Li et al., http://www.jcb.org/cgi/content/full/jcb.201408118/DC1

Spc97-TAP purification

	Protein ID	Coverage (%)		Animal homologs
		Meiosis	Mitosis	
γ-tubulin complex	Tub4	75	76	TubG1
	Spc97	66	65	TUBGPC2
	Spc98	55	62	TUBGPC3
Linkers	Spc110	64	63	Kendrin
	Spc72	46	43	TACC
	Cmd1	88	97	Calmodulin
Core & satellite	Nud1	58	58	Centriolin
	Spc42	67	69	
	Spc29	61	64	
	Cnm67	69	70	
Half bridge	Kar1	22	18	
	Sfi1	20	12	hSfi1
	Cdc31	69	70	Centrin3
	Mps3	38	29	SUN protein
Membrane anchor	Nbp1	41	46	
	Bbp1	67	63	
	Mps2	40	45	KASH protein
	Ndc1	27	24	
Meiotic plaque	Mpc54	55	0	
	Spo21	30	0	
	Ady3	14	0	
	Spo74	15	0	
	Don1	19	0	
Others	Bfa1	55	57	
	Bub2	60	66	
	Cdc5	3	25	Polo kinase
	Cdc14	55	55	Cdc14
	Cdc15	0	18	
	Mlp2	0	3	
	Ndj1	36	0	
	Pom152	2	13	
	Tub1	25	32	
	Tub2	43	36	

Figure S1. **Extended list of peptides recovered by mass spectrometry of Spc97-TAP samples.** Known animal homologues are shown to the right of the list.

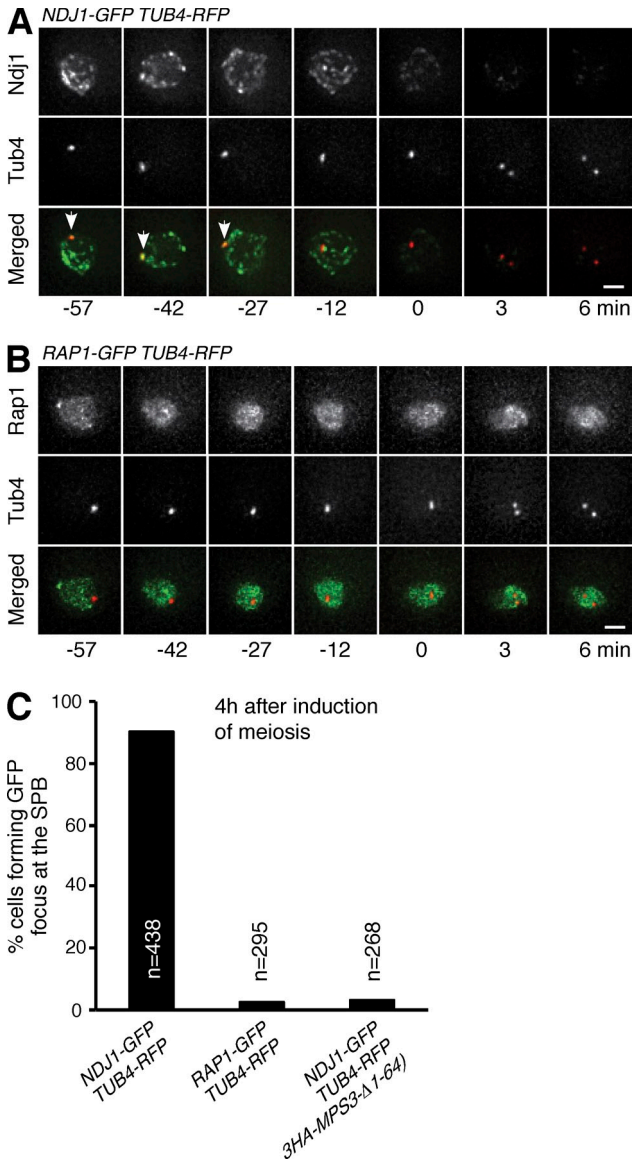

Figure S2. **Localization of Ndj1 and Rap1 in budding yeast meiosis.** Yeast cells were induced to undergo synchronous meiosis, and time-lapse fluorescence microscopy was performed as in Fig. 1 G. (A) Ndj1 dynamics during meiosis I. (B) Rap1 dynamics during meiosis I. (C) Quantification of Ndj1 and Rap1 localization during meiosis. Strains HY4086, HY4494, and HY4865 were used. Rap1-GFP and Ndj1-GFP are shown in green, Tub4-RFP in red. Bars, 2 µm.

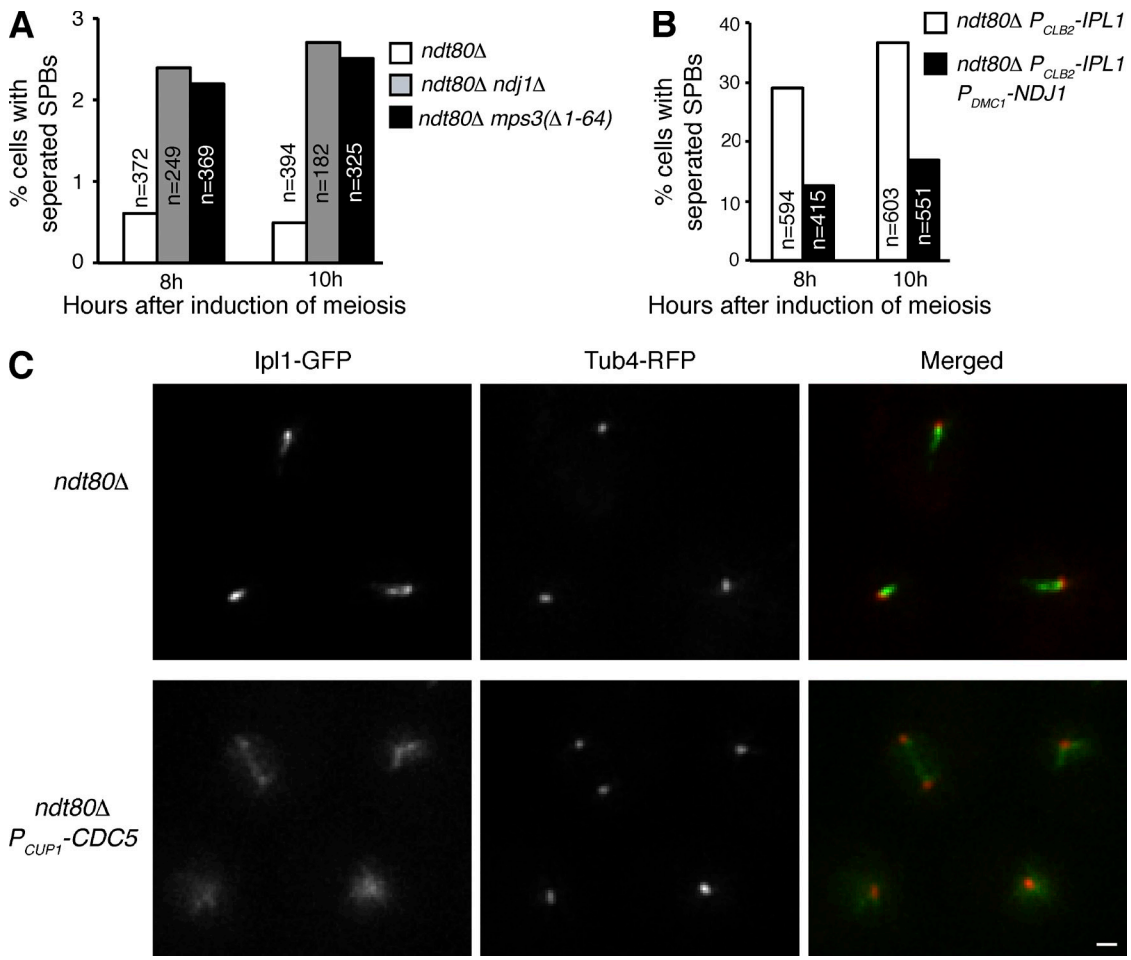

Figure S3. **Ndj1 and Cdc5 regulate SPB separation during yeast meiosis.** (A) SPB separation in cells arrested at prophase I by way of *ndt80Δ*. Yeast cells were induced to undergo synchronous meiosis, aliquots were withdrawn at indicated time points, and SPB separation was determined using fluorescence microscopy. SPB is marked by Tub4-RFP. (B) Overproduction of Ndj1 in cells arrested at prophase I but with Ipl1 depleted. Evaluation of SPB separation was performed as in A. Four copies of P_{DMC1}-*NDJ1* were introduced in *ndt80Δ* P_{CLB2}-*IPL1* P_{DMC1}-*NDJ1* cells. Strains used: HY4506 and HY4864. (C) Ectopic expression of *CDC5* promotes SPB separation. Yeast cells were induced to undergo synchronous meiosis, and then prepared for time-lapse fluorescence microscopy. Ipl1-GFP is shown in green; SPB, marked by Tub4-RFP, is shown in red. To induce *CDC5* expression, 60 mM $CuSO_4$ was added to the culture media 4 h after induction of meiosis. Note that SPBs separated prematurely in the presence of Cdc5. Strains used: HY2459 and HY4877. Bar, 2 μm.

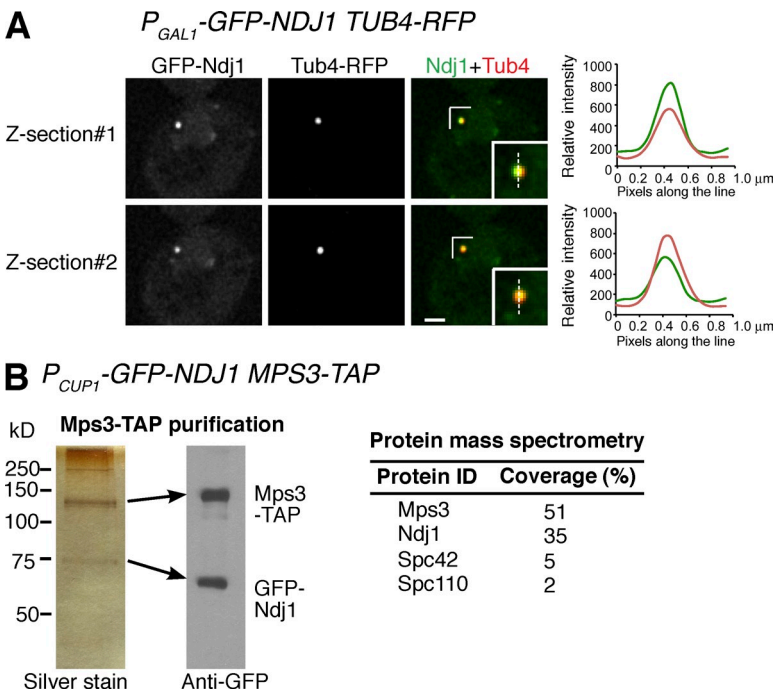

Figure S4. **Localization of Ndj1 and Tub4 in vegetative yeast cells.** (A) Fluorescence microscopy of GFP-Ndj1 and Tub4-RFP. Yeast cells (HY4128) were grown in synthetic complete medium with 3% galactose to induce the expression of P_{GAL1}-*GFP-NDJ1*. Two continuous optical sections are shown. Insets show 2× magnification of the area of interest. GFP-Ndj1, green; Tub4-RFP, red. Bar, 2 µm. A line scan of fluorescence intensity of GFP-Ndj1 and Tub4-RFP is shown to the right. Note that GFP-Ndj1 colocalizes with Tub4-RFP. The cell shown is from a single representative experiment out of four repeats; $n > 50$. (B) Ectopically produced Ndj1 binds to Mps3 in vegetative yeast cells. To produce Ndj1 at a lower protein level and therefore to generate viable cells, we used the P_{CUP1}-*GFP-NDJ1* construct. Yeast cells (HY4254-B) were grown to mid log phase in the presence 50 µM $CuSO_4$ and harvested for affinity protein purification as in Fig. 1 C. An anti-GFP antibody was used to probe GFP-Ndj1. This antibody also recognizes Mps3-TAP. Arrows point to the same protein bands determined by silver staining and immunoblotting. Representative peptides identified by protein mass spectrometry are shown in the table to the right.

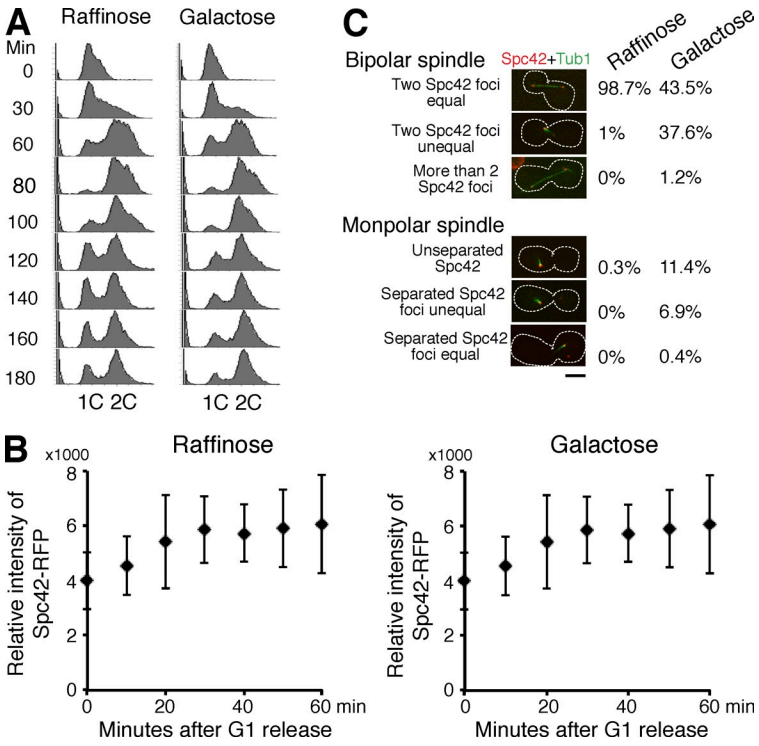

Figure S5. **Cell progression and SPB duplication in vegetative cells with ectopic expression of Ndj1.** Yeast cells grown in raffinose were arrested at G1 with alpha factor; addition of 3% galactose induced the expression of P_{GAL1}-*NDJ1* as shown in Fig. 5 D. (A and B) Cell aliquots were withdrawn at indicated times upon removal of the alpha factor and prepared for FACS (A) and fluorescence microscopy (B). Spc42 was tagged with RFP and used as an SPB marker. (A) FACS analysis of DNA synthesis. (B) The intensity of Spc42-RFP in vegetative yeast cells. (C) Spindle morphology in large budded yeast cells. Cells were divided into two major categories: with a bipolar spindle and with a monopolar spindle. Tub1, the α-tubulin in yeast, was marked with GFP (green); Spc42 was marked with RFP (red). About 200 cells were counted for each treatment. Note that the percentage of cells for each category varies from that of Fig. 5 F, which indicates experimental variations of the P_{GAL1}-*NDJ1* expression level in vegetative yeast cells. Bar, 2 μm.

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

Name	Background	Genotype
HY1635	SK1, diploid	his3Δ200, leu2-k, ura3, TUB4-RFP::HIS5/ his3Δ200, leu2-k, ura3, TUB4-RFP::HIS5
HY2459	SK1, diploid	leu2, ura3, his3, IPL1-GFP::HIS5, TUB4-RFP::HIS5, ndt80Δ::KAN/leu2, ura3, his3, IPL1-GFP::HIS5, TUB4-RFP::HIS5, ndt80Δ::KAN
HY3674	SK1, diploid	his3Δ200, leu2-k, ura3, SPC97-TAP::HIS5, SPC72-GFP::HIS5/ his3Δ200, leu2-k, ura3, SPC97-TAP::HIS5, SPC72-GFP::HIS5
HY3813	SK1, diploid	his3Δ200, leu2-k, ura3, NDJ1-TAP::HIS5, MPS3-GFP::HIS5/ his3Δ200, leu2-k, ura3, NDJ1-TAP::HIS5, MPS3-GFP::HIS5
HY3848	SK1, diploid	his3Δ200, leu2-k, ura3, NDJ1-3HA::HIS5, MPS3-TAP::HIS5/ his3Δ200, leu2-k, ura3, NDJ1-3HA::HIS5, MPS3-TAP::HIS5
HY3859	SK1, diploid	his3Δ200, leu2-k, ura3, NDJ1-GFP::HIS5, SPC42-RFP::HIS5/ his3Δ200, leu2-k, ura3, NDJ1-GFP::HIS5, SPC42-RFP::HIS5
HY3871	SK1, diploid	his3Δ200, leu2-k, ura3, lys2, ho::LYS2, MPS3-3HA::HIS5/ his3Δ200, leu2-k, ura3, lys2, ho::LYS2, MPS3-3HA::HIS5
HY3881	SK1, diploid	his3Δ200, leu2-k, ura3, NDJ1-GFP::HIS5, MPS3-RFP::HIS5/ his3Δ200, leu2-k, ura3, NDJ1-GFP::HIS5, MPS3-RFP::HIS5
HY3911	SK1, diploid	his3Δ200, leu2-k, ura3, P_{CLB2}-3HA-MPS3::NAT, NDJ1-GFP::HIS5, SPC42-RFP::HIS5/ his3Δ200, leu2-k, ura3, P_{CLB2}-3HA-MPS3::NAT, NDJ1-GFP::HIS5, SPC42-RFP::HIS5
HY3937	SK1, diploid	his3Δ200, leu2-k, ura3, lys2, ho::LYS2, NDJ1-3HA::HIS5, MPS3-V5::HIS5/ his3Δ200, leu2-k, ura3, lys2, ho::LYS2, NDJ1-3HA::HIS5, MPS3-V5::HIS5
HY3945	SK1, diploid	his3Δ200, leu2-k, ura3, ndj1Δ::HB, TUB4-RFP::HIS5/ his3Δ200, leu2-k, ura3, ndj1Δ::HB, TUB4-RFP::HIS5
HY3973	SK1, diploid	ura3, leu2, MPS3-V5::His5, ndt80 Δ::KAN, NDJ1-3HA::HIS5/ ura3, leu2, MPS3-V5::HIS5, ndt80 Δ:: KAN, NDJ1-3HA::HIS5
HY4031	SK1, diploid	ura3, leu2, P_{CLB2}-CDC20:: KAN, NDJ1-3HA::HIS5, MPS3-V5::HIS5/ ura3, leu2, P_{CLB2}-CDC20:: KAN, NDJ1-3HA::HIS5, MPS3-V5::HIS5
HY4074	SK1, diploid	his3Δ200, leu2-k, ura3, lys2, ho::LYS2, NDJ1-3HA::HIS5, P_{CUP1}-CDC5::KAN, ndt80Δ::KAN/his3Δ200, leu2-k, ura3, lys2, ho::LYS2, NDJ1-3HA::HIS5, P_{CUP1}-CDC5::KAN, ndt80Δ::KAN
HY4086	SK1, diploid	his3Δ200, leu2-k, ura3, NDJ1-GFP::HIS5, TUB4-RFP::HIS5/ his3Δ200, leu2-k, ura3, NDJ1-GFP::HIS5, TUB4-RFP::HIS5
HY4113	SK1, diploid	his3Δ200, leu2-k, ura3, NDJ1-GFP::HIS5, TUB4-RFP::HIS5, P_{CLB2}-CDC20:: KAN/ his3Δ200, leu2-k, ura3, NDJ1-GFP::HIS5, TUB4-RFP::HIS5, P_{CLB2}-CDC20:: KAN
HY4115	SK1, diploid	his3Δ200, leu2-k, ura3, NDJ1-GFP::HIS5, TUB4-RFP::HIS5, ndt80::KAN/ his3Δ200, leu2-k, ura3, NDJ1-GFP::HIS5, TUB4-RFP::HIS5, ndt80::KAN
HY4133	SK1, diploid	his3Δ200, leu2-k, ura3, spo11Δ::HB, TUB4-RFP::HIS5/ his3Δ200, leu2-k, ura3, spo11Δ::HB, TUB4-RFP::HIS5
HY4204	SK1, diploid	his3Δ200, leu2-k, ura3, ndj1Δ::HB, spo11Δ::HB, TUB4-RFP::HIS5/ his3Δ200, leu2-k, ura3, ndj1Δ::HB, spo11Δ::HB, TUB4-RFP::HIS5
HY4383	SK1, diploid	arg4, leu2, TUB4-RFP::HIS5, ura3::pGPD1-GAL4(848).ER::URA3, PGAL1-GFP-CSM4::LEU2 (pHG345)/arg4, leu2, TUB4-RFP::HIS5, ura3::pGPD1-GAL4(848).ER::URA3, PGAL1-GFP-CSM4::LEU2
HY4393	SK1, diploid	his3Δ200, leu2-k, ura3, lys2, ho::LYS2, NDJ1-3HA::HIS5, MPS3-V5::HIS5/ his3Δ200, leu2-k, ura3, lys2, ho::LYS2, NDJ1-TAP::HIS5, MPS3-V5::HIS5
HY4412	SK1, diploid	*his3Δ200, leu2-k, ura3, lys2, ho::LYS2, NDJ1-3HA::HIS5, KAN::P_{CUP1}-MPS3(Δ1-64)-V5::HIS5/ his3Δ200, leu2-k, ura3, lys2, ho::LYS2, NDJ1-3HA::HIS5, KAN::P_{CUP1}-MPS3(Δ1-64)-V5::HIS5*
Hy4418	SK1, diploid	his3Δ200, leu2-k, ura3, MPS3-GFP::HIS5, TUB4-RFP::HIS5/ his3Δ200, leu2-k, ura3, MPS3-GFP::HIS5, TUB4-RFP::HIS5
HY4419	SK1, diploid	his3Δ200, leu2-k, ura3, MPS3-GFP::HIS5, TUB4-RFP::HIS5, ndj1Δ::HB / his3Δ200, leu2-k, ura3, MPS3-GFP::HIS5, TUB4-RFP::HIS5, ndj1Δ::HB
HY4494	SK1, diploid	his3Δ200, leu2-k, ura3, lys2, ho::LYS2, RAP1-GFP::HIS5, TUB4-RFP::HIS5/ his3Δ200, leu2-k, ura3, lys2, ho::LYS2, RAP1-GFP::HIS5, TUB4-RFP::HIS5
HY4506	SK1, diploid	ura3, leu2, his4, P_{CLB2}-IPL1::KAN, NDJ1-3HA::HIS5, ndt80Δ::HB/ura3, leu2, his4, P_{CLB2}-IPL1::KAN, NDJ1-3HA::HIS5, ndt80Δ::HB
HY4654	SK1, diploid	his4, P_{CLB2}-IPL1::KAN, TUB4-RFP::HIS5, ndt80Δ::HB, P_{DMC1}-GFP-NDJ1::LEU2, P_{DMC1}-GFP-NDJ1::URA3/his4, P_{CLB2}-IPL1::KAN, TUB4-RFP::HIS5, ndt80Δ::HB, P_{DMC1}-GFP-NDJ1::LEU2, P_{DMC1}-GFP-NDJ1::URA3
HY4803	SK1, diploid	his3Δ200, leu2-k, ura3, P_{CUP1}-CDC5::KAN, ndt80Δ::KAN, TUB4-RFP::HIS5, P_{DMC1}-GFP-NDJ1::LEU2, P_{DMC1}-GFP-NDJ1::URA3/ his3Δ200, leu2-k, ura3, P_{CUP1}-CDC5::KAN, ndt80Δ::KAN, TUB4-RFP::HIS5, P_{DMC1}-GFP-NDJ1::LEU2, P_{DMC1}-GFP-NDJ1::URA3
HY4852	SK1, diploid	his3Δ200, leu2-k, ura3, NDJ1-GFP::HIS5, TUB4-RFP::HIS5, csm4Δ::HB / his3Δ200, leu2-k, ura3, NDJ1-GFP::HIS5, TUB4-RFP::HIS5, csm4Δ::HB
HY4860	SK1, diploid	his3Δ200, leu2-k, ura3, TUB4-RFP::HIS5, P_{DMC1}-GFP-NDJ1(pHG286)::LEU2, P_{DMC1}-GFP-NDJ1::URA3/ his3Δ200, leu2-k, ura3, TUB4-RFP::HIS5, P_{DMC1}-GFP-NDJ1::LEU2, P_{DMC1}-GFP-NDJ1::URA3
HY4861	SK1, diploid	his3Δ200, leu2-k, ura3, spo11Δ::HB, TUB4-RFP::HIS5, P_{DMC1}-GFP-NDJ1::LEU2, P_{DMC1}-GFP-NDJ1::URA3/ his3Δ200, leu2-k, ura3, spo11Δ::HB, TUB4-RFP::HIS5, P_{DMC1}-GFP-NDJ1::LEU2, P_{DMC1}-GFP-NDJ1::URA3
HY4864	SK1, diploid	his3Δ200, leu2-k, ura3, spo11Δ::HB, P_{CUP1}-MPS3(Δ1-64)::KAN, TUB4-RFP::HIS5/ his3Δ200, leu2-k, ura3, spo11Δ::HB, P_{CUP1}-MPS3(Δ1-64)::KAN,TUB4-RFP::HIS5
HY4865	SK1, diploid	*his3Δ200, leu2-k, ura3, spo11Δ::HB, P_{CUP1}-MPS3(Δ1-64)::KAN, TUB4-RFP::HIS5, P_{DMC1}-GFP-NDJ1::LEU2, P_{DMC1}-GFP-NDJ1::URA3/ his3Δ200, leu2-k, ura3, spo11Δ::HB, P_{CUP1}-MPS3(Δ1-64)::KAN, TUB4-RFP::HIS5, P_{DMC1}-GFP-NDJ1::LEU2, P_{DMC1}-GFP-NDJ1::URA3*

Table S1. **Yeast strains used in this study** *(Continued)*

Name	Background	Genotype
HY4254-B	SK1, haploid	MATα, his3Δ200, leu2-k, ura3, lys2, ho::LYS2, MPS3-TAP::HIS5, P_{CUP1}-GFP-NDJ1::LEU2
HY4877	SK1, diploid	leu2, ura3, his3, IPL1-GFP::HIS5, TUB4-RFP::HIS5, ndt80Δ::KAN, P_{CUP1}-CDC5/leu2, ura3, his3, IPL1-GFP::HIS5, TUB4-RFP::HIS5, ndt80Δ::KAN, P_{CUP1}-CDC5
HY3799	S288C	MATα, his3Δ1, leu2Δ, met15Δ, ura3Δ, TUB4-RFP::HIS5
HY4128	S288C	MATα, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, TUB4-RFP::HIS5, P_{GAL1}-GFP-NDJ1::LEU2
HY4149	S288C	MATα, P_{CLB2}-MPS3(Δ1-64)::KAN, his3Δ1, leu2Δ, met15Δ, ura3Δ, TUB4-RFP::HIS5
HY4150	S288C	MATα, P_{CLB2}-MPS3(Δ1-64)::KAN, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, TUB4-RFP::HIS5, P_{GAL1}-GFP-NDJ1::LEU2
HY4179	S288C	MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, P_{GAL1}-GFP-NDJ1::LEU2, MPS3-RFP::HIS5
HY4217	S288C	MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, P_{GAL1}-GFP-NDJ1::LEU2, RAP1-RFP::HIS5
HY4249-A	S288C	MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, HTA1-RFP::HIS5, P_{GAL1}-GFP-NDJ1::LEU2
HY4376	S288C	MATa, leu2Δ0, met15Δ0, ura3Δ0, his3Δ1::SPC42-RFP::HIS5, TUB1-GFP::HIS5, P_{GAL1}-V5-NDJ1::URA3
HY4933	S288C	his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, pom152Δ::KAN, P_{GAL1}-GFP-NDJ1::LEU2
HY4947	S288C	his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, TUB4-RFP::HIS5, P_{GAL1}-GFP-NDJ1::LEU2, pom152Δ::KAN

Table S2. **Plasmids used in this study**

Plasmid	Description
pHG274	P_{CUP1}-GFP-NDJ, LEU2
pHG286	P_{DMC1}-GFP-NDJ1, LEU2
pHG302	P_{GAL}-GFP-NDJ1, LEU2
pHG335	P_{GAL}-V5-NDJ1, URA3
pHG389	P_{DMC1}-GFP-NDJ1, URA3

Table S3. **Spore viability in selected yeast strains**

Strains	Spore viability
Untagged wild type	100%
HY3859: *NDJ1-GFP*, *SPC42-RFP*	97.9%
HY3881: *NDJ1-GFP*, *MPS3-RFP*	95.8%
HY4086: *NDJ1-GFP*, *TUB4-RFP*	96.4%
HY4418: *MPS3-GFP*, *TUB4-RFP*	95.8%
GHY4494: *RAP1-GFP*, *TUB4-RFP*	96.2%