Electronic Supplementary Information for Park *et al*.

Figure S1. 5 K, 0.05 T Mössbauer spectrum of mitochondria isolated from anaerobically grown WT cells. The red line is a composite simulation, assuming terms and parameters given in Table S1. The blue line is a simulation of the NHHS Fe^{II} component.

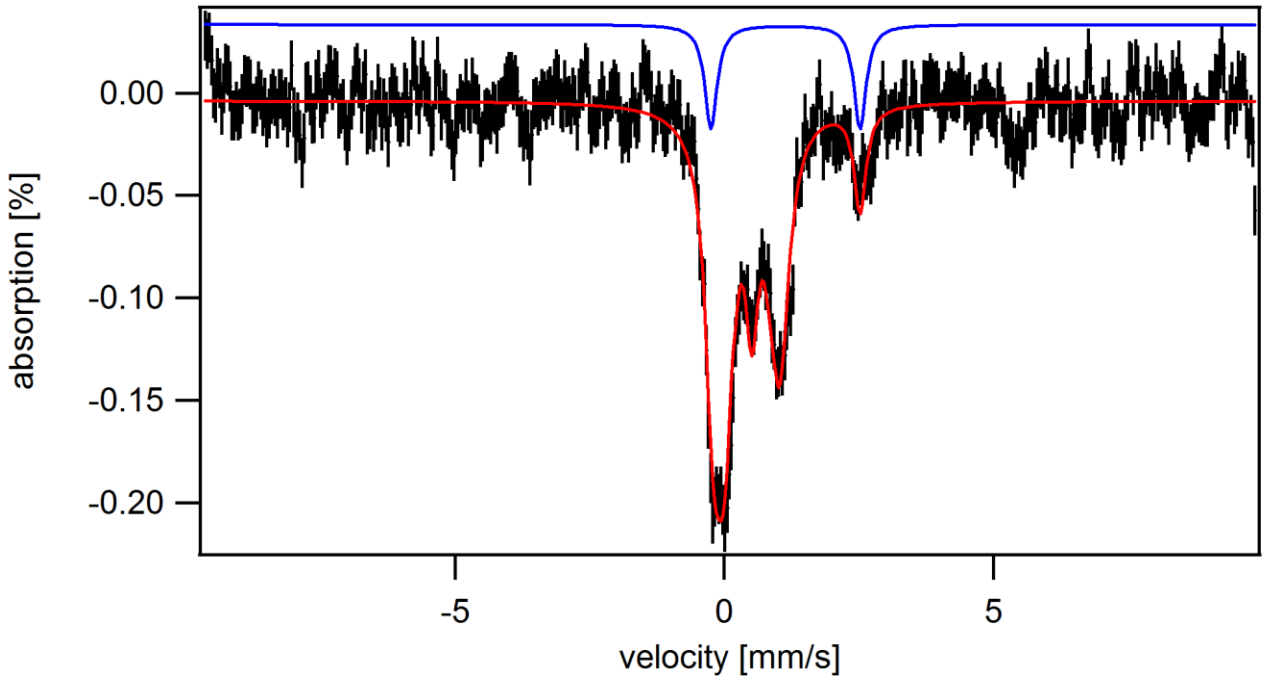

Figure S2. Mn and Fe traces from Experiment 1 of the first group of LC-ICP-MS studies. The inset shows SOD2 activity gels, Western blots against Sod2p, and SOD2 unit activities. (a) W303; (b) Δ*mtm1*.

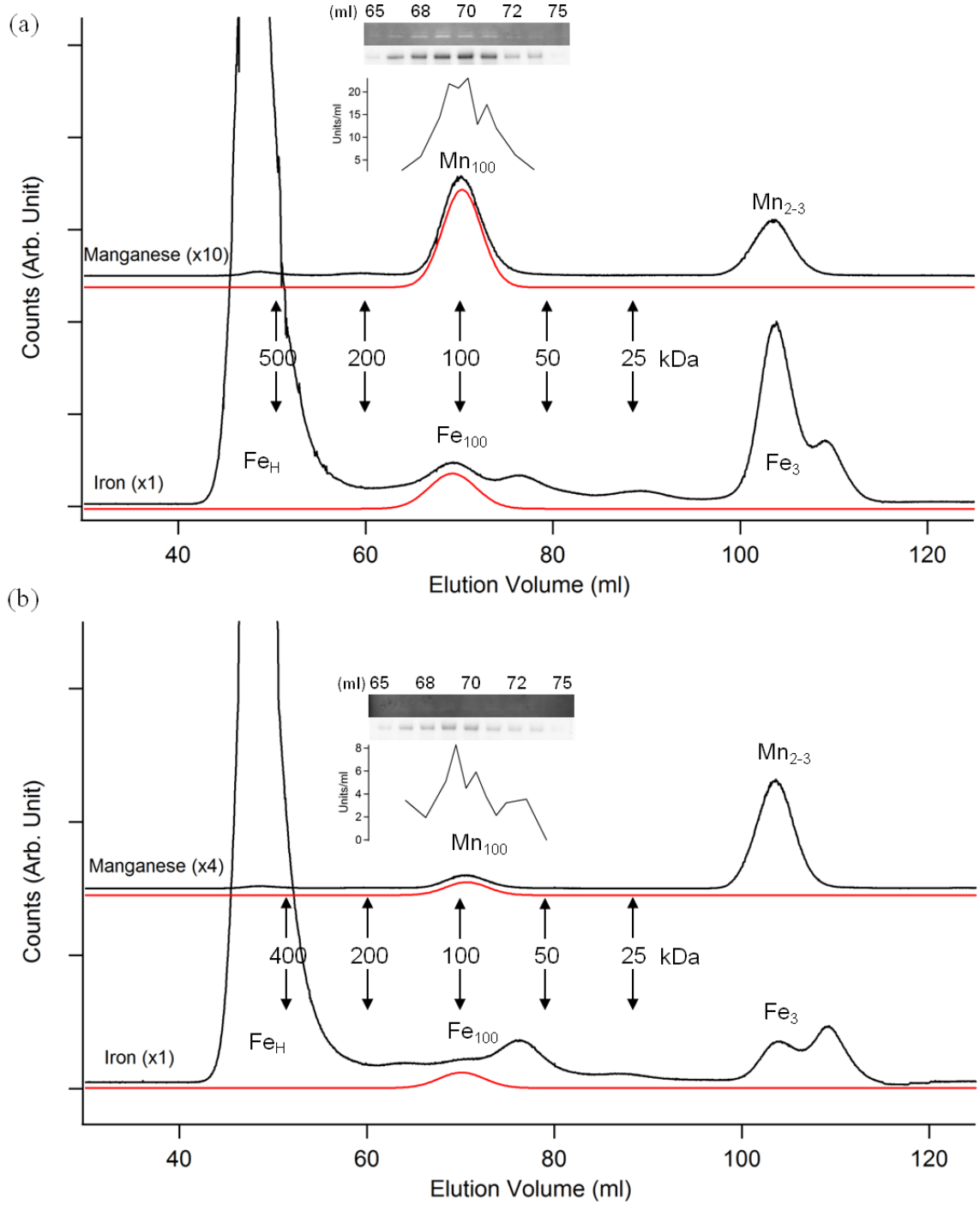

Figure S3. Mn and Fe traces from Experiment 3 (200 µM $MnCl_2$ supplemented in media) of the first group of LC-ICP-MS studies. The inset shows SOD2 activity gels, Western blots against Sod2p, and SOD2 unit activities determined spectrophotometrically. (a) W303; (b) Δ*mtm1*.

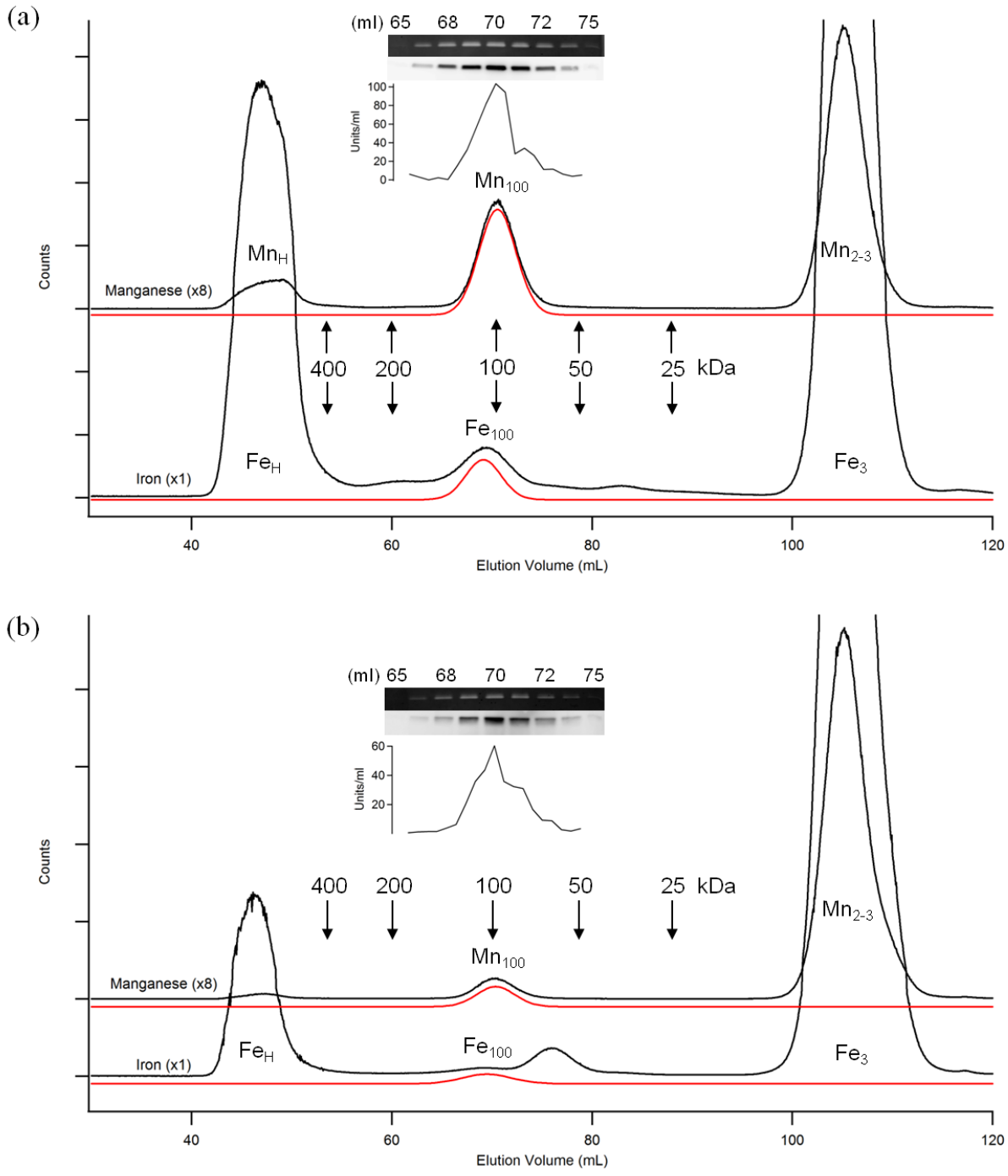

Figure S4. SOD activity and Western blot analysis of various samples. Lanes 1 and 2, reading from left to right, isolated mitochondria from Experiments 1 of the first group of LC-ICP-MS studies, before sonication. Lanes 3 – 6, pooled Sod2p-containing fractions from the Superdex 200 column in Experiments 1 and 2 (corresponding to Figures S2 and 6). Lanes 7 and 8 isolated mitochondria from Experiment 3 of the first group of LC-ICP-MS studies, before sonication. Lanes 9 – 10, pooled Sod2p-containing fractions from the Superdex 200 column in Experiment 3 (corresponding to Figure S3). Specific activities relative to wild type are shown for each lane. Top panels with dark background are images of native PAGE gels stained for NBT SOD2 activity assay. 2 – 3 bright bands represented MnSod2p tetramers migrating with different positions. Bottom panels with white background are images of Western blot membranes obtained from denaturing SDS-PAGE gels. Generally a single band was observed due to MnSod2p monomer.

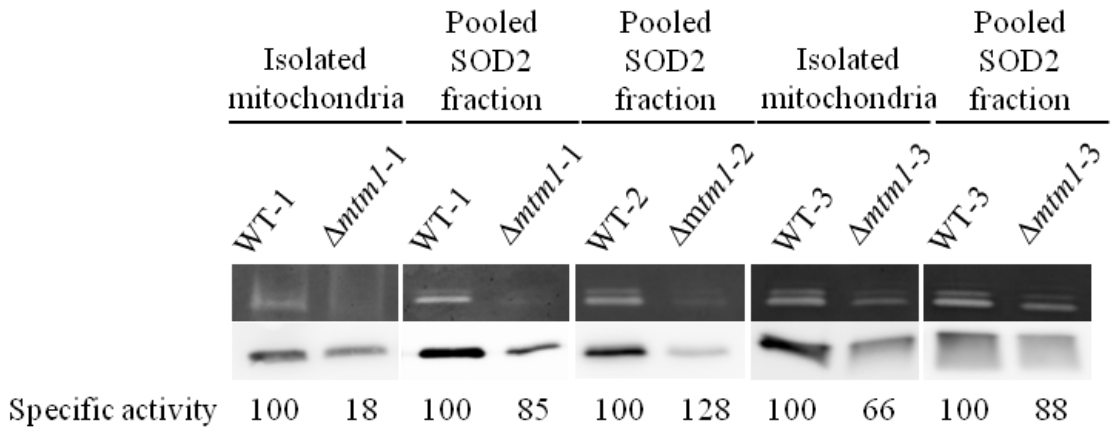

Table S1. Isomer shift (δ, mm/s), quadrupole splitting (ΔE_Q, mm/s), line width (Γ, mm/s), and percentage of Fe species as determined from Mössbauer spectra. Red-colored parameters were fixed when simulated.

Spectrum	Central doublet	NHHD Fe^{2+}	HS Fe^{2+} heme	$[Fe_2S_2]^{2+}$	Ferric nanoparticle	HS Fe^{3+} and unassigned species
W303/O_2 mitochondria (Fig. 1A)	0.44/1.23/0.47 59%	1.40/3.17/0.79 15%	0.96/2.26/0.27 5%	0.25/0.46/0.25 8%	0.52/0.63/0.26 6%	7%
Δ*mtm1*/O_2 mitochondria (Fig. 1B)	0.45/1.15/0.28 5%	1.44/2.89/0.23 2%	-	-	0.52/0.67/0.58 93%	-
Δ*mtm1*/O_2 mitochondria, dithionite-reduced (Fig. 1C)	0.45/1.15/0.48 5%	1.32/3.47/0.66 94%	-	-	0.52/0.67/0.35 1%	-
Δ*mtm1*/Ar mitochondria (Fig. 1E)	0.45/1.15/0.48 50%	1.19/2.67/0.76 26%	-	0.27/0.49/0.25 14%	0.52/0.67/0.34 4%	5%
W303/O_2 whole cell (Fig. 5A)	0.45/1.15/0.46 13%	1.30/3.10/0.70 2%	0.83/2.26/0.94 7%	0.27/0.49/0.50 3%	-	75%
Δ*mtm1*/O_2 whole cell (Fig. 5B)	1 ~ 2%	1.36/2.99/0.52 11%	-	-	0.53/0.58/0.58 69%	19%
W303/Ar whole cell (Fig. 5C)	0.45/1.15/0.83 9%	1.29/2.89/0.58 16%	0.83/2.58/0.28 3%	0.27/0.49/0.79 10%	0.52/0.63/0.47 8%	55%
Δ*mtm1*/Ar whole cell (Fig. 5D)	0.45/1.15/0.43 5%	1.32/2.98/0.60 20%	0.83/2.32/0.30 2%	0.27/0.49/0.46 8%	0.52/0.58/0.26 5%	61%
W303/Ar mitochondria (Fig. S1)	0.45/1.15/0.43 45%	1.14/2.77/0.27 12%	-	0.27/0.49/0.30 21%	0.52/0.63/0.19 2%	20%

Table S2. Spin quantification of EPR spectra of W303 and Δ*mtm1* mitochondria.

Spectrum	Succinate dehydrogenase g = 2.02, 1.93, 1.91	Rieske protein g = 2.02, 1.89, 1.75	Radical g = 2.00	Aconitase $[Fe_3S_4]^+$ g = 2.02, 2.01, 2.00	Non-heme HS Fe^{III} g = 4.3	HS heme Fe^{III} g = 6.4,5.3, 6.0
W303/O_2	0.9 µM	4 µM	0.2 µM	N/D[a]	2 µM	< 0.5 µM
Δ*mtm1*/O_2	0.2 µM	1 µM	N/D	N/D	2 µM	0.9 µM
W303/Ar	0.3 µM	0.3 µM	N/D	0.5 µM	2 µM	N/D
Δ*mtm1*/Ar	0.3 µM	0.2 µM	N/D	0.2 µM	13 µM	N/D

[a] not determined.

Table S3. Preparation of soluble mitochondrial fractions for LC-ICP-MS analysis. Percentages of metal, protein, and SOD2 activity in soluble mitochondrial fractions were measured relative to total mitochondrial contents. *Averages from two SEC experiments were used in the text to estimate: a) soluble Mn percentages ([44% + 32%]/2 = 38%, p. 10; [48% + 42%]/2 = 45%, p. 11); b) soluble Fe percentages ([24% + 25%]/2 = 25%, p. 11; [7% + 7%]/2 = 7%, p. 12); and c) soluble SOD2 activities ([23% + 32%]/2 = 28%, p. 10; [11% + 41%]/2 = 26%, p. 11).

Sample	Mn	Fe	Protein	SOD2 activity
W303-1 1st SEC Figure S2a	44%*	24%*	39%	23%*
W303-2 2nd SEC Figure 6a	32%*	25%*	18%	32%*
W303-3 3rd SEC (200 µM Mn) Figure S3a	15%	14%	7%	10%
W303-4 LMM (detergent-treated, passed 10 kDa cut-off) Figure 6c	17%	16%	-	-
W303-5 1st MonoQ	19%	11%	30%	56%

Figure 8				
W303-6 2nd MonoQ Figure 9a	26%	13%	29%	49%
Δ*mtm1*-1 1st SEC Figure S2b	48%*	7%*	39%	11%*
Δ*mtm1*-2 2nd SEC Figure 6b	42%*	7%*	30%	41%*
Δ*mtm1*-3 3rd SEC (200 µM Mn) Figure S3b	28%	8%	15%	31%
Δ*mtm1*-4 LMM (detergent-treated, passed 10 kDa cut-off) Figure 6c	16%	3%	-	-
Δ*mtm1*-5 1st MonoQ Figure 8	29%	20%	34%	43%
Δ*mtm1*-3 2nd MonoQ Figure 9b	38%	8%	40%	24%

Table S4. Peak analysis of HMM chromatograms. Percentage of each peak was calculated by fitting chromatograms. The numbers before the semicolons are the average of Experiments 1 and 2 (two numbers are given when the individual values were significantly different). The numbers after the semicolon refer to Experiment 3 in which cells were grown in 200 μM Mn in the medium.

Peak	Approximate Molecular mass (kDa)	% of Chromatogram intensity	
		W303	Δ*mtm1*
Mn_H	> 600	3 ; 9	2 ; 1
Mn_{200}	200	1 ; 0	0.4 ; 0
Mn_{100}	100	64 ; 23	9 ; 5
$Mn_{2\text{-}3}$	< 6 (2-3 using LMM column)	32 ; 68	89 ; 94
Fe_H	> 600	(67,17) ; 32	(79,15) ; 12
Fe_{200}	200	3 ; 2	2 ; 0
Fe_{100}	100	4 ; 3	2 ; 0.4
Fe_{60}	60	2 ; 1	4 ; 2
Fe_{35}	35	0 ; 1	0.2 ; 0.3
Fe_{20}	20	1 ; 0	1 ; 0
Fe_3	< 6 (3 using LMM column)	(19,74) ; 62	(8,79) ; 84

Table S5. **Concentrations of LMM Fe and Mn species in mitochondria.** Fractional intensities of chromatographic peaks were determined by dividing individual peak intensities by the sum of the intensities in the chromatogram. Concentrations of the LMM species within intact mitochondria were estimated by multiplying these fractions by the number of moles of metal in the solutions applied to the column, and dividing by the estimated mitochondrial volume (determined by dividing the total mg of proteins in the mitochondrial suspension by previously determined protein concentrations (mg/mL) of isolated mitochondria given in Table 1).

	Mitochondrial Mn and Fe concentrations corresponding to LMM chromatogram intensities (μM)	
LMM Peak	WT	Δ*mtm1*
Mn_{2-3}	0.2	0.8
Fe_3	9	20