Supplemental Materials

Molecular Biology of the Cell

Lewis *et al*.

Loss of major nutrient sensing and signaling pathways suppresses starvation lethality in electron transport chain mutants

Lewis AG, Caldwell R, Rogers JV, Ingaramo M, Wang RY, Soifer I, Hendrickson DG, McIsaac RS, Botstein D, Gibney PA

SUPPLEMENTAL INFORMATION

Supplemental Figure 1. Comparison of growth characteristics for final and intermediate ETC mutants.

Supplemental Figure 2. Transcription factor activities for gene expression data in Figure 3.

Supplemental Figure 3. Investigation of nutrient limitation in starvation-induced death of ETC mutants in YNB+glucose.

Supplemental Figure 4. Comparison of growth characteristics of individual suppressor mutants.

Supplemental Figure 5. Bud index data for ETC mutants and suppressor mutants used for gene expression data in Figure 6.

Supplemental Figure 6. Transcription factor activities for gene expression data used in Figure 6.

Supplemental Figure 7. Calibration curve for pHluorin2.

Supplemental Figure 8. Calibration curve for mScarlet.

Supplemental Figure 9. ETC suppressors partially restore intracellular pH homeostasis based on mScarlet fluorescent lifetime.

Supplemental Figure10. Transcriptional regulation in the absence of a single metabolic pathway gene.

Supplemental Figure 11. Comparison of RNA-Seq at t=0 for wild type, *nde1Δ nde2Δ ndi1Δ*, and *cox4Δ*.

Supplemental Figure 12. Confirmation of mitochondrial genome stability in ETC mutants *coq2Δ* and *cox4Δ*.

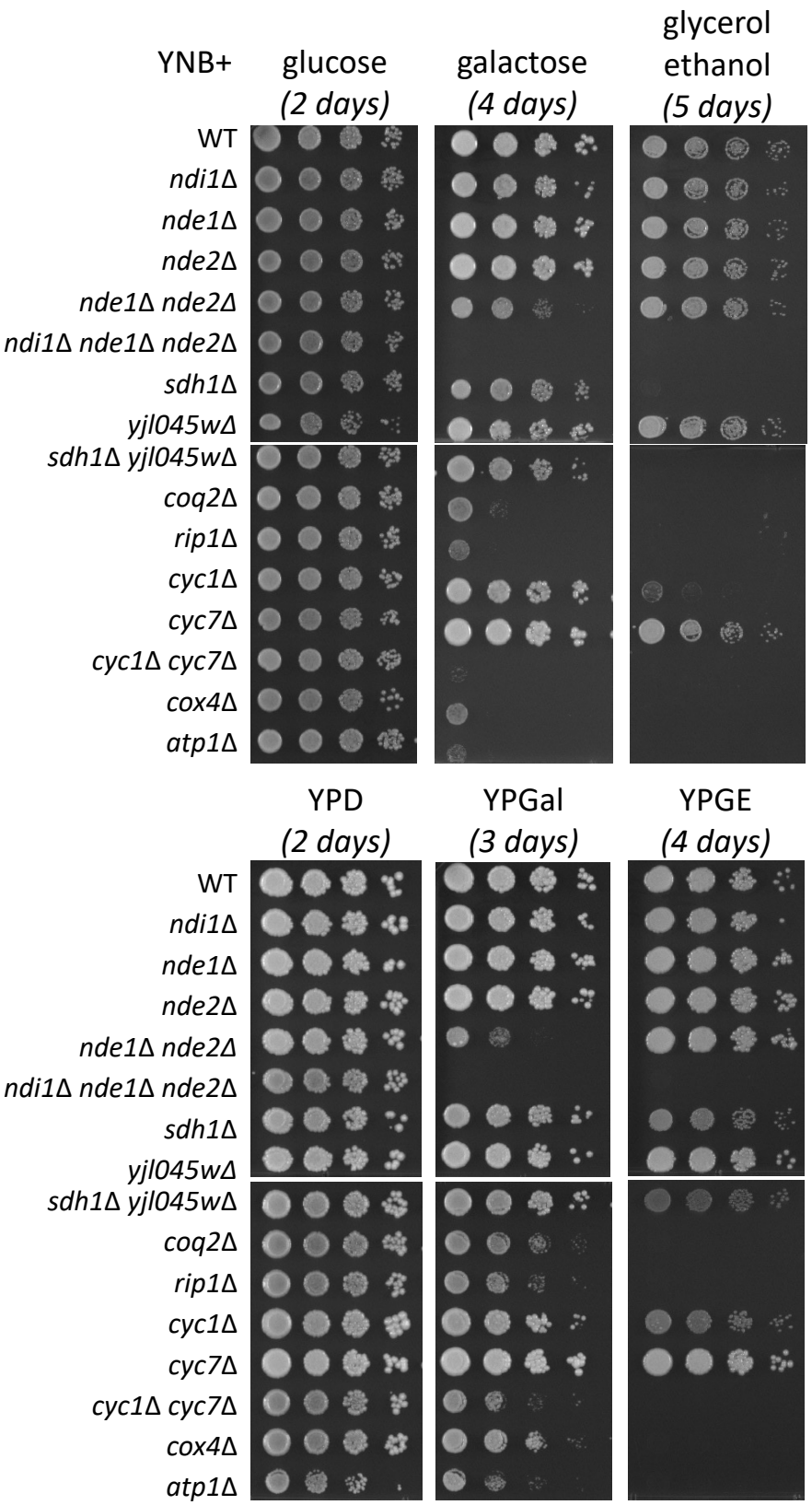
Supplemental Table 1. GO Term search results for clusters in Figure 3D.

Supplemental Table 2. GO Term searches for clusters in Figure 3E.

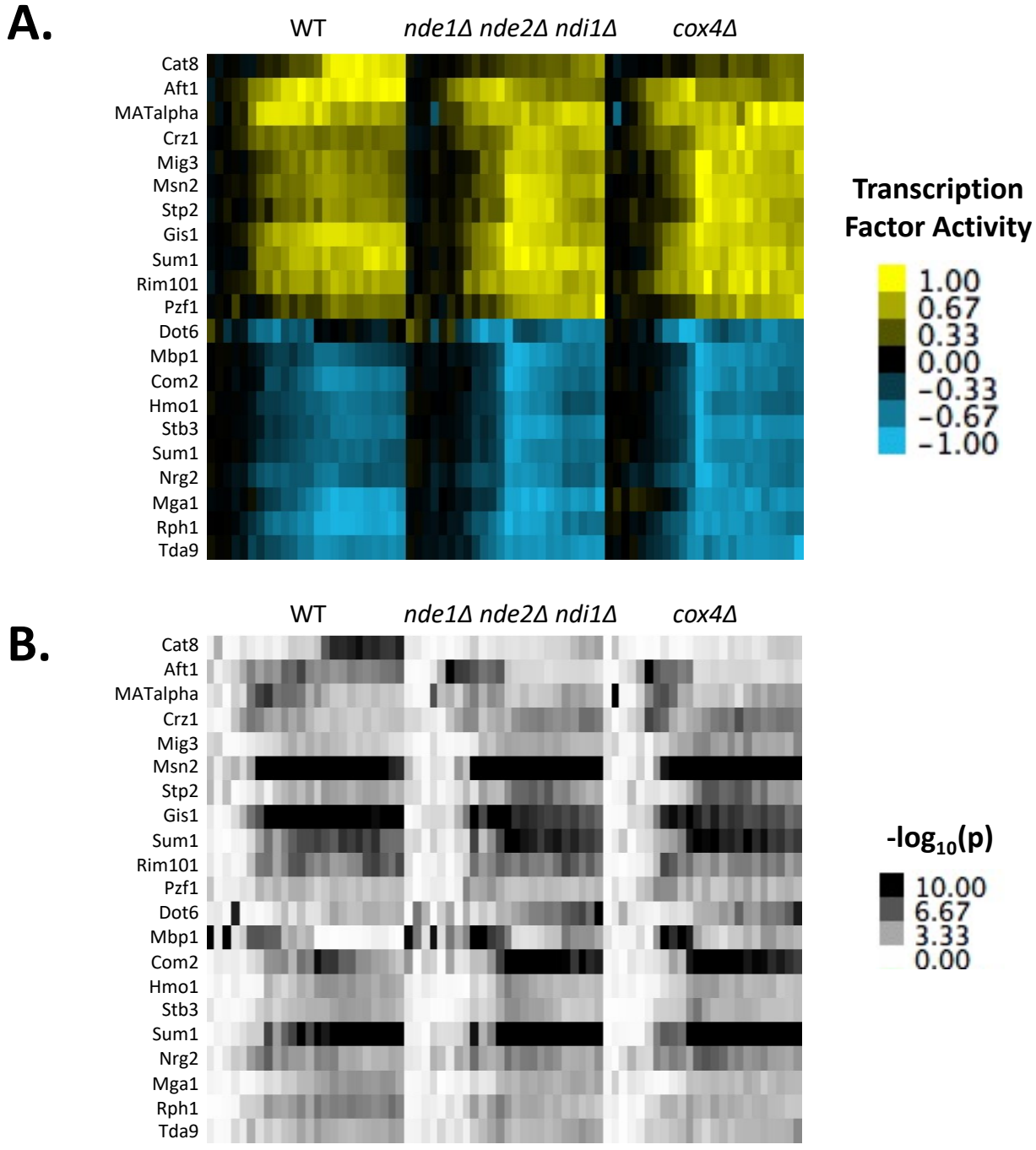
Supplemental Table 3. Measured mutation rate for each ETC mutant in YNB+glucose starvation.

Supplemental Table 4. Observed mutations in ETC suppressor strains.

Supplemental Table 5. Strains used in this study.

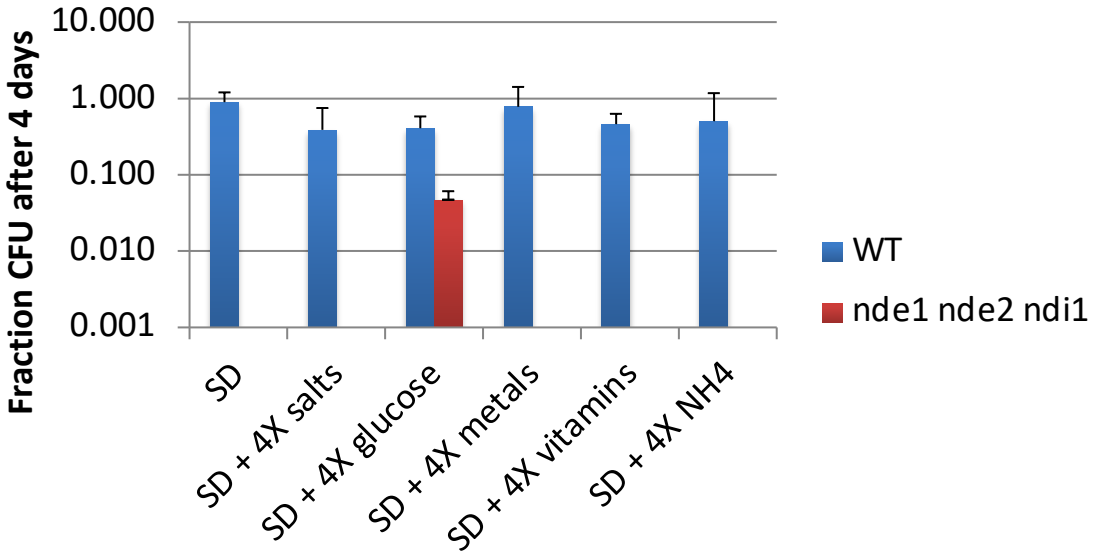


Supplemental Figure 1. Comparison of growth characteristics for final and intermediate ETC mutants. Strains were grown on specified types of growth medium at 30°C for the indicated amount of time before photographing. Before spotting, the initial dilution was normalized to an OD_{600} = 1.0 for each strain, and 10-fold serial dilutions were prepared. Shown is a representative example of three biological replicates.

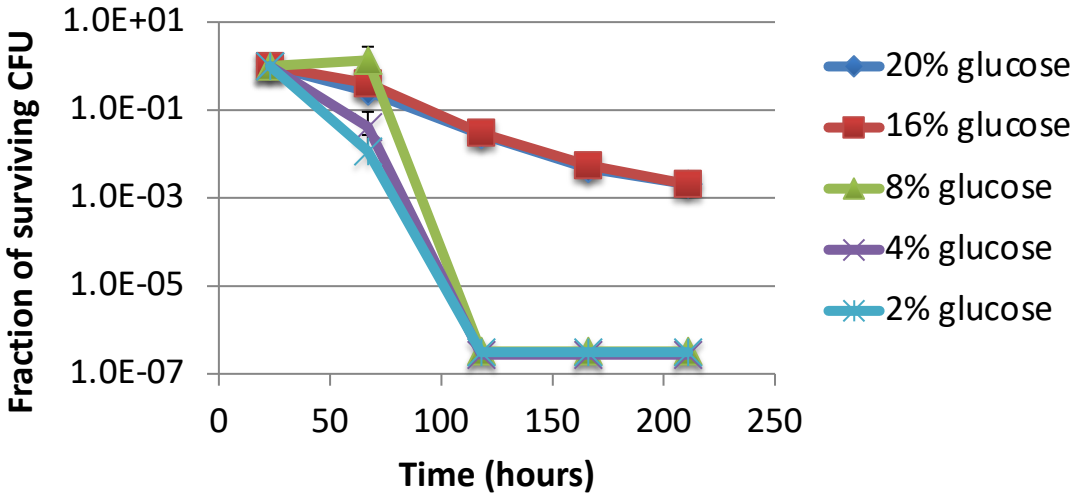


Supplemental Figure 2. Significant transcription factor activity estimations from REDUCE using Fig 3D RNA-Seq data. Transcription factor activities (**A**) and associated p-values (**B**). Transcription factor activities were calculated as described in Materials and Methods. Only significant transcription factors were included in this plot (based on having at least 4 time-points with uncorrected $p < 0.0001$), while the entire matrix of transcription factor activities is available as downloadable data. For each strain, the time-points are identical to those in Figure 3D (0, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20, 22, 24, 28, 32, 38, 46, and 58 hours).

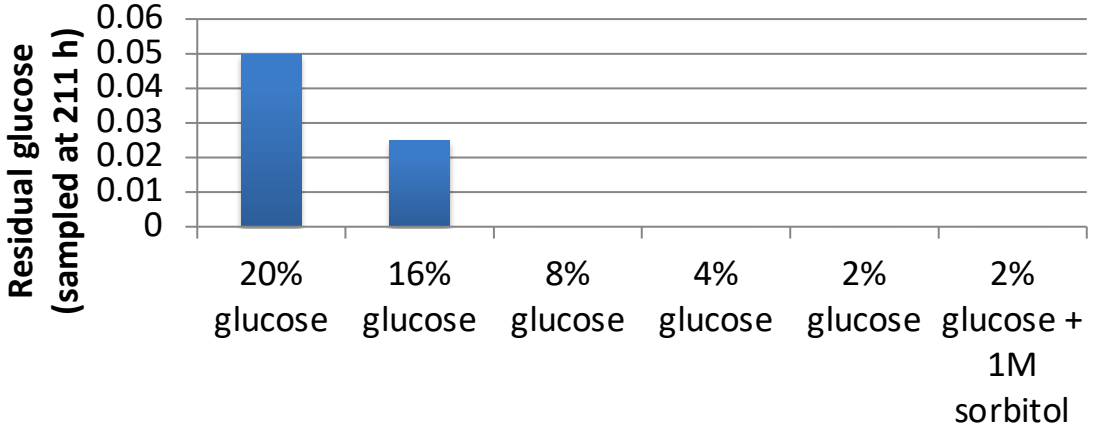
A.

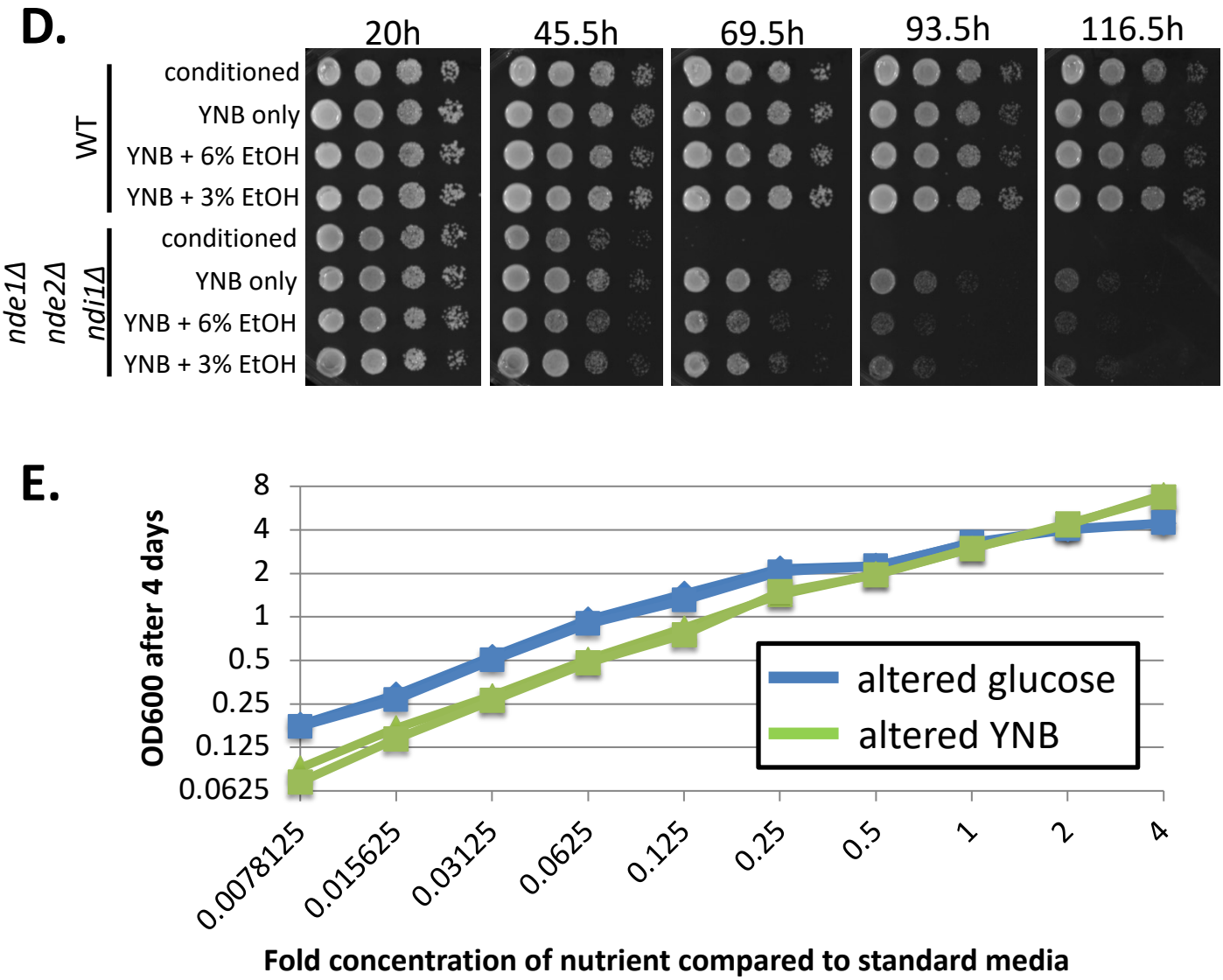

Fraction CFU after 4 days
10.000
1.000
0.100
0.010
0.001
SD
SD + 4X salts
SD + 4X glucose
SD + 4X metals
SD + 4X vitamins
SD + 4X NH4
WT
nde1 nde2 ndi1

B.

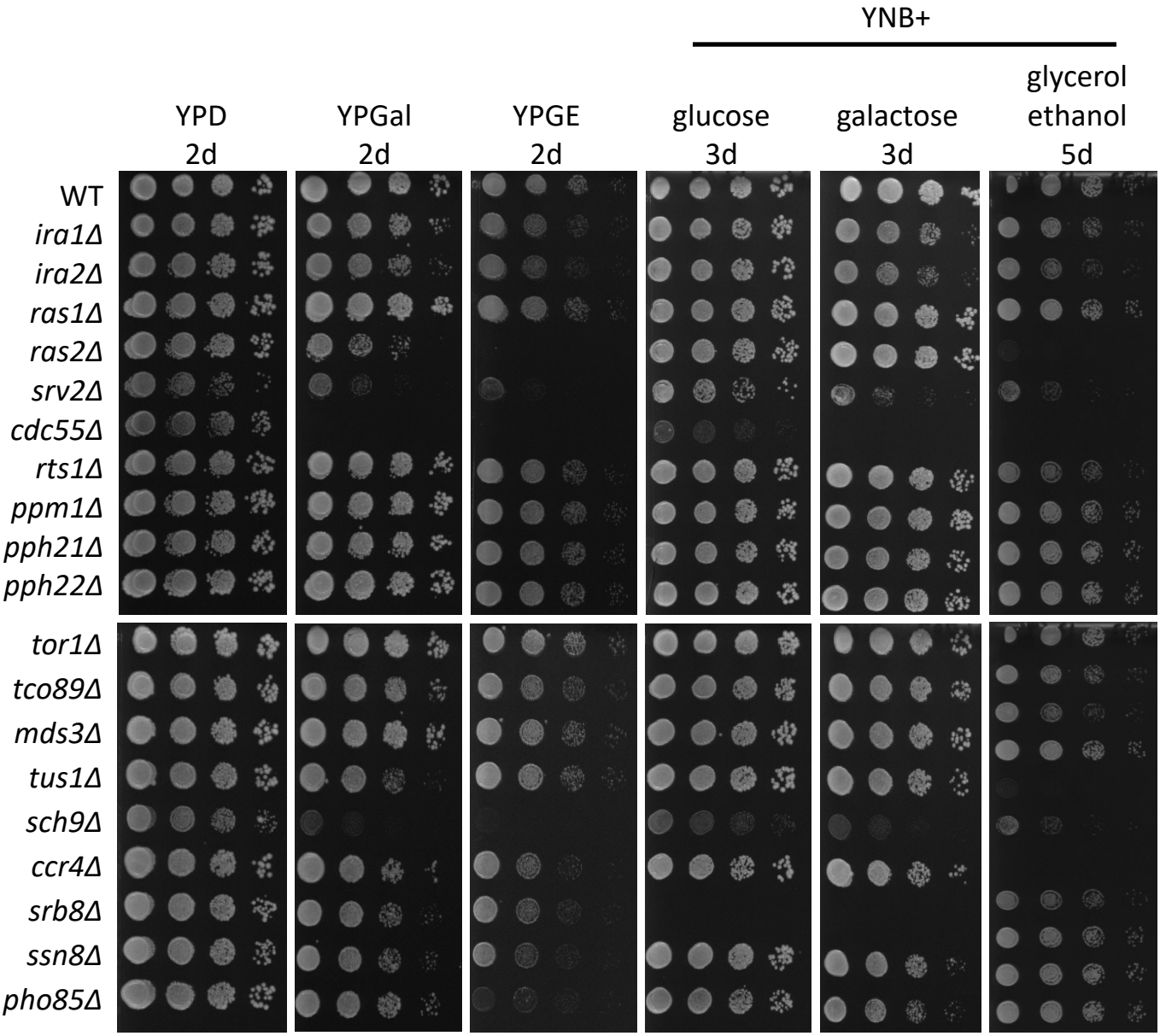

Fraction of surviving CFU
1.0E+01
1.0E-01
1.0E-03
1.0E-05
1.0E-07
0
50
100
150
200
250
Time (hours)
20% glucose
16% glucose
8% glucose
4% glucose
2% glucose

C.

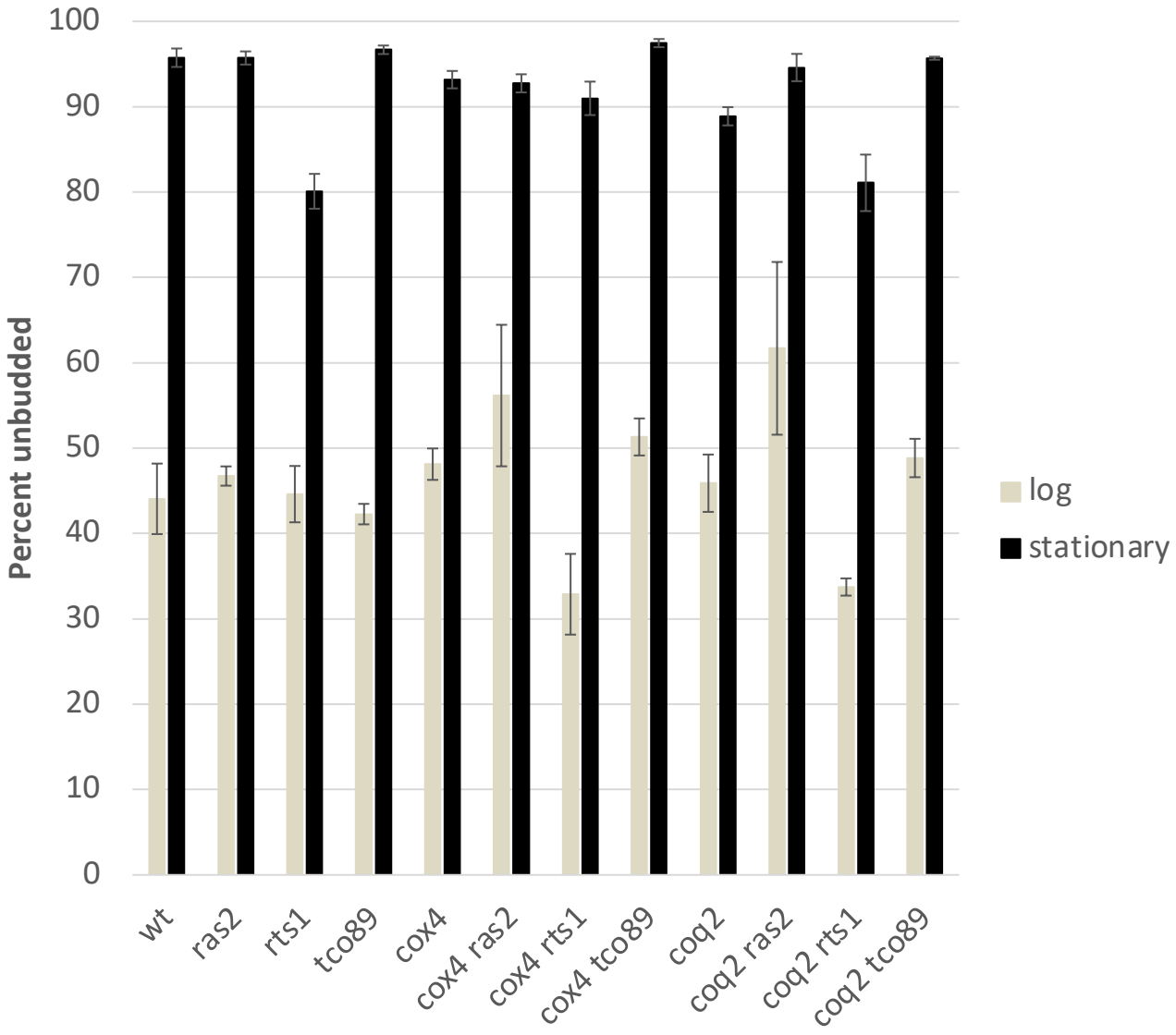

Residual glucose (sampled at 211 h)
0.06
0.05
0.04
0.03
0.02
0.01
0
20% glucose
16% glucose
8% glucose
4% glucose
2% glucose
2% glucose + 1M sorbitol



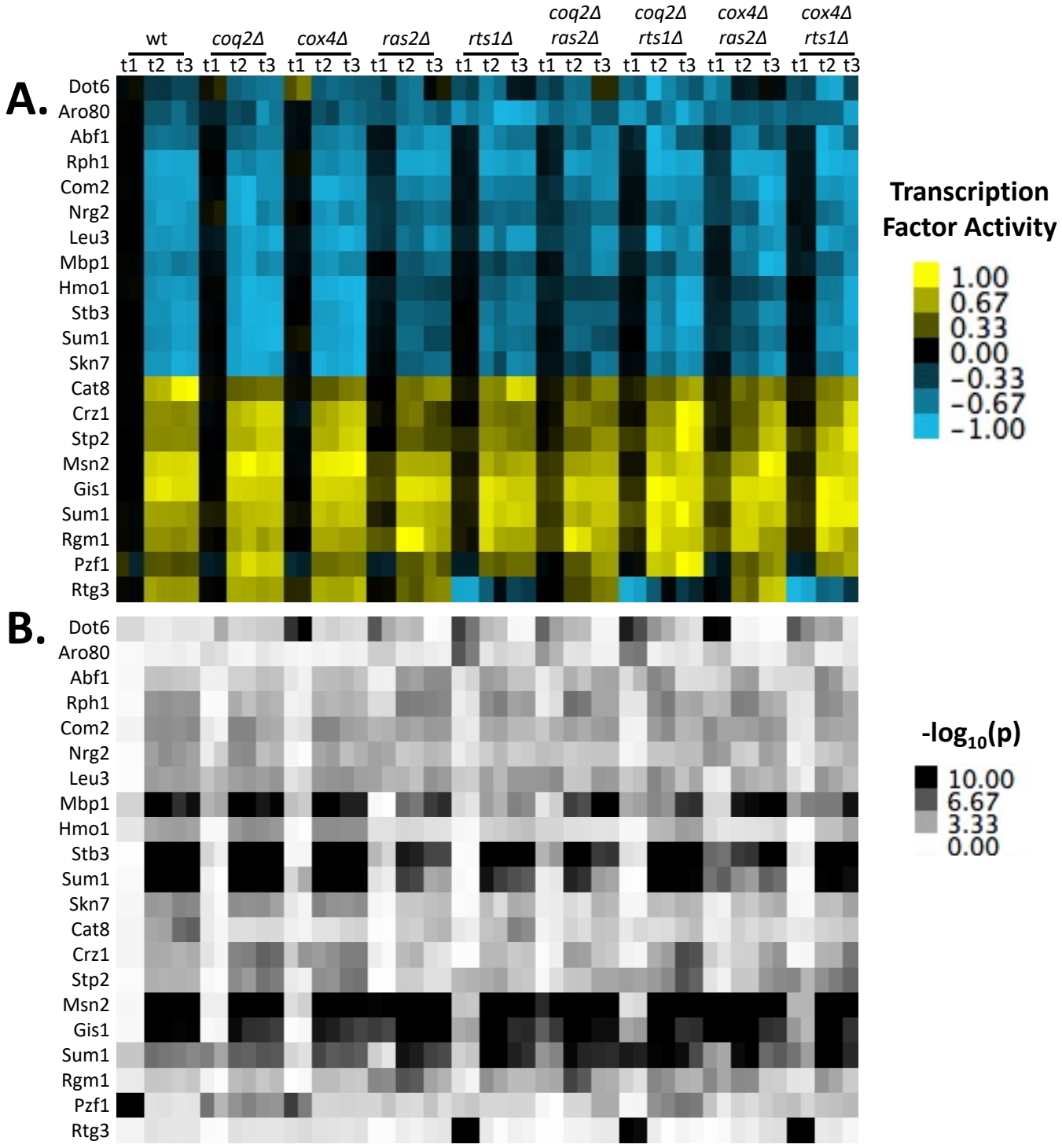
Supplemental Figure 3. Investigation of nutrient limitation in starvation-induced death of ETC mutants in standard YNB+glucose (SD) medium. A. Individual components were added back to YNB+glucose (SD) at 4X normal concentration, and survival was assessed after 4 days at 30°C. **B**. The *nde1Δ nde2Δ ndi1Δ* strain was grown in SD with varying (indicated) concentrations of glucose at 30°C and survival was measured at indicated timepoints. **C**. Residual media glucose from panel B was measured using a glucose testing strip. **D**. Indicated strains were grown overnight in SD. Cells were then spun-down, washed in 1 mL of sterile water, and resuspended in the indicated solutions (conditioned indicates original growth medium after being sterile filtered). Samples were taken at the indicated timepoints and 10-fold dilutions were spotted onto YPD to be grown at 30°C for 2 days. **E**. WT cells (cDBY0001) were inoculated into media containing variations in either glucose or YNB concentration, as indicated. Two biological replicates are shown for each. After 4 days of growth at 30°C, OD at 600nm was measured.



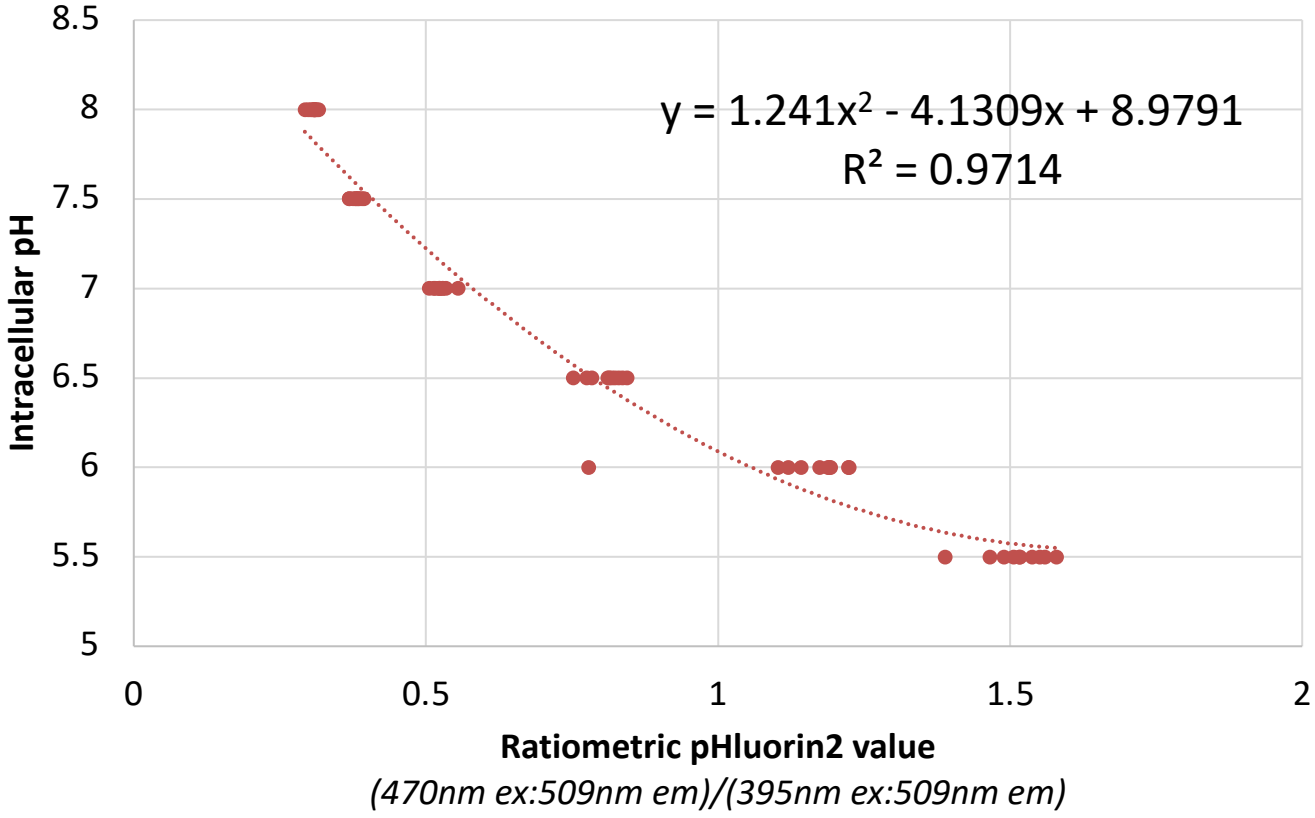
Supplemental Figure 4. Comparison of growth characteristics of individual suppressor mutants. Indicated strains were grown overnight in YPD, then washed 3X in sterile miliQ water and normalized to an OD600 of 1.0. 10-fold serial dilutions were performed and strains were spotted onto indicated media and grown at 30°C for the indicated time before photographing.



Supplemental Figure 5. Bud index data for ETC mutants and suppressor mutants used for gene expression data in Figure 6. Strains were grown in YNB+glucose as described in Materials and Methods; the stationary phase sample was taken after 3 days in YNB+glucose. Small bud, large bud, and unbudded cells were counted in a hemocytometer (at least 300 cells per biological replicate). Three biological replicates were performed for each strain. Error bars represent the standard deviation.

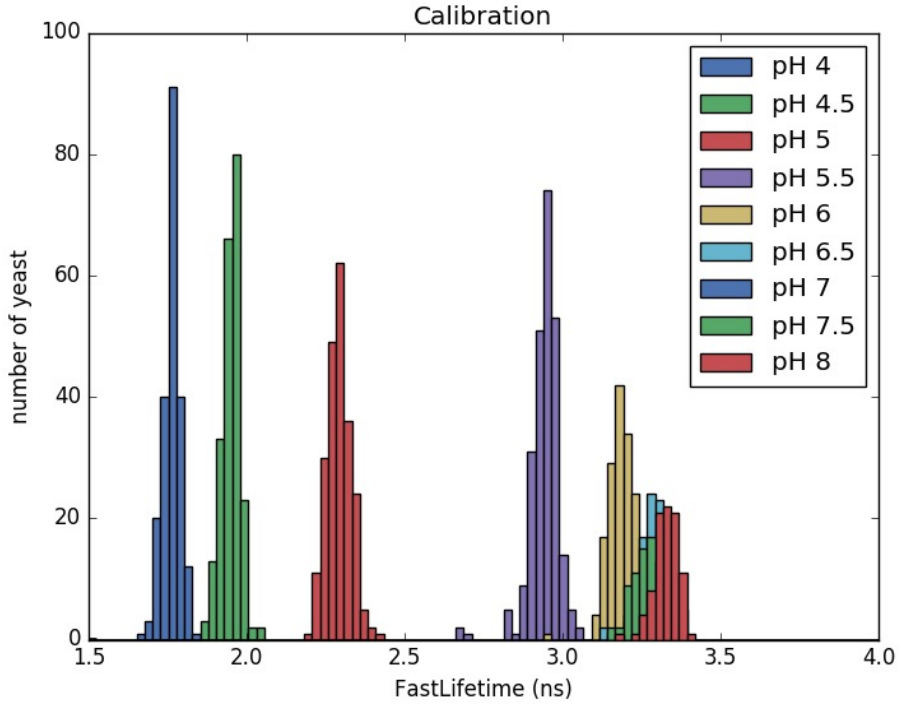


Supplemental Figure 6. Significant transcription factor activity estimations from REDUCE using Fig 6 RNA-Seq data. Transcription factor activities (**A**) and associated p-values (**B**). Transcription factor activities were calculated as described in Materials and Methods. Only significant transcription factors were included in this plot (based on having at least 4 time-points with uncorrected $p < 0.0001$), while the entire matrix of transcription factor activities is available as downloadable data. For each strain, two biological replicates are shown for each time-point.

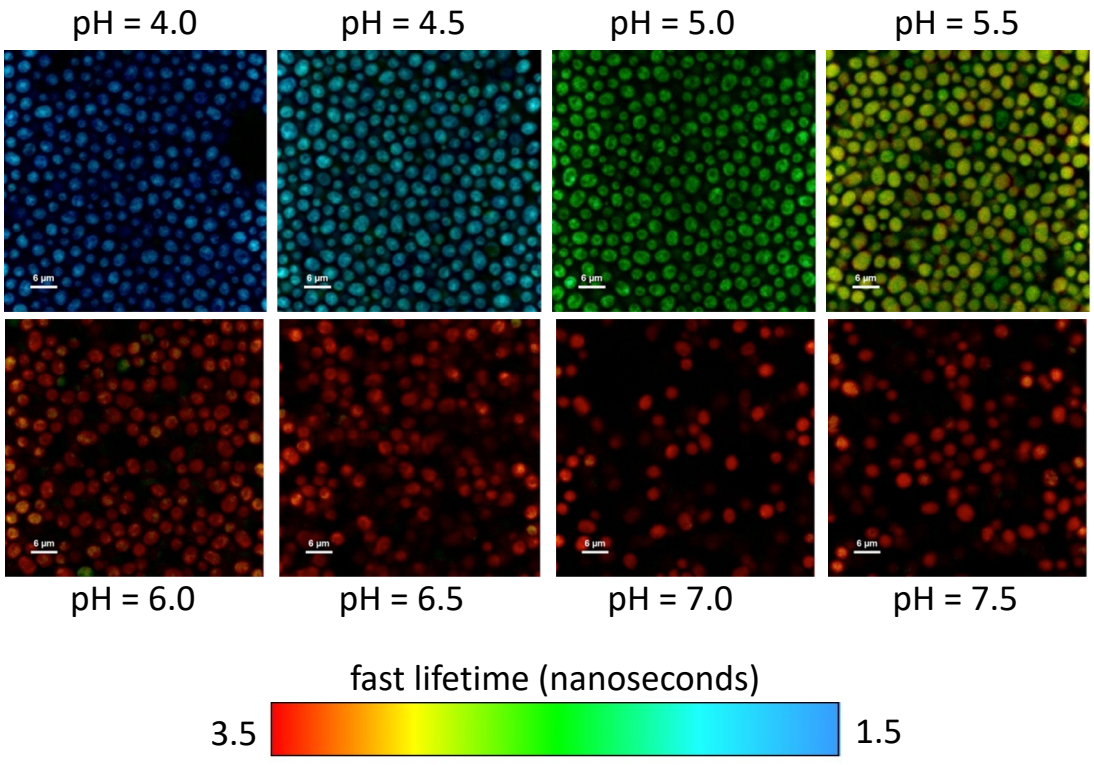


Supplemental Figure 7. Calibration curve for pHluorin2. pHluorin2 calibration was performed as described in Materials and Methods. Briefly, pHluorin2-containing strains from Figure 7 (wild type, *cox4Δ*, *cox4Δ ras2Δ*, *cox4Δ rts1Δ*, and *cox4Δ tco89Δ*) were grown in YNB+glucose, then permeabilized and resuspended in buffers at pH = 5.5, 6.0, 6.5, 7.0, 7.5, an 8.0. Fluorescence of these samples was measured using a Biotek plate reader. Calibrations were performed for each strain in biological duplicate, and all data was used to plot the calibration curve above. Inset: best fit polynomial curve equation, along with associated R^2 value.

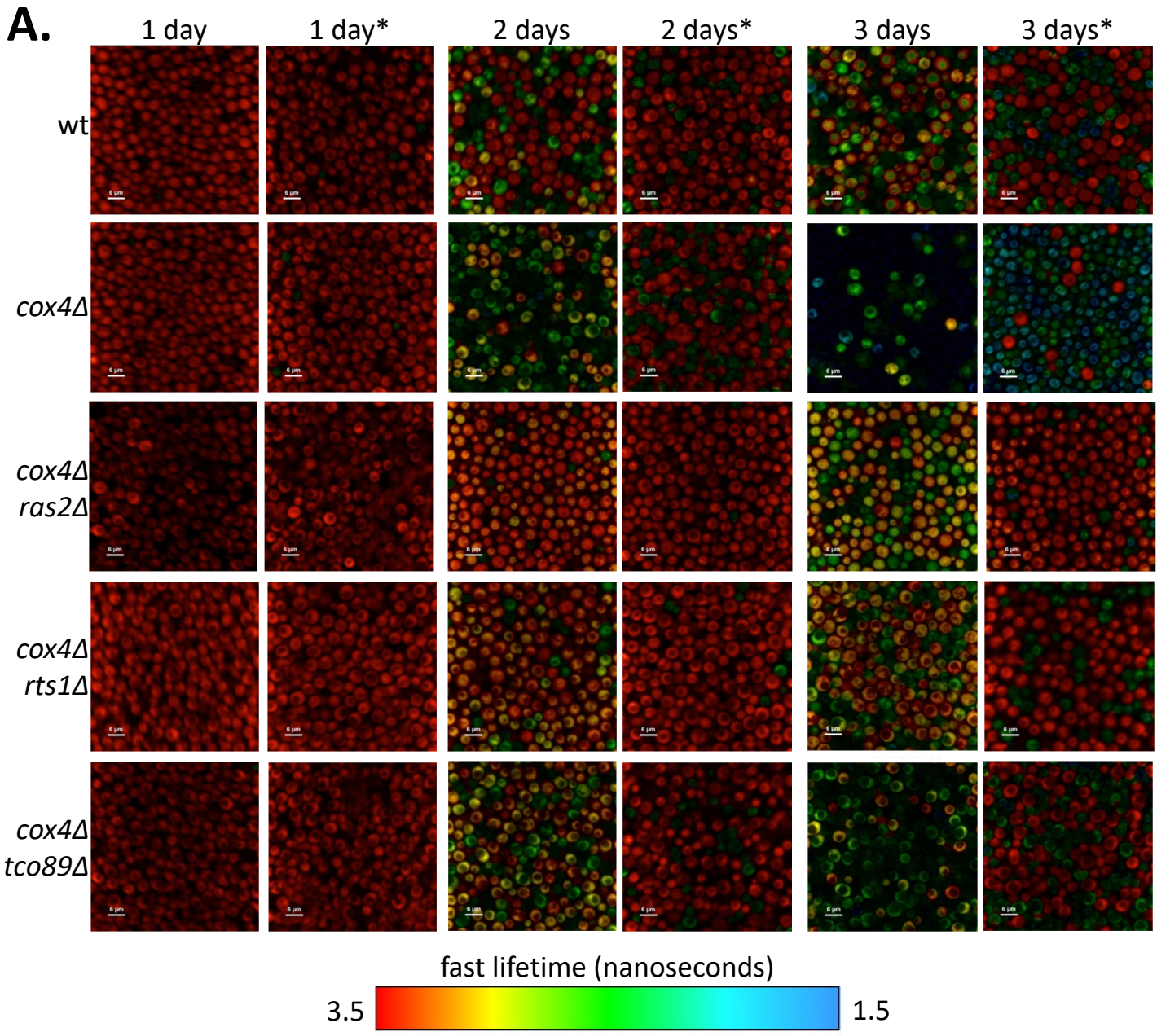
A.

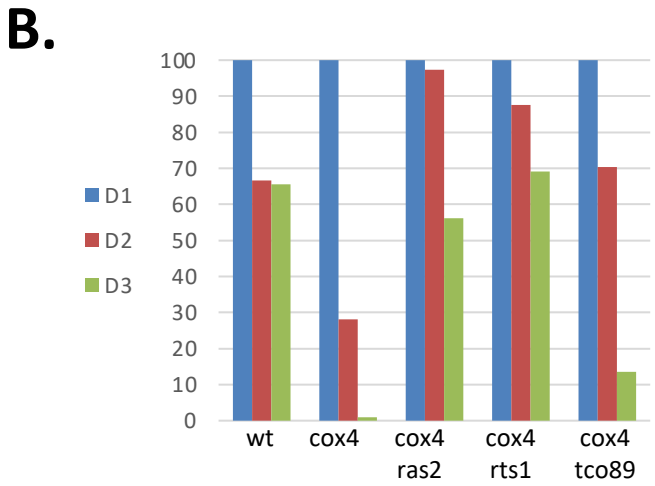


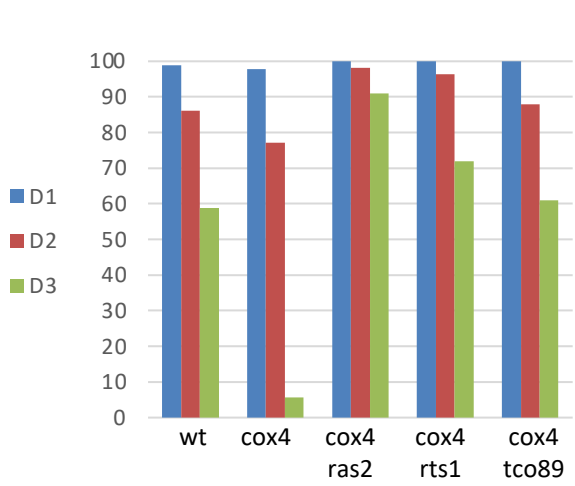
B.



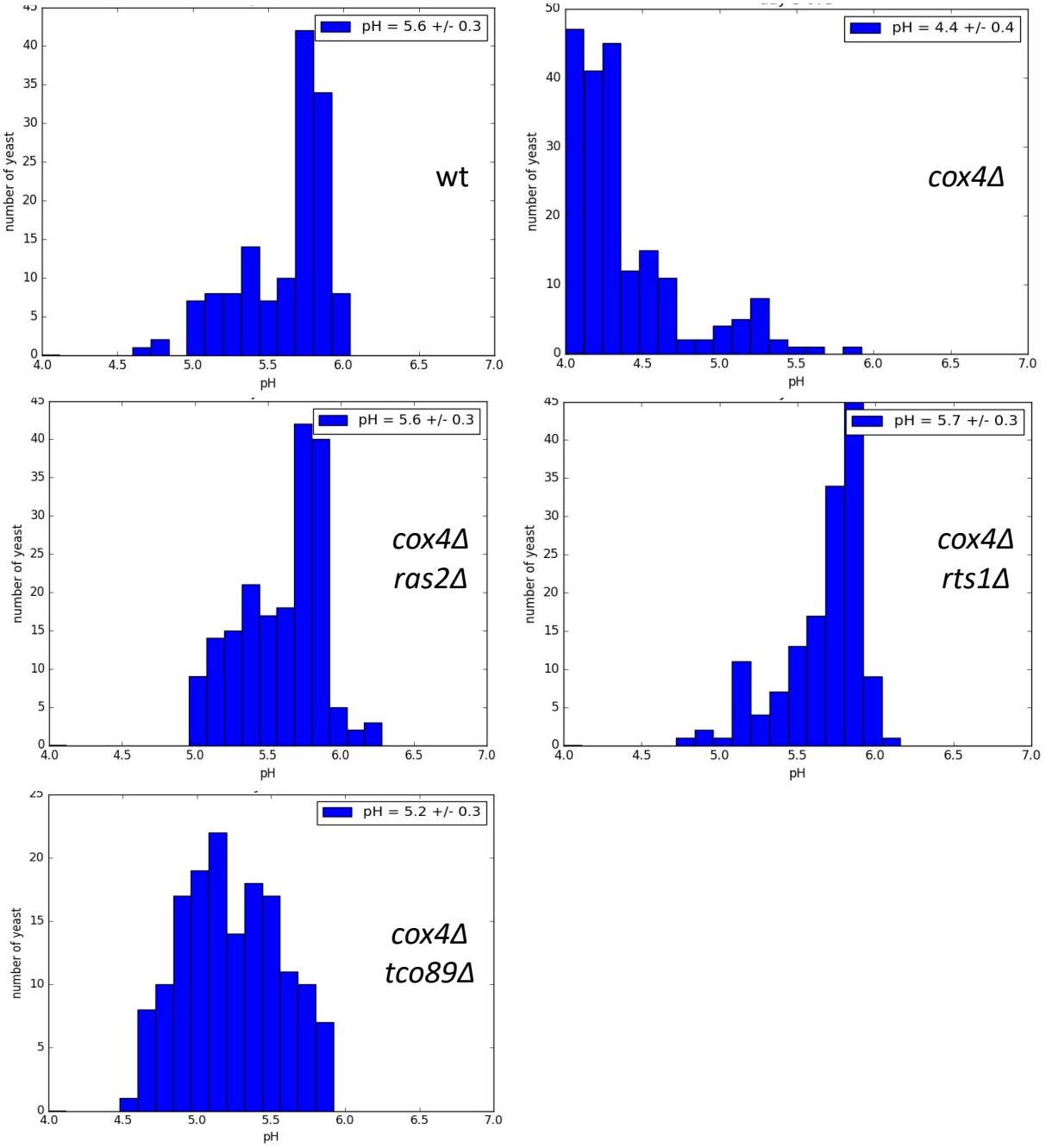
Supplemental Figure 8. Calibration curve for mScarlet. mScarlet calibration experiments were performed using fluorescence lifetime measurements to estimate intracellular pH as described in Materials and Methods. **A.** Plotted are mScarlet fluorescence lifetime measurements of individual cells when buffered at indicated pH values. **B**. Example of cells from each buffered pH (false colored based on fluorescence lifetime as indicated in color bar). Indicated size bar for each image represents 6 µm. Notably this fluorescent molecule would not work well above the pH range of 5.5 to 6.0.


A.
1 day
1 day*
2 days
2 days*
3 days
3 days*
wt
cox4Δ
cox4Δ ras2Δ
cox4Δ rts1Δ
cox4Δ tco89Δ
fast lifetime (nanoseconds)
3.5
1.5

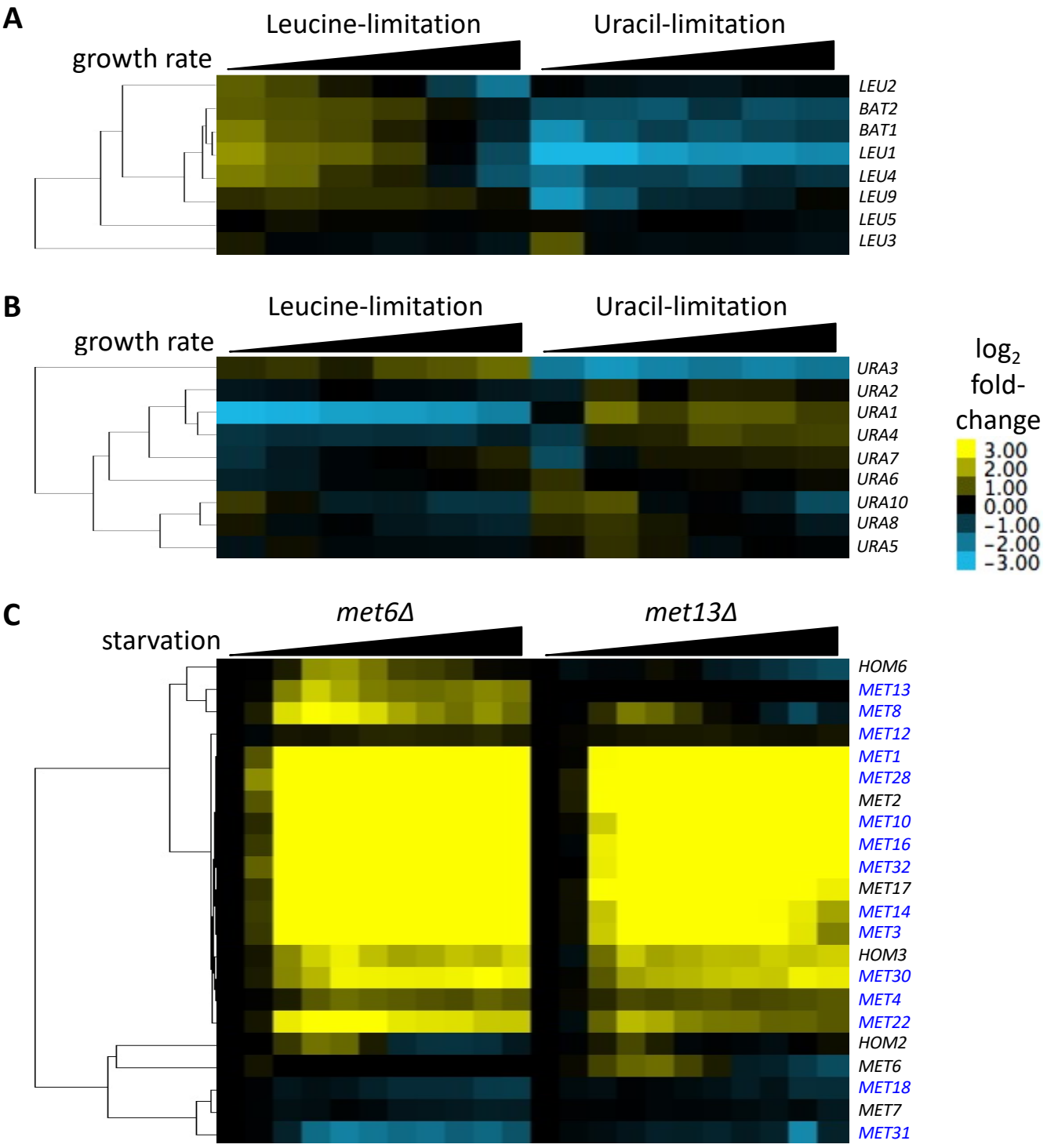

B.
100
90
80
70
60
50
40
30
20
10
0
D1
D2
D3
wt
cox4
cox4 ras2
cox4 rts1
cox4 tco89


C.
100
90
80
70
60
50
40
30
20
10
0
D1
D2
D3
wt
cox4
cox4 ras2
cox4 rts1
cox4 tco89

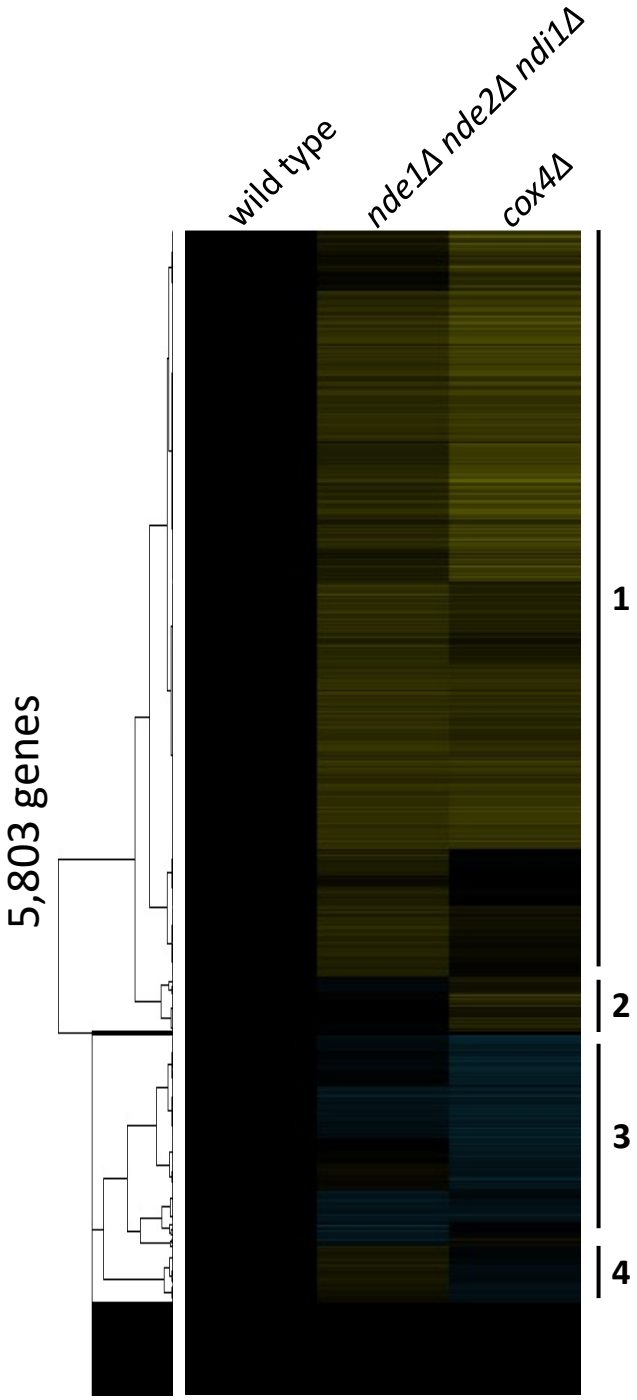
D.



Supplemental Figure 9. ETC suppressors partially restore intracellular pH homeostasis based on mScarlet fluorescent lifetime. A. Indicated strains were grown in YNB+glucose for 1, 2, or 3 days as indicated. False-colored images of cells shown based on fluorescent lifetime (see color bar). Recoverability of cells was assessed by resuspending in fresh medium for 30 minutes (samples indicated with *). **B**. Percent of samples on each day with intracellular pH > 5.5 (lifetime > 3 ns). **C**. Samples from each day were recovered as described in panel A, and percent with intracellular pH > 5.5 (lifetime > 3.5 ns) was measured. **D**. Histograms representing indicated strains with the distribution of individual cellular pH values after 3 days in YNB+glucose.



Supplemental Figure 10. Transcriptional regulation in the absence of a single metabolic pathway gene.
Data for panels A and B were adapted from Brauer *et al.* (Brauer *et al.,* 2008). In panels A and B, growth rates from left to right are 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 per hour. In panels A and B, expression values for each gene (row) were mean-centered, hierarchical clustering was performed using the Pearson correlation with average linkage. Data for panel C were adapted from Petti *et al*. (Petti *et al.,* 2011). In panel C, methionine starvation time-courses includes 0, 10, 30, 60, 90, 120, 150, 180, 210, 240, and 360 minute time-points. Each time-course was zero-normalized and hierarchical clustering was performed using the Pearson correlation with average linkage. Genes indicated in blue are not strictly part of the methionine biosynthesis pathway but are involved with regulation and other metabolic steps in sulfur metabolism.



ENRICHED PROCESSES

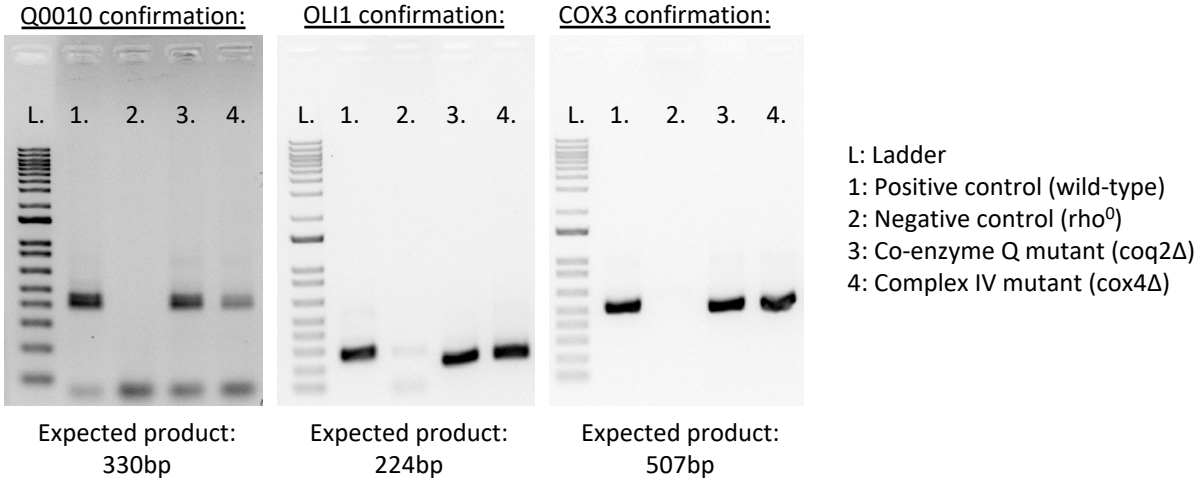
Cluster 1 (3,721 genes; 0.71)
response to chemical, transcription by RNAPII, transmembrane transport, mitotic cell cycle, lipid metabolism, etc.

Cluster 2 (270 genes; 0.85)
response to chemical, transcription by RNAPII, ion transport, transmembrane transport, regulation of organelle organization, etc.

Cluster 3 (1,052 genes; 0.44)
cytoplasmic translation, rRNA processing, response to chemical, transcription by RNAPII, ribosomal small subunit organization, etc.

Cluster 4 (280 genes; 0.89)
rRNA processing, transcription by RNAPII, mRNA processing, chromatin organization, etc.

Supplemental Figure 11. Comparison of RNA-Seq at t=0 for wild type, *nde1Δ nde2Δ ndi1Δ*, and *cox4Δ*. Samples shown are the t=0 samples from the RNA-Seq data in Figure 3. To examine how ETC mutant gene expression differs from WT, these t=0 samples were normalized to the WT t=0 sample. Indicated clusters were selected (gene number and correlation in parentheses) and GO Slim Mapper (www.yeastgenome.org) was used to identify top 5 enriched processes. The block of ~450 genes at the bottom of the heatmap are genes with raw TPM levels below the flooring limit; therefore all had the same, floored TPM value.



Supplemental Figure 12. Confirmation of mitochondrial genome stability in ETC mutants *coq2Δ* and *cox4Δ*. Genomic DNA was prepared from the indicated strains, and PCR was used to assess the presence/absence of three genes encoded in the yeast mitochondrial genome (*Q0010*, *OLI1*, and *COX3*). The following oligonucleotide pairs were used for each gene: Q0010 FOR (5' – CTATTCTATTGTGGGGGTCCCAATTAT – 3'), Q0010 REV (5' – CGAAACCGGGACCTCGGAGACG – 3'), OLI1 FOR (5' – GCAATTAGTATTAGCAGCTAAAT – 3'), OLI1 REV (5' – CACCGAATAATAATAAGAATGAAACC – 3'), COX3 FOR (5' – GACACATTTAGAAAGAAGTAGAC – 3'), COX3 REV (5' – CCTGATAAGGCTTTATTTCTATTACC – 3').

CLUSTER	CLUSTER CORRELATION	ONTOLOGY ASPECT	TERM	FDR	CORRECTED P-VALUE
I	0.52	PROCESS	cellular macromolecule metabolic process	0.00%	6.00E-06
I	0.52	PROCESS	regulation of cellular process	0.00%	3.97E-05
I	0.52	PROCESS	cellular protein modification process	0.00%	4.82E-05
I	0.52	PROCESS	protein modification process	0.00%	4.82E-05
I	0.52	PROCESS	biological regulation	0.00%	4.90E-05
I	0.52	PROCESS	regulation of biological process	0.00%	0.00048609
I	0.52	PROCESS	macromolecule modification	0.00%	0.00051589
I	0.52	PROCESS	cellular component assembly	0.00%	0.00078518
I	0.52	PROCESS	mannosylation	0.00%	0.00085415
I	0.52	PROCESS	protein-DNA complex subunit organization	0.00%	0.00087692
I	0.52	PROCESS	regulation of cellular metabolic process	0.00%	0.00167062
I	0.52	PROCESS	nucleosome positioning	0.00%	0.00167895
I	0.52	PROCESS	regulation of cellular component organization	0.00%	0.00182985
I	0.52	PROCESS	regulation of nucleobase-containing compound metabolic process	0.00%	0.00183969
I	0.52	PROCESS	glycosylation	0.00%	0.00215404
I	0.52	PROCESS	DNA conformation change	0.00%	0.00279288
I	0.52	PROCESS	regulation of cellular component biogenesis	0.00%	0.0029482
I	0.52	PROCESS	regulation of primary metabolic process	0.00%	0.0036733
I	0.52	PROCESS	actin filament-based process	0.00%	0.00370035
I	0.52	PROCESS	macromolecule biosynthetic process	0.00%	0.00430345
I	0.52	PROCESS	chromosome organization	0.00%	0.00456165
I	0.52	PROCESS	macromolecule metabolic process	0.00%	0.00493476
I	0.52	PROCESS	cellular component organization	0.00%	0.00512024
I	0.52	PROCESS	actin cytoskeleton organization	0.00%	0.00527353
I	0.52	PROCESS	regulation of biosynthetic process	0.00%	0.00629926
I	0.52	PROCESS	lipid metabolic process	0.00%	0.006306
I	0.52	PROCESS	lipid biosynthetic process	0.00%	0.0068279
I	0.52	PROCESS	regulation of organelle organization	0.00%	0.00712312
I	0.52	PROCESS	regulation of metabolic process	0.00%	0.00801231
I	0.52	PROCESS	regulation of nitrogen compound metabolic process	0.00%	0.00804745
I	0.52	PROCESS	regulation of cellular biosynthetic process	0.00%	0.0085674
I	0.52	PROCESS	glycoprotein metabolic process	0.00%	0.00945538
I	0.52	FUNCTION	binding	0.00%	1.37E-05
I	0.52	FUNCTION	mannosyltransferase activity	0.00%	9.46E-05
I	0.52	FUNCTION	protein binding	0.00%	0.00078075
I	0.52	FUNCTION	hydrolase activity	0.00%	0.00453681
I	0.52	FUNCTION	catalytic activity	0.00%	0.00772466
I	0.52	COMPONENT	endomembrane system	0.00%	1.53E-08
I	0.52	COMPONENT	cell cortex	0.00%	7.28E-08
I	0.52	COMPONENT	cytoplasmic region	0.00%	7.28E-08
I	0.52	COMPONENT	cell cortex part	0.00%	3.14E-07
I	0.52	COMPONENT	intracellular membrane-bounded organelle	0.00%	3.28E-07
I	0.52	COMPONENT	membrane-bounded organelle	0.00%	4.64E-07
I	0.52	COMPONENT	intracellular	0.00%	1.93E-06
I	0.52	COMPONENT	intracellular part	0.00%	1.93E-06
I	0.52	COMPONENT	cortical cytoskeleton	0.00%	2.71E-06
I	0.52	COMPONENT	organelle	0.00%	6.11E-06
I	0.52	COMPONENT	intracellular organelle	0.00%	9.35E-06
I	0.52	COMPONENT	cortical actin cytoskeleton	0.00%	1.65E-05
I	0.52	COMPONENT	actin cytoskeleton	0.00%	6.97E-05
I	0.52	COMPONENT	cell part	0.00%	7.90E-05
I	0.52	COMPONENT	cell	0.00%	8.67E-05
I	0.52	COMPONENT	actin cortical patch	0.00%	0.00011801
I	0.52	COMPONENT	endocytic patch	0.00%	0.00017616
I	0.52	COMPONENT	endoplasmic reticulum	0.00%	0.00028527
I	0.52	COMPONENT	cytoskeleton	0.00%	0.00030208
I	0.52	COMPONENT	endoplasmic reticulum part	0.00%	0.00049319
I	0.52	COMPONENT	site of polarized growth	0.00%	0.00058408
I	0.52	COMPONENT	Golgi apparatus	0.00%	0.00083962
I	0.52	COMPONENT	protein complex	0.00%	0.00160961
I	0.52	COMPONENT	cytoskeletal part	0.00%	0.00242245
I	0.52	COMPONENT	organelle subcompartment	0.00%	0.00685782
I	0.52	COMPONENT	intracellular organelle part	0.00%	0.00822244
I	0.52	COMPONENT	organelle part	0.00%	0.00906805
II	0.59	PROCESS	*no significant GO terms (threshold: p < 0.01)*	*n/a*	*n/a*
II	0.59	FUNCTION	*no significant GO terms (threshold: p < 0.01)*	*n/a*	*n/a*
II	0.59	COMPONENT	*no significant GO terms (threshold: p < 0.01)*	*n/a*	*n/a*
III	0.71	PROCESS	*no significant GO terms (threshold: p < 0.01)*	*n/a*	*n/a*
III	0.71	FUNCTION	*no significant GO terms (threshold: p < 0.01)*	*n/a*	*n/a*
III	0.71	COMPONENT	chromosomal region	2.00%	0.00381208
IV	0.55	PROCESS	proteolysis	0.00%	0.00017452
IV	0.55	PROCESS	endocytosis	0.00%	0.00389046
IV	0.55	PROCESS	ubiquitin-dependent protein catabolic process	0.00%	0.00713191
IV	0.55	FUNCTION	signal transducer activity	0.00%	0.00322875
IV	0.55	FUNCTION	catalytic activity, acting on a protein	0.00%	0.00411976
IV	0.55	COMPONENT	membrane	0.00%	0.00282463
IV	0.55	COMPONENT	proteasome regulatory particle	0.00%	0.00283027
IV	0.55	COMPONENT	proteasome accessory complex	0.00%	0.00283027
V	0.52	PROCESS	*no significant GO terms (threshold: p < 0.01)*	*n/a*	*n/a*
V	0.52	FUNCTION	*no significant GO terms (threshold: p < 0.01)*	*n/a*	*n/a*
V	0.52	COMPONENT	*no significant GO terms (threshold: p < 0.01)*	*n/a*	*n/a*

Supplemental Table 1. GO Term search results for clusters in Figure 3D. GO term searches were performed on 11.26.2019 using the search tool incorporated into the Saccharomyces Genome Database (www.yeastgenome.org). A filter was applied to only include results with a P-value < 0.01. The background gene set for this search included only the 5,803 genes assessed in the accompanying RNA-Seq.

CLUSTER	CLUSTER CORRELATION	ONTOLOGY ASPECT	TERM	FDR	CORRECTED P-VALUE
A	0.78	PROCESS	mitochondrion organization	12.00%	0.00542053
A	0.78	FUNCTION	*no significant GO terms (threshold: p < 0.01)*	*n/a*	*n/a*
A	0.78	COMPONENT	*no significant GO terms (threshold: p < 0.01)*	*n/a*	*n/a*
B	0.46	PROCESS	mitochondrial translation	0.00%	1.52E-08
B	0.46	PROCESS	mitochondrial gene expression	0.00%	7.92E-08
B	0.46	PROCESS	protein metabolic process	2.67%	0.00845372
B	0.46	FUNCTION	structural constituent of ribosome	0.00%	1.34E-06
B	0.46	FUNCTION	structural molecule activity	0.00%	8.58E-06
B	0.46	COMPONENT	organellar ribosome	0.00%	1.81E-10
B	0.46	COMPONENT	mitochondrial ribosome	0.00%	1.81E-10
B	0.46	COMPONENT	mitochondrial matrix	0.00%	7.25E-09
B	0.46	COMPONENT	ribosomal subunit	0.00%	3.32E-06
B	0.46	COMPONENT	ribosome	0.00%	7.95E-06
B	0.46	COMPONENT	organellar small ribosomal subunit	0.00%	3.39E-05
B	0.46	COMPONENT	mitochondrial small ribosomal subunit	0.00%	3.39E-05
B	0.46	COMPONENT	mitochondrial part	0.00%	7.90E-05
B	0.46	COMPONENT	organellar large ribosomal subunit	0.00%	0.00012441
B	0.46	COMPONENT	mitochondrial large ribosomal subunit	0.00%	0.00012441
B	0.46	COMPONENT	mitochondrion	0.00%	0.00013571
B	0.46	COMPONENT	small ribosomal subunit	0.17%	0.00507698
C	0.57	PROCESS	organellar ribosome	0.00%	1.81E-10
C	0.57	PROCESS	mitochondrial ribosome	0.00%	1.81E-10
C	0.57	PROCESS	mitochondrial matrix	0.00%	7.25E-09
C	0.57	PROCESS	ribosomal subunit	0.00%	3.32E-06
C	0.57	PROCESS	ribosome	0.00%	7.95E-06
C	0.57	PROCESS	organellar small ribosomal subunit	0.00%	3.39E-05
C	0.57	PROCESS	mitochondrial small ribosomal subunit	0.00%	3.39E-05
C	0.57	PROCESS	mitochondrial part	0.00%	7.90E-05
C	0.57	PROCESS	organellar large ribosomal subunit	0.00%	0.00012441
C	0.57	PROCESS	mitochondrial large ribosomal subunit	0.00%	0.00012441
C	0.57	PROCESS	mitochondrion	0.00%	0.00013571
C	0.57	PROCESS	small ribosomal subunit	0.17%	0.00507698
C	0.57	FUNCTION	oxidoreductase activity	2.00%	0.00381601
C	0.57	COMPONENT	mitochondrion	0.00%	1.82E-09
C	0.57	COMPONENT	mitochondrial part	0.00%	1.24E-08
C	0.57	COMPONENT	cytoplasm	0.00%	5.85E-07
C	0.57	COMPONENT	cytoplasmic part	0.00%	4.94E-06
C	0.57	COMPONENT	mitochondrial inner membrane	0.00%	5.29E-05
C	0.57	COMPONENT	mitochondrial envelope	0.00%	7.17E-05
C	0.57	COMPONENT	intracellular organelle	0.00%	8.14E-05
C	0.57	COMPONENT	organelle	0.00%	8.33E-05
C	0.57	COMPONENT	organelle inner membrane	0.00%	9.50E-05
C	0.57	COMPONENT	mitochondrial membrane	0.00%	0.00045897
C	0.57	COMPONENT	intracellular	0.00%	0.00090536
C	0.57	COMPONENT	intracellular part	0.00%	0.00090536
C	0.57	COMPONENT	organelle envelope	0.00%	0.00365059
C	0.57	COMPONENT	envelope	0.00%	0.00365059
C	0.57	COMPONENT	mitochondrial matrix	0.00%	0.00381188
C	0.57	COMPONENT	membrane-bounded organelle	0.00%	0.00389896
C	0.57	COMPONENT	organelle membrane	0.00%	0.005496
C	0.57	COMPONENT	cell part	0.00%	0.00566276
C	0.57	COMPONENT	cell	0.00%	0.00572536
C	0.57	COMPONENT	intracellular membrane-bounded organelle	0.20%	0.00709674

Supplemental Table 2. GO Term search results for clusters in Figure 3E. GO term searches were performed on 11.26.2019 using the search tool incorporated into the Saccharomyces Genome Database (www.yeastgenome.org). A filter was applied to only include results with a P-value < 0.01. The background gene set for this search included the entire set of genes available in SGD (7,166 genes).

Strain	Cultures with no suppressors	Number of generations	Mutation rate (mutations per cell division)
nde1Δ nde2Δ ndi1Δ	64/72	1.38E+07	8.5536E-09
coq2Δ	34/72	1.36E+07	5.5308E-08
rip1Δ	42/72	1.42E+07	3.788E-08
cyc1Δ cyc7Δ	45/72	1.43E+07	3.2796E-08
cox4Δ	15/72	1.38E+07	1.1392E-07
atp1Δ	21/64	1.56E+07	7.164E-08

Supplemental Table 3. Measured mutation rate for each ETC mutant in YNB+glucose starvation. A fluctuation assay was performed as described by *Lang et al*. (Lang, 2018). Mutation rates were calculated using the P_0 method ($u = -\ln(P_O)/N$; u = mutations per genome per generation, P_0 = probability that a mutation does not occur in the entire culture, N = number of generations that have occurred). For comparison, mutation to 5-FOA resistance is 5.43×10^{-8} mutations per genome per generation, and mutation to canavanine resistance is 1.52×10^{-7} mutations per genome per generation (Lang and Murray, 2008).

Gene	Essential	Observed Mutations	Confirmed
RAS2	no	D112Y[A] [1], Q295ns [1], I107L [1], V14L [2], D173fs [2], N221fs[B] [2], T42I [2], ΔM145-Q156 [2], M79I [3], D112V [3], M1I[C] [3], ΔT31-E44 [3], S291fs [3], R48G [4], Y178ns [4], E161fs [4], N221fs [4], ΔK23-V120 [4], G67R [4], A66V [5], ΔA157-S188 [5], ΔE83-E174 [5], Q253ns [5], E70ns [6], E76Q [6], T42K [6]	yes
CYR1	yes	A679D [1], Q1509ns [1], F711C [2], Q936H [2], F771S [2], R681H [2], L1983S [2], K959E [2], L1923Q [2], Q1736K [2], R681C [3], E1924K [3], S1676I [3], E1710G [4], E1659V [4], E1994ns [6], K1738T [6]	n.t.
CDC55	no	D76ns [2], ΔA18-T42 [2], Q157ns [2], E240ns [2], ΔA18-T42 [3], W92ns [3], V115fs [3], ΔK160-I236 [4], D203fs [4], ΔA18-T42 [4], ΔN356-G359 [4], Y179ns [4], D342fs [4], R339ns [5], ΔA18-T42 [5], N126fs [5], D262Y [5], ΔL112-N119 [5], ΔT36-A73 [5], D34fs [5], K139fs [6], L78ns [6], F288fs [6], T517fs [6], N406fs [6], S11fs [6], A41fs [6], L209ns [6], ΔL200-end[D] [6]	yes
IRA1	yes[E]	A1657V [6]	no
IRA2	no	S2769ns[F] [1], ΔN2996-S3016 [1], L1851S[G] [2], ΔD2998-S3016 [5], L2530fs[H] [6]	no
CDC25	yes	T1415I [1], E1540K [2], V1384F[I] [2], G1460C [2], ΔL624-S672[J] [2], P1306R [3], ΔI855-D896[J] [4], P1306L [5], E1328K [6], T1415K [6], Promoter SNP[K] [6]	n.t.
RAS1	no	D126N [3], A25P [5], D126N [6]	no
TPD3	no[L]	ΔQ86-E89 [5], G431ns [6]	n.t.
RTS1	no	ΔH492-L503[J] [2]	yes
SRV2	no	M8I [5]	yes
TUS1	no	Y954H [6]	yes
MDS3	no	E1023fs [6]	yes
TCO89	no	ΔE221-D251 [6]	yes
PHO85	no	ΔF158-A176[J]	yes
SRB8	no	K818ns [6]	yes
TIR1	no	ΔS144-S150 [6]	n.t.
PMA1	yes	P536T [5]	n.t.
GCS1	no	R130H [2]	n.t.
CMD1	yes	Promoter SNP[M] [2]	n.t.
CCR4	no	I176-Q191 [5]	yes
CDC39	yes	S1903 [3]	n.t.
MRP51	no	A138V [3]	n.t.
SSN8	no	G3fs [4]	yes
SST2	no	T268T [4]	n.t.
BUB3	no	D323N [4]	n.t.

Supplemental Table 4. Observed mutations in ETC suppressor strains.
(legend on following page)

Supplemental Table 4. Observed mutations in ETC suppressor strains. These are mutations we detected with high confidence. A subset was tested based on multiple mutations in the same gene or multiple mutations in the same/similar pathway. In the 'Essential' column, we note whether this gene has been annotated as essential in SGD. In the 'Confirmed' column, 'yes' indicates that deletion of this gene recapitulated the suppression phenotype, 'no' indicates that the deletion of this gene did not recapitulate the suppression phenotype, and n.t. indicates that this gene was not further tested for suppression activity. After each listed mutation, the number in brackets indicates the parent strain (1: *nde1Δ nde2Δ ndi1Δ*, 2: *coq2Δ*, 3: *rip1Δ*, 4: *cyc1Δ cyc7Δ*, 5: *cox4Δ*, and 6: *atp1Δ*). For each allele, 'ns' indicates a nonsense allele (stop codon insertion), 'fs' indicates a frameshift allele, and Δ indicates a gene deletion between the listed amino acids (identified in-frame deletions are noted). Precise nucleotide changes are available upon request.

Footnotes:
A: in addition to *ira2*-S2769ns
B: complex mutation event (G291D, N221fs, N223Y)
C: next ATG is out of frame (therefore this results in a frameshift)
D: deletion extends beyond the end of the gene
E: this gene appeared to be non-essential in our genetic background
F: in addition to *ras2*-D112Y
G: in addition to *cdc25*-V1384F
H: in addition to SNP in *CDC25* promoter at -51
I: in addition to *ira2*-L1851S
J: appears to be an in-frame deletion
K: SNP in promoter at -51, in addition to *ira2*-L2530fs
L: this gene appeared to be essential in our genetic background, and therefore wasn't tested further
M: SNP in promoter at -51 (T -> C)

STRAIN DESIGNATION	NAME IN FIGURES	GENOTYPE	SHOWN IN FIGURE	REF
cDBY0001	WT	S288C/FY derivative. MAT**a** prototroph. *HAP1*$^{+}$	1, 2, 3, 4, 7, S1, S2, S3, S4, S7, S8, SF1, SF2, SF3, SF4, SF7, SF8	See Materials and Methods
cDBY0002	WT	S288C/FY derivative. MATα prototroph. *HAP1*$^{+}$	5	See Materials and Methods
cDBY0083	*nde1Δ nde2Δ ndi1Δ*	MAT**a** *HAP1*$^{+}$ *ndi1*Δ::kanMX *nde1*Δ::hphMX *nde2*Δ::bleMX	1, 2, 3, SF1, SF2, SF8, ST3	This study
cDBY0114	*sdh1Δ yjl045wΔ*	MAT**a** *HAP1*$^{+}$ *sdh1*Δ::kanMX *yjl045w*Δ::hphMX	1, 2, SF1	This study
cDBY0037	*coq2Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX	1, 2, 4, SF1, SF4, ST3	This study
cDBY0041	*rip1Δ*	MAT**a** *HAP1*$^{+}$ *rip1*Δ::kanMX	1, 2, SF1, ST3	This study
cDBY0073	*cyc1Δ cyc1Δ*	MAT**a** *HAP1*$^{+}$ *cyc1*Δ::kanMX *cyc7*Δ::hphMX	1, 2, SF1, ST3	This study
cDBY0045	*cox4Δ*	MAT**a** *HAP1*$^{+}$ *cox4*Δ::kanMX	1, 2, 3, 4, 7, SF1, SF4, SF7, SF8, ST3	This study
cDBY0065	*atp1Δ*	MAT**a** *HAP1*$^{+}$ *atp1*Δ::kanMX	1, 2, SF1, ST3	This study
cDBY0595	*coq2Δ ira1Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX *ira1*Δ::natAC	4	This study
cDBY0603	*coq2Δ ira2Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX *ira2*Δ::natAC	4	This study
cDBY0610	*coq2Δ ras1Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX *ras1*Δ::natAC	4	This study
cDBY0611	*coq2Δ ras2Δ*	MATα *HAP1*$^{+}$ *coq2*Δ::kanMX *ras2*Δ::natAC	4, SF4	This study
cDBY0618	*coq2Δ srv2Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX *srv2*Δ::natAC	4	This study
PGY365	*coq2Δ cdc55Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX *cdc55*Δ::natAC	4	This study
cDBY0612	*coq2Δ rts1Δ*	MATα *HAP1*$^{+}$ *coq2*Δ::kanMX *rts1*Δ::natAC	4, SF4	This study
cDBY0608	*coq2Δ ppm1Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX *ppm1*Δ::natAC	4	This study
cDBY0607	*coq2Δ pph21Δ*	MATα *HAP1*$^{+}$ *coq2*Δ::kanMX *pph21*Δ::natAC	4	This study
cDBY0647	*coq2Δ pph22Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX *pph22*Δ::natAC	4	This study
cDBY0624	*coq2Δ tor1Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX *tor1*Δ::natAC	4	This study
cDBY0622	*coq2Δ tco89Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX *tco89*Δ::natAC	4, SF4	This study
cDBY0598	*coq2Δ mds3Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX *mds3*Δ::natAC	4	This study
cDBY0626	*coq2Δ tus1Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX *tus1*Δ::natAC	4	This study
cDBY0616	*coq2Δ sch9Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX *sch9*Δ::natAC	4	This study
cDBY0592	*coq2Δ ccr4Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX *ccr4*Δ::natAC	4	This study
cDBY0614	*coq2Δ srb8Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX *srb8*Δ::natAC	4	This study
cDBY0620	*coq2Δ ssn8Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX *ssn8*Δ::natAC	4	This study
cDBY0605	*coq2Δ pho85Δ*	MAT**a** *HAP1*$^{+}$ *coq2*Δ::kanMX *pho85*Δ::natAC	4	This study
cDBY0561	*cox4Δ ira1Δ*	MAT**a** *HAP1*$^{+}$ *cox4*Δ::kanMX *ira1*Δ::natAC	4	This study
cDBY0563	*cox4Δ ira2Δ*	MAT**a** *HAP1*$^{+}$ *cox4*Δ::kanMX *ira2*Δ::natAC	4	This study
cDBY0573	*cox4Δ ras1Δ*	MAT**a** *HAP1*$^{+}$ *cox4*Δ::kanMX *ras1*Δ::natAC	4	This study
cDBY0575	*cox4Δ ras2Δ*	MAT**a** *HAP1*$^{+}$ *cox4*Δ::kanMX *ras2*Δ::natAC	4, 7, SF4, SF7	This study
cDBY0583	*cox4Δ srv2Δ*	MAT**a** *HAP1*$^{+}$ *cox4*Δ::kanMX *srv2*Δ::natAC	4	This study
PGY310	*cox4Δ cdc55Δ*	MAT**a** *HAP1*$^{+}$ *cox4*Δ::kanMX *cdc55*Δ::natAC	4	This study
cDBY0577	*cox4Δ rts1Δ*	MAT**a** *HAP1*$^{+}$ *cox4*Δ::kanMX *rts1*Δ::natAC	4, 7, SF4, SF7	This study
cDBY0571	*cox4Δ ppm1Δ*	MAT**a** *HAP1*$^{+}$ *cox4*Δ::kanMX *ppm1*Δ::natAC	4	This study
cDBY0567	*cox4Δ pph21Δ*	MAT**a** *HAP1*$^{+}$ *cox4*Δ::kanMX *pph21*Δ::natAC	4	This study
cDBY0569	*cox4Δ pph22Δ*	MAT**a** *HAP1*$^{+}$ *cox4*Δ::kanMX *pph22*Δ::natAC	4	This study
cDBY0588	*cox4Δ tor1Δ*	MAT**a** *HAP1*$^{+}$ *cox4*Δ::kanMX *tor1*Δ::natAC	4	This study
cDBY0586	*cox4Δ tco89Δ*	MAT**a** *HAP1*$^{+}$ *cox4*Δ::kanMX *tco89*Δ::natAC	4, 7, SF4, SF7	This study
PGY360	*cox4Δ mds3Δ*	MAT**a** *HAP1*$^{+}$ *cox4*Δ::kanMX *mds3*Δ::natAC	4	This study
cDBY0590	*cox4Δ tus1Δ*	MAT**a** *HAP1*$^{+}$ *cox4*Δ::kanMX *tus1*Δ::natAC	4	This study
cDBY0579	*cox4Δ sch9Δ*	MAT**a** *HAP1*$^{+}$ *cox4*Δ::kanMX *sch9*Δ::natAC	4	This study

Supplemental Table 5. Strains used in this study.

STRAIN DESIGNATION	NAME IN FIGURES	GENOTYPE	SHOWN IN FIGURE	REF
cDBY0559	*cox4Δ ccr4Δ*	MATa *HAP1⁺ cox4Δ*::kanMX *ccr4Δ*::natAC	4	This study
cDBY0581	*cox4Δ srb8Δ*	MATa *HAP1⁺ cox4Δ*::kanMX *srb8Δ*::natAC	4	This study
cDBY0584	*cox4Δ ssn8Δ*	MATa *HAP1⁺ cox4Δ*::kanMX *ssn8Δ*::natAC	4	This study
cDBY0565	*cox4Δ pho85Δ*	MATa *HAP1⁺ cox4Δ*::kanMX *pho85Δ*::natAC	4	This study
DBY12031	*leu2Δ*	MATα *HAP1⁺ leu2Δ0*	5	See Materials and Methods
cDBY0426	*ras2Δ*	MATα *HAP1⁺ ras2Δ*::kanMX *leu2Δ0*	5	This study
cDBY0432	*srv2Δ*	MATα *HAP1⁺ srv2Δ*::kanMX *leu2Δ0*	5	This study
cDBY0417	*mds3Δ*	MATα *HAP1⁺ mds3Δ*::kanMX *leu2Δ0*	5	This study
cDBY0437	*tor1Δ*	MATα *HAP1⁺ tor1Δ*::kanMX *leu2Δ0*	5	This study
cDBY0428	*rts1Δ*	MATα *HAP1⁺ rts1Δ*::kanMX *leu2Δ0*	5	This study
cDBY0423	*ppm1Δ*	MATα *HAP1⁺ ppm1Δ*::kanMX *leu2Δ0*	5	This study
cDBY0415	*ccr4Δ*	MATα *HAP1⁺ ccr4Δ*::kanMX *leu2Δ0*	5	This study
cDBY0430	*srb8Δ*	MATα *HAP1⁺ srb8Δ*::kanMX *leu2Δ0*	5	This study
cDBY0434	*ssn8Δ*	MATα *HAP1⁺ ssn8Δ*::kanMX *leu2Δ0*	5	This study
cDBY0419	*pho85Δ*	MATα *HAP1⁺ pho85Δ*::kanMX *leu2Δ0*	5	This study
cDBY0081	*ndi1Δ*	MATa *HAP1⁺ ndi1Δ*::kanMX	SF1	This study
cDBY0051	*nde1Δ*	MATa *HAP1⁺ nde1Δ*::hphMX	SF1	This study
cDBY0061	*nde2Δ*	MATa *HAP1⁺ nde2Δ*::bleMX	SF1	This study
cDBY0077	*nde1Δ nde2Δ*	MATa *HAP1⁺ nde1Δ*::hphMX *nde2Δ*::bleMX	SF1	This study
cDBY0033	*sdh1Δ*	MATa *HAP1⁺ sdh1Δ*::kanMX	SF1	This study
cDBY0107	*yjl045wΔ*	MATa *HAP1⁺ yjl045wΔ*::hphMX	SF1	This study
cDBY0049	*cyc1Δ*	MATa *HAP1⁺ cyc1Δ*::kanMX	SF1	This study
cDBY0057	*cyc7Δ*	MATa *HAP1⁺ cyc7Δ*::hphMX	SF1	This study
cDBY0389	*ira1Δ*	MATa *HAP1⁺ ira1Δ*::natAC	SF3	This study
cDBY0391	*ira2Δ*	MATα *HAP1⁺ ira2Δ*::natAC	SF3	This study
cDBY0400	*ras1Δ*	MATa *HAP1⁺ ras1Δ*::natAC	SF3	This study
cDBY0402	*ras2Δ*	MATα *HAP1⁺ ras2Δ*::natAC	SF3, SF4	This study
cDBY0408	*srv2Δ*	MATa *HAP1⁺ srv2Δ*::natAC	SF3	This study
cDBY0387	*cdc55Δ*	MATa *HAP1⁺ cdc55Δ*::natAC	SF3	This study
cDBY0403	*rts1Δ*	MATa *HAP1⁺ rts1Δ*::natAC	SF3, SF4	This study
cDBY0398	*ppm1Δ*	MATa *HAP1⁺ ppm1Δ*::natAC	SF3	This study
cDBY0395	*pph21Δ*	MATa *HAP1⁺ pph21Δ*::natAC	SF3	This study
cDBY0397	*pph22Δ*	MATα *HAP1⁺ pph22Δ*::natAC	SF3	This study
cDBY0412	*tor1Δ*	MATα *HAP1⁺ tor1Δ*::natAC	SF3	This study
cDBY0410	*tco89Δ*	MATa *HAP1⁺ tco89Δ*::natAC	SF3, SF4	This study
cDBY0392	*mds3Δ*	MATa *HAP1⁺ mds3Δ*::natAC	SF3	This study
cDBY0413	*tus1Δ*	MATa *HAP1⁺ tus1Δ*::natAC	SF3	This study
cDBY0405	*sch9Δ*	MATa *HAP1⁺ sch9Δ*::natAC	SF3	This study
cDBY0386	*ccr4Δ*	MATa *HAP1⁺ ccr4Δ*::natAC	SF3	This study
cDBY0407	*srb8Δ*	MATα *HAP1⁺ srb8Δ*::natAC	SF3	This study
cDBY0409	*ssn8Δ*	MATα *HAP1⁺ ssn8Δ*::natAC	SF3	This study
cDBY0393	*pho85Δ*	MATa *HAP1⁺ pho85Δ*::natAC	SF3	This study
cDBY0653	WT (pHluorin)	MATa *HAP1+ can1Δ*::*TDH3*pr-ypHluorin2	SF3	This study

Supplemental Table 5. Strains used in this study.