

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 |
|---------------------------------------|--|---------------------------------|-----------|-----------|------------------------------|----------------------|----------------------------------|--------------------------|----------------------|
| | | | | | B/A | C/A | D/A | E/A | C/E |
| 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 |
| | | cystathionine | C02291 | HMDB00099 | 1.49 | 0.75 | 1.93 | 1.3 | 0.58 |
| | | S-adenosylmethionine (SAM) | C00019 | HMDB01185 | 1.31 | 1.49 | 1.67 | 0.8 | 1.85 |
| | | S-adenosylhomocysteine (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 |
| | | argininosuccinate | C03406 | HMDB01006 | 1.08 | 2.35 | 1.71 | 0.82 | 2.86 |
| | Creatine metabolism | creatine | C00300 | HMDB00064 | 0.63 | 1.31 | 0.49 | 0.91 | 1.43 |
| Butanoate metabolism | 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 | |

| | | | | | | | | | |
|-------------|--|---|--------------|-----------|-----------|------|------|------|------|
| | | 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 |
| | Pyrimidine metabolism, uracil containing | uracil | C00106 | HMDB00300 | 0.77 | 0.58 | 0.9 | 0.72 | 0.81 |
| | | 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 |
| | Hemoglobin and porphyrin metabolism | porphobilinogen | C00931 | HMDB00245 | 0.58 | 0.81 | 0.69 | 0.96 | 0.85 |
| | | 5-aminolevulinatate | 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 |
| | | phosphopantetheine | | | 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 ¹ | 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 |

¹Arrays 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.

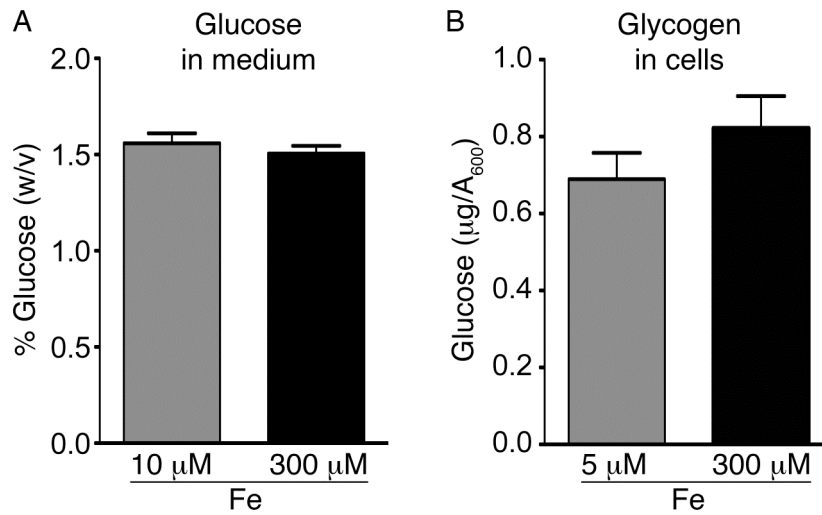


Figure S2. Lack of glucose depletion in medium and lack of change in glycogen storage in iron deficient cells. (A) No depletion of glucose in medium. Glucose levels in conditioned medium from steady state, low- and high-iron cells grown for metabolite analysis (Figure S1) was measured. (B) Similar levels of cellular glycogen in iron-deficient and iron-replete cells. Cells were grown in defined-iron medium containing 5 μM or 300 μM 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 ¹ | 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 |
| ACO1 ² | 1.71 | Aconitase | Glu |
| GLT1 | 5.97 | NAD(+)-dependent glutamate synthase (GOGAT) | Glu |
| ILV3 | 1.57 | Dihydroxyacid dehydratase | Leu, Ile, Val |
| MET5 | 1.57 | Sulfite reductase beta subunit | Cys, Met |
| LEU1 | 1.50 | Isopropylmalate isomerase | Leu |
| LYS4 | 2.12 | Homoaconitase | Lys |
| PUT4 | 1.81 | Proline permease | Pro |
| | | | Leu, Ile, Val, Met, Tyr, Phe, |
| ARO10 | 1.61 | Phenylpyruvate decarboxylase | Trp |

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

²Genes in bold face encode Fe-S cluster proteins.

Figure S3.

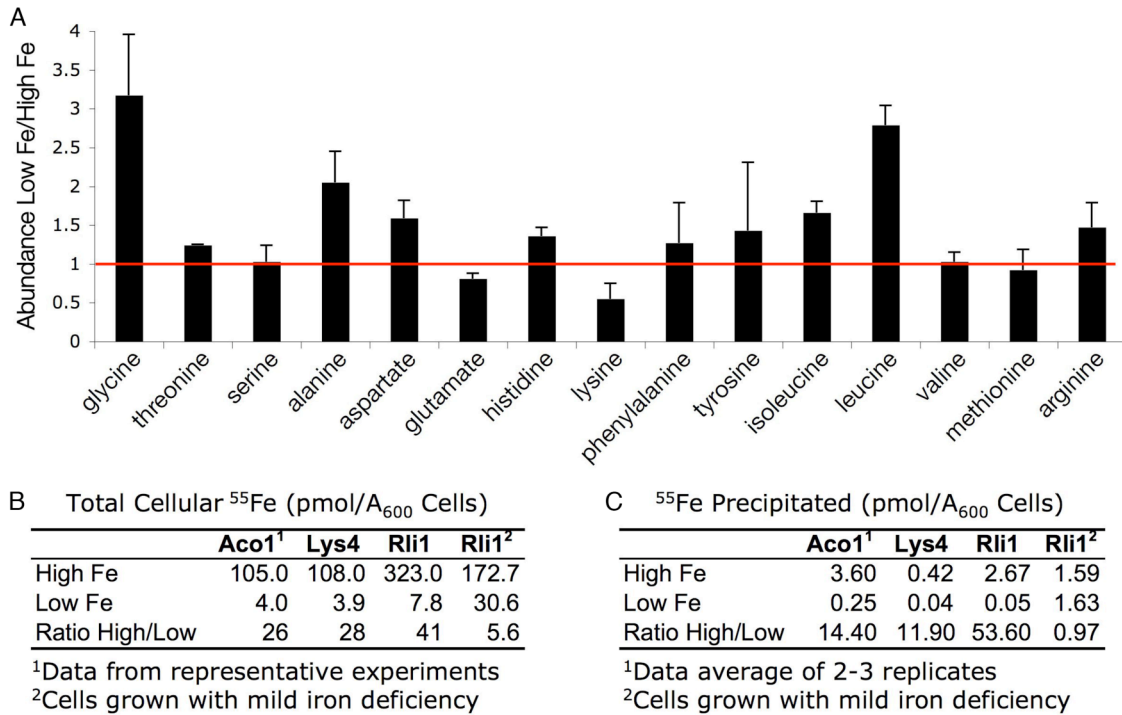


Figure S3. Lack of amino acid deficiencies in iron-deficient cells despite loss of iron-sulfur clusters. (A) Cells were grown in defined-iron medium containing 5-10 μM (Low Iron) or 300 μM (High Iron) ferrous iron for 24 hrs. Levels of amino acids were measured and expressed as a ratio. Values greater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