Supplementary Table S2:

Redox potentials of WT NfsB and mutants.

Titrations were performed with 50-100 μM protein in 50 mM phosphate buffer, pH 7.5, 500 mM KCl, 10% glycerol, in the absence of redox mediators, with two aliquots of the same enzyme preparation. The data was fitted to either 2 single electron transfer steps (equation 1) or a concerted 2 electron transfer step (equation 2) using Sigmaplot14 with equal weighting of all points**.**

Enzyme	**Fit to 2 single electron transfers**						**Fit to concerted electron transfer**			
	E_1 (mV)	**P**	**E_2 (mV)**	**P**	**Rsqr**	**Standard error**	**Em**	**P**	**Rsqr**	**Standard error**
WT	-231 ± 2.8	<0.0001	-204 ± 2.8	<0.0001	0.9975	0.0219	-217.8 ± 0.7	<0.0001	0.9924	0.0376
T41L/N71S	-215 ± 1.6	<0.0001	-252 ± 1.6	<0.0001	0.9968	0.0225	-233 ± 1.6	<0.0001	0.9635	0.0774
T41Q/N71S/F124T	-245 ± 2.6	<0.0001	-215 ± 2.6	<0.0001	0.9980	0.0194	-230 ± 0.7	<0.0001	0.9932	0.0354