		 ATP binding
hsp70S6k Sck1 Sck2 Gad8 Psk1	65 299 263 227 88	PECFELLRVLGKGGYGKVFQVRKVTGANTGRTGANKVLKKAMIVRNARDTAHTKAERNILEEVKHPFIVDLIYAFQT PEDFTALRLIGKGTFGQVYLVRKNDTNRIYAMKKISKKLIVR-KKEVTHTLGERNILVRTSLDESPFIVGLKFSFQT PDDFVPLKLIGKGTFGQVYLVRKKDTERVYAMKVLSKKVIVR-RKEVAHTVGERDILVQTSAADSPFIVALRFSFQT IDAFELLKVVGKGSFGKVMQVRKRDTSRIYADKTMKKAHIVS-RSEVDHTLAERTVDAQVNNPFIVPLKFSFQS PADFQPLTVLGEGSYGKVLLVKQKNTGRLFAQKQLKKASIVLRAKGLEQTKNERQILEEVRHPFICRLYYAFQD
hsp70s6k sck1 sck2 Gad8 Psk1	142 375 339 300 162	GGKLYLILETLSGGELEMQUEREGIFMEDTACFYLAEISMALGHLEQKGIIYRDLKPENI <mark>MU</mark> NHQGHVKUTDFGLCKESI ASDLYLITDYMSGGELFWHLQHEGRFPEQRAKFYIAELVLALEHLHKHDIIYRDLKPENILLDADGHIALCDFGLSKANL PKDLYLVTDYMAGGELFWHLQKSVRFPEERAKFYIAELLLALQALHKRGIVYRDLKPENILLDVQGHIALCDFGLSKANL PGKLYLVLAFVNGGELFHHLQREGCFDTYRAKFYIAELLVALECLHEFNVIYRDLKPENILLDYTGHIALCDFGLSKANL HDRLYLILQYAPGGELFSHLAEQRNLPEDVVAFYTAELTLALIHLHKLGIVYRDLKPENCLLDAEGHILLTDFGLSKVAE
hsp70s6k sck1 sck2 Gad8 Psk1	222 455 419 380 242	Activation loop HDGTVTHTFCGTIEYMAPEILMRS-GHNRAVDWWSLGALMYDMLTGAPPFTGENRKKTIDKILKCKLNLPPYLTQ-EARD SANATTNTFCGTTEYLAPEVLLEDKGYTKQVDFWSLGVLVFENCCGWSPFYAPDVQQMYRNIAFGKVRFPKGVLSSEGRS SVGTTTRTFCGTTDYLAPEVILDEAGYDMWVDFWSLGVLLYENTCGWSPFYADNTQQLYKNIVFGKVRFPRGLLSVEARD AKTDRTNTFCGTPEYLAPELLLG-HGYTKVVDWWTLGVLLYENITGLPPFYDENINEMYRKILQDPLRFPD-NIDEKAKD N-GADCRSFVGTEEYCAPEILLEQ-PYDHAVDWWSMGILIFDLLTGSPPFTANNHKRIMEKITRAKPNIPFYVTS-DARD
hsp70s6k Sck1 Sck2 Gad8 Psk1	300 535 499 458 319	Turn motif LLKKLLKRNAASRLGAGPGDAGEVQAHPFFRHINWEELLARKVEPPFKPLLQSEEDVSQEDSKFTRQTPVDSP FVRGLLNRNPNHRLGAVADTTELKEHPFFADINWDLLSKKKVQPPFKPNVQNDLDVSNFDKEFTNTNVKNIN-IVSN LIKLLLNRNPKHRLGAHGDVEEVMKHPFFDGIDWKKLAAKEISPPFKPIVEGEIDVSNFDVEFTNKAIDRDFS LLSGLLTRAPEKRLGSGGAQEIKNHPFFDDIDWKKLCAKKIQPPFKPSVESAIDTSNFDSEFTSEIP IINKFLKKNPKQRLGADGPEKGYDAIKKHRIVRRIDWNKLEKRMLPPPIVPCITNPEAAENFSVEFTKLPLSTTP
hsp7086k Sck1 Sck2 Gad8 Psk1	373 611 573 525 394	Hydrophobic motif DESTLSESANQVELGFTYVAPSVLESVKEKFSFEPKIRSPRRFIGSPRTPVSPVKFSPGDFWGRGASASTANPQT VDPANASTPLSNTIQDRFRGFTFVNKS-IDEQFQNLGLQENEETDNLHACRTTTHSSVNSINSHGNPRTVDANDPVADTV TDEMSTSAPLSSTVQNGFKGFTYIDASAMDEAFGYHNSNDSASSISSQDDYSKDNSDMDLNRANDEVFMGQ MDSVVADSHLSETVQQRFANWSYQRPTTIDTSDDINTIAPGSVIRPNFLNCEFLSNNAVSNH

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 ($tor2^{LP}psk1\Delta$), PJ001 ($tsc2\Delta$), and AN0190 ($psk1\Delta tsc2\Delta$) 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 ($tsc2\Delta$), AN0132 ($psk1\Delta$), and AN0190 ($psk1\Delta tsc2\Delta$) 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 ($pka1\Delta$) 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.

Strain	Genotype	Reference/source
972	h	Lab stock
JUp120 4	h ⁹⁰ FY155	S. Forsburg
JUp135 0	h^{90} tor 2^{LI310P} : kan MX	Lab stock
JY450	h ⁹⁰ ade6-M216 leu1	Lab stock
JZ633	h⁰º ade6-M216 leu1 ura4-D18 pka1∷ura4+	Lab stock
JX764	h ⁹⁰ sck1::his7 ⁺ sck2::ura4 ⁺ ade6-M210 leu1 his7- 366 ura4-D18	Lab stock
YO201	h ⁹⁰ psk1::kanMX ade6-M216 leu1	This study
YO202	h ⁹⁰ psk1::kanMX sck1::his7 ⁺ sck2::ura4 ⁺ ade6-M216 leu1 his7-366 ura4-D18	This study
AN012 9	h ⁹⁰ rps601::hphMX kanMX:rps602pro-myc-rps602 ⁺	Nakashima et al., 2010
AN013 2	h ⁻ psk1::hphMX	This study
AN013 3	h ⁹⁰ psk1::hphMX	This study
AN015 1	h ⁹⁰ sck1 ⁺ -3HA:hphMX	This study
AN015 3	h ⁹⁰ sck2 ⁺ -3HA:hphMX	This study
AN016 3	h ⁻ sck1 ⁺ -3HA:hphMX tor2 ⁺ :kanMX	This study
AN016 4	h ⁹⁰ sck1 ⁺ -3HA:hphMX tor2 ⁺ :kanMX	This study
AN016 6	h ⁹⁰ sck1 ⁺ -3HA:hphMX tor2 ^{L1310P} :kanMX	This study
AN016 7	h ⁻ sck1 ⁺ -3HA:hphMX tor2 ^{S1837E} :kanMX	This study
AN016 8	h ⁹⁰ psk1::hphMX rps601::hphMX kanMX:rps602pro-myc-rps602+	This study
AN017 0	h ⁹⁰ sck1::hphMX	This study
AN017 6	h ⁹⁰ gad8+-3HA:hphMX	This study
AN017 9	h ⁻ psk1 ⁺ -13myc:hphMX	This study
AN018 0	h ⁹⁰ psk1 ⁺ -13myc:hphMX	This study
AN018 1	h ⁻ psk1 ⁺ -13myc:hphMX tor2 ⁺ :kanMX	This study
AN018 2	h ⁹⁰ psk1 ⁺ -13myc:hphMX tor2 ⁺ :kanMX	This study
AN018 4	h ⁹⁰ psk1 ⁺ -13myc:hphMX tor2 ^{L1310P} :kanMX	This study
AN018 5	h ⁻ psk1 ⁺ -13myc:hphMX tor2 ^{S1837E} :kanMX	This study

Table S1. Yeast strains used in this study

AN018 9	8 h ⁹⁰ psk1::hphMX tor2L1310P:kanMX	This study	
AN019	h ⁻ psk1::hphMX tsc2::kanMX	This study	
AN020	$h^{90} sck2::hphMX$	This study	
AN021	1 h ⁻ psk1 ^{S248A} -13myc:hphMX	This study	
AN021	1 h ⁻ psk1 ^{T392A} -13myc:hphMX	This study	
AN021	1 h ⁻ psk1 ^{T415A} -13myc:hphMX	This study	
AN021	1 h ⁻ psk1 ^{T392E} -13myc:hphMX	This study	
AN021	1 h ⁻ psk1 ^{T415E} -13myc:hphMX	This study	
AN021	1 h ⁹⁰ psk1 ⁺ -13myc:hphMX tor2-ts6	This study	
AN021	1 h ⁹⁰ psk1 ⁺ -13myc:hphMX tor2-ts10	This study	
AN021	h ⁻ psk1 ^{K120A} -13myc:hphMX	This study	
AN023	³ h ⁻ psk1 ⁺ -13myc:hphMX pop3::kanMX	This study	
AN023	³ h ⁻ psk1 ⁺ -13myc:hphMX toc1::kanMX	This study	
AN023	³ h ⁻ psk1 ⁺ -13myc:hphMX tco89::kanMX	This study	
AN024	4 h ⁹⁰ psk1 ⁺ -13myc:hphMX ksg1-208	This study	
AN024	4 h ⁹⁰ psk1 ⁺ -13myc:hphMX ksg1-358	This study	
PJ001	h ⁻ tsc2::kanMX	Lab stock	