Supplementary Notes

Supplementary Note 1. Development of a stable and reproducible respiration culture condition.

To profile diverse yeast strains during respiratory growth, when mitochondrial OxPhos is highly active, we first needed to develop a distinct respiration condition suitable for large-scale investigation. Early log phase fermentation cultures repress mitochondrial respiration, cultures containing solely non-fermentable sugars preclude growth of respiration deficient yeast, and high glucose cultures grown past the diauxic shift are too biologically dynamic to allow reproducible sampling across a large scale study[43, 44]. To overcome these problems, we developed a culture system that includes low glucose (1 g/L) and high glycerol (30 g/L), enabling a short fermentation phase followed by a longer respiration phase. This respiration condition affords steady growth and a stable biological state—as reflected by a proteome that is constant over multiple hours (**Supplementary Fig. 1c–e**)—and, thus, an essential window for reproducible sample harvesting.

Supplementary Note 2. Δ*Gene*-specific phenotype detection.

To identify Δ*gene*-specific phenotypes, we broadly surveyed our data for characteristic outlier abundance measurements. For each profiled molecule (in both respiration and fermentation growth conditions) we separated potential Δ*gene*-specific measurements into two groups: positive $\log_2$ fold change ($\log_2$[Δ*gene*/WT]) and negative $\log_2$ fold change. These two sets were then plotted individually with $\log_2$ fold change and $-\log_{10}$(*p*-value [two-sided Student's *t*-test]) along the x- and y- axes, respectively. Data were normalized such that the largest $\log_2$ fold change and largest $-\log_{10}$(*p*-value) were set equal to 1. Considering the three largest fold changes where $P < 0.05$, we calculated the Euclidean distance to all neighboring data points and stored the smallest result. A requirement was imposed that all considered 'neighbors' have a smaller fold change than the data point being considered. It is anticipated that data points corresponding to Δ*gene*-specific phenotypes will be outliers in the described plots and have large associated nearest-neighbor Euclidean distances. The described routine yielded three separate distances, the largest of which was stored for further analysis. The results of this analysis and representative examples are highlighted (**Fig. 2, Supplementary Figs. 5** and **6**). We observed maximal Euclidean distances across a range of 0.006 to 1.25. We set a cutoff for classification as a 'Δ*gene*-specific phenotype' at 0.70 and report 714 molecules (4.6% of considered cases across both culture conditions) which exceed this threshold (**Supplementary Table 4**). This procedure provided a useful 'first pass' analysis and afforded a truncated set of leads, which were used to develop biological hypotheses.

Supplementary Note 3. Lack of effect of Dpl1p disruption on the Tyr-to-4-HB-to-CoQ pathway.

To test the idea that the CoQ biosynthesis and sphingolipid catabolism pathways are independent, we examined Δ*dpl1* yeast, which lack a known dihydrosphingosine phosphate lyase. Δ*dpl1* yeast show neither a $pABA^-$ respiratory growth phenotype nor CoQ deficiency (**Supplementary Fig. 7j,k**). These results demonstrate that disruption of the Tyr-to-4-HB pathway in Δ*hfd1* yeast is not downstream of a defect in sphingolipid metabolism. Furthermore, proteome analyses showed that Δ*hfd1* cultured without 4-HB and pABA are similar to Δ*coq8* yeast—but not Δ*dpl1* yeast—and adding 4-HB to Δ*hfd1* cultures returns their proteomes to WT-like profiles (**Supplementary Fig. 7l,m**).

Supplementary Note 4. Quantitative definition of the respiration deficiency response (RDR).

To quantitatively define the RDR, we categorized strains as respiration deficient (RD) or competent (RC) and examined differences between these two groups. Principal component analysis of the Y3K respiration dataset revealed marked separation of RD and RC strains (**Fig. 3c** and **Supplementary Fig. 8a**). The underlying phenotype changes that distinguish RD and RC strains include proteins, lipids, and metabolites (**Fig. 3d** and **Supplementary Table 5**). RDR perturbations include significant decreases in ATP synthase, TCA cycle, and MICOS proteins (**Fig. 3e,f** and **Supplementary Fig. 8b**), likely to decrease allocation of useless proteome mass to dysfunctional mitochondria[45]. Importantly, the RDR also includes a positive response, and numerous proteins—including protein folding, NADH metabolism, and proteasome assembly proteins—are significantly upregulated in RD strains (**Fig. 3e,f**). Numerous individual molecules—including lactate, alanine, 2-hydroxyglutarate, tyrosol, 4-HB, Gpx2p, and Ahp1p, among many others—are significantly perturbed in RD strains and strongly predictive of respiration deficiency (**Supplementary Fig. 8c,d**). Our quantitative assessment of the RDR highlights biochemical features of the cellular response to defects in mitochondrial respiration, and suggests that a multi-omic assessment of proteins, lipids, and metabolites could afford a highly specific biomarker panel for diseases affected by OxPhos deficiency.

Supplementary Note 5. RDR normalization procedure.

Δ*gene* strains were classified as RD (51) or respiration competent (RC) (123) based on observation of a common perturbation profile signature in the respiration culture condition. For each molecule we calculated an RDR score. This metric represents the proportion of RD Δ*gene* strains over which the molecule was consistently perturbed, relative to all RD Δ*gene* strains where the molecule was quantified. Across all RD Δ*gene* strains, 776 molecules were identified as having an RDR score > 0.95 (consistently perturbed across more than 95% of RD Δ*gene* strains where quantified) and classified as RDR-associated. (**Supplementary Table 6**). The individual measurements of these RDR-associated molecules were then mean normalized ('RDR-adjusted') using abundance values from RD Δ*gene* strains. This normalization procedure revealed characteristic deviations from the general RDR (**Supplementary Fig. 9**). Importantly, this procedure enables visualization of Δ*gene*-specific changes. For example, prior to RDR normalization, the expected decrease in Coq8p in Δ*coq8* yeast is obscured by RDR-associated proteins with large abundance changes (**Supplementary Fig. 9d**). RDR normalization not only uncovers the decrease in Coq8p, but a significant decrease in Coq5p, a functionally-related CoQ biosynthesis protein, also becomes readily apparent (**Supplementary Fig. 9d**).

Supplementary Note 6. Molecular defects of Δ*yjr120w* yeast.

To examine the molecular basis for the CoQ deficiency of Δ*yjr120w* yeast, we inspected our proteomics dataset, which revealed significant decreases in ATP synthase proteins, especially Atp2p (**Supplementary Fig. 10a**). Compared to other strains, the large decrease in Atp2p is unique to Δ*yjr120w* and Δ*atp2* (**Supplementary Fig. 10b**). A relationship between *yjr120w* and *atp2* is also suggested by their genetic proximity (**Supplementary Fig. 10c**). Plasmid overexpression of *atp2* rescues the Δ*yjr120w* respiratory growth defect (**Supplementary Fig. 10d**), indicating a functional relationship between *atp2* and *yjr120w in vivo*. A decrease in *atp2* mRNA in the Δ*yjr120w* strain is a component of the underlying mechanism (**Supplementary Fig. 10e**). Interestingly, CoQ deficiency was also observed in Δ*atp2* yeast (**Fig. 3h**).

Supplementary Note 7. Predicted enzymatic functions of Aim18p, Aro9p, and Aro10p.

Since 1907, yeast have been known to catabolize amino acids into fusel (German for 'bad liquor') alcohols through the Ehrlich pathway[46, 47], but the physiological roles for the enzymes involved—such as Aro9p and Aro10p—are not fully understood. Aro9p and Aro10p were previously thought to provide a simple catabolic route for extracting nitrogen from aromatic amino acids[48] (**Supplementary Fig. 14a**), but our MCNA unexpectedly indicated strong correlations between Aro9p, Aro10p, and proteins involved in mitochondrial respiration (**Fig. 4d,e**), suggesting a more complicated biological function that supports OxPhos. We hypothesized that this function might be in the Tyr-to-4-HB-to-CoQ pathway (**Supplementary Fig. 14b**), given the putative enzymatic activities of Aro9p and Aro10p in tyrosine and phenylalanine metabolism. Consistently, when cultured in pABA$^-$ media, Δ*aro9* and Δ*aro10* yeast are deficient in CoQ and PPHB (**Fig. 4f**).

Aim18p is a protein of undefined molecular function that has been detected in mitochondria[49] and potentially linked to mitochondrial inheritance (Altered Inheritance of Mitochondria, 'AIM') by large-scale studies in yeast[50]. Protein sequence alignments show that Aim18p contains a chalcone-flavone isomerase (CHI)-like domain (**Supplementary Fig. 14c**), whose homologs in plants typically function on aromatic small molecules (chalcones) (**Supplementary Fig. 14d**)[51-53]. Given the potential for this protein domain to catalyze modifications of aromatic small molecules, we hypothesized that Aim18p might function in the Tyr-to-4-HB pathway to produce the CoQ headgroup (**Supplementary Fig. 14d**). Consistently, when cultured in pABA$^-$ media, we observed deficiency of PPHB in Δ*aim18* yeast (**Fig. 4f**).

Supplementary Table Captions

Supplementary Table 1. Knockout yeast strains.
Table of single-gene deletion (Δ*gene*) yeast strains investigated in this study and their harvest culture densities. For each gene deleted in a strain studied, the first tab includes systematic yeast gene name, standard gene name, Entrez gene ID, UniProt ID, and human homolog(s). The second tab shows the culture densities upon harvest (growth phenotypes), and it includes the systematic yeast gene name, the standard gene name, the average culture densities at the harvest time point (mean, n = 3), and the corresponding standard deviations, fold changes (KO/WT), and *p*-values (Student's t-test) for respiration and fermentation cultures.

Supplementary Table 2. Profiled biomolecules.
Table of all 4505 molecules profiled in the study. Includes molecule type (protein, lipid, or metabolite), molecule name, standard gene name (for proteins) or standard lipid name, systematic gene name (for proteins), and UniProt ID (for proteins). For lipids, the numbers in parentheses indicate the number of carbons in the acyl tail(s) and the number of carbon-carbon double bonds in the chains (carbons:double_bonds).

Supplementary Table 3. Quantitative dataset.
Table containing quantitative measurements and descriptive statistics used throughout the Y3K study. Average fold changes in molecule abundances (mean $\log_2$[Δ*gene*/WT], n = 3) for all strains and all molecules in the respiration and fermentation datasets are shown on tabs labeled 'KO vs WT_Resp (ΔLFQ)' and 'KO vs WT_Ferm (ΔLFQ)'. Corresponding standard deviations, and *p*-values (2-tailed t-test [homostatic]) for all measured fold changes are shown on separate tabs labeled accordingly with '(Std. Dev.)' and '(P-Value).' Each tab contains a table with rows corresponding to molecules and columns corresponding to the 174 single gene knockout (Δ*gene*) strains profiled in the study.

Supplementary Table 4. Δ*gene*-specific phenotypes.
Table of unique Δ*gene*-phenotype relationships identified in this study. Includes molecule name (standard gene name followed by systematic gene name for all proteins), yeast deletion strain (standard gene name), calculated Euclidean distance, and associated growth condition (respiration or fermentation).

Supplementary Table 5. Respiration deficient strains vs respiration competent strains.
Table of average fold change in molecule abundances (mean $\log_2$[RD strains/RC strains]). Includes molecule identifiers (including UniProt IDs, symbols, and systematic names for proteins), average fold change (mean $\log_2$[RD strains/RC strains]), $-\log_{10}$(*p*-value), and select GO terms corresponding to those highlighted in **Fig. 3** and **Supplementary Fig. 8**.

Supplementary Table 6. Respiration deficient strains versus wild type.
Table of average fold change in molecule abundances (mean $\log_2$[RD strains/WT]). Includes molecule identifiers (including UniProt IDs, symbols, and systematic names for proteins), fraction of RD strains showing consistent perturbation of each molecule, RDR score (see Methods), and average fold change (mean $\log_2$[RD strains/WT]).

Supplementary Table 7. Δ*gene*–Δ*gene* perturbation profile correlations.
Table of Δ*gene*–Δ*gene* perturbation profile correlations (Pearson coefficients). Includes the gene knocked out of 'strain one' in the pairwise comparison, the gene knocked out of 'strain two' in the pairwise comparison, the Pearson coefficient, and the 'Ome' (proteome, lipidome, or metabolome) used for the regression analysis. Coefficients are only reported for Δ*gene*–Δ*gene* pairs meeting the criteria outlined in the Methods under the heading 'Regression analysis of phenotype changes'. Separate tabs are included for the respiration (resp), fermentation (ferm), and RDR-adjusted respiration (resp-RDR) datasets.

Supplementary Table 8. Molecule covariance network analysis results.
Table of 288,794 pairs of covariant molecules ($|\rho| \geq 0.58$ and Bonferroni-adjusted $P < 0.001$) identified in the Y3K dataset. Includes molecule names (standard protein name followed by systematic protein name) and types (protein, lipid, or metabolite) for 'molecule one' and 'molecule two', Spearman's correlation coefficients (ρ), and Bonferroni-adjusted *P*-values. Separate tabs are included for the respiration (resp), fermentation (ferm), and RDR-adjusted respiration (resp-RDR) datasets.

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