Supporting Information

The action of cytochrome b_5 on both CYP2E1 and CYP2C19 requires the anionic residues D58 and D65

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Table S1. Primers used for construction of his-tagged b_5 mutation cDNAs

Mutation	Primers	Sequence
K33G	b_5K33G-S[a]	5'-CTGCACCAC**GG**GGTGTACGATTTGAC-3'
	b_5K33G-AS[a]	5'-ATCGTACACC**CC**GTGGTGCAGGATC-3'
D36S	b_5D36G-S	5'-AAGGTGTACG**G**TTTGACCAAGTTTC-3'
	b_5D36G-AS	5'-CTTGGTCAAA**C**CGTACACCTTGTGG-3'
K39G	b_5K39G-S	5'-GATTTGACC**GG**GTTTCTGGAAGAGC-3'
	b_5K39G-AS	5'-TTCCAGAAAC**CC**GGTCAAATCGTAC-3'
E42G	b_5E42G-S	5'-AAGTTTCTGG**G**AGAGCATCCTGGTG-3'
	b_5E42G-AS	5'-AGGATGCTCT**C**CCAGAAACTTGGTC-3'
E48G	b_5E48G-S	5'-CCTGGTGG**A**G**G**AGAAGTTTTAAGGG-3'
	b_5E48G-AS	5'-TAAAACTTCT**C**C**T**CCACCAGGATGC-3'
E49G	b_5E49G-S	5'-GGTGGGGAAG**G**AGTTTTAAGGGAAC-3'
	b_5E49G-AS	5'-CCTTAAAACT**C**CTTCCCCACCAGGATG-3'
R52G	b_5R52G-S	5'-GAAGTTTTA**G**GGGAACAAGCTGGAGG-3'
	b_5R52G-AS	5'-AGCTTGTTCCC**C**TAAAACTTCTTCC-3'
E53G	b_5E53G-S	5'-GTTTTAAGGG**G**ACAAGCTGGAGGTG-3'
	b_5E53G-AS	5'-TCCAGCTTGT**C**CCCTTAAAACTTC-3'
Q54G	b_5Q54G-S	5'-TTAAGGGAA**GG**AGCTGGAGGTGACG-3'
	b_5Q54G-AS	5'-ACCTCCAGCT**CC**TTCCCTTAAAACT-3'
D58G	b_5D58G-S	5'-GCTGGAGGTG**G**CGCTACTGAGAAC-3'
	b_5D58G-AS	5'-CTCAGTAGCG**C**CACCTCCAGCTTG-3'
E61G	b_5E61G-S	5'-GACGCTACTG**G**GAACTTTGAGGATG-3'
	b_5E61G-AS	5'-CTCAAAGTTC**C**CAGTAGCGTCACCTC-3'
D65G	b_5D65G-S	5'-GAACTTTGAGG**G**TGTCGGGCACTC-3'
	b_5D65G-AS	5'-GTGCCCGACA**C**CCTCAAAGTTCTC-3'
R73G	b_5R73G-S	5'-GATGCC**G**GGGAAATGTCCAAAAC-3'
	b_5R73G-AS	5'-GGACATTTCCC**C**GGCATCTGTAGAG-3'
E47G	b_5E74G-S	5'-GCCAGGG**G**AATGTCCAAAACATTC-3'
	b_5E74G-AS	5'-TTTGGACATT**C**CCCTGGCATCTGTAG-3'
E48G+E49G	b_5E48G/E49G-S	5'-CCTGGTGG**A**G**G**AG**G**AGTTTTAAGGG-3'
	b_5E48G/E49G-AS	5'-TAAAACT**C**CT**C**C**T**CCACCAGGATGC-3'
D58G+D65G	b_5D58G+D65G-S	5′-G**G**CGCTACTGAGAACTTTGAGG**G**TGTC-3′
	b_5D58G/D65G-AS	5′-GACA**C**CCTCAAAGTTCTCAGTAGCG**C**C-3′

[a] The nucleotide changes that produce the indicated mutation(s) are in bold type. Nucleotide changes in both bold type and underlined were altered to introduce silent mutations, which avoid primers containing five consecutive G bases. “S” and “AS” denote sense and antisense primers, respectively.

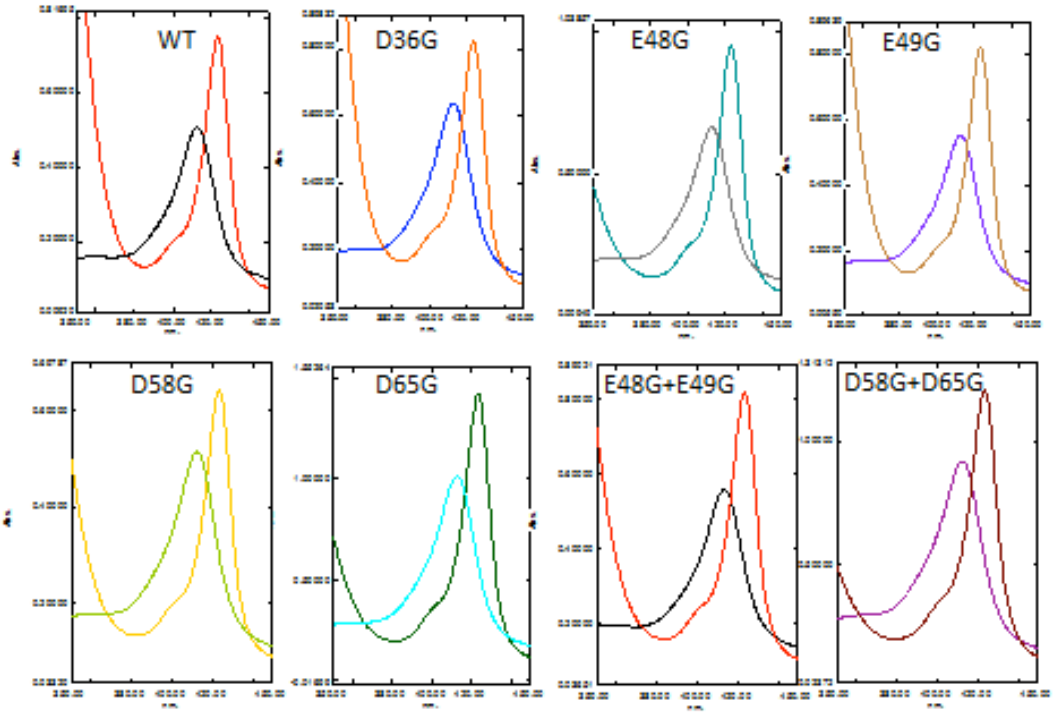

Figure S1. Typical absorbance spectra of the purified recombinant human cytochrome b_5 preparations. Characteristic spectra with maximum absorption wavelength for oxidized (412 nm, native) and reduced (424 nm, after adding solid sodium dithionite) forms are shown. Spectra were recorded with 4-8.5 μM cytochrome b_5 in 1 mL of 10 mM potassium phosphate buffer, pH 7.4; ordinates are 0.5-1.0 AU full scale.