

Supplementary Information

Supplementary results

Aconitase activity was reduced by approximately 50% in the lysine and quadruple alanine substitution mutants, and cytochrome c oxidase activity was about 60% of that of wild-type (Supplementary Table 2)

Supplementary methods

The mutations were obtained by PCR amplification of a *PvuII* fragment of the *YFH1* gene inserted in the pGEM vector using oligonucleotides containing the required base substitutions. The mutated fragment was cleaved by *PvuII* and substituted for the wild-type fragment in the pFL39/YFH1 plasmid. The mutagenized DNA inserts were entirely sequenced.

Supplementary Table 1. Phenotype of published frataxin mutants in the acidic ridge.

Organism	Mutation	<i>In vitro</i> phenotype			<i>In vivo</i> phenotype				Reference
		Iron binding* (%)	Aggregation [#] (%)	Ferroxidase [‡]	Cell growth [§]	Oxidative damage	Isu1 interaction	Aconitase activity	
<i>E. coli</i> CyaY	E18A/E33S	19 (2)	11		Not tested	Not tested	Not tested	Not tested	Adinolfi et al, 2002
	E18K/E19K/D22K	18 (1)	0						
<i>S. cerevisiae</i> Yfh1	D86N/E90Q/E93Q	Low	0	Not tested	wt	Not tested	Not tested	Not tested	Aloria et al, 2004
	D86A/E90A/E93A		0		wt		Yes	wt	
	D78N/D82N/E89Q	Not tested	Not tested		wt		Not tested	Not tested	
	D79N/D86N/E90Q	Not tested	Not tested		wt		Not tested	Not tested	
	E71A/E75A/E76A	96	Not tested	wt	wt	Not tested	Not tested	Not tested	Gakh et al, 2006
	D79A/D82A	95	wt	Decreased	wt	Increased	wt		
	E93A	49	42	wt	wt	Increased	Not tested		
	E93A/D97A/E103A	23	36	wt	wt	Increased	wt		

*Bound iron in mutants is expressed as percent of iron bound to wild-type CyaY monomer, Yfh1 aggregates and CyaY aggregates(brackets)

[#]Aggregation is expressed as the percentage of aggregated frataxin in the total frataxin. Under the experimental conditions used by the authors percent of aggregation was 60% for wild-type CyaY and 68% for wild-type Yfh1.

[‡]Ferroxidase activity has not been detected in CyaY.

[§]wt, wild-type.

Supplementary Table 2. Aconitase and cytochrome c oxidase activities in *yfh1* mutants

Strain	Aconitase*	Cytochrome c oxidase [#]
Wild-type	625	6.6
D86A/E89A	620	6.9
D86K/E89K	321 [‡]	4.3
D86A/E89A/D101A/E103A	246 [‡]	4.8

The activities were measured in mitochondria of raffinose grown cells.

*Aconitase activity is measured by following conversion of citrate to isocitrate at 240 nm and is expressed in nmol isocitrate/min/mg protein.

[#]Cytochrome c oxidase activity is expressed in μ M cytochrome c reduced/min/mg protein.

[‡]These values are not considered as significantly different.

Supplementary Table 3. Phenotypes of *yfh1* mutants in this study

Mutation	CyaY equivalent	*Growth + FeSO ₄	*Growth + H ₂ O ₂	Aconitase activity	Isu1 interaction
Wild-type		+	+	High	Strong
D86K	E19	+	+/-	High	Not tested
E89K	D22	+/-	+/-	Slightly decreased	Decreased
D101A	D31	+	+/-	High	Not tested
D86A/E89A	E19, D22	+	+/-	High	Not tested
D86K/E89K	E19, D22	-	-	Low	Low
D101A/E103A	D31, E33	+	+/-	Not tested	Not tested
D101K/E103K	D31, E33	-	-	Low	Not tested
D86A/E89A/D101A/E103A	E19, D22, D31, E33	-	-	Low	Low

*Cellular growth is wild-type (+), slightly decreased (+/-), or strongly inhibited (-).

Supplementary data to Figure 4

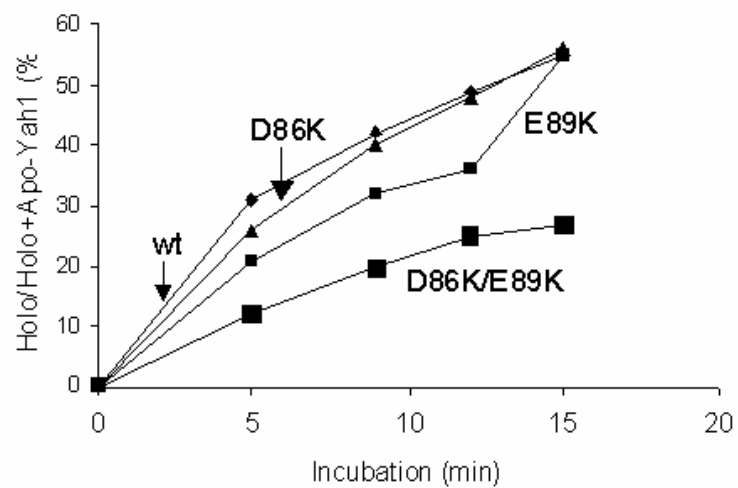
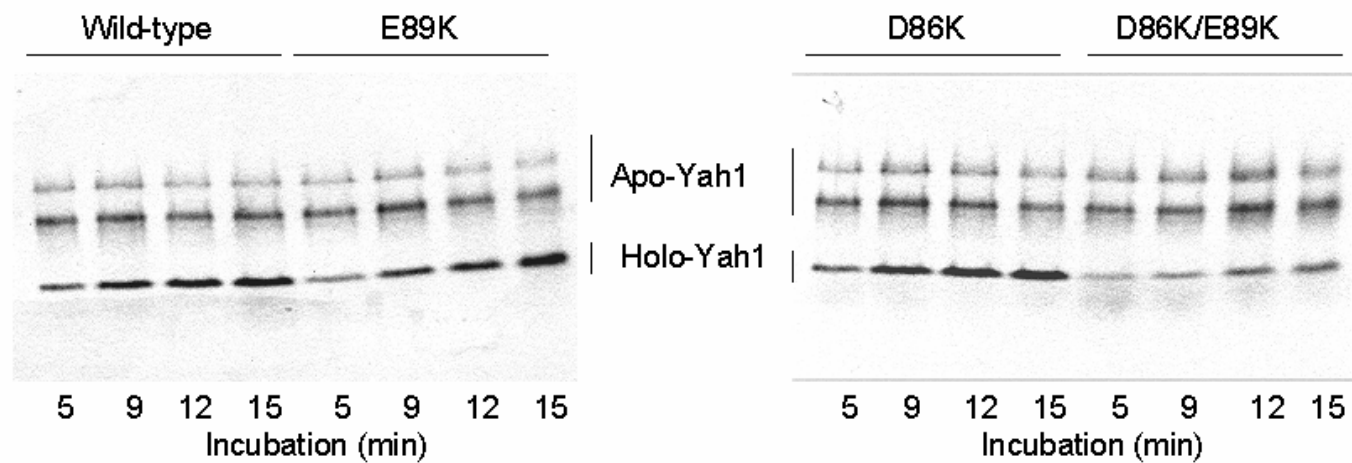


Figure 4

