

**SUPPLEMENTARY MATERIAL FOR**  
**THE METABOLIC RESPONSE TO IRON DEFICIENCY IN**  
**SACCHAROMYCES CEREVISIAE.**

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Table S1.

Metabolite Analysis of Yeast Grown in Defined-Iron Minimal Media

Super Pathway	Sub Pathway	Name	Kegg	HMDB	10 Fe / 4 hr / 100 Fe	10 Fe / 100 Fe	300 Fe 4 hr / 100 Fe	300 Fe / 100 Fe	10 Fe / 300 Fe
Amino acid	Glycine, serine and threonine metabolism	glycine	C00037	HMDB00123	0.91	1.84	0.62	0.76	2.44
		glycine	C00037	HMDB00123	1.03	1.52	0.73	0.68	2.22
		N-acetylglycine		HMDB00532	1.13	1.32	0.68	0.88	1.49
		serine	C00065	HMDB03406	0.9	0.94	1.03	1.07	0.88
		O-acetylhomoserine	C01077		1.02	1.48	0.91	0.93	1.59
		threonine	C00188	HMDB00167	1	1.42	0.94	0.89	1.59
		allo-threonine	C05519	HMDB04041	0.82	2.54	1.03	1.67	1.52
	Alanine and aspartate metabolism	alanine	C00041	HMDB00161	1.17	1.79	1.18	1.02	1.75
		aspartate	C00049	HMDB00191	0.92	0.94	1.13	0.95	0.99
		N-carbamoylaspartate	C00438	HMDB00828	0.37	1.4	0.54	0.67	2.08
		asparagine	C00152	HMDB00168	1.04	1.91	1.06	1.13	1.69
	Glutamate metabolism	glutamate	C00025	HMDB03339	0.96	0.74	1.09	1.03	0.72
		glutamine	C00064	HMDB00641	1.17	1	1.2	1.02	0.98
		N-acetylglutamate	C00624		1.06	0.46	1.2	1.01	0.46
	Histidine metabolism	histidine	C00135	HMDB00177	0.95	1.15	0.91	0.88	1.32
	Lysine metabolism	lysine	C00047	HMDB00182	0.66	0.78	0.79	0.93	0.83
		2-aminoadipate	C00956	HMDB00510	0.6	2.5	0.45	0.69	3.57
		saccharopine	C00449	HMDB00279	0.6	0.75	0.97	0.63	1.19
	Phenylalanine & tyrosine metabolism	phenylalanine	C00079	HMDB00159	0.89	1.58	1.24	1.16	1.35
		tyrosine	C00082	HMDB00158	0.97	1.98	1.44	1.44	1.37
		tyrosine	C00082	HMDB00158	0.97	1.8	1.47	1.36	1.33
	Tryptophan metabolism	kynurenine	C00328	HMDB00183	0.64	0.98	1.04	1.16	0.85
		tryptophan	C00078	HMDB00929	1.1	2.53	1.43	1.5	1.69
		3-hydroxykynurenine	C02794	HMDB00732	1.04	0.27	1.89	2.05	0.13
	Valine, leucine and isoleucine metabolism	isoleucine	C00407	HMDB00172	1.1	1.57	1.03	0.97	1.61
		leucine	C00123	HMDB00687	1.17	2.44	1.28	1.37	1.79
		valine	C00183	HMDB00883	1.28	1.58	1.07	0.93	1.69
	Cysteine, methionine, SAM, taurine metabolism	cysteine	C00097	HMDB00574	0.92	1.74	1.29	1.34	1.30
		cystathione	C02291	HMDB00099	1.49	0.75	1.93	1.3	0.58
		Sadenosylmethionine (SAM)	C00019	HMDB01185	1.31	1.49	1.67	0.8	1.85
		Sadenosylhomocysteine (SAH)	C00021	HMDB00939	1.11	1.07	1.46	1.19	0.91
		methionine	C00073	HMDB00696	0.91	1.22	1.16	1.13	1.08
		homocystine	C01817	HMDB00676	1.46	0.96	0.99	0.78	1.23
	Urea cycle; arginine-, proline-, metabolism	N,N-dimethylarginine (ADMA)	C03626	HMDB01539	0.85	1.2	1.25	1.18	1.02
		arginine	C00062	HMDB03416	1.05	1.13	1.08	1.01	1.11
		ornithine	C00077	HMDB03374	0.86	0.77	0.97	0.96	0.80
		urea	C00086	HMDB00294	0.62	0.69	0.75	0.46	1.49
		proline	C00148	HMDB00162	1.06	0.9	1.15	1.05	0.85
		citrulline	C00327	HMDB00904	1.08	1.17	1.17	1.1	1.06
		trans-4-hydroxyproline	C01157	HMDB00725	0.91	0.75	0.85	1.05	0.71
	Creatine metabolism	argininosuccinate	C03406	HMDB01006	1.08	2.35	1.71	0.82	2.86
		creatine	C00300	HMDB00064	0.63	1.31	0.49	0.91	1.43
		2-aminobutyrate	C02261	HMDB00581	0.98	1.21	0.95	0.86	1.41
	Polyamine metabolism	5-methylthioadenosine (MTA)	C00170	HMDB01173	1.79	2.43	2.19	1.73	1.41
		putrescine	C00134	HMDB01414	0.72	0.73	0.78	0.55	1.32
	Guanidino and acetamido metabolism	1-methylguanidine	C02294	HMDB01522	1.03	1.32	1.09	0.93	1.43
	Glutathione metabolism	glutathione, reduced (GSH)	C00051	HMDB00125	1.04	0.94	1.24	1.07	0.88
		5-oxoproline	C01879	HMDB00267	0.99	0.86	1.12	1	0.85
		glutathione, oxidized (GSSG)	C00127	HMDB03337	0.97	0.72	1.17	1	0.72

Peptide	Dipeptide	glycylproline		HMDB00721	1.09	<b>1.87</b>	1.18	<b>1.8</b>	1.04	
		prolylleucine			1.36	<b>3.16</b>	<b>1.84</b>	<b>2.24</b>	1.41	
	g-glutamyl	gamma-glutamylcysteine	C00669	HMDB01049	0.92	<b>0.77</b>	<b>1.22</b>	1.1	<b>0.70</b>	
		gamma-glutamylglutamine			<b>0.73</b>	<b>0.73</b>	1.08	1.11	0.65	
Carbohydrate	Aminosugars metabolism	N-acetylgalactosamine	C01074	HMDB00835	<b>0.7</b>	<b>0.67</b>	0.93	0.93	<b>0.71</b>	
		N-acetyl-beta-glucosaminylamine	C01239	HMDB01104	<b>0.61</b>	<b>0.44</b>	0.95	0.81	<b>0.54</b>	
	Fructose, mannose, galactose, starch, and sucrose metabolism	sorbitol (glucitol)	C00794	HMDB00107	<b>1.23</b>	1.24	<b>1.34</b>	1.17	1.05	
		2-isopropylmalate	C02504	HMDB00402	<b>2.2</b>	<b>12.87</b>	<b>1.47</b>	0.94	<b>14.29</b>	
	Glycolysis, gluconeogenesis, pyruvate metabolism	glycerate	C00258	HMDB00139	<b>0.69</b>	<b>0.56</b>	0.91	0.9	<b>0.63</b>	
		glucose-6-phosphate (G6P)	C00668	HMDB01078	1.07	<b>0.61</b>	0.87	1.29	<b>0.47</b>	
		glucose	C00267	HMDB00122	0.83	<b>0.28</b>	1.21	1.18	<b>0.24</b>	
		3-phosphoglycerate	C00197	HMDB00807	0.9	<b>0.43</b>	<b>1.29</b>	0.94	<b>0.45</b>	
		phosphoenolpyruvate (PEP)	C00074	HMDB00263	0.94	0.58	1.39	0.69	0.84	
		pyruvate	C00022	HMDB00243	<b>0.49</b>	<b>1.49</b>	<b>0.56</b>	0.73	<b>2.04</b>	
Lipid	Nucleotide sugars, pentose metabolism	ribitol (adonitol)	C00474	HMDB01851	0.8	<b>0.75</b>	0.82	<b>0.75</b>	1.00	
		gluconate	C00257	HMDB03373	1.22	<b>0.69</b>	1.01	0.71	0.96	
		ribose	C11476	HMDB00283	1.16	1.21	1.28	1.17	1.03	
		xylulose	C00310	HMDB00621	1.31	0.94	1.3	0.85	1.10	
	Glycerolipid metabolism	lyxose	C11476	HMDB03402	1.27	1.03	1.24	1.19	0.86	
		caprate	C01571	HMDB00511	<b>0.77</b>	0.6	<b>0.47</b>	<b>0.52</b>	1.15	
		laurate	C02679	HMDB00638	0.8	0.99	0.79	0.94	1.05	
		myristate	C06424	HMDB00806	0.88	<b>1.22</b>	1.11	1.19	1.03	
		palmitate	C00249	HMDB00220	0.99	<b>1.39</b>	1.13	1.2	1.16	
		palmitoleate	C08362	HMDB03229	1.06	0.96	<b>1.36</b>	1.29	<b>0.75</b>	
Energy	Fatty acid, monoene	oleate	C00712	HMDB00573	0.98	1.14	<b>1.41</b>	1.32	0.86	
		Carnitine metabolism	carnitine	C00487		1.21	1.19	1.11	1.22	0.98
	Glycerolipid metabolism	choline	C00114	HMDB00097	1.02	<b>0.5</b>	<b>0.68</b>	<b>0.4</b>	1.25	
		glycerol	C00116	HMDB00131	0.97	<b>0.5</b>	<b>0.67</b>	<b>0.52</b>	0.95	
		glycerol 3-phosphate (G3P)	C00093	HMDB00126	1.05	1.31	1.16	1.41	0.93	
		glycerophosphorylcholine (GPC)	C00670	HMDB00086	0.71	0.65	1.12	0.98	<b>0.66</b>	
	Inositol metabolism	inositol	C00137	HMDB00160	1.12	<b>1.47</b>	<b>1.38</b>	1.37	1.08	
		inositol 1-phosphate (I1P)	C01177	HMDB00213	1.17	<b>0.79</b>	1.11	1.2	<b>0.66</b>	
	Sphingolipid	sphinganine	C00836	HMDB00269	1.17	<b>8.87</b>	1	1	<b>9.09</b>	
	Monoacylglycerol	palmitoylglycerol (monopalmitin)			0.81	0.98	<b>1.64</b>	1.46	0.68	
Nucleotide	Sterol/Steroid	squalene	C00751	HMDB00256	<b>1.76</b>	<b>16.51</b>	1.01	0.9	<b>20.00</b>	
		ergosterol	C01694	HMDB00878	1.09	1.15	<b>1.55</b>	<b>1.35</b>	0.85	
		citrate	C00158	HMDB00094	0.71	<b>2.83</b>	<b>2.72</b>	<b>2.19</b>	1.28	
		alpha-keto-glutarate	C00026	HMDB00208	1.16	0.93	<b>1.34</b>	<b>1.56</b>	<b>0.60</b>	
		succinate	C00042	HMDB00254	0.83	<b>1.78</b>	0.89	1.04	<b>1.69</b>	
Energy	Krebb cycle	fumarate	C00122	HMDB00134	1.1	<b>1.65</b>	<b>1.39</b>	0.9	<b>1.82</b>	
		malate	C00149	HMDB00156	1.13	<b>1.87</b>	<b>1.83</b>	0.96	<b>1.96</b>	
		Oxidative phosphorylation	phosphate	C00009	HMDB01429	1.01	<b>1.33</b>	1.06	1.11	1.20
		xanthine	C00385	HMDB00292	1.09	0.91	1.13	0.95	0.96	
		hypoxanthine	C00262	HMDB00157	<b>0.84</b>	<b>0.55</b>	0.96	<b>0.86</b>	<b>0.65</b>	
	Purine metabolism, adenine containing	inosine	C00294	HMDB00195	0.94	<b>0.48</b>	0.91	<b>0.62</b>	0.78	
		adenine	C00147	HMDB00034	<b>1.38</b>	<b>1.8</b>	<b>1.71</b>	<b>1.39</b>	<b>1.28</b>	
		adenosine	C00212	HMDB00050	0.97	0.99	<b>1.41</b>	<b>1.35</b>	<b>0.74</b>	
		2'-deoxyadenosine	C00559	HMDB00101	0.99	<b>0.73</b>	<b>1.41</b>	<b>1.22</b>	<b>0.60</b>	
		adenosine 5'-monophosphate (AMP)	C00020	HMDB00045	1	1	1.03	0.86	1.18	
Nucleotide	Purine metabolism, guanine containing	adenosine 5'-diphosphate (ADP)		HMDB01341	1.4	0.53	1.32	0.94	0.57	
		guanine	C00242	HMDB00132	1.03	0.88	0.89	0.9	0.98	
		guanosine	C00387	HMDB00133	0.84	<b>0.49</b>	<b>0.56</b>	<b>0.51</b>	0.96	
			C00144	HMDB01397	1.11	<b>1.38</b>	0.7	<b>0.65</b>	<b>2.13</b>	

		monophosphate (GMP)							
	Pyrimidine metabolism, cytidine containing	cytidine	C00475	HMDB00089	1	0.96	1.09	0.96	1.01
	Pyrimidine metabolism, orotate containing	orotate		HMDB00226	0.66	1.71	1.13	1.51	1.14
		orotidine 5'-phosphate	C01103	HMDB00218	0.8	0.83	1.02	0.98	0.84
	Pyrimidine metabolism, thymine containing	thymidine		HMDB00273	0.91	0.87	1.46	1.09	0.80
		uracil	C00106	HMDB00300	0.77	0.58	0.9	0.72	0.81
	Pyrimidine metabolism, uracil containing	uridine	C00299	HMDB00296	1.97	1.24	1.99	1.41	0.88
		uridine 5'-monophosphate (UMP)	C00105	HMDB00288	1.62	1.39	1.15	0.87	1.59
Cofactors and vitamins	Hemoglobin and porphyrin metabolism	porphobilinogen	C00931	HMDB00245	0.58	0.81	0.69	0.96	0.85
		5-aminolevulinate	C00430	HMDB01149	0.64	1.15	0.48	0.83	1.39
	Nicotinate and nicotinamide metabolism	nicotinamide	C00153	HMDB01406	0.86	0.78	0.71	0.56	1.37
		nicotinamide ribonucleotide (NMN)		HMDB00229	0.59	0.78	1.5	1.01	0.77
		nicotinamide adenine dinucleotide (NAD+)	C00003	HMDB00902	0.98	1.25	0.99	0.92	1.35
		nicotinamide adenine dinucleotide reduced (NADH)	C00004	HMDB01487	1.26	1.03	1.14	1.22	0.85
		nicotinate	C00253	HMDB01488	1.01	0.85	1.17	1.07	0.79
	Pantothenate and CoA metabolism	pantothenate	C00864	HMDB00210	0.83	0.68	0.87	0.93	0.73
		phosphopantheine			1.04	0.91	1.17	0.92	1.00
	Pyridoxal metabolism	pyridoxal	C00250	HMDB01545	0.96	0.73	1.24	1.05	0.69
	Riboflavin metabolism	riboflavin (Vitamin B2)	C00255	HMDB00244	1.29	0.99	1.29	1.21	0.83
	Thiamine metabolism	thiamin (Vitamin B1)	C00378	HMDB00235	0.97	0.79	1.44	1.09	0.72
		5-(2-Hydroxyethyl)-4-methylthiazole	C04294		1.43	1.14	1.16	1.02	1.11
	Vitamin B6 metabolism	pyridoxine (Vitamin B6)	C00314	HMDB02075	1.03	0.95	1.16	1.12	0.85
Xenobiotics	Chemical	glycerol-2-phosphate	C02979	HMDB02520	1.02	1	1.36	1.27	0.79
	Drug	citramalate	C02612	HMDB00426	0.52	1.11	1.26	1.16	0.95
No Pathway	No Pathway	pyridine-4-carboxaldoxime			0.95	0.66	0.9	0.81	0.82

Sample Group	Growth Condition	Number of Samples
A	100 mM Iron	6
B	10 mM Iron, 4 hrs	6
C	10 mM Iron, 18 hrs	6
D	300 mM Iron, 4 hrs	6
E	300 mM Iron, 18 hrs	6

Data presented are paired comparisons as indicated and represent fold-change of mean values normalized to total protein of sample. Values with p<0.05 indicated in red or green box, data with p>0.05 and <0.1, blue text.

Green box indicates decreased fold-change, red indicates increased fold-change.

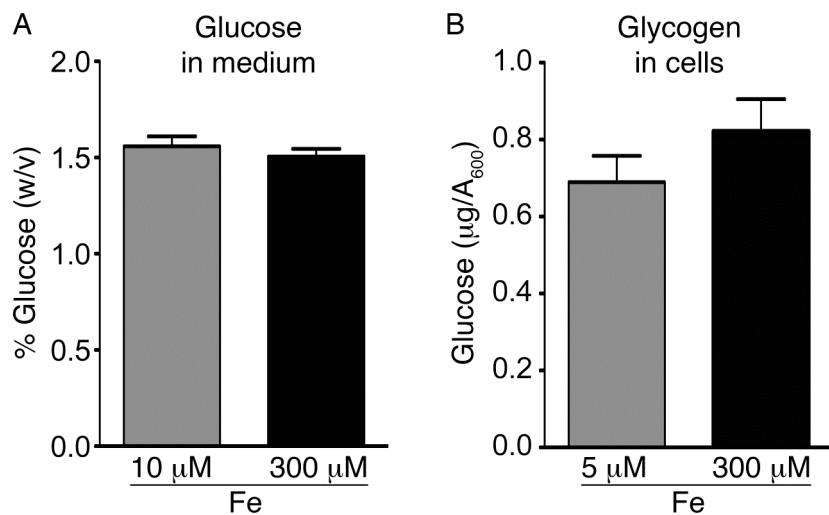
Table S2. Regulation of respiration-related transcripts in iron deficiency.

Respiration Transcripts Down-Regulated in Iron Deficiency		
Gene	Fold Change	Function
CYC1	13.25 <sup>1</sup>	Cytochrome c, isoform 1
CYT1	6.52	Cytochrome c1
CYC7	5.61	Cytochrome c isoform 2
COA2	4.72	Cytochrome oxidase assembly factor
RIP1	4.43	Ubiquinol-cytochrome-c reductase
YJL045W	3.70	Minor succinate dehydrogenase isozyme
SDH4	3.54	Membrane anchor subunit of succinate dehydrogenase
QCR10	3.42	Subunit of the ubiquinol-cytochrome c oxidoreductase
SDH3	3.41	Cytochrome b subunit of succinate dehydrogenase
COX6	2.82	Subunit VI of cytochrome c oxidase
QCR2	2.64	Subunit 2 of the ubiquinol cytochrome-c reductase
QCR8	2.59	Subunit 8 of ubiquinol cytochrome-c reductase complex
NDI1	2.52	NADH:ubiquinone oxidoreductase
COR1	2.46	Core subunit ubiquinol-cytochrome c reductase
QCR7	2.42	Subunit 7 of the ubiquinol cytochrome-c reductase
CBP4	2.34	Required for assembly of ubiquinol cytochrome-c reductase
CBP6	2.24	Mitochondrial translational activator of the COB mRNA
COX4	2.24	Subunit IV of cytochrome c oxidase
QCR9	2.03	Subunit 9 of the ubiquinol cytochrome-c reductase
CYB2	2.00	Cytochrome b2 (L-lactate cytochrome-c oxidoreductase)
COX5A	1.93	Subunit Va of cytochrome c oxidase
COX8	1.91	Subunit VIII of cytochrome c oxidase
CBP3	1.83	Required for assembly of ubiquinol cytochrome-c reductase
CYT2	1.85	Cytochrome c1 heme lyase
PET100	1.80	Assembly of cytochrome c oxidase
COX15	1.78	Hydroxylation of heme O to form heme A
RSF1	1.68	Protein required for respiratory growth
COX7	1.66	Subunit VII of cytochrome c oxidase
PET20	1.63	Required for respiratory growth under some conditions
COX17	1.55	Copper metallochaperone for Sco1p and Cox11p

TCA Cycle Transcripts Down-Regulated in Iron Deficiency		
Gene	Fold Change	Function
ACO1	1.71	Mitochondrial aconitase
ACO2	1.91	Putative mitochondrial aconitase isozyme
KGD1	1.64	Component of mitochondrial alpha-ketoglutarate dehydrogenase
SDH1	7.07	Flavoprotein subunit of succinate dehydrogenase
SDH2	5.01	Iron-sulfur protein subunit of succinate dehydrogenase
SDH3	3.41	Cytochrome b subunit of succinate dehydrogenase
SDH4	3.54	Membrane anchor subunit of succinate dehydrogenase

<sup>1</sup> Arrays were performed in triplicate and the average fold change is shown. Transcripts with 1.5-fold or greater change and p < 0.05 shown.

Figure S2.



**Figure S2. Lack of glucose depletion in medium and lack of change in glycogen storage in iron deficient cells.** (A) No depletion of glucose in medium. Glucose levels in conditioned medium from steady state, low- and high-iron cells grown for metabolite analysis (Figure S1) was measured. (B) Similar levels of cellular glycogen in iron-deficient and iron-replete cells. Cells were grown in defined-iron medium containing 5  $\mu\text{M}$  or 300  $\mu\text{M}$  ferrous iron and glycogen levels measured. Error bars indicate SEM.

Table S3. Regulation of amino acid-related transcripts in iron deficiency.

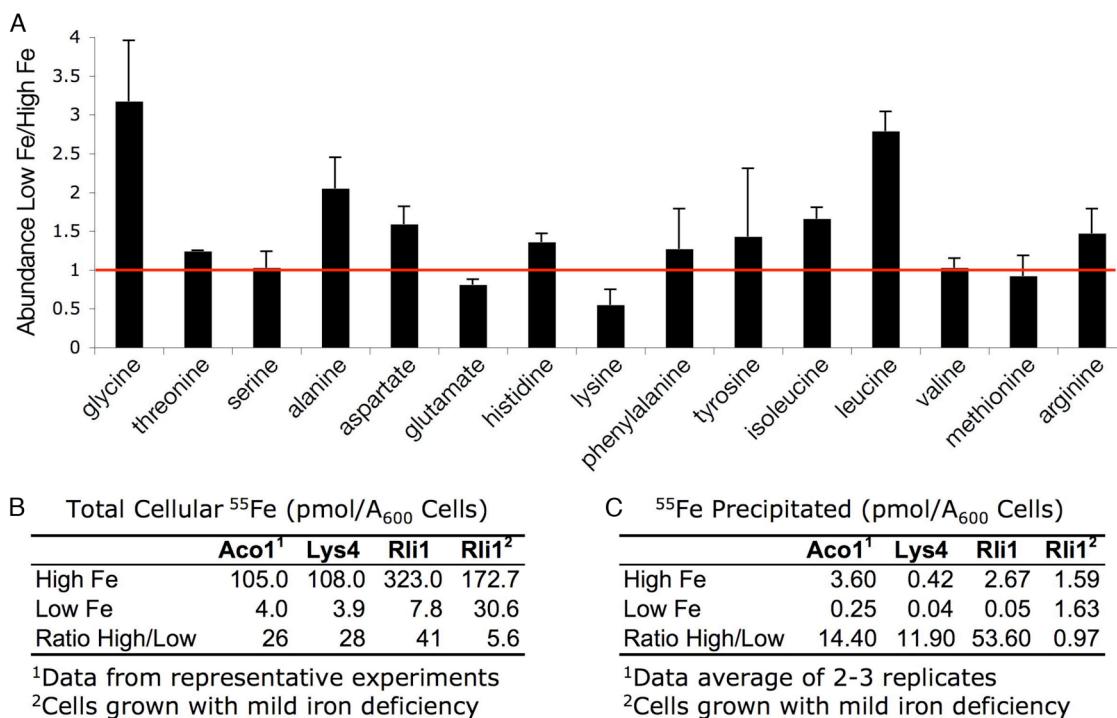
Amino Acid Transcripts Up-Regulated in Iron Deficiency			
Gene	Fold Change	Function	Amino Acid
PTR3	1.75 <sup>1</sup>	Amino acid sensor	Amino acids
ARG80	1.61	Transcription factor ARG genes	Arg
ARG3	1.55	Ornithine carbamoyltransferase	Arg
VBA3	3.51	Vacuolar basic amino acid permease	Arg, Lys
ALP1	1.87	Arginine transporter	Arg, Lys
GTO3	11.80	Omega class glutathione transferase	Cys, Met
YCT1	3.98	Cysteine-specific transporter	Cys, Met
ECM4	2.89	Omega class glutathione transferase	Cys, Met
MET2	2.52	L-homoserine-O-acetyltransferase	Cys, Met
MMP1	2.13	S-methylmethionine permease	Cys, Met
BDS1	1.87	Sulfatase	Cys, Met
SUL2	1.77	Sulfate permease	Cys, Met
LAP3	1.74	Cysteine aminopeptidase	Cys, Met
STR3	1.54	Cystathionine beta-lyase	Cys, Met
SNO1	2.60	Glutamine amido transferase	Gln
GDH3	4.52	Glutamate dehydrogenase	Glu
TMT1	4.73	Trans-aconitate methyltransferase	Leu
OAC1	1.72	Mitochondrial carrier, isopropylmalate transport	Leu
BAT2	2.42	Branched-chain amino acid aminotransferase	Leu, Ile, Val
BAP2	12.93	Leucine permease	Leu, Ile, Val
NMA2	2.00	Nicotinic acid mononucleotide adenylyltransferase	Trp
HBN1	5.34	Nitroreductases	Tyr, Phe, Trp
FRM2	4.18	Nitroreductases	Tyr, Phe, Trp

Amino Acid Transcripts Down-Regulated in Iron Deficiency			
Gene	Fold Change	Function	Amino Acid
<b>ACO1<sup>2</sup></b>	1.71	Aconitase	Glu
<b>GLT1</b>	5.97	NAD(+-)dependent glutamate synthase (GOGAT)	Glu
<b>ILV3</b>	1.57	Dihydroxyacid dehydratase	Leu, Ile, Val
<b>MET5</b>	1.57	Sulfite reductase beta subunit	Cys, Met
<b>LEU1</b>	1.50	Isopropylmalate isomerase	Leu
<b>LYS4</b>	2.12	Homoaconitase	Lys
PUT4	1.81	Proline permease	Pro
ARO10	1.61	Phenylpyruvate decarboxylase	Leu, Ile, Val, Met, Tyr, Phe, Trp

<sup>1</sup>Arrays were performed in triplicate and the average fold change is shown. Transcripts with 1.5-fold or greater change and p < 0.05 shown.

<sup>2</sup>Genes in bold face encode Fe-S cluster proteins.

Figure S3.



**Figure S3. Lack of amino acid deficiencies in iron-deficient cells despite loss of iron-sulfur clusters.** (A) Cells were grown in defined-iron medium containing 5-10  $\mu\text{M}$  (Low Iron) or 300  $\mu\text{M}$  (High Iron) ferrous iron for 24 hrs. Levels of amino acids were measured and expressed as a ratio. Values greater than 1 indicate accumulation in iron-deficient cells. Error bars indicate SEM. (B) Total cellular iron in cells labeled with  $^{55}\text{Fe}$  and (C) Iron bound to Fe-S enzymes from cells labeled with  $^{55}\text{Fe}$ . Strains expressing Aco1-TAP, Lys4-TAP, and Rli1-TAP were labeled with  $^{55}\text{Fe}$  in iron-sufficient medium, then cells were re-innoculated into either iron-chelated or iron-replete medium containing  $^{55}\text{Fe}$  of the same concentration and specific activity and grown for an additional 7 generations.