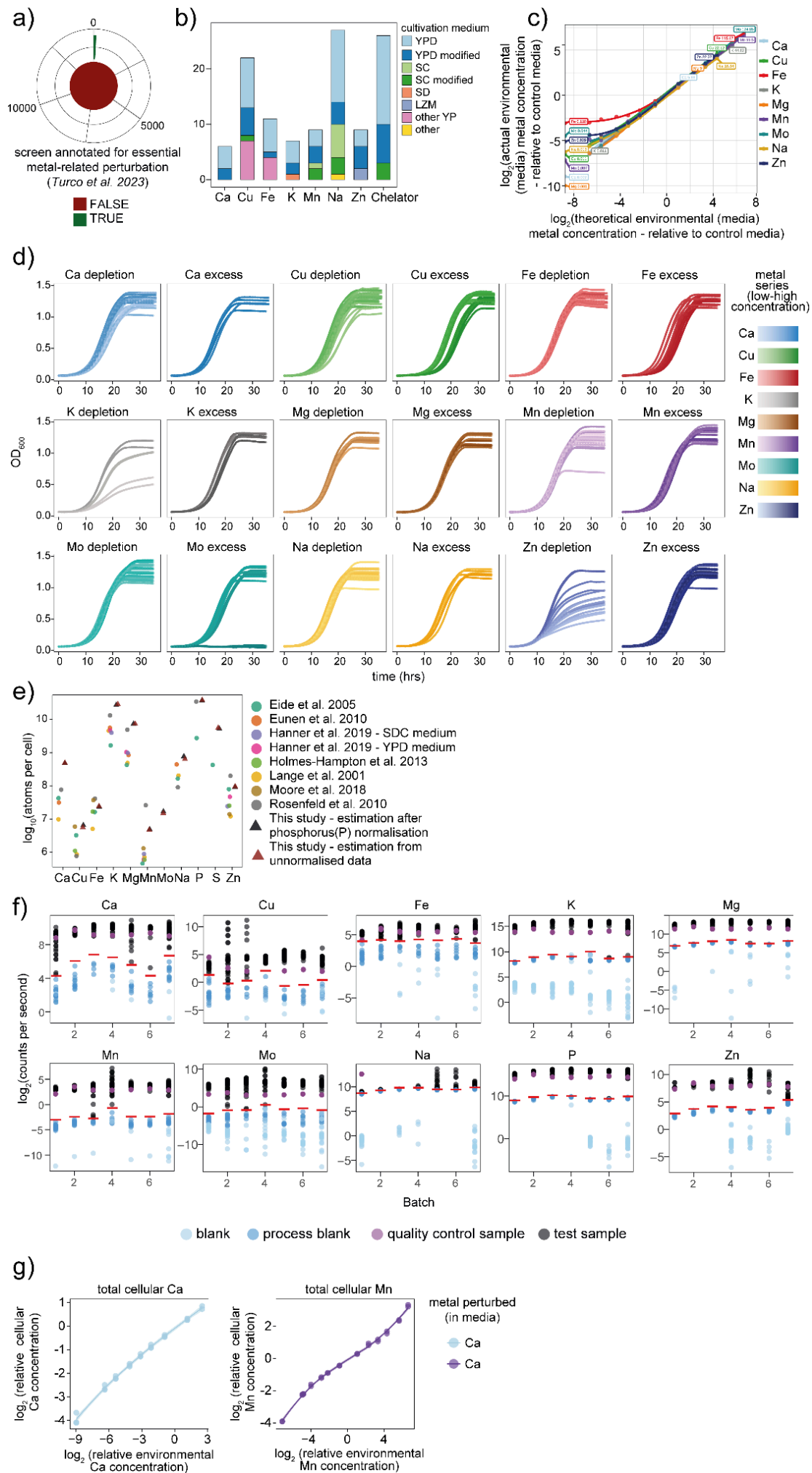


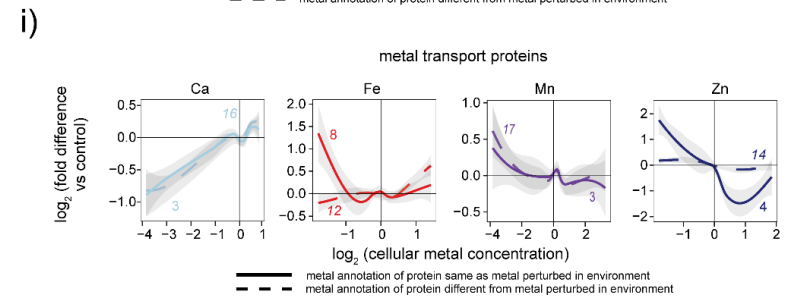
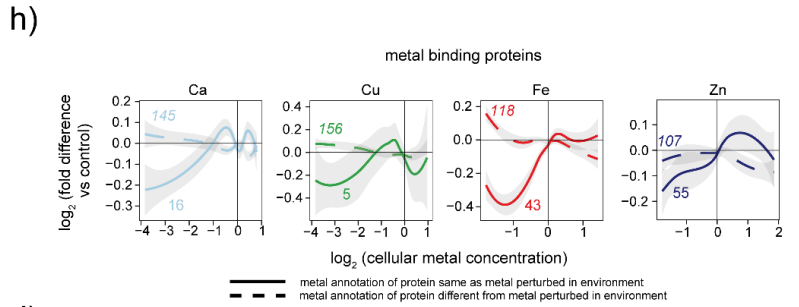
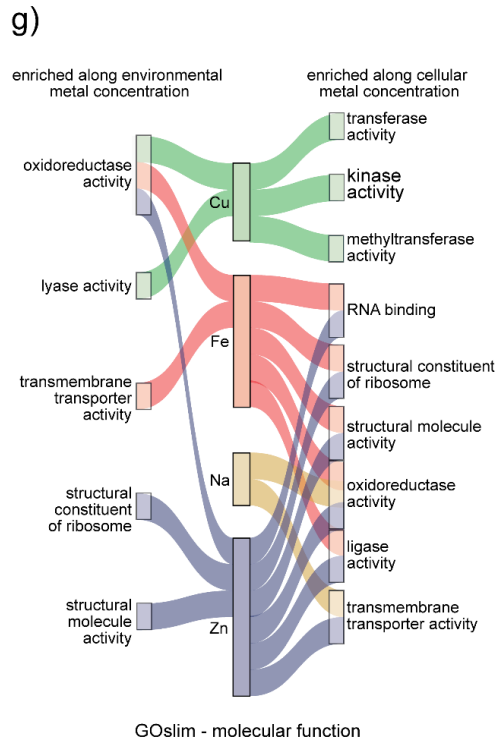
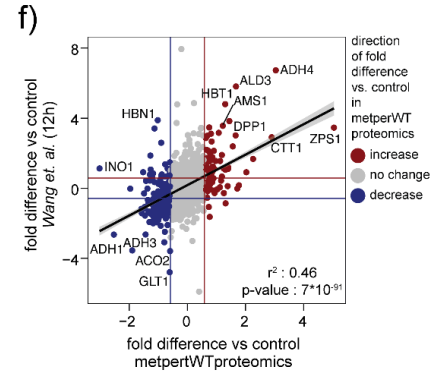
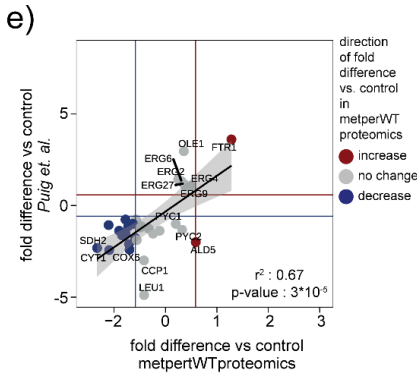
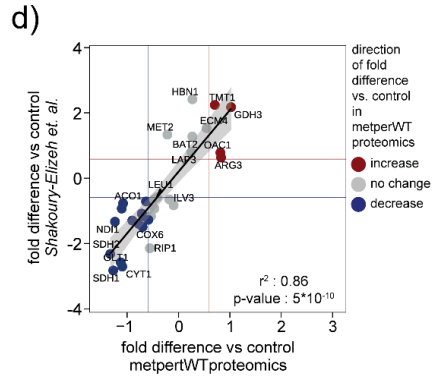
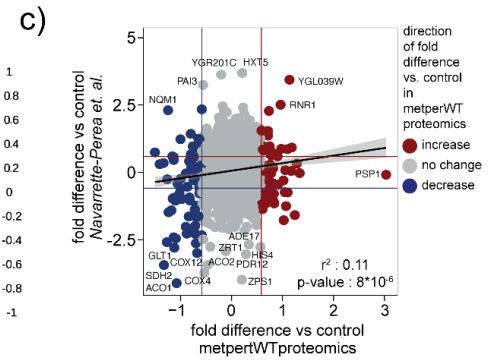
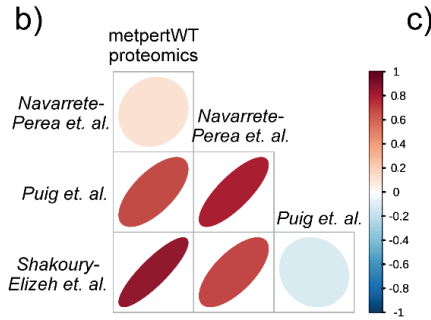
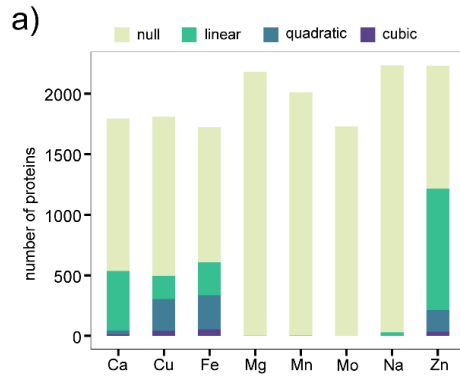
Supplementary Figure 1 : Summary of previous deletion mutant screens under metal perturbation conditions, and growth curve and metal concentration data collected in this work, related to Figure 1.

- a) The number of experiments compiled by ⁹ that represent the concentration perturbations of physiological relevant metal ions (green) compared to all other types of environmental perturbations (red).
- b) Cultivation media used for metal concentration perturbations in previous studies. The physiologically relevant metal or chelator condition is shown against the number of experiments in which the metal was perturbed. YPD, Yeast extract peptone dextrose; SC, synthetic complete; SD, synthetic defined (same as synthetic minimal media); LZM, Low zinc media; other YP, media composed of yeast extract and peptones but a different sugar source.
- c) Concentration of metal ions in each metal perturbation media, quantified by ICP-MS. Labels indicate the lowest and the highest relative metal concentrations that were measured.
- d) Growth curves of *S. cerevisiae* (WT BY4741+pHLUM) cells cultivated in media with perturbed metal concentrations.
- e) Estimates of the total cellular metal concentration for each metal quantified in this study and previous reports ^{20,66–68}.
- f) ICP-MS data collected across all batches for blanks, process blanks, quality control samples and test samples.
- g) Impact of environmental Ca and Mn concentration changes (x-axis) on intracellular Ca and Mn cellular concentration changes of the same metal (y-axis). Colour indicates environmental metal perturbation.



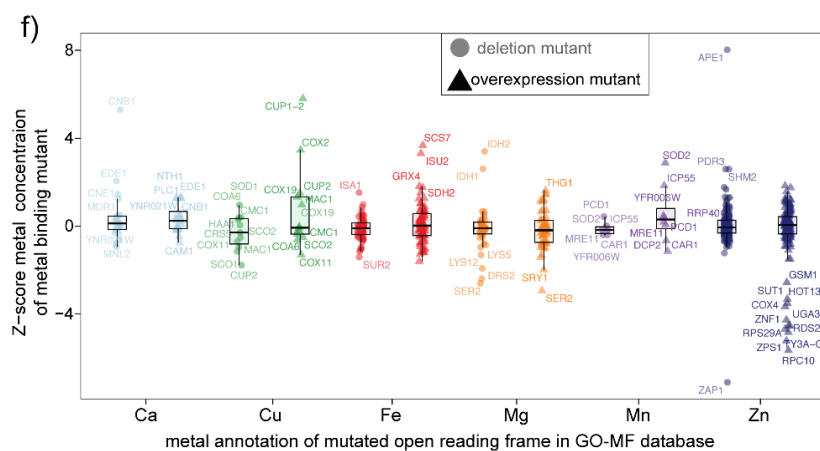
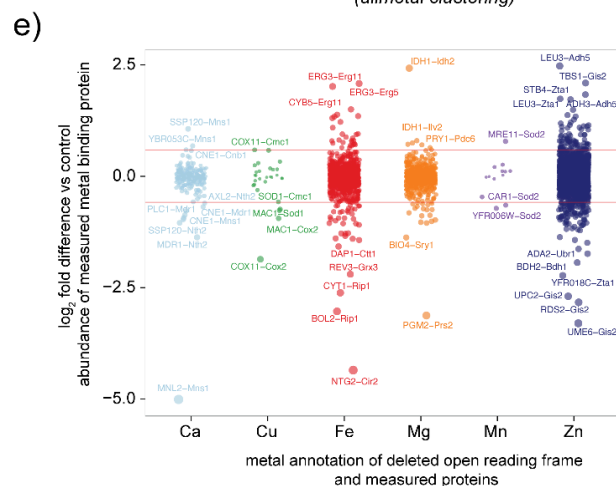
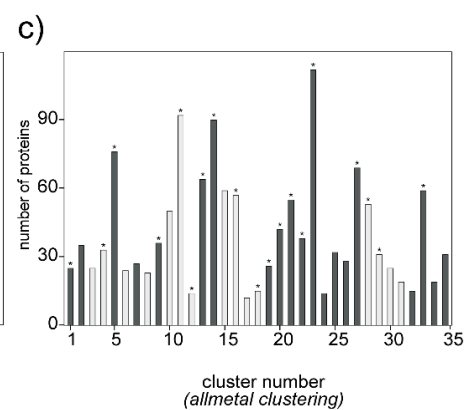
Supplementary Figure 2 : Summary of statistical analysis of proteomics data, comparison of proteomics data from this study to previous studies, summary of gene set enrichments of proteomics data and abundance of metal binding proteins along metal perturbation series. Related to Figure 2

- a) Number of proteins classified as having no significant abundance change (null model) or following a linear, quadratic or cubic protein abundance profile along an environmental concentration series for each metal.
- b) Summary of correlation between the quantitative proteomics dataset described in this study and previous work ¹¹⁻¹³ on Fe depletion. Right leaning ellipses and red colour indicate a positive correlation. Left leaning ellipses and blue colour indicate a negative correlation.
- c) - e) Correlation between quantitative transcriptome or protein abundance data from Fe depletion samples acquired in this study and previous works: ¹³ (c), ¹¹ d), and ¹² (e).
- f) Correlation between quantitative transcriptome or protein abundance data from Zn depletion samples acquired in this study and ¹⁴.
- g) GOslim - molecular function terms enriched in groups of proteins significantly differentially abundant along each environmental (left) and cellular (right) metal perturbation series. The colour indicates the metal that was perturbed in the environment or measured in cells.
- h) & i) Average abundance of metal binding proteins (h) or metal transporters (i) in *S. cerevisiae* cells along measurement cellular metal concentration across all metal perturbation series. Numbers in upright font indicate the total number of proteins bearing annotations for the same metal being perturbed, those in italics indicate the total number of proteins that have an annotation that is different from the metal being perturbed.
- j) & k) Protein abundance changes of proteins implicated in generating proton motive force across cellular and organellar membranes along environmental (j) and cellular (k) metal concentration series.



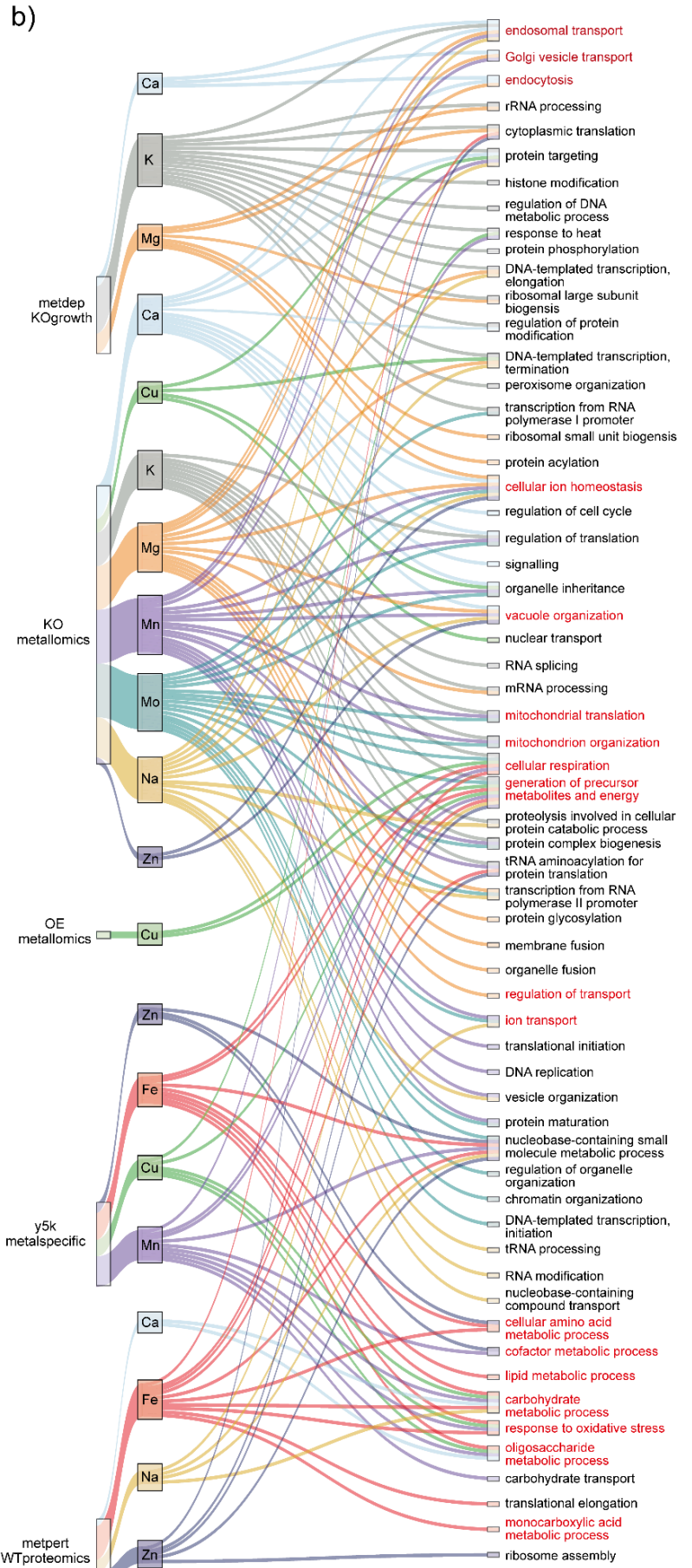
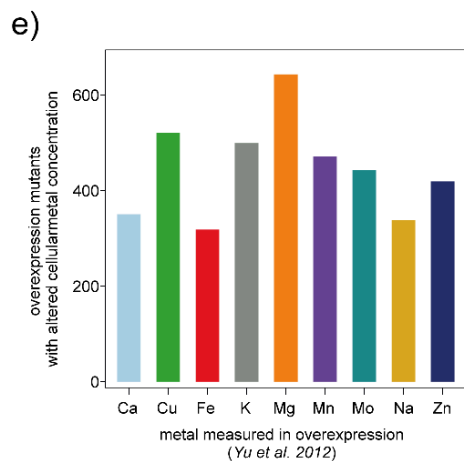
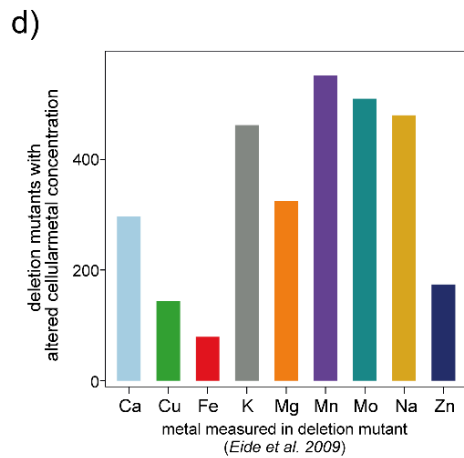
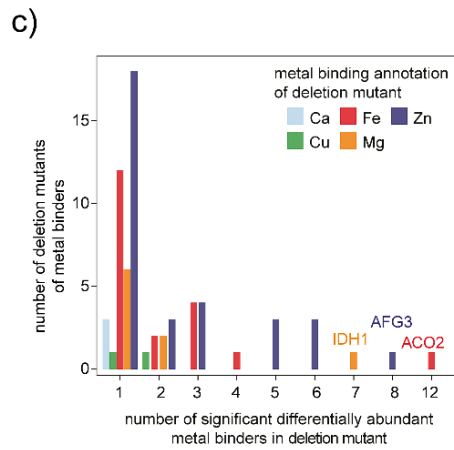
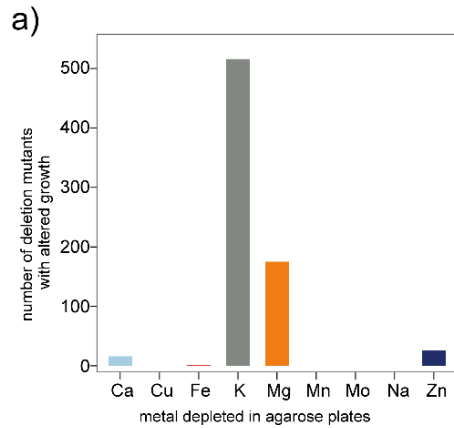
Supplementary Figure 3 : Summary of ensemble clustering analysis (related to Figure 3) and metal related genes and proteins in deletion and overexpression mutant screens (related to Figure 4)

- a) The ensemble clustering pipeline. Created in BioRender. Grüning, N. (2025) <https://BioRender.com/x41qj66>
- b) &
- c) The number of poorly characterised proteins in each cluster obtained via *metal-wise* (b) and all-metal (c) ensemble clustering. The filled bars indicate whether any gene function term was enriched in the cluster; an asterisk (*) indicates that at least one poorly characterised protein (UniProt annotation score < 3) is present in the cluster.
- d) Effect of deletion of open reading frames (ORFs) encoding metal-binding proteins on the growth of *S. cerevisiae* on metal depletion agarose media. Colours indicate the depleted metal and metal binding annotation of the deleted gene, as the dataset was filtered to retain values where the metal depleted matched the metal binding annotation of the mutant.
- e) Effect of the deletion of ORFs encoding metal-binding proteins on the abundance of other proteins annotated to bind the same metal. Colours indicate the metal annotations of the deleted genes and measured proteins. Labels next to some of the points indicate the gene deleted (in capitals) followed by the protein measured (in title case). Only those points which represent an absolute \log_2 fold-difference of the measured protein more than 1.5 and could be labelled without overlapping text are annotated with gene names.
- f) Effect of deletion and overexpression of ORFs encoding metal-binding proteins on the cellular concentration of the metal each ORF is annotated to bind in the GO database. Colours indicate both the metal binding annotation of the mutated gene as well as metal measured from mutant cells.



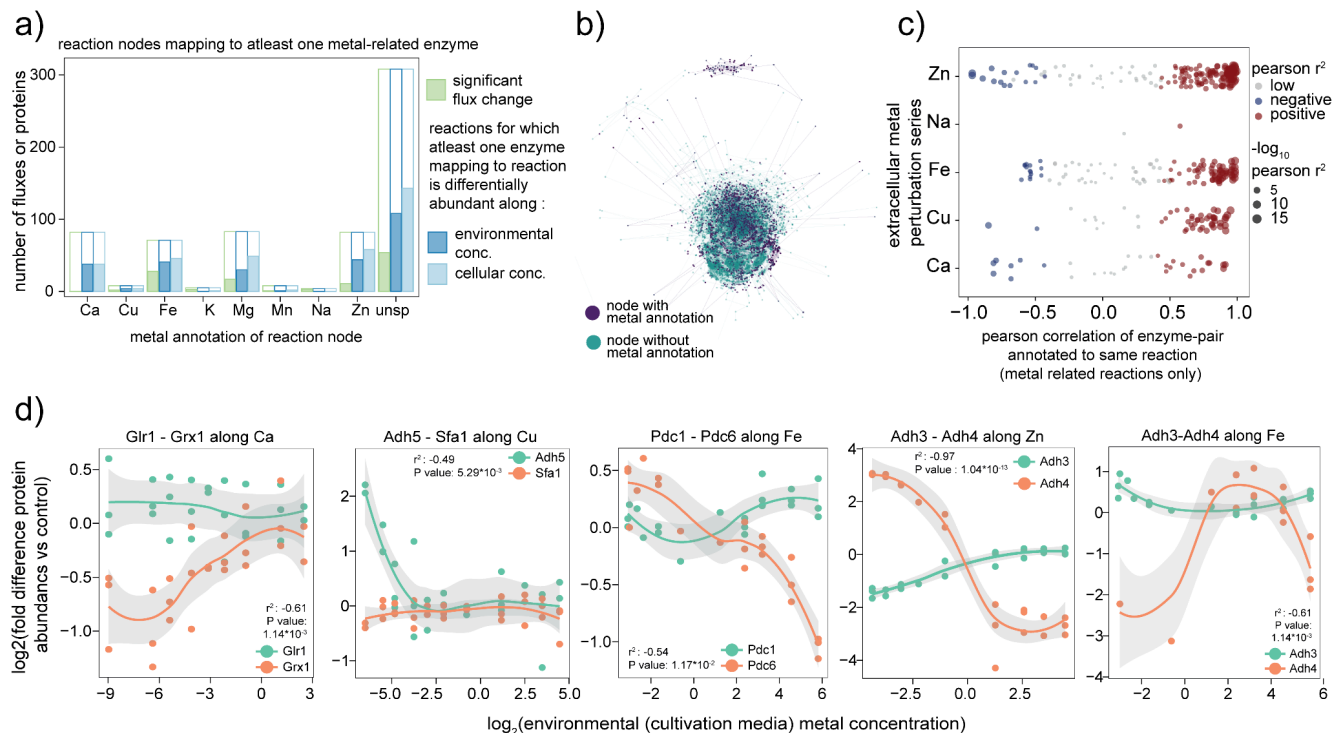
Supplementary Figure 4 : Summary of metal related proteins or genes identified as significantly impacted in growth or protein abundance in deletion and overexpression mutant screens, and gene set terms enriched in significantly impacted genes across all datasets. Related to Figure 4.

- a) The number of metal-gene interactions identified by cultivating the prototrophic haploid deletion (BY4741+pHLUM) collection of *S. cerevisiae* on agarose plates containing depleted amounts of metals.
- b) GOslim - biological process terms enriched in genetic interactions with metals or differentially abundant proteins identified in each dataset. Red colour indicates the metal for which a process was enriched in the interacting genes or differentially abundant proteins. Deletion mutant libraries have been abbreviated as KO for knock-out and overexpression mutants as OE.
- c) The number of metal-gene interactions identified based on metallomics data from *S. cerevisiae* deletion mutants acquired by ²⁶ and analysed by ⁴⁵.
- d) Number of metal-gene interactions identified based on metallomics data from *S. cerevisiae* overexpression mutants acquired by ⁴⁴ and analysed by ⁴⁵.
- e) Distribution of metal binding proteins differentially abundant in deletion mutants of metal binding proteins. The colour indicates the metal binding annotation of the protein encoded by the knocked-out gene.



Supplementary Figure 5 : Summary of reaction nodes mapping to metal related enzymes, and abundances of enzyme pairs catalysing the same reactions along metal concentration series. Related to Figure 5

- Significantly altered fluxes based on FBA simulations (green), differentially abundant proteins identified along environmental metal concentration (blue) and cellular metal concentration (light blue) at reaction nodes bearing at least one metal binding annotation. “unsp”, unclear which metal binds the enzyme. The outer rectangle of the bars indicates the number of fluxes or proteins that were quantified; the inner filled bar indicates the number of significantly altered fluxes or proteins (filled up fraction of bar).
- Visualisation of the directed bi-partite graph representation of the *S. cerevisiae* Yeast8 GEM with H^+ and H_2O filtered out. Each metabolite and reaction are represented by a unique node in the graph. Links in the graph are directed (from substrate metabolite to reaction and from reaction to product) with irreversible reactions added twice (with links in both directions).
- Correlations between the abundances of enzyme pairs that catalyse the same reaction along environmental metal concentration series.
- Selected examples of enzyme pairs mapping to metal-related reaction nodes that exhibit negative correlations in protein abundance.



Supplementary Table Titles

Main

Supplementary Table 1 : Metal concentrations in all test conditions for growth, metallomics and proteomics

Supplementary Table 2 : Theoretical and experimentally quantified metal concentration in media

Supplementary Table 3 : Growth metrics of *S. cerevisiae* cells cultivated in media with varying metal concentrations

Supplementary Table 4 : Measured relative metal concentrations of *S. cerevisiae* cells cultivated in media with varying metal concentrations

Supplementary Table 5 : Cellular metal buffering capacity against environmental excess and depletion

Supplementary Table 6 : Correlations between metal perturbed in cultivation media and cellular concentration of each metal quantified in cell extracts

Supplementary Table 7: Number of proteins identified as differentially abundant along environmental and cellular metal concentration series

Supplementary Table 8: Results of the statistical analysis to identify proteins changing in abundance along each altered environmental metal concentration series

Supplementary Table 9 : Results of the statistical analysis to identify proteins changing in abundance along each measured cellular metal concentration

Supplementary Table 10 : Gene set terms enriched in proteins differentially abundant along each metal concentration series

Supplementary Table 11 : Responses of signalling pathways to changes in metal availability

Supplementary Table 12 : Responses of protein complexes to changes in metal availability

Supplementary Table 13 : Responses of metabolic pathways (KEGG database) to changes in metal availability

Supplementary Table 14 : Assignment of ORF to clusters based on ensemble clustering and gene set terms enriched in each cluster

Supplementary Table 15 : Results of growth screen to identify genetic interactions with metal depletion using the haploid knockout deletion mutant collection

Supplementary Table 16 : Gene set terms enriched in proteins or genes identified as significantly affected across all datasets

Supplementary Table 17 : Proteomes profiles of knockout deletion mutant strains (reported by *Messner et al 2023*) filtered for proteins that have metal related annotations in GO-Molecular Function database.

Supplementary Table 18 : Summary of differentially abundant proteins in metal-related knockout deletion mutant proteomes quantified by *Messner et. al 2023*

Supplementary Table 19 : Number of datasets in which each poorly characterised ORF was identified as significantly affected

Supplementary Table 20 : Number of datasets in which each combination of a metal and a poorly characterised ORF was identified as having an interaction

Supplementary Table 21 : Fraction of reaction nodes at each shortest distance from nearest metal requiring reaction node in a graph representation of the Yeast8 genome scale metabolic model

Supplementary Table 22 : Metal binding proteins in metabolic pathways

Supplementary Table 23 : Fraction of metabolite nodes at each shortest distance to the nearest metal requiring reaction node in a graph representation of the Yeast8 genome scale metabolic model

Supplementary Table 24 : Pearson correlation coefficients of protein abundance of isozymes along environmental metal concentration series

Methods

Supplementary Table M1 : Details of liquid cultivation media preparation from ultra pure chemical stocks

Supplementary Table M2 : 96-well plate layouts for growth, metallomics and proteomics characterisation of *S. cerevisiae* cells cultivation in liquid media with varied metal concentrations

Supplementary Table M3 : Metal composition of calibration curve for ICP-MS measurements

Supplementary Table M4 : 96-well plate layouts for ICP-MS measurements of cultivation media

Supplementary Table M5 : Volume of cell culture transferred for ICP-MS analysis and reconstituted after HNO₃ digestion of cell pellet to arrive at a 10% HNO₃ (v/v) concentration.

Supplementary Table M6 : Mean, standard deviation and coefficient of variation of ICP-MS based metal quantification of replicates in each metal-perturbation cultivation media

Supplementary Table M7 : Comparison of estimated metal concentrations in WT *S. cerevisiae* cells cultivated in standard cultivation conditions with data from previous studies.

Supplementary Table M8 : Details of the non-linear liquid chromatography gradient used in the LC-MS/MS based proteomics experiment

Supplementary Table M9 : Details of the composition of agarose media with varying concentrations of metals used for growth screen of haploid knockout deletion mutants

Supplementary Table M10 : Supplementary Note on preparation of Mg and K perturbation media.

Supplementary Table M11 : Supplementary Note on chelators in media preparation.

Supplementary Table M12 : ICP-MS acquisition parameter.

Supplementary Table M13 : DIA-NN parameters for processing proteomics raw files.

Supplementary Table M14 : Code summary table - maps each R, python and MATLAB script in github repository to the analysis conducted, Figures and Supplementary Figures in the manuscript.