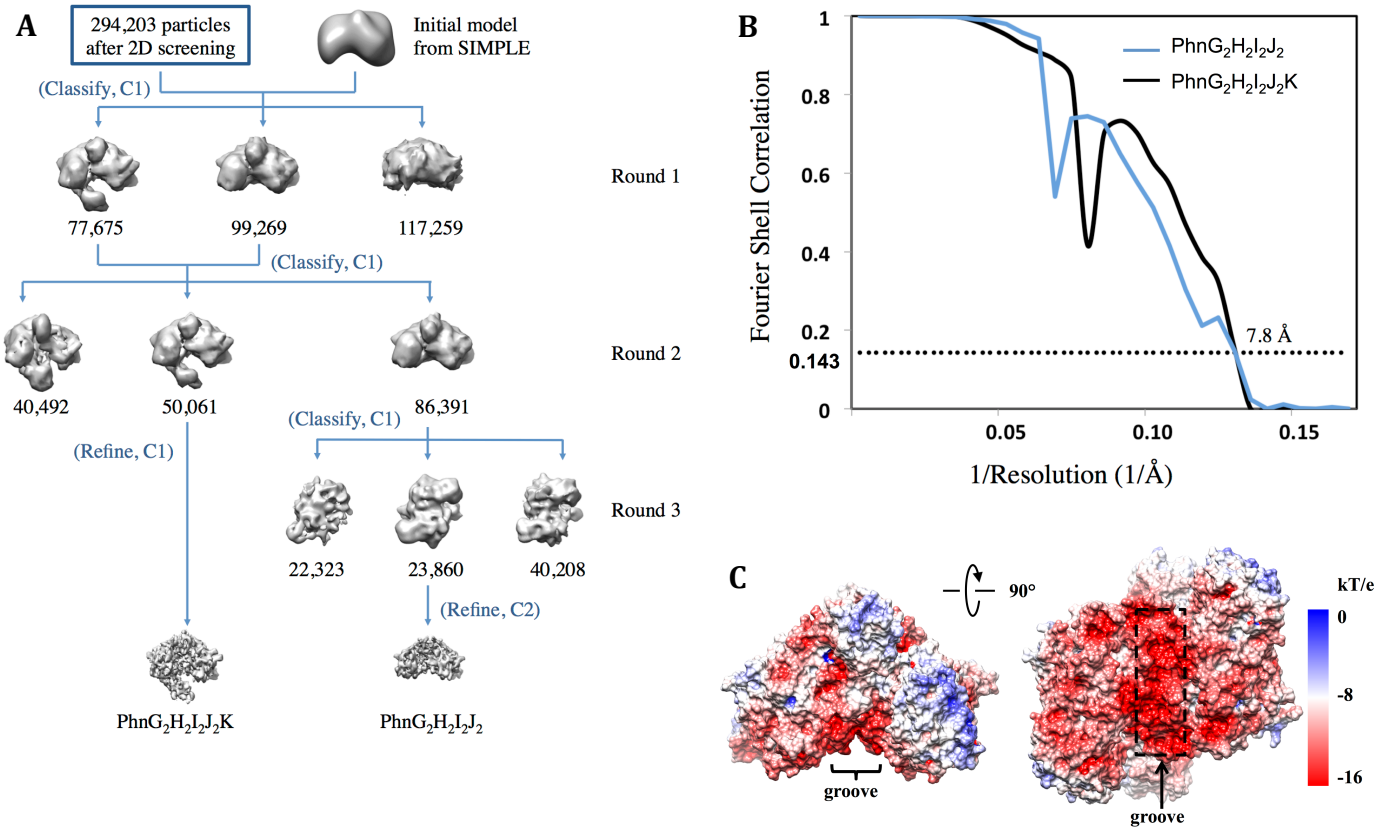

Figure S1, related to **Figures 1 and 2**. (A) Hierarchical unsupervised classification of particles in Relion. "(Classify, C1)" denotes unsupervised 3D classification without any symmetry constraints. "(Refine, C1)" denotes auto-refinement without any symmetry constraints. "(Refine, C2)" denotes auto-refinement with C2 symmetry constraint. Initial models for all classifications or refinements were low-pass filtered to 60Å. In round 1 and round 2, particles were downscaled by 4 times and separated mainly by the presence or absence of PhnK. In round 3, particles were downscaled by 2 times. All maps are shown with the original handedness and in the original order of the output. Refinement against the particles without downscaling, did not improve the final resolution of the maps. (B) Fourier shell correlations of the reconstructions of $PhnG_2H_2I_2J_2$ and $PhnG_2H_2I_2J_2K$. (C) Electrostatic surface of $PhnG_2H_2I_2J_2$ shows a negatively charged groove. The electrostatic surface is calculated based on the PDB structure 4XB6.

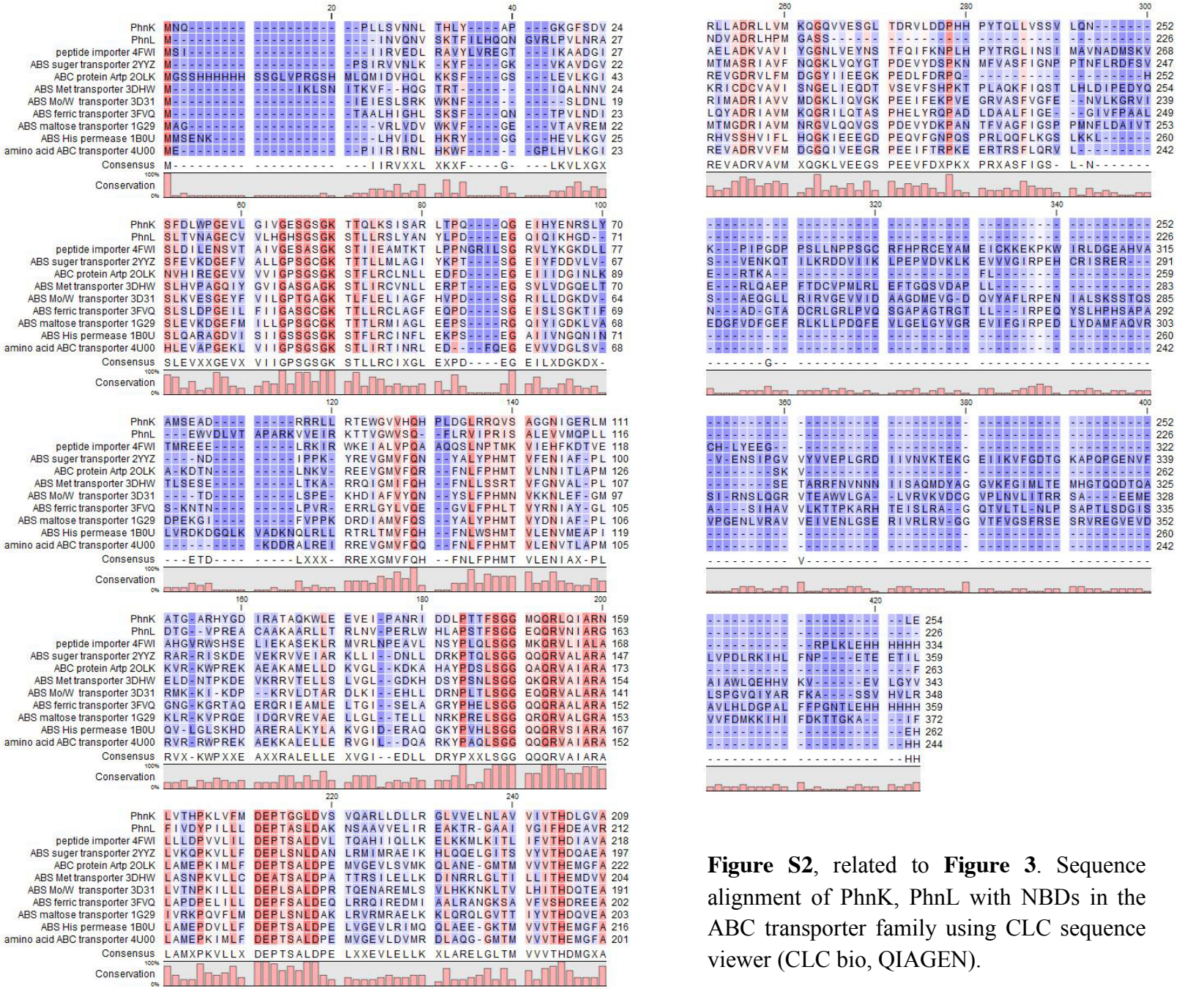

Figure S2, related to **Figure 3**. Sequence alignment of PhnK, PhnL with NBDs in the ABC transporter family using CLC sequence viewer (CLC bio, QIAGEN).

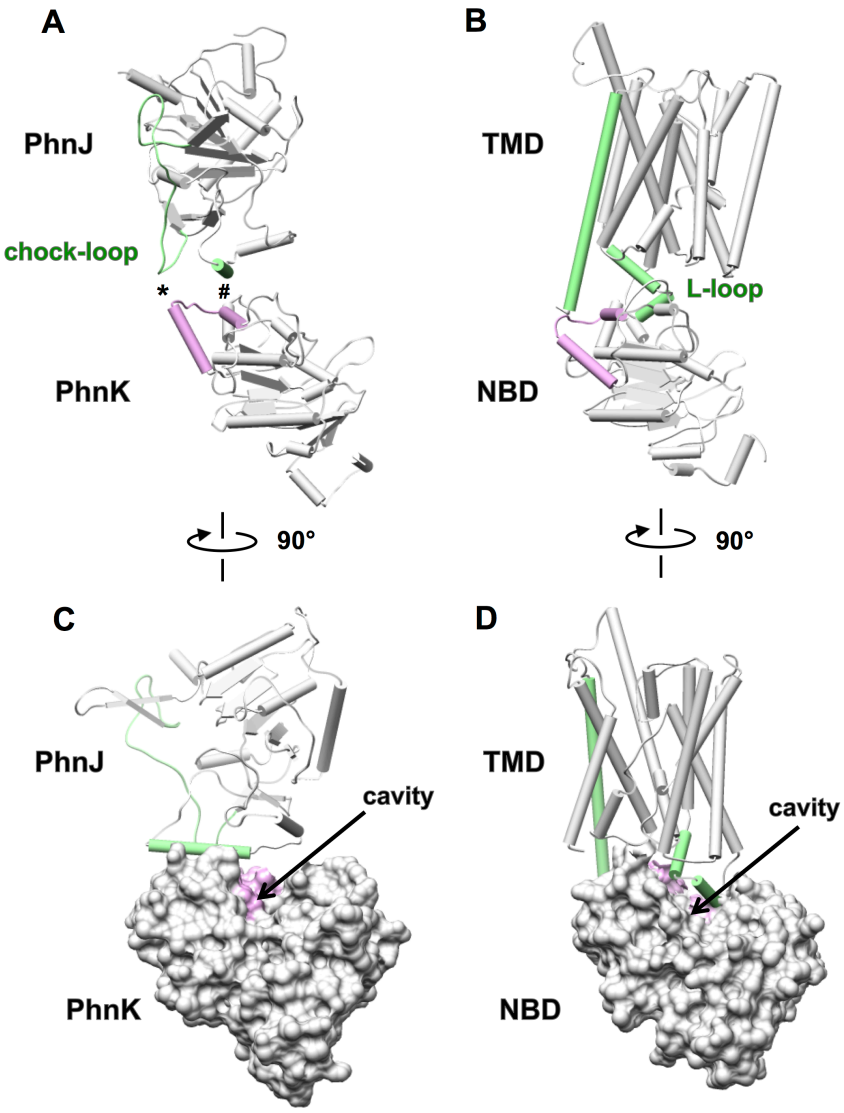

Figure S3, related to **Figure 3**. The interaction mode between PhnK and PhnJ (A, C) was compared with the interaction between a classical NBD and the corresponding TMD (B, D; PDB id: 1L7V). Purple color: Helices 3 and 4 of PhnK interacting with PhnJ, and their corresponding helices in the NBD. Green color: the chock-loop and Helix 6 of PhnJ, the L-loop and a helix (at the similar position of the chock-loop) of TMD. PhnK and NBD in (C) and (D) are in the surface representation to show the cavity for L-loop binding in ABC transporters.

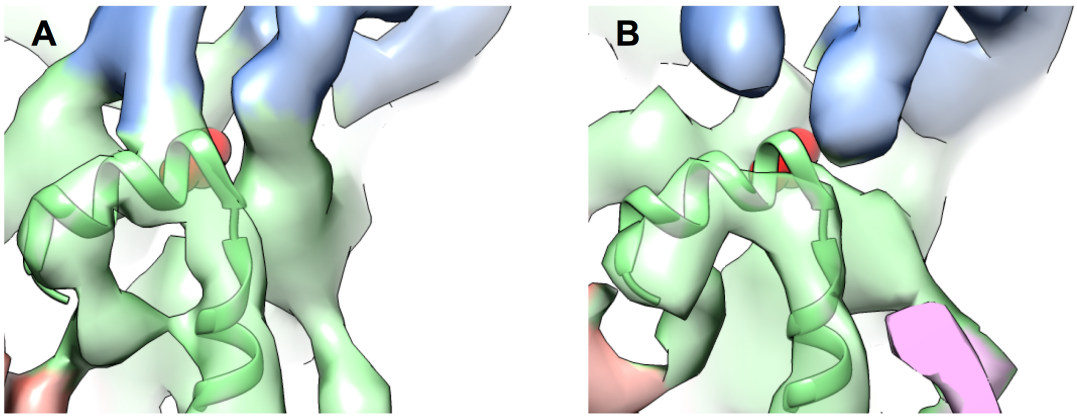

Figure S4, related to **Figure 4**. Comparison between our density map of $PhnG_2H_2I_2J_2K$ and α-helices 5 and 6 of PhnJ in the crystal structure (PDB id: 4XB6) for the PhnK apo side (A) and bound side (B), respectively. The color code and orientation are the same as Figure 4.