Electronic Supplemental Information for Nguyen et al. 2020

Chromatographic detection of low-molecular-mass metal complexes in the cytosol of *Saccharomyces cerevisiae*

Table S1. Yeast strains used in this study.

Strain	Genotype	Source
W303 wild type	*MAT α ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1*	1
*fet3*Δ	*MAT α ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 Δfet3::KanMX*	Current study
*mrs3*Δ *mrs4*Δ	*MAT α ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 Δmrs3::HIS3 Δmrs4::LEU2*	2
*ccc1*Δ	*MAT α ade2 can1 his3 leu2 trp1 ura3 Δccc1::HIS3*	2
*vma2*Δ	*MAT α ade2-1 his3-1,14 leu2-3,112 trp 1-1 ura3-1 Δvma2:: HIS3*	Current study
*cox17*Δ	*MAT a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 cox17::HphMX4*	3
*cup1*Δ	*MAT α trp1-1 gall met13 can1 cup1S ura3-50 Ade$^-$ His$^-$ Δcup1::URA3*	4
CUP1R	*MAT α trp1 gal1Δ his3-532 ade1-100 leu2-3 leu2-112 CUP1R*	4

References:

1. Moore, MJ., Wofford, JD., Dancis, A., Lindahl, PA. Recovery of *mrs3Δmrs4Δ Saccharomyces cerevisiae* cells under iron-sufficient conditions and the role of Fe_{580}. *Biochemistry*, 2018, **57**, 672–683

2. Li L., Kaplan J. A Mitochondrial-Vacuolar Signaling Pathway in Yeast That Affects Iron and Copper Metabolism *J. Biol. Chem.* 2004, **279**, 33653–33661

3. Ghosh, A., Pratt, AT., Soma, S., Theriault, SG., Griffin, A., Trivedi, PP., Vishal, VM. Mitochondrial disease genes COA6, COX6B and SCO2 have overlapping roles in COX2 biogenesis. *Hum Mol Genet*. 2016, **25**, 660–671

4. Hamer, DH., Thiele, DJ. Lemontt, JE. Function and Autoregulation of Yeast Copper Thionein. *Science*. 1985, **228**, 685–690

Appendix A: Generation of the *fet3*Δ deletion strain

The *fet3*Δ strain was derived from the W303 background, created by PCR amplifying the KanMX cassette and then using that amplicon to delete the *FET3* gene by homologous recombination. The forward and reverse primers used for amplification were GCA TAG GAA ACG AAG AGG ACC CCA GTG TAA GGA AGA GTA GGA CAT GGA GGC CCA GAA TAC, and CCG AAA AAA AAA AAA CAG GTT AAC CGC AAA ATA CAT GAT CCA GTA TAG CGA CCA GCA TTC, respectively.

Figure S1: Western Blot of 6 isolated cytosol batches. Each lane was loaded with 22 μg protein. "WC", whole cells; "Cyt.", isolated cytosol. Batches A, B, E, and F were isolated from WT cells grown in MM with the supplementation of 40 μM Fe and 10 μM Cu. Batches C and D were from cells grown under the same condition but with 1 μM Fe. Primary antibodies are described in *Experimental Procedures*. PGK, Kar2, CPY, and VDAC are markers for cytosol, ER, vacuoles, and mitochondria, respectively.

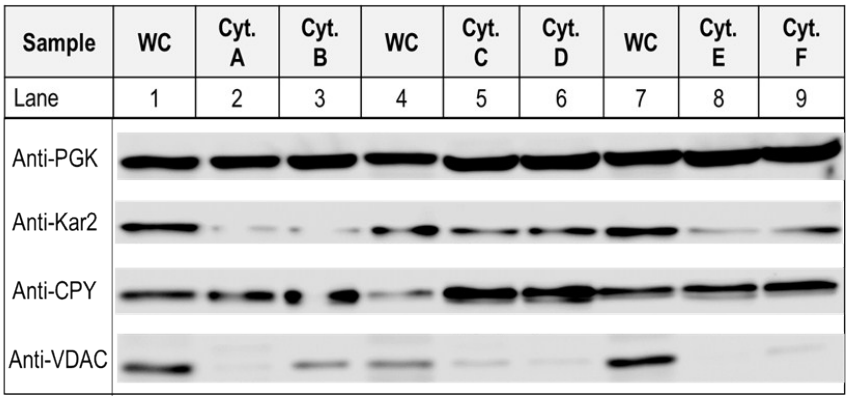


Figure S2: Iron chromatograms of FTS of independent cytosol batches isolated from WT cells supplemented with 40 µM of Fe and 100 µM of Fe in the growth media.

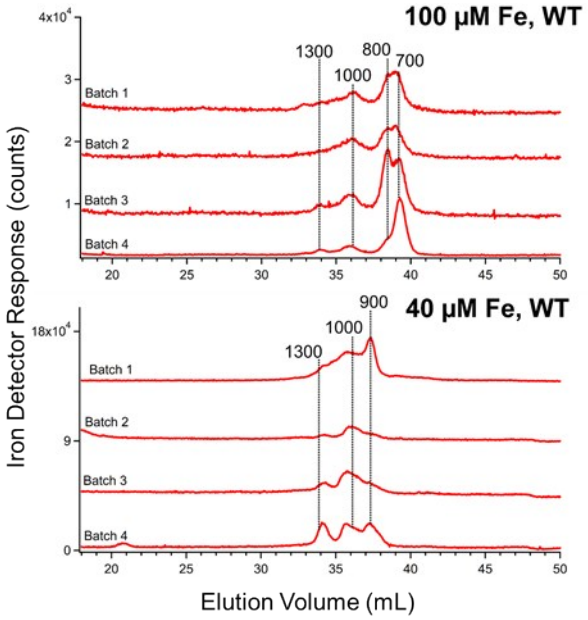
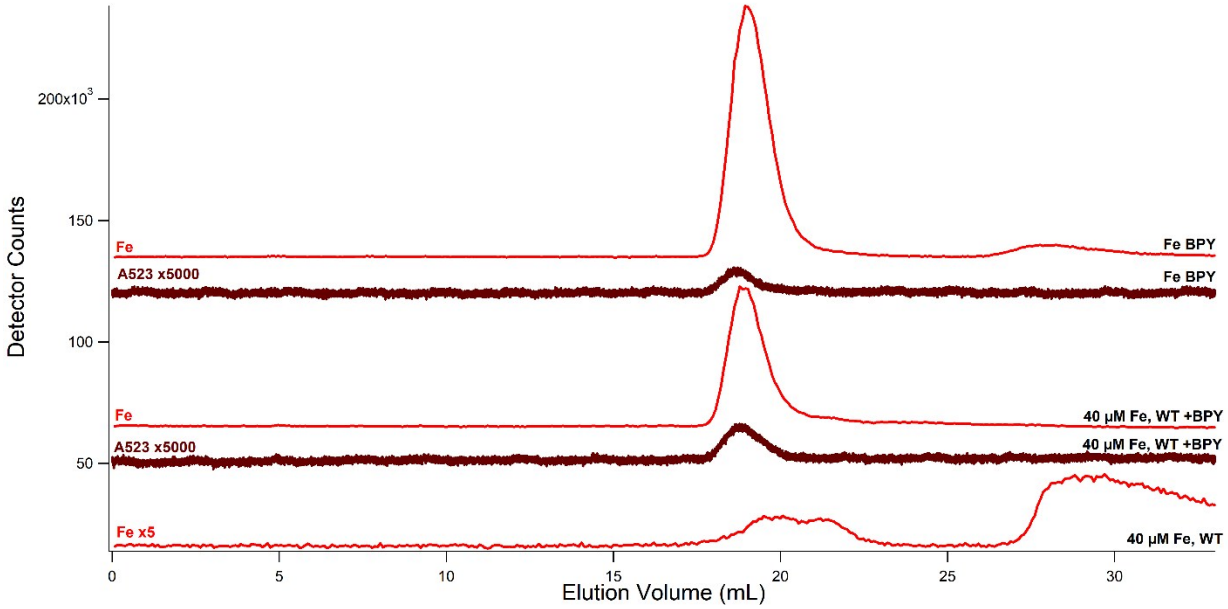


Figure S3: Iron-detected chromatograms of samples incubated with 2,2-bipyridine (BPY). Cytosolic FTS was incubated with 100 µM 2,2-bipyridine at 37 ˚C for 2 hours. Top traces: The $Fe(BPY)_3$ standard composed of 5 µM $FeSO_4$ and 10 µM BPY incubated in the same conditions. Absorbance at 523 nm, reflecting the complex, is shown below as the dark maroon line. Middle traces: Iron trace of cytosolic FTS isolated from WT cells grown on 40 µM Fe^{III} citrate after exposure to BPY. A523 nm trace is shown below in the dark maroon line. Bottom trace: Iron trace of the same sample as used in the middle trace before treatment with BPY.



$Fe(BPY)_3$ has an absorbance maximum at 523 nm. The same elution volume peak and A523 peak is shown in both sample and standard traces, which indicates that the $Fe(BPY)_3$ complex was made in both cases. There is some overlap of the $Fe(BPY)_3$ peak and the peaks at 19.5 mL due to LMM Fe complexes in the cytosol FTS, but the broad iron peak starting at 27 mL is clearly absent in the middle trace after BPY treatment.