TABLE S1

Residues in the transmission interface identified as second-site suppressors

Original mutation	Suppressor mutation	Location
S558Y (TMH2)	N242K	Near or in Q-loop of NBD1
	E244G	Q-loop of NBD1
	D246Δ	Q-loop of NBD1
	P596L	ICL1
	S597T / I	ICL1
	M679L	TMH5
	G1233D	ECL4
N242K (near or in the Q-loop)	M649L	TMH4 near ICL2
	V656L	ICL2
	A666G	TMH5 near ICL2
	L806F	ECL1
	K1016I	NBD2 deviant signature
E244G (Q-loop)	V656L	ICL2

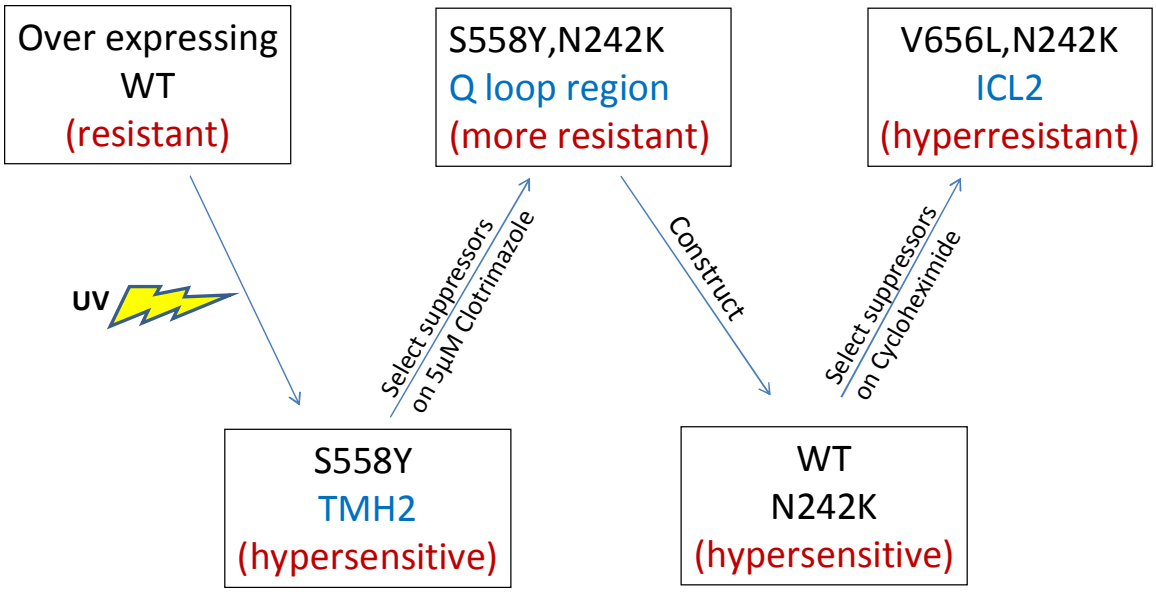

FIG. S1. Isolation of V656L as part of a suppressor screen. The N242K mutation restores drug resistance when coupled to the transmission-defective mutant S558Y. In an otherwise WT background, however, N242K is hypersensitive to cyh. Suppressors of cyh sensitivity were sought as described in Ananthaswamy *et al.* (2010-see the Supporting Information for that paper). One suppressor resulted from a V656L mutation

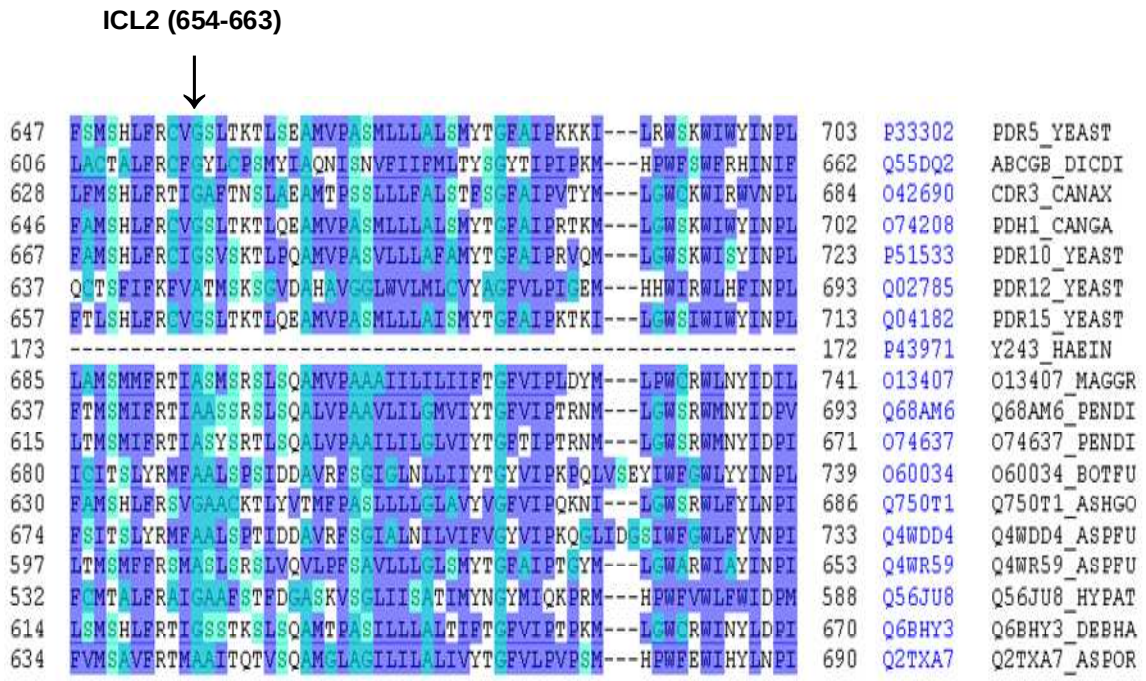

FIG. S2. Alignment of a region of the Pdr fungal subfamily that includes ICL2. The Pdr5 amino acid sequence was compared to 17 other members of the Pdr5 ABC transporter subfamily using the Swiss Protein Database alignment tool (www.unitprot.com). The non-polar residues are shaded purple, the tiny residues green, and tiny, non-polar residues are turquoise. In Pdr5 (top line), ICL2 is predicted to run from Arg-654 to Thr-662.

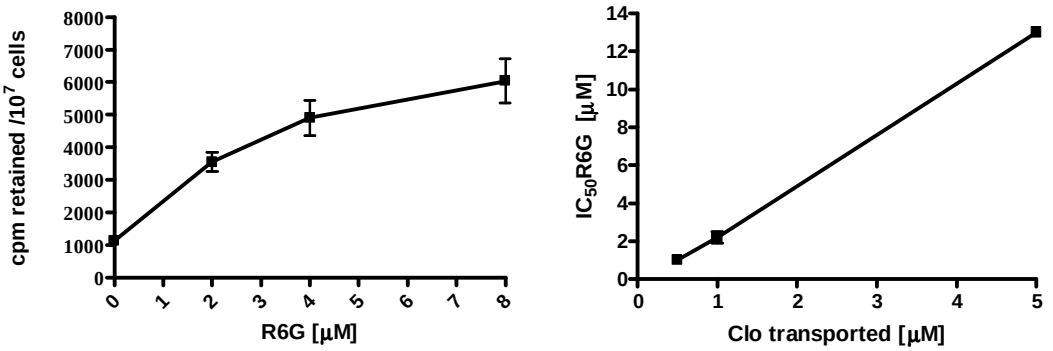

FIG. S3. R6G is a competitive inhibitor of clo transport. The ability of R6G to inhibit 0.5, 1.0 and 5.0 µM clo transport was tested using a steady-state whole cell efflux assay previously described (22). [^{3}H]-clo (20 Ci/mmol) was purchased from American Radiolabeled Chemicals. A. Results from the 1.0 µM clo efflux experiment (n=3) showing retained clo as a function of R6G concentration. In these experiments, the activity retained in the Δ*pdr5* control was ~ 7000 cpm / 10^7 cells. Complete, concentration-dependent inhibition of efflux by R6G was also observed at 0.5 µM and 5.0 µM clo. B. Plot of IC_{50} concentration of R6G (y axis) versus clo efflux concentration. The nearly linear plot is indicative of a competitive inhibitor.

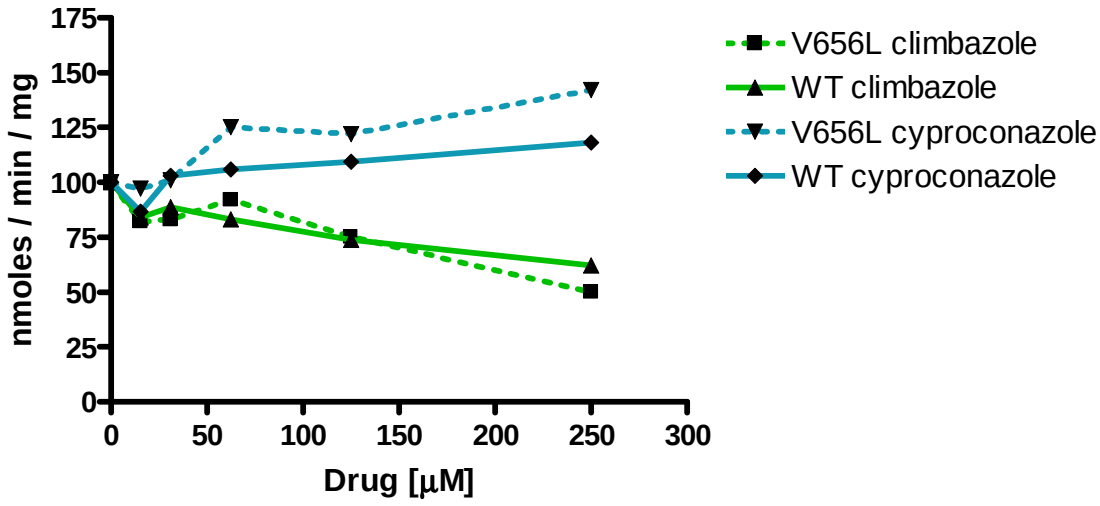

FIG. S4. The effect of climbazole and cyproconazole on V656L ATPase activity. The ATPase activity of PM vesicles derived from WT and V656L double-copy strains was assayed as described in Fig. 5 using 3 mM ATP in each reaction.