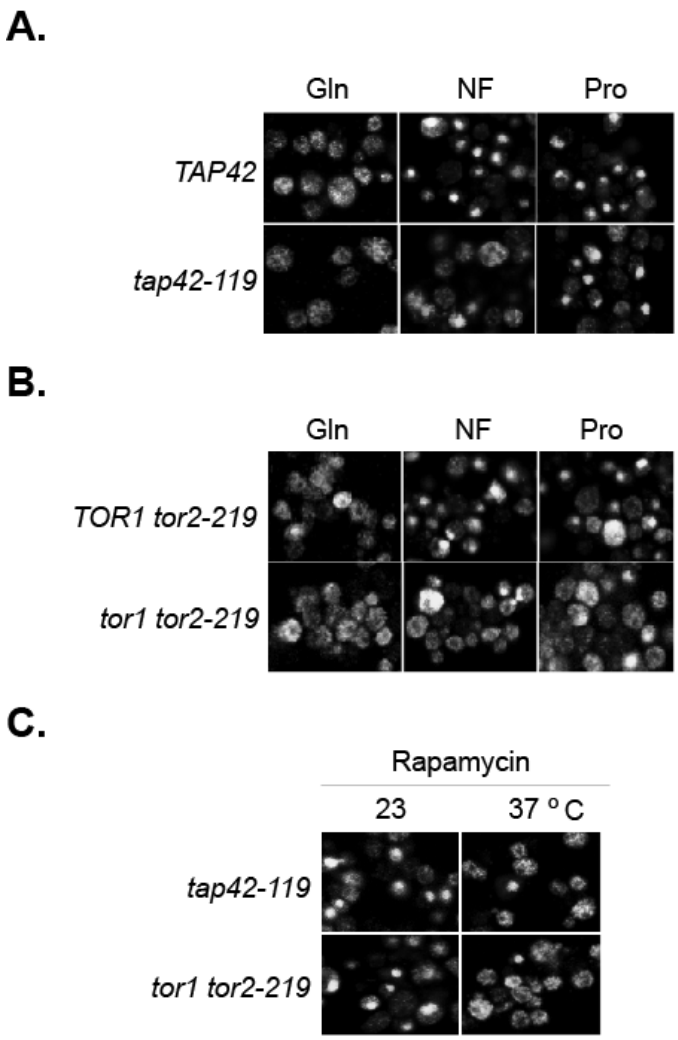
[Supplementary Information]

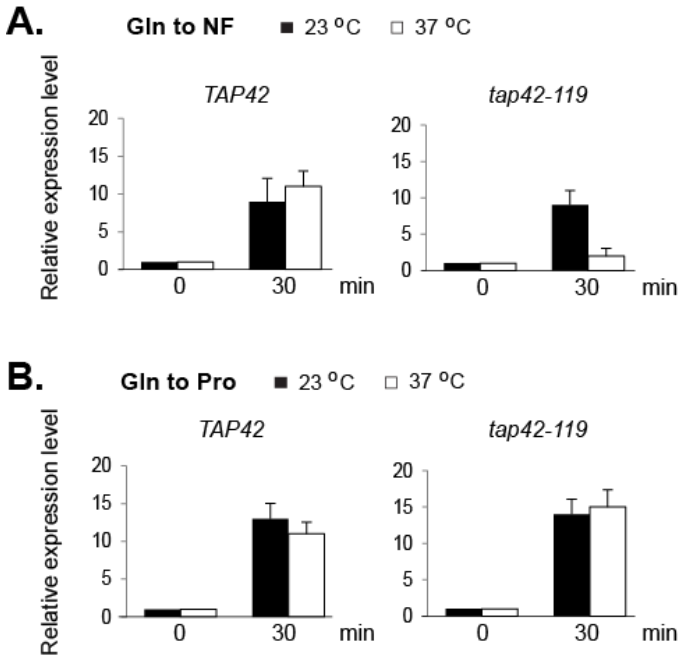
Target of rapamycin complex 1 and Tap42-Associated Phosphatases Are Required for Sensing Changes in Nitrogen Conditions in the Yeast *Saccharomyces cerevisiae*

Contents:

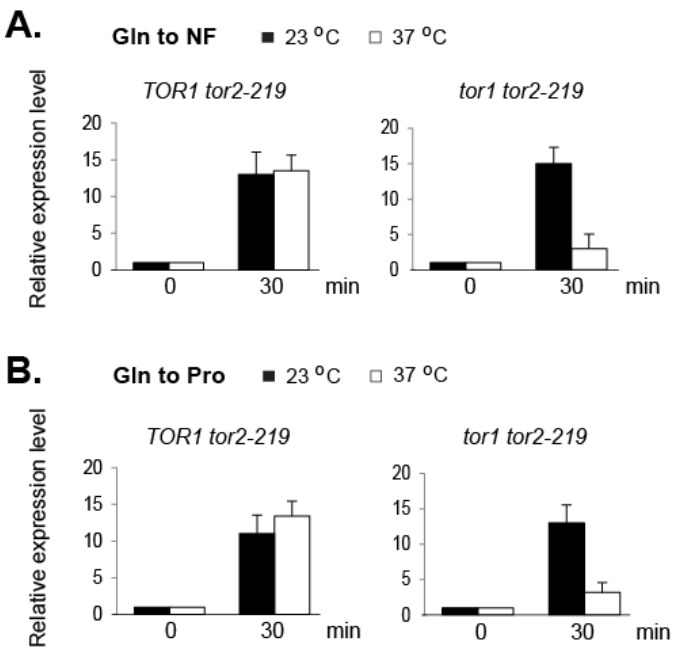
1. Supplemental Figures



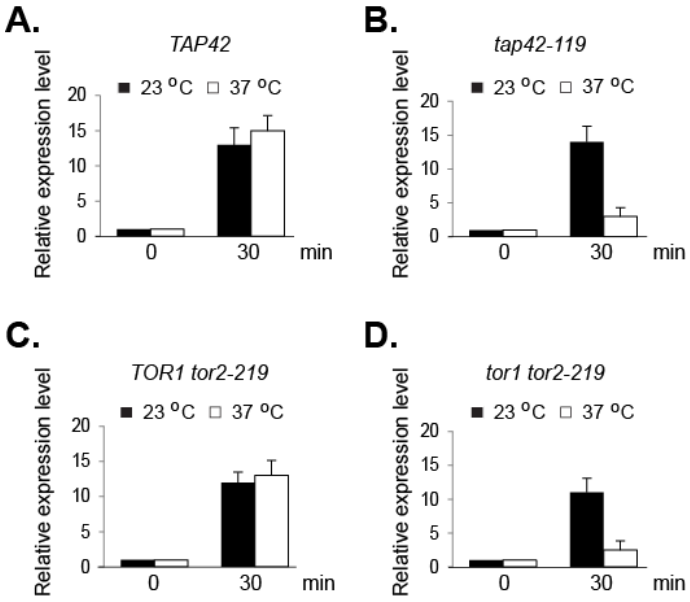
Supplemental Figure 1. Gln3 distribution in wild type and mutant *tor* and *tap42* cells under different nitrogen conditions. Exponentially growing cells expressing *GLN3*-myc in glutamine (Gln) medium were shifted from 23 to 37^{o}C for 2 hr and then transferred to nitrogen-free (NF) or proline (Pro) medium for 30 min. **A.** *TAP42* (Y339) and *tap42-119* (Y351)**. B.** *TOR1 tor2-219 and tor1 tor2-219***. C.** Exponentially growing *tap42-119* and *tor1 tor2-219* cells expressing *GLN3*-myc in glutamine (Gln) medium were shifted from 23 to 37^{o}C for 2 hr and then treated with rapamycin for 30 min. Gln3-myc distribution in the cells was examined by immunofluorescent microscopy.



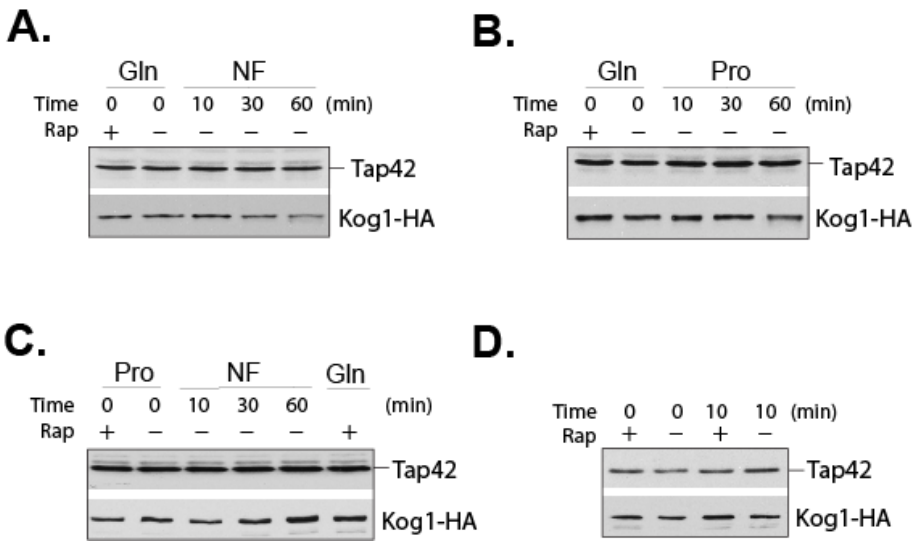
Supplemental Figure 2. Tap42 is dispensable for nitrogen downshift-induced expression of *GLN1*. Wild type (Y339) and *tap42-119* mutant (Y351) cells expressing *GLN3*-myc were grown at 23°C to early log phase in glutamine (Gln) medium. The cultures were divided and incubated for two additional hours with one half at 37°C and the other half at 23°C. Cells were then transferred to nitrogen-free (**A**) or proline (**B**) medium and collected before (time 0) and 30 min after the transfer. Expression levels of *GLN1* were analyzed by qRT-PCR and shown as relative values of treated vs. untreated samples. The experiment was repeated three times. The relative expression levels are shown as mean ± SD.



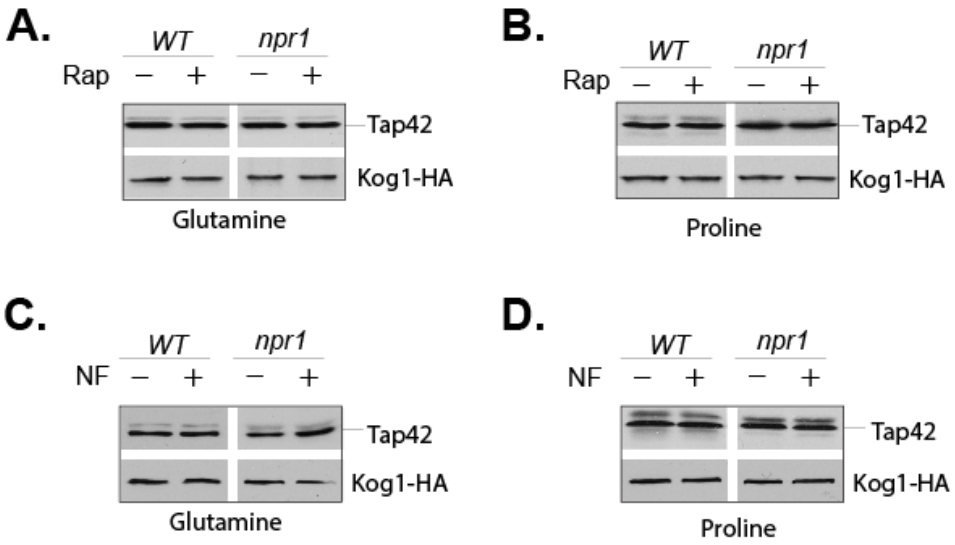
Supplemental Figure 3. TORC1 is required for nitrogen limitation or downshift-induced *GLN1* expression. The *tor2-219* (Y511) and *tor1 tor2-102* mutant (Y442) cells expressing *GLN3*-myc were grown at 23°C to early log phase in glutamine (Gln) medium. The cultures were divided and incubated for two additional hours with one half at 37°C and the other half at 23°C. Cells were then transferred to nitrogen-free (**A**) or proline (**B**) medium and collected before (time 0) and 30 min after the transfer. Expression levels of *GLN1* were analyzed by qRT-PCR and shown as relative values of treated vs. untreated samples. The experiment was repeated three times. The relative expression levels are expressed as mean ± SD



Supplemental Figure 4. Both Tap42 and TORC1 are required for rapamycin-induced expression of *GLN1*. Cells expressing *GLN3*-myc were grown at 23°C to early log phase in glutamine (Gln) medium. The cultures were divided, with one half shifted to 37°C and the other half remained at 23°C. Upon incubation for additional 2 hr, cells were treated with rapamycin and collected before (time 0) and 30 after addition of the drug. Expression levels of *GLN1* were analyzed by qRT-PCR and shown as relative values of treated vs. untreated samples. The experiment was repeated three times. The relative expression levels are expressed as mean ± SD. **A**. Wild type (Y339), **B**. *tap42-119* mutant (Y351), **C**. *TOR1 tor2-219* (Y511), and **D**. *tor1 tor2-219* mutant (Y442).



Supplemental Figure 5. **Expression levels of Tap42 and Kog1-HA in cells shown in Figure 4 of the main text.** Cells expressing *KOG1*-HA (Y1032) and *GLN3*-myc grown in glutamine medium (Gln) were treated with rapamycin for 30 min (Rap +) or transferred to nitrogen–free (NF) (**A**) or proline (Pro) medium (**B**) for indicated times. **C**. Cells grown in proline medium were treated with rapamycin for 30 min (Rap +) or transferred to nitrogen-free (NF) medium. Cells grown in glutamine medium were treated with rapamycin and included as a control (Gln Rap+). **D**. Cells grown in glutamine medium were treated with rapamycin (+) or drug vehicle (-) before and after being shifted to proline medium for 10 min. The expression levels of Tap42 and Kog1-HA in the cells were examined by western blotting. The association of Tap42 with Kog1-HA in the treated cells was assayed by immunoprecipitation and shown in Figure 4 of the main text.



Supplemental Figure 6. Expression levels of Tap42 and Kog1-HA in cells shown in Figure 6 of the main text. Wild type (Y1032) and *npr1* deletion (Y1709) cells expressing *KOG1*-HA grown in glutamine (**A**) or proline medium (**B**) were treated with rapamycin (Rap +) or drug vehicle (Rap -) for 30 min. The same pairs of cells grown in glutamine (**C**) or proline medium (**D**) were transferred to nitrogen-free (NF) medium and collected before and 30 min after the transfer. The expression levels of Tap42 and Kog1-HA in the cells were examined by western blotting. The association of Tap42 with Kog1-HA in the treated cells was assayed by immunoprecipitation and shown in Figure 6 of the main text.

2. Supplemental Methods

Quantitative RT-PCR __Total yeast cell RNA was isolated using the Qiagen RNeasy MiniKit (Qiagen, Chatsworth, CA). cDNA was generated from the isolated RNA using iScript Reverse Transcrition kit (Bio-Rad, Hercules, CA) following manufacturer's instructions. cDNA was analyzed by RT-PCR for levels of *GLN1* and *ACT1* using previously reported primers as follows (Crespo *et al.*, 2002): *GLN1*, 5′-CGTTTGGATCGATGGTACTG-3′ and 5′-CGCAAACAGTTTCACACATG-3′; *ACT1*, 5′-ATGGATTCTGAGGTTGCTGC-3′ and 5′-ACCTTCATGGAAGATGGAGC-3′. The expression level of *GLN1* was normalized to that of *ACT1* in the same sample.

3. Supplemental References:

Crespo, J.L., T. Powers, B. Fowler & M.N. Hall, (2002) The TOR-controlled transcription activators GLN3, RTG1, and RTG3 are regulated in response to intracellular levels of

glutamine. *Proceedings of the National Academy of Sciences of the United States of America* **99**: 6784-6789.