SUPPLEMENTARY DATA

In order to address the question of *in vivo* oligomerization of Yfh1 we attempted to detect the presence of oligomeric wt and 86/90/93A mutant Yfh1 in mitochondria containing high levels of iron. Mitochondria were purified from *erv1*^{ts} cells expressing wt or *86/90/93A YFH1*. Gel filtration analysis of mitochondrial lysates indicated that the vast majority of wt and mutant Yfh1 eluted in a peak at a position corresponding to their monomeric form (supplementary figure 1). Minimal amounts of wt Yfh1 (less than 2% of total) was present in fraction 8. However, fraction 8 contains all the material above the separation range of the gel filtration column. No Yfh1 was detected in fractions adjacent, but within the column separation range. Also, no Yfh1 oligomers were found in the analogous fractions in the *in vitro* experiments (Figure 2 of main text). Therefore, although it is possible that this small amount of material contains functional oligomers, we favor the interpretation that the material in fraction 8 is either non-specific aggregates or Yfh1 present in mitochondrial fragments not cleared in the initial centrifugation.

SUPPLEMENTARY METHODS

Δ*yfh1erv1*^{ts} haploid strain (this study) containing wt or *86/90/93A YFH1* in pRS316 plasmid (Voisine *et al.,* 2000; this study) was grown at 25°C to an approximate O.D. of 0.2. The temperature was shifted to 37°C for 7 hours, cells were harvested and mitochondria were purified (Gambill *et al.,* 1993). 5 mg of mitochondria were lysed by incubation for 10 min on ice in 10 mM HEPES-KOH pH 7.4, 0.375% Triton X-100 and 1X Complete EDTA Free Protein Inhibitor Cocktail (Roche) buffer. Membrane debris was removed by centrifugation at 20,817 x g, 4°C for 15 min. Mitochondrial soluble lysate was fractionated on a Superdex-200 column

(Amersham Biosciences) equilibrated in 10 mM HEPES pH 7.4, 100 mM NaCl. Yfh1 was detected by immunoblot analysis of the column fractions.

SUPPLEMENTARY REFERENCES

Gambill, B.D., Voos, W., Kang, P.J., Miao, B., Langer, T., Craig, E.A. and Pfanner, N. (1993) A dual role for mitochondrial heat shock protein 70 in membrane translocation of preproteins. *J. Cell Biol.,* **123**, 109-117.

Voisine, C., Schilke, B., Ohlson, M., Beinert, H., Marszalek, J. and Craig, E.A. (2000) Role of the mitochondrial Hsp70s, Ssc1 and Ssq1, in the maturation of Yfh1. *Mol. Cell. Biol.,* **20**, 3677-3684.

SUPPLEMENTARY FIGURE LEGEND

Supplementary Fig. 1 Yfh1 is present as a monomer *in vivo*. Mitochondrial lysates purified from a Δ*yfh1erv1*^{ts} strain containing wt or *86/90/93A YFH1* in a plasmid were fractionated on a Superdex-200 column. The presence of wt or 86/90/93A Yfh1 in the indicated fractions was detected by immunoblot analysis. Elution positions of thyroglobulin (669 kDa), BSA (67 kDa), ovalbumin (43 kDa) and ribonuclease A (13.7 kDa) are indicated.

Supplementary figure 1