Supplemental material

Table S1 Primers used in this study.

Oligonucleotide	Primer sequence(5'-3')	Function of underlined base
sav7469-F	GGAATTCCATATGACAGAGCCCGGTACGT	*Nde*I
sav7469-R	CCGCTCGAGTTACCAGGTCACGGGGAG	*Xho*I
sav7469-pdx-pdr-F	CGCCATATGACAGAGCCCGGTACGTCCGTGT	*Nde*I
sav7469-pdx-pdr-R	CGGACTAGTTTACCAGGTCACGGGGAGTTCCAGC	*Spe*I
fprD-F	CATGCCATGGGCGTGGTCGACGCGGAT	*Nco*I
fprD-R	CCGCTCGAGGGGGGTCAGGGTCTCC	*Xho*I
fdxH-F	CATGCCATGGGCATGCACAACGACAGCAAC	*Nco*I
fdxH-R	CCGCTCGAGGCTTGCCGACCGC	*Xho*I
fprD-Duet-F	CGCGGATCCGCGTGGTCGACGCGGAT	*Bam*HI
fprD-Duet-R	CCCAAGCTTTCAGGGGGTCAGGGTCTCC	*Hind*III
fdxH-Duet-F	CGCCATATGGCATGCACAACGACAGCAAC	*Nde*I
fdxH-Duet-R	CCGCTCGAGTCAGCTTGCCGACCGC	*Xho*I
sav7469-RhFRED-F	GGATGCTGGAACTCCCCGTGACCTGGGTGCTGCACCGGCATCAACCG	Overlap sequence for gene fusion
sav7469-RhFRED-R	GATCTCAGTGGTGGTGGTGGTGGTGTCAGAGTCGCAGGGCCAG	Overlap sequence for gene fusion

Table S2 Strains and plasmids used in this study.

E. Coli strain or plasmid	Characteristic (s)	Source or reference
Strains		
DH5α	Cloning host	Novagen
BL21(DE3)	Expression host	Novagen
BL21(DE3)-condon plus RIL	Expression host	Novagen
CYP105D7-FdxH-FprD	Recombinant *E. coli* strain co- expression CYP105D7, FdxH and FprD	This work
Plasmids		
pET11-*sav7469-pdx-pdr*	Vector for coexpression of CYP105D7, Pdx and Pdr	Previous study
pET28b-*sav7469*	Vector for heterologous expression of CYP105D7	This work
pET28b-*sav7469-RhFRED*	Vector for coexpression of CYP105D7 and RhFRED	This work
pCDFDuet-1-*fdxH-fprD*	Vector for coexpression of FdxH and FprD	This work

Table S3 Primers of mutants used in this study.

Oligonucleotide	Primer sequence(5'-3')
R70A	AGCGGCTTTCCTCCGACGCGACGCTGCCCAGGTTCCC
	GGGAACCTGGGCAGCGTCGCGTCGGAGGAAAGCCGCT
R81A	TCCCCGCGACCACCGAGGCGTTCGAGGCCGTACGCAC
	GTGCGTACGGCCTCGAACGCCTCGGTGGTCGCGGGGA
R86A	AGCGGTTCGAGGCCGTAGCGACCCGCCGGGTGGCGCT
	AGCGCCACCCGGCGGGTCGCTACGGCCTCGAACCGCT
R88A	TCGAGGCCGTACGCACCGCGCGGGTGGCGCTGCTCGG
	CCGAGCAGCGCCACCCGCGCGGTGCGTACGGCCTCGA
R89A	AGGCCGTACGCACCCGCGCGGTGGCGCTGCTCGGTGT
	ACACCGAGCAGCGCCACCGCGCGGGTGCGTACGGCCT
R190A	CCGAGGTCCAGGACGCCGCGGCCCAACTGGACGACTA
	TAGTCGTCCAGTTGGGCCGCGGCGTCCTGGACCTCGG

Fig. S1 Maps for recombinant plasmids. (A) Co-expression system of CYP105D7 and variants with redox proteins, Pdx and Pdr. (B) Self-sufficient system of CYP105D7-RhFRED. (C) Co-expression system of CYP105D7 with its natural redox proteins, FdxH and FprD.

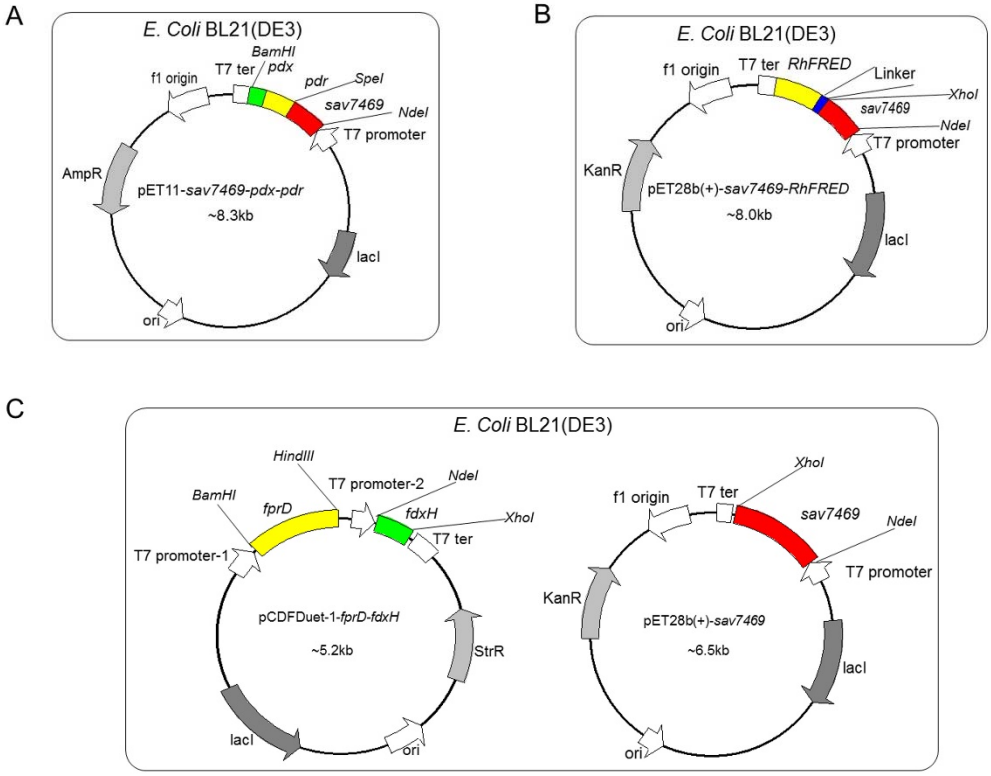


Fig. S2 Spectral analysis of CYP105D7 wild type and its mutants. Spectra are shown for the oxidized (solid line), dithionite-reduced (dashed line), and CO-bound (chain line) forms of the enzyme. Inset: reduced CO difference spectrum. Spectral features of N-terminal His6-tagged CYP105D7 wild type (A), R70A (B), R81A (C), R86A (D), R88A (E), R89A (F), R190A (G), R70A/R81A (H), R70A/R190A (I) were determined.

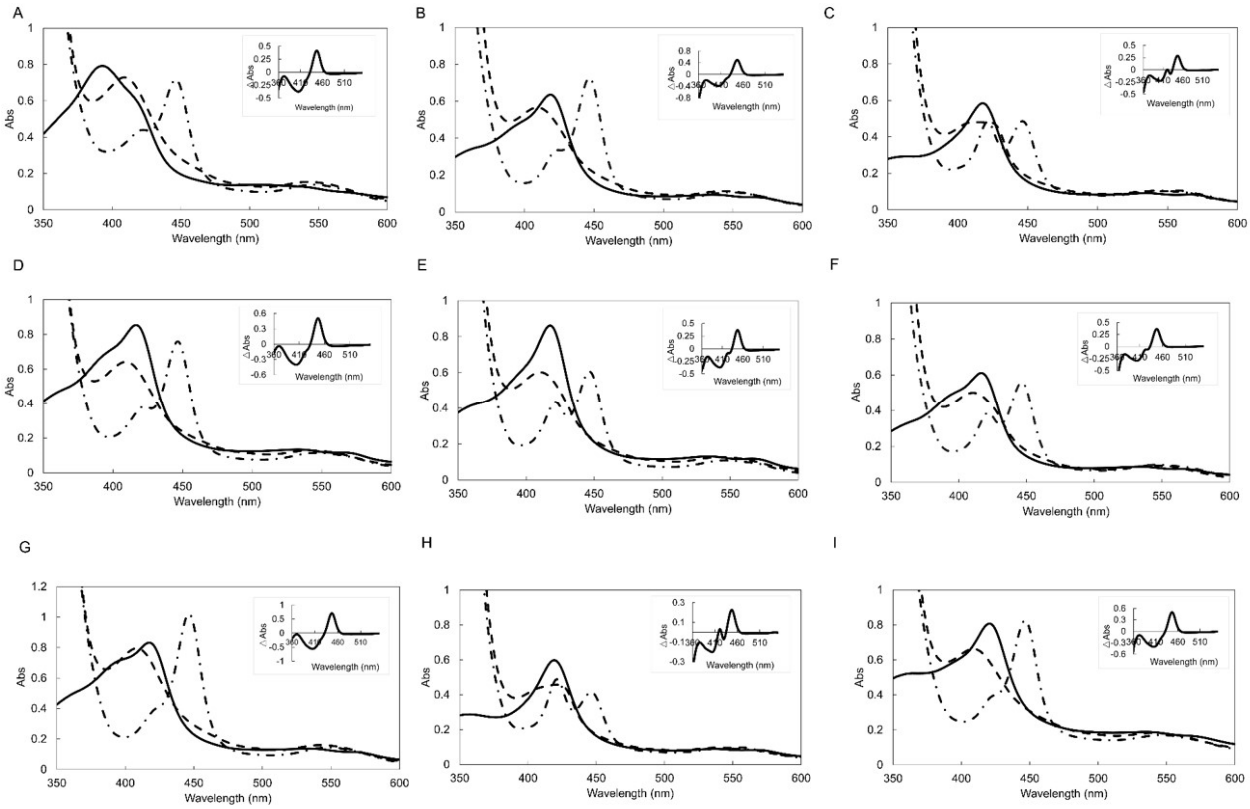
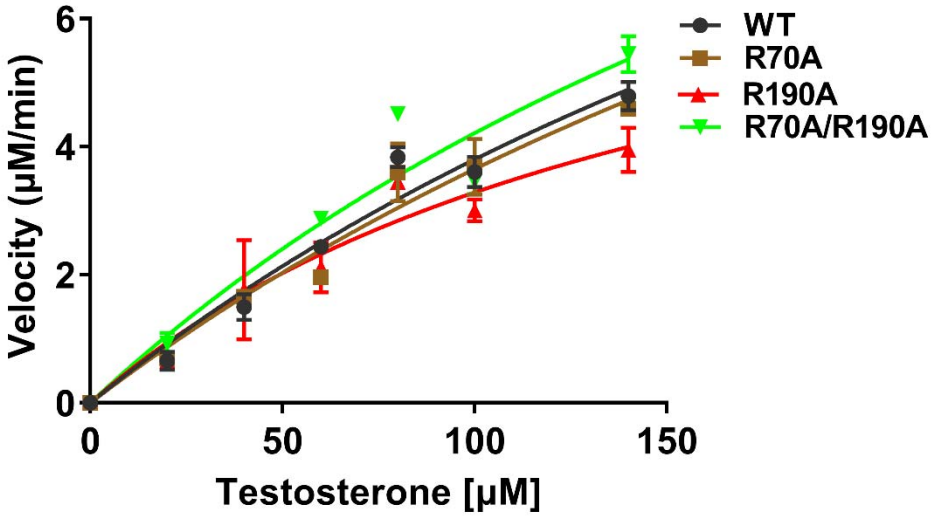


Fig. S3 Determination of steady-state kinetic parameters of CYP105D7 and its mutants (R70A, R190A and R70A/R190A) hydroxylation of testosterone. The vertical and horizontal axes show the initial velocity of the reaction and concentration of the substrate, respectively. The error bars in this figure represent three independent experiments with standard deviations.



Purification and identification of biotransformation of steroid compounds. The purified hydroxylated products were dissolved in Chloroform-*d* for NMR anaylsis. Products were identified by ^{1}H NMR and ^{13}C NMR spectroscopy on a Bruker 500 spectrometer or a JEOL 600 spectrometer, and HRESIMS data were acquired on an Agilent 6230 TOF LC/MS spectrometer.

2β-hydroxytestosterone

^{13}C NMR (125 MHz, Chloroform-*d*) δ 199.9, 175.3, 118.8, 81.7, 68.7, 50.6, 50.4, 43.5, 41.6, 39.6, 36.5, 36.0, 34.7, 33.1, 30.6, 23.5, 23.0, 22.7, 11.4.

^{1}H NMR (600 MHz, Chloroform-*d*) δ 5.81 (d, *J* = 1.3 Hz, 1H), 4.18 (dd, *J* = 13.9, 5.6 Hz, 1H), 3.66 (t, *J* = 8.6 Hz, 1H), 2.53 (dddd, *J* = 13.7, 12.3, 5.0, 1.5 Hz, 1H), 2.48 (dd, *J* = 13.7, 5.6 Hz, 1H), 2.25 (ddd, *J* = 12.3, 4.4, 2.6 Hz, 1H), 2.11 – 2.03 (m, 1H), 1.98 (ddq, *J* = 11.8, 4.7, 2.7 Hz, 1H), 1.88 (ddd, *J* = 12.8, 4.0, 2.8 Hz, 1H), 1.79 (dq, *J* = 13.5, 3.8 Hz, 1H), 1.70 (dtd, *J* = 11.9, 10.6, 4.1 Hz, 1H), 1.61 – 1.55 (m, 1H), 1.55 – 1.51 (m, 2H), 1.50 – 1.42 (m, 1H), 1.39 (ddd, *J* = 12.5, 10.3, 3.9 Hz, 1H), 1.32 – 1.29 (m, 1H), 1.19 (s, 3H), 1.13 (td, *J* = 12.9, 4.2 Hz, 1H), 1.05 – 0.95 (m, 2H), 0.79 (s, 3H); HRESIMS $[M+Na]^{+}$ calcd for $C_{19}H_{28}O_3$: 327.1936, found: 327.1931.

2β-hydroxyprogesterone

^{13}C NMR (125 MHz, Chloroform-d) δ 209.2, 199.7, 174.9, 118.8, 68.5, 63.4, 56.0, 49.9, 44.4, 41.4, 39.4, 38.6, 35.8, 34.9, 33.0, 31.5, 24.3, 22.9, 22.9, 22.8, 13.5.

^{1}H NMR (600 MHz, Chloroform-*d*) δ 5.82 (s, 1H), 4.19 (dd, J = 14.34, 5.38 Hz, 1H), 2.57 – 2.52 (m, 2H), 2.48 (dd, J = 13.8, 5.5 Hz, 1H), 2.29 – 2.24 (m, 1H), 2.23 – 2.17 (m, 1H), 2.13 (s, 3H), 2.11 – 2.08 (m, 1H), 2.02 – 1.97 (m, 1H), 1.85 – 1.81 (m, 1H), 1.70 – 1.65 (m, 3H), 1.58 – 1.52 (m, 2H), 1.49 – 1.45 (m, 2H), 1.25 (s, 1H), 1.24 – 1.19 (m, 1H), 1.18 (s, 3H), 1.11 – 1.04 (m, 1H), 0.66 (s, 3H); HRESIMS $[M+Na]^{+}$ calcd for $C_{21}H_{30}O_3$: 353.2093, found: 353.2089.

16β-hydroxyprogesterone

^{13}C NMR (125 MHz, Chloroform-d) δ 213.1, 199.5, 170.7, 124.1, 72.2, 66.0, 55.7, 54.1, 44.0, 39.0, 38.8, 38.7, 36.8, 35.8, 34.5, 33.9, 32.8, 29.8, 20.8, 17.4, 14.8.

^{1}H NMR (600 MHz, Chloroform-d) δ 5.74 (s, 1H), 4.61 – 4.56 (m, 1H), 2.47 – 2.39 (m, 2H), 2.38-2.35 (m, 1H), 2.35 – 2.33 (m, 1H), 2.32 – 2.29 (m, 1H), 2.29 – 2.26 (m, 1H), 2.22 (s, 3H), 2.09 – 2.00 (m, 2H), 1.87 (d, J = 18.2 Hz, 1H), 1.78 – 1.72 (m, 1H), 1.71 – 1.61 (m, 2H), 1.52 (dd, J = 13.0, 4.1 Hz, 1H), 1.44 – 1.36 (m, 1H), 1.25 (s, 3H), 1.15 – 1.09 (m, 1H), 1.21(s, 3H),1.12-1.09 (m, 1H), 1.06-0.99 (m, 1H), 0.90-0.85 (m, 1H); HRESIMS $[M+Na]^{+}$ calcd for $C_{21}H_{30}O_3$: 353.2093, found: 353.2088.

2β-hydroxy-4-Androstene-3,17-dione

^{13}C NMR (125 MHz, Chloroform-d) δ 219.9, 199.7, 174.2, 119.1, 68.3, 50.8, 50.0, 48.0, 41.5, 39.4, 35.8, 35.3, 33.8, 32.8, 31.3, 22.9, 22.2, 21.9, 14.0.

^{1}H NMR (600 MHz, Chloroform-d) δ 5.76 (s, 1H), 4.23 – 4.13 (m, 1H), 2.56 – 2.45 (m, 1H), 2.45 – 2.37 (m, 2H), 2.36 – 2.33 (m, 1H), 2.09 – 2.01 (m, 2H), 2.00 – 1.95 (m, 1H), 1.94 – 1.86 (m, 2H), 1.83 – 1.76 (m, 1H), 1.66 – 1.60 (m, 1H), 1.58 – 1.50 (m, 2H), 1.45 – 1.41 (m, 1H), 1.35 – 1.27 (m, 2H), 1.22 (s, 3H), 1.17 – 1.11 (m, 1H), 1.00 (s, 3H); HRESIMS $[M+Na]^{+}$ calcd for $C_{19}H_{26}O_3$: 325.1780, found: 325.1779.

16β-hydroxy-4-Androstene-3,17-dione

^{13}C NMR (125 MHz, Chloroform-d) δ 219.7, 199.2, 169.9, 124.1, 74.8, 53.8, 46.5, 45.0, 38.2, 35.4, 33.9, 33.6, 32.3, 31.3, 31.1, 30.7, 19.9, 17.1, 14.6.
^{1}H NMR (600 MHz, Chloroform-d) δ 5.83 (d, J = 1.2 Hz, 1H), 4.18 (dd, J = 13.9, 5.6 Hz, 1H), 2.60 – 2.54 (m, 1H), 2.50 – 2.45 (m, 2H), 2.34 – 2.29 (m, 1H), 2.12 (d, J = 9.1 Hz, 1H), 2.10 – 2.06 (m, 1H), 1.96 – 1.92 (m, 1H), 1.88 (t, J = 3.0 Hz, 1H), 1.87 – 1.82 (m, 1H), 1.61 – 1.57 (m, 1H), 1.56 (d, J = 3.6 Hz, 1H), 1.55 – 1.51 (m, 1H), 1.48 – 1.43 (m, 1H), 1.35 – 1.32 (m, 1H), 1.31 – 1.24 (m, 1H), 1.20 (s, 3H), 1.13 (qd, J = 13.0, 4.3 Hz, 1H), 0.97 – 0.92 (m, 1H), 0.92 (s, 3H); HRESIMS $[M+Na]^+$ calcd for $C_{19}H_{26}O_3$: 325.1780, found: 325.1781.