SUPPLEMENTAL FIGURES

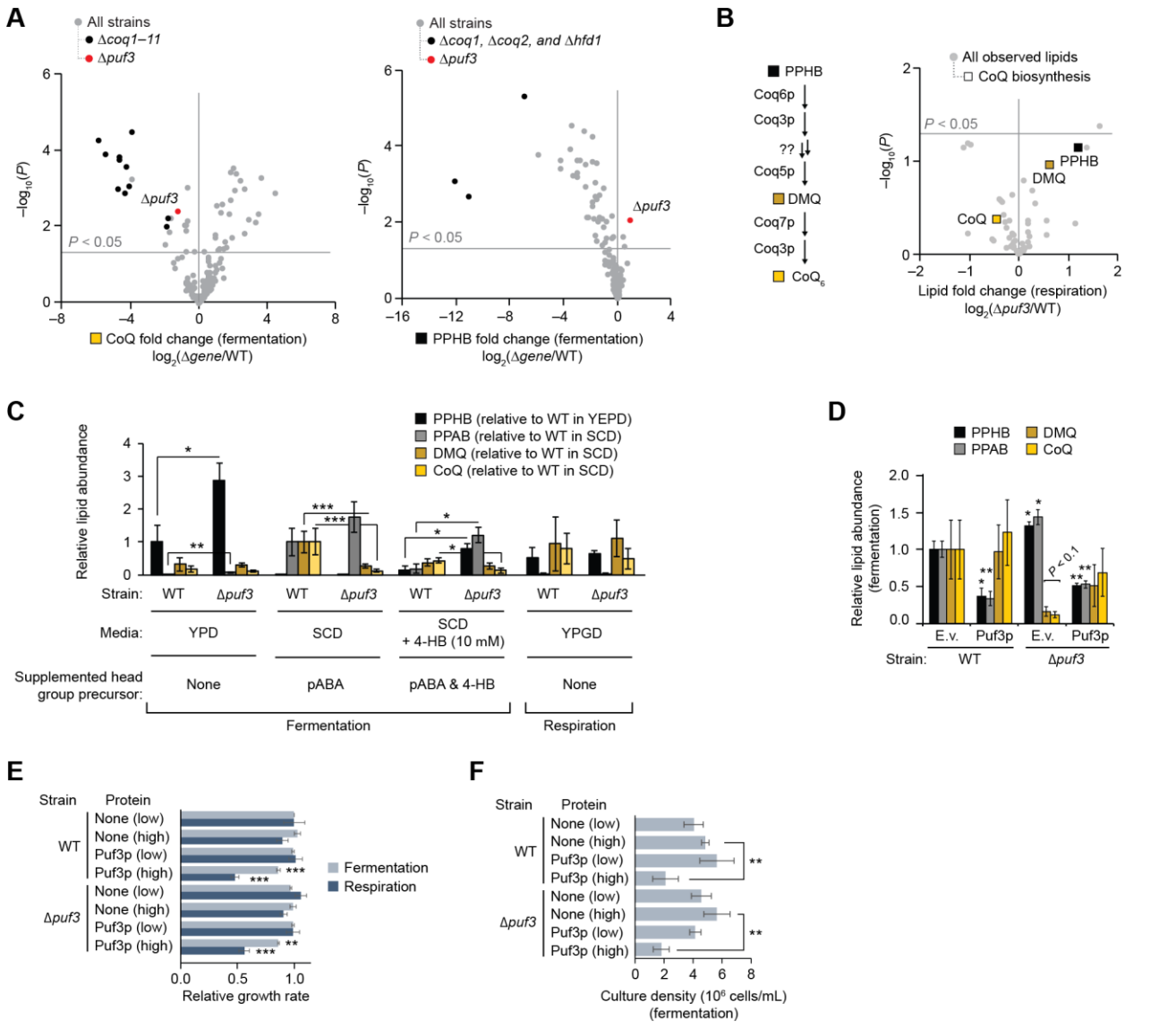

Figure S1, related to Figure 1. Puf3p Regulates CoQ Biosynthesis

(A) Relative abundances of CoQ and PPHB (mean, *n* = 3) versus statistical significance (*P*) across all yeast strains in the Y3K data set (Stefely et al., 2016a) (fermentation culture condition).

(B) Lipid abundances in Δ*puf3* yeast compared to WT (mean, *n* = 3) versus statistical significance (*P*, respiration condition). Raw data from the Y3K data set (Stefely et al., 2016a).

(C) Relative lipid abundances in WT and Δ*puf3* yeast cultured under a variety of standard yeast growth media.

(D) Relative lipid abundances in yeast transformed with high-copy plasmids overexpressing Puf3p (or empty vector, e.v.) and cultured in fermentation media (mean ± SD, *n* = 3). Bonferroni corrected **P* < 0.05; ***P* < 0.01.

(E) Growth rates of WT or Δ*puf3* yeast transformed with plasmids overexpressing the proteins shown and cultured in either fermentation or respiration media (mean ± SD, *n* = 3).

(F) Culture densities of WT or Δ*puf3* yeast transformed with plasmids overexpressing the proteins shown and cultured in fermentation media (mean ± SD, $n = 3$) at the time point of harvest for the lipid analyses shown in Figure 1C and panel (C) of this figure.
Two-sided Student's *t*-test for all panels. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

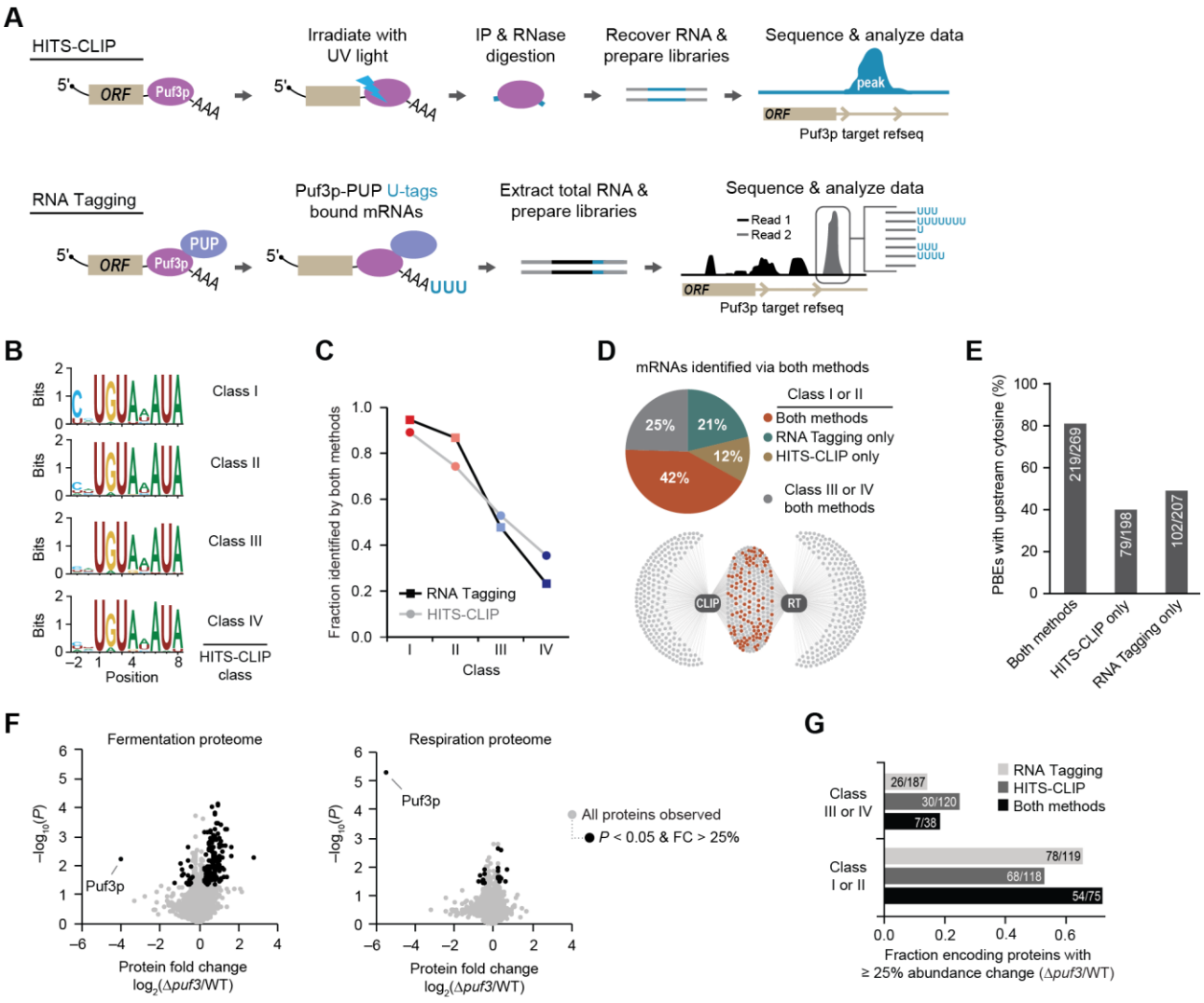

Figure S2, related to Figure 2. Integration of HITS-CLIP, RT, & Proteomics Defines Puf3p Targets

(A) Schematic of HITS-CLIP and RNA Tagging (RT) methods.

(B) Position-weight matrices of PBEs identified under peaks for HITS-CLIP classes.

(C) Overlap of RNAs identified as bound by Puf3p via both RNA Tagging and HITS-CLIP versus target class for the indicated method.

(D) Class composition of Puf3p-bound mRNAs identified via both RNA Tagging (RT) and HITS-CLIP. The network map (bottom) shows Puf3p-bound mRNAs (dots) detected by RT and/or HITS-CLIP (CLIP) (edges). Puf3p-bound mRNAs present in class I or II of both methods are indicated in orange.

(E) Percent of PBEs with upstream cytosine (–1 or –2 position).

(F) Relative protein abundances in Δ*puf3* yeast compared to WT (mean, n = 3) versus statistical significance in fermentation and respiration conditions. Proteins with fold change (FC) > 25% and $P < 0.05$ (160 and 24 proteins, respectively) are highlighted (two-sided Student's *t*-test).

(G) Fraction of genes with at least a 25% change in protein abundance ($P < 0.05$, two-sided Student's *t*-test) for the indicated groups. Analyses were limited to genes with proteins detected in the Y3K proteomics study (Stefely et al., 2016a), which is the denominator of each ratio. This figure includes new, integrated analyses of publicly available raw data from the RT (Lapointe et al., 2015), HITS-CLIP (Wilinski et al., 2017), and Y3K multi-omic (Stefely et al., 2016a) data sets generated in our labs.

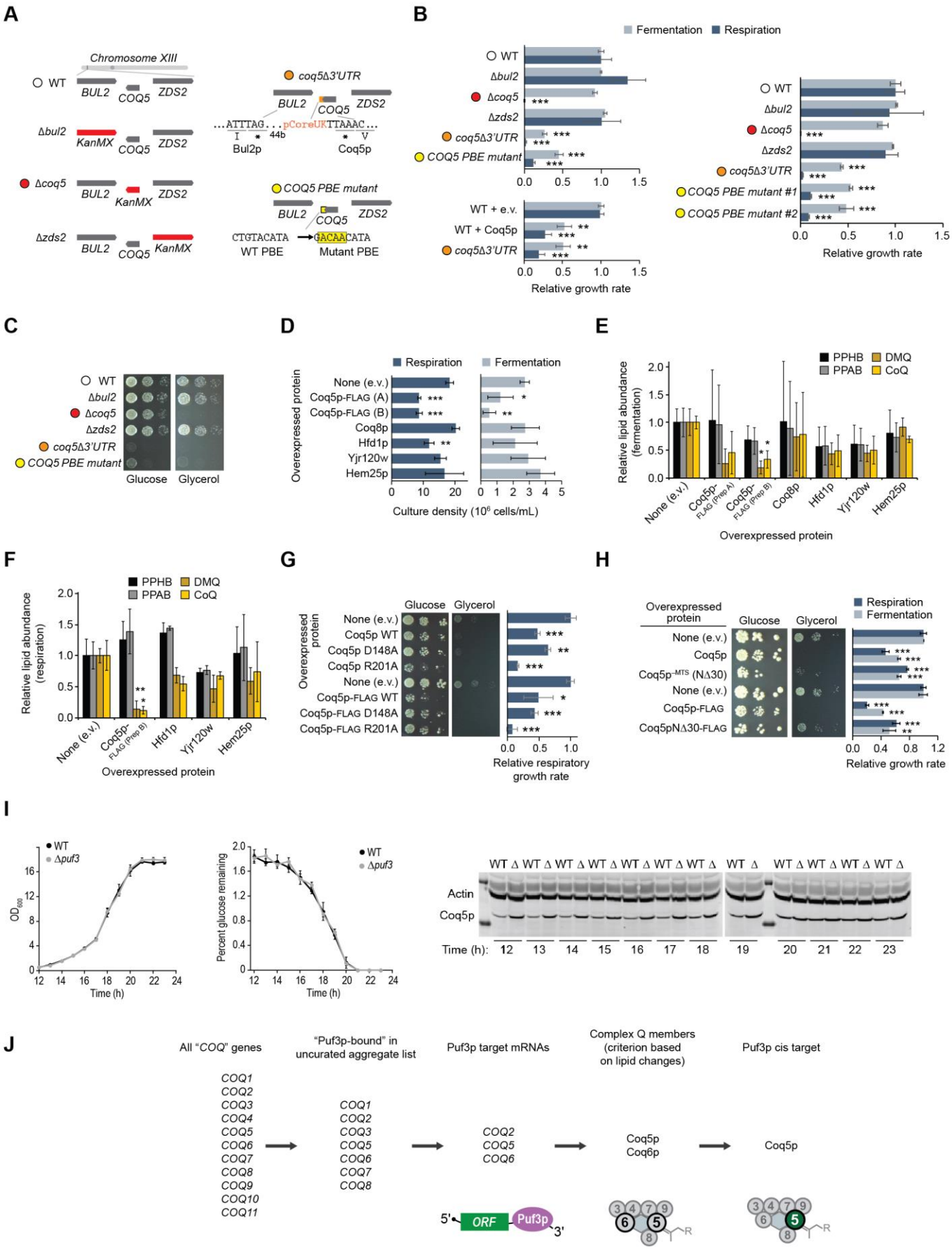
A
Chromosome XIII
WT
BUL2
COQ5
ZDS2
Δbul2
KanMX
Δcoq5
Δzds2
coq5Δ3'UTR
...ATTTAG...pCoreURTTAAAC...
Bul2p
Coq5p
COQ5 PBE mutant
CTGTACATA
GACAACATA
WT PBE
Mutant PBE
B
Fermentation
Respiration
WT + e.v.
WT + Coq5p
COQ5 PBE mutant
COQ5 PBE mutant #1
COQ5 PBE mutant #2
Relative growth rate
C
Glucose
Glycerol
D
Overexpressed protein
None (e.v.)
Coq5p-FLAG (A)
Coq5p-FLAG (B)
Coq8p
Hfd1p
Yjr120w
Hem25p
Culture density (10^6 cells/mL)
E
PPHB
PPAB
DMQ
CoQ
Relative lipid abundance (fermentation)
F
Relative lipid abundance (respiration)
G
Coq5p WT
Coq5p D148A
Coq5p R201A
Coq5p-FLAG WT
Coq5p-FLAG D148A
Coq5p-FLAG R201A
Relative respiratory growth rate
H
Coq5p
Coq5p-MTS (NΔ30)
Coq5p-FLAG
Coq5pNΔ30-FLAG
I
WT
Δpuf3
OD600
Time (h)
Percent glucose remaining
Actin
Coq5p
J
All "COQ" genes
"Puf3p-bound" in uncurated aggregate list
Puf3p target mRNAs
Complex Q members (criterion based on lipid changes)
Puf3p cis target
COQ1
COQ2
COQ3
COQ4
COQ5
COQ6
COQ7
COQ8
COQ9
COQ10
COQ11
Coq5p
Coq6p
ORF
Puf3p

Figure S3, related to Figure 3. Puf3p Regulates the CoQ Biosynthesis Enzyme Coq5p

(A) Scheme of genetic alterations in the yeast strains used in (B) and (C).

(B) Yeast strain growth rates in either fermentation or respiration media (mean ± SD, $n = 3$). $^{**}P < 0.01$; $^{***}P < 0.001$.

(C) Serial dilutions of yeast strains cultured on solid media containing either glucose or glycerol.

(D) Densities of yeast cultures at the time point of harvest for the lipid quantitation experiments depicted in Figure 3C and panels (E) and (F) of this figure.

(E) and (F) Relative lipid abundances in WT yeast transformed with plasmids overexpressing the proteins shown and cultured in respiration media (E) or fermentation media (F) (mean ± SD, $n = 3$). Bonferroni corrected $^{*}P < 0.05$; $^{**}P < 0.01$ compared to empty vector (e.v.) control.

(G) and (H) Left, serial dilutions of WT yeast transformed with plasmids overexpressing the proteins shown and cultured on solid media containing either glucose or glycerol. Right, growth rates of WT yeast transformed with plasmids overexpressing the proteins shown and cultured in liquid media (mean ± SD, $n = 3$).

(I) Yeast densities, percent glucose remaining, and endogenous Coq5p abundance in WT and Δ*puf3* (Δ) yeast across the diauxic shift at the indicated time points (h). (mean ± SD, $n = 3$ for yeast densities and percent glucose remaining). One representative western blot is shown. Actin was used as a loading control.

(J) Scheme of how Coq5p was identified as a key Puf3p target responsible for the Δ*puf3* CoQ deficiency.

For all panels, *P*-values were determined by a two-sided Student's *t*-test. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

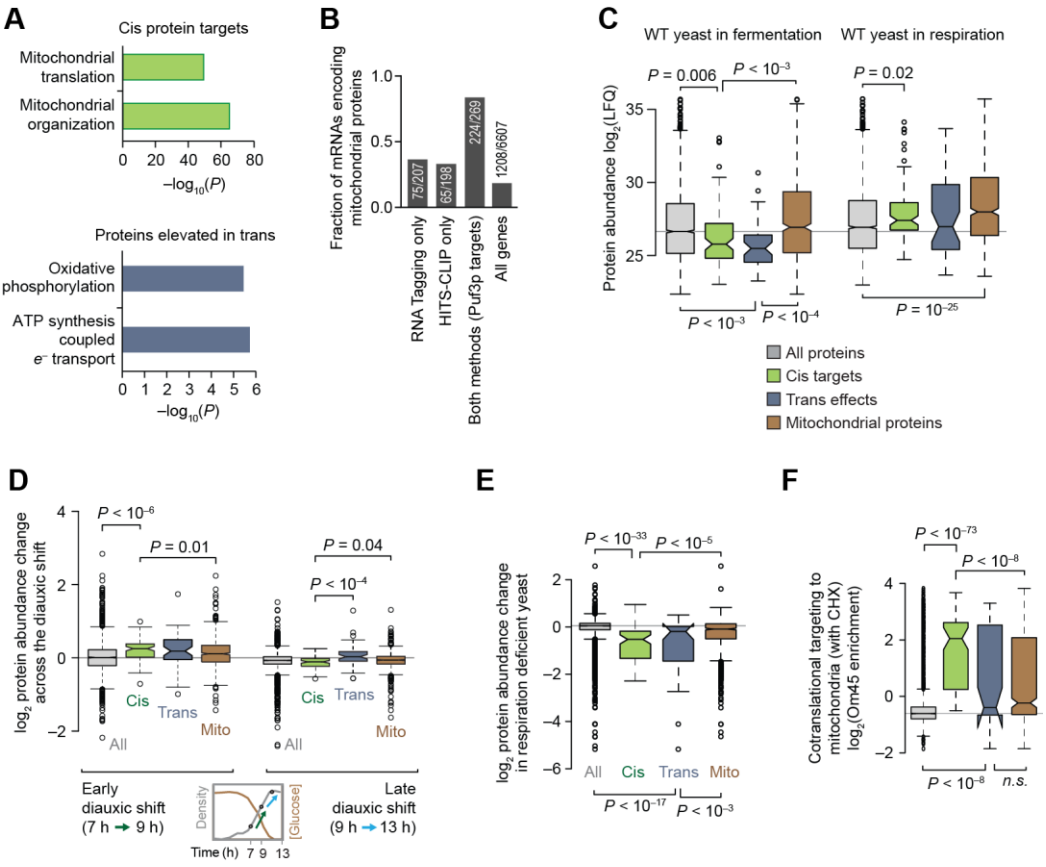

Figure S4, related to Figure 4. Puf3p Targets Share Distinctive Properties and Dynamics

(A) Gene Ontology (GO) terms enriched in cis Puf3p targets (top) or in elevated Puf3p trans effect proteins (bottom).

(B) Fraction of genes annotated as encoding mitochondrial proteins for the indicated groups.

(C)–(F) Box plots comparing the distributions of various gene and protein properties for all proteins observed in the Y3K Δ*puf3* proteomics data set (gray), cis Puf3p targets (green), trans Puf3p effects (blue), and mitochondrial proteins (brown). Center lines indicate medians, limits indicate 25th and 75th percentiles, whiskers extend 1.5 times the interquartile range, outliers are represented by dots, and *P* values were determined with a Student's *t*-test (two-tailed, homostatic). Protein set sizes: all proteins *n* = 3152, mitochondrial proteins *n* = 715, cis targets *n* = 91, trans effect proteins *n* = 49.

(C) Protein abundances (Stefely et al., 2016a) for each group of proteins shown. LFQ, label free quantitation value.

(D) Protein abundance changes (Stefely et al., 2016b) across the diauxic shift.

(E) Protein abundance changes (Stefely et al., 2016a) in respiration deficient yeast compared to respiration competent yeast.

(F) Measure of cotranslational targeting to mitochondria in yeast treated with cycloheximide (CHX) (Williams et al., 2014).

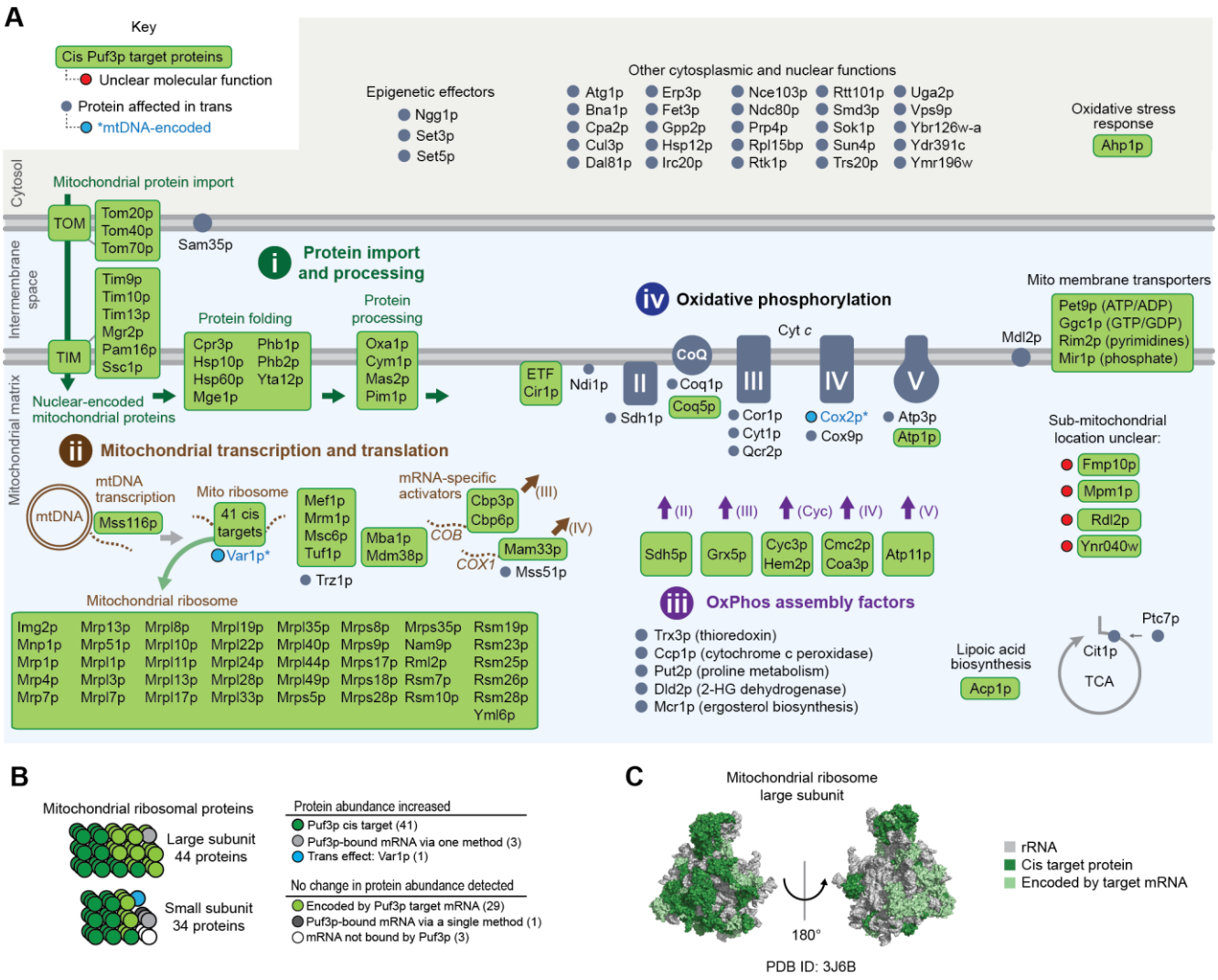

Figure S5, related to Figure 4. Puf3p Targets Pathways of Mitochondrial Biogenesis Proteins

(A) Cartoon indicating all identified cis Puf3p targets and trans effects.

(B) Cartoon of mitochondrial ribosomal proteins with the effect by Puf3p indicated.

(C) Surface representation of the large subunit of the yeast mitochondrial ribosome (PDB: 3J6B) (Amunts et al., 2014) indicating Puf3p targets.

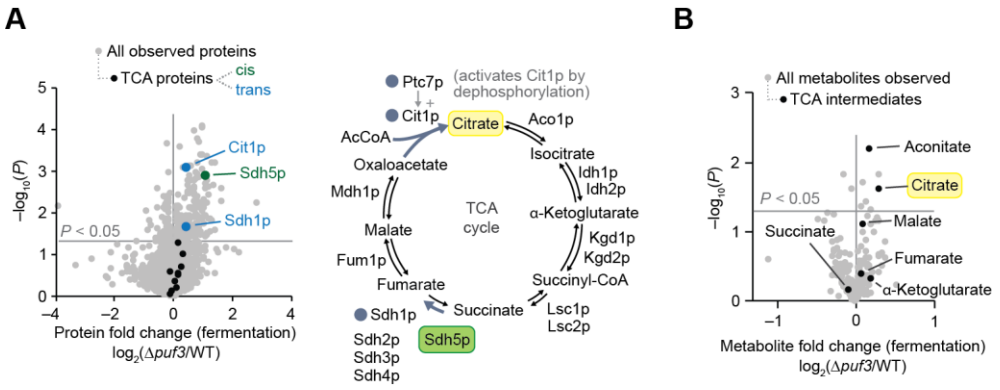

Figure S6, related to Figure 4. Puf3p Regulates TCA Cycle Proteins

(A) Relative protein abundances in Δ*puf3* yeast compared to WT highlighting TCA proteins (left), and scheme of the TCA cycle highlighting proteins significantly ($P < 0.05$) elevated in Δ*puf3* yeast (right).

(B) Relative metabolite abundances in Δ*puf3* yeast compared to WT (mean, $n = 3$) versus statistical significance (fermentation condition), highlighting TCA cycle metabolites. Raw data from the Y3K data set (Stefely et al., 2016a).

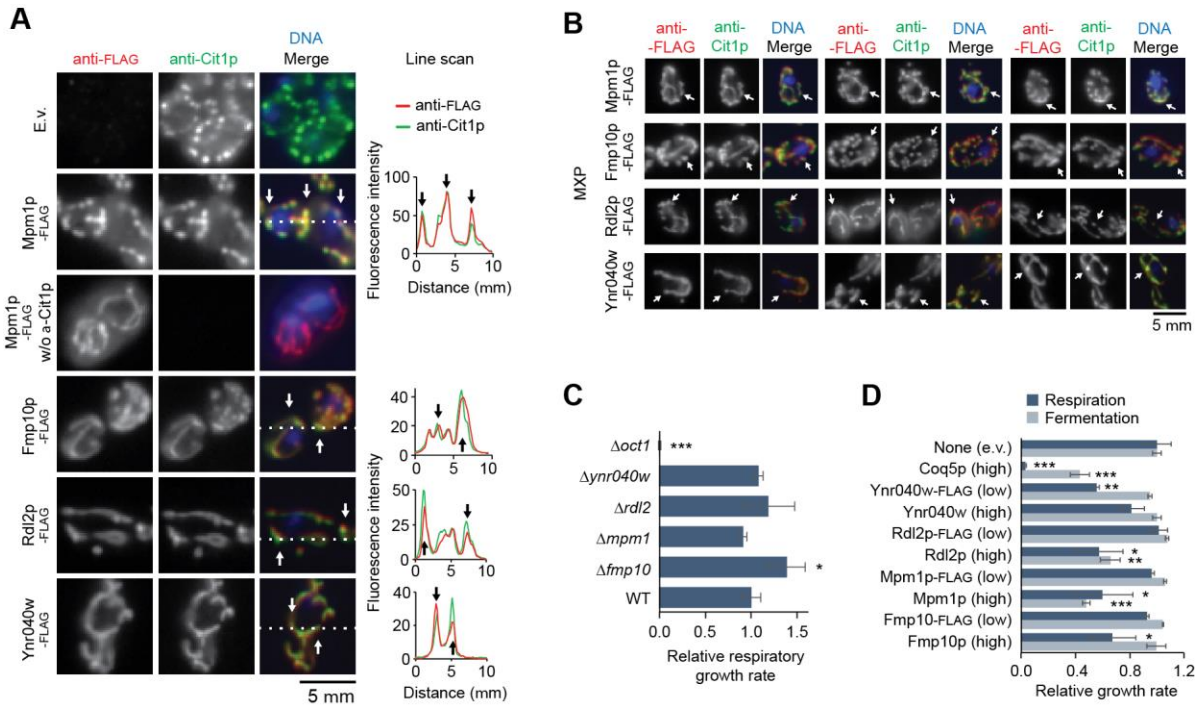

Figure S7, related to Figure 4. Uncharacterized Puf3p Targets Localize to Mitochondria

(A) Fluorescent immunocytochemistry analysis of yeast transformed with the indicated FLAG tagged proteins. An antibody against Cit1p was used to localize mitochondria. Arrows indicate landmarks on the line scan (dotted line on image). Scale bar, 5 μm.

(B) Fluorescent immunocytochemistry analysis of WT yeast transformed with the indicated FLAG tagged mitochondrial uncharacterized proteins (MXPs). Arrows indicate spatial landmarks across each channel. Scale bar, 5 μm.

(C) Relative growth rates of yeast strains cultured in respiration media (mean ± SD, *n* = 3).

(D) Relative growth rates of WT yeast transformed with plasmids overexpressing the proteins shown and cultured in either fermentation or respiration media (mean ± SD, *n* = 3). High or low copy number plasmids are indicated.

Two-sided Student's *t*-test for all panels. **P* < 0.05; ***P* < 0.01; ****P* < 0.001