

SUPPORTING INFORMATION

**Table 1S. List of primers used for site-directed mutagenesis.**

<b>Primer Name</b>	<b>Sequence (5' → 3')</b>
C79S Forward	CCGTGGTCGTGCTGTCTGGGACGG
C79S Reverse	CCGTCCCAGACAGCACGACCACGG
C152S Forward	GAGGAGGCCCGGTCTCTGGTGGAGG
C152S Reverse	CCTCCACCAGAGACCGGGCCTCCTC
E60C Forward	TCCTTCCTGCGGCTCCGATGTAAATACGGGGACGTGTTC
E60C Reverse	GAACACGTCCCCGTATTTACATCGGAGCCGCAGGAAGGA
H226C Forward	CCCGGGCTTCCTAAAGTGCTTTCCTGGCACGCAC
H226C Reverse	GTGCGTGCCAGGAAAGCACTTTAGGAAGCCCGGG
Y484C Forward	CGTGCCCCCGAGCTGCCAGATCCGCTTC
Y484C Reverse	GAAGCGGATCTGGCAGCTCGGGGGCACG
V267C Forward	CTAGGGATTTTCATCGACTGCTACCTGCTCCGCATGG
V267C Reverse	CCATGCGGAGCAGGTAGCAGTCGATGAAATCCCTAG
R133C Forward	CGGAGATTCTCCCTGGCCACCATGTGCGACTTCGGCATGGGGAAGCGGAGC
R133C Reverse	GCTCCGCTTCCCATGCCGAAGTCGCACATGGTGGCCAGGGAGAATCTCCG
L270C Forward	AGGGATTTTCATCGACGTCTACCTGTGCCGCATGGAAAAAGACAAGTCCGA
L270C Reverse	TCGGACTTGTCTTTTTCCATGCGGCACAGGTAGACGTCGATGAAATCCCT
L420C Forward	GCCACTTTCTAGATGCCAACGGGGCATGCAAGAGGAATGAAGGCTTTATGCCCTT
L420C Reverse	AAGGGCATAAAGCCTTCATTCCTCTTGCATGCCCCGTTGGCATCTAGAAAGTGGC

**Table 2S. CYP2B4 proximal residues, identified by Bridges et al. and this study, to be involved in CPR binding and their corresponding residues in P450BM3.**

<b>CYP2B4</b>	<b>P450BM3</b>
R443	L408
R422	-
R133	V121
K433	Q398
K139	L127
R126	N102
R122	K98
M137	V125
F135	I123
V267	H237
L270	L239

“-” = no equivalent residue identified in P450BM3

**Figure 1S. Kinetics for the reduction of the CYP2B4 V267C and L270C proximal variants under saturating and subsaturating amounts of CPR in the presence of NADPH and BNZ.**

CYP2B4, CPR and DLPC were reconstituted for 1 hr on ice and mixed with NADPH in a stopped-flow apparatus. After mixing, the final concentration of CYP2B4 was 0.75  $\mu\text{M}$ , NADPH was 50  $\mu\text{M}$  while BNZ was 1mM. Experiments were conducted as described under “Experimental Procedures” with a saturated solution of CO in both syringes. For convenience, all the absorbance data are offset to the same baseline.



