Supplementary Data

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SI Experimental Procedures

XAS Data Collection and Analysis

Duplicate mitochondria samples were loaded into Lucite cuvettes with 40 μm Kapton windows and rapidly frozen in liquid nitrogen. XAS data were collected at SSRL on beamline 9-3 (3 GeV, ~ 90 mA), using a Si(220) double-crystal monochromator with a Rh-coated mirror upstream of the monochromator for harmonic rejection. Spectra were measured using 10 eV increments in the pre-edge region (6880-7090 eV), 0.2 eV for the edge region (7090-7140 eV), 0.05 $Å^{-1}$ increments for the early EXAFS region (1.62 – 7.2 $Å^{-1}$), and 0.05 $Å^{-1}$ increments for the late EXAFS region (7.2 – 12 $Å^{-1}$) with integration times of 1 s in the pre-edge and edge regions and 1-25 s (k^3 weighted) in the EXAFS region for a total scan time of ~ 46 min. For all samples, energy calibration was performed using an iron foil as an internal standard, with the first inflection point of the foil spectrum defined as 7111.30 eV. XAS data were collected as fluorescence excitation spectra using a thirty-element Ge solid-state detector array, equipped with a 3 μm Mn filter and Soller slits focused on the Ge detector. Specifics of the data collection for each sample are detailed below. For all samples, each channel of each scan was examined independently for glitches and good channels were averaged to give the final spectrum. Average files for duplicate mitochondria samples were calculated using multiple scans.

XANES data were normalized by fitting data to the McMaster absorption coefficients (1, 2) below and above the edge using a single background polynomial and scale factor. The EXAFS background correction was performed by fitting a three-region cubic spline for all samples. The data were then converted to *k*-space using

$$k = \sqrt{\frac{2m_e\left(E - E_0\right)}{\hbar^2}},$$

using E_0 = 7130 eV. EXAFS data fitting was performed using k^3-weighted data over ranges described in the table below (Table S1).

EXAFS data can be described by the following equation

$$\chi(k) = \sum_s \frac{N_s S_s(k) A_s(k)}{kR_{as}^2} \exp\left(-2k^2\sigma_{as}^2\right) \exp\left(\frac{-2R_{as}}{\lambda}\right) \sin\left(2kR_{as} + \phi_{as}(k)\right),$$

where $\chi(k)$ is the fractional modulation in the absorption coefficient above the edge; N_s is the number of scatters at a distance R_{as}; $A_s(k)$ is the backscattering amplitude; σ_{as}^2 is the root-mean-square variation in R_{as}; $\phi_{as}(k)$ is the phase shift experienced by the photoelectron wave in passing through the potentials of the absorbing atom; λ is the mean free path of the photoelectron and backscattering atoms; and $S_s(k)$ is a scale factor specific to the absorber-scatterer pair (3). The sum is taken over all scattering interactions. The program Feff version 7.02 (4) was used to calculate amplitude and phase functions, $A_s(k)\exp(-2R_{as}/\lambda)$ and $\phi_{as}(k)$ for an iron-oxygen interaction at 2.0 Å, and an iron-iron interaction at 3.0 Å. Data were analyzed in k-space using the program EXAFSPAK (5). For all data, S_s was fixed at 0.9.

EXAFS Fitting

Table S2 summarizes the EXAFS data fitting results, and the k^3 weighted EXAFS spectra and fits are shown in Figure S1. Due to a high signal-to-noise ratio, the EXAFS data was transformed over a truncated k range (k_{max} = 8.3–9.7); thus, it was necessary to constrain several fitting parameters. Fourier transforms of the EXAFS data (not shown) show an intense first-shell peak, which can be fit to O/N scatters at 1.99 Å. Valence bond sums predict a Fe–O/N bond distance of 1.99 Å to be consistent with either tetracoordinate Fe(II) or hexacoordinate Fe(III) (6, 7). Therefore, coordination number values were constrained from 4–6 in the fits of the EXAFS data and Debye-Waller factors were constrained to CN × 10^{-3}. Only one freely variable parameter was used to fit the first shell of the EXAFS data, R, whose value of 1.99 Å varied only ± 0.02 Å across all fitting attempts. For those samples with a second shell scattering peak above the noise level (all samples except 2*grx5*Δ and 2*rho*+Fe), this peak was fit by freely varying all associated parameters (CN, R, and σ^2). The addition of second shell scattering invariably improved all associated data fitting attempts, though its inclusion did not dramatically alter the fitting results of the first shell.

SI references

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Supplementary Figure Legends

Figure S1: EXAFS spectra

A comparison of the k^3-weighted EXAFS spectra and the $n = 5$ fits to these data for all six mitochondria samples. EXAFS spectra are shown in black solid lines (——), while the fits are shown in red dotted lines (••••••••).

Figure S2: Quantitation of Figures 5C, 6A, and 6B

Normalization of *S. cerevisiae* Sod2p activity and measurement of Isu polypeptide levels. Sod2p activity was normalized for expression levels of the Sod2p polypeptide where values for WT = 100 (A and upper panels of B and C). Isu levels are reported as percent of WT values with WT = 100 (B and C, lower panels). Both bands corresponding to Sod2p activity were quantitated using ImageQuant TL software v2005. Sod2p and Isu polypeptide levels were quantitated using Odyssey quantitation software (version 1.2). Results from Figure 5C are represented in panel A. Panels B and C represent results from Figures 6A and 6B respectively.

Table S1: Data collection parameters

	1*grx5*Δ	2*grx5*Δ	1*mtm1*Δ	2*mtm1*Δ	1*rho*+Fe	2*rho*+Fe
number of scans	19	19	18	16	26	22
temperature (K)	17.5	8.2	15	8	8	11.5
total count rate (kHz)	7.1	4.5	10.9	9.5	8.0	9.1
Fe Kα count rate (Hz)	800	667	733	733	733	733
useful counts at k = 12 Å	3.0×10^5	2.5×10^5	2.7×10^5	2.7×10^5	2.7×10^5	2.7×10^5
# of averaged channels	14	16	16	16	15	15
k_{max} (Å^{-1}) for data fitting	9.7	9.7	9.5	9.5	9.7	8.2

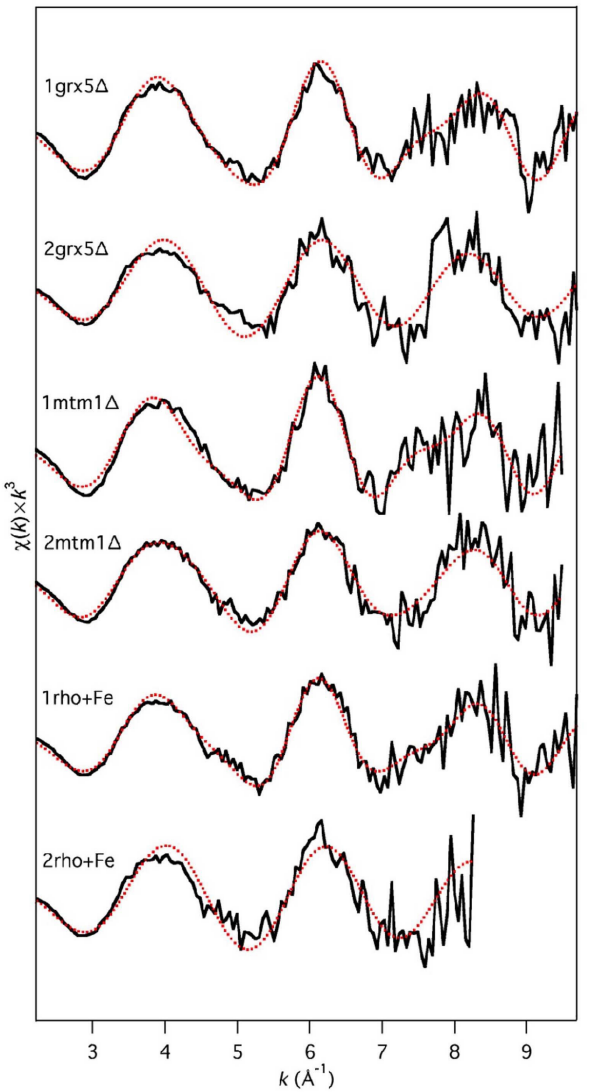
Table S2: EXAFS fitting results for mitochondria samples

		First Shell: O/N			Second Shell: Fe				**E_0 = 7130 eV**
	N_{idp} [a]	CN [b]	R (Å)	$\sigma^2 \times 10^3$ [b]	CN	R (Å)	$\sigma^2 \times 10^3$	$\xi' \times 10^2$ [c]	ΔE_0 (eV) [b]
1*grx5*Δ	10.5	4	1.99	4.0	1.3	3.02	6.5	3.2	-11.00
		5	1.99	5.0	1.4	3.02	6.9	3.5	-11.00
		6	1.99	6.0	1.4	3.02	6.9	4.4	-11.00
2grx5 Δ	5.7	4	1.98	4.0				10	-11.00
		5	1.98	5.0				10	-11.00
		6	1.98	6.0				11	-11.00
1*mtm1* Δ	10.2	4	2.00	4.0	2.9	3.03	11.3	7.3	-11.00
		5	2.00	5.0	3.0	3.03	11.4	7.2	-11.00
		6	2.00	6.0	3.0	3.03	11.5	7.6	-11.00
2*mtm1* Δ	10.2	4	1.98	4.0	2.3	3.01	6.0	6.5	-11.00
		5	1.98	5.0	2.4	3.01	6.0	5.9	-11.00
		6	1.98	6.0	0.9	3.05	4.5	6.2	-11.00
1*rho*+Fe	10.5	4	1.99	4.0	2.5	3.04	11.6	5.9	-11.00
		5	1.99	5.0	2.8	3.04	12.6	5.6	-11.00
		6	1.99	6.0	2.8	3.04	12.8	6.0	-11.00
2*rho*+Fe	3.9	4	1.97	4.0				18	-11.00
		5	1.97	5.0				18	-11.00
		6	1.97	6.0				20	-11.00

[a] number of independent data points: $N_{idp} = (2\ \Delta k\ \Delta R)/\pi$.
[b] CN and σ^2 factors were constrained in fits of the first shell. ΔE_0 was constrained for all fits.
[c] ξ'= mean-square deviation between divided by $(N_{idp} - N_{var})$, where N_{var} is the number of variables in the fit.

Supplementary Figure S1

EXAFS spectra



Supplementary Figure S2

Quantitation of Figures 5C, 6A and 6B