

SUPPORTING INFORMATION

Figure 1

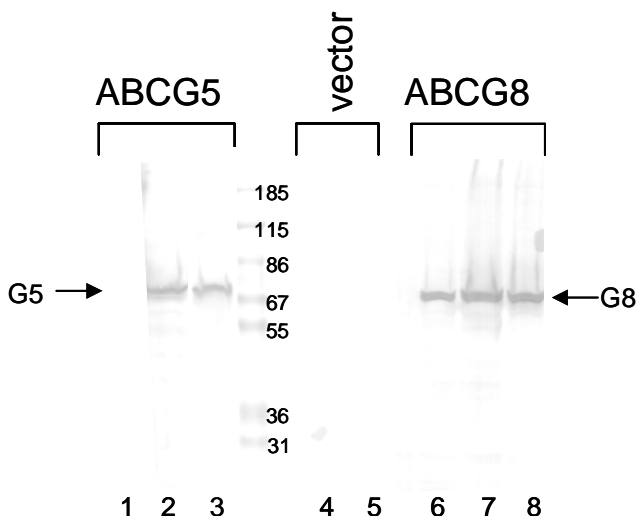


Figure 1: Expression of ABCG5 and ABCG8. Membrane preparations of *P. pastoris* transformants (see Experimental Procedures) were resolved on SDS-gels, and analyzed by Western blot using a monoclonal antibody against the C-terminal RGS-His₄ epitope. The names of the respective gene products are given on top of the blots; vector lanes are control transformants with the “empty” vector and are not expected to cross-react with the antibody. Transformants shown in lanes 1, 2, 4, and 6 were selected on low concentrations of Zeocin (100 µg/ml), all others on high concentrations of Zeocin (1000 µg/ml). Intensities of ABCG5 and ABCG8 on the western blots reflect variations in expression levels of the proteins. The numbers between lanes 3 and 4 correspond to the positions of Mw protein markers in kDa. Calculated MW of ABCG5 and ABCG8 are 82 kDa and 79 kDa, respectively.

Figure 2

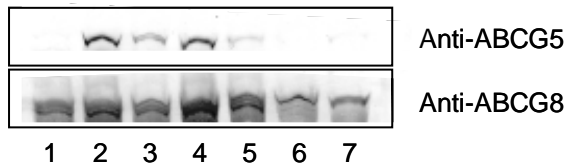


Figure 2: **Co-expression of ABCG5/G8.** Membrane preparations were analyzed as in Fig. 1 except that a polyclonal anti-ABCG5 and a monoclonal anti-ABCG8 antibody were used for Western blotting. All transformants were selected on 500 $\mu\text{g/ml}$ of Zeocin.

Figure 3

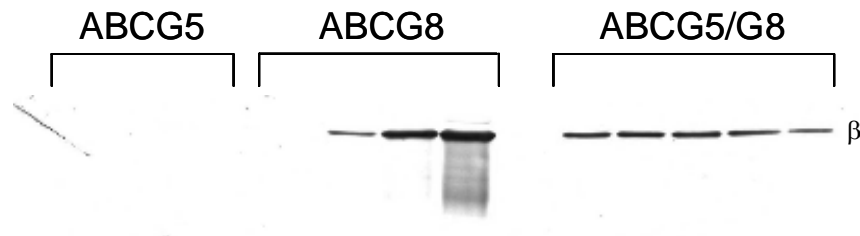


Figure 3: **Co-purification of F₁-ATPase in different preparation of ABCG5, ABCG8, and ABCG5/G8.** 10 μg of purified proteins from three ABCG5, four ABCG8, and five different ABCG5/G8 preparations were resolved on SDS gels. After protein transfer, the membranes were probed with an anti-F₁-ATPase antibody. This polyclonal antibody, although raised against the whole F₀F₁-ATPase from *E. coli*, only cross-reacted with the β -subunit of the F₁-ATPase from *P. pastoris*. Notably, F₁-ATPase co-purified with ABCG8 in three out of four preparations but not with ABCG5, and to a lesser extent with ABCG5/G8.