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Supporting information for article:

Three-dimensional structure of the human breast cancer resistance protein (BCRP/ABCG2) in an inward-facing conformation

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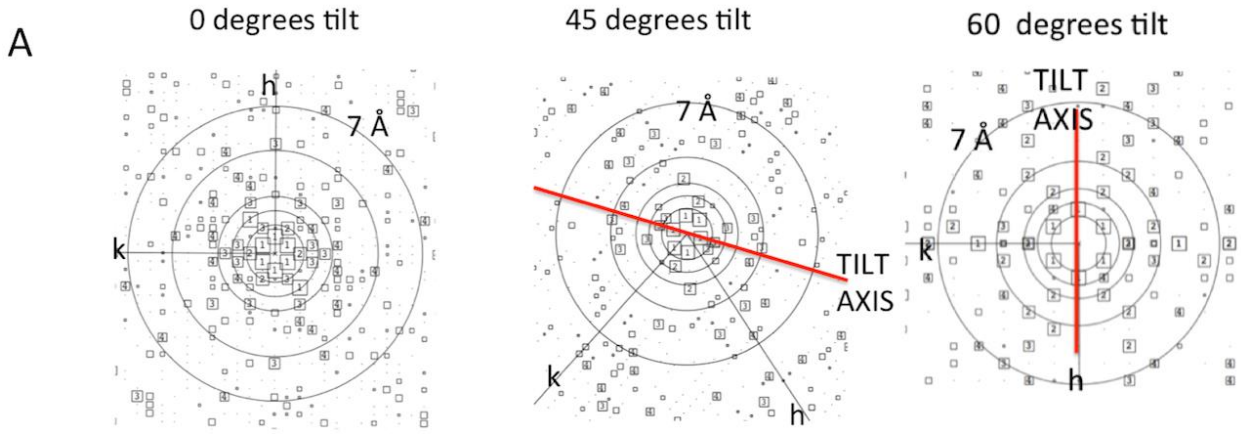

Figure S1 Fourier transforms of typical images recorded at the tilt angles 0º, 45º and 60º from the left panel to the right panel. The size of the squares and the numbers correspond to the IQ values of the spots. The rings depict the resolution bins at 3.6, 2.4, 1.8, 1.2 and 0.7 nm (7 Å) resolution, respectively. The axes give the directions of the reciprocal lattice vectors *h* and *k*. The 60 º plot has several high signal-to-noise ratio spots (IQ 2). These are not as dense because of the high tilt of the crystal resulting in some loss of resolution due to stability problems at higher tilt. Most of the images were close to focus, explaining the lack of Thon rings.

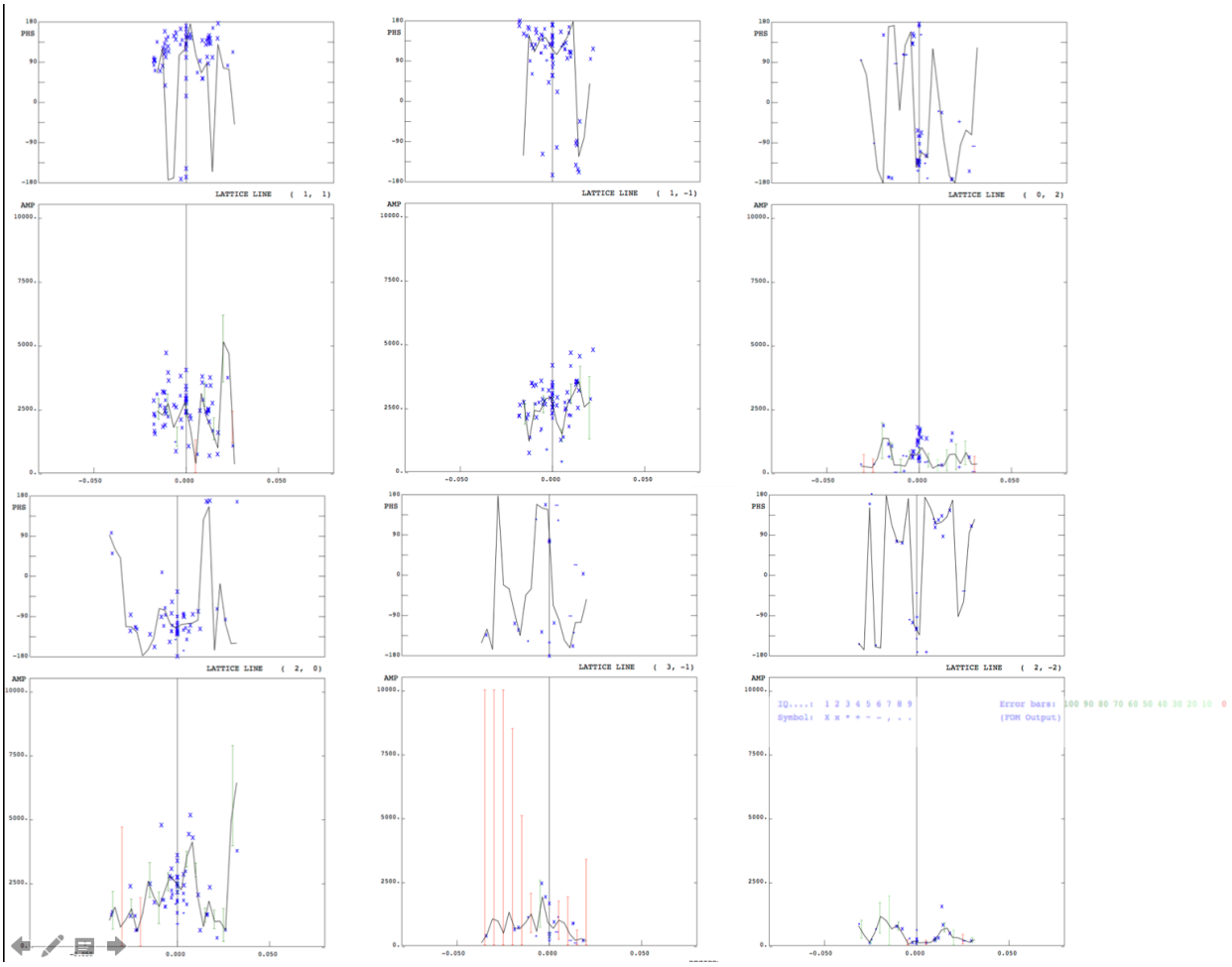

Figure S2 Phases (panel rows 1 and 3) and amplitudes (panel rows 2 and 4) of six exemplar lattice lines plotted against the reciprocal lattice coordinate z*. The Z resolution (horizontal axis) is 1.3 nm.

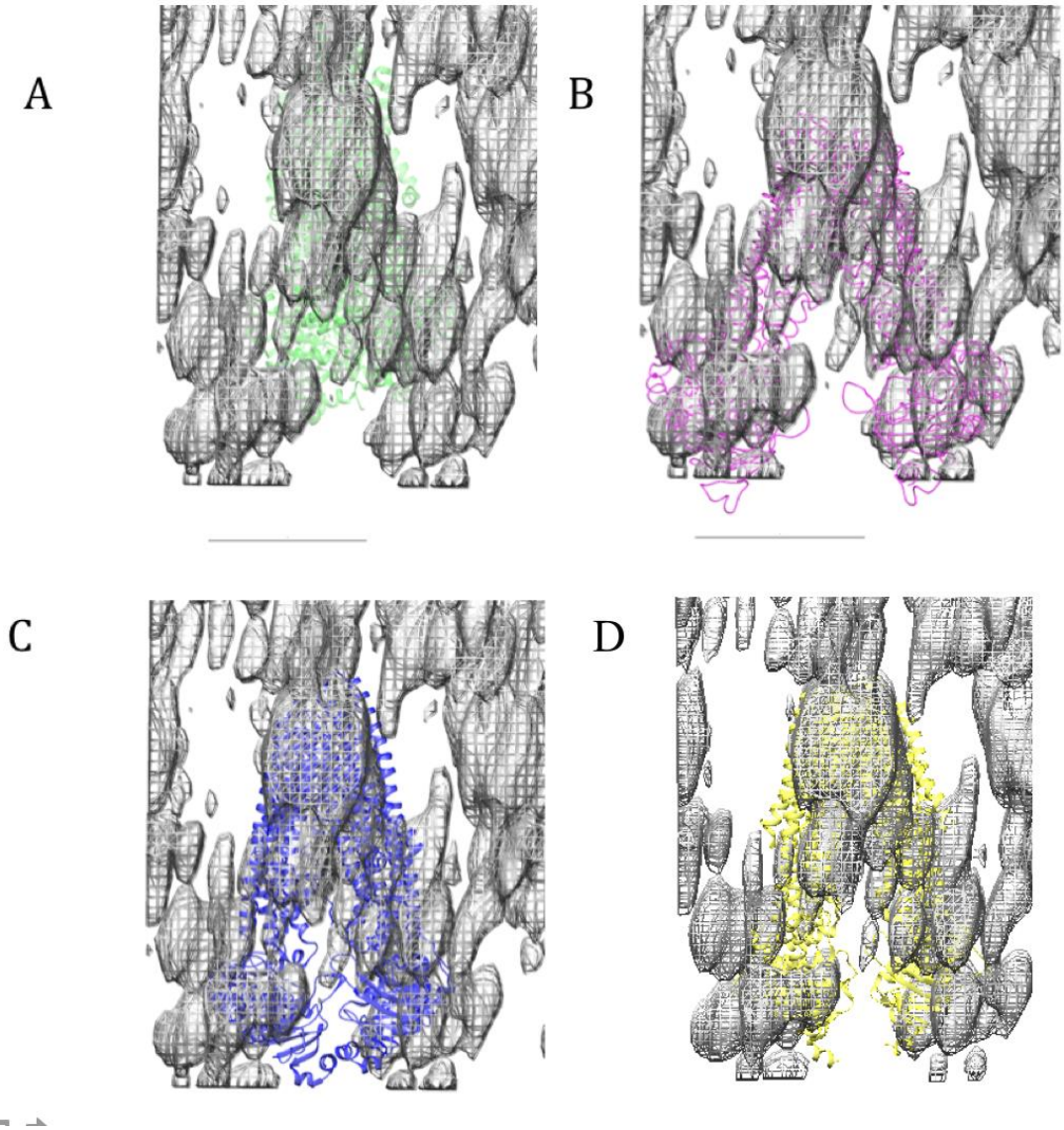

Figure S3 Fitting of alternative ABC transporter structures to the ABCG2 map. **A**) Sav1866 (2HYD, outward-facing) fitted with a correlation coefficient of 0.34. **B**) MsbA (3B5W, inward-facing open apo) fitted with a correlation coefficient of 0.42. **C**) Murine ABCB1 (3G60, inward-facing closed apo) fitted with a correlation coefficient of 0.47. In particular, the fit for this ABCB1 is significantly better than that for Sav1866. **D**) Human mitochondrial ABCB10 (3ZDQ, inward-facing closed apo) fitted with a correlation coefficient of 0.53. The scale bar represents 6 nm.

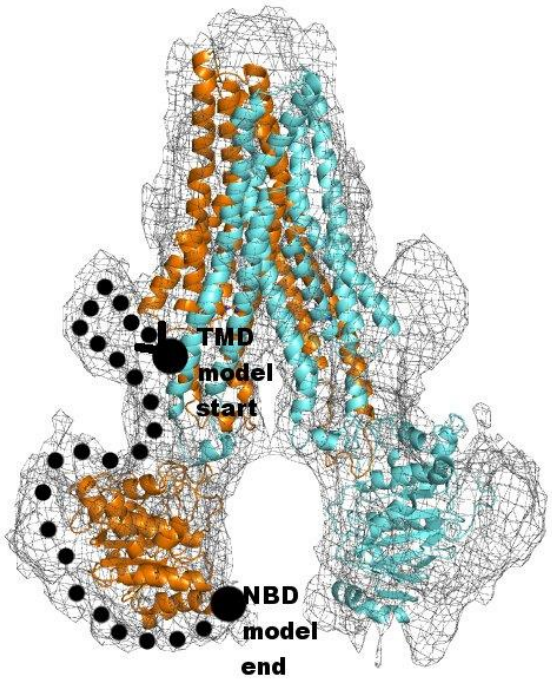

Figure S4 The linker region (the dotted line) connecting C-terminus of the NBD and N-terminus of the TMD possibly accounts for the "beak" regions and the unmatched regions in the NBDs between the refined ABCG2 model and the ABCG2 map.

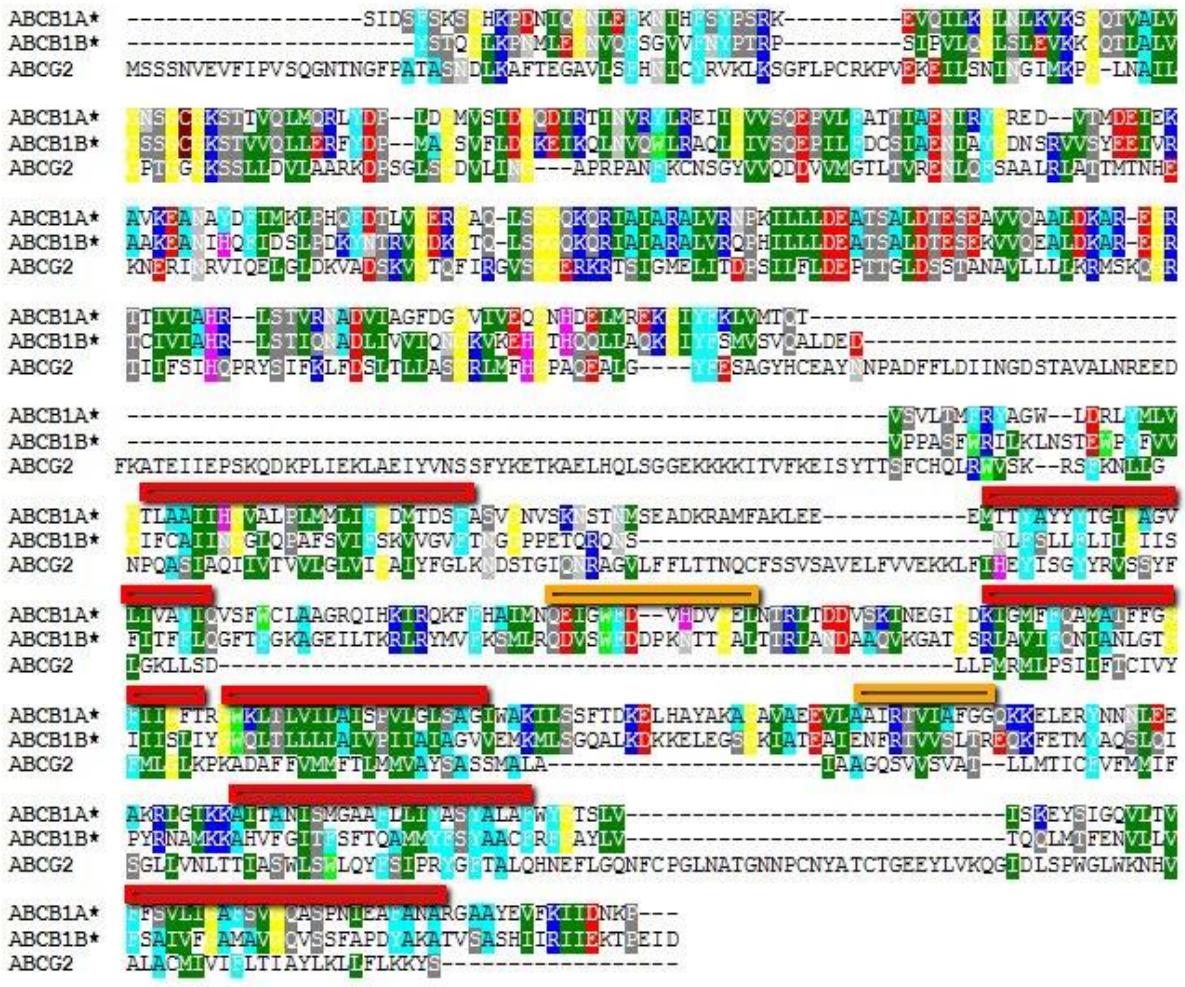

Figure S5 Shown is the sequence alignment between human ABCG2 and the two halves of murine ABCB1 (PDB code: 3G5U), illustrating the difference in intracellular loops (ICLs) between the two ABC transporters. ABCB1A* and ABCB1B* represent the first and second halves of ABCB1 and were created by cutting the ABCB1 protein sequence to two halves and then changing the order of TMD and NBD in order to get amino acid sequences that can be directly aligned with that of ABCG2. Amino acid identity and similarity were identified using the BLOSUM62 software and are highlighted with colors. Putative TM helices are highlighted with red lines above the sequences. Two coupling helices of each half of the ABCB1 molecule within the long ICLs that have direct contacts with the NBDs are highlighted with brown lines above the sequences. This sequence alignment suggests that, in contrast to ABCB1, each ABCG2 molecule contains only one long ICL with a potential coupling helix. This illustration is similar to what we previously published (Rosenberg et al., 2010).