Supporting Information

A Salt Bridge in Intracellular Loop 2 is Essential for Folding of Human P-glycoprotein.

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Contents

1. Experimental Procedures.

Expression and Maturation of Mutants – Mutations were introduced into the human P-gp cDNA as described previously (*1*). The mutants contained an A52 epitope tag at their C-terminal ends for use in whole cell immunoblot assays (*2*). The presence of the epitope tag distinguished the mutant proteins from any endogenous P-gp.

Mutants were expressed in HEK 293 cells for 18 h in the presence or absence of 10 mM cyclosporine A. Expression in the presence of drug substrates like cyclosporine A promotes maturation of processing mutants (*3, 4*). Whole cell extracts were subjected to immunoblot analysis using monoclonal antibody A52.

Glycosylation of P-gp can be used to differentiate mature human P-gp (170 kDa) from the immature 150 kDa protein (*2*). SDS extracts of cells expressing the mutant protein were treated with Endo H_f or PNGase F (New England Biolabs, Mississauga, ON, Canada) and samples subjected to immunoblot analysis as described previously (*5*).

Purification of P-gp and Measurement of ATPase Activity – Histidine-tagged P-gps were expressed in HEK 293 cells and then isolated by nickel-chelate chromatography as described previously (*6*). Recovery of P-gp was monitored by immunoblot analysis with rabbit anti-P-gp polyclonal antibody (*6*). A sample of the isolated histidine-tagged P-gp was mixed with an equal

volume of 10 mg/ml sheep brain phosphatidylethanolamine (Type II-S, Sigma) that had been washed and suspended in TBS (pH 7.4). ATPase activity was measured in the presence of various concentrations of verapamil.

Modeling of human ICL2 – The human ICL2 structures (*7, 8*) were viewed using Pymol (*9*).

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2. Figures.

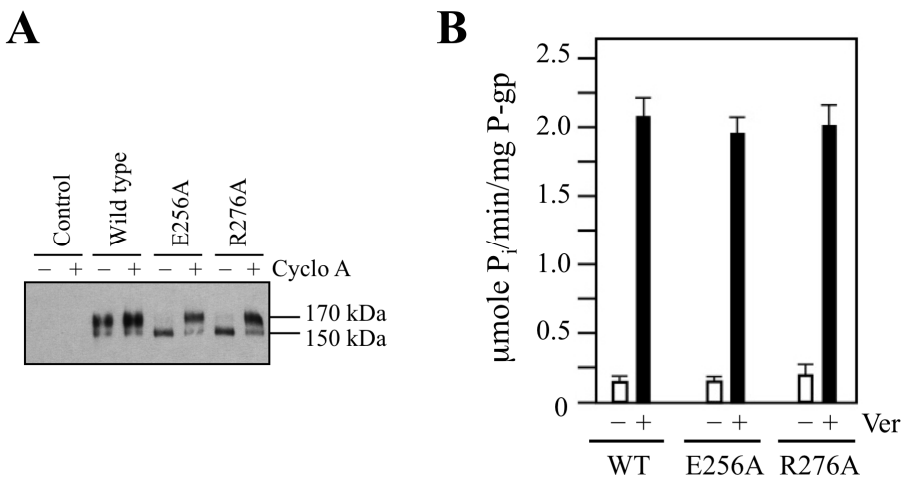

Figure S1. Rescue of salt bridge mutants. (A) Wild-type P-gp or mutants E256A or R276A were expressed in the presence (+) or absence (-) of cyclosporine A (Cyclo A) and whole cell SDS extracts subjected to immunoblot analysis. (B) ATPase activities of P-gps expressed in cyclosporin A were measured in the presence (+) or absence (-) of 0.6 mM verapamil (Ver). Each value is the mean ± S.D. (n=3).

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