SUPPORTING INFORMATION

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

Primer	Sequence $(5' \rightarrow 3')$
Name	
C79S	CCGTGGTCGTGCTGTCTGGGACGG
Forward	
C79S	CCGTCCCAGACAGCACGACCACGG
Reverse	
C152S	GAGGAGGCCCGGTCTCTGGTGGAGG
Forward	
C152S	CCTCCACCAGAGACCGGGCCTCCTC
Reverse	
E60C	TCCTTCCTGCGGCTCCGATGTAAATACGGGGACGTGTTC
Forward	
E60C	GAACACGTCCCCGTATTTACATCGGAGCCGCAGGAAGGA
Reverse	
H226C	CCCGGGCTTCCTAAAGTGCTTTCCTGGCACGCAC
Forward	
H226C	GTGCGTGCCAGGAAAGCACTTTAGGAAGCCCGGG
Reverse	
Y484C	CGTGCCCCGAGCTGCCAGATCCGCTTC
Forward	
Y484C	GAAGCGGATCTGGCAGCTCGGGGGGCACG
Reverse	
V267C	CTAGGGATTTCATCGACTGCTACCTGCTCCGCATGG
Forward	
V267C	CCATGCGGAGCAGGTAGCAGTCGATGAAATCCCTAG
Reverse	
R133C	CGGAGATTCTCCCTGGCCACCATGTGCGACTTCGGCATGGGGAAGCGGAGC
Forward	
R133C	GCTCCGCTTCCCCATGCCGAAGTCGCACATGGTGGCCAGGGAGAATCTCCG
Reverse	AGGGATTTCATCGACGTCTACCTGTGCCGCATGGAAAAAGACAAGTCCGA
L270C Forward	AUUUATTICATCUACUTCTACCTUTUCCUCATUUAAAAAUACAAUTCCUA
L270C	TCGGACTTGTCTTTTTCCATGCGGCACAGGTAGACGTCGATGAAATCCCT
Reverse	
L420C	GCCACTTTCTAGATGCCAACGGGGCATGCAAGAGGAATGAAGGCTTTATGCCCTT
Forward	
L420C	AAGGGCATAAAGCCTTCATTCCTCTTGCATGCCCCGTTGGCATCTAGAAAGTGGC
Reverse	
ICC VUISC	

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.

CYP2B4	P450BM3
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 μM, NADPH was 50 μM 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.

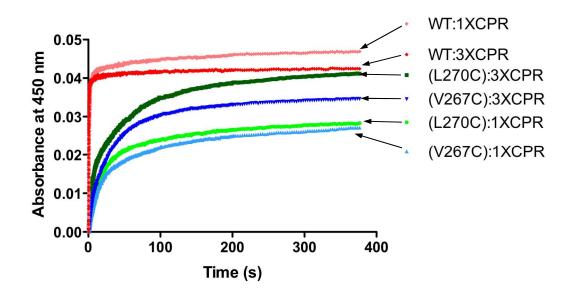


Figure 2S. EMBOSS Pairwise align algorithm output of CYP2B4 (first line in each

sequence pair) and P450BM3 (second line in each sequence pair) amino acid sequences.

EMBOSS 001	1 MEFSLLLLLAFLAGLLLLLFRGHPKAHGRLPPGPSPLPVLGNLLQMDRKG	₅₀ ← CYP2B4
- EMBOSS_001	:.: . :. 1LLNTDKP-	²⁶ ← P450BM3
- EMBOSS_001	51 LLRSFLRLREKYGDVFTVYLGSRPVVVLCGTDAIREALVD	90
EMBOSS_001	:::.::.:. :: . . :.:: .: : 27 -VQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKEACDESRFDKN	71
EMBOSS_001	91QAEAFSGRGKIAVVDPIFQGYGVIFANGERWRALRRFSL	129
EMBOSS_001	. :.:::: : 72 LSQALKFVRDFAGDGLFTSWTHEKNWKKAHNILLPSFSQQA	112
EMBOSS_001	130ATMRDFGMGKRSVEERIQEEARCLVEELRKSKGALLDNTLLFHSI	174
EMBOSS_001 113 MKGYHAMMVDIAVQLVQKWERLNADEHIEVPEDMTRL	149
EMBOSS_001	175 TSNIICSIVFGKRFDYKDPVFLRLLDLFFQSFSLISSFSSQVFELFSG .:. : :: :	222
EMBOSS_001	.:. . : : : 150 TLDTIGLCGFNYRFNSFYRDQPHPFITSMVRALDEAMNK	188
EMBOSS_001	223 FLKHFPGTHRQIYRNLQEINTFITQSVEKHRATLDPSNPRDFID 	266
EMBOSS_001	189 LQRANPDDPAYDENKRQFQEDIKVMNDLVDKIIADRKASGEQSDDLLT	236
EMBOSS_001	267 VYLLRMEKDKSDPSSEFHHQNLILTVLSLFFAGTETTSTLRYGFLLMLK	316
EMBOSS_001		283
EMBOSS_001	317 YPHVTERVQKEIEQVIGSHRP-PALDDRAKMPYTDAVIHEIQRLGDLIP-	364
EMBOSS_001	. .::: :::. :: :: 284 NPHVLQKAAEEAARVLVDPVPSYKQVKQLKYVGMVLNEALRLWPTAPA	331
EMBOSS_001	365 FGVPHTVTKDTQFRG-YVIPKNTEVFPVLSSALHDPR .:: .:. !! . .	400
EMBOSS_001	332 FSLYAKEDTVLGGEYPLEKGDELM-VLIPQLHRDKTIWGDDVEEFRPE	378
EMBOSS_001	401 YFKTPNTFNPGHFLDANGALKRNEGFMPFSLGKRVCLGEGIARTELFLFF . :. : . . .	450
EMBOSS_001	379 RFENPSAI-PQHAFKPFGNGQRACIGQQFALHEATLVL	415
EMBOSS_001	451 TTILQNFSIASPVPPE-DIDLTPRESGVGNVP-PSYQ :::::	485
EMBOSS_001	416 GMMLKHFDFEDHTNYELDIKETLTLKPEGFVVKAKSKKIPLGGIPSPSTE	465
EMBOSS_001	486IRFLAR	491
EMBOSS_001	466 QSAKKVRKKAENAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVA	515
EMBOSS_001	491	491
EMBOSS_001	516 TLDSHAGNLPREGAVLIVTASYNGHPPDNAKQFVDWLDQASADEVKGVRY	565
EMBOSS_001	491	491
EMBOSS_001	566 SVFGCGDKNWATTYQKVPAFIDETLAAKGAENIADRGEADASDDFEGTYE	615
EMBOSS_001	491	491
EMBOSS_001	$616\ {\tt EWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDSAADMPLAKMHGAFST}$	665
EMBOSS_001	491	491
EMBOSS 001	666 NVVASKELQQPGSARSTRHLEIELPKEASYQEGDHLGVIPRNYEGIVNRV	715