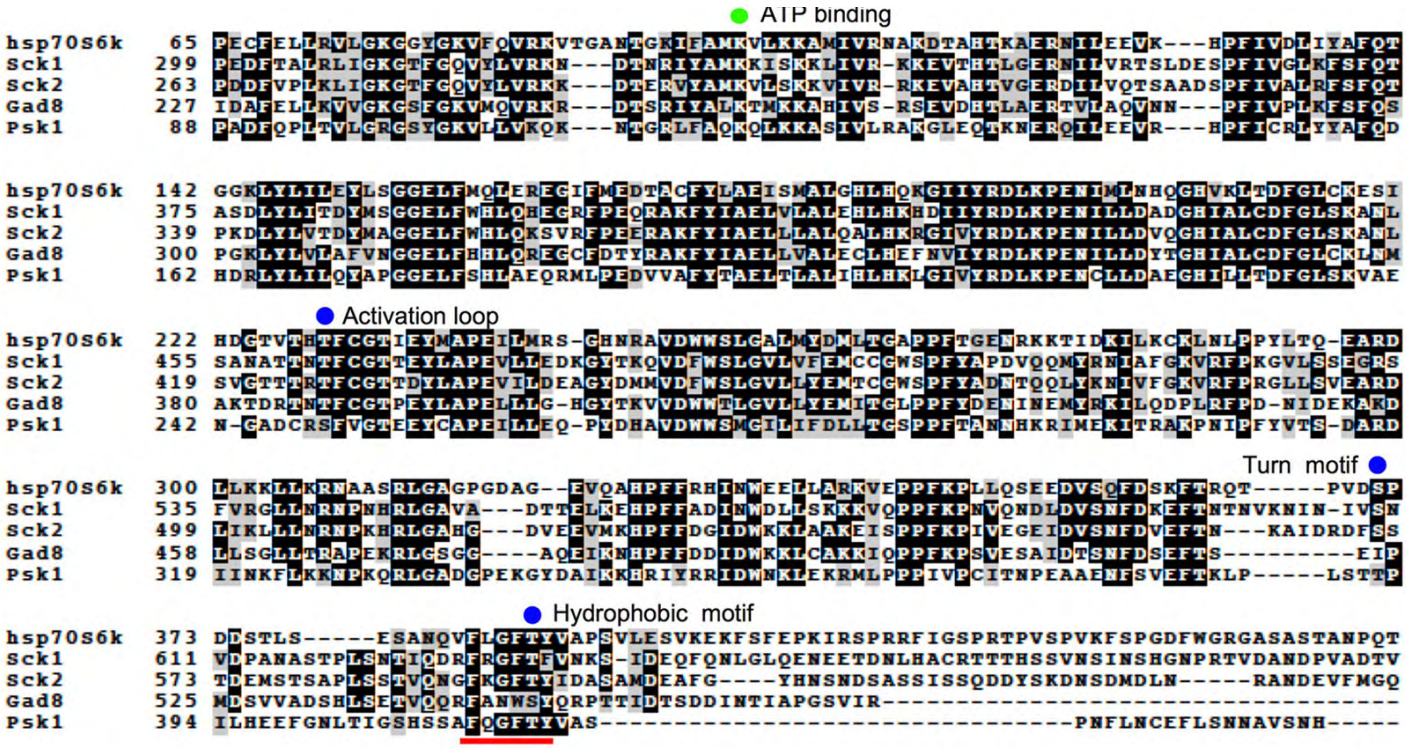


**Fig. S1. Alignment of the predicted amino acid sequences of human p70S6K1 and its homologs in fission yeast.** Amino acid sequences of human S6K1 (hsp70s6k, AAA36411) and its homologs in fission yeast (Sck1, NP_593754; Sck2, NP_594840; Gad8, NP_588010; Psk1, NP_587830) are aligned. The identical residues and conservative substitutions are highlighted in black and boxed in light gray, respectively. The red underline indicates the hydrophobic motif. Blue circles show predicted phosphorylation residues of human p70s6k in the activation loop, the turn motif, and the hydrophobic motif. The green circle indicates a predicted lysine residue binding to ATP. Numbers on the left indicate amino acids.

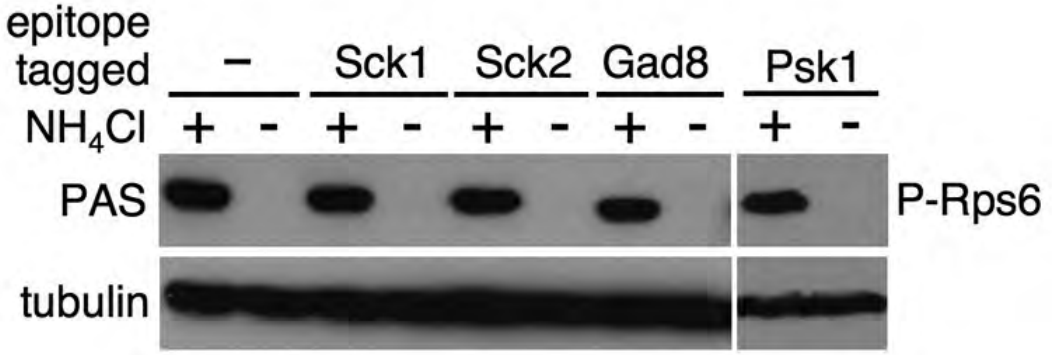


**Fig. S2. Nitrogen-dependent phosphorylation of Rps6 in cells expressing the epitope-tagged AGC kinases as indicated.** Protein extracts described in Fig. 1B were subjected to immunoblotting of Rps6 phosphorylation and tubulin with the indicated antibodies. Tubulin is shown as a loading control.

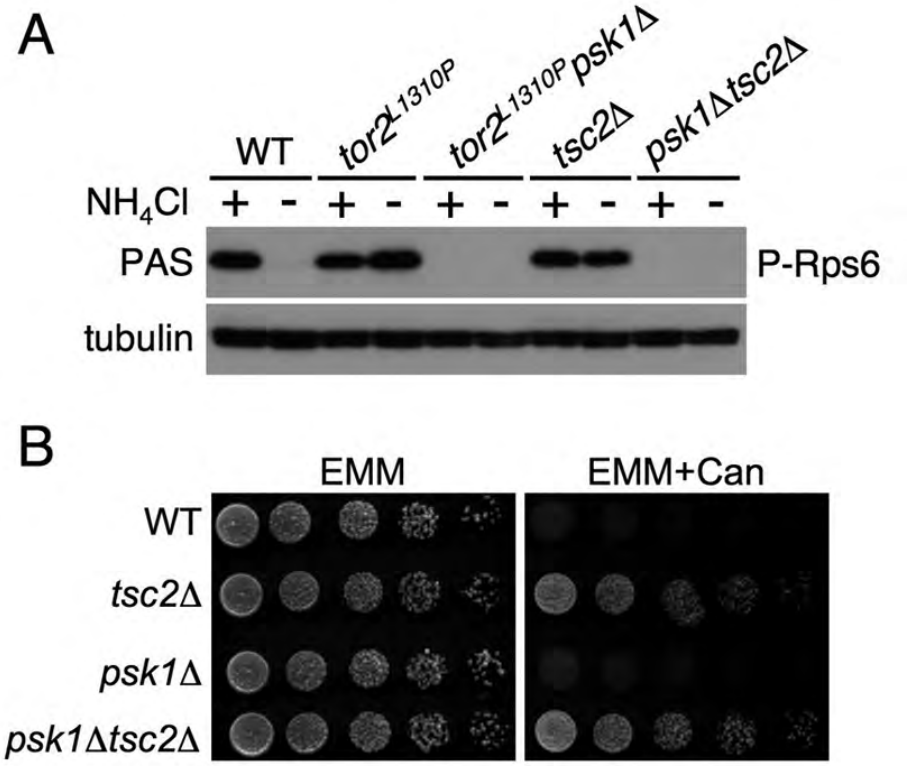


**Fig. S3. Psk1 is a downstream effector of the TSC-TORC1 pathway to regulate Rps6 phosphorylation but not to participate in canavanine resistance in the *tsc2* disruptant.** (**A**) Cells of JUp1204 (WT), JUp1350 (*tor2*$^{L1310P}$), AN0189 (*tor2*$^{LP}$*psk1*Δ), PJ001 (*tsc2*Δ), and AN0190 (*psk1*Δ*tsc2*Δ) were washed twice and incubated in EMM with (+) or without (-) ammonium for 15 minutes. Cell extracts were probed with the indicated antibodies. (**B**) Cells of 972 (WT), PJ001 (*tsc2*Δ), AN0132 (*psk1*Δ), and AN0190 (*psk1*Δ*tsc2*Δ) were serially diluted 5-fold and spotted onto EMM with or without 60 μg/ml canavanine (Can). Cells were incubated at 30°C for 2 days (EMM) or 3 days (EMM+Can).

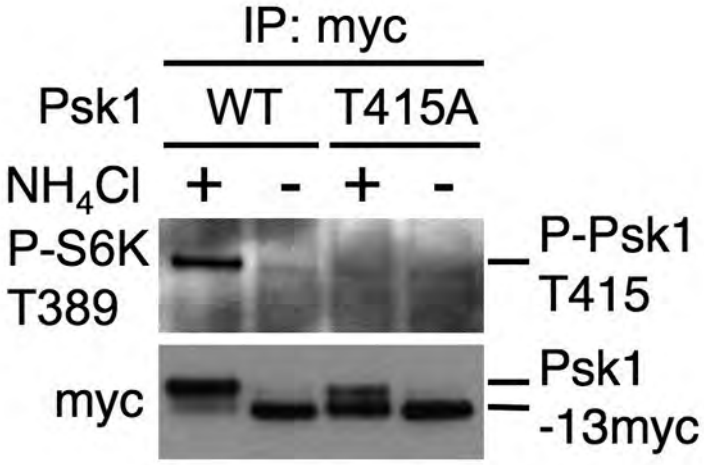


**Fig. S4. Phosphorylation of the hydrophobic motif in Psk1 is regulated in response to nitrogen source availability.** Cells of AN0179 (WT) and AN0212 (T415A) in YES (+) were washed and cultured in EMM-N for 30 minutes (-). Protein extracts were probed with the indicated antibodies.

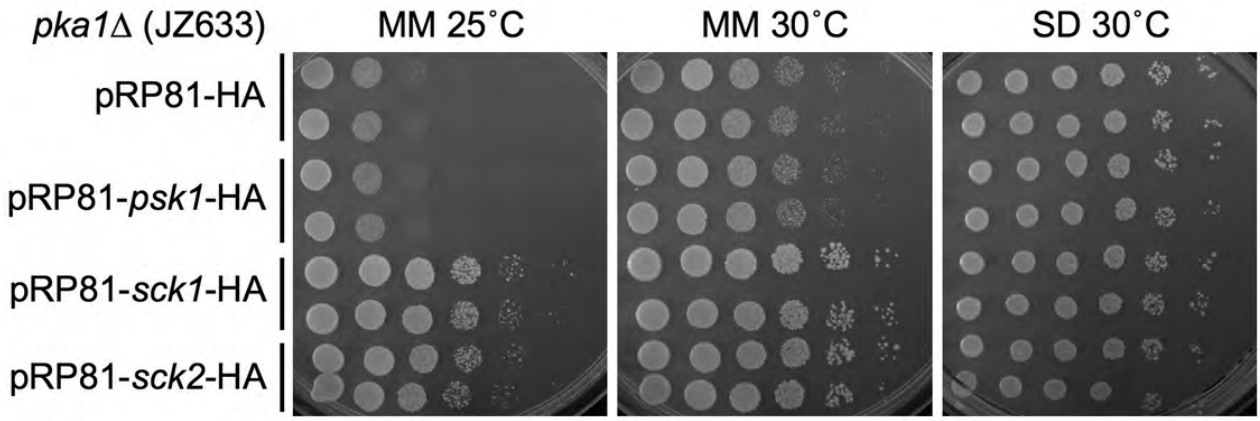


**Fig. S5. Psk1 has no redundant function with Pka1.** Serial 10-fold dilutions of JZ633 (*pka1*Δ) cells carrying an empty vector (pREP81), pREP81-*psk1*$^+$, pREP81-*sck1*$^+$ or pREP81-*sck2*$^+$ were spotted onto SD medium where expression of genes on the plasmids was repressed at 30°C, or minimal medium (MM) where expression of genes on the plasmids was induced at 25°C and 30°C for 4 days.

**Table S1. Yeast strains used in this study**

| | Strain | Genotype | Reference/source |
|---|---|---|---|
| | 972 | *h⁻* | Lab stock |
| 4 | JUp120 | *h⁹⁰ FY155* | S. Forsburg |
| 0 | JUp135 | *h⁹⁰ tor2$^{L1310P}$:kanMX* | Lab stock |
| | JY450 | *h⁹⁰ ade6-M216 leu1* | Lab stock |
| | JZ633 | *h⁹⁰ ade6-M216 leu1 ura4-D18 pka1::ura4⁺* | Lab stock |
| | JX764 | *h⁹⁰ sck1::his7⁺ sck2::ura4⁺ ade6-M210 leu1 his7-366 ura4-D18* | Lab stock |
| | YO201 | *h⁹⁰ psk1::kanMX ade6-M216 leu1* | This study |
| | YO202 | *h⁹⁰ psk1::kanMX sck1::his7⁺ sck2::ura4⁺ ade6-M216 leu1 his7-366 ura4-D18* | This study |
| 9 | AN012 | *h⁹⁰ rps601::hphMX kanMX:rps602pro-myc-rps602⁺* | Nakashima et al., 2010 |
| 2 | AN013 | *h⁻ psk1::hphMX* | This study |
| 3 | AN013 | *h⁹⁰ psk1::hphMX* | This study |
| 1 | AN015 | *h⁹⁰ sck1⁺-3HA:hphMX* | This study |
| 3 | AN015 | *h⁹⁰ sck2⁺-3HA:hphMX* | This study |
| 3 | AN016 | *h⁻ sck1⁺-3HA:hphMX tor2⁺:kanMX* | This study |
| 4 | AN016 | *h⁹⁰ sck1⁺-3HA:hphMX tor2⁺:kanMX* | This study |
| 6 | AN016 | *h⁹⁰ sck1⁺-3HA:hphMX tor2$^{L1310P}$:kanMX* | This study |
| 7 | AN016 | *h⁻ sck1⁺-3HA:hphMX tor2$^{S1837E}$:kanMX* | This study |
| 8 | AN016 | *h⁹⁰ psk1::hphMX rps601::hphMX kanMX:rps602pro-myc-rps602⁺* | This study |
| 0 | AN017 | *h⁹⁰ sck1::hphMX* | This study |
| 6 | AN017 | *h⁹⁰ gad8⁺-3HA:hphMX* | This study |
| 9 | AN017 | *h⁻ psk1⁺-13myc:hphMX* | This study |
| 0 | AN018 | *h⁹⁰ psk1⁺-13myc:hphMX* | This study |
| 1 | AN018 | *h⁻ psk1⁺-13myc:hphMX tor2⁺:kanMX* | This study |
| 2 | AN018 | *h⁹⁰ psk1⁺-13myc:hphMX tor2⁺:kanMX* | This study |
| 4 | AN018 | *h⁹⁰ psk1⁺-13myc:hphMX tor2$^{L1310P}$:kanMX* | This study |
| 5 | AN018 | *h⁻ psk1⁺-13myc:hphMX tor2$^{S1837E}$:kanMX* | This study |

| | | |
|---|---|---|
| AN0189 | $h^{90}$ *psk1::hphMX tor2L1310P:kanMX* | This study |
| AN0190 | $h^{-}$ *psk1::hphMX tsc2::kanMX* | This study |
| AN0203 | $h^{90}$ *sck2::hphMX* | This study |
| AN0210 | $h^{-}$ *psk1*$^{S248A}$*-13myc:hphMX* | This study |
| AN0211 | $h^{-}$ *psk1*$^{T392A}$*-13myc:hphMX* | This study |
| AN0212 | $h^{-}$ *psk1*$^{T415A}$*-13myc:hphMX* | This study |
| AN0213 | $h^{-}$ *psk1*$^{T392E}$*-13myc:hphMX* | This study |
| AN0216 | $h^{-}$ *psk1*$^{T415E}$*-13myc:hphMX* | This study |
| AN0217 | $h^{90}$ *psk1*$^{+}$*-13myc:hphMX tor2-ts6* | This study |
| AN0218 | $h^{90}$ *psk1*$^{+}$*-13myc:hphMX tor2-ts10* | This study |
| AN0219 | $h^{-}$ *psk1*$^{K120A}$*-13myc:hphMX* | This study |
| AN0233 | $h^{-}$ *psk1*$^{+}$*-13myc:hphMX pop3::kanMX* | This study |
| AN0237 | $h^{-}$ *psk1*$^{+}$*-13myc:hphMX toc1::kanMX* | This study |
| AN0238 | $h^{-}$ *psk1*$^{+}$*-13myc:hphMX tco89::kanMX* | This study |
| AN0243 | $h^{90}$ *psk1*$^{+}$*-13myc:hphMX ksg1-208* | This study |
| AN0245 | $h^{90}$ *psk1*$^{+}$*-13myc:hphMX ksg1-358* | This study |
| PJ001 | $h^{-}$ *tsc2::kanMX* | Lab stock |