

**Supplementary Table 1.** Primer sets used to generate mutants

Primer	Sequences (5'-3')
p450LA_F	TCTAGA ctggatcatcatgctccgga
p450LA_R	aag ctt ggt acc gaa ttc ctctcgtttcgtatcgcg
p450RA_F	gaa ttc ggt acc aag ctt gagatccgatcgacggcagg
p450RA_R	TCTAGA ggcgtagcggtagatcttgctg
gRNA (5'-3')	GAGACATCTTTGAAGACAAacgctcaggtgaatgtggggccgagtttagagctagaatagcaagttaaaataaggctagtcggtatcaact-gaaaaagtggcaccgagtcggtgcttttttagcataacccttggggcctctaaacgggctctgaggggtttttggctgctcctcggtcggacgtgctctacgggcacct-taccgagccgtcggctgtgacacggacggatcgggcgaactggcggatgctgggagaagcgcgctgctgtacggcgcgaccgggtgctggagcccctc-gcgagcgggtgtaaacctctgtgaatggcctgttcgggtgctttttatatacggctgccagataaggctgcagcatctggcggctaccgctatgatcggggcgttctg-caattcttagtgcgagtatctgaaaggggatacggcgtgatacggacggtcatgtttAAGTCTTCTTTCACGTGGC

**Supplementary Table 2.** Bacterial strains and plasmids used in this study

Strains/Plasmids	Description
<i>E. coli</i>	
DH5a	F-, $\Phi$ 80dlacZ $\Delta$ M15, (lacZYA-argF)U169, <i>deoR</i> , <i>recA1</i> , <i>endA1</i> , <i>hsdR17</i> (rk-,mk+), <i>phoA</i> , <i>supE44</i> , $\lambda$ -, <i>thi-1</i> , <i>gyrA96</i> , <i>relA1</i>
ET12567/pUZ8002	<i>dam-13::Tn9 dcm-6 hsdM</i> , carries RK2 derivative with defective <i>oriT</i> for plasmid mobilization, Kan <sup>r</sup>
<i>Streptomyces globisporus</i> SP6C4	
SP6C4. <i>lanM</i>	SP6C4 <i>lanM</i> :: Apra <sup>r</sup>
SP6C4. <i>tsrD</i>	SP6C4 <i>tsrD</i> :: Apra <sup>r</sup>
SP6C4. <i>P450</i>	SP6C4 <i>P450</i> :: Apra <sup>r</sup>
SP6C4. <i>P450. lanM</i>	SP6C4 <i>P450 lanM</i> :: Apra <sup>r</sup>
SP6C4. <i>P450. tsrD</i>	SP6C4 <i>P450 tsrD</i> :: Apra <sup>r</sup>
Plasmid/Cosmid	
pCRISPomyces-2	Am <sup>r</sup> , <i>oriT</i> , <i>rep</i> <sup>pSG5(ts)</sup> , <i>ori</i> <sup>ColE1</sup> , <i>sSpcas9</i> , sgRNA cassette
pBHA	Am <sup>r</sup> , pUC <i>ori</i> , gRNA clone
pCRSP- <i>lanM</i>	pCRISPomyces-2 + $\Delta$ <i>lanM</i> editing template, <i>lanM</i> sgRNA – <i>sSpcas9</i>
pCRISP- <i>tsrD</i>	pCRISPomyces-2 + $\Delta$ <i>tsrD</i> editing template, <i>tsrD</i> sgRNA – <i>sSpcas9</i>
pCRISP- <i>P450</i>	pCRISPomyces-2 + $\Delta$ <i>P450</i> editing template, <i>P450</i> sgRNA – <i>sSpcas9</i>