JCB

Supplemental material

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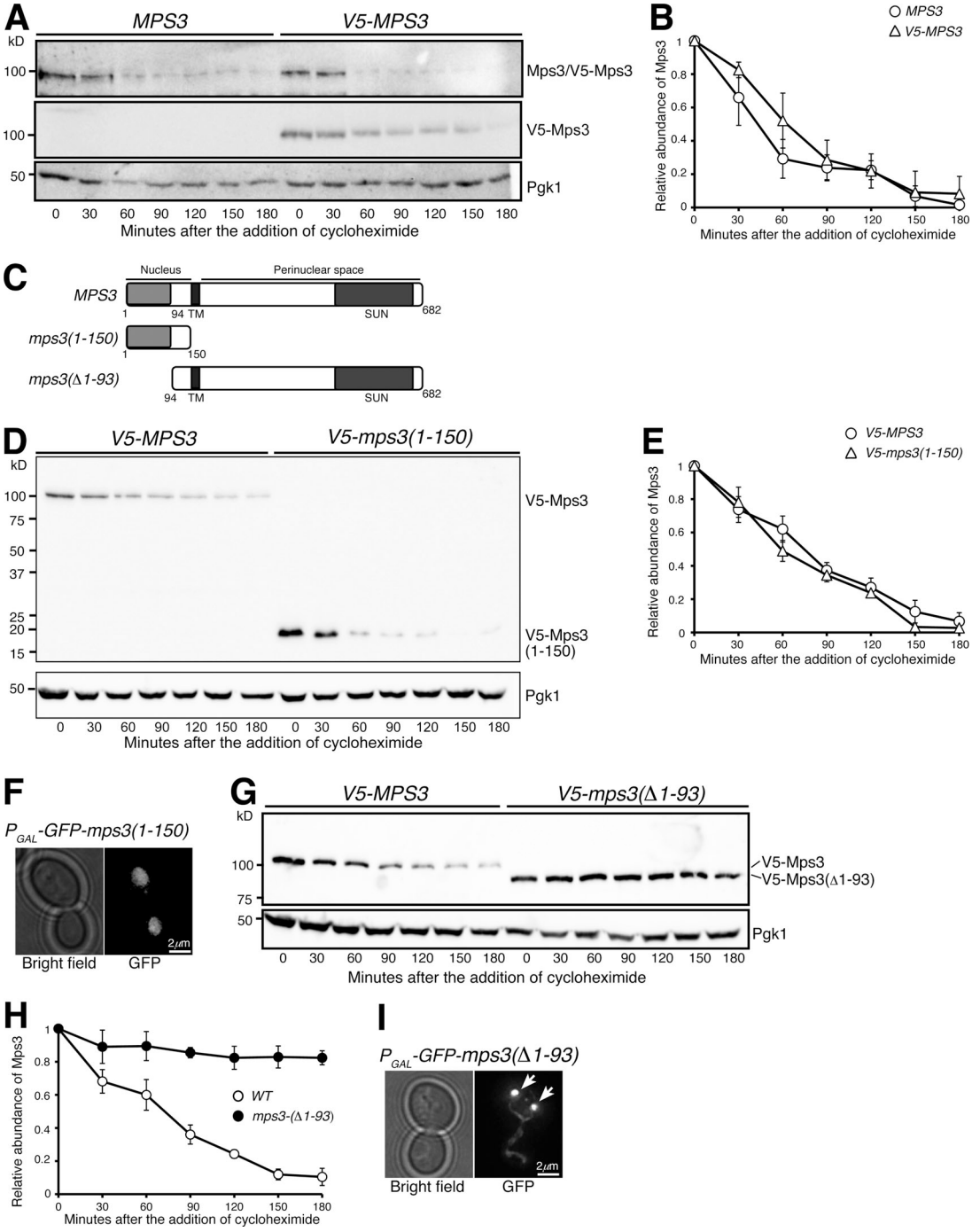

Figure S1. **Mps3 localization and its degradation. (A and B)** Protein half-life of Mps3. Yeast cells were grown to the exponential phase, then CHX was added to the culture medium. Cell aliquots were withdrawn at indicated times, and protein extracts were prepared for Western blotting. A polyclonal antibody against Mps3 and a V5 antibody were used to probe Mps3 and V5-Mps3. The V5 antibody only recognizes V5-Mps3. The level of Pgk1 serves as a loading control. Time zero is the point of CHX addition. The expression of *V5-MPS3* was under the control of the endogenous MPS3 promoter. Quantification of Mps3 protein abundance is shown in B (n = 5), with error bars representing the standard deviation. **(C–F)** Localization and stability of the N terminus of Mps3. A schematic representation of Mps3 protein domains and mutants is shown in C. TM, transmembrane domain. CHX experiment was performed as in A to determine protein stability (D and E). Quantification of protein abundance is shown in E (n = 3), with error bars representing the standard deviation. The expression of both *V5-MPS3* and *V5-MPS3(1–150)* were under the control of the endogenous MPS3 promoter. The expression of *GFP-MPS3(1–150)* was under the control of the galactose promoter (F). Note that GFP-Mps3(1–150) accumulates inside the yeast nucleus upon galactose induction (t = 180 min). **(G–I)** The N terminus regulates Mps3 stability. CHX experiment was performed as in A to determine protein stability (G and H). Quantification of protein abundance is shown in H (n = 4), with error bars representing the standard deviation. The galactose promoter was used to express *GFP-MPS3(Δ1-93)* as shown in I. Note that Mps3(Δ1–93) remains at the SPB (arrows) and the nuclear periphery, but is highly stable.

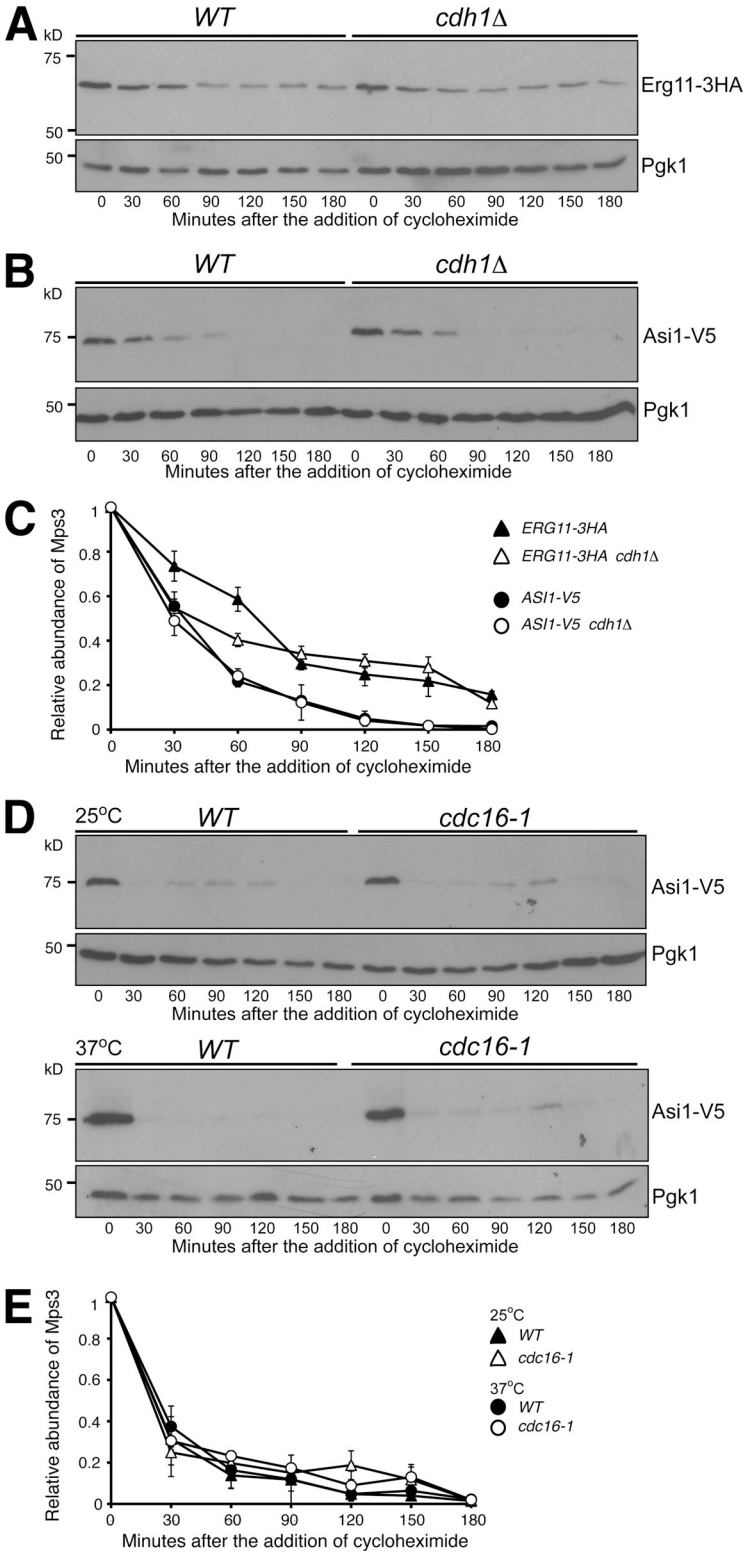

Figure S2. **Degradation of Erg11 and Asi1 is independent of Cdh1 and APC/C. (A–C)** Degradation of Erg11 and Asi1 is independent of Cdh1. The relative protein abundance of Erg11 (A) and Asi1 (B) in WT and *cdh1Δ* cells was analyzed after arresting cells in G1 using α factor (10 µg/ml) for 2 h and then performing CHX chase as described in Fig. 5 B. Quantification of the relative protein abundance is shown in panel C (n = 3), with error bars representing the standard deviation. Note that the Pgk1 loading control in A is the same as that in Fig. 5 B. **(D and E)** Degradation of Asi1 is independent on Cdc16. Cells were grown at 25°C to the exponential phase, and CHX was then added as shown in Fig. 5 E. To inactivate *cdc16-1*, cells were shifted to 37°C for 1 h before the addition of CHX. Quantification of Asi1-V5 protein abundance is shown in E (n = 3), with error bars representing the standard deviation. Note there is no change in stability between the permissive and nonpermissive temperatures.

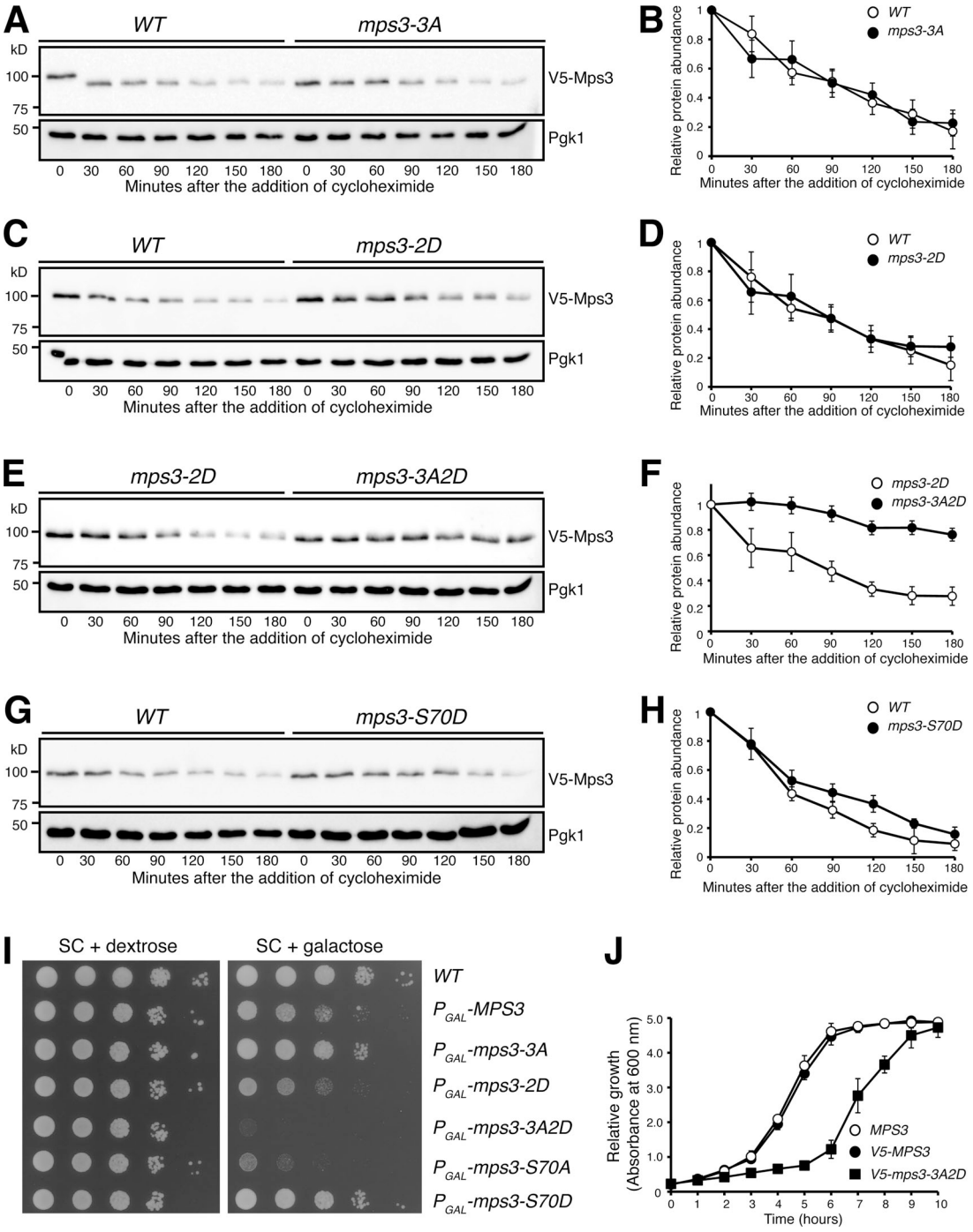

Figure S3. **Redundant destruction motifs located at the N terminus of Mps3. (A and B)** The effect of the KEN box on Mps3 protein stability. Yeast cells were prepared for CHX chase and analyzed as described in Fig. 1 A. Quantification of Mps3 protein abundance is shown in B (n = 3), with error bars representing the standard deviation. **(C and D)** The effect of the putative D box on Mps3 protein stability. Quantification of Mps3 protein abundance is shown in D (n = 3), with error bars representing the standard deviation. **(E and F)** The KEN and D boxes are redundant in regulating Mps3 degradation. Quantification of Mps3 protein abundance is shown in F (n = 3), with error bars representing the standard deviation. **(G and H)** Phosphorylation at S70 plays a role in Mps3 stability. Quantification of Mps3 protein abundance is shown in H (n = 3), with error bars representing the standard deviation. **(I)** Overproduction of stabilized Mps3 causes cell lethality. Yeast cells were grown overnight in YPD liquid medium to reach saturation, 10-fold diluted, spotted onto SC plates with either 2% glucose or 2% galactose, and then incubated at 30°C for ∼2 d. **(J)** Disruption of the KEN and D boxes within Mps3's N terminus results in a cell proliferation defect. Yeast cells were grown overnight in YPD liquid medium to reach saturation, diluted to OD (optical density, λ = 600 nm) of 0.2, and continued to grow with vigorous shaking at 30°C. Cell proliferation was determined by optical density every hour for 10 h. Error bars represent the standard deviation from three experimental trials. The expression of *V5-MPS3* and *mps3-3A2D* both were under the control of the endogenous *MPS3* promoter. Note that the proliferation of *mps3-3A2D* cells lags behind the wild-type and *V5-MPS3* cells.

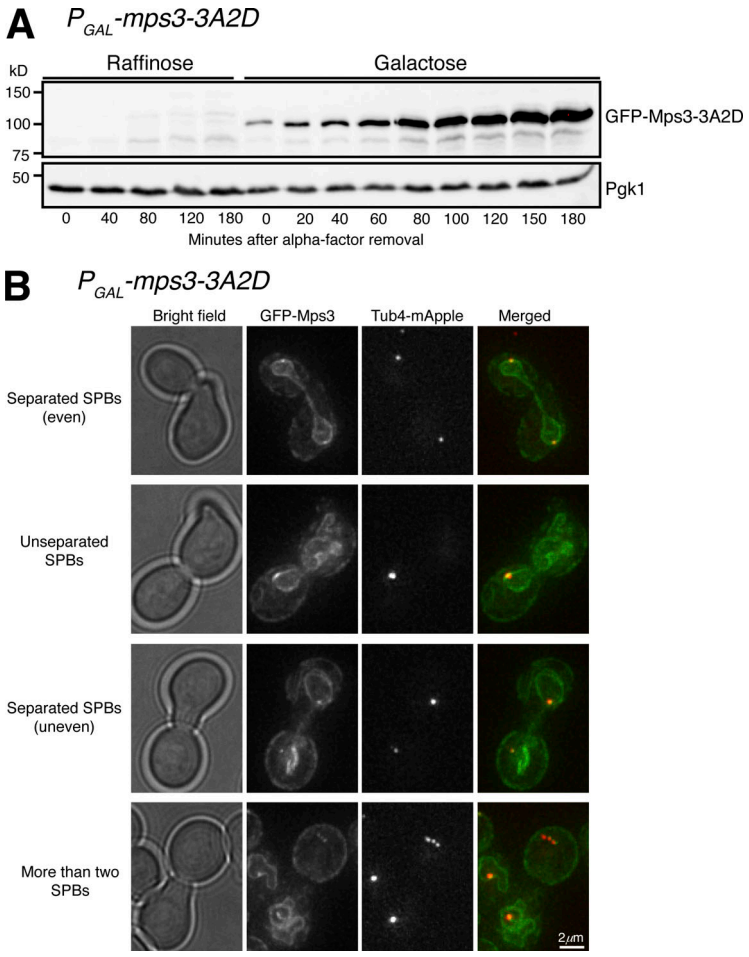

Figure S4. **Overexpression of *mps3-3A2D* leads to defective SPB separation. (A)** Protein level of Mps3-3A2D upon galactose induction of P_{GAL1}-*mps3-3A2D*. Yeast cells were grown in raffinose and arrested at G1 with α-factor as shown in Fig. 6 I. To induce the *GAL1-10* promoter, galactose was added to the culture medium 30 min before the removal of α-factor. Cell aliquots were withdrawn at the indicated times and prepared for Western blot. Time zero refers to the point of α-factor removal. The level of Pgk1 serves as a loading control. **(B)** Representative images showing the localization of GFP-Mps3 and Tub4-mApple 180 min after galactose induction. Aliquots were withdrawn and prepared for live-cell fluorescence microscopy. Tub4-mApple marks the SPB. Four categories of SPB separation in large budded cells were classified, the first being normal SPB separation in which SPBs separated evenly, and the remaining three being types of defective SPB separation: unseparated SPBs (one Tub4-mApple spot), uneven separation of SPBs (two unequal Tub4-mApple spots), and more than two SPBs (more than 2 Tub4-mApple spots in a single cell).

Table S1. **Genetic screen with targeted gene deletions of the ubiquitin system**

Systematic name	Standard name
YAL002W	VPS8
YBL058W	SHP1
YBL104C	SEA4
YBR062C	n/a
YBR082C	UBC4
YBR114W	RAD16
YBR158W	AMN1
YBR203W	COS111
YBR273C	UBX7
YBR280C	SAF1
YCR059C	YIH1
YCR066W	RAD18
YDL013W	SLX5
YDL074C	BRE1
YDL091C	UBX3
YDL190C	UFD2
YDR049W	VMS1
YDR059C	UBC5
YDR069C	DOA4
YDR092W	UBC13
YDR103W	STE5
YDR128W	MTC4
YDR131C	n/a
YDR143C	SAN1
YDR152W	GIR2
YDR219C	MFB1
YDR255C	RMD5
YDR265W	PEX10
YDR266C	HEL2
YDR283C	GCN2
YDR306C	n/a
YDR313C	PIB1
YDR330W	UBX5
YDR457W	TOM1
YEL012W	UBC8
YER068W	MOT2
YER100W	UBC6
YER116C	SLX8
YGL003C	CDH1
YGL058W	UBC2
YGL131C	SNT2
YGL141W	HUL5
YGR003W	CUL3

Table S1. **Genetic screen with targeted gene deletions of the ubiquitin system (*Continued*)**

Systematic name	Standard name
YGR133W	UBC10
YGR184C	UBR1
YHL010C	ETP1
YHR115C	DMA1
YIL030C	SSM4/DOA10
YIL097W	FYV10
YJL047C	RTT101
YJL048C	UBX6
YJL149W	DAS1
YJL157C	FAR1
YJL204C	RCY1
YJL210W	PEX2
YJR036C	HUL4
YJR052W	RAD7
YJR090C	GRR1
YKL010C	UFD4
YKL034W	TUL1
YKL213C	DOA1
YKR017C	HEL1
YLR032W	RAD5
YLR097C	HRT3
YLR148W	PEP3
YLR224W	UCC1
YLR247C	IRC20
YLR306W	UBC12
YLR352W	LUG1
YLR368W	MDM30
YLR427W	MAG2
YML013W	UBX2
YML068W	ITT1
YML088W	UFO1
YMR022W	UBC7
YMR026C	PEX12
YMR067C	UBX4
YMR119W	ASI1
YMR231W	PEP5
YMR247C	RKR1
YMR258C	ROY1
YNL008C	ASI3
YNL023C	FAP1
YNL116W	DMA2
YNL230C	ELA1
YNL311C	SKP2

Table S1. **Genetic screen with targeted gene deletions of the ubiquitin system (*Continued*)**

Systematic name	Standard name
YOL013C	HRD1
YOL054W	PSH1
YOL138C	RTC1
YOR080W	DIA2
YOR191W	ULS1
YOR339C	UBC11
YPR093C	ASR1

n/a, not available.

Table S2. **Yeast strains used in this study**

Strain	Background	Genotype	Experiment
BY4741	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0*	Parental strain
HY5850	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, V5-MPS3*	Figs. 1 A; 3, C–E and H; 4, and E; 5 B; 6, C, E, and G; S1, A, C, and F; and S3, A, C, G, and J
HY6017	S288C	*MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, V5-MPS3, cdc48Δ::KANMX6, pRS316-cdc48-6::HIS3*	Fig. 1 C
HY6102	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, V5-MPS3, pdr5Δ::NATMX6*	Fig. 1 E
HY5854	W303	*MATa, ura3-1, his3-11,15, leu2-3,112, trp1-1, ade2-1, can1-100, psi+, TOR1-1, fpr1::NATMX6, RPN11-FRB-GFP::KANMX6, V5-MPS3*	Fig. 2, B and E
HY5856	W303	*MATa, ura3-1, his3-11,15, leu2-3,112, trp1-1, ade2-1, can1-100, psi+, TOR1-1, fpr1::NATMX6, RPN11-FRB-GFP::KANMX6, RPL13A-2xFKBP12::TRP1, V5-MPS3*	Fig. 2, B and D
HY5855	W303	*MATa, ura3-1, his3-11,15, leu2-3,112, trp1-1, ade2-1, can1-100, psi+, TOR1-1, fpr1::NATMX6, RPN11-FRB-GFP::KANMX6, PMA1-2xFKBP12::TRP1, V5-MPS3*	Fig. 2, E and G
HY4671	S288C	*MATα, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, ade2::P_{MFA1}-HIS3, MPS3::P_{GAL1}-GFP-MPS3::LEU2*	Fig. 3 A
HY4238	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, MPS3::P_{GAL1}-GFP-MPS3::LEU2*	Figs. 3 B; 5, A and G; 6 B; 7, F and J; and S3 I
HY5788	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, ubc6Δ::NATMX6*	Fig. 3 B
HY6124	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, ubc6Δ::NATMX6, MPS3::P_{GAL1}-GFP-MPS3::LEU2*	Fig. 3 B
HY5064	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, ubc7Δ::KANMX6*	Fig. 3 B
HY5122	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, ubc7Δ::KANMX6, MPS3::P_{GAL1}-GFP-MPS3::LEU2*	Fig. 3 B
HY5793	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, ubc6Δ::NATMX6, ubc7Δ::KANMX6*	Fig. 3 B
HY6013	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, ubc6Δ::NATMX6, ubc7Δ::KANMX6, MPS3::P_{GAL1}-GFP-MPS3::LEU2*	Fig. 3 B
HY5662	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, doa4Δ::KANMX6, pRS202*	Fig. 3 B
HY6239	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, doa4Δ::KANMX6, P_{CUP1}-UBI4*	Fig. 3 B
HY5496	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, doa4Δ::KANMX6, MPS3::P_{GAL1}-GFP-MPS3::LEU2, pRS202*	Fig. 3 B
HY6237	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, doa4Δ::KANMX6, MPS3::P_{GAL1}-GFP-MPS3::LEU2, P_{CUP1}-UBI4*	Fig. 3 B
HY6022	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, ubc6Δ::NATMX6, V5-MPS3*	Fig. 3 D
HY5963-2C	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, ubc7Δ::KANMX6, V5-MPS3*	Fig. 3 C
HY6016	S288C	*MATa, his3Δ200, leu2-3,112, trp1-1, lys2-801, ura3-52, ubc6Δ::NATMX6, ubc7Δ::KANMX6, V5-MPS3*	Fig. 3 E
HY6350	W303	*MATa, his3Δ200, leu2-3,112, met15Δ0, lys2-801, trp1-1 ura3-52, gal2, ubc1Δ::HIS3, V5-MPS3*	Fig. 3 G
HY6056	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, ubc4Δ::KANMX6, V5-MPS3*	Fig. 3 H
HY6057	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, hrdΔ1::KAN, V5-MPS3*	Fig. 4 A
HY5907	S288C	*MATα, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, doa10Δ::KANMX6, V5-MPS3*	Fig. 4 C
HY6015	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, asi3Δ::KANMX6, ERG11-3HA::HIS5, V5-MPS3*	Fig. 4 C
HY6082-8A	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, asi3Δ::KANMX6, doa10Δ::NATMX6, hrd1Δ::KANMX6, ERG11-3HA::HIS5, V5-MPS3*	Fig. 4 E
HY5047	S288C	*MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, cdh1Δ::KANMX6*	Figs. 5 A and 7 J
HY5068	S288C	*MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, cdh1Δ::KANMX6, MPS3::P_{GAL1}-GFP-MPS3::LEU2*	Figs. 5 A and 7, F and J
HY5901	S288C	*MATa, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, cdh1Δ::KAN, V5-MPS3*	Fig. 5 B
HY5905-3A	W303	*MATa, ura3-1, his3-11,15, leu2-3,112, trp1-1, ade2-1, can1-100, V5-MPS3*	Figs. 3 G and 5 E
HY5962-4B	W303	*MATa, ura3-1, his3-11,15, leu2-3,112, trp1-1, ade2-1, can1-100, V5-MPS3, cdc20-1*	Fig. 5 D
HY5905-2A	W303	*MATa, ura3-1, his3-11,15, leu2-3,112, trp1-1, ade2-1, can1-100, V5-MPS3, cdc16-1*	Fig. 5 E

Table S2. **Yeast strains used in this study (*Continued*)**

Strain	Background	Genotype	Experiment
JBY332	W303	*MATa, ura3-1, his3-11,15, leu2-3,112, trp1-1, ade2-1, can1-100, cdc16-1*	Figs. 5 G and 7 K
HY6075	W303	*MATa, ura3-1, his3-11,15, leu2-3,112, trp1-1, ade2-1, can1-100, MPS3::P_{GAL1}-GFP-MPS3::LEU2*	Fig. 5 G
HY6076	W303	*MATa, ura3-1, his3-11,15, leu2-3,112, trp1-1, ade2-1, can1-100, MPS3::P_{GAL1}-GFP-MPS3::LEU2, cdc16-1*	Fig. 5 G
HY6309	W303	*MATa, ura3-1, his3-11,15, leu2-3,112, trp1-1, ade2-1, can1-100, V5-MPS3, cdc14-1*	Fig. 5 H
HY6135-1A	W303	*MATa, ura3-1, his3-11,15, leu2-3,112, trp1-1, ade2-1, can1-100, V5-MPS3, cdc15-2*	Fig. 5 H
HY4358	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, MPS3::P_{GAL1}-GFP-mps3-S70A::LEU2*	Figs. 6 C and S3 I
HY5349	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, MPS3::P_{GAL1}-GFP-mps3-S70D::LEU2*	Figs. 6 C and S3 I
HY6095	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, MPS3::P_{GAL1}-GFP-mps3-3A2D::LEU2*	Figs. 6 C and S3 I
HY4456	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, MPS3::P_{GAL1}-GFP-mps3-NC::LEU2*	Figs. 6 C and S3 I
HY6060	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, V5-mps3-3A2D*	Figs. 6 C and S3, E and J
HY5851	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, V5-mps3-NC*	Fig. 6 E
HY6059	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, V5-mps3-S70A*	Fig. 6 G
HY6197	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, Tub4-mApple::HIS5, MPS3::P_{GAL1}-GFP-mps3-3A2D::LEU2*	Figs. 6, I–K; and S4, A and B
HY5310	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, HEH2::P_{GAL1}-GFP-HEH2::LEU2*	Fig. 7, B and J
HY5513	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, cdh1Δ::KANMX6, HEH2::P_{GAL1}-GFP-HEH2::LEU2*	Fig. 7 J
HY6116	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, HEH2::P_{GAL1}-GFP-mps3(1-94)-HEH2::LEU2*	Fig. 7, B, D, and J
HY6155-1A	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, cdh1Δ::KANMX6, HEH2::P_{GAL1}-GFP-mps3(1-94)-HEH2::LEU2*	Fig. 7, D and J
HY6221	W303	*MATa, ura3-1, his3-11,15, leu2-3,112, trp1-1, ade2-1, can1-100, HEH2::P_{GAL1}- GFP-mps3(1-94)-HEH2::LEU2, cdc16-1*	Fig. 7, H and K
HY6220	W303	*MATa, ura3-1, his3-11,15, leu2-3,112, trp1-1, ade2-1, can1-100, HEH2::P_{GAL1}- GFP-mps3(1-94)-HEH2::LEU2*	Fig. 7 K
HY6238	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, V5-mps3-150::URA3*	Fig. S1 C
HY6336	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, P_{GAL1}-GFP-mps3-150::URA3*	Fig. S1 E
HY5814	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, V5-mps3(Δ1-93)*	Fig. S1 F
HY5614-A	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, MPS3::P_{GAL1}-GFP-mps3(Δ1-93)::LEU2*	Fig. S1 H
HY5936	W303	*MATa, ura3-1, his3-11,15, leu2-3,112, trp1-1, ade2-1, can1-100, ASI1-V5::HIS5*	Fig. S2 D
HY6047-1D	W303	*MATa, ura3-1, his3-11,15, leu2-3,112, trp1-1, ade2-1, can1-100, ASI1-V5::HIS5, cdc16-1*	Fig. S2 D
HY5932	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, ASI1-V5::HIS5*	Fig. S2 B
HY5961-5D	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, ASI1-V5::HIS5, cdh1Δ::KAN*	Fig. S2 B
HY5678	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, ERG11-3HA::HIS5*	Figs. 4 E and S2 A
HY6153-2D	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, ERG11-3HA::HIS5, cdh1Δ::KAN*	Fig. S2 A
HY6179	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, V5-mps3-3A*	Fig. S3 A
HY6178	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, V5-mps3-2D*	Fig. S3, C and E
HY6211	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, V5-mps3-S70D*	Fig. S2 G
HY6177	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, MPS3::P_{GAL1}-GFP-mps3-2D::LEU2*	Fig. S2 I
HY6138	S288C	*MATa, his3Δ1, leu2Δ0, met15Δ0, lys2Δ0, ura3Δ0, MPS3::P_{GAL1}-GFP-mps3-3A::LEU2*	Fig. S2 I

Table S3. **Plasmids used in this study**

Plasmid name	Construct feature
pHG553	P_{MPS3}-V5-MPS3, URA3
pHG323	P_{GAL1}-GFP-MPS3, LEU2
pRS202	2μ, URA3
pHG638	P_{CUP1}-6xHIS-UBI4, URA3
pHG607	P_{MPS3}-V5-mps3-S70A, URA3
pHG358	P_{GAL1}-GFP-mps3-S70A, LEU2
pHG608	P_{MPS3}-V5-mps3-S70D, URA3
pHG376	P_{GAL1}-GFP-mps3-S70D, LEU2
pHG609	P_{MPS3}-V5-mps3-3A2D, URA3
pHG616	P_{GAL1}-GFP-mps3-3A2D, LEU2
pHG557	P_{MPS3}-V5-mps3-nc, URA3
pHG348	P_{GAL1}-GFP-mps3-nc, LEU2
pHG623	P_{MPS3}-V5-mps3-3A, URA3
pHG628	P_{GAL1}-GFP-mps3-3A, LEU2
pHG655	P_{MPS3}-V5-mps3-2D, URA3
pHG644	P_{GAL1}-GFP-mps3-2D, LEU2
pHG558	P_{MPS3}-V5-mps3(Δ1-93), URA3
pHG446	P_{GAL1}-GFP- mps3(Δ1-93), LEU2
pRT1166	cdc48-6, HIS3
pHG572	P_{GAL1}-GFP-MPS3(1-94)-HEH2, LEU2
pHG479	P_{GAL1}-GFP-HEH2, LEU2
pHG634	P_{MPS3}-V5-mps3-150, URA3
pHG687	P_{GAL1}-GFP-mps3-150, URA3

Table S4. **Primers used in this study**

Primer name	Sequence information (5′ to 3′)
CDC48-colonyF	TGGGTGTGTTTGCTTCCATT
CDC48-colonyR	ATGTCGAAATTATGCCTGGC
PDR5-deletionF	CCGAGGCCAAGCTTAACGATAACGTCAACGACGTTACTAGCTCAGGGGCATGATGTGACT
PDR5-deletionR	CACTACGTTCCCCAACGTTTTCGGGGTCATTTGCATTCTTTTCAGTTAGCTCGTTTTCGACACTGGAT
UBC6-deletionF	TAGTAATGGCTACAAAGCAGGCTCACAAGAGATTGACGAAAGAGTCAGGGGCATGATGTGACT
UBC6-deletionR	CATTTCATAAAAAGGCCAACCAAAAACAAAAAAATAGCGATAGCTCGTTTTCGACACTGGAT
UBC7-colonyF	CGATGCACACGCATATTTGTT
UBC7-colonyR	TTGGCCACAGAAAATTTGAGG
DOA10-deletionF	TGCATAAGGTGGCAAACGAGGAAACAGATACCGCCACTTTCAGGGGCATGATGTGACT
DOA10-deletionR	GAACCATTGAATGAACAGCACAGTTGCTTGGAAAAAGAACGTGCTCGTTTTCGACACTGGAT
ERG11-tagF	CAAGATCATCTGGGAAAAGAGAAATCCAGAACAAAAGATCGCGGCCGCTCTAGAACTAGT
ERG11-tagR	GTACAACTTCTCTCTTTTTCTGTTTTTTTTTTTTTTCTCAGTTACAAACCCCCTCGAGGTCGACGGTA
CDH1-deletionF	TCCACAAACCTGAACCCATTCATGAATAATACGCCTTCCTCCTCCCCACTCAGGGGCATGATGTGACT
CDH1-deletionR	CCAATATCGCAATGTTTCATCTCCAGCCCCAGAAACCACCGTGGTTCCAGCTCGTTTTCGACACTGGAT
ASI1-tagF	AGTAAAGTTCATGGGTACTGTAAGGTTCATCCTGTTTCAGATAGTAAAGCGGCCGCTCTAGAACTAGT
ASI1-tagR	CTCCCAAACGAAAAACCTCTTTTAGATACCATGCAAAAGTTCTTAAACCCCCTCGAGGTCGACGGTA