Supporting Information

Cross-linking Mass Spectrometry and Mutagenesis Confirm the Functional Importance of Surface Interactions between CYP3A4 and Holo/Apo Cytochrome b_5

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FIGURE S1. CO-ferrous binding difference spectra of wild-type CYP3A4 and its mutants. Spectra were measured in buffer containing 50 mM KPi, 20% glycerol, pH 7.4. Enzyme concentrations in the buffer are as the following: WT: 0.48 μM (blue); K96A: 0.36 μM (green); K421A: 0.25 μM (purple); R446A: 0.47 μM (pink); Triple: 0.22 μM (gray).

FIGURE S2. Testosterone binding affinity with CYP3A4 wild-type and mutants. Binding affinity of testosterone with CYP3A4 wild-type were measured by spectral changes induced by titration of increasing concentrations of testosterone, with equal volumes of vehicle (acetonitrile) added to the reference cuvette each time. The CYP3A4 enzyme was 1 μM and the buffer was 50 mM KPi, 20% glycerol, pH 7.4. The ΔA is the change of absorbance difference between 390 nm and 418 nm. ■ WT; ▲ K96A; ♦ K421A;● R446A; □ Triple.

FIGURE S1.

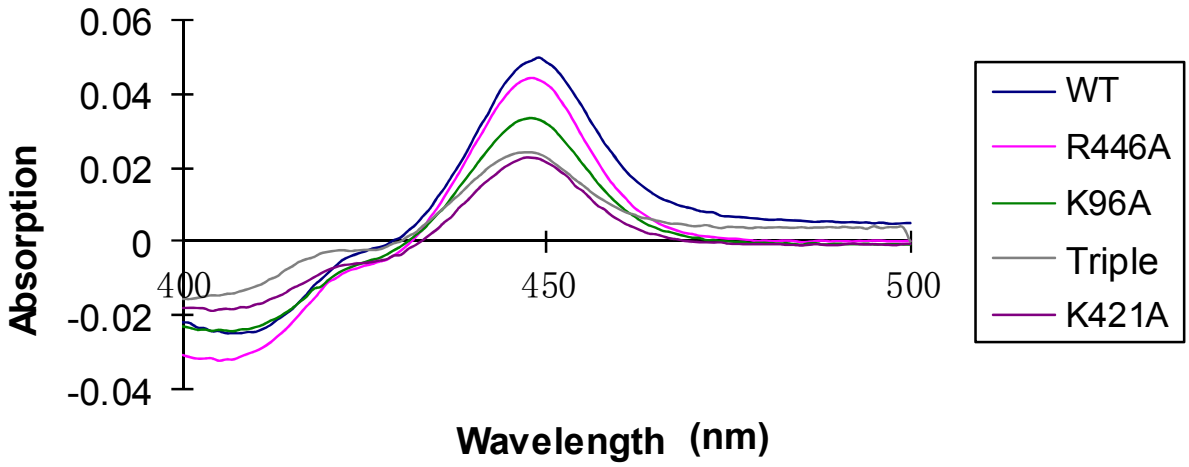
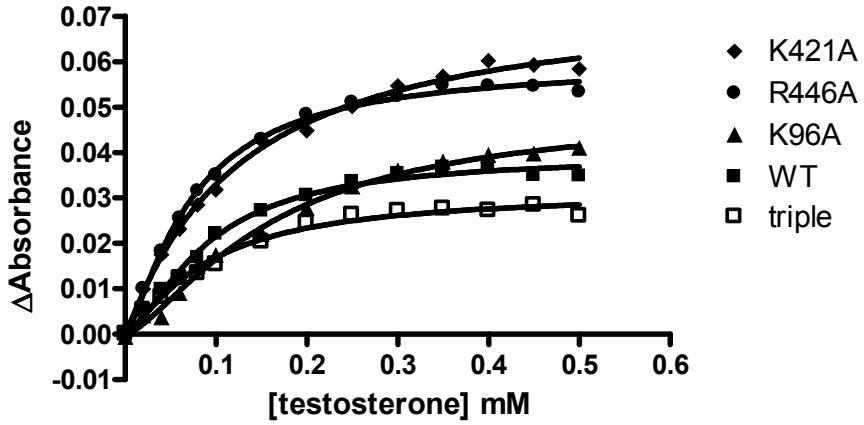

0.06
0.04
0.02
0
-0.02
-0.04
Absorption
400
450
500
Wavelength (nm)
WT
R446A
K96A
Triple
K421A

FIGURE S2.


0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
ΔAbsorbance
0.1
0.2
0.3
0.4
0.5
0.6
[testosterone] mM
K421A
R446A
K96A
WT
triple