**SUPPLEMENTARY INFORMATION**

**Lee C-D and Tu BP, "Glucose-responsive phosphorylation of the PUF protein Puf3 regulates the translational fate of its bound mRNAs and its association with RNA granules"**

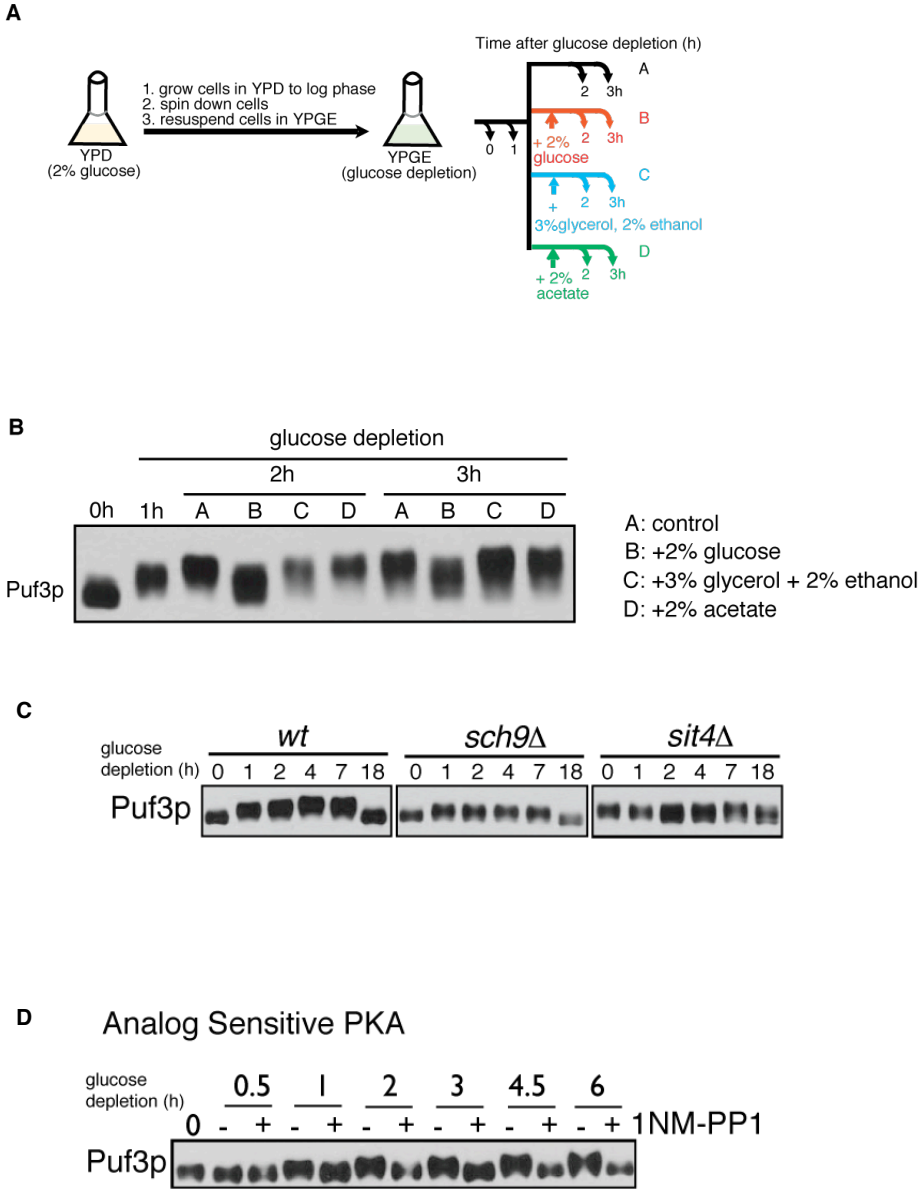



**Figure S1 (refers to Figure 1). Puf3p phosphorylation is inhibited by glucose but not simpler carbon sources, and is regulated by nutrient-responsive kinases and phosphatases**

(A) Schematic view of carbon source addition experiments. Cells were grown in YPD, switched to glucose depletion medium (YPGE), and then the culture was split into four after 1 h. One culture was kept in YPGE, while others were supplemented with either 2% glucose, 3% glycerol plus 2% ethanol, or 2% acetate. Samples were collected at the indicated time points.

(B) Puf3p phosphorylation was assessed by Western blot.

(C) The *sch9*Δ mutant exhibited reduced Puf3p phosphorylation under glucose depletion and the *sit4*Δ mutant exhibited phosphorylated Puf3p even in glucose medium.

(D) The PKA analog-sensitive mutant, after treatment with the 1NM-PP1 inhibitor, exhibited reduced Puf3p phosphorylation under glucose depletion.

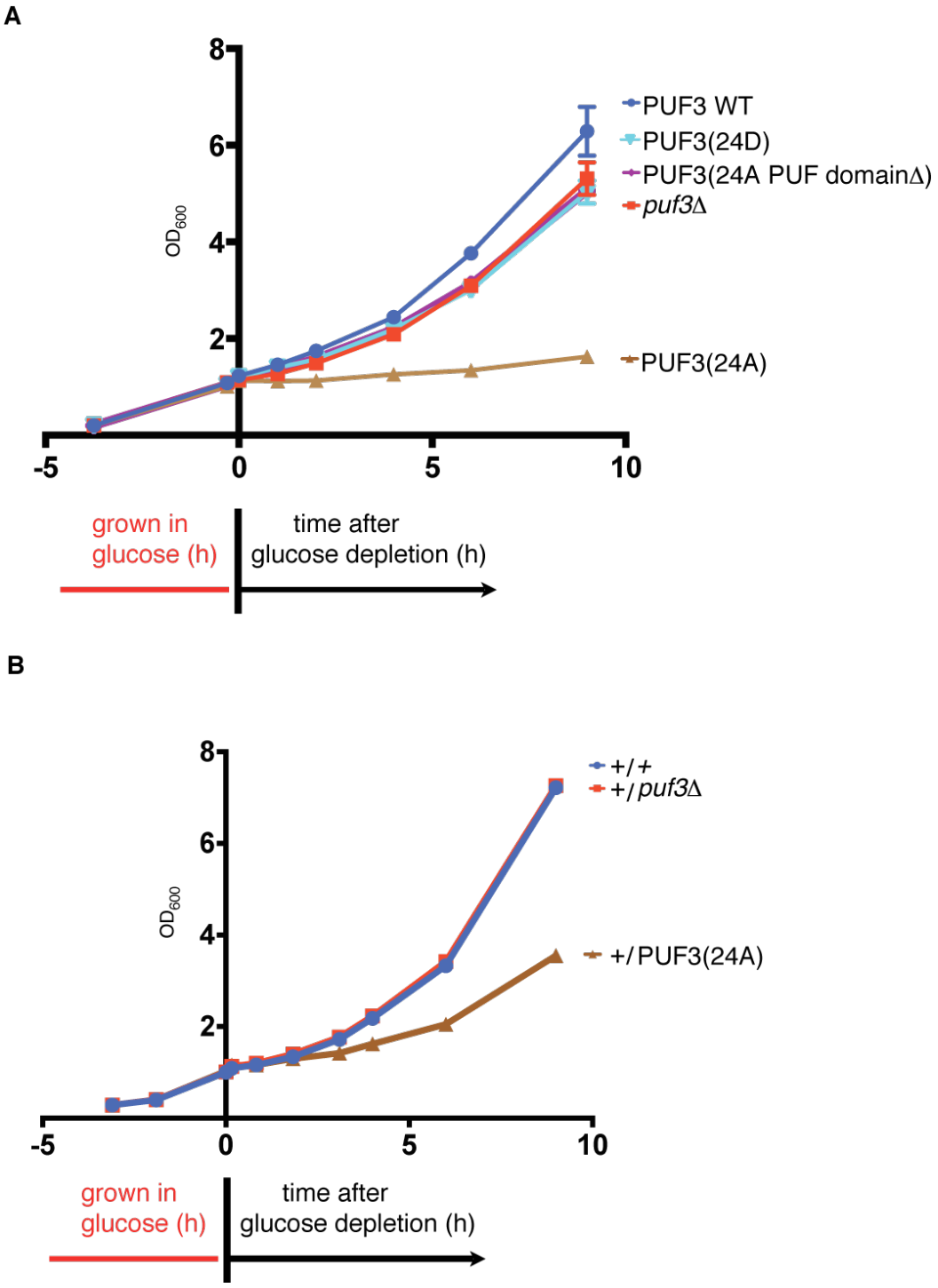
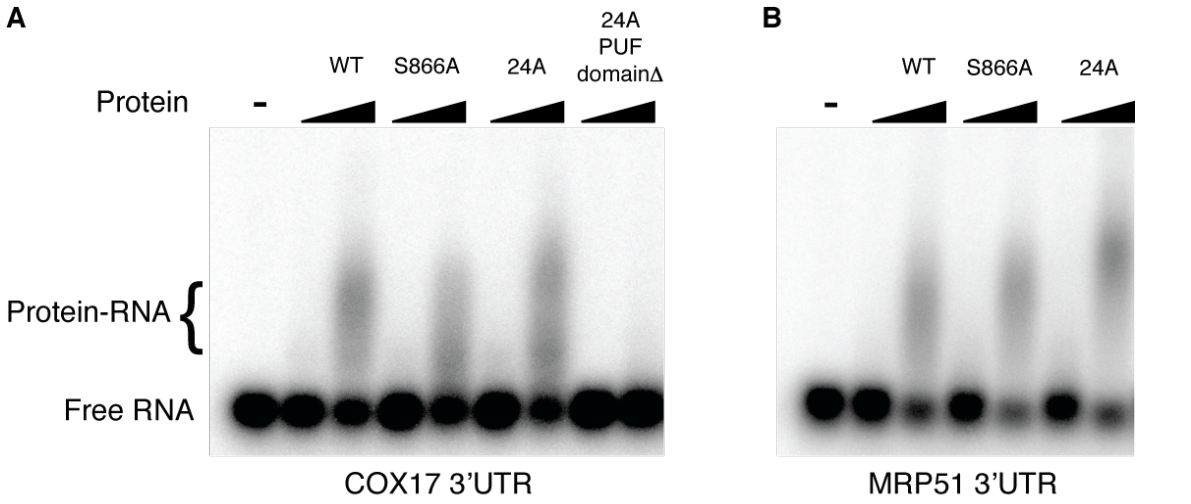
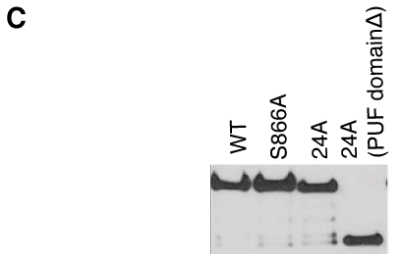


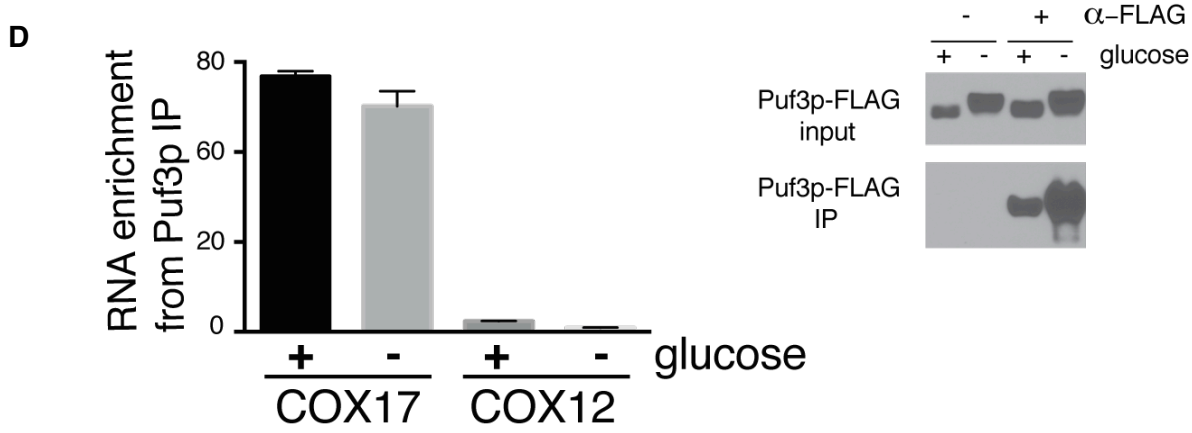

**Figure S2 (refers to Figure 2). Various mutants in *PUF3* have a similar growth rate compared to WT in glucose medium**

(A) The indicated haploid mutants of *PUF3* grow comparably to WT in glucose medium.

(B) The indicated diploid mutants grow comparably to WT in glucose medium.


A
Protein
-
WT
S866A
24A
24A PUF domainΔ
Protein-RNA
Free RNA
COX17 3'UTR
B
-
WT
S866A
24A
MRP51 3'UTR


C
WT
S866A
24A
24A (PUF domainΔ)


D
RNA enrichment from Puf3p IP
80
60
20
0
+
-
+
-
glucose
COX17
COX12
-
+
α–FLAG
+
-
+
-
glucose
Puf3p-FLAG input
Puf3p-FLAG IP

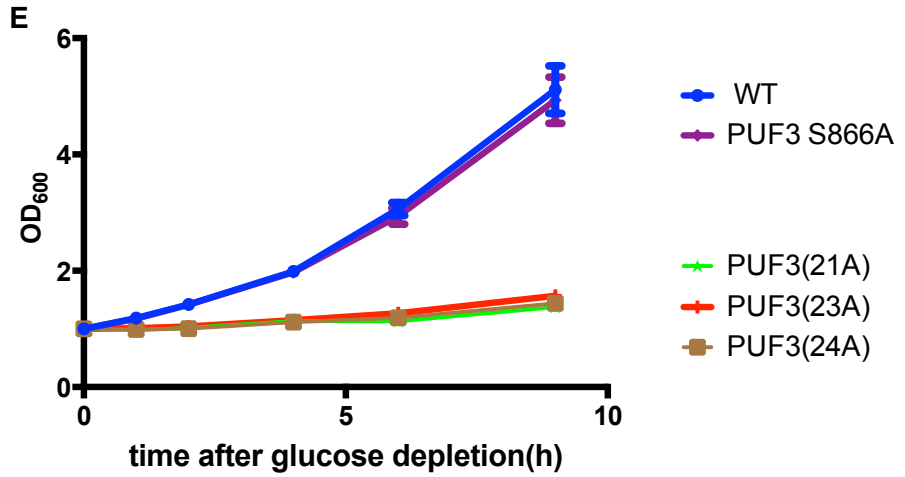

E
6
4
2
0
OD600
0
5
10
time after glucose depletion(h)
WT
PUF3 S866A
PUF3(21A)
PUF3(23A)
PUF3(24A)

**Figure S3 (refers to Figure 2). RNA-binding and growth properties of Puf3p variants**
(A) The indicated Puf3p variants were tagged with FLAG at the endogenous locus, immunoprecipitated from yeast extracts, and then tested for their ability to bind $^{32}\alpha$-UTP-labeled COX17 3'UTR (237nt in length) using EMSA. First lane is RNA only control. A 10-fold increase in protein was loaded in the second lane for each variant. Only Puf3p(24A PUF domainΔ) exhibited a defect in RNA binding.
(B) MRP51 is a Puf3p target. Same as in (A), except the RNA probe is $^{32}\alpha$-UTP-labeled MRP51 3'UTR (237nt in length).
(C) Protein input of Puf3p variants.
(D) Left: Puf3p binds comparably to a COX17 target mRNA in both glucose and glucose depletion conditions as assayed by native ribonucleoprotein IP (RIP). COX12, which is not a target mRNA, is included as a control. RNA fold enrichment was calculated by the amount of pull-down RNA with α-FLAG conjugated dynabead divided by the pull-down RNA with non-conjugated dynabead. This value was then normalized by the amount of pull-down Puf3p. Puf3p amount was quantified by ImageJ. Right: Puf3p pull-down by non-conjugated dynabead or α-FLAG conjugated dynabead. Puf3p was detected by α-FLAG Western blotting.
(E) Growth rates of the indicated Puf3p variants following glucose depletion. 23A mutant has residue 866 changed back to Ser, and 21A contains residues 515, 563, 866 changed back to Ser. Note the 21A and 23A mutants exhibit the same severe growth defect as 24A in glucose depletion, while the single S866A mutant exhibits no growth defect compared to WT.

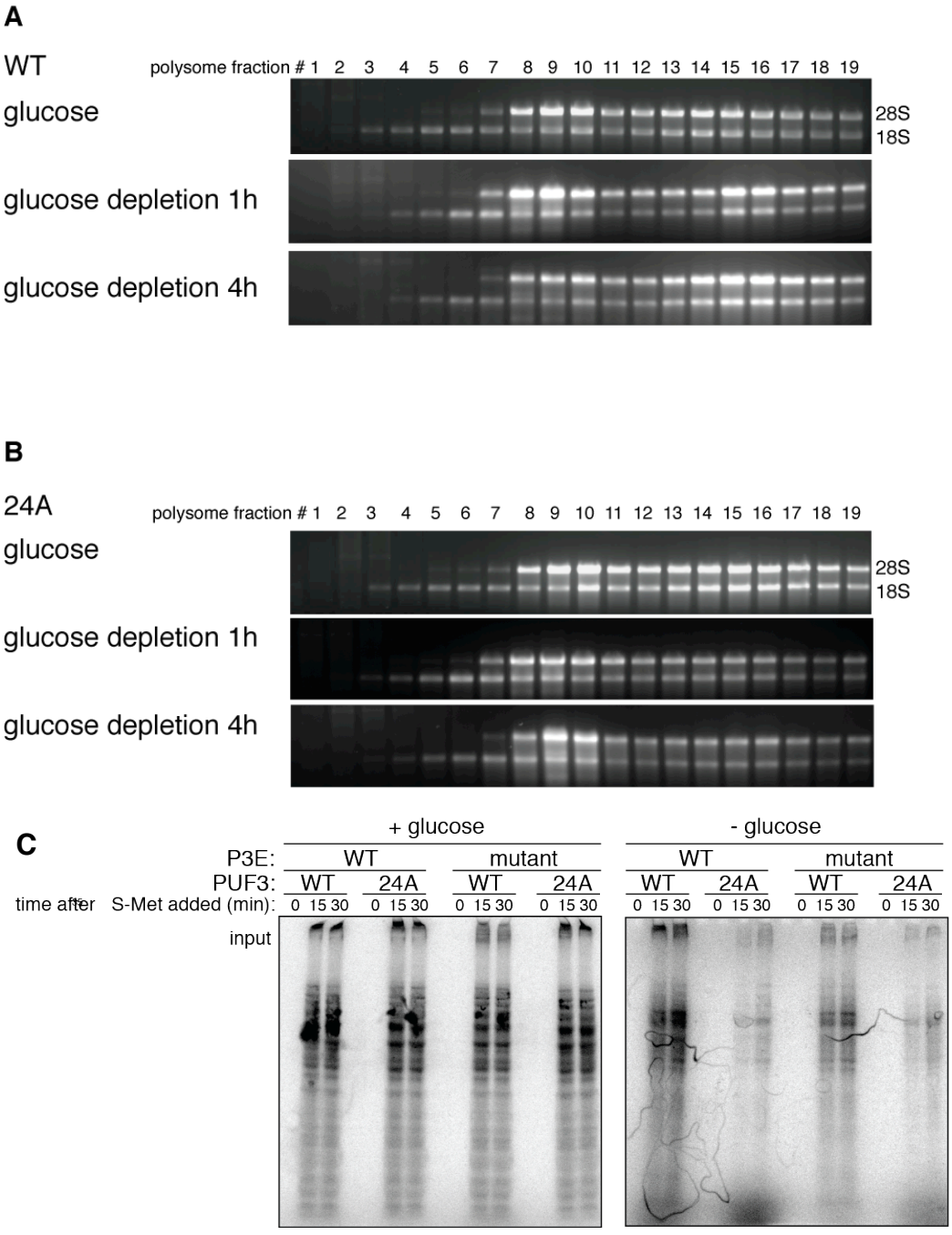



**Figure S4 (refers to Figures 3 and 4). PUF(24A) exhibits different rRNA distribution patterns and compromises global translation only following glucose depletion**

(A) rRNA distribution in polysome profiles in WT PUF3.

(B) rRNA distribution in polysome profiles in PUF3(24A). Note the accumulation of rRNA in inactive monosome fractions upon glucose depletion in the mutant.

(C) Total input in $^{35}$S-Met pulse-labeling assay shows no difference between WT and PUF(24A) in glucose medium. In contrast, PUF3(24A) cells exhibit a substantial decrease in global translation upon glucose depletion.

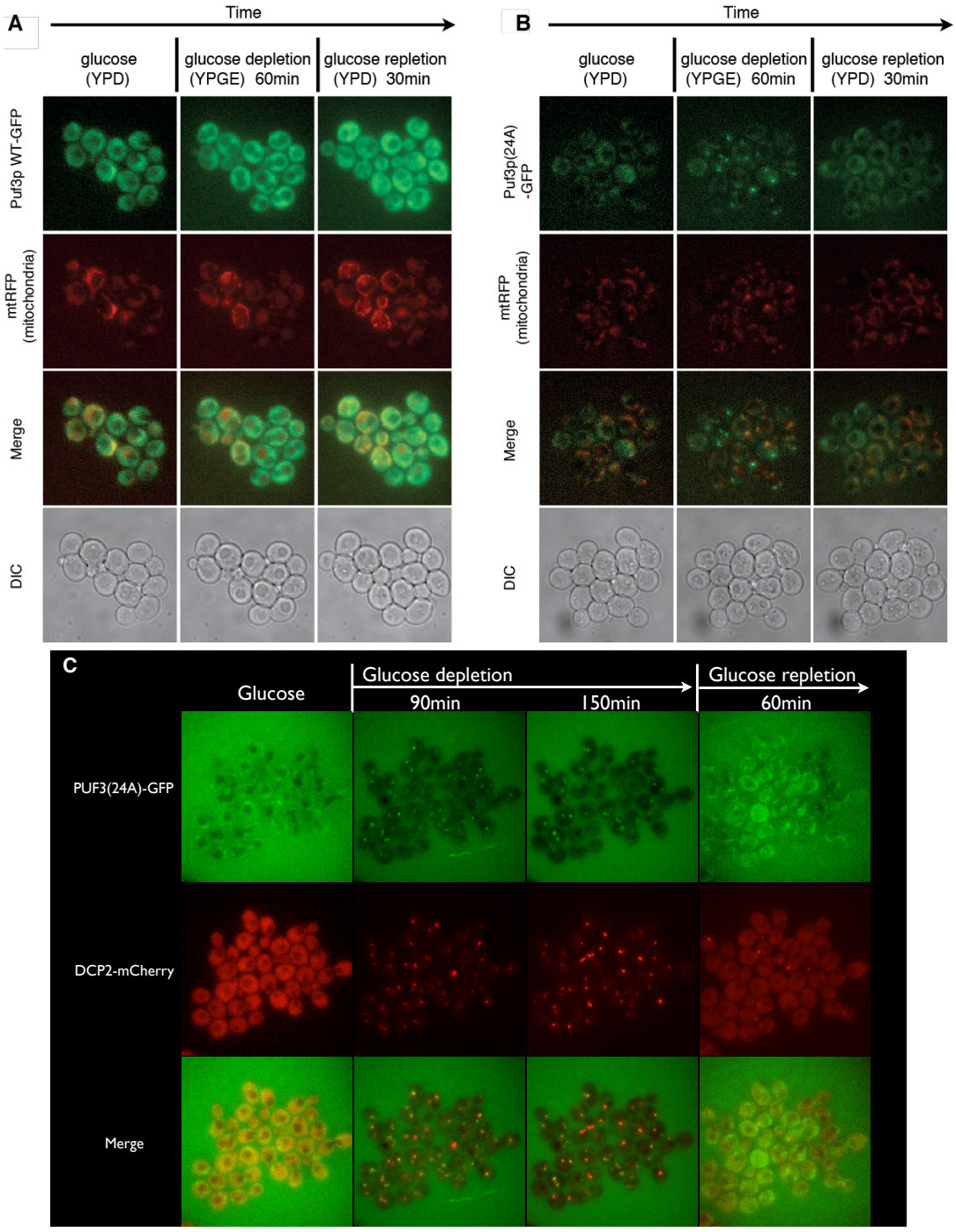



**Figure S5 (refers to Figure 5). Puf3p(24A) forms foci and exhibits partial co-localization with Dcp2p, a marker of p-bodies, following glucose depletion in YP medium**

(A) Live cell imaging of cells expressing Puf3p-GFP and the mitochondrial marker mtRFP, before and after switch to glucose depletion medium for the indicated times. Puf3p-GFP was uniformly distributed in the cytosol and not specifically localized to mitochondria regardless of glucose availability in YP medium.

(B) Live cell imaging of cells expressing Puf3p(24A)-GFP and the mitochondrial marker mtRFP. Note that Puf3p(24A) forms foci only after switch to glucose depletion medium and some foci localized to the vicinity of mitochondria. After glucose repletion, Puf3p(24A) redistributed to the cytosol in YP medium.

(C) Approximately ~50% of the Puf3p(24A) foci exhibited co-localization with Dcp2p following glucose depletion. Note that Dcp2p was not present in foci in glucose medium.

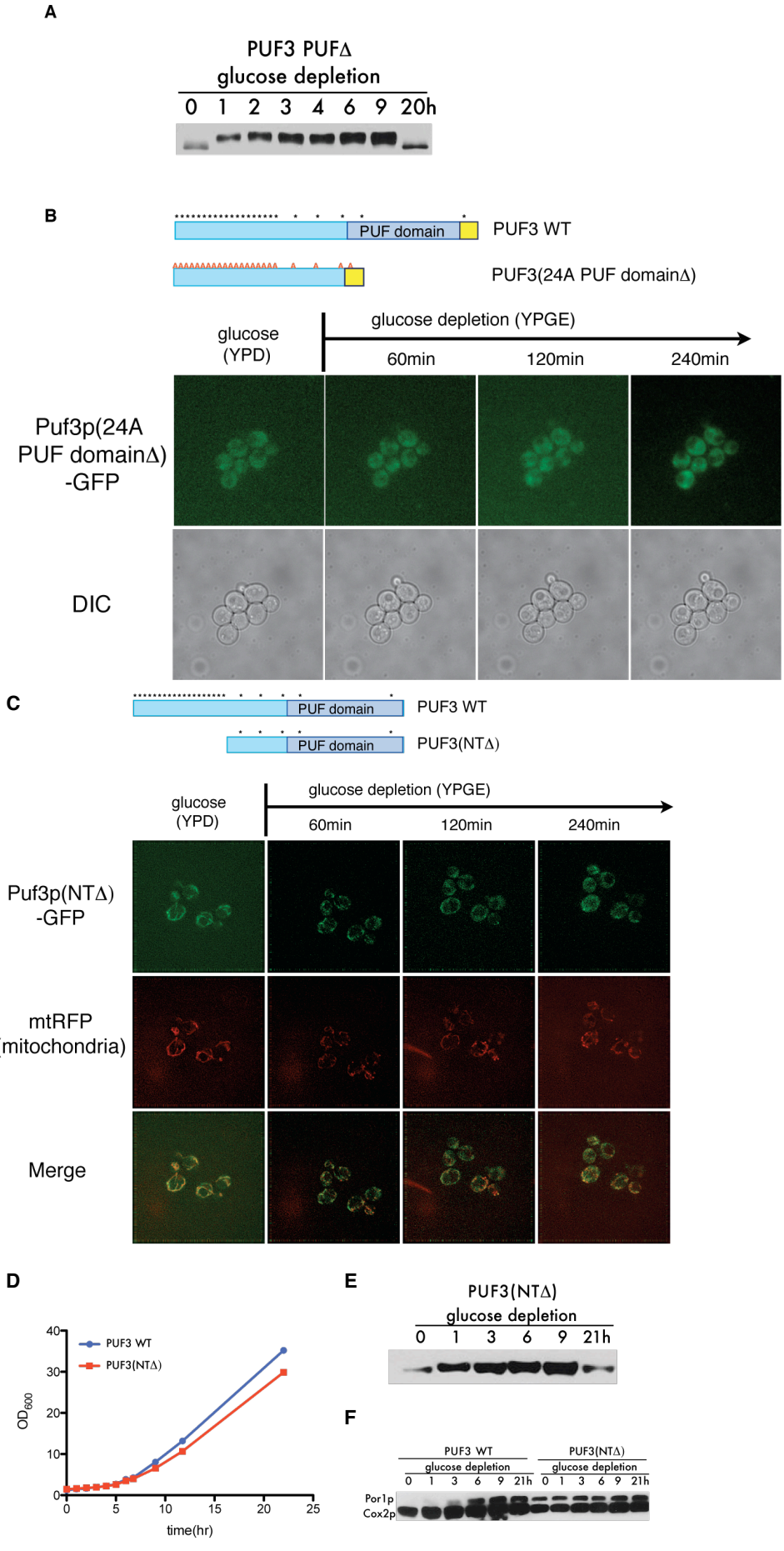

A
PUF3 PUFΔ
glucose depletion
0 1 2 3 4 6 9 20h
B
PUF domain
PUF3 WT
PUF3(24A PUF domainΔ)
glucose
(YPD)
glucose depletion (YPGE)
60min
120min
240min
Puf3p(24A
PUF domainΔ)
-GFP
DIC
C
PUF domain
PUF3 WT
PUF domain
PUF3(NTΔ)
glucose
(YPD)
glucose depletion (YPGE)
60min
120min
240min
Puf3p(NTΔ)
-GFP
mtRFP
(mitochondria)
Merge
D
PUF3 WT
PUF3(NTΔ)
OD600
time(hr)
E
PUF3(NTΔ)
glucose depletion
0 1 3 6 9 21h
F
PUF3 WT
PUF3(NTΔ)
glucose depletion
glucose depletion
0 1 3 6 9 21h 0 1 3 6 9 21h
Por1p
Cox2p

**Figure S6 (refers to Figure 5). Truncation studies suggest Puf3p might be a modular protein, and the N-terminal region of Puf3p is subject to phosphorylation to regulate localization**

(A) PUF domain deletion does not noticeably affect the dynamic phosphorylation and protein level of Puf3p.

(B) Live cell imaging of cells expressing Puf3p(24A PUF domain**Δ**)-GFP, which lacks the PUF domain that is required for mRNA-binding. Puf3p(24A PUF domain**Δ**)-GFP no longer forms foci and appears largely cytosolic regardless of glucose availability.

(C) Live cell imaging of cells expressing Puf3p NT**Δ**-GFP, which lacks the N-terminal region that is highly phosphorylated upon glucose depletion. Puf3p NT**Δ**-GFP localizes to mitochondria and no longer responds to glucose availability.

(D) NT deletion of Puf3p has a slight growth defect in non-fermentable carbon sources.

(E) Puf3p(NTΔ) does not have obvious gel shift upon glucose depletion.

(F) NT deletion of Puf3p abolishes its ability to properly regulate mitochondrial biogenesis.

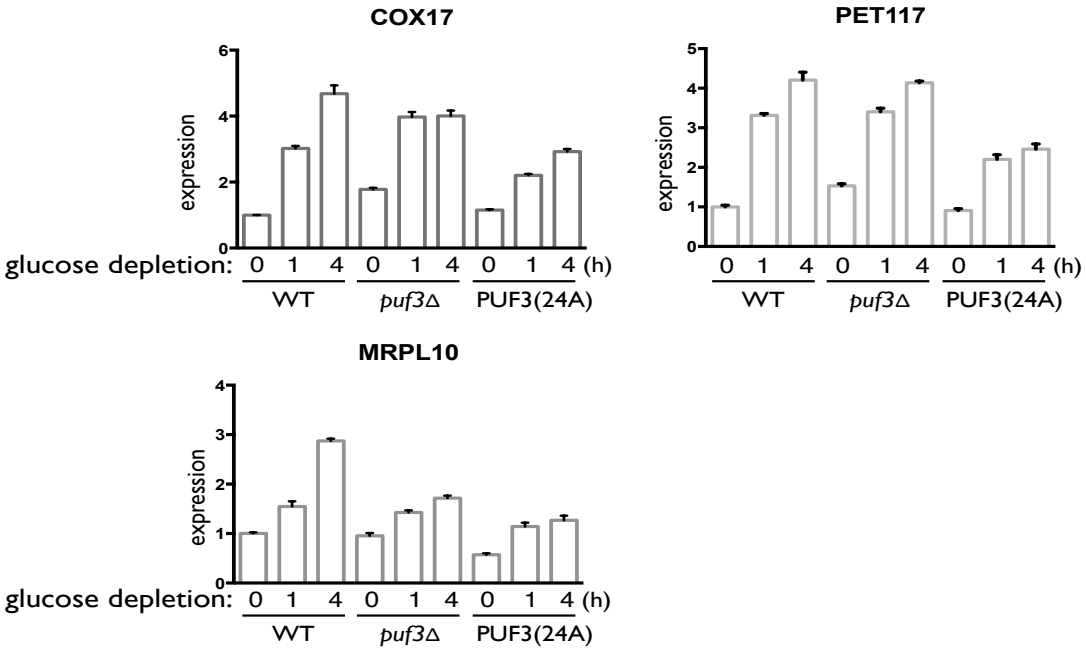



**Figure S7 (refers to Figures 2, 5, and 6). The PUF3(24A) phosphomutant does not cause enhanced mRNA degradation**

Several endogenous mRNAs containing a Puf3p-binding element in their 3'UTRs were examined by qRT-PCR in WT, *puf3Δ* and PUF3(24A) strains. These mRNAs increase following glucose depletion in both WT and PUF3(24A) strains, suggesting PUF3(24A) toxicity is not simply caused by enhanced mRNA degradation.

**Table S1 (refers to Figures 1-6). Yeast strains used in this study**

| Strain name | Genotype | Origin | strain background |
|---|---|---|---|
| YL27 | MATa *PUF3*-FLAG-KanMX6 | this study | Prototrophic CEN.PK |
| YL32 | MATa *puf3*::KanMX6 | this study | Prototrophic CEN.PK |
| YL46 | MATa *ho::*$P_{ADH1}$-Mito-RFP-KanMX6 | this study | Prototrophic CEN.PK |
| YL122 | MATa *PUF3*-FLAG-KanMX6 *sch9*::NatNT2 | this study | Prototrophic CEN.PK |
| YL128 | MATa *PUF3*-FLAG-NatMX6 *sit4*::KanMX6 | this study | Prototrophic CEN.PK |
| PKA AS mutant | MATa *trp1 leu2 ura3 his3 can1* GAL+ psi+ , ADE+ , *tpk1-M164G, tpk2-M147G, tpk3-M165G* | Hao and O'Shea, 2011 | W303 |
| YL133 | MATa *trp1 leu2 ura3 his3 can1* GAL+ psi+ , ADE+ , *tpk1-M164G, tpk2-M147G, tpk3-M165G PUF3*-Flag-KanMX6 | this study | W303 |
| YL203 | MATa *PUF3*-FLAG-Kan *snf3*::HygMX6 | this study | Prototrophic CEN.PK |
| YL205 | MATa *PUF3*-FLAG-Kan *rgt2*::HygMX6 | this study | Prototrophic CEN.PK |
| YL230 | MATa *PUF3(NTΔ)*-FLAG-NatNT2 | this study | Prototrophic CEN.PK |
| YL248 | MATa *PUF3(PUF domainΔ)*- FLAG -NatNT2 | | |
| YL249 | MATa *ho*::$P_{ADH1}$-Mito-RFP-KanMX6 *PUF3(NTΔ)*-yEGFP-NatNT2 | this study | Prototrophic CEN.PK |
| YL281 | MATa *ho*::$P_{ADH1}$-Mito-RFP-KanMX6 *PUF3*-yEGFP-NatNT2 | this study | Prototrophic CEN.PK |
| YL578 | MATa *PUF3*-FLAG-Kan *rgt2*::HygMX6 *snf3*::NatMX6 | this study | Prototrophic CEN.PK |
| YL611 | MAT a/α *PUF3*/*puf3*::KanMX6 | this study | Prototrophic CEN.PK |
| YL632 | MATa *PUF3(24A)*-FLAG-NatNT2 | this study | Prototrophic CEN.PK |
| YL633 | MAT a/α *PUF3*/*PUF3(24A)*-FLAG-NatNT2 | this study | Prototrophic CEN.PK |
| YL646 | MATa *PUF3(24D)*-FLAG-NatNT2 | this study | Prototrophic CEN.PK |
| YL648 | MATa *PUF3(24A PUF domainΔ)*-FLAG-NatNT2 | this study | Prototrophic CEN.PK |
| YL668 | MATa ho::$P_{ADH1}$ -Mito-RFP $P_{ACT1}$-GEV $P_{GAL1}$-COX4(1~21)-yEGFP-MRP51 3'UTR-HygMX6 | this study | Prototrophic CEN.PK |

| | | | |
|---|---|---|---|
| YL670 | MATa ho::$P_{ADH1}$ -Mito-RFP $P_{ACT1}$-GEV $P_{GAL}$1-COX4(1~21)-yEGFP-MRP51 3'UTR-HygMX6 *PUF3(24A)*-FLAG-NatNT2 | this study | Prototrophic CEN.PK |
| YL672 | MATa ho::$P_{ADH1}$ -Mito-RFP $P_{ACT1}$-GEV $P_{GAL}$1-COX4(1~21)-yEGFP-MRP51* 3'UTR-HygMX6 | this study | Prototrophic CEN.PK |
| YL674 | MATa ho::$P_{ADH1}$ -Mito-RFP $P_{ACT1}$-GEV $P_{GAL1}$-COX4(1~21)-yEGFP-MRP51* 3'UTR-HygMX6 *PUF3(24A)*-FLAG-NatNT2 | this study | Prototrophic CEN.PK |
| YL700 | MATa *PUF3(24A)*-yEGFP-KanMX6 DCP2-mCherry-HygMX6 | this study | Prototrophic CEN.PK |
| YL721 | MATa ho::$P_{ADH1}$-Mito-RFP-KanMX6 *PUF3(24A)*-yEGFP-NatNT2 | this study | Prototrophic CEN.PK |
| YL768 | MATa *PUF3(24A PUF domainΔ)*-yEGFP-KanMX6 | this study | Prototrophic CEN.PK |
| YL931 | MATa *PUF3(21A)*-FLAG-NatNT2 | this study | Prototrophic CEN.PK |
| YL932 | MATa *PUF3(23A)*-FLAG-NatNT2 | this study | Prototrophic CEN.PK |

*PUF3(PUF NTΔ)* denotes *PUF3* with amino acids 2-258 deleted

*PUF3(PUF domainΔ)* denotes *PUF3* with amino acids 538-844 deleted

*PUF3(24A)* denotes 24 serine/threonine phosphorylation sites identified in this study were mutated to alanine

*PUF3(24A PUF domainΔ)* denotes *PUF3(24A)* with amino acids 538-844 deleted

*PUF3(24D)* denotes 24 serine/threonine phosphorylation sites identified in this study were mutated to aspartate

*PUF3(21A)* denotes 21 serine/threonine phosphorylation sites except S515, S563, and S866 identified in this study were mutated to alanine

*PUF3(23A)* denotes 23 serine/threonine phosphorylation sites except S866 identified in this study were mutated to alanine

3'UTR denotes 1~237bp sequence downstream of ORF

MRP51 3'UTR * denotes one TGTAAATA motif in MRP51 3'UTR has been mutated to ACACAATA

**Table S2 (refers to Figures 1-6). qRT-PCR primers and RNA probe sequence information**

| Primer or probe name | Sequence |
|---|---|
| PL272 (ACT1 F) | CCCAGGTATTGCCGAAAGAATGC |
| PL273 (ACT1 R) | GGAAGATGGAGCCAAAGCGG |
| PL274 (COX17 F) | CCAGAAAAGGAGGAGCGGGATA |
| PL275 (COX17 R) | CGAAGCCATAACCCTTCATGCAC |
| PL276 (COX12 F) | CCCCAACAAAACCAAACAAAGCA |
| PL277 (COX12 R) | TCCAAAAGACCTTGCACGGAG |
| PL579 (MRPL10 F) | CGCCGGTATTAGCATTTCAA |
| PL580 (MRPL10 R) | CCATCTGACGGTTTCAATTGTC |
| PL581 (PET117 F) | TGCATCAGGGTCCGATAAAA |
| PL582 (PET117 R) | CCGTTTGTCTTTCTCAACCTCTT |
| PL863 (PGK1 F) | TCATTGGTGGTGGTGACACT |
| PL864 (PGK1 R) | GCAACACCTGGCAATTC |
| COX17 3'UTR | TCTTACTGACAGTCTGCCGACAACCATTTCTTGTATATATAAGAATAGGTATTCAC AAACTATAGTATATACTACCTGTAAATATGTGCGATGCACAATTAACATTACCTC ATCACTACTACACCACTTCTACTGCTAGCACTGTCCTCTTGTGCTTGGCCCCTTAA GAGTGTTCTAAGACCACGTGACCAGAAAAGGACGTATCACGTGACACAAACCTA ATAACTTTTTTAGGAA |
| MRP51 3'UTR | GAAGAGAAAAAAAAAAATGTGAACAATCAATGTATATTCAGAGTTCTGTAAATAA ATAAAGAAAATAAAGTTTACATATTACTAAGGATTTTTGTCGCCTATTTTTACTAT TTTTCAGGTGAAATGAAACGTTTTATATCACATTTTGCTATGATAACAAAGTTATT ATGATTTTTATGTAGCCTATATTATTGACGCGTTGTTATAGCATTCTATGAATTTG CCTGTCATTTTCCAC |

Note: The highlighted regions denote Puf3 binding elements (P3E)