SUPPLEMENTAL INFORMATION

Interaction of human drug-metabolizing CYP3A4 with small inhibitory molecules

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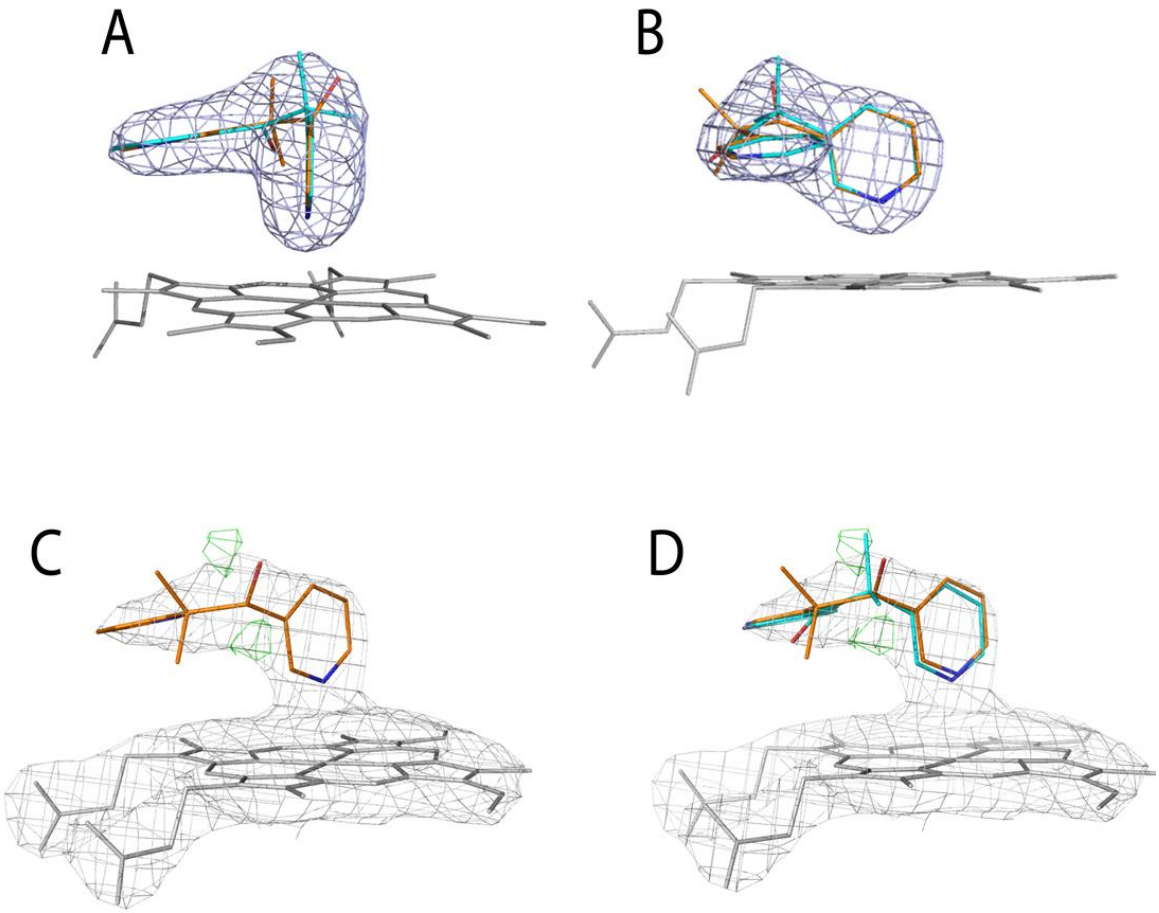

Figure S1. ***A*** and ***B***, Two possible orientations of metyrapone in the CYP3A4 active site. Only the conformer depicted in cyan can perfectly fit into a polder F_o-F_c difference map (contoured at 8σ level). ***C*** and ***D***, $2F_o$-F_c and F_o-F_c electron density maps (in gray and green mesh contoured at 2σ and 3σ levels, respectively) calculated for the alternative conformer of metyrapone. Bulks of positive density (in green) indicate the predicted location of methyl groups. The cyan conformer not only fits better but also preferably binds to CYP3A4 (occupancy of 0.87 versus 0.13 for the yellow conformer). Simultaneous fitting of two metyrapone conformers did not improve the R/R_{free} factors and, therefore, only the cyan conformer was modeled in the 6MA6 structure.

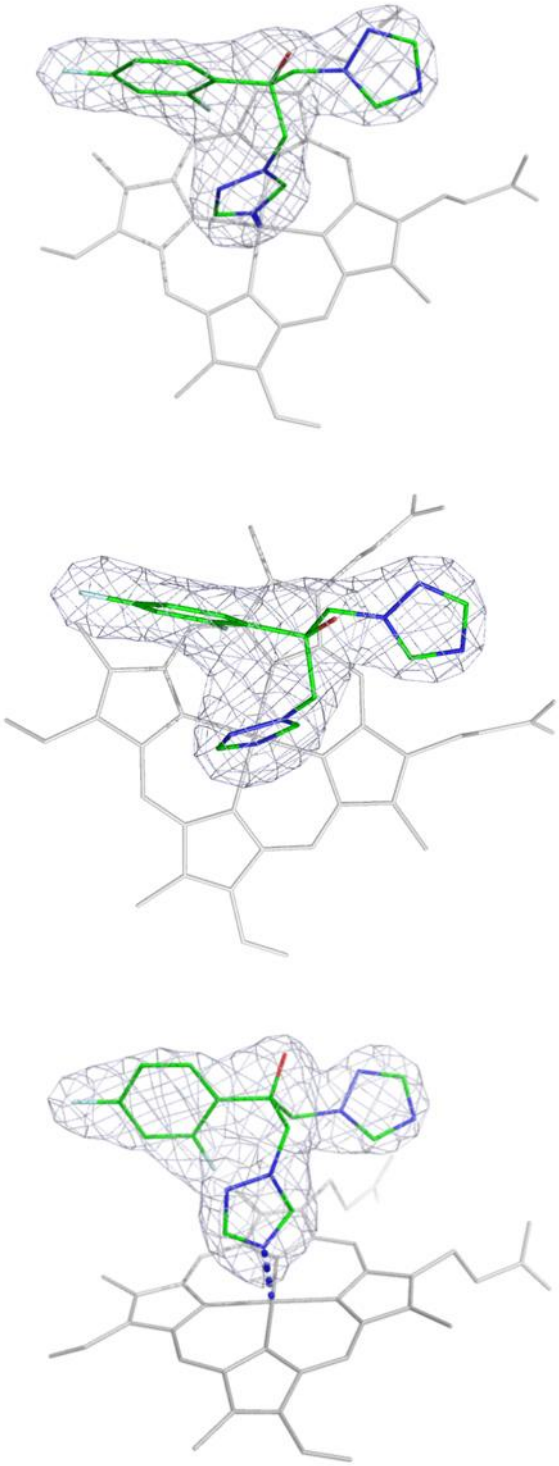

Figure S2. Different views at the heme-bound fluconazole to demonstrate an unambiguous identification of the ligand orientation. Gray mesh is a polder F_o-F_c difference map contoured at 8σ level.

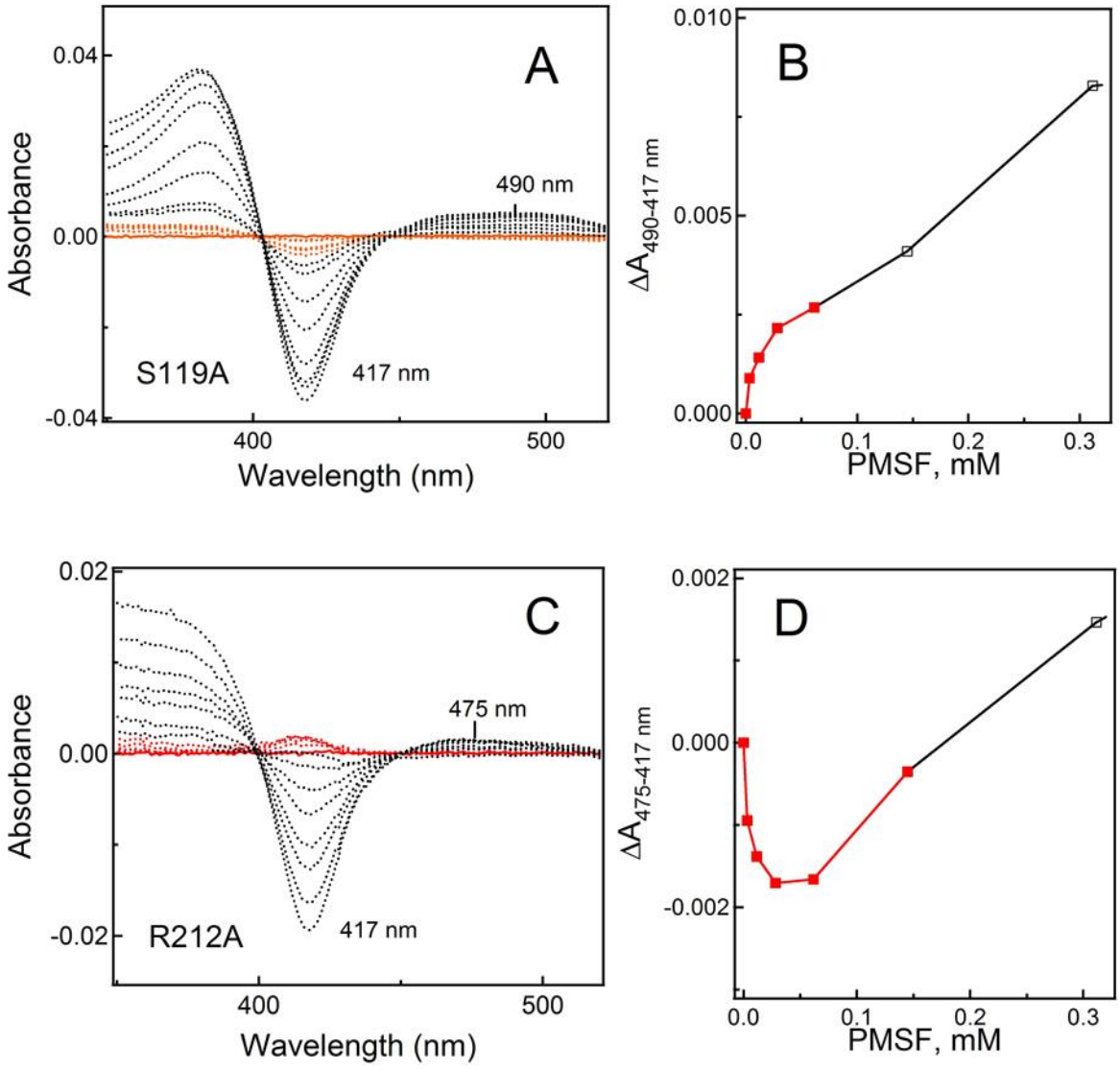

Figure S3. ***A*** and ***C***, Difference absorbance spectra recorded during titration of 2.5 μM S119A and R212A CYP3A4 with PMSF (3 mM final concentration). ***B*** and ***D***, Corresponding titration plots for the low ligand concentration range (0.003, 0.012, 0.028, 0.062, 0.145 and 0.312 mM). Similar to WT CYP3A4, two spectral phases were observed during titration of both mutants, suggesting the presence of two distinct PMSF binding sites. However, in R212A CYP3A4, there was an initial increase rather than a decrease in the Soret band.

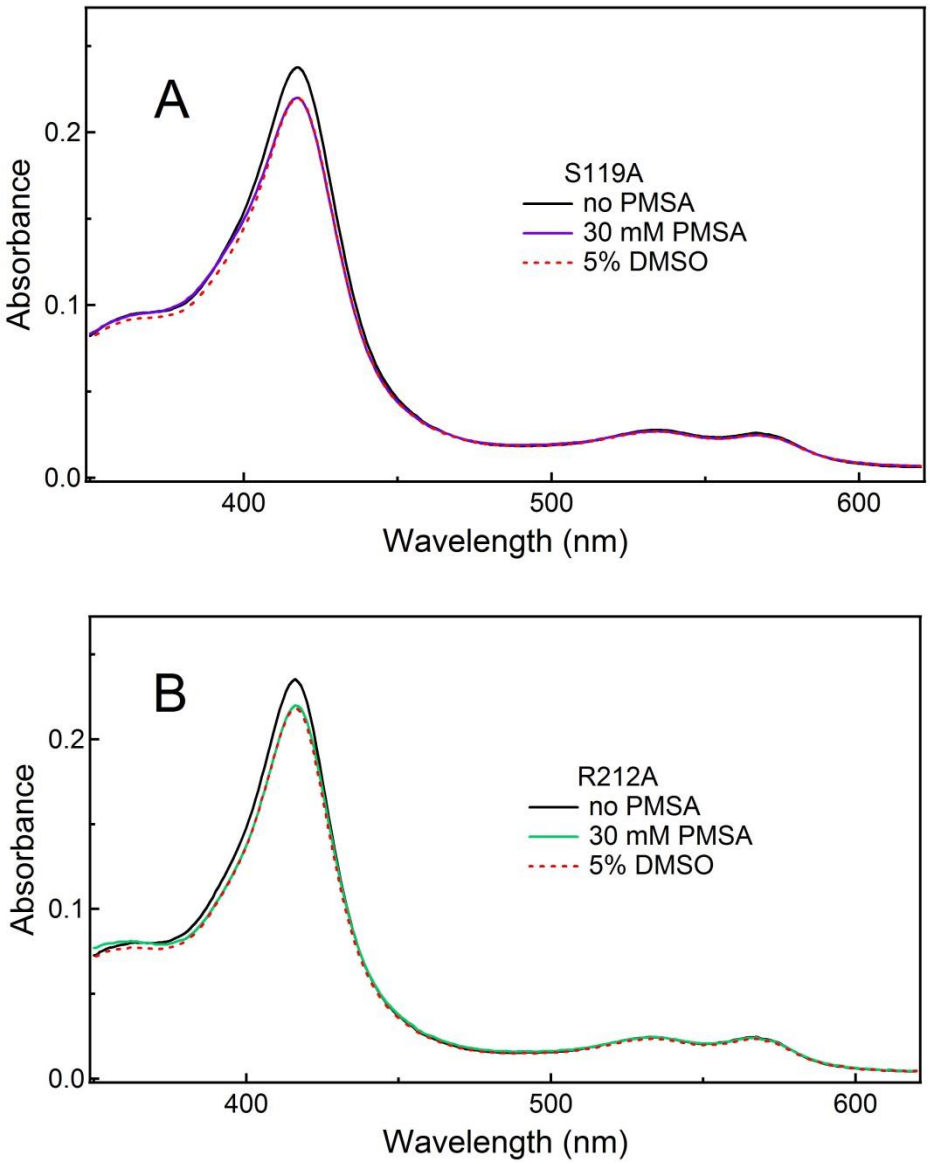

Figure S4. Absorbance spectra of S119A and R212A CYP3A4 in the absence and presence of 30 mM PMSA or 5% DMSO. Spectral changes induced by the solvent alone are nearly identical and suggest that both mutations preclude PMSA from binding in the vicinity of the heme.