Electronic Supplementary Material (ESI) for Metallomics
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Supplementary material accompanying the paper:

On the molecular relationships between high-zinc tolerance and aconitase (Aco1) in *Saccharomyces cerevisiae*

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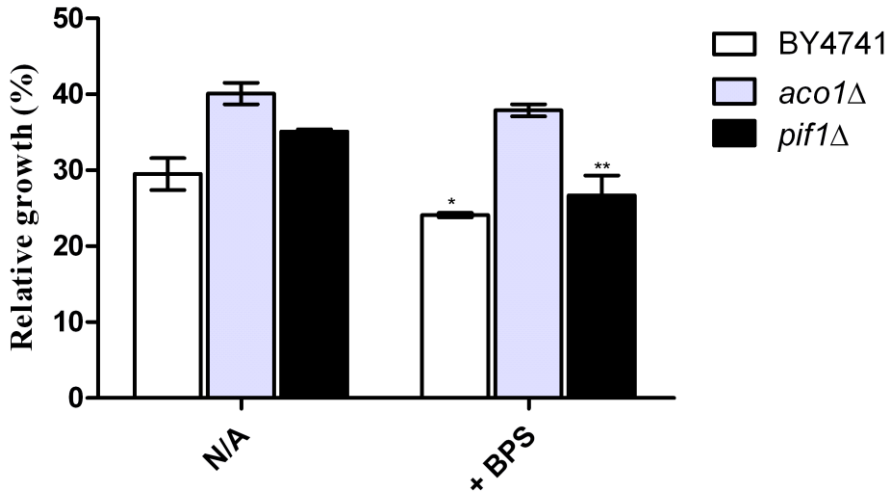
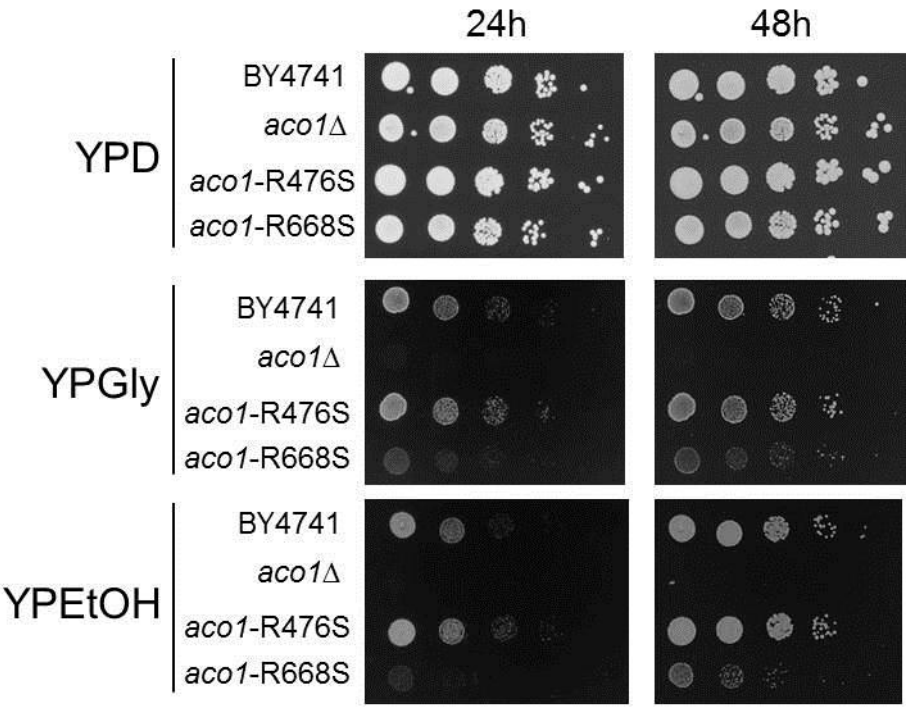


Figure S1. Effect of iron availability on zinc tolerance of wild type, *aco1*Δ and *pif1*Δ cells. Wild type strain BY4741 (white bars), *aco1*Δ (grey bars) and the *pif1*Δ (black bars) were inoculated at OD_{600} 0.1 on liquid YPD medium containing 5 mM $ZnCl_2$ (+Zn), or YPD medium containing 0.5 mM BPS, with addition of 5 mM $ZnCl_2$ (+BPS). Growth was monitored at 600 nm after 48 h. For each mutant strain, data correspond to relative growth regarding the culture without zinc supplementation. The results are means of three independent cultures, and the error bars indicate ± S.D. *P < 0.05; **P < 0.05, ***P < 0.01 (as assessed with the Newman-Keuls Multiple Comparison Test).

A)



B)

	Aco1 prot/mitochondrial protein (µg/µg)	Aco1 activity (Units/mg mitochondrial protein)
BY4741	0.218	1.017
aco1-R476S	0.195	1.066
aco1-R668S	0.196	0.059 **

Figure S2. A. Effect of the carbon source in the growth of different *aco1* mutants. Growth in different carbon sources was analysed in the BY4741, *aco1*Δ, *aco1*-R476S and *aco1*-R668S strains. Cultures were grown in YPD medium overnight at 30 ºC and then they were diluted in fresh YPD medium to an OD_{600} of 0.1. Cultures were further grown to an exponential phase (OD_{600} 0.3-0.5) and then were diluted and spotted into YPD (2% glucose), YPGly (3% glycerol) or YPEtOH (2% ethanol). Plates were allowed to grow for 48 h. **B.** Aconitase activity and Aco1 protein content were measured in all the indicated strains. Cells were grown in YPD medium overnight at 30 ºC and diluted in fresh YPD medium to an OD600 of 0.1 and both cultures were grown for 24h. Results are means of three independent cultures, and the error bars indicate ± S.D. *P < 0.05; **P < 0.05, ***P < 0.01 (as assessed with the Newman-Keuls Multiple Comparison Test).

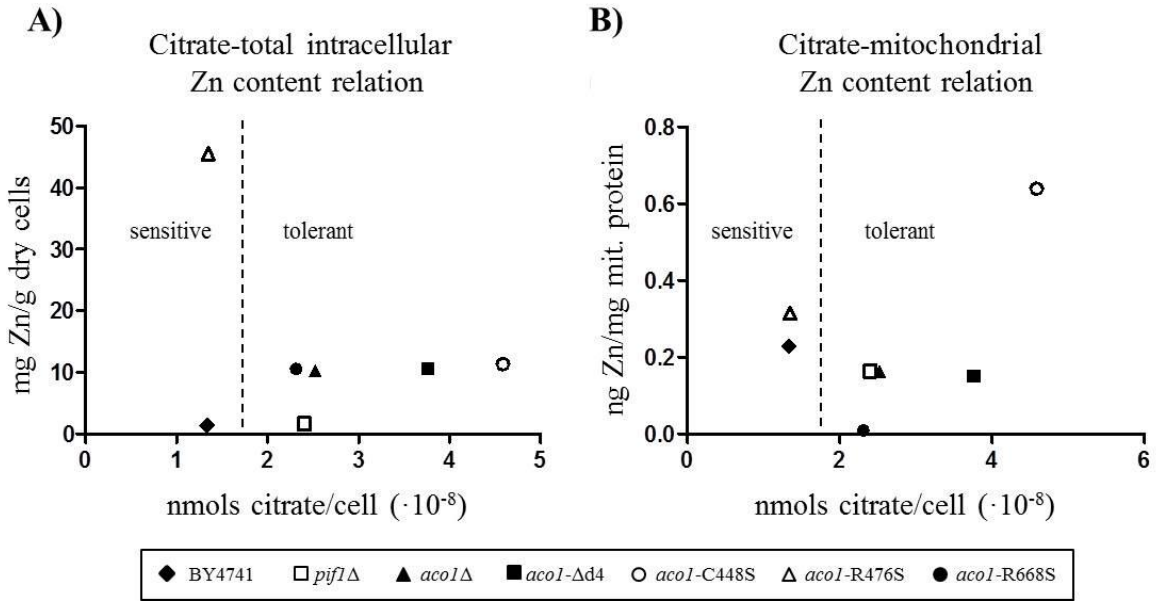
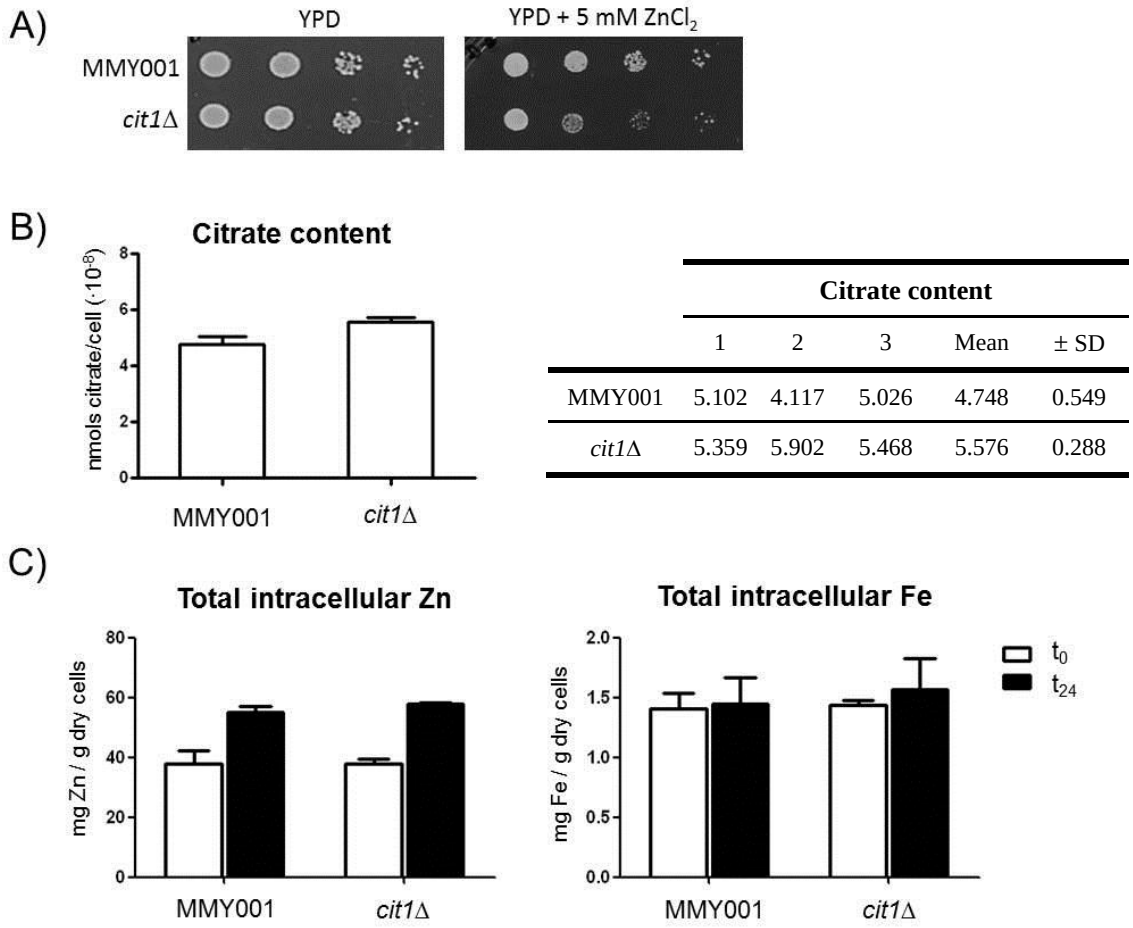


Figure S3. Citrate, zinc content and zinc tolerance relationship in different strains. **A**. Representation of the relation of citrate content (data in table S7) and total intracellular zinc content (t_{24}, data in table S8) in BY4741, *pif1*Δ, *aco1*Δ and the different *ACO1* mutants. **B**. Representation of the relation of citrate content (data in table S7) and mitochondrial zinc content (t_{24}, data in table S8) in BY4741, *pif1*Δ, *aco1*Δ and the different *ACO1* mutants. The representation with other time points yields equivalent plots.



	Citrate content				
	1	2	3	Mean	± SD
MMY001	5.102	4.117	5.026	4.748	0.549
*cit1*Δ	5.359	5.902	5.468	5.576	0.288

		Total intracellular Zn					Total intracellular Fe				
		1	2	3	Mean	± SD	1	2	3	Mean	± SD
MMY001	t_0	33.410	37.558	42.160	37.709	4.377	1.551	1.378	1.281	1.403	0.137
	t_{24}	57.242	53.224	54.157	54.874	2.103	1.600	1.193	1.542	1.445	0.220
*cit1*Δ	t_0	36.096	38.707	38.687	37.830	1.502	1.441	1.474	1.391	1.435	0.042
	t_{24}	57.343	57.957	58.136	57.812	0.416	1.554	1.320	1.830	1.568	0.255

Figure S4. Characterization of the *cit1*Δ mutant in MYY001 background. **A.** Effect of high zinc levels. MMY001 and *cit1*Δ strains were grown in YPD medium overnight at 30 ºC and then diluted in fresh YPD to an OD_{600} of 0.1. Cultures were grown to an exponential phase (OD_{600} 0.3-0.5) and were then diluted and spotted into YPD and YPD + 5mM $ZnCl_2$ plates which were allowed to grow for 72 h. **B.** Measurements of the citrate content. Citrate content was measured from 60 absorbance units of cells in mid-log phase and early-stationary phase. Cultures were grown in YPD medium overnight at 30 ºC and then diluted in fresh YPD medium to an OD_{600} of 0.1 and they were grown to an exponential phase (OD_{600} 0.3-0.5), when samples were collected and frozen at -80 ºC. Results are means of three independent cultures, and the error bars indicate ± S.D. No significant differences regarding the wild type strain are observed. **C.** Intracellular zinc and iron content measurements. Cells were grown in YPD medium overnight at 30 ºC and diluted in fresh YPD to an OD_{600} of 0.1, then growth was allowed until to an OD_{600} ~0.4 and (t_0, white bars) and for further 24 hours (t_{24}, black bars). Results are means of three independent cultures, and the error bars indicate ± S.D. No significant differences regarding the wild type strain are observed. (MMY001 and *cit1*Δ strains were kindly provided by Dr. McAlister-Henn, University of Texas, USA).

Table S1. *S. cerevisiae* trains used in this work

STRAIN	GENOTYPE	COMMENTS	SOURCE/REFERENCE
BY4741	*MAT**a** his3*Δ1 *leu2*Δ0 *met15*Δ0 *ura3*Δ0	Wild type	Euroscarf
*pif1*Δ	BY4741 *pif1::kanMX*		Euroscarf
*aco1*Δ	BY4741 *aco1::kanMX*		Euroscarf
MML830	W303-1[a] *tetR*[1]*-Ssn6::LEU2*	Integration of PCM225 in W303-1A	15
p*tetO*$_7$*-ACO1*	W303-1A *tetO*$_7$*-aco1::kanMX4 tetR*[1] *–Ssn6::LEU2*	Promoter substitution of *ACO1* in MML830	This work
pif1Δ (MML)	MML830 *pif1*::*trp1*		This work
p*tetO*$_7$*-ACO1-pif1Δ*	W303-1A *tetO*$_7$*-aco1::kanMX4 pif1::trp1 tetR*[1] *–Ssn6::LEU2*	Conditional double mutant	This work
CS1304-3A (*aco1*-R476S)	BY4741 *aco1Δ::kan, ade2::pUC-URA3/4-ade2-aco1[R476S]*	Single point mutant of *ACO1* with diminished DNA binding capacity.	11
CS1309-5D (*aco1*-R668S)	BY4741 *aco1Δ::kan, ade2::pUC-URA3/4-ade2-aco1[R668S]*	Single point mutant of *ACO1* with diminished DNA binding capacity.	11
aco1-C448S	BY4741 *aco1Δ::kan, ade2::pUC-URA3/4-ade2-aco1[C448S]*	Single point mutant of *ACO1* with not aconitase activity.	This work
aco1-Δd4	BY4741 *aco1::kanMX* + *ACO1-Δd4/pRS316*	ACO1 without the fourth domain, no aconitase activity or DNA binding capacity.	This work
BY4741+ pRS316			This work
BY4741+ pVT-U			This work
*aco1*Δ + pVT-U	BY4741 *aco1::kanMX*		This work
*aco1*Δ + *ACO1*/pVT-U	BY4741 *aco1::kanMX*		This work
*pif1*Δ + pVT-U	BY4741 *pif1::kanMX*		This work
*pif1*Δ + *ACO1*/pVT-U	BY4741 *pif1::kanMX*		This work

Table S2. Oligonucleotides used for mutant construction.

PRIMERS	SEQUENCES
ACO1-Fwd1	5´-GATCA*GGATCC*GAATTCCAAAGGCTCACAAATGGC-3´
ACO1-Rv1	5´- GCTCG*CTCGAG*TTATAACAATGTTTTCATTATTTC-3´
ACO1-ΔD4-Rv	5´-GCTCG*CTCGAG*TTAAGCATCCTTACCATCCCAAGG-3´
ACO1-C448S-Primer	5´-TGGCAAACGCCTGTGGCCCATCTATTGGTCAATGGGATCGTA-3´
ACO1-C448S-SeqFw	5´-GCTGTTGCTAATAACTGGCCATTGGA-3´
ACO1-C448S-SeqRv	5´- CCACATGGTGATGGTTTGCCTCA-3´
ACO1-tetO7-Fwd	5´-TTTCTTAGTTATTACATAGTAGAACGAAGGTAATAAATACCAGCTGAA GCTTCGTACGC-3´
ACO1-tetO7-Rv	5´- GAACAATGGGTCTCTTGATGGCAGAACGTGCAGACAGCATATAGGCCA CTAGTGGATCTG-3´
Aco1-tetO7-CkFwd	5´-TTCGGAAAGGCAAGCACAAAAAGG-3´
Aco1-tetO7-CkRv	5´-GATGGCAGAACGTGCAGACAGCAT-3´
Aco1-tetO7-WhFwd	5´-TTCGGAAAGGCAAGCACAAAAAGG-3´
Aco1-tetO7-WhRv	5´-TTATTTCTTCTCATCGGCCTTAAT-3´
ACO1-Fwd2	5´-ATGCTGTCTGCACGTTCTGCCATC-3´
ACO1-Rv2	5´-TTATTTCTTCTCATCGGCCTTAAT-3´
PIF1-TRP1-disrup Fwd	5’-TCCATTGAGCGATTAGCTTACTTGTATCAATCAATTTTACCGGA TCCCCGGGTTAATTAA-3’
PIF1-TRP1-disrup Rv	5’-GCAGTTTGTATTCTATATAACTATGTGTATTAATATGTACGAAT TCGAGCTCGTTTAAAC-3’
PIF1-TRP1-check Fwd	5’- TACTGGTATTGCCTCGAT-3′
PIF1-TRP1-check Rv	5’-CTATGTATCTCGGCCTTG-3′

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Table S3. Genes related to Fe metabolism analysed by RT-PCR.

GENE	FUNCTION	PRIMERS
AFT1	Transcription factor involved in iron utilization and homeostasis	Fwd: TTCAATCCGGCTGACATAGA Rv: TCACATACTTCAACTCGCCGT
FTR1	High affinity iron permease; involved in the transport of iron across the plasma membrane.	Fwd: AGTGTTTAACGTGGCCGTTT Rv: AAGAGAGTCGGCTTTAACGTG
FIT2	Mannoprotein involved in the retention of siderophore-iron in the cell wall.	Fwd: CTCAACTATTTTCGGAGCTAC Rv: AGAAATCAAACCAAGGGCCA
FIT3	Mannoprotein involved in the retention of siderophore-iron in the cell wall.	Fwd: TTCTCTTCCGCTTTGGTTCT Rv: CAATAACATGACGGCAGCAA
ARN1	Transporter of siderophore-iron complexes.	Fwd: TCTCGCGATCCTGTTAAGGA Rv: CCCGTTTAAAAGTGAATTTTG
FET3	Ferro-O2-oxidoreductase; required for high-affinity iron uptake Also involved in the resistance to copper ion toxicity.	Fwd: TGCTCTCTATAGCCGTTTTGC Rv: GAAGAACCGTTTGGCTTTAGT
FET5	Multicopper oxidase, membrane protein that may have a role in iron transport.	Fwd: TTGTTCTACTCGTTCGTGTGG Rv: CCGCGAGAAACCTCTATTTCA
MRS3	Mitochondrial iron transporter; mediates Fe^{2+} transport across inner mitochondrial membrane; low-iron conditions.	Fwd: TGGTAGAAAACTGTCGAGTA Rv: CGTCATTAGGAAATGTTTTGC
MRS4	Mitochondrial iron transporter; mediates Fe^{2+} transport across inner mitochondrial membrane; low-iron conditions.	Fwd: GAACTGTCAATAGCTGAGGA Rv: AAAATGCTTAGCACATTCA
CCC1	Putative vacuolar Fe^{2+}/Mn^{2+} transporter; it prevents mitochondrial iron accumulation.	Fwd: CACTAAAGAACGCAGTGGTGA Rv: TAACAAAGAACCAAGCCGCA
ACO1	Aconitase enzyme of the tricarboxylic acid (TCA) cycle and also independently required for mitochondrial genome maintenance.	Fwd: GCAGAATGTGGAAACGCTGGA Rv: CACCAGTATATACATTTTTAA
ACT1	Actin, structural protein involved in cell polarization, endocytosis, and other cytoskeletal funcions	Fwd: CTGAATTAACAATGGATTCTG Rv: AGTAACACCATCACCGGAAT

Table S4. Raw data corresponding to the results shown and analysed in Figure S1 (relative growth (%) regarding the culture without zinc supplementation).

	Treatment	N/A					+BPS				
	Replicates	1	2	3	Mean	± SD	1	2	3	Mean	± SD
STRAIN	BY4741	31.58	27.48	28.56	29.21	2.12	23.82	24.44	24.22	24.15	0.33
	*aco1*Δ	41.51	38.71	39.87	40.03	1.41	38.75	37.12	37.78	37.88	0.83
	*pif1*Δ	35.35	34.75	35.03	35.08	0.30	24.13	29.36	25.86	26.45	2.66

Table S5. Raw data corresponding to the results shown and analysed in Figure 5 (A and B).
A. Aconitase activity in aconitase U/mg protein. **B.** Aco1 protein content in µg Aco1/10^6 cells.

		A) ACONITASE ACTIVITY						
	Strain	BY4741	*pif1*Δ	*aco1*Δ	*aco1*-Δd4	*aco1*-C448S	*aco1*-R476S	*aco1*-R668S
t_{24}	Replicate	0.416	0.019	0.005	0.013	0.013	0.317	0.369
		0.444	0.015	0.013	0.010	0.012	0.325	0.344
		0.411	0.016	0.008	0.009	0.009	0.279	0.348
	Mean	0.424	0.017	0.009	0.011	0.011	0.307	0.354
	± SD	0.018	0.002	0.004	0.002	0.002	0.025	0.013
t_{24} +Zn	Replicate	0.112	0.060	0.008	0.010	0.009	0.171	0.120
		0.118	0.044	0.006	0.008	0.009	0.137	0.099
		0.088	0.041	0.012	0.012	0.012	0.140	0.093
	Mean	0.106	0.048	0.009	0.010	0.010	0.149	0.104
	± SD	0.016	0.010	0.003	0.002	0.002	0.019	0.015

		B) Aco1 PROTEIN						
	Strain	BY4741	*pif1*Δ	*aco1*Δ	*aco1*-Δd4	*aco1*-C448S	*aco1*-R476S	*aco1*-R668S
t_{24}	Replicate	11.330	10.136	0.076	9.285	9.734	7.448	11.557
		9.699	8.744	0.110	7.565	8.005	9.236	13.379
		9.423	8.812	0.090	7.837	9.515	8.996	11.824
	Mean	10.151	9.230	0.092	8.229	9.085	8.560	12.253
	± SD	1.030	0.785	0.017	0.925	0.941	0.981	0.984
t_{24} +Zn	Replicate	17.105	11.713	0.090	9.085	12.356	12.698	21.586
		14.565	14.568	0.102	7.476	10.538	10.263	17.957
		15.002	15.424	0.095	7.308	10.893	10.427	18.785
	Mean	15.557	13.901	0.096	7.956	11.262	11.129	19.443
	± SD	1.358	1.943	0.006	0.981	0.964	1.361	1.902

Table S6. Raw data corresponding to the results shown and analysed in Figure 6 (A and B). **A.** Aconitase activity in aconitase U/mg protein. **B.** Aco1 protein content in µg Aco1/10^6 cells.

	A) ACONITASE ACTIVITY							
Strain	BY4741	BY4741+ pVT-U	*aco1*Δ	*aco1*Δ+ pVT-U	*aco1*Δ+ pVT-U/*ACO1*	*pif1*Δ	*pif1*Δ + pVT-U	*pif1*Δ + pVT-U/*ACO1*
Replicate	0.416	0.435	0.003	0.011	0.349	0.021	0.060	0.425
	0.444	0.448	0.009	0.008	0.428	0.008	0.056	0.488
	0.411	0.428	0.008	0.007	0.378	0.023	0.019	0.439
Mean	0.424	0.437	0.009	0.009	0.385	0.017	0.045	0.451
± SD	0.018	0.011	0.004	0.002	0.039	0.008	0.023	0.033

	B) Aco1 PROTEIN							
Strain	BY4741	BY4741+ pVT-U	*aco1*Δ	*aco1*Δ+ pVT-U	*aco1*Δ+ pVT-U/*ACO1*	*pif1*Δ	*pif1*Δ + pVT-U	*pif1*Δ + pVT-U/*ACO1*
Replicate	11.150	9.332	0.156	0.124	8.744	10.858	11.354	14.731
	9.687	9.332	0.109	0.196	10.825	10.712	9.954	11.697
	9.563	8.147	0.094	0.083	8.975	12.733	11.742	13.254
Mean	10.249	8.937	0.120	0.134	9.515	11.434	11.016	13.227
± SD	1.082	0.685	0.032	0.057	1.141	1.127	0.942	1.517

Table S7. Raw data corresponding to the results shown and analysed in Figure 7 (citrate content in nmols of citrate/cell $\cdot 10^{-8}$).

	CITRATE CONTENT						
Strain	BY4741	*pif1*Δ	*aco1*Δ	*aco1*-Δd4	*aco1*-C448S	*aco1*-R476S	*aco1*-R668S
Replicate	1.084	2.089	2.447	4.154	5.454	1.224	2.624
	1.709	2.745	2.638	3.516	3.592	1.682	2.089
	1.215	2.375	2.486	3.623	4.715	1.139	2.226
Mean	1.336	2.403	2.524	3.764	4.587	1.348	2.313
± SD	0.329	0.329	0.102	0.342	0.937	0.292	0.278

Table S8. Raw data corresponding to the results shown and analysed in Figure 8 (for **A** and **C**, mg of metal/g dry cells and for **B** and **D**, ng metal/mg mitochondrial protein).

		A) TOTAL INTRACELLULAR Zn						
Strain		BY4741	*pif1*Δ	*aco1*Δ	*aco1*-Δd4	*aco1*-C448S	*aco1*-R476S	*aco1*-R668S
t_0	Replicate	1.336	0.776	9.073	8.124	7.911	18.279	10.326
		1.082	0.356	8.857	7.896	8.109	18.287	10.172
		0.571	1.148	8.996	7.953	7.925	18.305	11.004
	Mean	0.996	0.760	8.975	7.991	7.981	18.290	10.499
	± SD	0.390	0.396	0.110	0.119	0.110	0.013	0.441
t_{24}	Replicate	1.920	2.410	10.185	10.567	11.402	45.635	10.270
		1.042	1.693	10.400	10.815	11.434	45.448	10.417
		1.174	0.867	10.208	10.581	11.148	45.467	10.947
	Mean	1.379	1.657	10.264	10.654	11.328	45.517	10.545
	± SD	0.473	0.772	0.118	0.139	0.157	0.103	0.356
t_{24+Zn}	Replicate	26.381	18.845	417.397	414.568	398.312	315.322	454.169
		20.269	20.911	419.108	424.211	428.416	329.064	467.549
		23.711	15.052	427.359	412.325	421.893	326.767	491.747
	Mean	23.454	18.269	421.397	417.035	416.207	323.718	471.155
	± SD	3.064	2.972	5.210	6.315	15.837	7.361	19.047

		B) MITOCHONDRIAL Zn						
Strain		BY4741	*pif1*Δ	*aco1*Δ	*aco1*-Δd4	*aco1*-C448S	*aco1*-R476S	*aco1*-R668S
t_0	Replicate	0.413	1.009	0.354	0.445	0.075	0.167	0.194
		0.338	1.031	0.339	0.391	0.038	0.142	0.256
		0.345	1.205	0.378	0.412	0.022	0.148	0.217
	Mean	0.365	1.082	0.357	0.416	0.045	0.152	0.222
	± SD	0.041	0.107	0.020	0.027	0.027	0.013	0.031
t_{24}	Replicate	0.226	0.175	0.147	0.145	0.660	0.357	0.031
		0.198	0.139	0.149	0.191	0.655	0.302	0.058
		0.259	0.180	0.189	0.122	0.607	0.287	0.066
	Mean	0.228	0.165	0.162	0.153	0.641	0.315	0.052
	± SD	0.031	0.022	0.024	0.035	0.029	0.037	0.018
t_{24+Zn}	Replicate	2.897	7.787	50.025	44.259	4.265	0.953	27.531
		3.052	7.085	45.198	47.263	3.975	1.218	26.987
		2.714	7.172	46.144	47.951	3.887	1.178	24.682
	Mean	2.888	7.348	47.122	46.491	4.042	1.116	26.400
	± SD	0.169	0.383	2.558	1.963	0.198	0.143	1.512

		C) TOTAL INTRACELLULAR Fe						
Strain		BY4741	*pif1*Δ	*aco1*Δ	*aco1*-Δd4	*aco1*-C448S	*aco1*-R476S	*aco1*-R668S
t_0	Replicate	0.128	0.163	1.128	1.568	1.075	1.655	1.770
		0.099	0.199	1.156	1.602	1.108	1.723	1.706
		0.056	0.112	1.151	1.578	1.115	1.662	1.720
	Mean	0.095	0.158	1.145	1.583	1.099	1.680	1.732
	± SD	0.036	0.044	0.015	0.017	0.021	0.037	0.033
t_{24}	Replicate	0.137	0.246	3.501	3.681	3.504	1.201	1.931
		0.151	0.282	3.653	3.802	3.591	1.526	1.158
		0.132	0.331	3.420	3.874	3.723	1.152	1.765
	Mean	0.140	0.286	3.525	3.786	3.606	1.293	1.618
	± SD	0.010	0.043	0.118	0.098	0.110	0.203	0.407
t_{24+Zn}	Replicate	0.155	0.176	1.308	1.596	1.399	1.408	2.895
		0.189	0.184	1.343	1.610	1.417	1.430	2.845
		0.138	0.155	1.328	1.651	1.448	1.409	2.002
	Mean	0.160	0.172	1.327	1.619	1.421	1.416	2.581
	± SD	0.026	0.015	0.018	0.029	0.026	0.012	0.502

		D) MITOCHONDRIAL Fe						
Strain		BY4741	*pif1*Δ	*aco1*Δ	*aco1*-Δd4	*aco1*-C448S	*aco1*-R476S	*aco1*-R668S
t_0	Replicate	0.293	2.405	0.245	0.271	0.007	0.067	0.062
		0.254	2.116	0.281	0.189	0.011	0.048	0.061
		0.269	2.095	0.287	0.201	0.008	0.045	0.085
	Mean	0.272	2.205	0.271	0.220	0.009	0.053	0.069
	± SD	0.020	0.173	0.023	0.044	0.002	0.012	0.014
t_{24}	Replicate	0.133	0.715	0.185	0.158	0.072	0.257	0.196
		0.155	0.706	0.171	0.149	0.097	0.241	0.235
		0.128	0.744	0.142	0.145	0.103	0.296	0.221
	Mean	0.139	0.722	0.166	0.151	0.091	0.265	0.217
	± SD	0.014	0.020	0.022	0.007	0.016	0.028	0.020
t_{24+Zn}	Replicate	0.230	0.801	0.287	0.258	1.564	0.339	0.996
		0.257	0.714	0.272	0.303	1.209	0.310	1.157
		0.238	0.739	0.213	0.227	1.316	0.361	1.113
	Mean	0.242	0.751	0.257	0.263	1.363	0.337	1.089
	± SD	0.014	0.045	0.039	0.038	0.182	0.026	0.083

Table S9. Raw data corresponding to the results shown and analysed in Figure 10 (succinate dehydrogenase activity measurements in BT-formazan (µmols)/cell units relative to WT strain).

	SUCCINATE DEHYDROGENASE ACTIVITY						
Strain	BY4741	*pif1*Δ	*aco1*Δ	*aco1*-Δd4	*aco1*-C448S	*aco1*-R476S	*aco1*-R668S
Replicate	1.132	0.036	0.091	0.396	0.296	1.174	0.431
	1.005	0.036	0.043	0.278	0.399	1.851	0.611
	0.884	0.016	0.075	0.375	0.495	1.508	0.607
Mean	1.007	0.029	0.070	0.350	0.397	1.511	0.550
± SD	0.124	0.011	0.024	0.063	0.099	0.338	0.103