from 4914

429   ctf corrected micrographs (Figure S5B-E). After 2D classification, 469397 particles were subjected

430   to initial model building. This model was used as a 3D reference to perform 3D classification

431   (k=5). A single class comprising 174415 particles was refined to 5.4 Å, the corresponding particles

432   unbinned and re-refined. The nanodisc density was subtracted within Relion, followed by 3D

433   classification (k=3). The highest resolution class comprising 51780 particles was refined to 4.3

434   Angstroms and used for 3D reference-based particle picking for a larger data set comprising the

435   initial 4914 movies and a new set of 4474 ctf corrected micrographs. A total of 3773280 particles

436   were picked and subjected to multiple rounds of 2D classification in Relion 4.0-beta. 898916

437   particles were subjected to 3D classification (K=3) and a single highest resolution class consisting

438   of 407424 particles was refined to 5.4 Å. The refined coordinates were then used to re-extract the

439   respective particles without binning (0.889 Å/ pixel) and refined again before 3D classification

440   (K=5). A single class comprising 177230 particles was selected and subjected to 3D refinement,

441   Bayesian polishing, and postprocessing/B-factor sharpening to yield a final map at 3.7 Å.

442   **Model building and refinement**

443   Model building was done in coot 0.9.5[50] using a combination of Map 1 and its local

444   resolution filtered variant and Map 2. Both Map 1 and Map 2 displayed significant conformational

445   heterogeneity in the second half of ABCA7, with the quality of density in Map 1 allowing

446   placement of a TMD2 model guided in part by the homologous ABCA1 structure. Density

447   attributed to inter-TMD phospholipids was clearest in Map 1. Map 2 revealed very poor and

448   discontinuous density for TMD2-NBD2 but significantly better density for TMD1-NBD1 and the

449   majority of ECD1 and ECD2, allowing for de novo model building. The model for ECD was also

450   guided by the presence of nine glycosylation sites (N78, N98, N312, N340, N1335, N1381, N1386,

451 N1457, & N1518) as well as 4 disulfide bond pairs. Density for the lid region of the ECD was

452 missing in both maps. Model building for both NBDs was guided by structures of the homologous

453 transporters TM287[51], ABCG2, and ABCA1, where density features did not allow for de novo

454 model building. We observed extra density at the nucleotide binding sites for both NBDs despite

455 their open conformation. The structure of the RD was based on a homology model of the predicted

456 RD structure in ABCA4[25]. The quality of density for the ABCA7 RD allowed rigid body placement

457 for the entire domain. Restrained real space refinement of the model was carried out in Phenix

458 1.19.1[52] using automatically generated secondary structure restraints. Structural figures were

459 prepared in UCSF Chimera v. 1.13.1[53] , ChimeraX v. 1.2.5[54], and PyMOL 2.4.1 (The PyMOL

460 Molecular Graphics System, Version 1.8 Schrödinger, LLC).

461 The model for ABCA7$_{PE}$ was generated by rigid body placement of the ABCA7$_{BPL}$ model

462 followed by real space refinement in phenix as described above. The model for ABCA7$_{DIGITONIN}$

463 was generated by rigid body fitting each TMD, NBD, ECD, and RD into its postprocessed map

464 followed my manual adjustment of sidechains as allowed for the map. Non proteinaceous EM

465 density was modeled as a CHS and Digitonin (ligand ID Y01) molecule. The corrected model was

466 real space refined against the postprocessed map. The model for ABCA7$_{EQ-ATP}$ was built starting

467 with a homology model based on the ATP bound structure of ABCA4 (PDB 7LKZ), followed by

468 manual adjustment of the structure as required and permitted by the map. The ECD was replaced

469 by a rigid body fitted model of the ECD from the ABAC7$_{Digitonin}$ structure. This model was then

470 refined against both the postprocessed map, and its local resolution filtered counterpart.

471 **MD simulations**

472 We employed MD simulations to capture the arrangement and dynamics of the lipid bilayer

473 induced by the experimentally derived open conformation of ABCA7$_{PE}$, which was used as the

21

474 starting model in all the simulations. For system setup, a C-terminal carboxylate capping group,

475 an N-terminal ammonium capping group and all the hydrogen atoms were added using the PSFGEN

476 plugin of VMD (Visual Molecular Dynamics)[56]. The resulting all-atom (AA) model was then

477 converted to a coarse-grained (CG) Martini model using the MARTINIZE protocol

478 (http://www.cgmartini.nl/), using an elastic network on atom pairs within a 10-Å cutoff. The

479 Orientations of Proteins in Membranes (OPM) database[57] was used to identify and align the

480 transmembrane region of the protein with the membrane normal. The protein was embedded in

481 two distinct lipid bilayers (palmitoyl-oleoyl-phosphatidyl-ethanolamine (POPE) and cholesterol

482 with a molar ratio of 4:1 (POPE/Chol), and palmitoyl-oleoyl-phosphatidyl-choline (POPC) and

483 cholesterol with a molar ratio of 4:1 (POPC/Chol), respectively). The protein secondary structure

484 was defined from the AA model and was maintained throughout the CG simulations by the applied

485 elastic network. To increase the sampling of lipid-protein interactions and improve statistics, four

486 independent copies of the CG protein were placed at a distance of 200 Å in a large lipid bilayer

487 $(400\times400$ Å^2). The system was then solvated and ionized with 150 mM salt using INSANE[58].

488 The systems were simulated employing GROMACS 2021.3[59,60]. A 20-fs timestep was

489 employed in all the simulations. The temperature was maintained at 310 K with a velocity-

490 rescaling thermostat[61] employing a coupling time constant of 1 ps. A semi-isotropic 1 bar pressure

491 was maintained using the Berendsen barostat [62] with a compressibility and relaxation time constant

492 of 3×10^{-4} bar and 5 ps, respectively. The systems were energy minimized for 1,000 steps, followed

493 by short equilibration runs of 18 ns, while restraints were applied to lipid bilayer headgroups and

494 protein backbones. During this time the restraints on bilayer headgroups were reduced gradually

495 from k = 200 kJ.mol^{-1}.nm^{-2} to zero, whereas the protein backbones' restraints (k = 1,000 kJ.mol$^-$

496 ^{1}.nm^{-2}) were kept constant. Each system was then simulated for 2 μs, with restraints only applied

497 to the protein backbones, resulting in an aggregate sampling of 8 μs (4 copies × 2 μs). All the

498 systems were simulated following the same MD protocol.

499 All the molecular images were generated using VMD[56]. The membrane deformation

500 induced by ABCA7 was quantified by calculating the z distance of the lipid phosphate moieties

501 (PO_4 bead type in MARTINI) with respect to the bilayer midplane, over the last 1 μs of each

502 trajectory. The generated histogram (binned in 2×2 $Å^2$ bins) in each leaflet illustrates the spatial

503 distribution of the height of the lipid head groups within each leaflet. We quantified the differential

504 movement of POPE and POPC within the protein lumen by calculating the number of

505 phospholipids located within the TMDs. If the PO_4 bead of a phospholipid was within 22.5 Å and

506 12.5 Å in x and y, respectively, with respect to a protein's center in the x-y plane (membrane plane),

507 then the phospholipid was considered to be within the TMD lumen (Figure S9C).

508 **Acknowledgments**

Author Contributions

AA conceived the research. LTML, JRT, and AA performed all experiments. MD simulations were conducted and analyzed by SD, SP, and ET. TK participated in assay design. LTML, JRT, and AA wrote the manuscript with input from all other authors.

Declaration of Interests

SP is currently an employee of Loxo Oncology @ Lilly and is a shareholder of stock in Eli Lilly and Co. The rest of the authors declare no competing interests.

Data and materials availability:

The cryo-EM Maps have been deposited at the Electron Microscopy Databank (EMDB) under accession codes EMD-AAAAA (ABCA7$_{BPL}$ Map 1), EMD-BBBBB (ABCA7BPL Map 2), EMD-CCCCC (ABCA7$_{PE}$), EMD-DDDDD (ABCA7$_{EQ-ATP}$), and EMD-EEEEE (ABCA7DI$_{GITONIN}$). The associated atomic coordinates have been deposited at the Protein Data bank (PDB) under accession codes 1WWW (ABCA7$_{BPL}$), 2XXX(ABCA7$_{PE}$), 3YYY, (ABCA7$_{EQ-ATP}$), and 4ZZZ (ABCA7DI$_{GITONIN}$).

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701 **Figures and Legends**
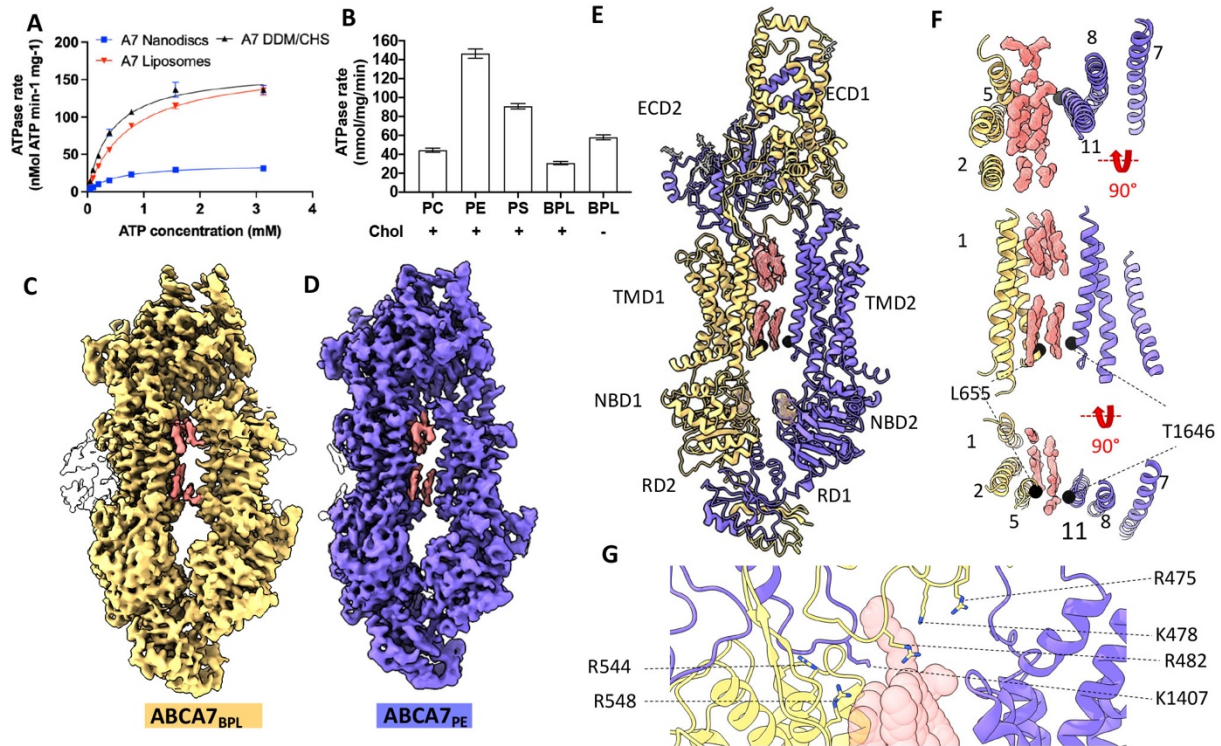
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703 **Figure 1. Functional characterization and structure of human ABCA7 incorporated in**
704 **nanodiscs (A)** ATPase activity of ABCA7 at different ATP concentrations and **(B)** different
705 nanodisc phospholipid/cholesterol compositions. Cholesterol (20%) presence is indicated by + or
706 -. Experimental replicates (n)=3 and error bars represent standard deviation (s.d.). **(C)** Cryo-EM
707 map of ABCA7$_{BPL}$ (yellow) at 3.6 Å resolution and **(D)** ABCA7$_{PE}$ at 4.0 Å resolution(purple).
708 Density for protein is shown in yellow (0.025 contour) and that for modeled lipid acyl chains
709 (0.035 contour) shown in pink (TMD luminal lipids) and white (peripherally associated lipids).
710 **(E)** ABCA7$_{PE}$ shown in ribbon format with Half 1 colored yellow and Half 2 purple, along with
711 density for TMD lipids (pink 0.025 contour) and bound nucleotide (yellow 0.035 contour). Acyl
712 chains and glycans are shown as pink and grey sticks, respectively. **(F)** TMD lumen with density
713 for TMD lipids (pink 0.025 contour) viewed from the extracellular side (top), membrane plane
714 (middle) and cytoplasmic side (bottom). Cα atoms for the cytoplasmic gate are shown as black
715 spheres. **(G)** View of the TMD-ECD interface with select residues oriented towards lipids
716 (transparent red spheres) shown.

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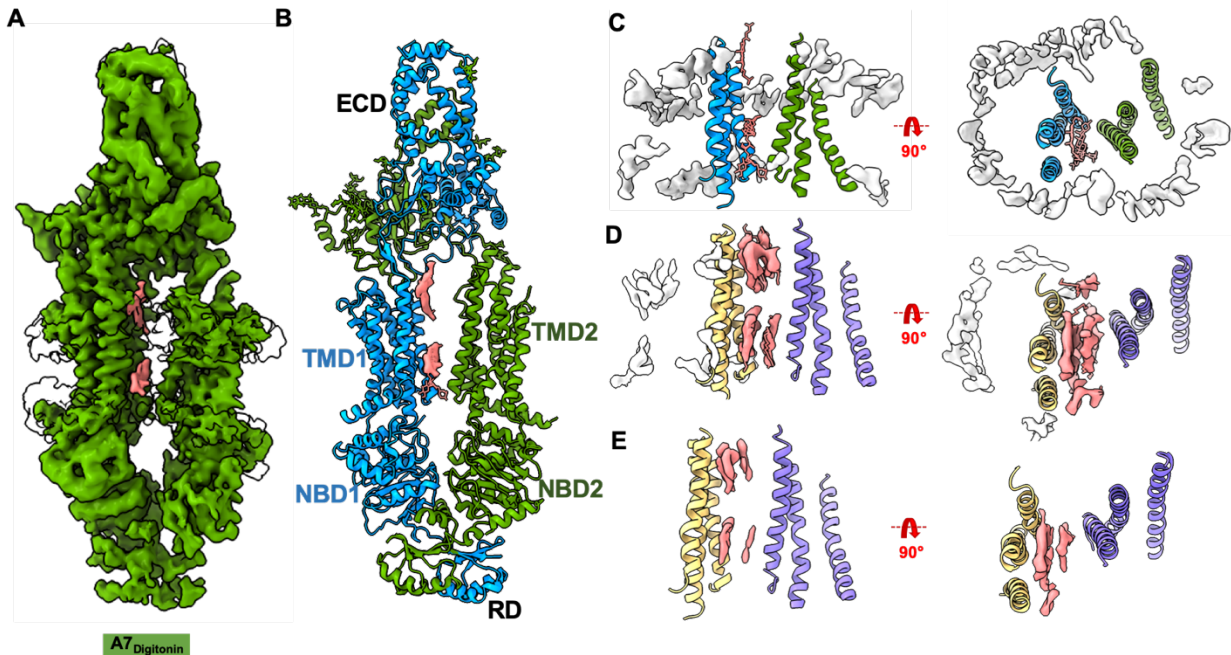
Figure 2. Cryo-EM structure of ABCA7$_{\text{DIGITONIN}}$. **(A)** Cryo-EM map of human ABCA7$_{\text{Digitonin}}$ at 3.9 Å with density for protein shown in green (0.013 contour) and extraneous density likely belonging to detergent shown in pink (0.013 contour). **(B)** Cryo-EM structure of human ABCA7$_{\text{DIGITONIN}}$ shown in ribbon format with each half colored differently (blue and green). **(C)** TMD lumen of ABCA7$_{\text{DIGITONIN}}$ with density for bulk micelle shown in white at 0.015 contour where detergent density inside TMDs disappears. Sticks for unmodeled detergent molecules are shown for which density is visible at the lower contour of 0.013. **(D)** TMD lumen of ABCA7$_{\text{PE}}$ with density (0.035 contour) for TMD lipids (pink) and peripherally associated ordered lipids (white) shown. **(E)** Same as D with higher density contour of 0.046 where density of peripherally associated lipids is absent but that of luminal lipids remains.

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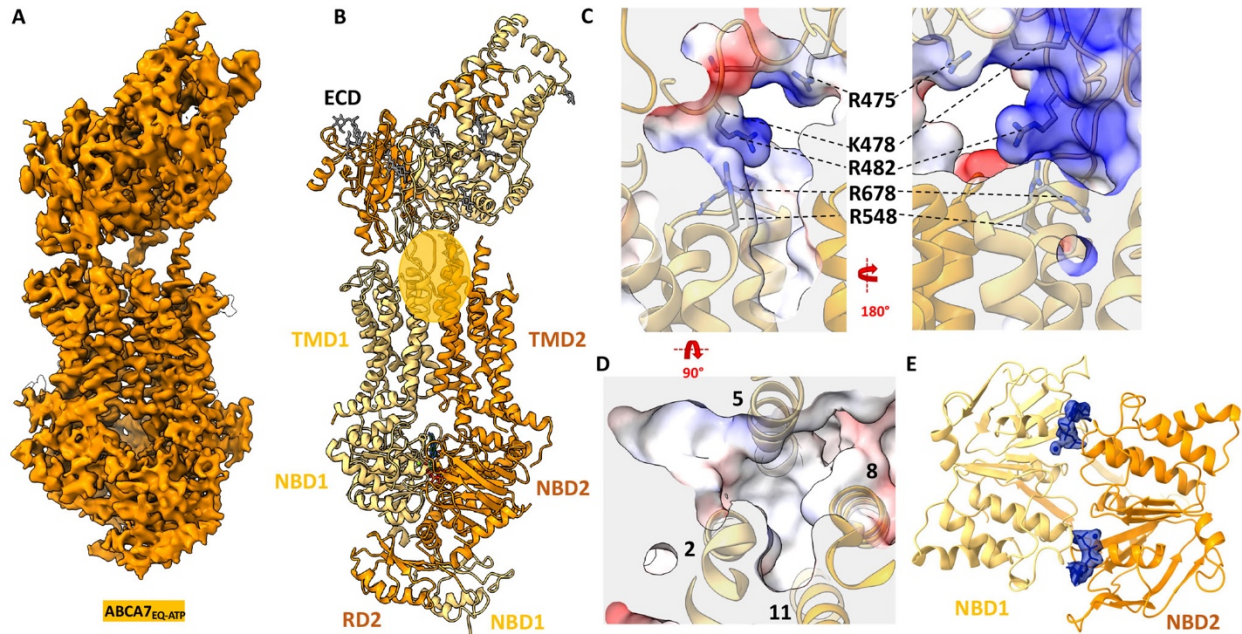
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Figure 3. Cryo-EM structure of ABCA7$_{EQ-ATP}$. (A) Cryo-EM map of human ABCA7$_{EQ-ATP}$ in BPL/Chol nanodiscs at 3.7 Å resolution (orange density, 0.02 contour). **(B)** Cryo-EM structure of ABCA7$_{EQ-ATP}$ shown in ribbon format with half 1 and half 2 colored yellow and orange, respectively. The transparent orange oval demarcates the observed exit pocket. **(C)** Central slice of electrostatic surface map showing surface details of exit pocket in two different orientations **(D)** 4-TM bundle forming the exit pocket. **(E)** NBDs viewed from the extracellular side with density for bound nucleotide (blue sticks) shown in blue (0.02 contour).

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	TMD1	TMD2	TMD1/ NBD1	TMD2/ NBD2	ECD1/2	RD1/2	
ABCA7$_{EQ\text{-}ATP}$	1.389	0.791	2.457	1.741	0.636	N/A	RMSD (Å)
ABCA7$_{DIGITONIN}$	0.523	0.817	0.739	0.941	0.606	1.171	RMSD (Å)
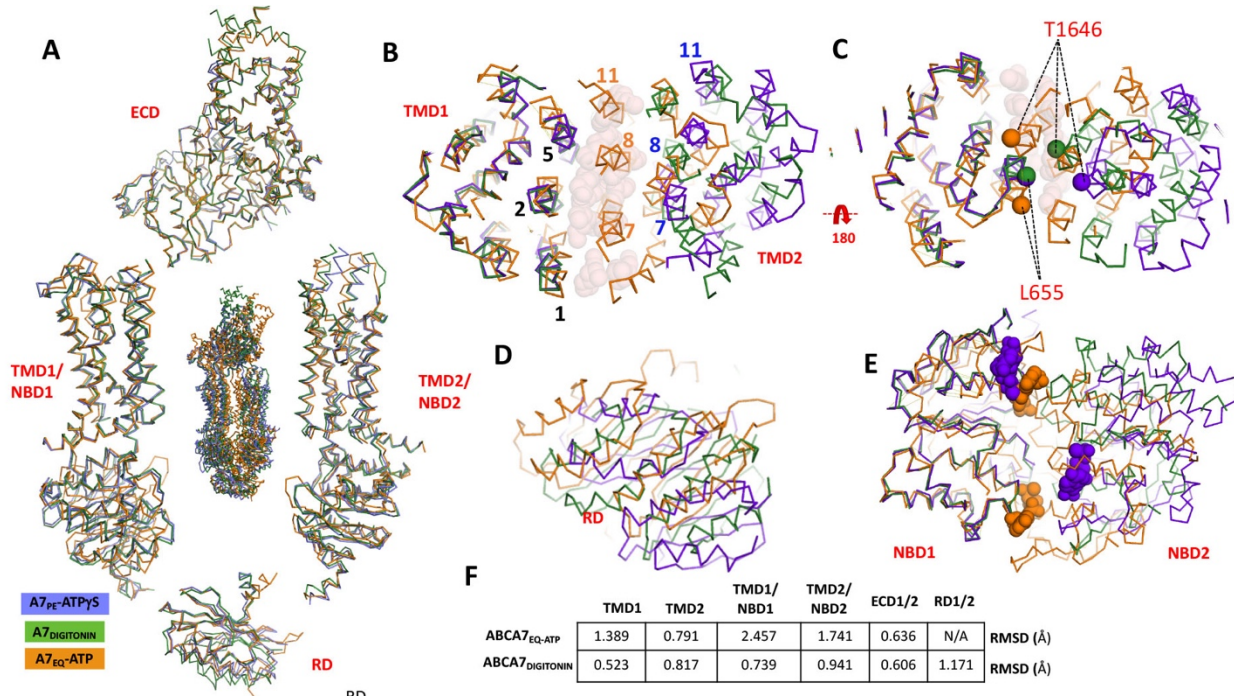
739 **Figure 4. Comparison of open and closed conformations of ABCA7 (A)** Overall structural

740 alignment of the three ABCA7 conformations (center) along with individual alignments of rigid

741 body pairs TMD1-NBD1, TMD2-NBD2, ECD, and RD (**B**) Overall alignment of the three ABCA7

742 conformations showing only TMD1 and TMD2 viewed from the extracellular side using the

743 TMD1-NBD1 pair as an alignment reference. TMs lining the TMD pathway are numbered (**C**)

744 Same as panel B, viewed from the cytoplasmic side with Cα atoms of gate forming residues shown

745 as spheres (**D**) Same as panels B/C showing only RDs viewed from the cytosolic side. (**E**) Same

746 as panel D showing just NBDs and bound nucleotides viewed from the extracellular side. ATPγS

747 and ATP-Mg^{2+} are shown as purple and orange spheres, respectively. (**F**) Root mean square

748 deviations (RMSD) of aligned atoms of ABCA7$_{BPE}$ vs ABCA7$_{EQ\text{-}ATP}$ and ABCA7$_{DIGITONIN}$.

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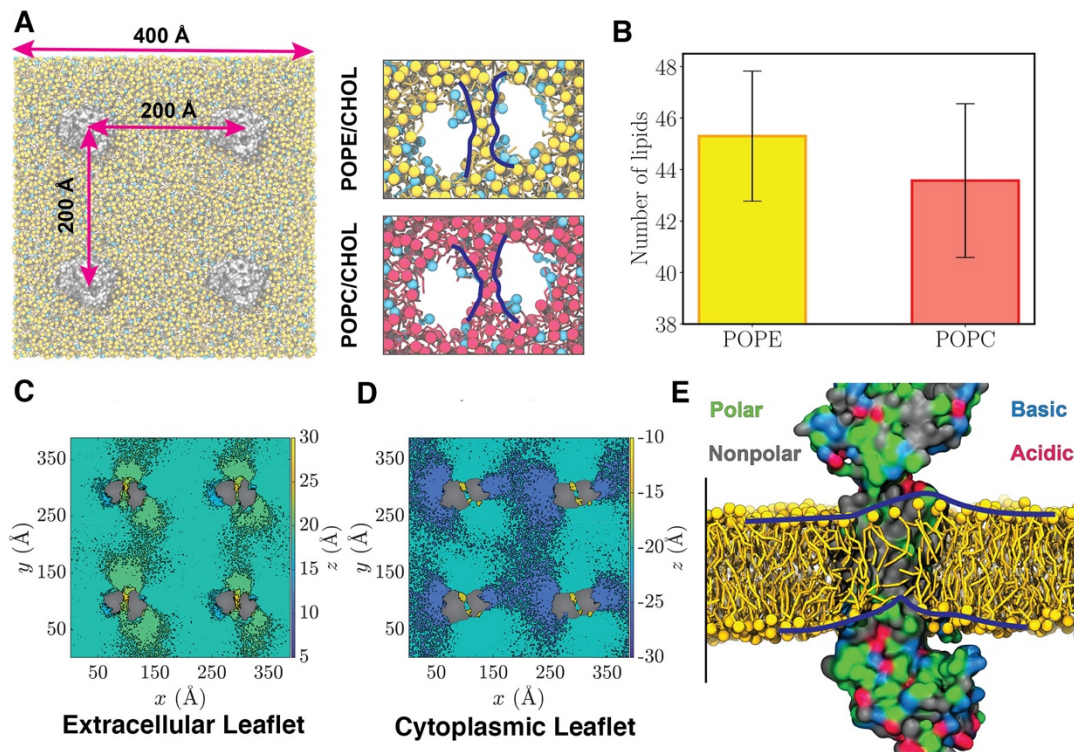
Figure 5. MD Simulations of ABCA7 in Lipid Bilayers. (A) Left: A representative simulation system with four copies of ABCA7 (silver), taken from the POPE/cholesterol (yellow/cyan) lipid patch. Right: the phospholipid belt (blue lines) formed in (top) the POPE/cholesterol (yellow/cyan) and (bottom) POPC/cholesterol (red/cyan) membranes ($t = 2$ μs). (B) The total count of phospholipids (sum of all four simulated proteins in a patch) partitioned in the TMD lumen for POPE/cholesterol and POPC/cholesterol bilayers (averaged over time ± standard deviation). (C/D) Heatmaps representing the average height (z values) of POPE headgroup with respect to the membrane midplane in the extracellular (C) and cytoplasmic (D) leaflets. Phospholipids are observed to climb the protein and form a dome-like configuration in the TMD lumen. (E) Snapshot of lipids partitioned in the TMD lumen, taken from the POPE trajectory at $t = 2$ μs. Polar, nonpolar, basic, and acidic residues are colored green, gray, blue, and red, respectively. TMD2 is hidden for a clearer view of the luminal dome-like lipid configuration (outlined by blue lines).

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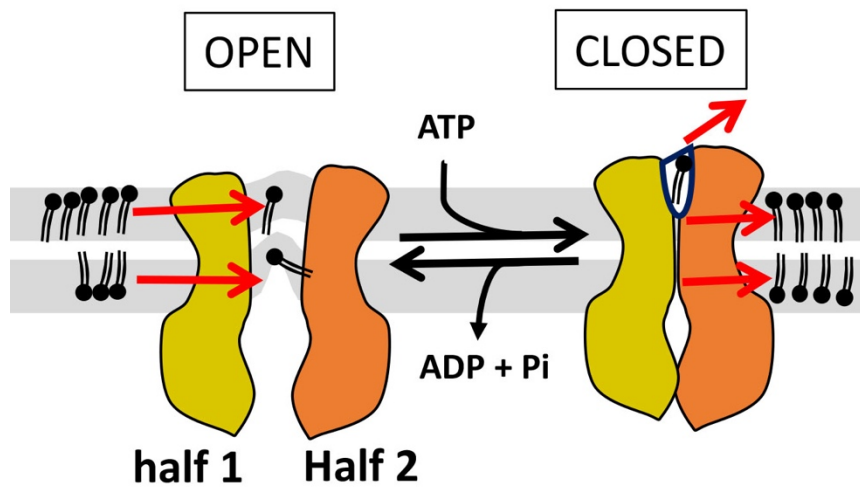
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765 **Figure 6. Proposed model of ABCA7 conformational transitions and lipid interactions.** The

766 membrane bilayer leaflets are shown in grey. Red arrows indicate movement of lipids (black)

767 under the influence of TMD conformation. The ECD and RD have been omitted for simplicity as

768 has any reference to potential lipid flipping from the cytoplasmic to extracellular leaflets.
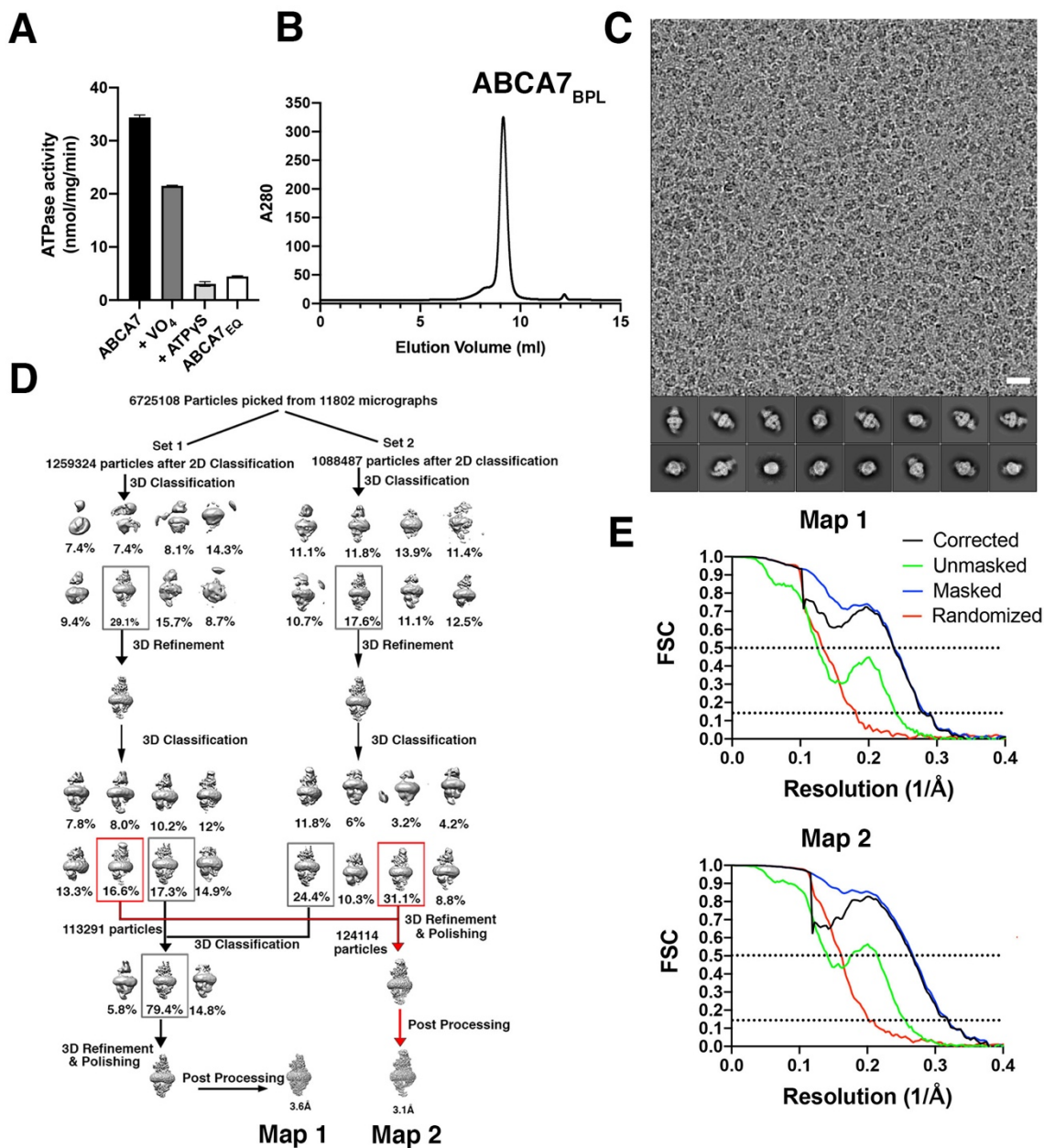
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Figure S1. ABCA7$_{BPL}$ characterization and Cryo-EM data processing. A ATPase data for nanodisc reconstituted ABCA7 with and without sodium orthovanadate (VO4) or ATPγS. N=3 and error bars represent s.d **B** SEC peak of ABCA7$_{BPL}$ sample for 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.

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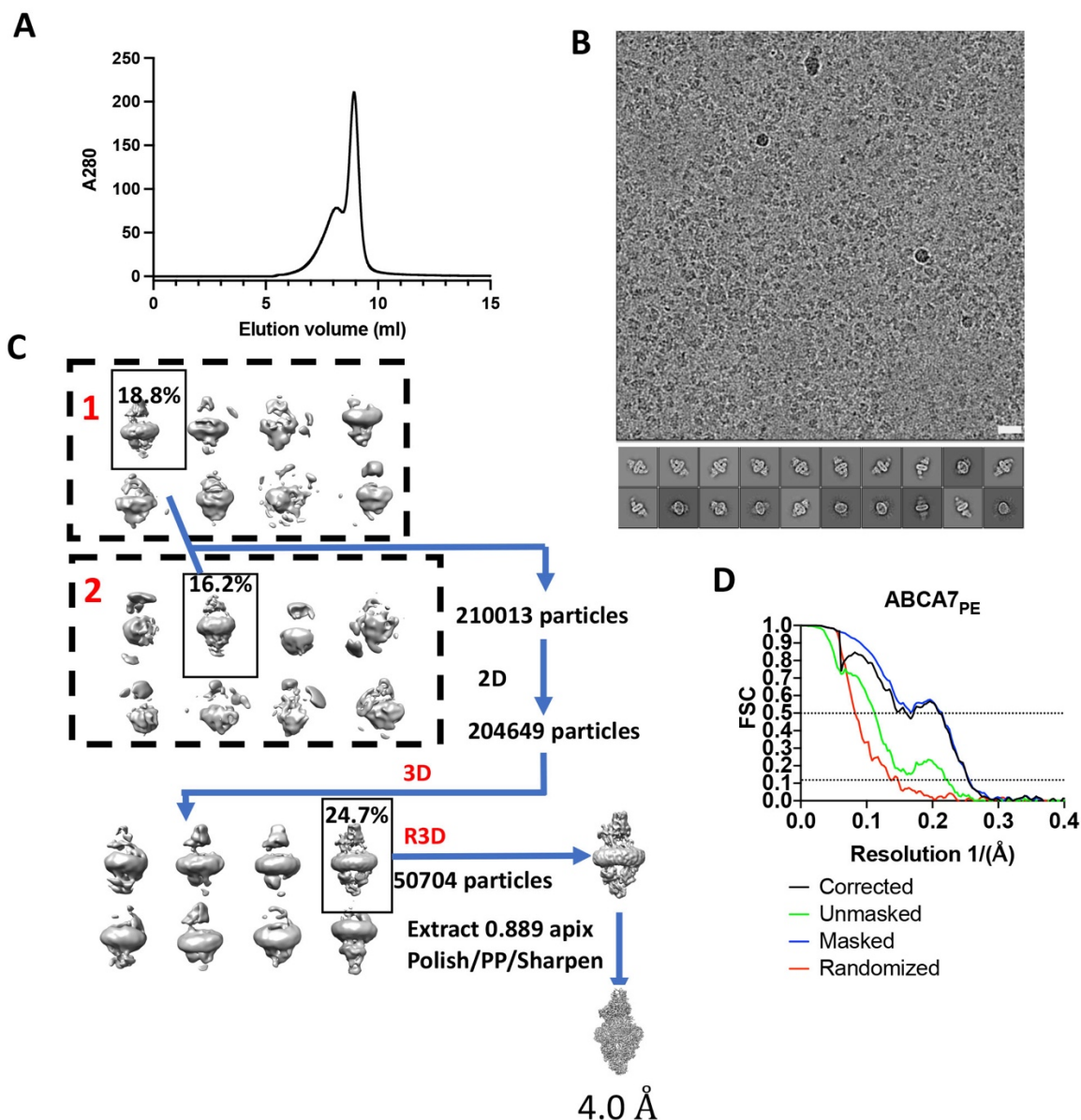
Figure S2. ABCA7PE Cryo-EM data processing. A Size exclusion chromatography micrograph of cryo-EM sample showing monodisperse ABCA7PE nanodisc (main peak). **B** Representative micrograph at -2.5 μm defocus and 2D classes. Scale bar = 20 nm. **C** cryo-EM processing workflow. Dashed boxes demarcate Subsets 1 and 2. Solid boxes indicate 3D classes used for further refinement. C2D = 2D Classification, C3D=3D classification, R3D = 3D refinement **D** Fourier shell correlation (FSC) curves for ABCA7PE Dotted lines indicate position 0.143 and 0.5 cutoff criteria for resolution estimates.
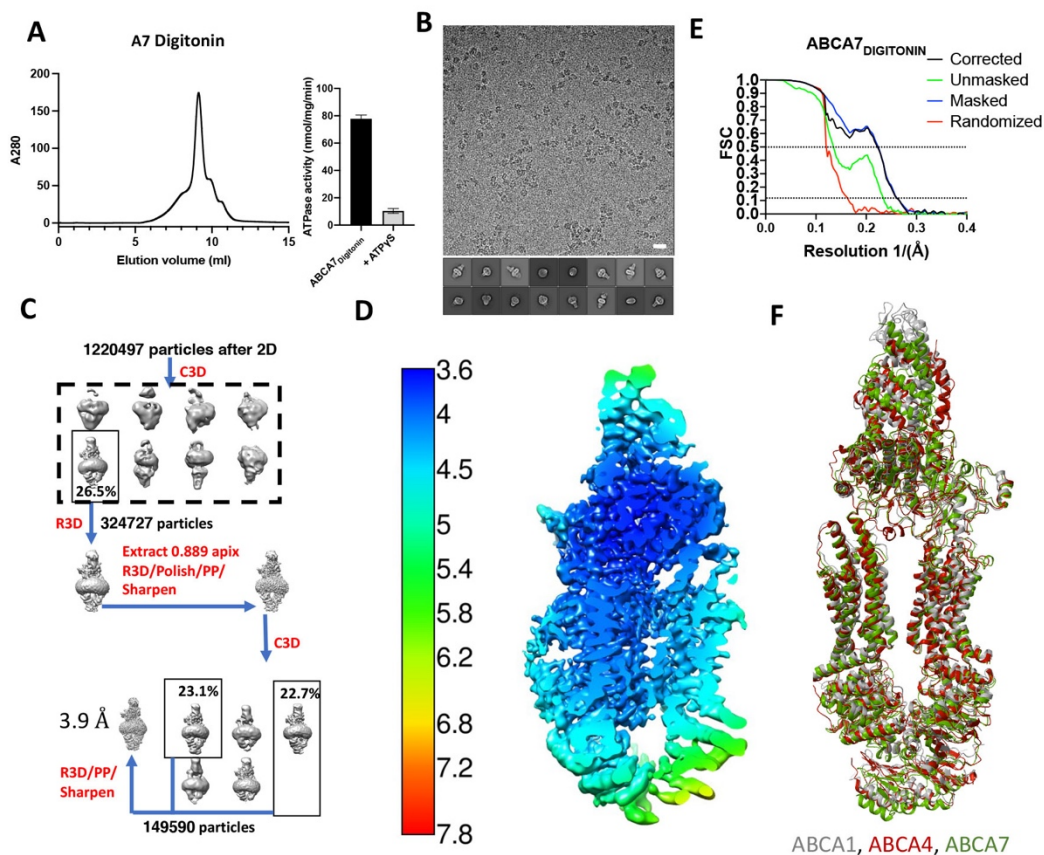
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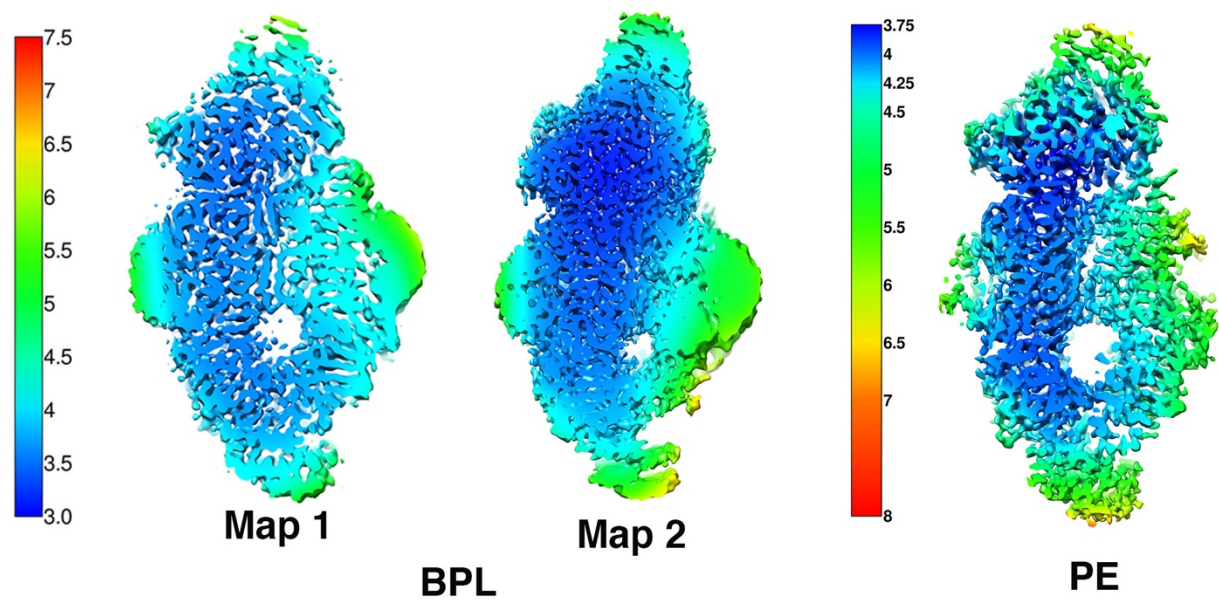
Figure S3. ABCA7$_{BPL}$ and ABCA7$_{PE}$ local resolution filtered maps. Color keys are indicated on the left of each set of maps with numbers representing resolution (Å).

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Figure S4 ABCA7$_{DIGITON}$ 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. **F** Superposition of ABCA7 (green, this manuscript), ABCA1 (grey, PDB 5XJY), and ABCA4 (red, PDB 7LKP) structures in digitonin.
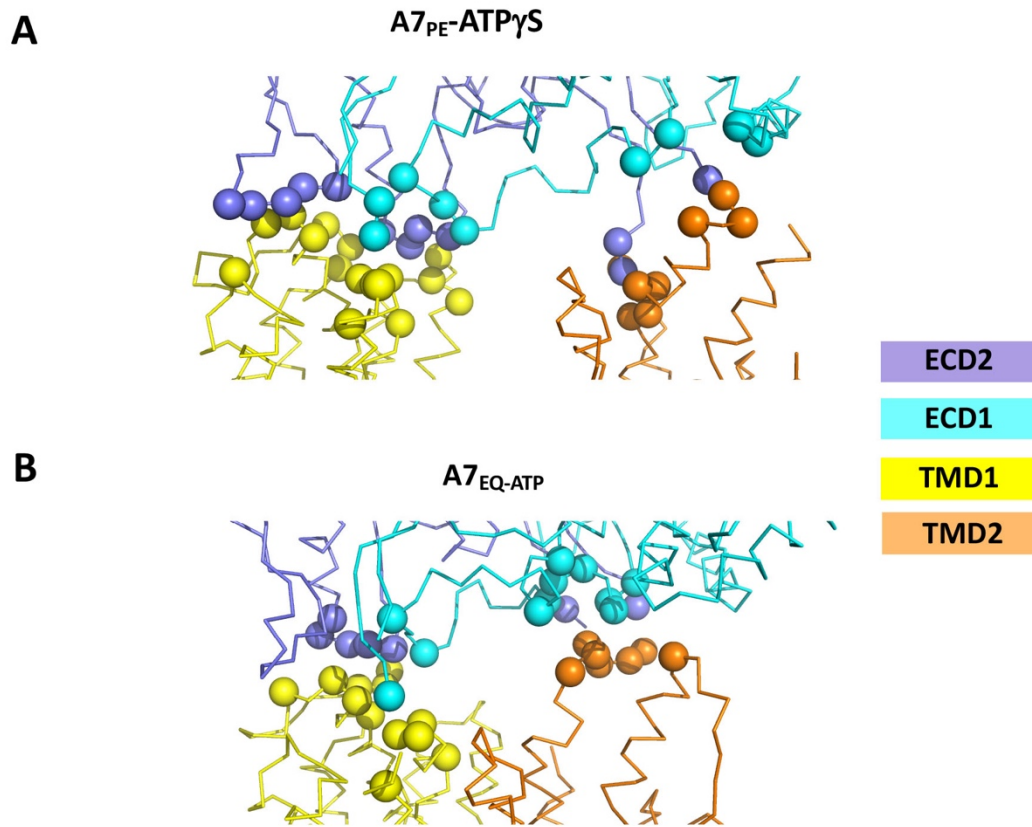
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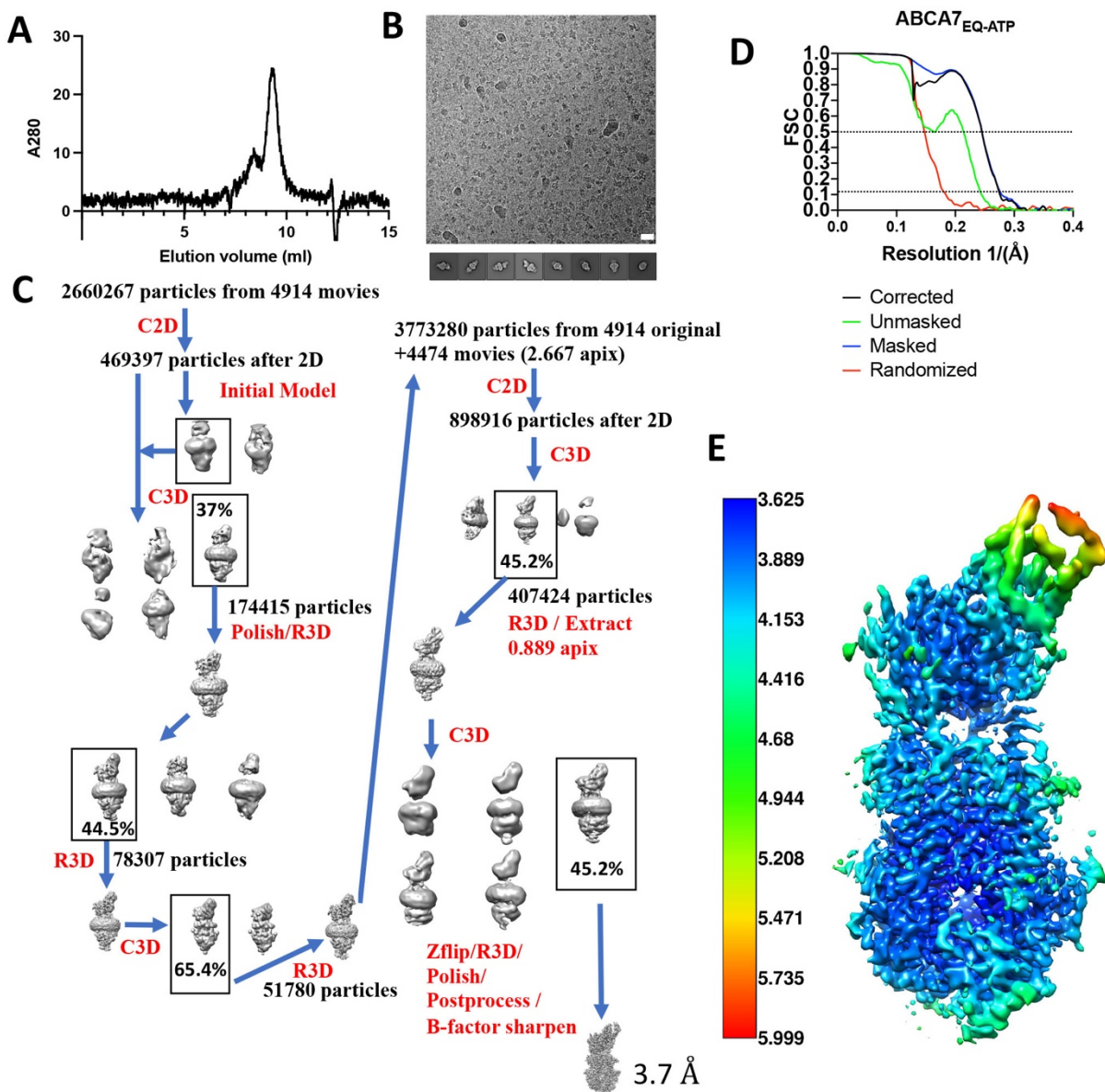
Figure S5. ABCA7$_{EQ-ATP}$ cryo-EM processing. **(A)** SEC peak for ABCA7$_{EQ-ATP}$ in nanodiscs. **B** Representative EM micrograph (-2.5 defocus) and rep 2D classes for ABCA7$_{EQ-ATP}$ sample. **C** Cryo-EM data processing pipeline. C2D = 2D Classification, C3D=3D classification, R3D = 3D refinement. **D** FSC curves. **E** Local resolution colored EM map.
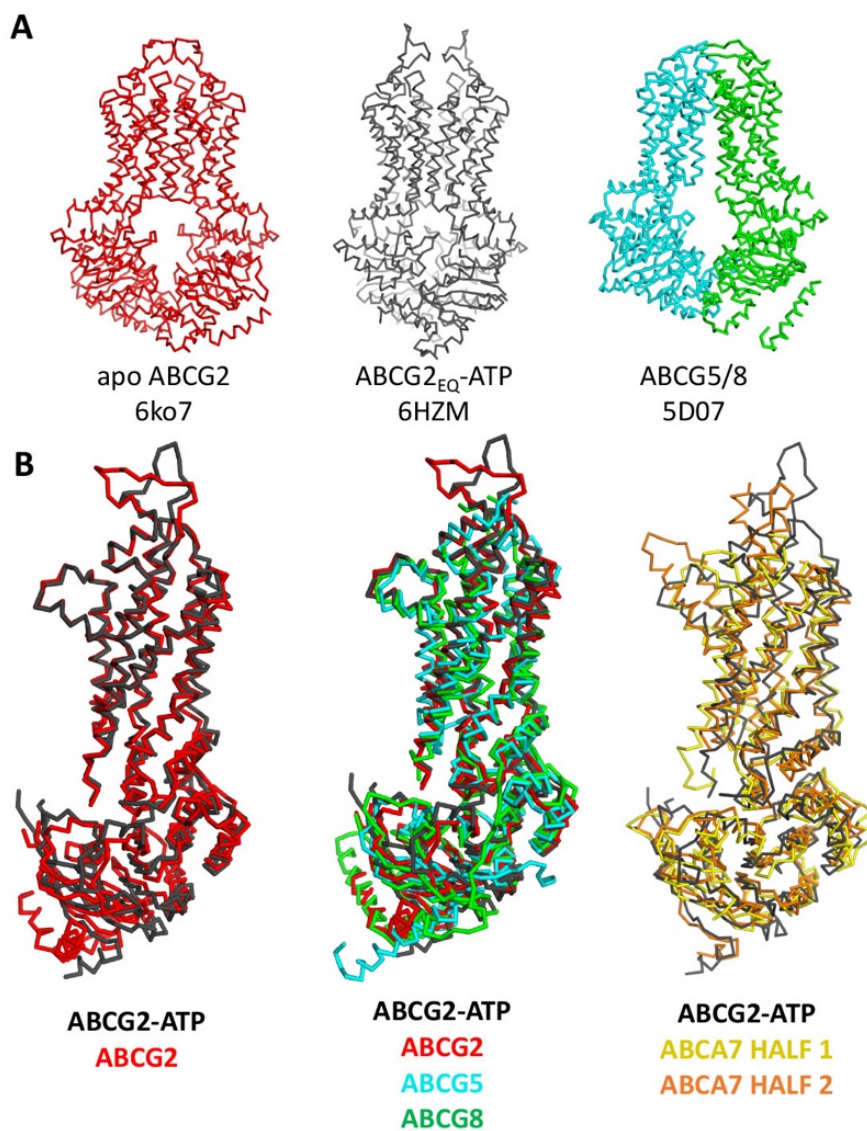
Figure S6. TMD-ECD interfaces of open and closed form ABCA7. (A) The TMD-ECD binding interfaces of ABCA7$_{PE}$ with TMD1 and TMD2 and with Cα for residues in TMD1 and TMD2 within 5Å of either ECD or vice versa shown as spheres. **(B)** The same analysis for the TMD-ECD binding interfaces of ABCA7$_{EQ-ATP}$ with closed cavity.

Figure S7. Conservation of ABCA and ABCG family structural elements (A) Ribbon representation of select ABCG family transporter structures and their respective PDB IDs including apo open ABCG2 (red), closed ATP bound structure of ABCG2$_{EQ}$ (black), and ABCG5/G8 (cyan and green, respectively) (**B**) Alignment of NBD-TMD pairs from open and closed conformations of ABCG2 (left), ABCG2 and ABCG5/G8 (center), and closed ABCG2 with TMD-NBD pairs from ABCA7 half 1 (gold) and half 2 (orange).

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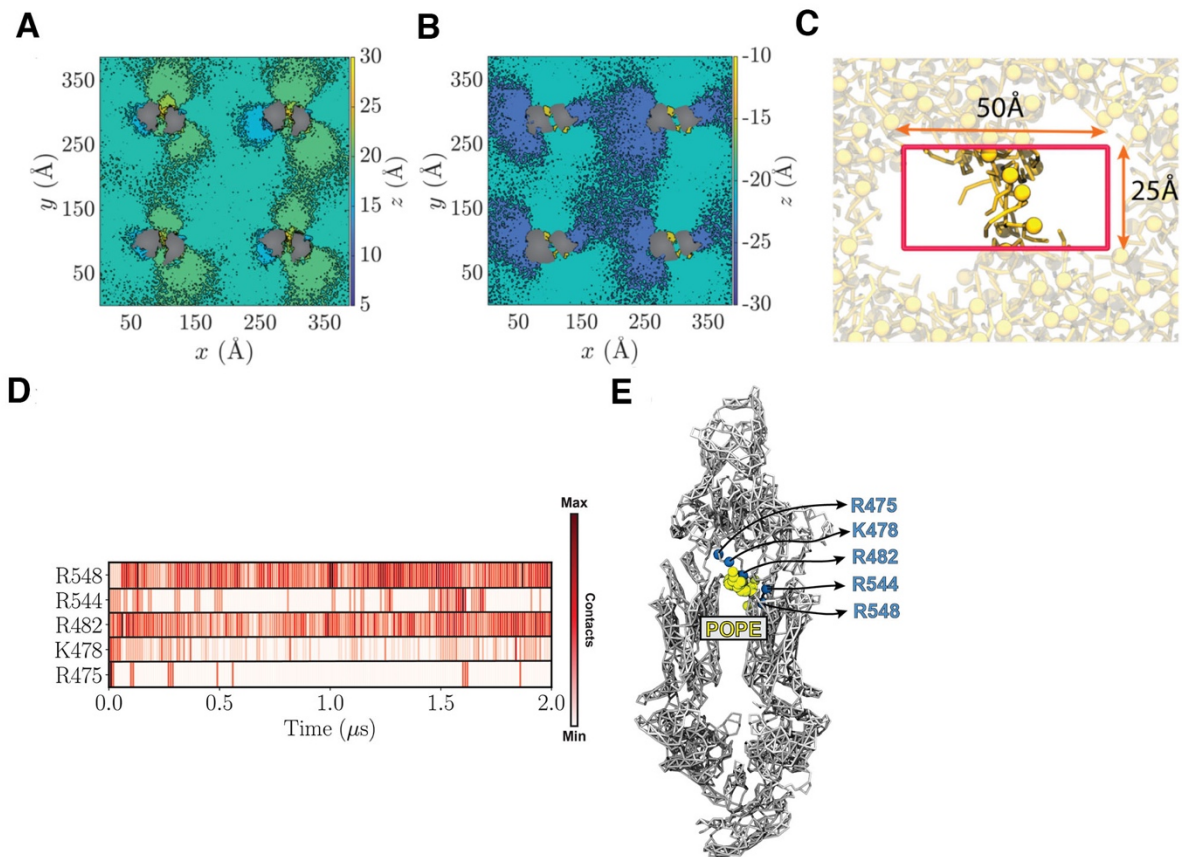
Figure S8. Lipid configuration induced by protein copies in a POPC bilayer and criteria used for identifying luminal lipids in MD simulations. (A/B) POPC headgroup height calculated for extracellular (A) and cytoplasmic (B) leaflets. **(C)** Dimension and position of the box used to calculate the number of phospholipids (yellow) partitioned in the TMD leaflets. The box is centered at the protein center and has dimensions of 50 Å and 25 Å in *x* and *y* directions, respectively. The ABCA7 transporter is hidden for clarity. **(D)** Contact map, based on the number of POPE headgroups in proximity of each residue throughout the simulation. **(E)** Accumulation of POPE headgroups (yellow spheres) in close proximity of most frequently contacted residues throughout the simulation.

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Figure S9. 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).

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hsABCA7    1  MAFWTQLMLLLWKNFMYRRRQPVQLLVELLWPLFLFFILVAVRHSHPPLEHHECHFPNKPLPSAGTVPWL
hsABCA1    1  MACWPQLRLLLWKNLTFRRRQTCQLLLEVAWPLFIFLILISVRLSYPPYEQHECHFPNKAMPSAGTLPWV
hsABCA4    1  MGFVRQIQLLLWKNWTLRKRQKIRFVVELVWPLSLFLVLIWLRNANPLYSHHECHFPNKAMPSAGMLPWL

hsABCA7   71  QGLICNVNNTCFPQLTPGEEPGRLSNFNDSLVSRLLADARTVLGGASAHRTLAGLGKLIATLRA......
hsABCA1   71  QGIICNANNPCFRYPTPGEAPGVVGNFNKSIVARLFSDARRLLYSQKDTSMKDMRKVLRTLQQI.....
hsABCA4   71  QGIFCNVNNPCFQSPTPGESPGIVSNYNNSILARVYRDFQELLMNAPESQHLGRIWTELHILSQFMDTLR

hsABCA7  135  ................................ARSTAQPQPTKQSPLEPPMLDVAELLLTSLLRTESL.
hsABCA1  136  ....KKSSSNLKLQDFLVDNETFSGFLYHNLSLPKSTVDKMLRADVILHKVFLQGYQLHLTSLL.CNGSKS
hsABCA4  141  THPERIAGRGIRIRDILKDEETLTLFLIKNIGLSDSVVYLLINSQVRPEQFAHGVPDLALKDIACSEALL

hsABCA7  170  ...................GLALGQAQ...................................EPLHSLLEA
hsABCA1  201  EEMIQLGDQEVSE.....LCGLPREKLAAAERVLRSNMDILKPILRTLNSTSPFPSKEL.AEATKTLLHS
hsABCA4  211  ERFIIFSQRRGAKTVRYALCSLSQGTLQWIEDTLYANVDFFK.LFRVLPTLLDSRSQGINLRSWGGILSD

hsABCA7  187  AEDLAQELLALRSLVELRALLQRPRGTSGP......LELLSEALCSVRGPSSTVGPSLNWYEASDLMELV
hsABCA1  265  LGTLAQELFSMRSWSDMRQEVMFLTNVNSSSSSTQIYQAVSRIVCGHPEGGGLKIKSLNWYEDNNYKALF
hsABCA4  280  MSPRIQEFIHRPSMQDLLWVTRPLMQNGGPETFTKLMGILSDLLCGYPEGGGSRVLSFNWYEDNNYKAFL

hsABCA7  251  G....QEPESALPDSSLSPACSELIGALDSHPLSRLLWRRLKPLILGKLLFAPDTPFTRKLMAQVNRTFE
hsABCA1  335  GGNGTEEDAETFYDNSTTPYCNDLMKNLESSPLSRIIWKALKPLLVGKILYTPDTPATRQVMAEVNKTFQ
hsABCA4  350  GIDSTRKDPIYSYDRRTTSFCNALIQSLESNPLTKIAWRAAKPLLMGKILYTPDSPAARRILKNANSTFE

hsABCA7  317  ELTLLRDVREVWEMLGPRIFTFMNDSSNVAMLQRLLQMQDEGRRQPRPGGRDHM..EALRSFLDPG....
hsABCA1  405  ELAVFHDLEGMWEELSPKIWTFMENSQEMDLVRMLLDSRDNDHFWEQQLDGLDWTAQDIVAFLAKHPEDV
hsABCA4  420  ELEHVRKLVKAWEEVGPQIWYFFDNSTQMNMIRDTLGNPTVKDFLNRQLGEEGITAEAILNFLYKGPRES

hsABCA7  381  ....SGGYSWQDAHADVGHLVGTLGRVTECLSLDKLEAAPSEAALVSRALQLLAEHRFWAGVVFLGPEDS
hsABCA1  475  QSSNGSVYTWREAFNETNQAIRTISRFMECVNLNKLEPIATEVWLINKSMELLIDERKFWAGIVF.....
hsABCA4  490  QADDMANFDWRDIFNITDRTLRLVNQYLECLVLDKFESYNDETQLTQRALSLLEENMFWAGVVF......

hsABCA7  447  SDPTEHPTPDLGPGHVRIKIRMDIDVVTRTNKIRDRFWDPGPAADPLTDLRYVWGGFVYLQDIVERAAVR
hsABCA1  539  ..TGITPGSIELPHVKYKIRMDIDNVERTNKIKDGYWDPGPRADPFEDMRYVWGGFAYLQDVVEQAIIR
hsABCA4  554  ..PDMYPWTSSLPPHVKYKIRMDIDVVEKTNKIKDRYWDSGPRADPVEDFRYIWGGFAYLQDMVEQGITR

hsABCA7  517  VLSGANPRAGLYLQQMPYPCYVDDVFLRVLSRSLPLFLTLAWIYSVTLTVKAVVREKETRLRDTMRAMGL
hsABCA1  607  VLTGTEKKTGVYMQQMPYPCYVDDIFLRVMSRSMPLFMTLAWIYSVAVIIKGIVYEKEARLKETMRIMGL
hsABCA4  622  SQVQAEAPVGIYLQQMPYPCFVDDSFMIILNRCFPIFMVLAWIYSVSMTVKSIVLEKELRLKETLKNQGV

hsABCA7  587  SRAVLWLGWFLSCLGPFLLSAALLVLVLKLGDILPYSHPGVVELFLAAFAVATVTQSFLLSAFFSRANLA
hsABCA1  677  DNSIIWFSWFISSLIPLLVSAGLLVVILKLGNLLPYSDPSVVEFLSVFAVVTILQCFLISTLFSRANLA
hsABCA4  692  SNAVIWCTWFLDSFSIMSMSIFLLTIFIMHGRILHYSDPFILFLFLLAFSTATIMLCFLLSTFFSKASLA
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hsABCA7   657  AACGGLAYFSLYLPYVLCVAWRDRLPAGGRVAASLLSPVAFGFGCESLALLEEQGEGAQWHNVGTRPT.A
hsABCA1   747  AACGGIIYFTLYLPYVLCVAWQDYVGFTLKIFASLLSPVAFGFGCEYFALFEEQGIGVQWDNLFESPVEE
hsABCA4   762  AACSGVIYFTLYLPHILCFAWQDRMTAELKKAVSLLSPVAFGFGTEYLVRFEEQGLGLQWSNIGNSPTEG


hsABCA7   726  DVFSLAQVSGLLLLDAALYGLATWYLEAVCPGQYGIPEPWNFPFRRSYWCG........PRPPKSPAPCP
hsABCA1   817  DGFNLTTSVSMMLFDTFLYGVMTWYIEAVFPGQYGIPRPWYFPCTKSYWFG........EESDEKSHPGS
hsABCA4   832  DEFSFLLSMQMMLLDAAVYGLLAWYLDQVFPGDYGTPLPWYFLLQESYWLGGEGCSTREERALEKTEPLT


hsABCA7   788  TPLD........PKVLVEEAPPGLSPGVSVRSLEKRFPGSPQPALRGLSLDFYQGHITAFLGHNGAGKTT
hsABCA1   879  NQKR.......ISEICMEEEPTHLKLGVSIQNLVKVYRDGMKVAVDGLALNFYEGQITSFLGHNGAGKTT
hsABCA4   902  EETEDPEHPEGIHDSFFEREHPGWVPGVCVKNLVKIFEPCGRPAVDRLNITFYENQITAFLGHNGAGKTT


hsABCA7   850  TLSILSGLFPPSGCSAFILGHDVRSSMAAIRPHLGVCPQYNVLEDMLTVDEHVWFYGRLKGLSAAVVGPE
hsABCA1   942  TMSILTGLFPPTSGTAYILGKDIRSEMSTIRQNLGVCPQHNVLEDMLTVEEHIWFYARLKGLSEKHVKAE
hsABCA4   972  TLSILTGLLPPTSGTVLVGGRDIETSLDAVRQSLGMCPQHNILEHHLTVAEHMLFYAQLKGKSQEEAQLE


hsABCA7   920  QDRLLQDVGL.VSKQSVQTRHLSGGMQRKLSVATAFVGGSQVVILDEPTAGVDPASRRGIWELLLLKYREG
hsABCA1   1012 MEQMALDVGLPSSKLKSKTSQLSGGMQRKLSVALAFVGGSKVVILDEPTAGVDPYSRRGIWELLLLKYRQG
hsABCA4   1042 MEAMLEDTGL.HHKRNEEAQDLSGGMQRKLSVAIAFVGDAKVVILDEPTSGVDPYSRRSIWDLLLKYRSG


hsABCA7   989  RTLILSTHHLDEAELLGDRVAVVAGGRLCCCGSPLFLRRHLGSCYYLTLVKARLPLTTNEKADTDMEGSV
hsABCA1   1082 RTIILSTHHMDEADVLGDRIAIISHGKLCCVGSSLFLKNQLGTGYYLTLVKKDVESSLSSCRNSSSTVSY
hsABCA4   1111 RTIIMSTHHMDEADLLGDRIAIIAQGRLYCSGTPLFLKNCFGTGLYLTLVRK.MKNIQSQRKGSEGTCSC


hsABCA7   1059 DTRQEKKNGSQGSRVGTPQ...........LLALVQHWVPGARLVEELPHELVLVLPYTGAHDGSFATL
hsABCA1   1152 LKKEDSVSQSSSDAGLGSDHESDTLTIDVSAISNLIRKHVSEARLVEDIGHELTYVLPYEAAKEGAFVEL
hsABCA4   1180 SSKGFSTTCPAHVDDLTPEQVLDG...DVNELMDVVLHHVPEAKLVECIGQELIFLLPNKNFKHRAYASL


hsABCA7   1117 FRELDTRLAELRLTGYGISDTSLEEIFLKVVEECAADTDMEDCSC.GQHLCTGIAGLDVTLRLKMPPQET
hsABCA1   1222 FHEIDDRLSDLGISSYGISETTLEEIFLKVAEESGVDAETSDGCTLPARRNRRAFGDKQSCLRPFTEDDAA
hsABCA4   1247 FRELEETLADLGLSSFGISDTPLEEIFLKVTEDSDSGPLFAGC...AQQKRENVNPRHPCLGP.REKAGQ


hsABCA7   1186 ALENGEPAGSAPETDQGSG.....PDAVG..RVQCWALTRQQLQALLLKRFLLARRSRRGLFAQIVLPAL
hsABCA1   1292 DPNDSDIDPESRETDLLSGM.....DGKGSYQVKGWKLTQQQFVALLWKRLLIARRSRKGFFAQIVLPAV
hsABCA4   1313 TPQDSNVCSPGAPAAHPEGQPPPEPECPGPQLNTCTQLVLQHVQALLVKRFQHTIRSHKDFLAQIVLPAT


hsABCA7   1249 FVGLALVFSLIVPPFGHYPALRLSPTMYGAQVSFFSEDAPGDPGRARLLEALLQEAG..........LEE
hsABCA1   1357 FVCIALVFSLIVPPFGKYPSLELQPWMYNEQYTFVSNDAPEDTGTLELLNALTKDPGFGTRCMEGNPIPD
hsABCA4   1383 FVFLALMLSIVIPPFGEYPALTLHPWIYGQQYTFFSMDEPGSEQFTVLADVLLNKPGFGNRCLKEGWLPE


hsABCA7   1309 PPVQHSSHRFSAPEVPAEVAKVLASGNWTPESPSPACQCSRPGARRLLPDCPAAAGGPPPPQAVTGSGEV
hsABCA1   1427 TPCQAGEEEWTTAPVPQTIMDLFQNGNWTMQNPSPACQCSSDKIKKMLPVCPPGAGGLPPPQRKQNTADI
hsABCA4   1453 YPC.GNSTPWKTPSVSPNITQLFQKQKWTQVNPSPSCRCSTREKLTMLPECPEGAGGLPPPQRTQRSTEI
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hsABCA7  1379  VQNLTGRNLSDFLVKTYPRLVRQGLKTKKWVNEVRYGGFSLCGRDP.GLPSGQELGRSVEELWALLSPLP
hsABCA1  1497  LQDLTGRNISDYLVKTYVQIIAKSLKNKIWVNEFRYGGFSLGVSNTQALPPSQEVNDAIKQMKKHLKLAK
hsABCA4  1522  LQDLTDRNISDFLVKTYPALIRSSLKSKFWVNEQRYGGISIGGKLPVVPITGEALVGFLSDLGRIMNVSG

hsABCA7  1448  GGALDRVLKNLTAWAHSLDAQDSLKIWFNNKGWHSMVAFVNRASNAILRAHLPPGPARHAHSITTLNHPL
hsABCA1  1567  DSSADRFLNSLGRFMTGLDTKNNVKVWFNNKGWHAISSFLNVINNAILRANLQKGENPSHYGITAFNHPL
hsABCA4  1592  GPITREASKEIPDFLKHLETEDNIKVWFNNKGWHALVSFLNVAHNAILRASLPKDRSPEEYGITVISQPL

hsABCA7  1518  NLTKEQLSEGALMASSVDVLVSICVVFAMSFVPASFTLVLIEERVTRAKHLQLMGGLSPTLYWLGNFLWD
hsABCA1  1637  NLTKQQLSEVALMTTSVDVLVSICVIFAMSFVPASFVVFLIQERVSKAKHLQFISGVKPVIYWLSNFVWD
hsABCA4  1662  NLTKEQLSEITVLTTSVDAVVAICVIFSMSFVPASFVLYLIQERVNKSKHLQFISGVSPTTYWVTNFLWD

hsABCA7  1588  MCNYLVPACIVVLIFLAFQQRAYVAPANLPALLLLLLLYGWSITPLMYPASFFFSVPSTAYVVLTCINLF
hsABCA1  1707  MCNYVVPATLVIIIBICFQQKSYVSSTNLPVLALLLLLYGWSITPLMYPASFVFKIPSTAYVVLTSVNLF
hsABCA4  1732  IMNYSVSAGLVVGIFIGFQKKAYTSPENLPALVALLLLYGWAVIPMMYPASFLFDVPSTAYVALSCANLF

hsABCA7  1658  IGINGSMATFVLELFSDQK.LQEVSRILKQVFLIFPHFCLGRGLIDMVRNQAMADAFERLCDRQFQSPLR
hsABCA1  1777  IGINGSVATFVLELFTDNK.LNNINDILKSVFLIFPHFCLGRGLIDMVKNQAMADALERFGENRFVSPLS
hsABCA4  1802  IGINSSAITFILELFENNRTLLRFNAVLRKLLIVFPHFCLGRGLIDLALSQAVTDVYARFGEEHSANPFH

hsABCA7  1727  WEVVGKNLLAMVIQGPLFLLFTLLLQHRSQLLPQPRVRSLPLLGEEDEDVARERERVVQGATQGDVLVLR
hsABCA1  1846  WDLVGRNLFAMAVECVVFFLITVLIQYRFFIRPRPVNAKLSPLNDEDEDVRRERQRILDGGGQNDILEIK
hsABCA4  1872  WDLIGKNLFAMVVECVVYFLLTLLVQRHFFLSQWIAEPTKEPIVDEDDDVAEERQRIITGGNKTDILRLH

hsABCA7  1797  NLTKVYRGQRMPAVDRLCLGIPPGECFGLLGVNGAGKTSTFRMVTGDTLASRGEAVLAGHSVAREPSAAH
hsABCA1  1916  ELTKIYRRKRKPAVDRICVGIPPGECFGLLGVNGAGKSSTFKMLTGDTTVTRGDAFLNKNSILSNIHEVH
hsABCA4  1942  ELTKIYPGTSSPAVDRLCVGVRPGECFGLLGVNGAGKTTTFKMLTGDTTVTSGDATVAGKSILTNISEVH

hsABCA7  1867  LSMGYCPQSDAIFELLTGREHLELLARLRGVPEAQVAQTAGSGILARLGLSWYADRPAGTYSGGNKRKLAT
hsABCA1  1986  QNMGYCPQFDAITELLTGREHVEFFALLRGVPEKEVGKVGEWAIRKLGLVKYGEKYAGNYSGGNKRKLST
hsABCA4  2012  QNMGYCPQFDAIDELLTGREHLYLYARLRGVPAEEIEKVANWSIKSLGLTVYADCLAGTYSGGNKRKLST

hsABCA7  1937  ALALVGDPAVVFLDEPTTGMDPSARRFLWNSLIAVVREGRSVMLTSHSMEECEALCSRLAIMVNGRFRCL
hsABCA1  2056  AMALIGGPPVVFLDEPTTGMDPKARRFLWNCALSVVKEGRSVVLTSHSMEECEALCTRMAIMVNGRFRCL
hsABCA4  2082  AIALIGCPPLVLLDEPTTGMDPQARRMLWNVIVSIIREGRAVVLTSHSMEECEALCTRLAIMVKGAFRCM

hsABCA7  2007  GSPQHLKGRFAAGHTLTLRVPAAR......SQPAAAFVAAEFPGAELREAHGGRLRFQLPPGGRCALARV
hsABCA1  2126  GSVQHLKNRFGDGYTIVVRIAGSN....PDLKPVQDFFGLAFPGSVLKEKHRNMLQYQLP.SSLSSLARI
hsABCA4  2152  GTIQHLKSKFGDGYIVTMKIKSPKDDLLPDLNPVEQFFQGNFPGSVQRERHYNMLQFQVS.SS..SLARI

hsABCA7  2071  FGELAVHGAEHGVEDFSVSQTMLEEVFLYFSKDQGKDEDTEEQKEAGVGVDPAPGLQHPKRVSQFLDDPS
hsABCA1  2191  FSILSQSKKRLHIEDYSVSQTTLDQVFVNFAKDQSDDDHLKD.....LSLHKNQTVVDVAVLTSFLQDEK
hsABCA4  2219  FQLLLSHKDSLLIEEYSVTQTTLDQVFVNFAKQQTESH...ID.....LPLHPRAAGASRQAQD.......
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hsABCA7   2141   TAETVL
hsABCA1   2256   VKESYV
hsABCA4          ......
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849   **Figure S10 Sequence alignment of human (hs) ABCA7, ABCA1, and ABCA4.**

Dataset	ABCA7$_{BPL}$		ABCA7$_{PE}$	ABCA7$_{DIGITONIN}$	ABCA7$_{EQ-ATP}$
Magnification	96k		96k	96k	96k
Pixel Size (Å)	0.895		0.889	0.889	0/889
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	Map4	Map5
EMDB ID	EMD-22996	EMD-22998	EMD-A	EMD-B	EMD-C
# 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					
Refined Coordinates	ABCA7$_{BPL}$		ABCA7$_{PE}$	ABCA7$_{DIGITONIN}$	ABCA7$_{EQ-ATP}$
PDB ID	AAAA		BBBB	CCCC	DDDD
# Residues/Non-hydrogen Atoms	1801/14761		1846/14749	1856/4662	1871/14748
Glycans	16		16	26	16
Ligands	21		20		2
R.M.S deviations					
Bond Length (Å)	0.003		0.003	0.003	0.003
Bond Angles (°)	0.616		0.600	0.678	0.645
MolProbity Statistics					
MolProbity Score	1.68		1.72	1.83	1.77
Clashscore	8.05		8.16	11.40	9.83
Poor rotamers (%)	0.00		0.00	0.00	0.00
Ramachandran statistics					
Favored (%)	96.38		95.84	96.24	96.22
Allowed (%)	3.62		4.16	3.76	3.78
Outliers (%)	0.00		0.00	0.00	0.00

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851 **Table S1 Data collection and refinement statistics.**

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