

**The A2357T substitution in the kinase domain of yeast
Tor1 confers growth-promoting TORC1 activity
independent of Gtr1/2 and Pib2**

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Supplementary information

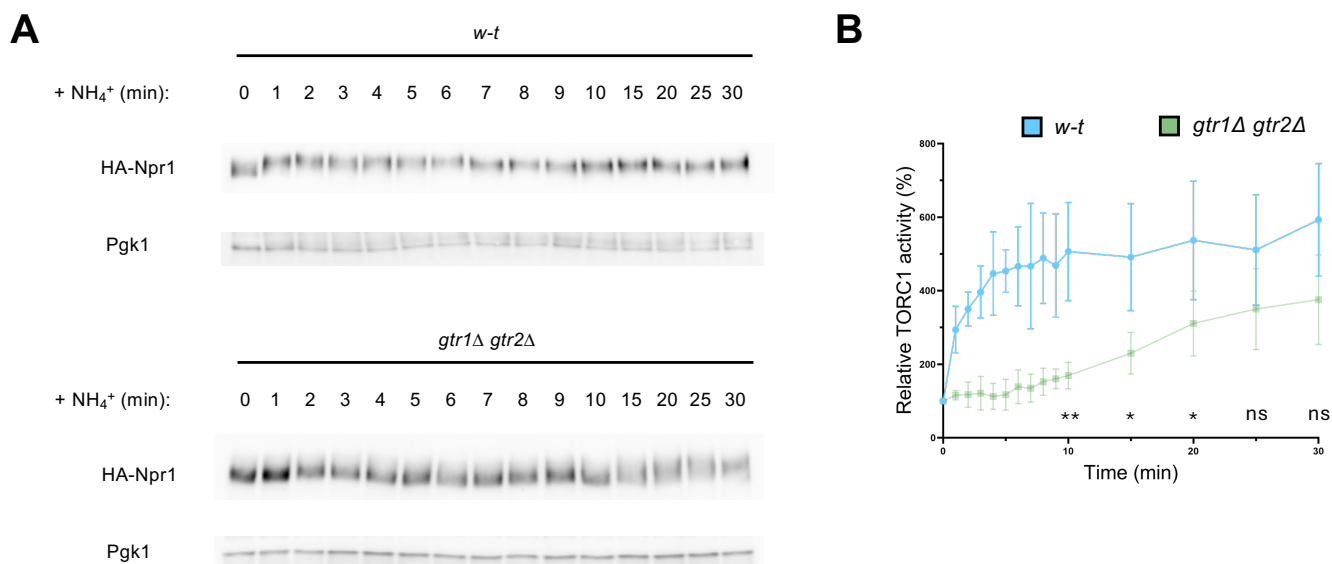


Figure S1. Role of Gtr1-Gtr2 in early and late activation of yeast TORC1 upon NH₄⁺ addition.

(A) Immunoblot analysis of HA-Npr1 in cell extracts prepared from *w-t* and *gtr1Δ gtr2Δ* cells before and various times after addition of NH₄⁺ (5 mM). Cells were initially grown in glucose proline medium. Cell extracts were prepared and immunoblotted with anti-HA and anti-Pgk antibodies. The original blots are included in Supplementary Figures (Fig. S10E). **(B)** Quantification, from 4 independent experiments performed as illustrated in A, of the stimulating effect of NH₄⁺ on HA-Npr1 phosphorylation. Bars represent means ± S.D. Asterisks indicate significant differences assessed by the two-tailed paired *t*-test. ns: not significant; * *P* < 0.05; ** *P* < 0.01.

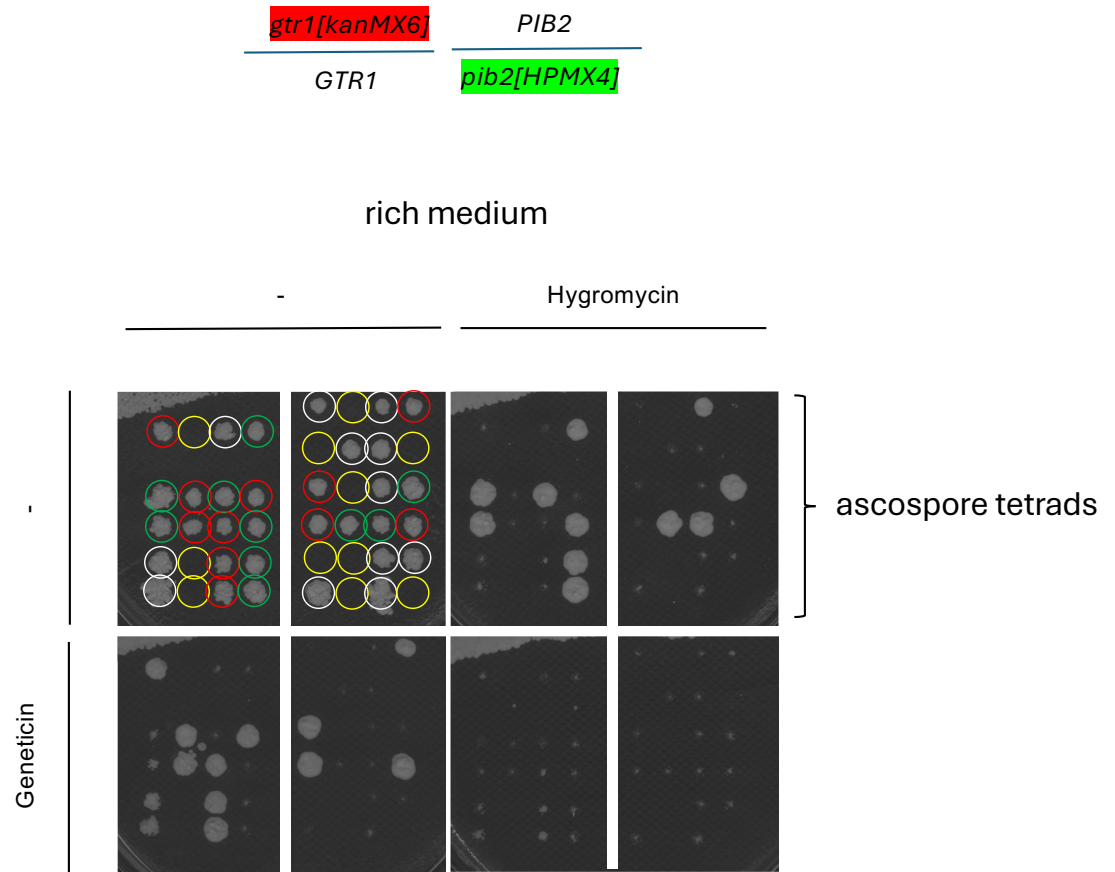
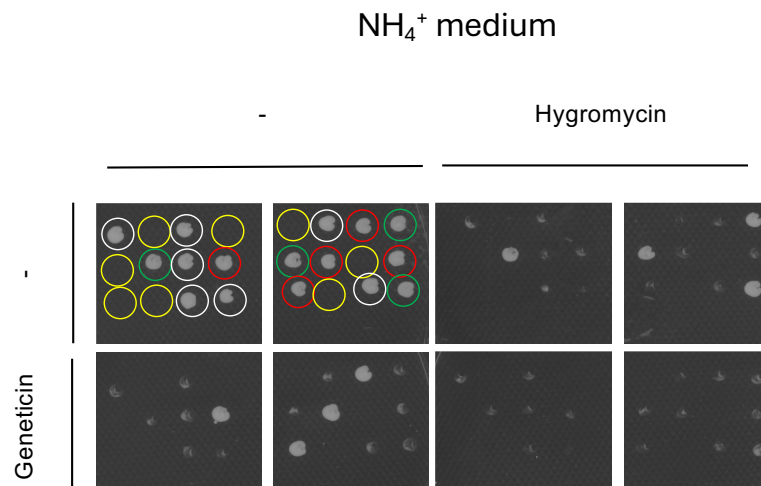
A**B**

Figure S2. Simultaneous deletion of *GTR1* and *PIB2* causes synthetic lethality in the $\Sigma 1278b$ strain background.

Analysis after germination on amino-acid-rich medium **(A)** or minimal NH₄⁺ medium **(B)** of haploid obtained by crossing the *gtr1* Δ [KANMX6] (geneticin resistant) and *pib2* Δ [HPMX4] (hygromycin resistant) mutants. The deduced genotypes of the four ascospores are labelled as follows: *w-t* (white), *gtr1* Δ (red), *pib2* Δ (green), *gtr1* Δ *pib2* Δ (yellow).

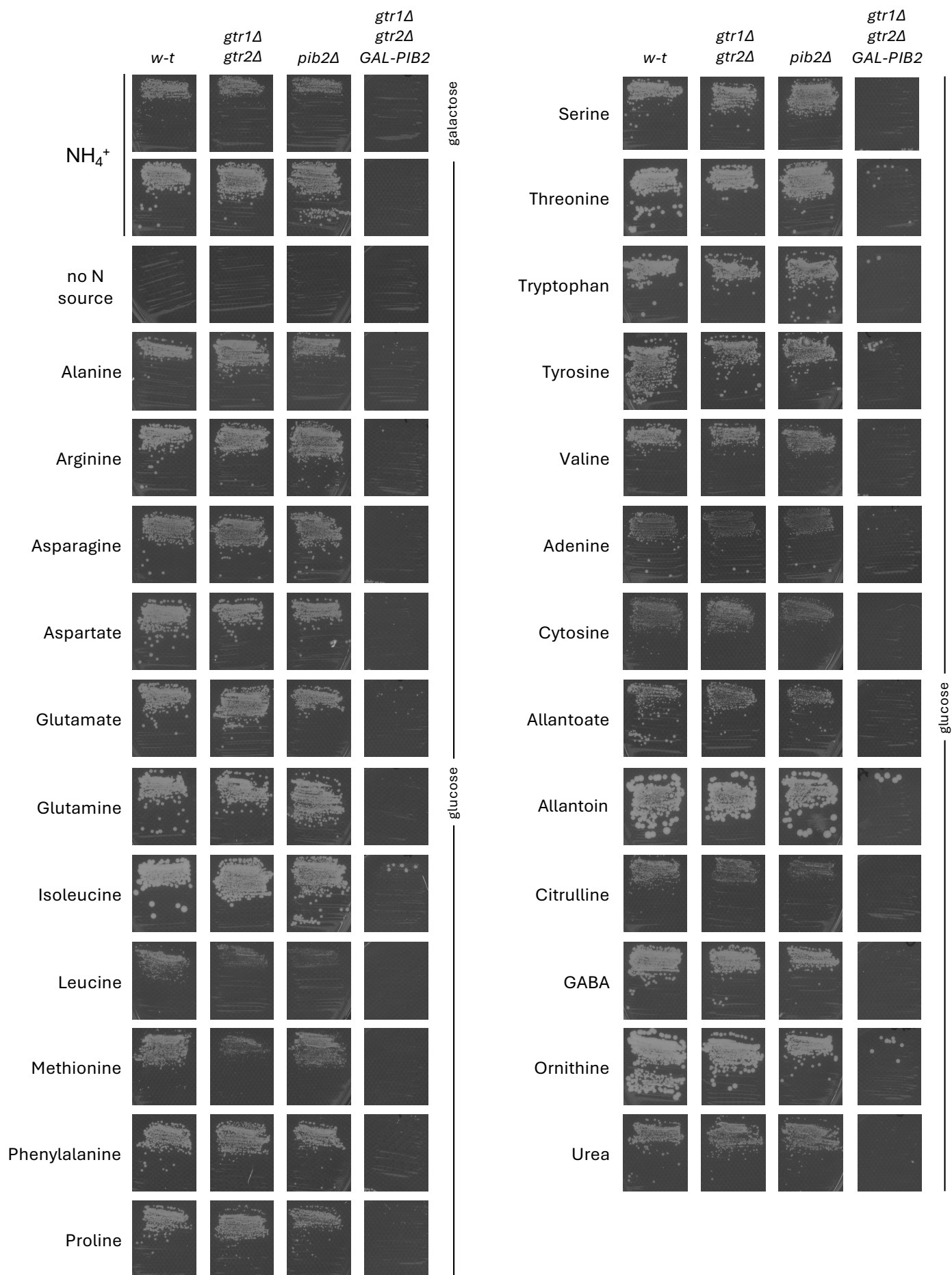


Figure S3. Simultaneous lack of Gtr1/2 and Pib2 expression is incompatible with growth, whatever the nitrogen source.

w-t, *gtr1Δ gtr2Δ*, *pib2Δ* and *gtr1Δ gtr2Δ GAL-PIB2* cells were spread on minimal medium with the indicated nitrogen compound as sole nitrogen source (10 mM), glucose (or galactose) as sole carbon source, and uracil (100 μM). For some conditions, the nitrogen source concentration was adjusted: NH_4^+ (5 mM), adenine (1 mM), methionine (6,7 mM), phenylalanine (5 mM), threonine (5 mM), tyrosine (5 mM), allantoate (3 mM), and allantoin (3 mM). Single colonies of the *gtr1Δ gtr2Δ GAL-PIB2* strain visible on certain media correspond to suppressor mutants.

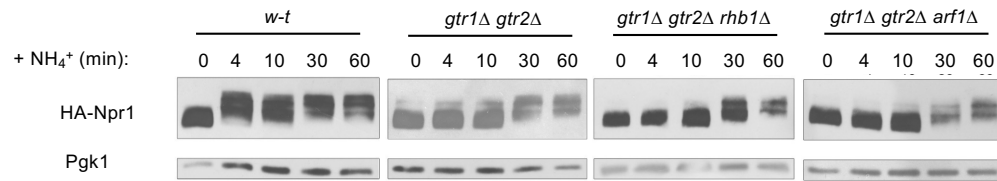


Figure S4. Role of Rhb1 and Arf1 in Gtr1-Gtr2-independent late activation of TORC1 upon NH₄⁺ addition.

Immunoblot analysis of HA-Npr1 in cell extracts prepared from the indicated strains before and various times after addition of NH₄⁺ (5 mM). Cells were initially grown in glucose proline medium. Cellular extracts were prepared and immunoblotted with anti-HA and anti-Pgk antibodies. The original blot is included in Supplementary Figures (Fig. S10F).

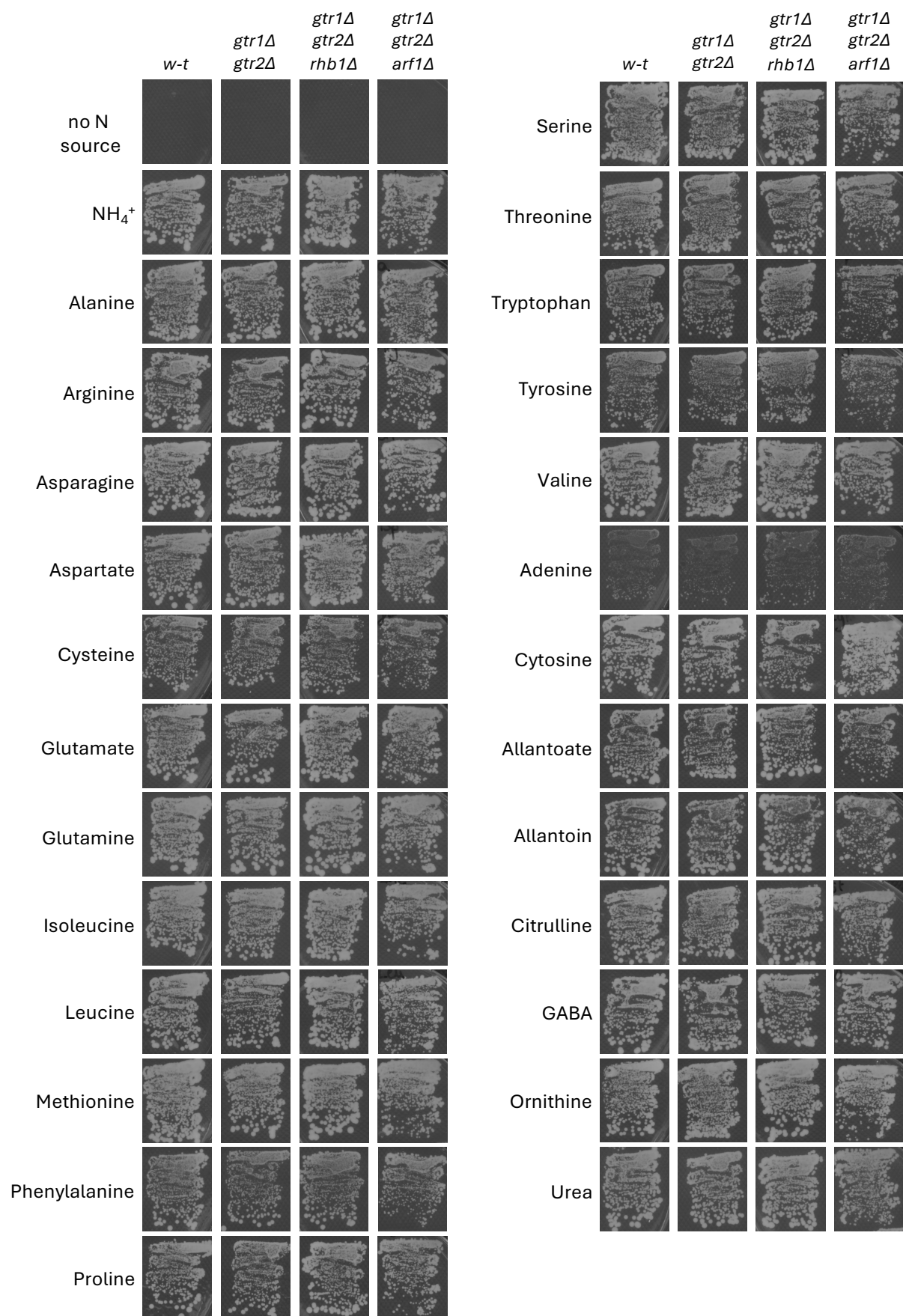


Figure S5. The Rhb1 and Arf1 small GTPases are dispensable for growth of the *gtr1Δ gtr2Δ* strain, whatever the nitrogen source.

Strains with the indicated genotypes were grown on solid minimal medium containing glucose as sole carbon source and the indicated nitrogen compounds as sole nitrogen source (10mM). For some conditions, the nitrogen source concentration was adjusted as in Fig. S3.

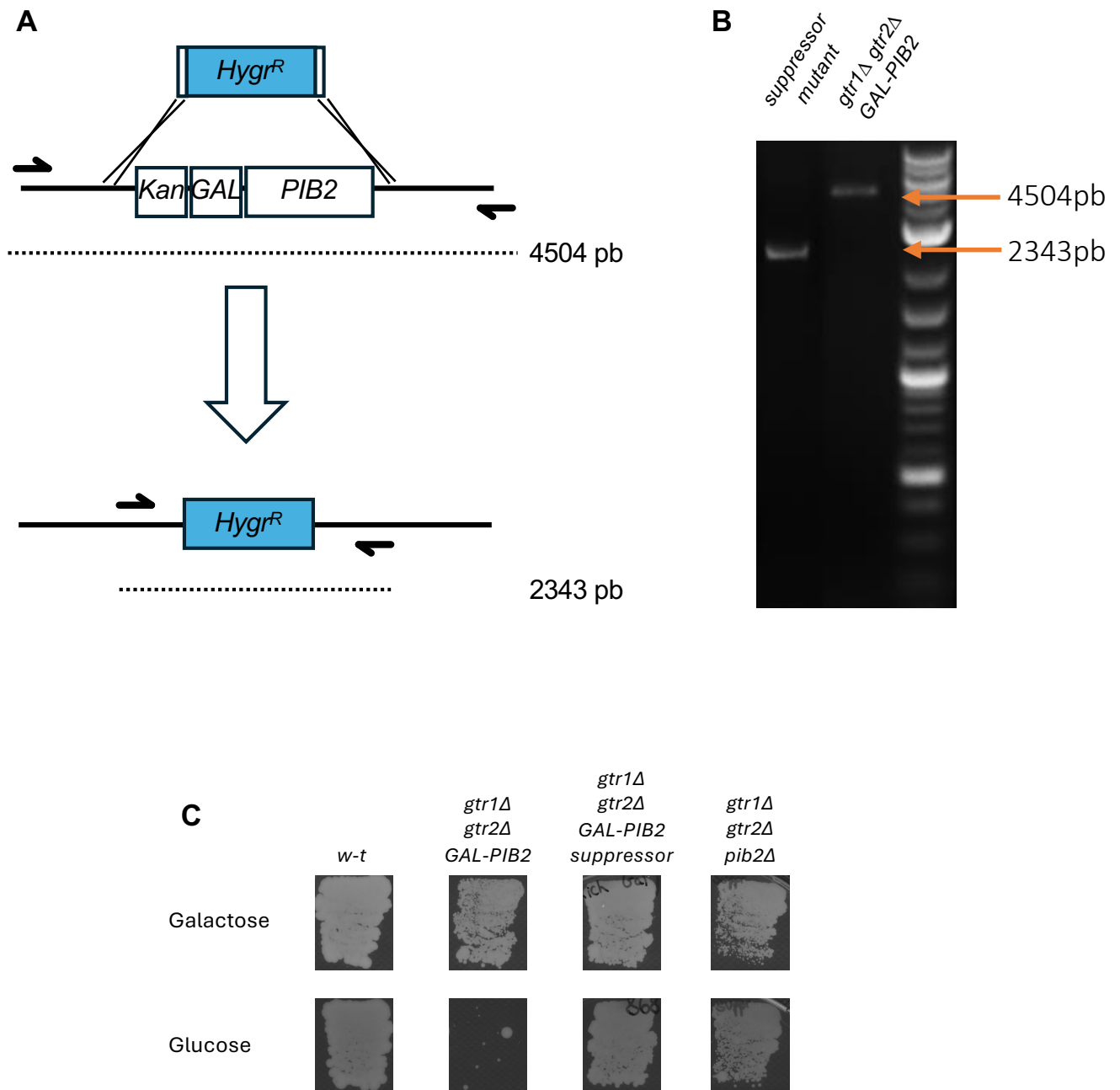


Figure S6. Deletion of the *GAL-PIB2* construct in the suppressor mutant isolated from the *gtr1Δ gtr2Δ GAL-PIB2* strain.

(A) Schematic illustration of the *GAL-PIB2* construct and its replacement with the hygromycin resistance gene. (B) Electrophoretic migration of PCR products obtained from the suppressor mutant and the parental *gtr1Δ gtr2Δ GAL-PIB2* strain, using primers as illustrated in A. (C) Comparative growth of the *w-t*, *gtr1Δ gtr2Δ GAL-PIB2*, *gtr1Δ gtr2Δ GAL-PIB2*-derived suppressor mutant and *gtr1Δ gtr2Δ pib2Δ* derived from the suppressor mutant on solid amino-acid-rich medium containing glucose or galactose as the carbon source.

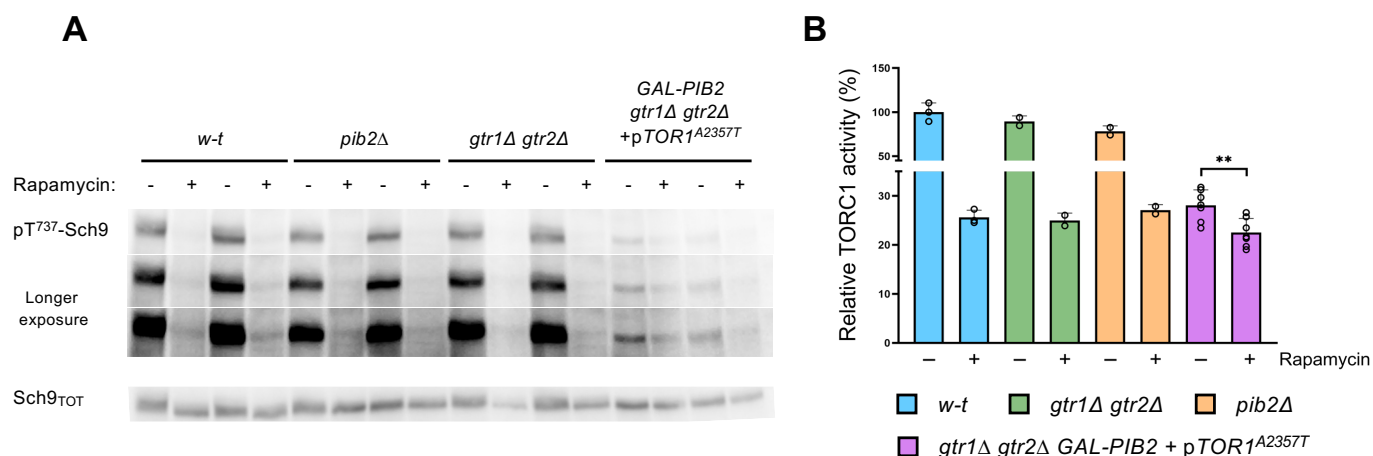


Figure S7. The low TORC1 activity in the *gtr1Δ gtr2Δ GAL-PIB2 + pTOR1^{A2357T}* strain is sensitive to rapamycin. (A)

The *w-t*, *pib2Δ*, and *gtr1Δ gtr2Δ* strains, and the *gtr1Δ gtr2Δ GAL-PIB2* strain transformed with a plasmid containing *TOR1^{A2357T}*, were grown in amino acid-rich medium. Once the cultures reached exponential phase, cells were treated with rapamycin (200ng/ml). Samples were collected before and 20min after this treatment. Cell extracts were prepared and immunoblotted with anti-HA and anti-Pgk antibodies. The original blot is included in Supplementary Figures (Fig. S10G). **(B)** Quantification of T⁷³⁷-Sch9 phosphorylation normalized to the total Sch9 level. The TORC1 activity of *w-t* is set at 100%. Bars represent means $\pm$ S.D. Asterisks indicate significant differences assessed by the two-tailed paired *t*-test. ** *P* < 0.01.

Gene view

The gene view histogram is a graphical view of mutations across MTOR. These mutations are displayed at the amino acid level across the full length of the gene by default. Restrict the view to a region of the gene by dragging across the histogram to highlight the region of interest, or by using the sliders in the filters panel to the left. [Show more](#)

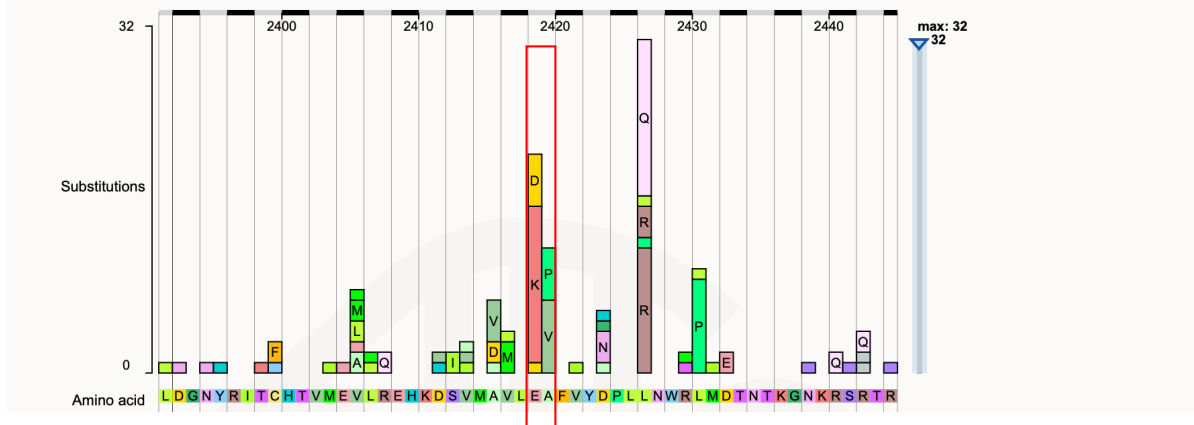


Figure S8. Missense mutations causing substitutions in ²⁴¹⁹Glu-Ala²⁴²⁰ of mTOR are prevalent in cancer tissues. The Glu-Ala residues and the corresponding substitutions are highlighted in red, results from the COSMIC database (<https://cancer.sanger.ac.uk/cosmic/>).

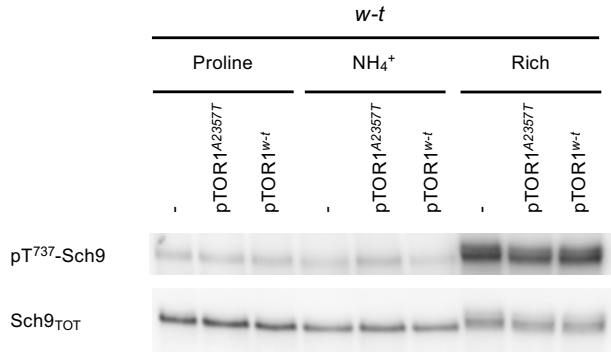
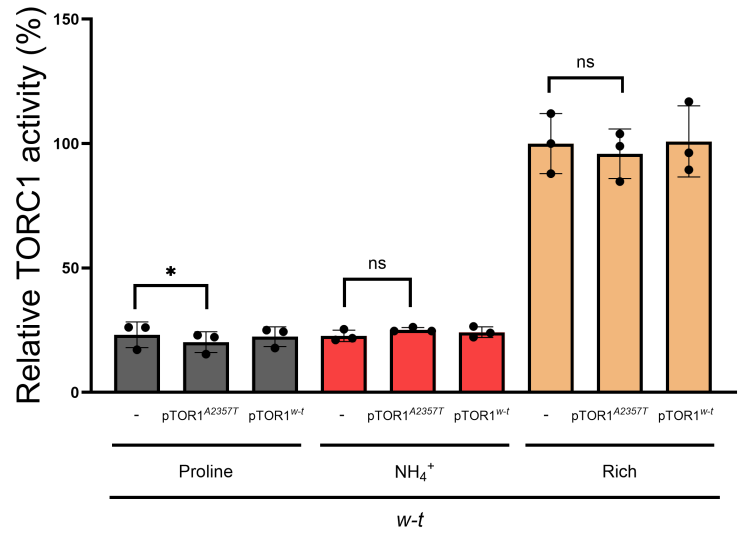
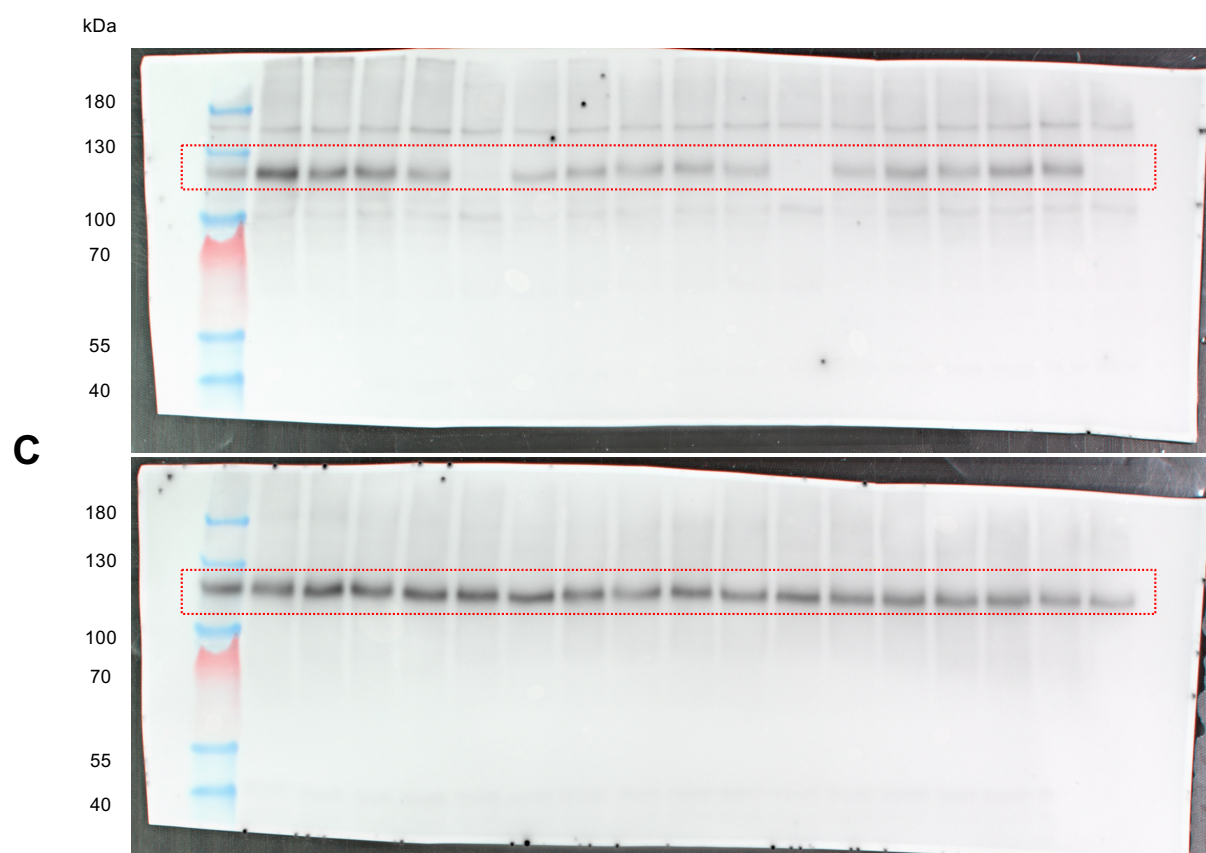
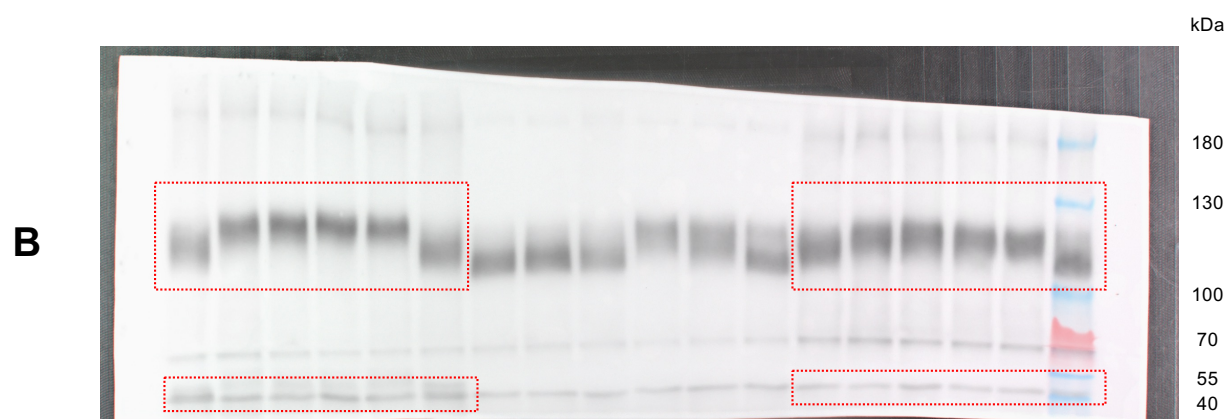
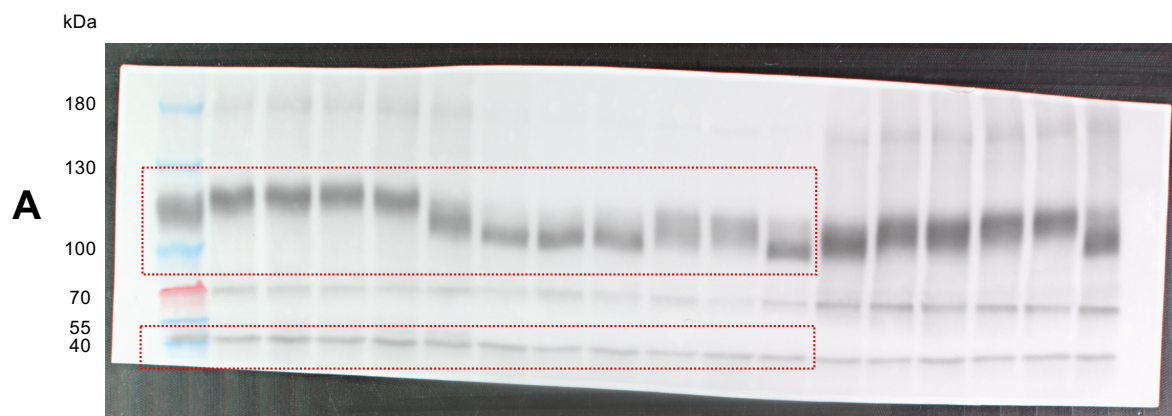
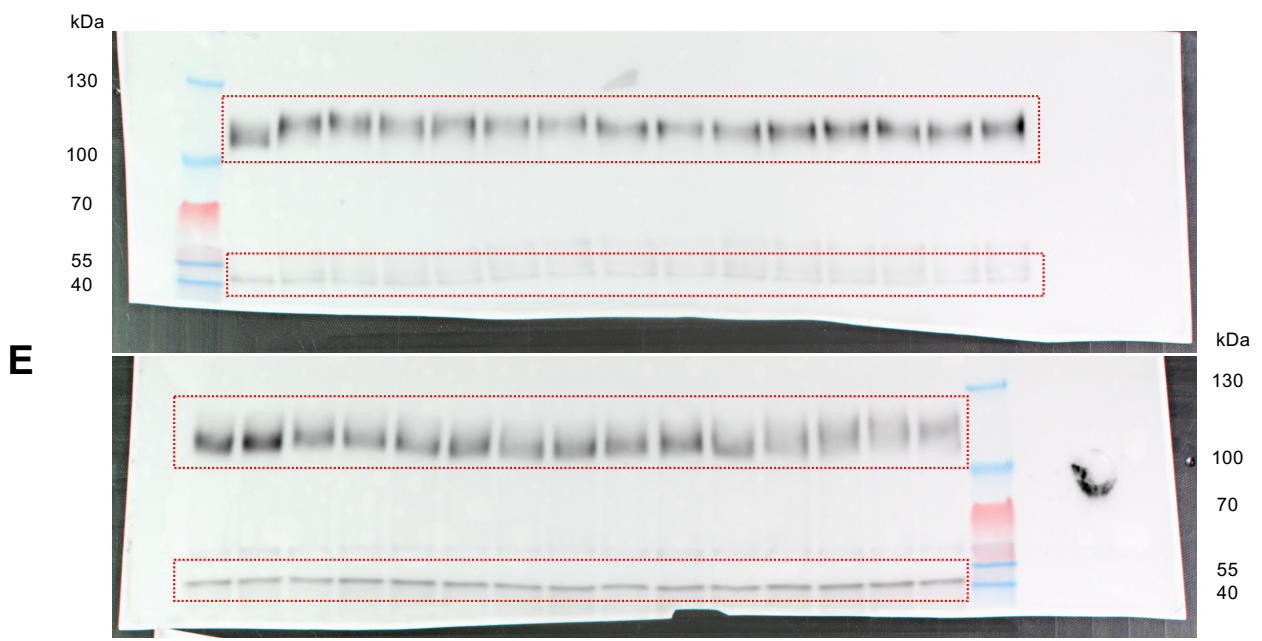
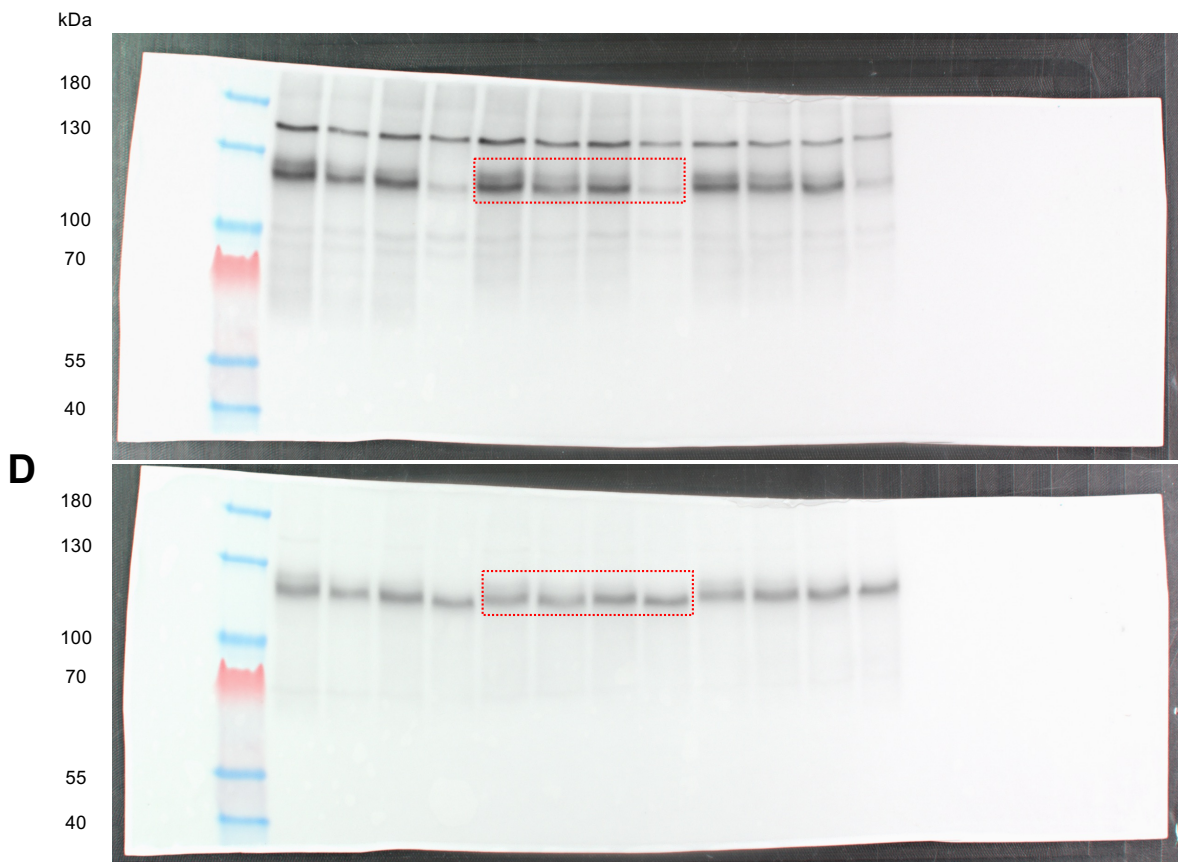
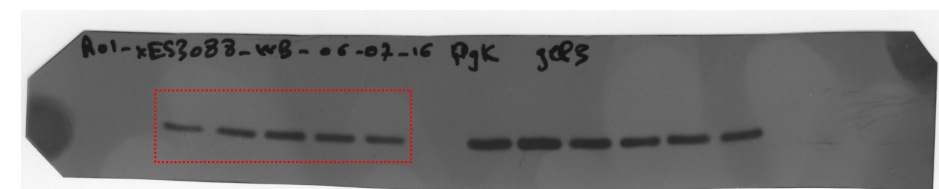
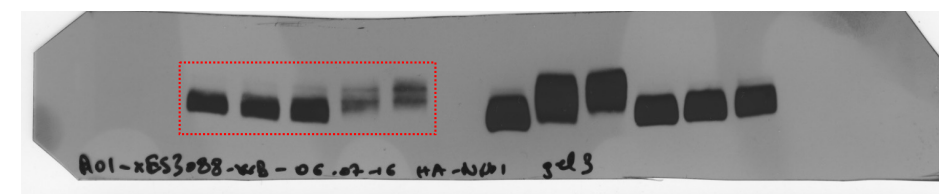
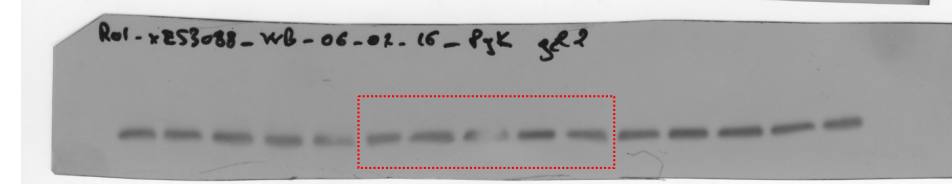
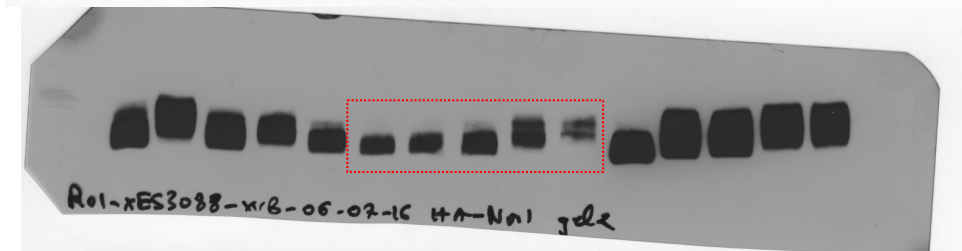
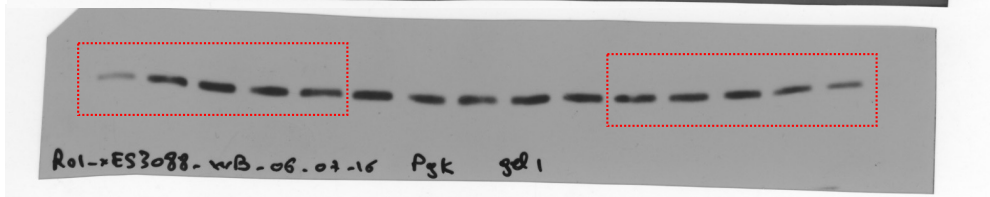
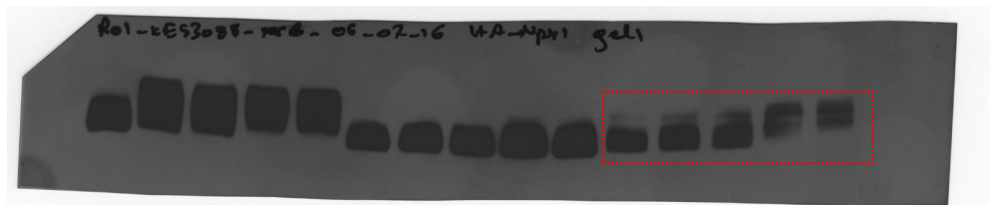
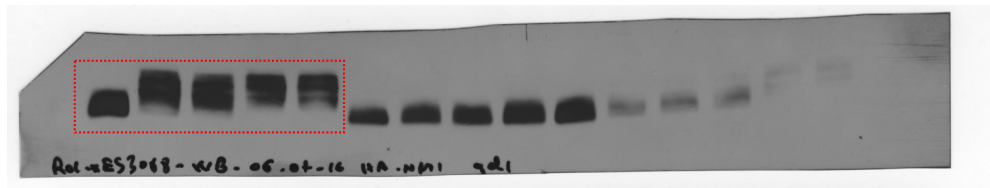
A**B**

Figure S9. Assessing TORC1 activity in a *w-t* strain expressing *TOR1* or *TOR1*^{A2357T} carried on a plasmid.

(A) Immunoblotting performed to assess the T⁷³⁷-Sch9 phosphorylation state of *w-t* cells transformed either with an empty plasmid (-) or a low-copy plasmid containing either *TOR1* or *TOR1*^{A2357T}. Cells were grown in amino-acid-rich medium or minimal medium containing proline or NH₄⁺ as sole nitrogen source. Culture samples were collected during the exponential growth phase. The original blot is included in Supplementary Figures (Fig. S10H). **(B)** Quantification of relative TORC1 activity, based on three biological replicates. Relative TORC1 activity corresponds to the phosphorylated T⁷³⁷-Sch9 signal normalized to the total Sch9 signal. The TORC1 activity in *w-t* cells transformed with the empty plasmid and growing on amino-acid rich medium was set at 100%. Bars represent means $\pm$ S.D. Asterisks indicate significant differences assessed by the two-tailed paired *t*-test. ns: not significant; * *P* < 0.05.







F

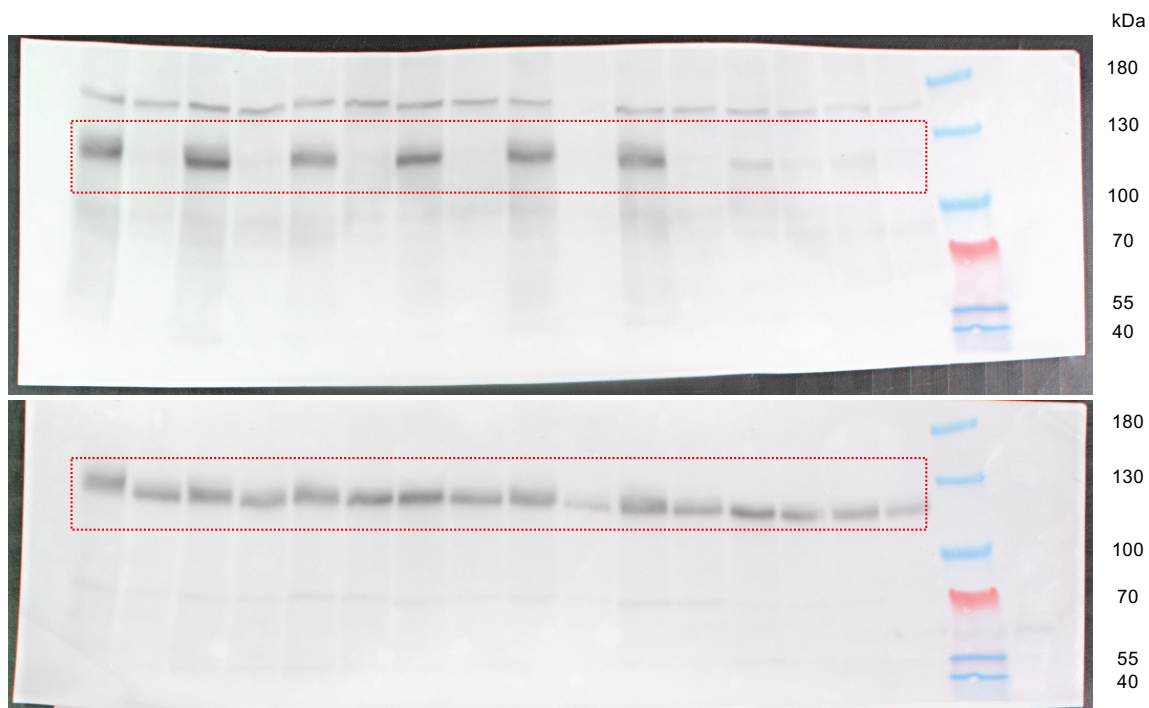
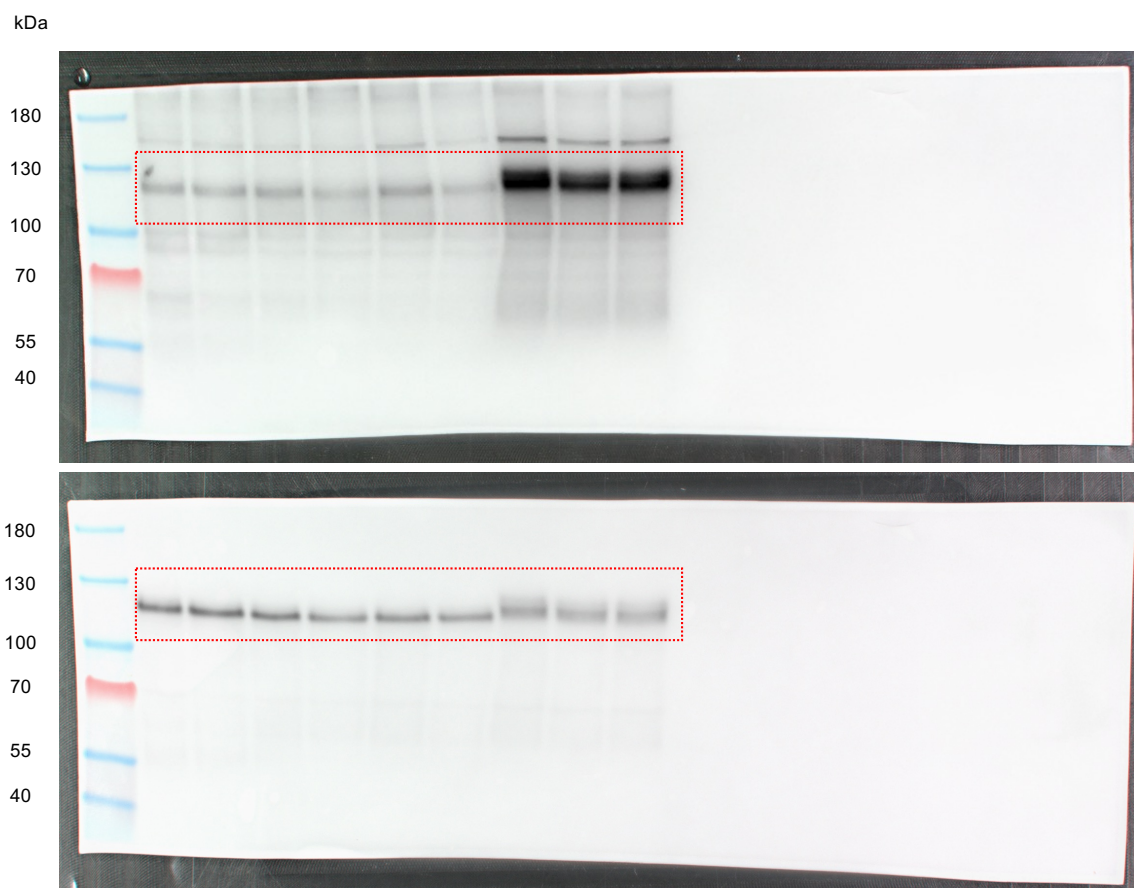
G**H**

Figure S10. Original immunoblots of figures displayed in this article.

Highlighted in red are the parts of the immunoblots that were used to construct the following figures. **(A)** Fig.1A **(B)** Fig.1B **(C)** Fig.1C **(D)** Fig.3F **(E)** Fig. S1A **(F)** Fig.S4 **(G)** Fig. S7A **(H)** Fig. S9A