

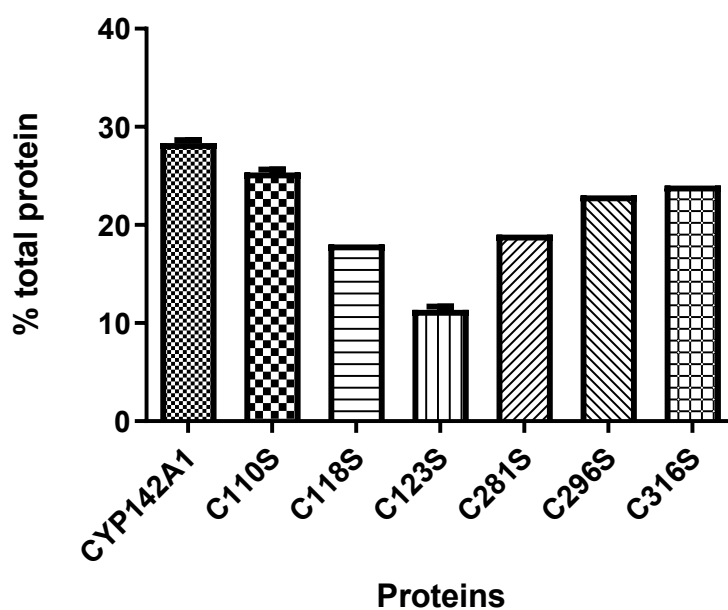
## Roles of Cysteine in the Structure and Metabolic Function of *Mycobacterium tuberculosis* CYP142A1

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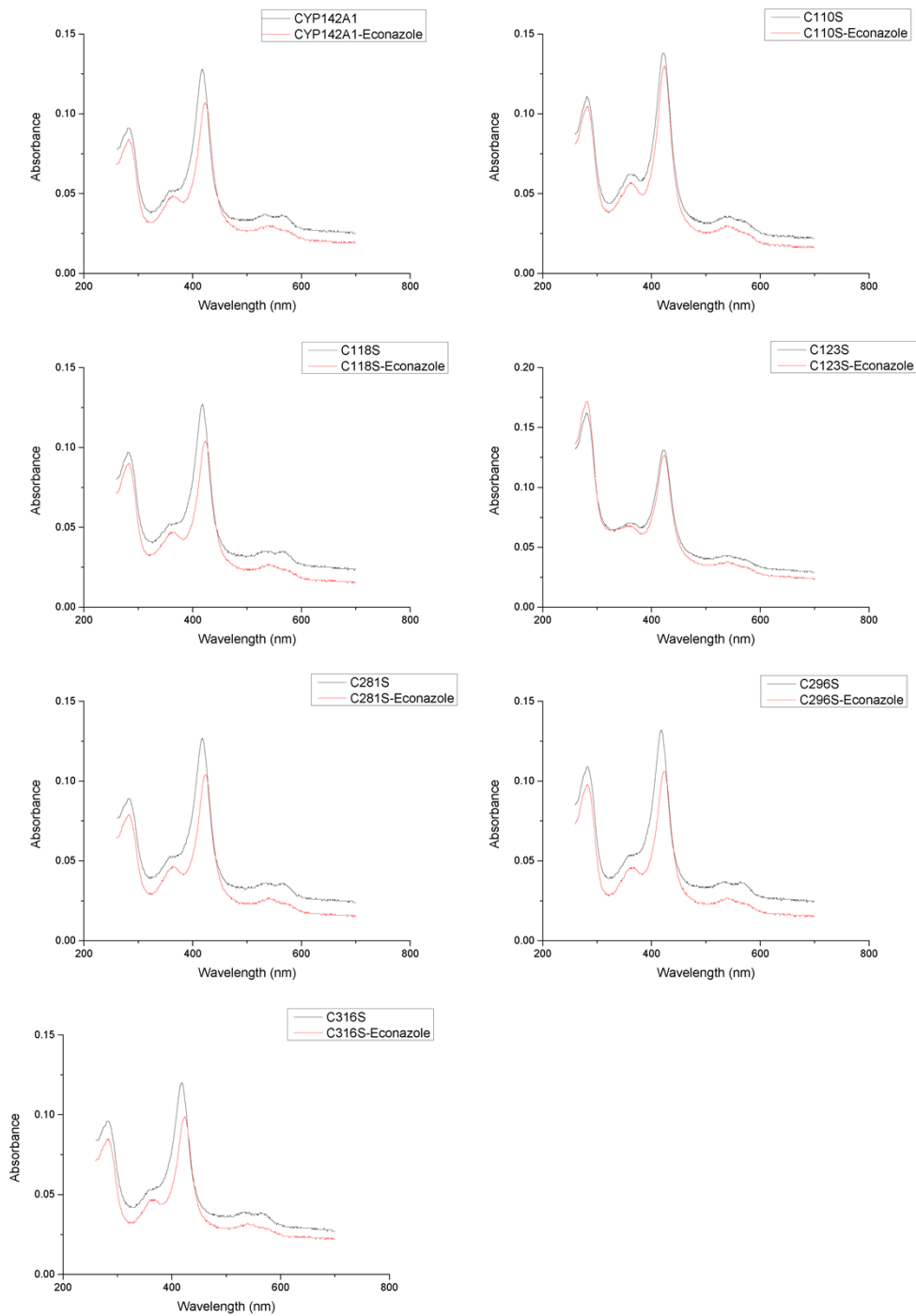
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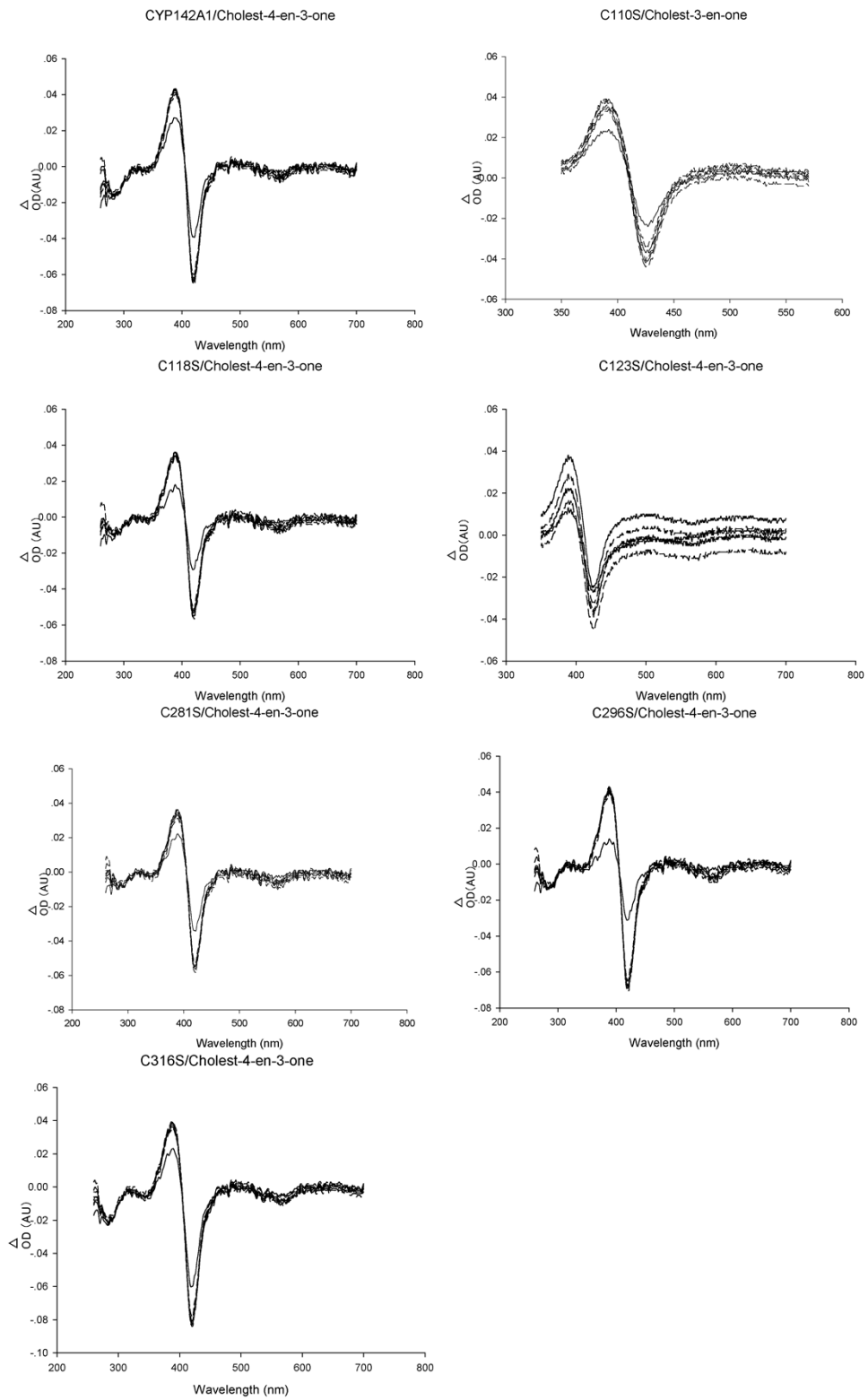
Fig. S1~7



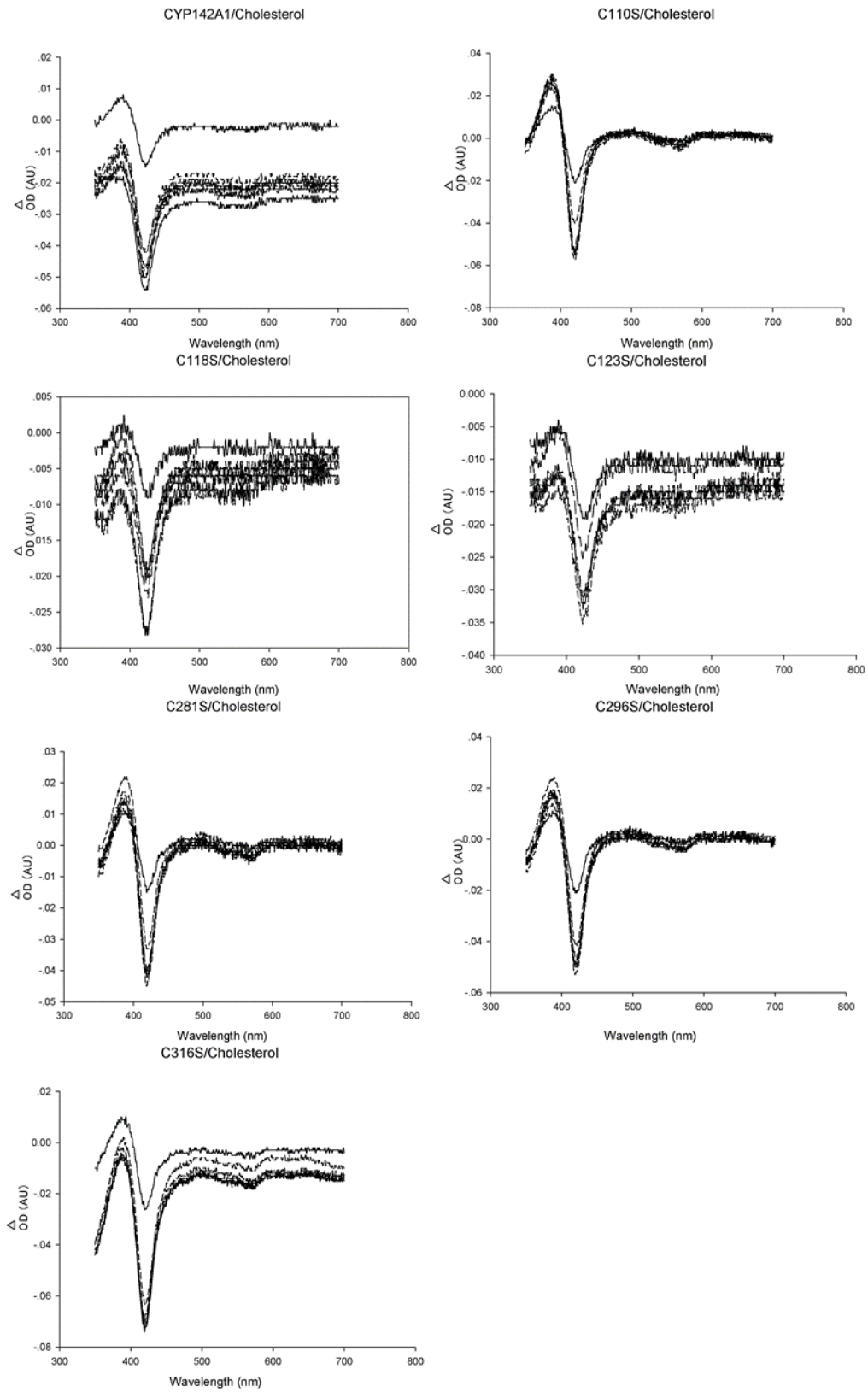
**Fig. S1.** Effects of single-cysteine mutations on the expression of CYP142A1 in *E. coli*. All values were normalized and shown as a percentage of the expression level of total protein.



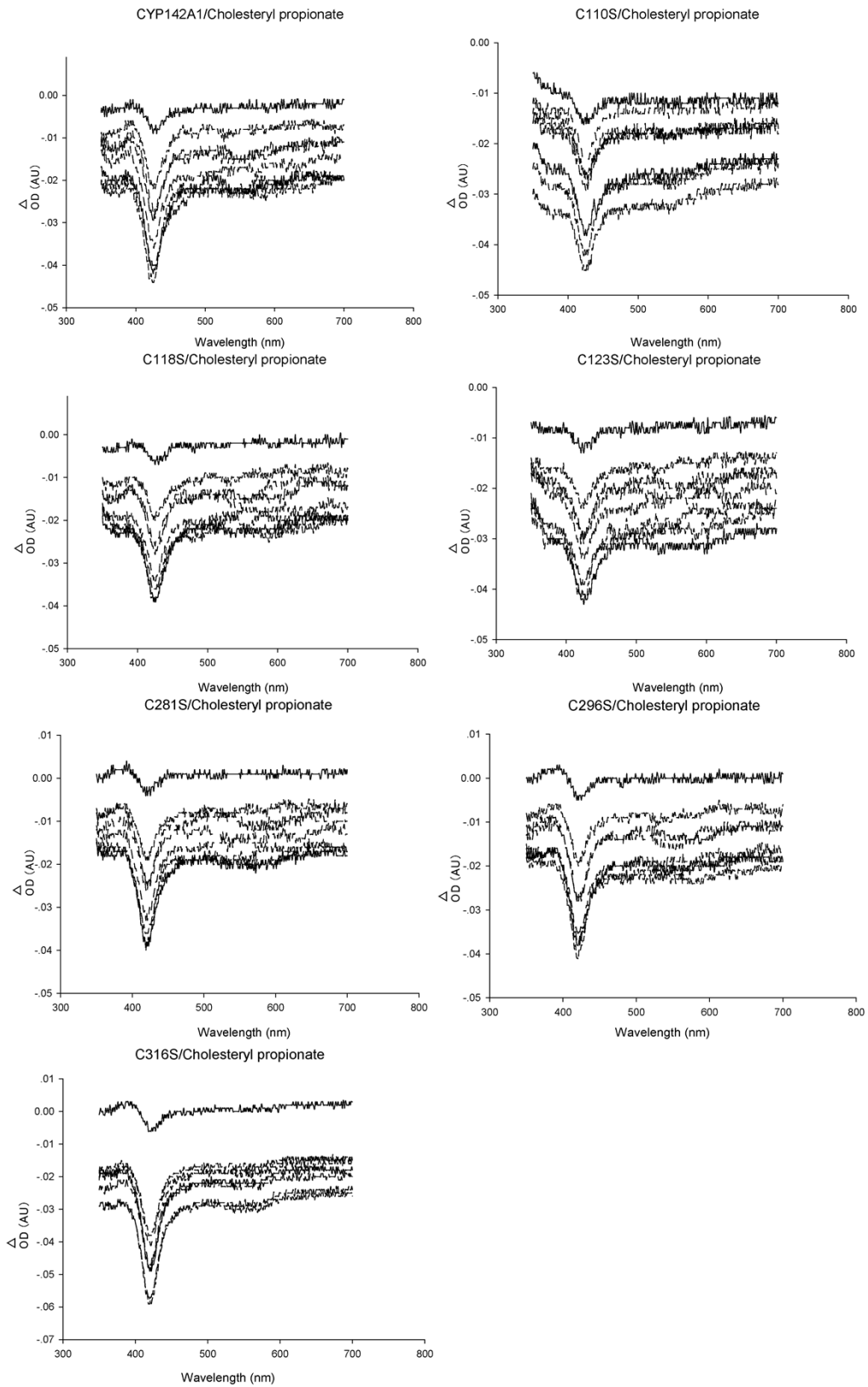
**Fig. S2.** UV-visible absorption features of substrate-free recombinant CYP142A1 and cysteine-to-serine mutants and of enzymes bound to econazole nitrate (100 nM).



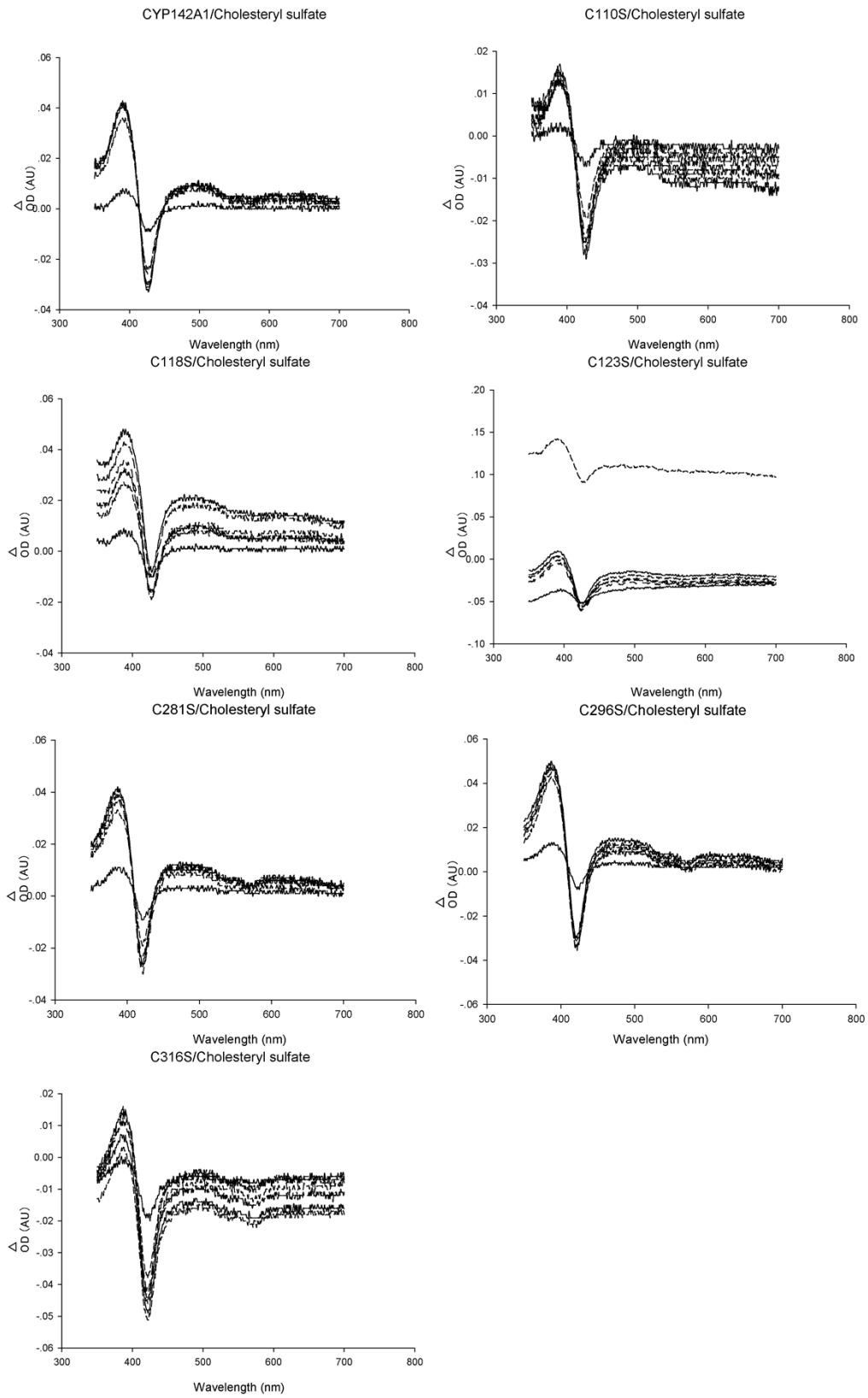
**Fig. S3.** Titration spectra of recombinant wild-type CYP142A1 and non-central cysteine mutants against cholest-4-en-3-one.



**Fig. S4.** Titration spectra of recombinant wild-type CYP142A1 and non-central cysteine mutants against cholesterol.

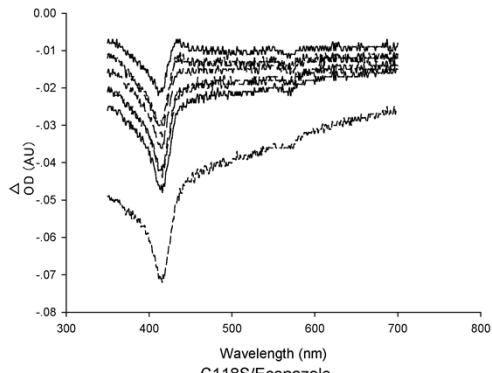


**Fig. S5.** Titration spectra of recombinant wild-type CYP142A1 and non-central cysteine mutants against cholesteryl propionate.

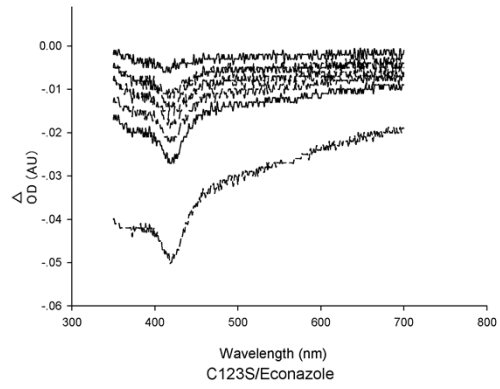


**Fig. S6.** Titration spectra of recombinant wild-type CYP142A1 and non-central cysteine mutants against cholesteryl sulfate.

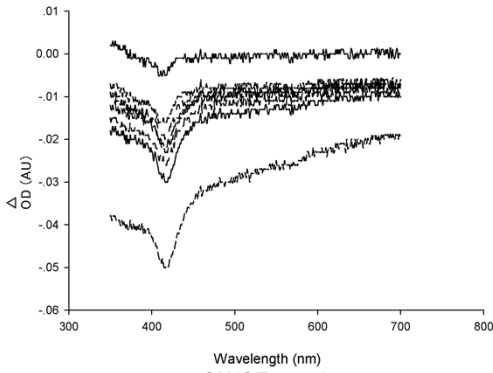
CYP142A1/Econazole



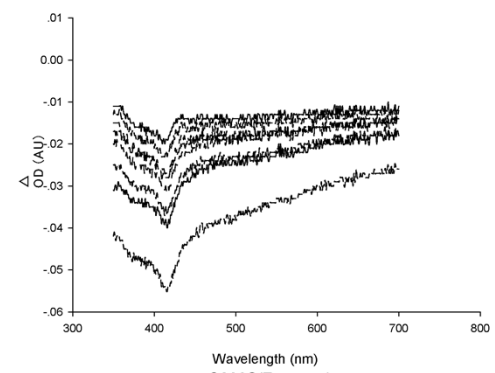
C110S/Econazole



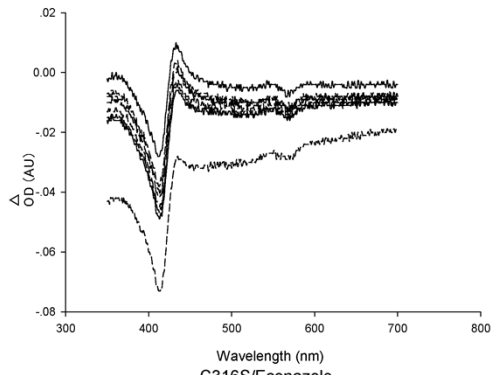
C118S/Econazole



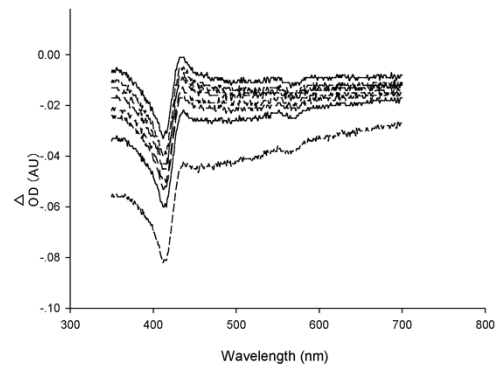
C123S/Econazole



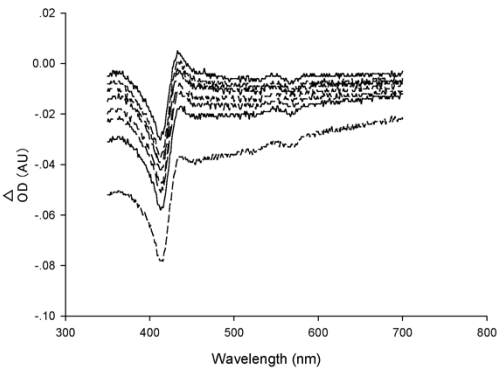
C281S/Econazole



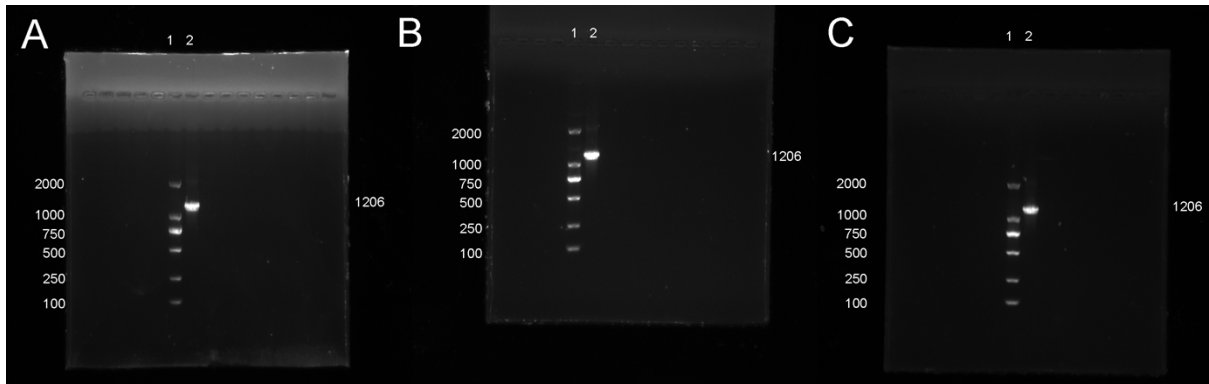
C296S/Econazole



C316S/Econazole



**Fig. S7.** Titration spectra of recombinant wild-type CYP142A1 and the non-central cysteine mutants against econazole nitrate.



**Fig. S8.** Whole gel electrophoresis of *cyp142* gene cloned by PCR for three replicates. Lane 1, DL2000 marker (100, 250, 500, 750, 1,000, and 2,000 bp); Lane 2 *cyp142*(1206bp). A, PCR for the first time; B, PCR for the second time; C, PCR for the third time.

**Table S1~2**

**Table S1.** Primers used in *cyp142* clone and site-mutations.

Name	Sequence(5'-3')
<i>cyp142</i> F	GGCATATGACTGAAGCTCCGGACGT
<i>cyp142</i> R	TCTCGAGGCCAGCGGGGCTC
pet30aF	GGTGTCTGGGGCGCAGCCATGACCCAGTCACGTAGCG
pet30aR	TCATGGCTGCGCCCCGACACCCGCCAACACCCGCTG
C110S F	GCCGCGCTGTCTGACACCCTGATCGACGCCGTGTGC
C110S R	CAGGGTGT <b>CAG</b> ACAGCGGCAATCGACGCCTCCTT
C118S F	GACGCCGTGT <b>CAG</b> AACCGGCGGAGTGTGACTTCGTG
C118S R	GCCGCGTTCT <b>GAC</b> ACGGCGTCGATCAGGGTGTCAACA
C123S F	CGCGGCGAGTCTGACTTCGTGCGGGACCTGGCCGC
C123S R	CACGAAGT <b>CAG</b> ACTCGCCGCGTTCGCACACGGCGT
C281S F	AAGAACATGTCTCGGGTGTGACCGGGATACCGAG



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C281S R CAACACCCG**AG**ACATGTTCTTTACCGGGGCGGTCCA

C296S F ACGGCGTT**GCT**GCCGGCGAGAAGATGATGCTGCTC

C296S R CTCGCCGGC**AG**ACAACGCCGTGCCGTGAAACTCGGT

C316S F GCGGTTTT**CT**CAGAACCGGAAAAGTTTGATGTTCCAG

C316S R TTCCGGTT**CT**GAGAAAACCGCCTCGTCCAAGTTCGC

C340T F ACGCATTT**CA**CTCTGGGCAATCAGCTGGCCCGGTTG

C340T R ATTGCC**CA**GTGAAATGCGTGCCGAAGCCAAACGC

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**Table S2.** Molecular weight of CYP142A1 and cysterine mutants measured by ESI-MS.

Name	Molecular weight
CYP142A1	45461.87
C110S	45441.45
C118S	45441.68
C123S	45446.04
C281S	45442.46
C296S	45440.73
C316S	45443.07

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