Supplementary Information

Pka1 contributes to the canavanine response and controls E3 ubiquitin ligase Pub1 localization

Ami Kuroki and Yasuhiro Matsuo[1]*

*Corresponding author: ymatsuo@life.shimane-u.ac.jp

Supplementary Figures 1-7

Supplementary Tables 1-2

Supplementary Table S1 *S. pombe* strains used in this study

Strain	Genotype	Source
PR109	*h⁻ leu1-32 ura4-D18*	[1]
YMP43	*h⁻ leu1-32 ura4-D18 git3::ura4*	[1]
YMP39	*h⁻ leu1-32 ura4-D18 gpa2::ura4*	[1]
YMP28	*h⁻ leu1-32 ura4-D18 cyr1::ura4*	[1]
YMP40	*h⁻ leu1-32 ura4-D18 cgs1::ura4*	[1]
YMP36	*h⁻ leu1-32 ura4-D18 pka1::ura4*	[1]
YMP58	*h⁻ leu1-32 ura4-D18 cyr1::LEU2 cgs1::ura4*	[1]
YMP923	*h⁻ leu1-32 ura4-D18 cat1-GFP(S65T)-FLAG-6His-kanMX6*	This study
YMP935	*h⁻ leu1-32 ura4-D18 cgs1::ura4 cat1-GFP(S65T)-FLAG-6His-kanMX6*	This study
YMP938	*h⁻ leu1-32 ura4-D18 pka1::ura4 cat1-GFP(S65T)-FLAG-6His-kanMX6*	This study
FY23873 (P2-7G)	*h+ ade6-M21x leu1-32 ura4-D18 cat1::kanMX6*	NBRP, [2]
YMP1012	*h⁻ leu1-32 ura4-D18 cat1::kanMX6*	This study
YMP1014	*h⁻ leu1-32 ura4-D18 pka1::ura4 cat1::kanMX6*	This study
AKP1	*h⁻ leu1-32 ura4-D18 aat1-GFP(S65T)-FLAG-6His-kanMX6*	This study
AKP2	*h⁻ leu1-32 ura4-D18 cgs1::ura4 aat1-GFP(S65T)-FLAG-6His-kanMX6*	This study
AKP4	*h⁻ leu1-32 ura4-D18 pka1::ura4 aat1-GFP(S65T)-FLAG-6His-kanMX6*	This study
AKP5	*h⁻ leu1-32 ura4-D18 aat1::kanMX6*	This study
AKP6	*h⁻ leu1-32 ura4-D18 pka1::ura4 aat1::kanMX6*	This study
YMP887	*h⁻ leu1-32 ura4-D18 pka1::ura4 pub1::natMX6*	This study
YMP178	*h⁻ leu1-32 ura4-D18 pka1::natMX6*	[3]
YMP860	*h⁻ leu1-32 ura4-D18 any1::hphMX6*	This study

YMP883	*h⁻ leu1-32 ura4-D18 pka1::natMX6 any1::hphMX6*	This study
KNP63	*h⁻ leu1-32 ura4-D18 pub1::natMX6*	[4]
YMP890	*h⁻ leu1-32 ura4-D18 pka1::hphMX6 pub1::natMX6*	This study
YMP878	*h⁻ leu1-32 ura4-D18 any1-GFP(S65T)-hphMX6*	This study
YMP891	*h⁻ leu1-32 ura4-D18 cgs1::natMX6 any1-GFP(S65T)-hphMX6*	This study
YMP884	*h⁻ leu1-32 ura4-D18 pka1::natMX6 any1-GFP(S65T)-hphMX6*	This study
FY14741 (H07/G06)	*h^{90} ade6-216 leu1-32 lys1-131 ura4-D18 pub1-GFP-HA-kanMX6*	NBRP, [5]
YMP908	*h⁻ leu1-32 ura4-D18 pub1-GFP(S65T)-HA-kanMX6*	This study
YMP909	*h⁻ leu1-32 ura4-D18 cgs1::ura4 pub1-GFP(S65T)-HA-kanMX6*	This study
YMP910	*h⁻ leu1-32 ura4-D18 pka1::ura4 pub1-GFP(S65T)-HA-kanMX6*	This study

References

1. Matsuo Y, Kawamukai M. cAMP-dependent protein kinase involves calcium tolerance through the regulation of Prz1 in *Schizosaccharomyces pombe*. Biosci Biotechnol Biochem. 2017;81:231–41. https://doi.org/10.1080/09168451.2016.1246171.

2. Chen JS, Beckley JR, McDonald NA, Ren L, Mangione M, Jang SJ, et al. Identification of new players in cell division, DNA damage response, and morphogenesis through construction of *Schizosaccharomyces pombe* deletion strains. G3 Bethesda. 2014;5:361–70. https://doi.org/10.1534/g3.114.015701.

3. Naozuka G, Kawamukai M, Matsuo Y. Pps1, phosphatidylserine synthase, regulates the salt stress response in *Schizosaccharomyces pombe*. Mol Genet Genomics. 2024;299:43. https://doi.org/10.1007/s00438-024-02135-4.

4. Nishino K, Kushima M, Matsuo Y, Matsuo Y, Kawamukai M. Cell Lysis in *S. pombe ura4* Mutants Is Suppressed by Loss of Functional Pub1, Which Regulates the Uracil

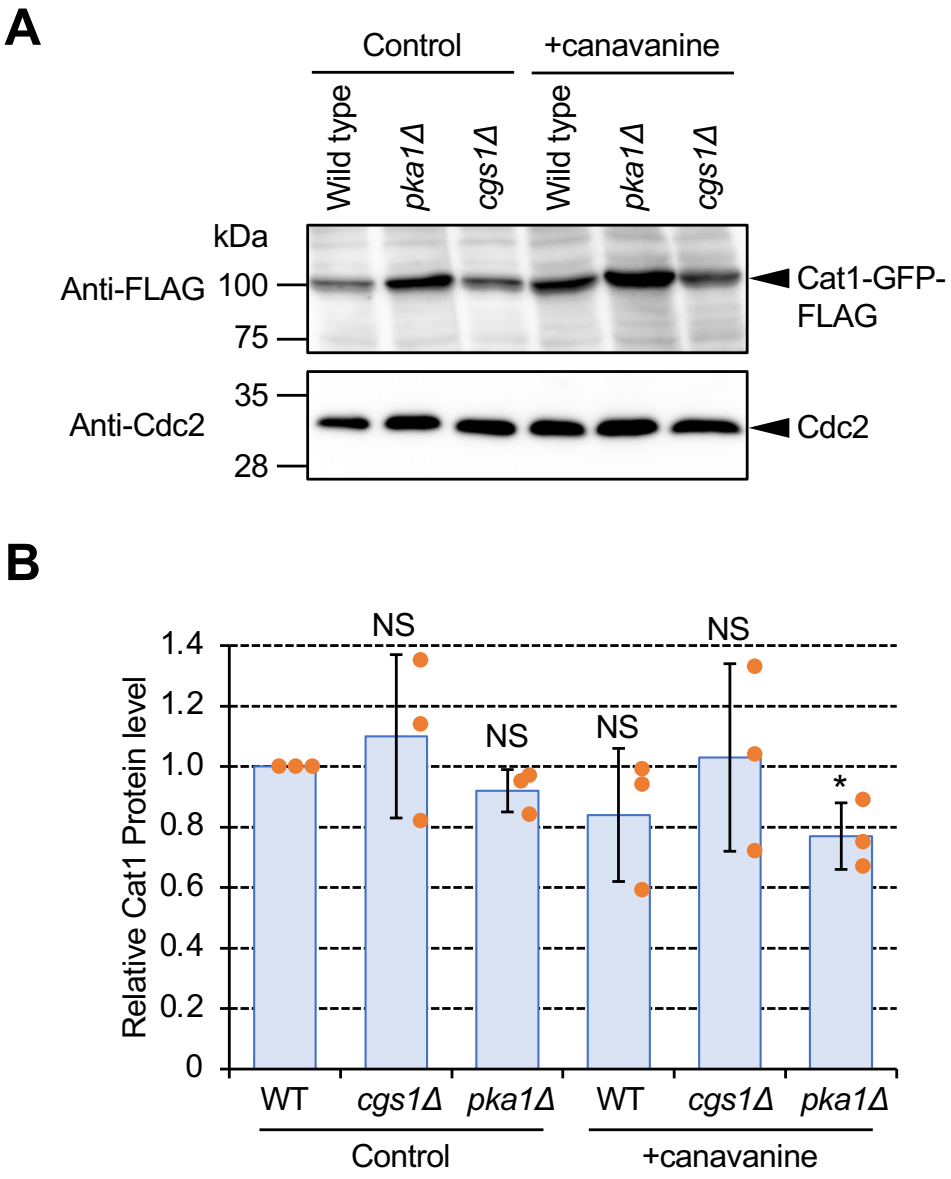
Transporter Fur4. PloS One. 2015;10:e0141796. https://doi.org/10.1371/journal.pone.0141796.

5. Hayashi A, Ding D-Q, Tsutsumi C, Chikashige Y, Masuda H, Haraguchi T, et al. Localization of gene products using a chromosomally tagged GFP-fusion library in the fission yeast *Schizosaccharomyces pombe*. Genes Cells Devoted Mol Cell Mech. 2009;14:217–25. https://doi.org/10.1111/j.1365-2443.2008.01264.x.

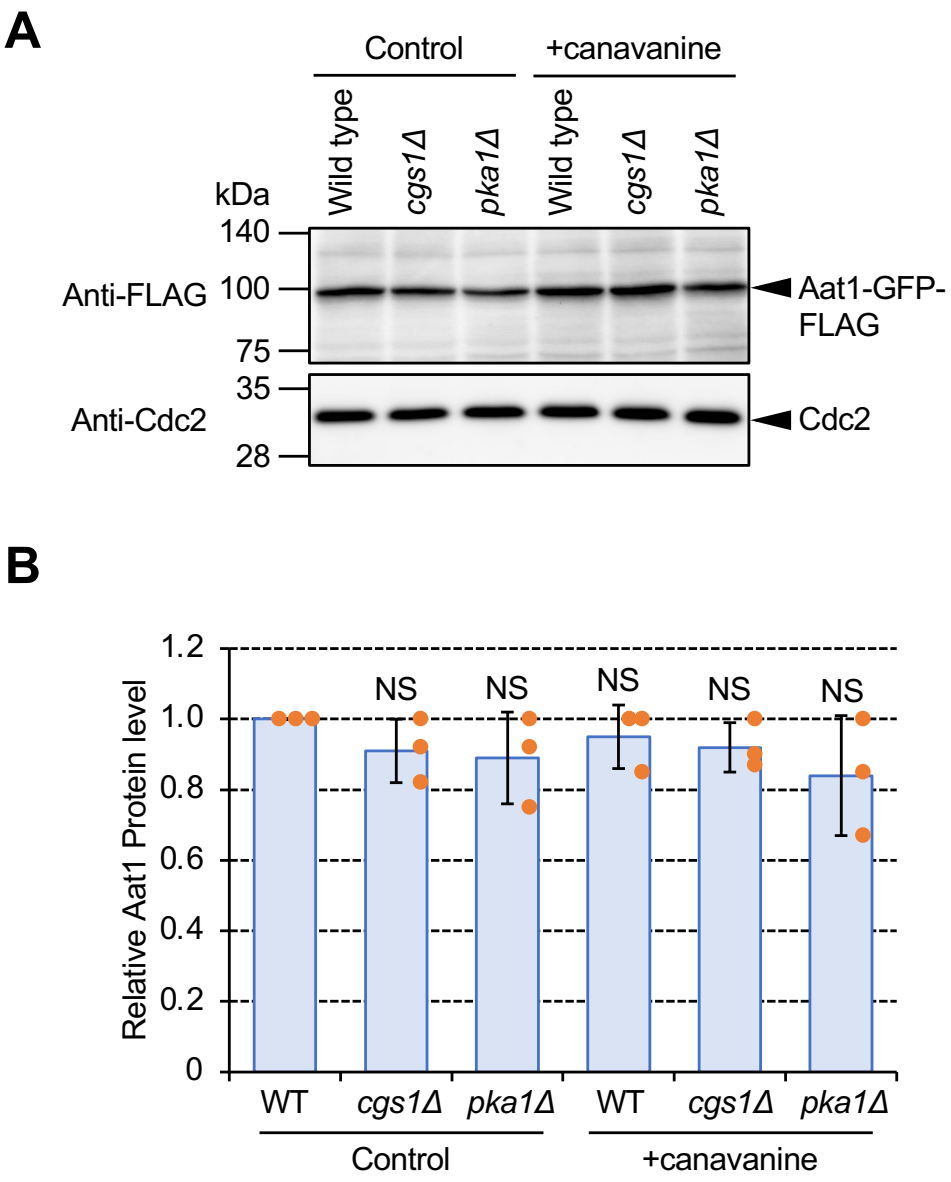
Supplementary Table S2 Oligonucleotide primers used for making strains in this study

Primer name	Sequence
ANY1KOW	5'-CATCAATTGGATTACTATTAC-3'
ANY1KOX	5'-GGGGATCCGTCGACCTGCAGCGTACGAGTAATAAGAAAATCTAAGCGT-3'
ANY1KOY	5'-GTTTAAACGAGCTCGAATTCATCGATGTGGGTCGGATGTAACCGGAAG-3'
ANY1KOZ	5'-CTACGCATAACTTGTCTATCGT-3'
ANY1TAGW	5'-GTAAAACCTCGACCTATTGAAG-3'
ANY1TAGX	5'GGGGATCCGTCGACCTGCAGCGTACGAGGTATAGGCAGGCGGAGCGGG-3'
CAT1TAGW	5'-CTTGGCGTTTAAGAAACAAG-3'
CAT1TAGX	5'-GGGGATCCGTCGACCTGCAGCGTACGAACAGAAAACCGAACTGATTTT-3'
CAT1TAGY	5'-GTTTAAACGAGCTCGAATTCATCGATAATCGATGAAATTTCCTTGAG-3'
CAT1TAGZ	5'-CTTGTGCTTACGATGTTCGCTTC-3'
AAT1TAGW	5'-CAAGGTCATTCCTTAAAGGAG-3'
AAT1TAGX	5'-GGGGATCCGTCGACCTGCAGCGTACGAGCAAACGGTATTAATTATTTT-3'
AAT1TAGY	5'-GTTTAAACGAGCTCGAATTCATCGATTCAGAGTTTGGATCATCTCAG-3'
AAT1TAGZ	5'-ACTAATACTAGATTTACAATG-3'

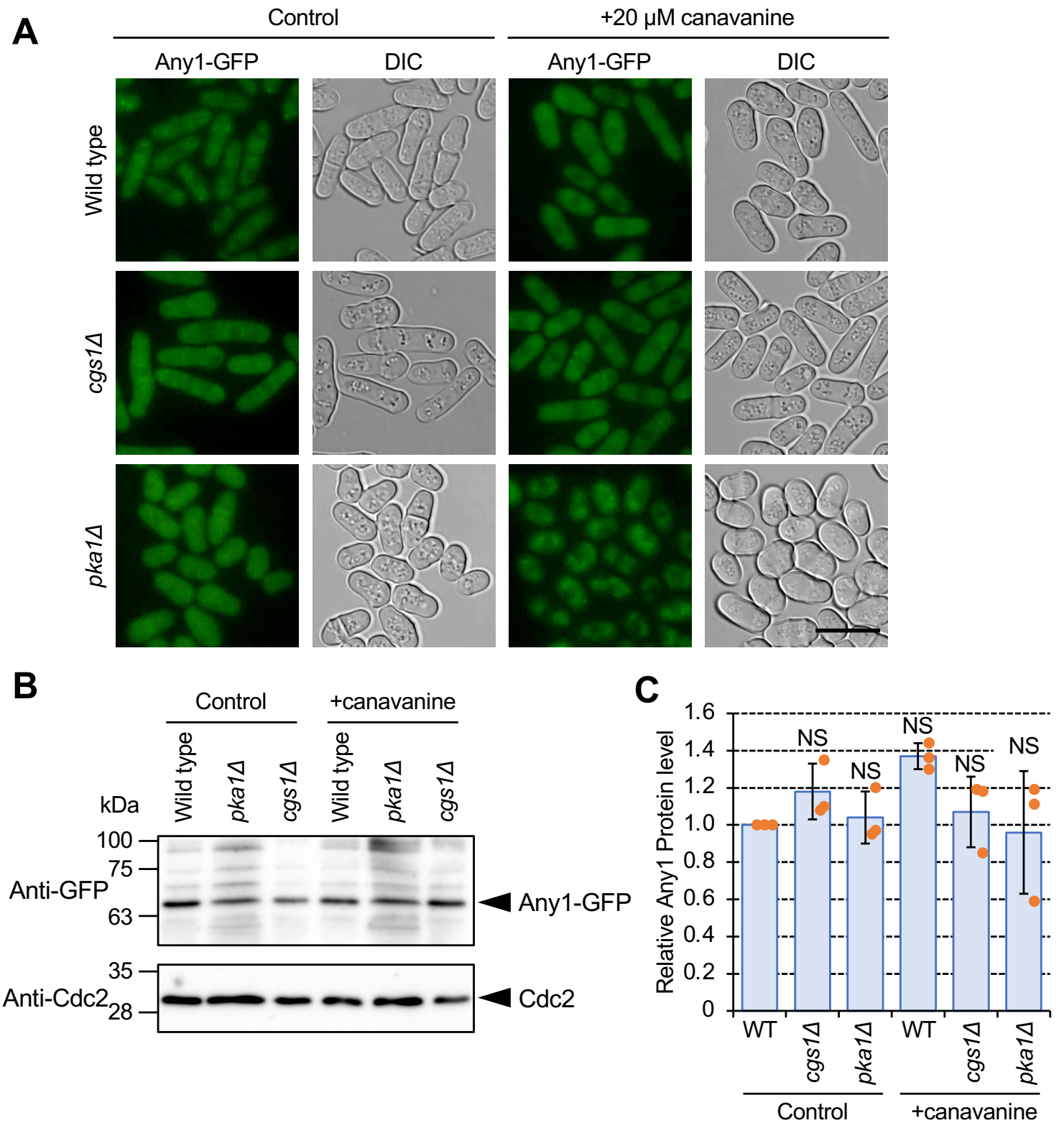
AAT1KOW	5'-CTTGAGTGCAGCTTATATTAG-3'
AAT1KOX	5'-GGGGATCCGTCGACCTGCAGCGTACGATTGAAAATTACAAGTAGTAC-3'
ANY1-SF	5'-TATGTCGACATGCCATTAAAACTTGCACTA-3'
ANY1-BR	5'-ACAGGATCCCTAGGTATAGGCAGGCGGAG-3'
ANY1(P116W G117W)-F	5'-TTGGCTTTTCTGGTGGTCGCTTCCTG-3'
ANY1(P116W G117W)-R	5'-CAGGAAGCGACCACCAGAAAAGCCAA-3'
ANY1(K263R)-F	5'-TCGGGATGGAGGTTCATTGAT-3'
ANY1(K263R)-R	5'-ATCAATGAACCTCCATCCCGA-3'
ANY1(4A)-R1	5'-GAGCGGGTCCTACACTTTCAGCAACTGCCGGGCATTCTTC-3'
ANY1(4A)-R2	5'-ACAGGATCCCTAGGTAGCGGCAGCCGGAGCGGGTCCTAC-3'



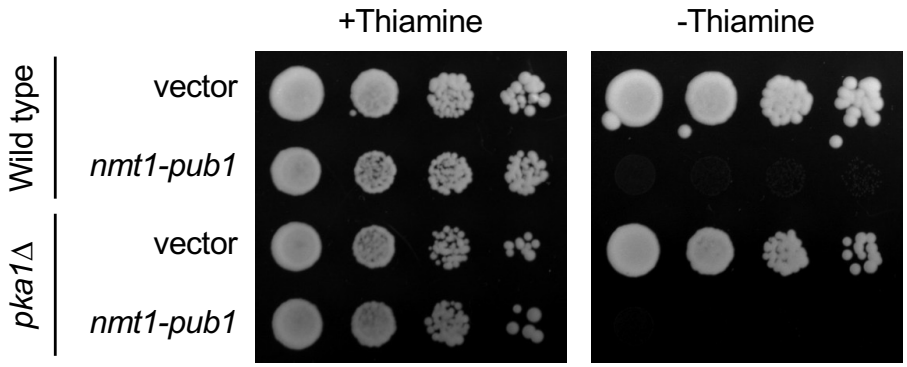
Supplementary Fig. S1 - Cat1 expression is not altered in the *pka1*Δ strain. (A) Cat1-GFP-FLAG (YMP923), *cgs1*Δ Cat1-GFP-FLAG (YMP935), and *pka1*Δ Cat1-GFP-FLAG (YMP938) cells were cultured as described in Fig. 2A. Cat1-GFP-FLAG was detected using an anti-FLAG antibody. An anti-Cdc2 antibody was used as an internal loading control. Full-length blots are presented in Supplementary Fig. S7. (B) Expression levels of Cat1-GFP-FLAG were quantified and normalized to those in the wild-type strain. Data are presented as the mean ± S.D. of three independent experiments (n = 3), with individual data points shown. Statistical significance was assessed by one-way ANOVA followed by Dunnett's multiple-comparisons test. NS and asterisk indicate no significant difference and $P < 0.05$, respectively, compared with the wild-type strain under normal conditions.



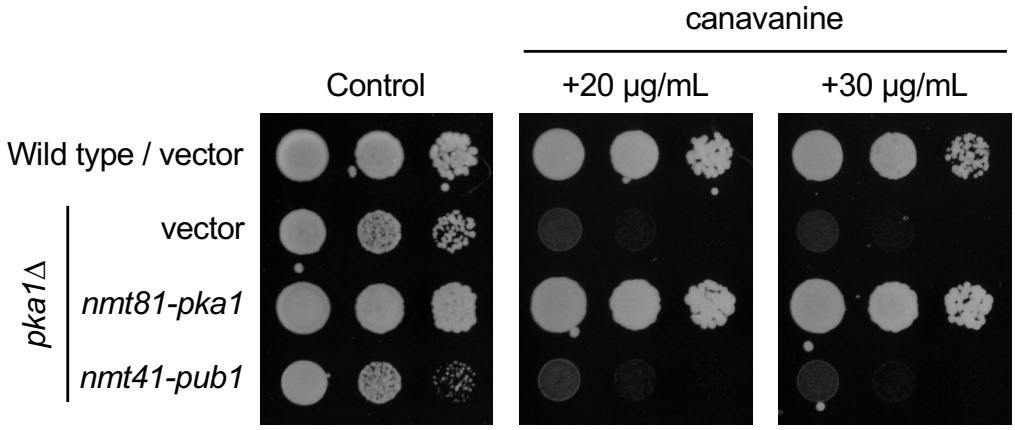
Supplementary Fig. S2 - Aat1 expression is not altered in the *pka1*Δ strain. (A) Aat1-GFP-FLAG (AKP1), *cgs1*Δ Aat1-GFP-FLAG (AKP2), and *pka1*Δ Aat1-GFP-FLAG (AKP4) cells were cultured as described in Fig. 2A. Aat1-GFP-FLAG was detected using an anti-FLAG antibody. An anti-Cdc2 antibody was used as an internal loading control. Full-length blots are presented in Supplementary Fig. S7. (B) Expression levels of Aat1-GFP-FLAG were quantified and normalized to those in the wild-type strain. Data are presented as the mean ± S.D. of three independent experiments (n = 3), with individual data points shown. Statistical significance was assessed by one-way ANOVA followed by Dunnett's multiple-comparisons test. NS indicates no significant compared with the wild-type strain under normal conditions.



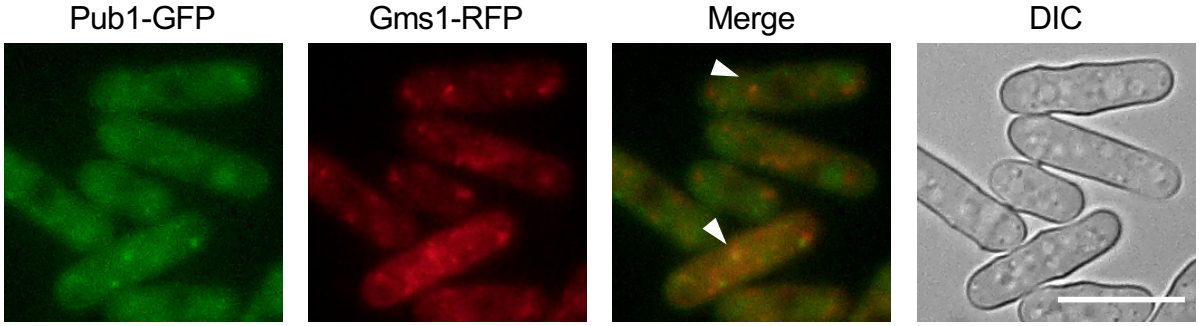
Supplementary Fig. S3 - Any1 localization and expression are not altered in the *pka1*Δ strain. (A) Any1-GFP (YMP878), *cgs1*Δ Any1-GFP (YMP891), and *pka1*Δ Any1-GFP (YMP884) cells were cultured as described in Fig. 2A. Any1-GFP localization was observed by fluorescent microscopy. Scale bar, 10 μm. (B) Any1-GFP (YMP878), *cgs1*Δ Any1-GFP (YMP891), and *pka1*Δ Any1-GFP (YMP884) cells were cultured as described in Fig. 2A. Any1-GFP was detected using an anti-GFP antibody. An anti-Cdc2 antibody was used as an internal loading control. Full-length blots are presented in Supplementary Fig. S7. (C) Expression levels of Any1-GFP were quantified and normalized to those in the wild-type strain. Data are presented as the mean ± S.D. of three independent experiments (n = 3), with individual data points shown. Statistical significance was assessed by one-way ANOVA followed by Dunnett's multiple-comparisons test. NS indicates no significant compared with the wild-type strain under normal conditions.



Supplementary Fig. S4 - Pub1 overexpression causes a growth defect. Wild-type (PR109) and *pka1*Δ (YMP36) cells harboring pREP3X (vector) or pREP3X-pub1 were cultured in EMMU medium for 1 day at 30˚C to induce expression from the *nmt1* promoter. Culture dilutions were prepared as described in Fig. 1A, and each dilution was spotted onto EMMU plates with or without thiamine. All plates were incubated for 6 days at 30˚C.

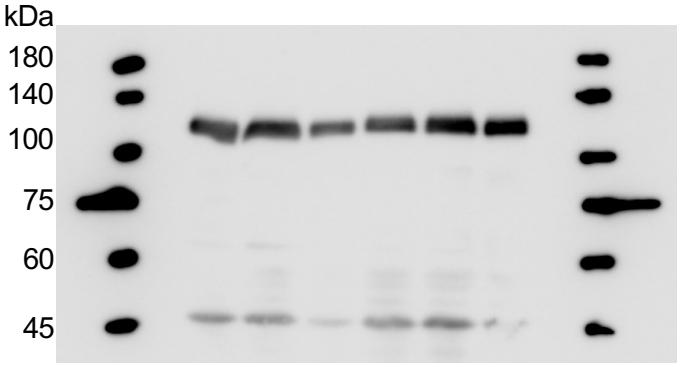


Supplementary Fig. S5 - Pub1 overexpression does not rescue the canavanine-sensitive phenotype of the *pka1*Δ strain. Wild-type (PR109) and *pka1*Δ (YMP36) cells harboring pREP41X (vector), pREP81-pka1, or pREP41X-pub1 were cultured in EMMU medium for 1 day at 30˚C to induce expression from the *nmt* promoter. Culture dilutions were prepared as described in Fig. 1A, and each dilution was spotted onto EMMU plates with or without canavanine. All plates were incubated for 6 days at 30˚C.

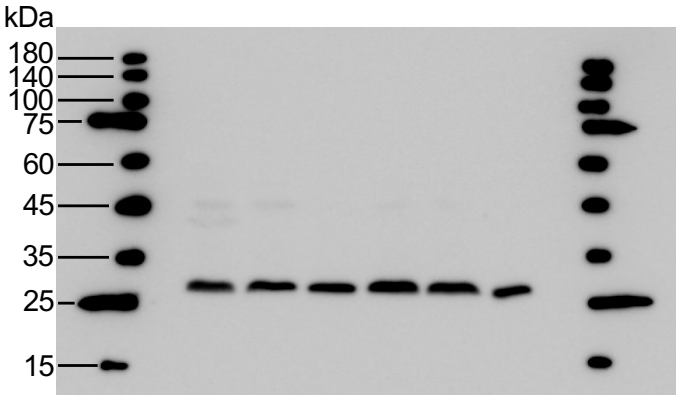


Supplementary Fig. S6 - Pub1-GFP partially colocalizes with the Golgi marker Gms1-RFP. Pub1-GFP (YMP908) cells expressing Gms1-RFP were cultured as described in Fig. 2A. Pub1-GFP and Gms1-RFP were visualized by fluorescent microscopy. Scale bar, 10 µm.

Fig. 7C

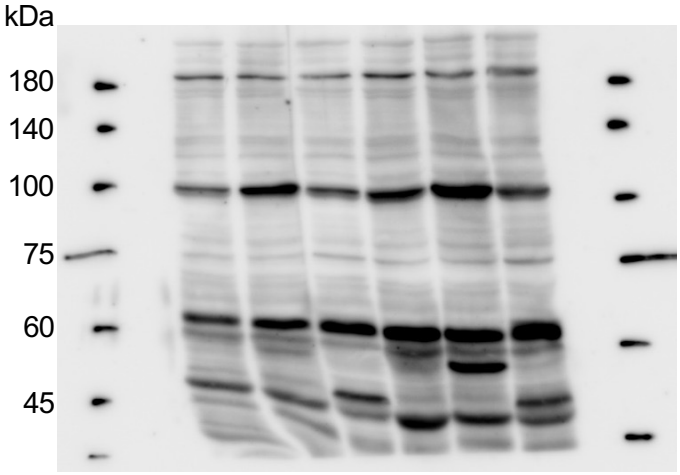


Anti-HA

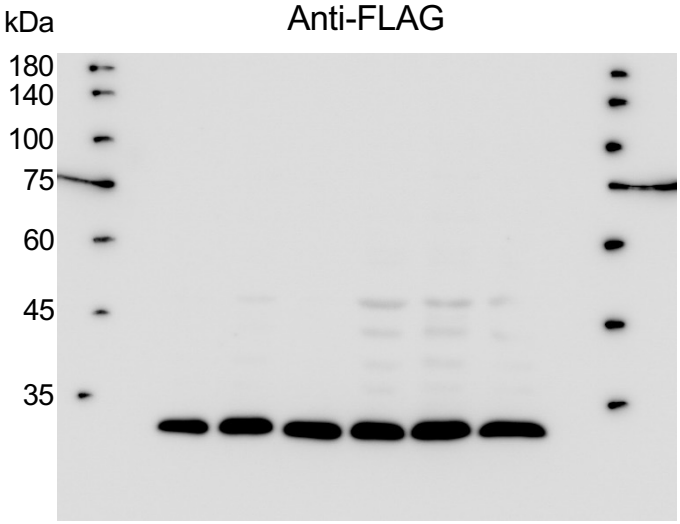


Anti-Cdc2

Supplementary Fig. S1A

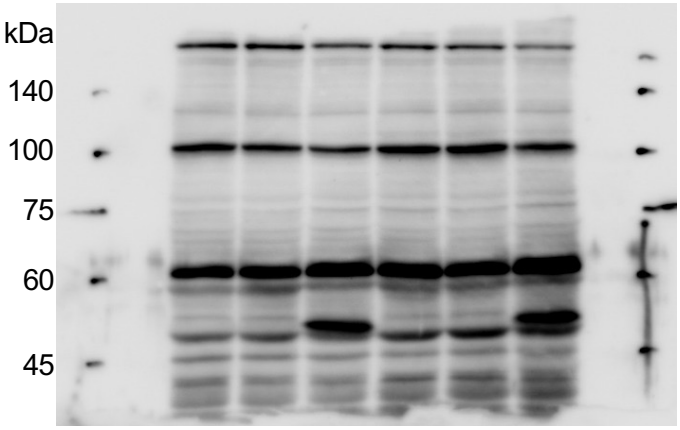


Anti-FLAG

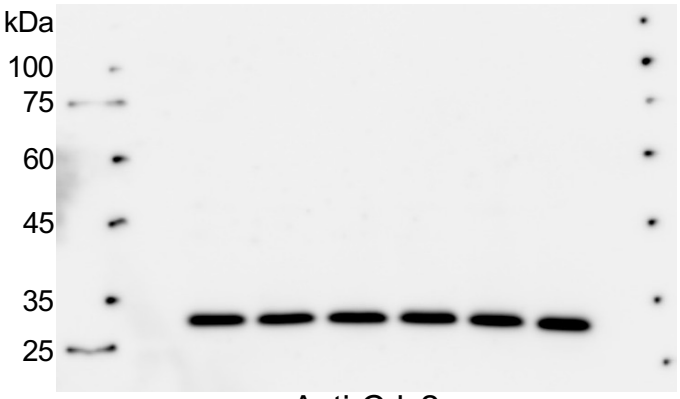


Anti-Cdc2

Supplementary Fig. S2A

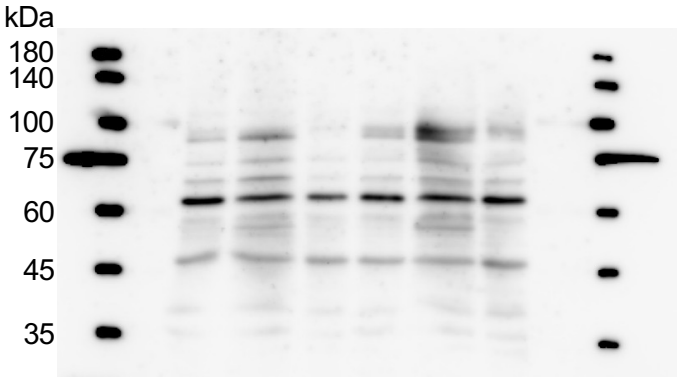


Anti-FLAG

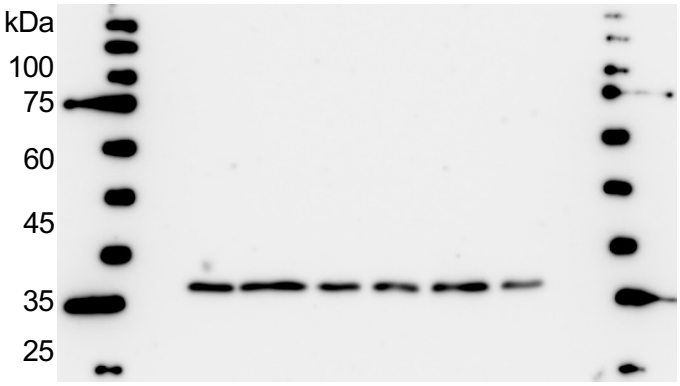


Anti-Cdc2

Supplementary Fig. S3B



Anti-GFP



Anti-Cdc2

Supplementary Fig. S7 Full scans of uncropped immunoblots. Full scans of the immunoblots shown in Fig. 7C and Supplementary Figs. S1A, S2A, and S3B are presented.