Section 1: New biological insights for "iron responsive genes" and "iHKG" identified in this study (Supplementary Note S7)

1) Iron responsive genes in the light of other datasets from previously published works

To assess the relevance of our list of iron responsive genes (637 genes, **Supplementary Data S2**), we collected from the GEO database the transcriptomics data presented in Gerwien *et al.* (2016)[1]. Starting from the raw datasets available on the GEO webpage[2] (accession number GSE84816), we used the GEO2R tool[3] to compare the four different groups of samples as described in the original article of Gerwien *et al.* 2016 (**Figure S7.1**, below). These groups are named "WT 4h vs WT 0h", "*aft1Δ* 4h vs WT 4h", "*sef1Δ* 4h vs WT 4h" and "*ftr1Δ* 4h vs WT 4h". We retrieved for each gene the final LogFC values (identical to those presented **Figure S7.1**) and the statistical parameters for differential expressions which were calculated with the LIMMA R package. Detailed output from GEO2R are available here (GitHub repository). Note that with GEO2R, the exact same protocol as the one we used to search for differentially expressed genes in our own transcriptomics data was applied (see **Material & Methods** in the main text).

In our study, "iron responsive genes" are genes which we observed to be differentially expressed in at least one of the conditions C1, C2, C3 or C4. "Iron homeostasis key genes" (iHKG) are subsets of iron responsive genes, which are de-regulated in conditions (C1 or C2) and (C3 or C4). Our hypothesis is that these genes have functions important for the cell to counterbalance external fluctuations in iron availability, in any direction (low or high). In that respect, "Type I" are iHKG with opposite deregulation in low and high iron conditions and "Type II" are iHKG with constant (or parallel) deregulation in low and high iron conditions (see **Figure 1**, main text).

For all these genes, we searched for differential expression in the Gerwien et al. datasets (2016) and verified if they were cited in the review of Devaux et al. (2019)[4]. Results are presented **Table S7.1** (below). From our list of 637 iron responsive genes, 478 (75%) were found to be differentially expressed in the dataset "WT 4h vs WT 0h" (adjusted p-value lower than 0.01), 223 (35%) were found to be differentially expressed in the dataset "*aft1Δ* 4h vs WT 4h", 1 gene was found to be differentially expressed in dataset "*sef1Δ* 4h vs WT 4h" (gene CAGL0D06424g|ACO1) and 4 were found to be differentially expressed in dataset "*ftr1Δ* 4h vs WT 4h" (CAGL0E01727g|YPS3, CAGL0I01408g|CYC1, CAGL0I06050g|INO1 and CAGL0I06743g|FTR1). Additionally, 51 genes were cited in the review of Devaux et al. (2019). The detailed lists of genes in each category in available in **Supplementary Data S2** (available here, GitHub repository). Altogether, we could not find significant information for only 110 genes, which represents 17% of our list of iron responsive genes. If the analysis is restricted to iHKG of Type I and II, we found 27 genes (10 of Type I and 17

[1] A Novel Hybrid Iron Regulation Network Combines Features from Pathogenic and Nonpathogenic Yeasts. Gerwien F, Safyan A, Wisgott S, Hille F, Kaemmer P, Linde J, Brunke S, Kasper L, Hube B. mBio. 2016 Oct 18;7(5). pii: e01782-16. doi: 10.1128/mBio.01782-16. PMID: 27795405

[2] https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE84816

[3] https://www.ncbi.nlm.nih.gov/geo/info/geo2r.html

[4] The regulation of iron homeostasis in the fungal human pathogen Candida glabrata. Devaux F, Thiébaut A. Microbiology. 2019 Oct;165(10):1041-1060. doi: 10.1099/mic.0.000807. Epub 2019 May 3. PMID: 31050635

of Type II) with no reference (**Table S7.2**, below). This demonstrates the overall consistency of our results with emblematic studies previously published in the field.

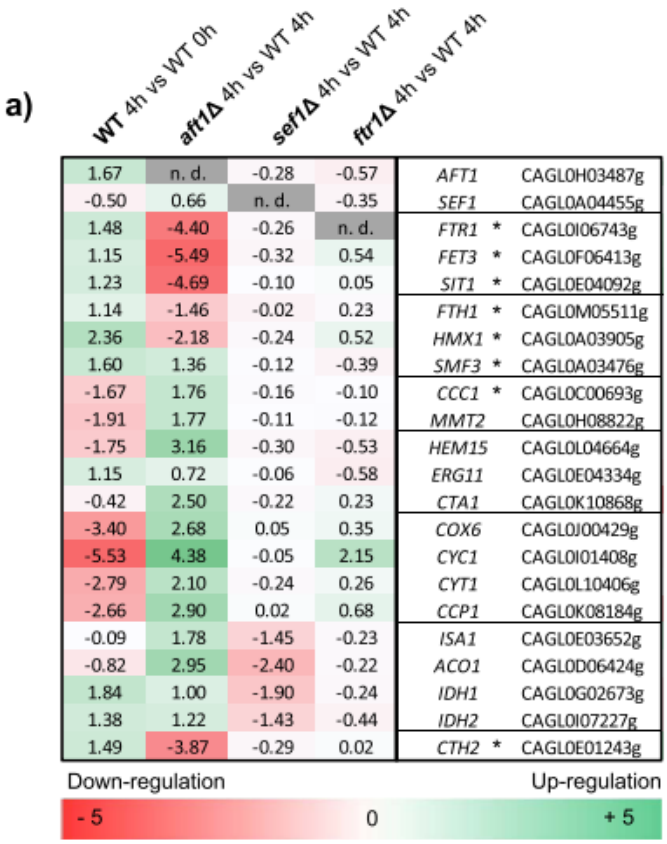

Figure S7.1: Screenshot of the figure that presents the transcriptomics results, in the original article of Gerwien et al. (2016). Overall, transcriptomics data comprises four groups of comparisons named "WT 4h vs WT 0h", "*aft1Δ* 4h vs WT 4h", "*sef1Δ* 4h vs WT 4h" and "*ftr1Δ* 4h vs WT 4h". They are shown here in columns. Rows correspond to a subset of iron-associated genes (see Gerwien et al. (2016) for more details). The numerical values correspond to LogFC values quantifying the relative mRNA levels of genes in the wildtype (WT) strain (4 hours of iron starvation versus 0 hour) or in the strain deleted for genes AFT1 (Δaft1), SEF1 (Δsef1), FTR1 (Δftr1) (4 hours of iron starvation in the deleted strains versus 4 hours of iron starvation in the WT strain). These values were reproduced with GEO2R, starting from the microarray raw data available in the GEO database (accession number GSE81816). LogFC values for all genes together with LIMMA statistical output parameters can be found here.

	WT 4h vs WT 0h (Gerwien et al. 2016)	**aft1Δ 4h vs WT 4h (Gerwien et al. 2016)**	**sef1Δ 4h vs WT 4h (Gerwien et al. 2016)**	**ftr1Δ 4h vs WT 4h (Gerwien et al. 2016)**	**Cited in Devaux et al. (2019)**	**No reference in other datasets**
iron responsive genes	478	223	1	4	51	110
iHKG - Type I	55	45	1	1	17	10
iHKG - Type II	116	37	0	2	5	17

Table S7.1: Iron responsive genes in other datasets. Number of genes identified in our study (637 iron responsive genes, 73 iHKG – Type I and 141 iHKG – Type II) which are differentially expressed in Gerwien *et al.* datasets (2016) or cited in the review of Devaux *et al.* (2019). The data can be queried with the interactive viewer : https://thomasdenecker.github.io/iHKG/OtherDatasets.html.

ORF	Gene name CGD	Description (CGD)	Orthologous gene (SCERE)	Functional category (this study)	Subcategory (this study)	iHKG Type
CAGL0J04466g		Ortholog(s) have role in fungal-type cell wall organization, protein complex oligomerization and cell cortex of cell tip, membrane raft, plasma membrane localization	PUN1	MEMBRANE / CELL WALL	_	Type I
CAGL0L00759g	HIS1	ATP phosphoribosyltransferase; protein abundance increased in ace2 mutant cells		METABOLISM	AMINO ACID METABOLISM	Type I
CAGL0J03212g	ALD5	Putative mitochondrial aldehyde dehydrogenase (NAD+); protein abundance increased in ace2 mutant cells	ALD5	REDOX SIGNALING	NAD(P)/NAD(P)H DEPENDANT ENZYME	Type I
CAGL0K06259g	TSA1	Thiol-specific antioxidant protein; predicted thioredoxin peroxidase involved in oxidative stress response; protein abundance decreased in ace2 mutant cells	TSA2	REDOX SIGNALING	THIOL REDOX	Type I
CAGL0M05995g		Ortholog(s) have lipid droplet localization	PET10	REDOX SIGNALING	METABOLISM	Type I
CAGL0H09592g		Putative GPI-linked cell wall protein		STRESS RESPONSE	_	Type I
CAGL0H09614g		Putative GPI-linked cell wall protein	TIR1	STRESS RESPONSE	_	Type I
CAGL0A01199g	DIP5	Putative dicarboxylic amino acid permease	DIP5	TRANSPORT / TRAFFICKING	PLASMA MEMBRANE	Type I
CAGL0D02640g		Has domain(s) with predicted transmembrane transporter activity, role in transmembrane transport and integral component of membrane, membrane localization		TRANSPORT / TRAFFICKING	PLASMA MEMBRANE	Type I
CAGL0I10147g	PWP1	Protein with 32 tandem repeats; putative adhesin-like protein; belongs to adhesin cluster II		UNCLASSIFIED	_	Type I
CAGL0J00649g		Ortholog(s) have homoserine kinase activity and role in homoserine metabolic process, threonine biosynthetic process	THR1	IRON-SULFUR CLUSTER SYNTHESIS AND ASSEMBLY	_	Type II
CAGL0K06677g	MET8	Putative bifunctional dehydrogenase and ferrochelatase; gene is upregulated in azole-resistant strain	MET8	IRON-SULFUR CLUSTER SYNTHESIS AND ASSEMBLY	_	Type II
CAGL0I06644g		Putative GPI-linked cell wall protein	SPI1	MEMBRANE / CELL WALL	_	Type II

CAGL0L01551g		Ortholog(s) have role in ascospore formation, cellular response to biotic stimulus, cellular response to chemical stimulus, cellular response to glucose starvation and cellular response to neutral pH, more	SUR7	MEMBRANE / CELL WALL	_	Type II
CAGL0A04829g		Putative hexokinase isoenzyme 2; protein differentially expressed in azole resistant strain	HXK1	METABOLISM	ENERGY PRODUCTION (NON RESPIRATORY)	Type II
CAGL0E05654g		Ortholog(s) have phosphatidylglycerol phospholipase C activity, role in cell-abiotic substrate adhesion, glycerophospholipid catabolic process, phosphatidylglycerol catabolic process and lipid droplet, mitochondrion localization	PGC1	METABOLISM	FATTY ACID AND LIPIDS	Type II
CAGL0G01826g		Ortholog(s) have role in ribosomal large subunit assembly and cytosolic large ribosomal subunit localization	RPL11A	METABOLISM	RIBOSOMAL BIOGENESIS	Type II
CAGL0G09064g		Ortholog(s) have role in glycerol biosynthetic process and cytosol, nucleus localization	YIG1	METABOLISM	ENERGY PRODUCTION (NON RESPIRATORY)	Type II
CAGL0G09130g		Ortholog(s) have role in maturation of LSU-rRNA, ribosomal large subunit biogenesis and cytoplasm, cytosolic large ribosomal subunit, nucleolus localization	RPL7B	METABOLISM	RIBOSOMAL BIOGENESIS	Type II
CAGL0J03234g		Ortholog(s) have role in maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	RPS24A	METABOLISM	RIBOSOMAL BIOGENESIS	Type II
CAGL0M12551g		Ortholog(s) have role in energy reserve metabolic process	RGI2	REDOX SIGNALING	METABOLISM	Type II
CAGL0E01991g		Ortholog(s) have structural constituent of ribosome activity, role in rRNA export from nucleus, ribosomal small subunit biogenesis and cytosolic small ribosomal subunit localization	RPS19A	REGULATION	TRANSLATION	Type II
CAGL0G08019g		Ortholog(s) have role in cellular response to biotic stimulus, cellular response to starvation and filamentous growth of a population of unicellular organisms in response to biotic stimulus, more	YDR090C	TRANSPORT / TRAFFICKING	PLASMA MEMBRANE	Type II
CAGL0F04191g		Ortholog of S. cerevisiae : YBL029C-A, C. albicans SC5314 : C1_02060W_A, C. dubliniensis CD36 : Cd36_01920, C. parapsilosis CDC317 : CPAR2_108200 and C. auris B8441 : B9J08_000493	YBL029C-A	UNCLASSIFIED	_	Type II

CAGL0I04328g		Ortholog of S. cerevisiae : YJL133C-A and Saccharomyces cerevisiae S288C : YJL133C-A	YJL133C-A	UNCLASSIFIED	_	Type II
CAGL0K00110g	AWP2	Putative adhesin; identified in cell wall extracts by mass spectrometry; belongs to adhesin cluster V; predicted GPI-anchor		UNCLASSIFIED	_	Type II
CAGL0K07205g		Protein of unknown function		UNCLASSIFIED	_	Type II

Table S7.2: Detailed list of genes identified as iHKG (Type I and Type II) and for which no reference was found in Gerwien et al. (2016) or Devaux et al. (2019).

The biological consistency of this list of genes is interesting. We found genes with functions related to plasma membrane and cell wall organization (e.g. CAGL0J04466g, CAGL0A06644g, CAGL0L01551g), redox activities (CAGL0J03212g|ALD5, CAGL0K06259g|TSA1, CAGL0M05995g) and iron-sulfur clusters (CAGL0J00649g and CAGL0K06677g|MET8). We were also able to find two genes, CAGL0A01199g|DIP5 and CAGL0K06259g|TSA1, with a DNA binding site (TGCACCC) for the transcription factor Aft1 (see the next section). These observations merit additional experiments to be further investigated, but to our knowledge, it is the first time these genes are functionally described as part of the "Iron regulon" of *Candida glabrata*, on the basis of experiments carried out directly in this species (no information was transferred from the model yeasts *S. cerevisiae* and *C. albicans*).

2) Search for potential target genes of the transcription factor Aft1 in iron responsive genes

The transcription factor Aft1 is well-described in the literature, as one of the main regulators of iron homeostasis in *C. glabrata*[5]. To search for potential target genes of Aft1 in our list of iron responsive genes, we used the datasets “WT 4h vs WT 0h” and “*aft1Δ* 4h vs WT 4h” (see previous paragraph). These datasets are very complementary since they allow (*i*) to identify the genes whose expression is modified in the wild type strain at 4 h 00 of iron deficiency, and (*ii*) to identify the genes whose expression is affected by the deletion of the gene encoding the transcription factor Aft1. In this context, the genes which are differentially expressed in both datasets are good candidates to be direct targets of the Aft1 transcription factor.

Figure S7.2 (below) shows the number of iron responsive genes and iHKG (Type I and II) in each category. It is interesting to observe that more than a quarter (27%) of the iron responsive genes are identified in the WT dataset and in the *aft1Δ* dataset. Notably, this percentage decreases to 20% considering iHKG – Type II but increases to 50% considering only the iHKG – Type I. This observation underlines a specificity for iHKG - Type I regarding the underlying transcriptional regulatory processes. To go further in this observation, we searched for enriched DNA motifs in promoter sequences of iHKG – Type I genes in the one hand, and iHKG – Type II genes on the other hand. We applied the RSAT web tool with default parameters. Pictures of the motifs ranked at first positions by RSAT in iHKG - Type I and iHKG - Type II are presented in **Figure S7.3** (below).

[5] The regulation of iron homeostasis in the fungal human pathogen Candida glabrata. Devaux F, Thiébaut A. Microbiology. 2019 Oct;165(10):1041-1060. doi: 10.1099/mic.0.000807. Epub 2019 May 3. PMID: 31050635

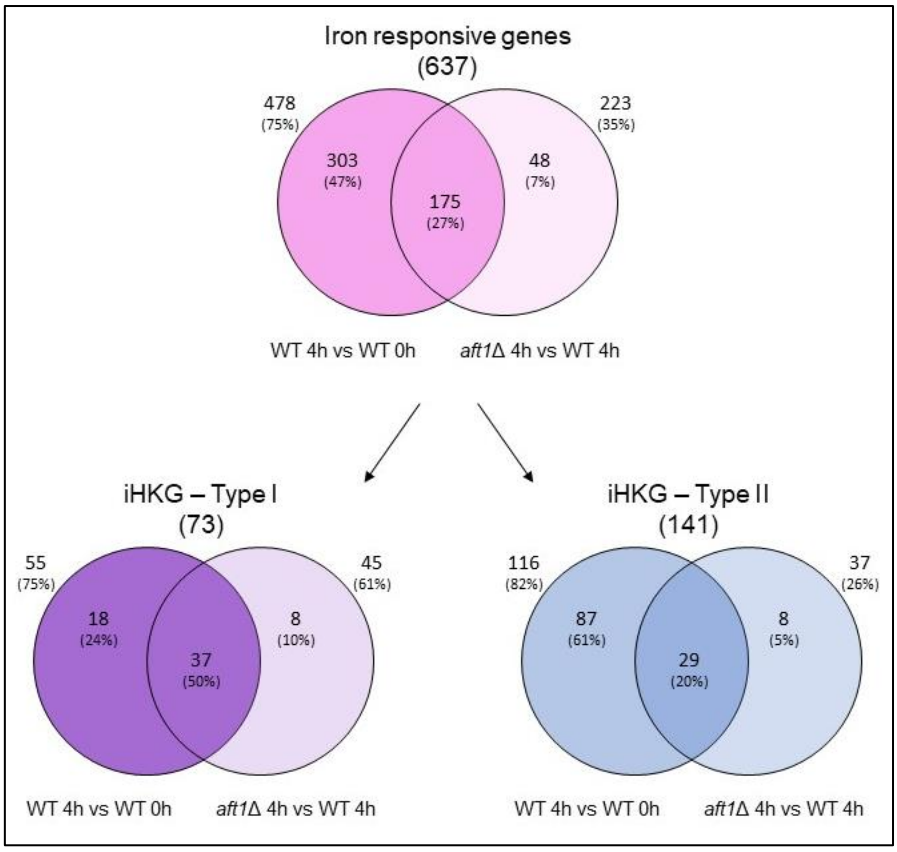

Figure S7.2: Identification of potential target genes of the transcription factor Aft1. Venn diagram showing the number of genes, which are differentially expressed in the datasets "WT 4h vs WT 0h" and "aft1Δ 4h vs WT 4h". Typically, a target gene of Aft1 should exhibit differential expression in both datasets.

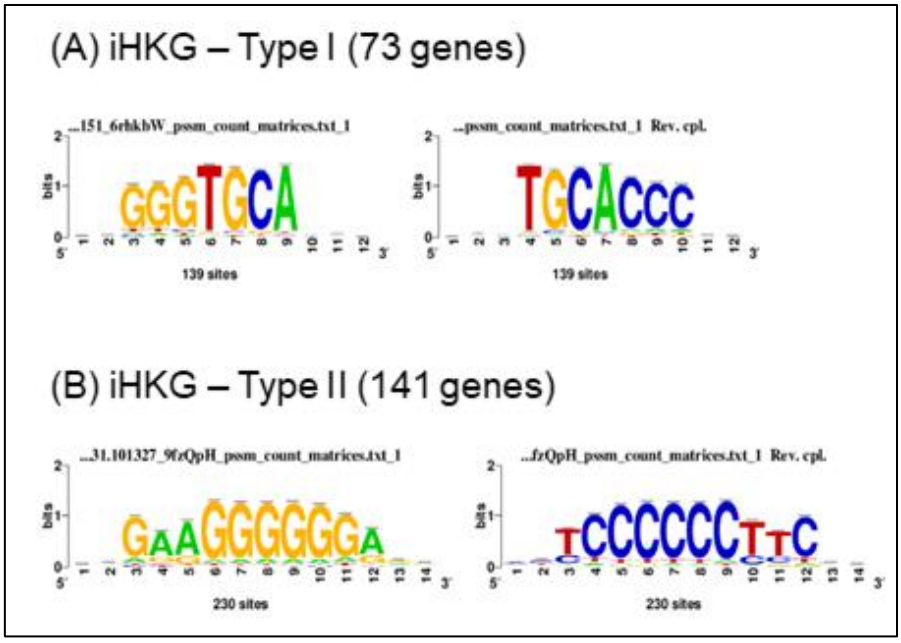

Figure S7.3: PSSM count matrices ranked at first position by RSAT, analysing promoter sequences (1 kb) of iHKG – Type I (A) or iHKG – Type II (B). Detailed results are available here (GitHub repository). Full documentation as well as tutorials for RSAT can be found at http://pedagogix-tagc.univ-mrs.fr/rsat/.

In agreement with our previous observations, the motifs identified for the two sets of iHKG are different. In type II genes the first ranked motif comprises the sequence AGGG which is the STRE element, *i.e.* the DNA binding site of Msn2[6] and Msn4[7] transcription factors, whereas in Type I genes the first ranked motif is TGCACCC (and its reverse complement GGGTGCA) that corresponds precisely to the DNA binding site of Aft1[8].

To conclude this part, our analyses highlight the very important role of the transcription factor Aft1 in the underlying regulatory control of iHKG - Type I genes.

3) Enhancement of the interactive web viewer, allowing the interrogation of multi-omics datasets (transcriptomics and proteomics)

Recently (Denecker et al., 2019[9]), we analysed several "omics" datasets (transcriptomics and proteomics) obtained in *C. glabrata* under alkaline pH conditions. The transcriptomics datasets were collected from Linde et al. (2015)[10] and proteomics datasets from Lelandais et al. (2019)[11]. Transcriptomics and proteomics provide interesting complementary multi-omics information. Also, it is known that under alkaline pH conditions, iron solubility is reduced. The adaptation of cells to an alkaline pH should therefore require (at least in part) an adaptation to iron starvation. In this context, we thought it was interesting to explore the "iron responsive genes" in this multi-omic data. With this idea in mind, we created a web page in the interactive web viewer: https://thomasdenecker.github.io/iHKG/OtherDatasets.html.

This page facilitates queries of these additional data sets (**Figure S7.2**, below). As an illustration, we searched for the list of genes presented **Table S7.2** in proteomics (mass spectrometry) and transcriptomics (RNAseq) datasets (Dataset 3 and Dataset 4, **Figure S7.2)**. We observed important deregulations (logFC > 1 or logFC < -1) for 10 genes (**Table S7.3**, below). Again, further experimental investigations are required to fully validate these observations, but such cross investigation of multi-omics datasets can provide a strong indication of the potential role of the genes, as good candidates to be involved in iron homeostasis processes in the pathogenic yeast *Candida glabrata*.

[6] http://www.yeastract.com/view.php?existing=protein&proteinname=Msn2p
[7] http://www.yeastract.com/view.php?existing=protein&proteinname=Msn4p
[8] http://www.yeastract.com/view.php?existing=protein&proteinname=Aft1p

[9] Pixel: a content management platform for quantitative omics data. Denecker T, Durand W, Maupetit J, Hébert C, Camadro JM, Poulain P, Lelandais G. PeerJ. 2019 Mar 27;7:e6623. doi: 10.7717/peerj.6623. eCollection 2019. PMID: 30944779

[10] Defining the transcriptomic landscape of Candida glabrata by RNA-Seq. Linde J, Duggan S, Weber M, Horn F, Sieber P, Hellwig D, Riege K, Marz M, Martin R, Guthke R, Kurzai O. Nucleic Acids Res. 2015 Feb 18;43(3):1392-406. doi: 10.1093/nar/gku1357. Epub 2015 Jan 13. PMID: 25586221

[11] Label-free quantitative proteomics in Candida yeast species: technical and biological replicates to assess data reproducibility. Lelandais G, Denecker T, Garcia C, Danila N, Léger T, Camadro JM. BMC Res Notes. 2019 Aug 1;12(1):470. doi: 10.1186/s13104-019-4505-8. PMID: 31370875

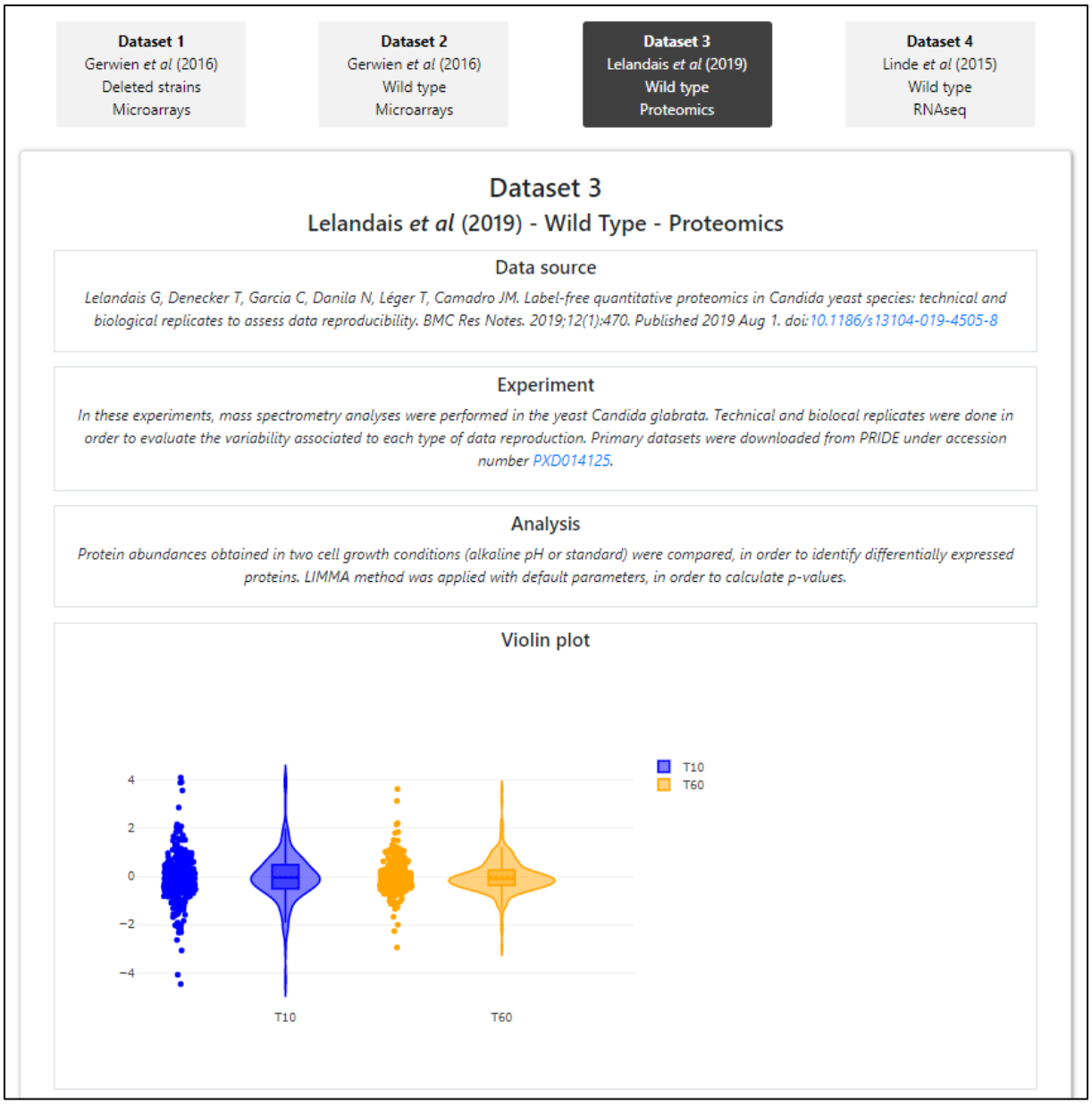

Figure S7.3: Screenshot of the page (iHKG viewer) to query iron responsive genes in other datasets. Four different datasets are available. Datasets 1 and 2 are transcriptomics results obtained with microarray technology, Dataset 3 is proteomics results obtained with mass spectrometry technology and Dataset 4 is transcriptomics results obtained with RNAseq technology. Data source, summary of experiments and analyses are explained on the webpage: https://thomasdenecker.github.io/iHKG/OtherDatasets.html.

Gene name (CGLAB)	Orthologous gene (SCERE)	Functional category (this study)	iHKG	T60 (proteomics, Lelandais et al.)	pH8 vs pH4 (RNAseq, Linde et al.)
CAGL0H09614g	TIR1	STRESS RESPONSE	Type I	#N/A	4.50
CAGL0H09592g		STRESS RESPONSE	Type I	#N/A	3.66
CAGL0G09064g	YIG1	METABOLISM	Type II	#N/A	3.37
CAGL0M12551g	RGI2	REDOX SIGNALING	Type II	#N/A	2.94
CAGL0K06259g	TSA2	REDOX SIGNALING	Type I	0.07	2.59

CAGL0I04328g	YJL133C-A	UNCLASSIFIED	Type II	#N/A	2.43
CAGL0A04829g	HXK1	METABOLISM	Type II	0.22	1.69
CAGL0L01551g	SUR7	MEMBRANE / CELL WALL	Type II	-1.24	1.00
CAGL0K06677g	MET8	IRON-SULFUR CLUSTER SYNTHESIS AND ASSEMBLY	Type II	-0.16	0.46
CAGL0K00110g		UNCLASSIFIED	Type II	-0.23	0.38
CAGL0A01199g	DIP5	TRANSPORT / TRAFFICKING	Type I	#N/A	0.34
CAGL0K07205g		UNCLASSIFIED	Type II	#N/A	0.30
CAGL0J03212g	ALD5	REDOX SIGNALING	Type I	-0.07	0.15
CAGL0G09130g	RPL7B	METABOLISM	Type II	-0.14	0.01
CAGL0E01991g	RPS19A	REGULATION	Type II	-0.04	-0.06
CAGL0J03234g	RPS24A	METABOLISM	Type II	-0.10	-0.12
CAGL0G01826g	RPL11A	METABOLISM	Type II	-0.16	-0.15
CAGL0F04191g	YBL029C-A	UNCLASSIFIED	Type II	#N/A	-0.17
CAGL0J00649g	THR1	IRON-SULFUR CLUSTER SYNTHESIS AND ASSEMBLY	Type II	0.27	-0.31
CAGL0M05995g	PET10	REDOX SIGNALING	Type I	0.50	-0.39
CAGL0D02640g		TRANSPORT / TRAFFICKING	Type I	#N/A	-0.43
CAGL0L00759g		METABOLISM	Type I	0.13	-0.52
CAGL0I10147g		UNCLASSIFIED	Type I	#N/A	-0.56
CAGL0J04466g	PUN1	MEMBRANE / CELL WALL	Type I	2.21	-0.78
CAGL0G08019g	YDR090C	TRANSPORT / TRAFFICKING	Type II	#N/A	-0.98
CAGL0E05654g	PGC1	METABOLISM	Type II	#N/A	-0.99
CAGL0I06644g	SPI1	MEMBRANE / CELL WALL	Type II	#N/A	-3.91

Table S7.3: Exploration of the list of genes presented Table S7.2 in multi-omics datasets (proteomics and RNAseq). Numerical values correspond to logFC values collected for the proteomics analyses described in Lelandais et al. (2019) and the RNAseq analyses described in Linde et al. (2015). More detailed information is provided at https://thomasdenecker.github.io/iHKG/OtherDatasets.html. Values > 1 or < - 1 are shown with grey background.