Supplementary Material

Conformational change and human cytochrome *c* function: mutation of residue 41 modulates caspase activation and destabilizes Met-80 coordination

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Table S1. Comparison of ^{1}H chemical shifts of oxidized G41T cytochrome *c* with the reported shifts for non-native conformations of oxidized horse cytochrome *c*

Protein	Proposed Axial Ligation	δ (ppm)				Ref.
		8-CH_3	5-CH_3	3-CH_3	1-CH_3	
Horse cyt *c*	His/Lys	23.9	22.2	11.9	13.2	1
G41T	His/Lys	24.3	22.6			this work
Horse cyt *c*	His/His	25.7	18.6	13.5		1
G41T	His/His	25.2	18.9			this work

Reference

1. Russell, B S, Melenkivitz, R, and Bren, K L. (2000) Proc Natl Acad Sci U S A 97:8312-8317

Table S2. Chemical shifts (ppm) of selected protons in reduced human cytochrome *c* variants

	WT	G41S	G41T
L35			
HN	7.01	7.02	7.04
α-H	3.65	3.63	3.63
β-H	2.14	2.11	2.16
β-H	1.55	1.56	1.54
γ-H	1.31	1.32	1.27
δ-CH_3	0.87	0.99	0.97
δ-CH_3	0.69	0.69	0.70
I57			
HN	6.55	6.56	6.59
α-H	4.41	4.41	4.40
β-H	1.80	1.77	1.73
γ-H1	0.93	0.85	0.85
γ-H2	0.42	0.36	0.52
γ-CH_3	0.70	0.71	0.68
δ-CH_3	-0.70	-0.76	-0.68
W59			
ε3-H	7.61	7.60	7.60
ζ3-H	5.68	5.66	5.66
η2-H	6.68	6.67	6.68
ζ2-H	7.07	7.07	7.07
G60			
NH	7.95	7.93	7.90
α1-H	4.21	4.22	4.22
α2-H	3.92	3.90	4.01
L64			
HN	8.95	8.96	8.97
α-H	4.38	4.39	4.39
β-H	2.11	2.13	2.13
γ-H	1.28	1.29	1.29
δ-CH_3	0.75	0.80	0.74
δ-CH_3	0.50	0.52	0.53

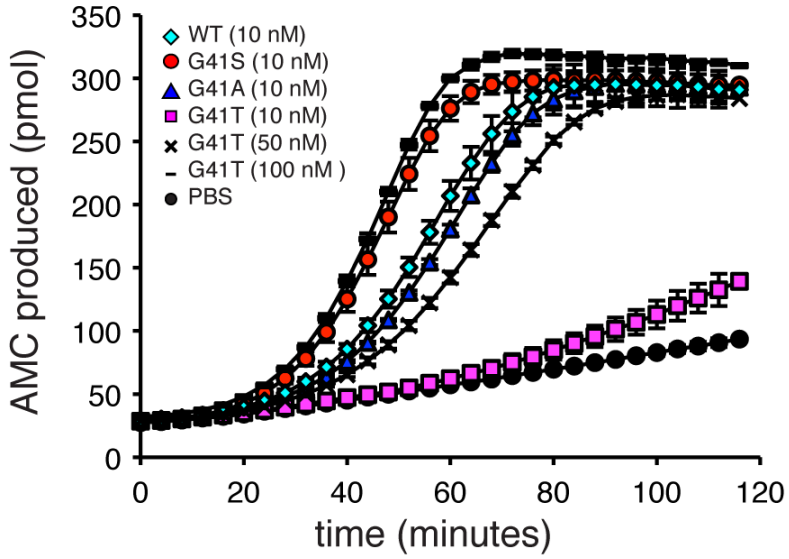

Fig. S1 Changes at residue 41 in cytochrome *c* modulate caspase activation. Cleavage of the caspase 3 substrate acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin was monitored at 37°C in U937 cytosol with the addition of 10 nM wild-type (*WT*), G41S, G41A or G41T, 50 nM G41T, 100 nM G41T cytochromes *c* or phosphate-buffered saline (*PBS*) at pH 7.25. All reactions contained 1 mM dATP and 5 mM dithiothreitol. n = 3 ± SD. This figure reproduces figure 2, showing error bars. *AMC* 7-amino-4-methylcoumarin

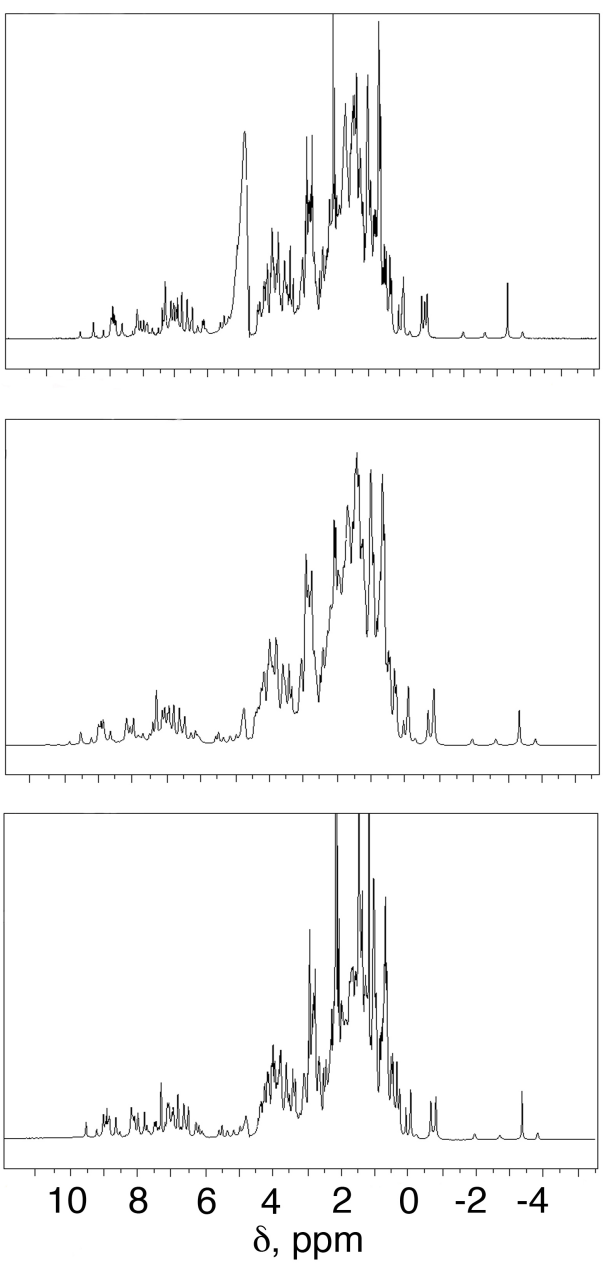

Fig. S2 One-dimensional NMR spectra of reduced wild-type (top), G41S (middle), and G41T (bottom) cytochromes *c*. Samples contain 1 mM protein in 45 mM sodium phosphate, 10% D_2O, pH 7.0, 25 ˚C. The characteristic upfield-shifted resonances indicating axial Met ligation between -2 and -4 ppm are visible in each spectrum.