Supplementary Material

Figure S1:

Liquid drug resistance assay

Drug susceptibility of yeast cells expressing Pdr5 WT (black line), no Pdr5 (*⊿Pdr5*, dashed grey line*)* and the S1360F mutant (grey line) towards different drugs. The microdilution method was performed as described in Experimental Procedures. (**A**) Susceptibility towards increasing FK506 concentrations. The optical density OD_{600} of each well on the microtiter plate was determined after incubation for 48h at 30°C and plotted against the corresponding FK506 concentration. (**B - D**) Drug resistance pattern towards different substrates in the presence of FK506. (**B**) Fluconazole (41,8 µM), (**C**) Ketoconazole (1,5 µM) and (**D**) R6G (15,7 µM) in the presence of increasing FK506 concentrations.

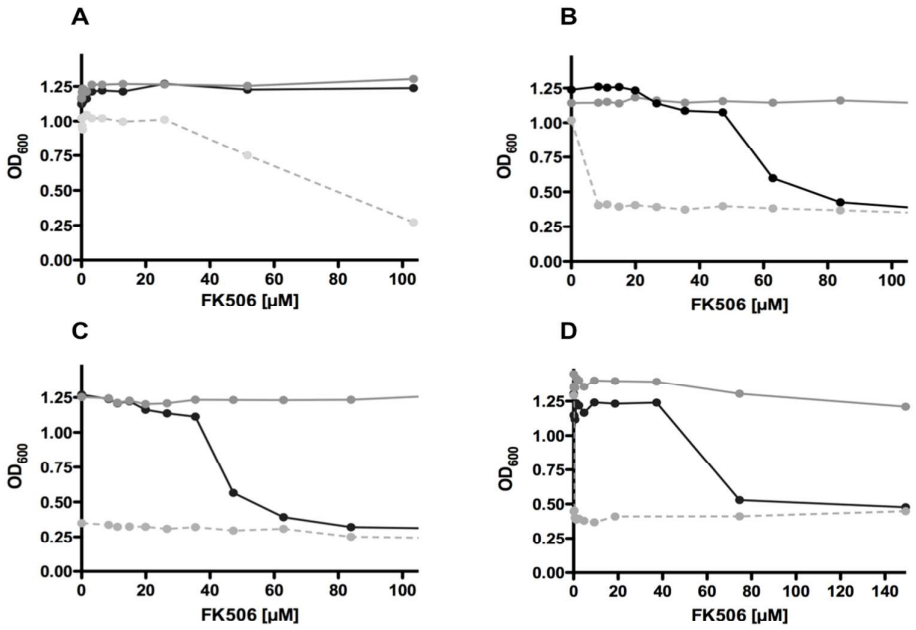

Figure S2:

FK506 does not interfere with ATP binding.

Isolated plasma membranes of Pdr5 WT (A) or the S1360F mutant (B) were labeled with 5 µM [α- or γ-^{32}P]-8-azido-ATP in the presence or the absence of non-labeled nucleotides and FK506 under non-hydrolyzing conditions as described in Experimental Procedures. The upper lanes show autoradiograms after cross-linking of γ-^{32}P- or α-^{32}P-8-azido-ATP to Pdr5 WT (A) and the S1360F mutant (B), while the lower panel displays a representative silver-stained SDS-PAGE analysis of one of the experiments of the upper panel. The positions of Pdr5 and Pma1 are indicated. Densitometric analysis of the autoradiograms and correlation to the amount of protein used in the individual experiments did not show any influence of FK506 on the binding of ATP.