

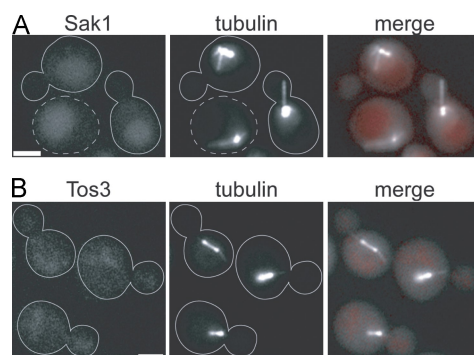
Moore et al., <http://www.jcb.org/cgi/content/full/jcb.201006092/DC1>

Figure S1. **Localization of Sak1 and Tos3.** (A) Localization of Sak1. Tandem RFP/tidimer2 was fused to the carboxy terminus of Sak1 by integration at the endogenous *SAK1* locus. Cells also express GFP-Tub1. Strain: yJC7003. (B) Localization of Tos3. Monomeric YFP/mCitrine was fused to the carboxy terminus of Tos3 by integration at the endogenous *TOS3* locus. Cells also express CFP-Tub1. Dashed lines indicate unbudded cells, and solid lines indicate budded cells. Strain: yJC7073. Bars, 2 μ m.

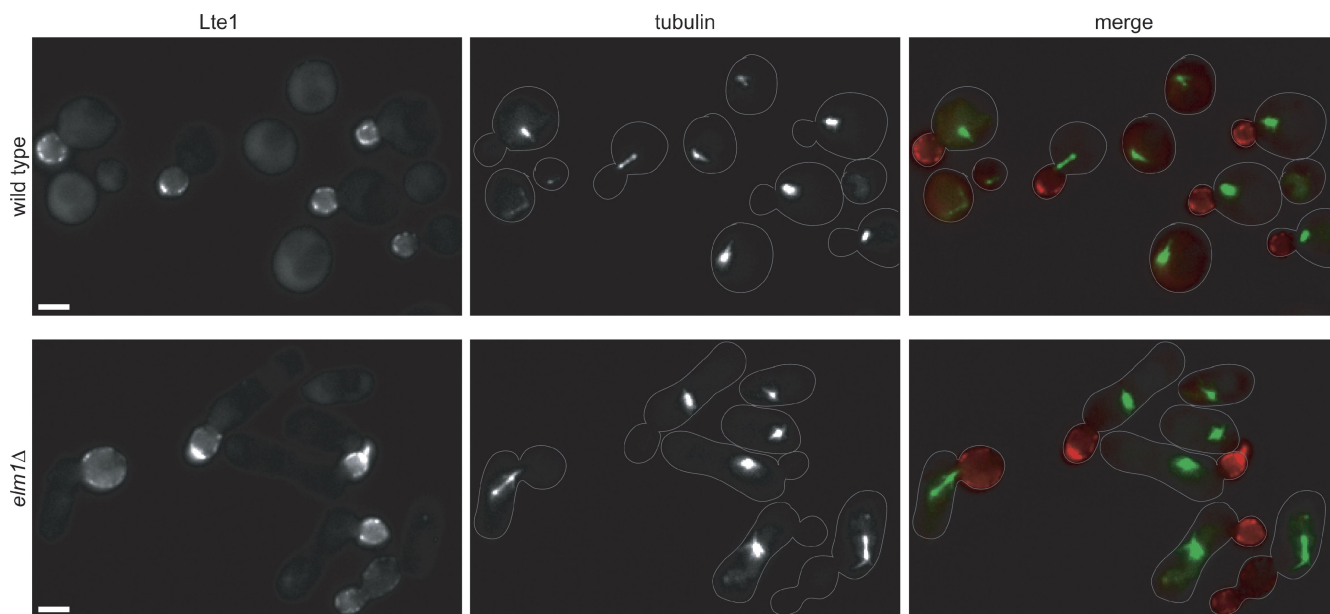


Figure S2. **Lte1 localization in *elm1Δ* mutants.** Three copies of GFP were fused to the carboxy terminus of Lte1 by integration at the endogenous locus (Castillon et al., 2003) and crossed into wild-type or *elm1Δ* mutant cells expressing microtubules labeled with mCherry-Tub1 (plasmid pAK011). Images were collected using a wide-field microscope. Solid lines indicate outlines of cells. Strains: wild type, yJC6390 and *elm1Δ*, yJC6389. Bars, 2 μ m.

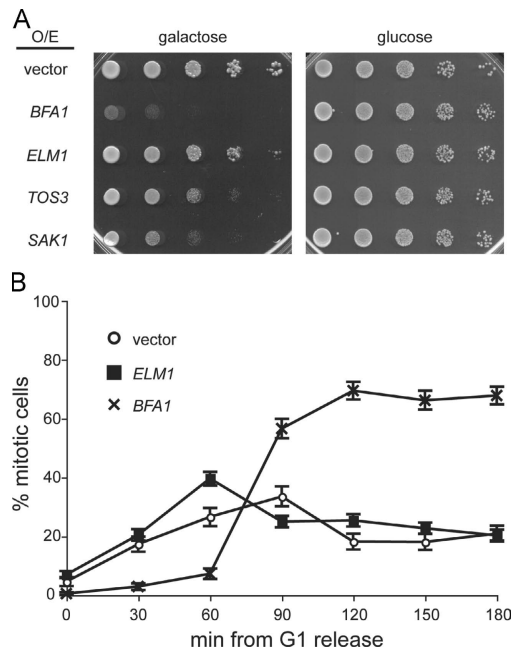


Figure S3. ***ELM1* overexpression does not inhibit mitotic exit.** (A) Growth assay on plates. High-copy plasmids containing *BFA1*, *ELM1*, *TOS3*, or *SAK1* under the control of a galactose-inducible promoter were transformed into a wild-type strain background, and a 10-fold dilution series was spotted onto media selective for plasmid retention and containing either galactose to induce expression or glucose to inhibit expression (see Materials and methods). O/E, overexpression. (B) Cell cycle analysis. Strains used in A were arrested with α factor and released into new media containing galactose to induce expression of plasmid-borne genes (see Materials and methods). After release, samples were collected at 30-min intervals and fixed, and the proportions of cells containing anaphase-length spindles were determined. Error bars are the standard error of proportion. Strain: yJC3677. Plasmids: pGAL-*BFA1*, pBJ1652; pGAL-*ELM1*, pBJ1695; pGAL-*TOS3*, pBJ1649; pGAL-*SAK1*, pBJ1650; and vector, pBJ216.

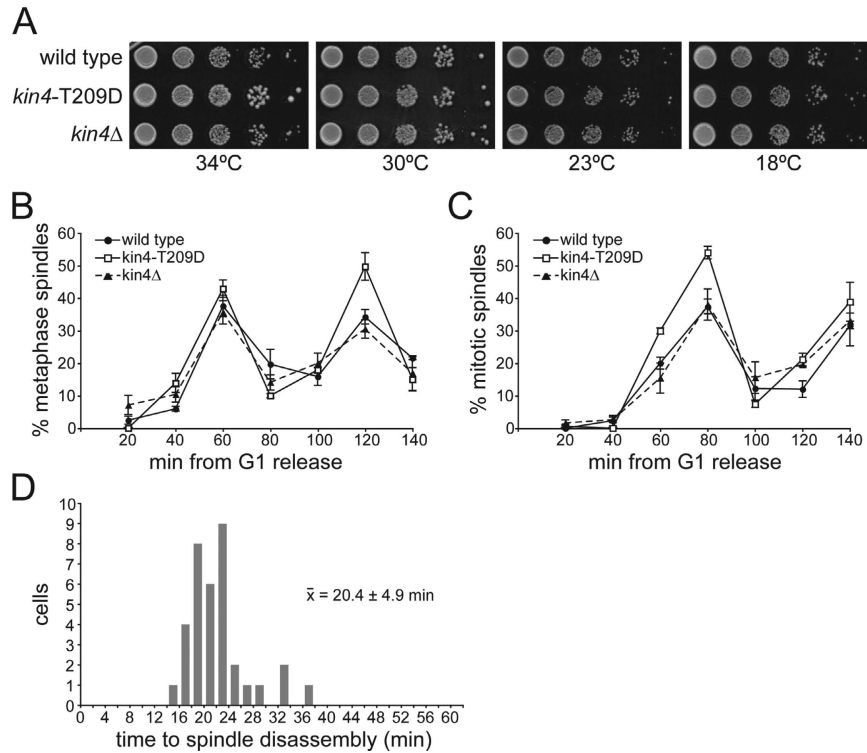


Figure S4. **Phosphomimetic Kin4-T209D is not sufficient to prolong mitosis.** Strains: wild type, yJC5920; *kin4-T209D*, yJC7084; and *kin4Δ*, yJC7805. (A) Growth assay on plates. Wild type, *kin4-T209D*, and *kin4Δ* strains were grown to saturation in rich media and diluted 1:4, cell number was normalized by OD₆₀₀, and a 10-fold dilution series was spotted onto rich media. Cells were allowed to grow for 2 d at 34°C, 30°C, and 23°C or for 7 d at 18°C. (B and C) Cell cycle analysis. Strains used in A were arrested with α factor and released into new media. After release, samples were collected at 20-min intervals and fixed, and the numbers of cells containing metaphase-length spindles ($\leq 2 \mu\text{m}$; B) or anaphase-length spindles ($> 2 \mu\text{m}$; C) were determined based on GFP-labeled microtubules. These are depicted as a percentage of total cells. Values are the means of three counts of at least 50 cells. Error bars are the standard error of the means. (D) Timing of spindle disassembly after one SPB moves into the bud from videos of GFP-tubulin collected on a confocal microscope. Strain: *kin4-T209D dyn1Δ*, yJC7080, $n = 35$.

Table S1. Strains and plasmids used in this study

Strains	Genotype	Source
yJC2295/YEF473A	MATa ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	Bi and Pringle, 1996
yJC2296/YEF473B	MATα ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	Amberg et al., 1997
yJC2297/YEF473	MATa/α ura3-52/ura3-52 lys2-801/lys2-801 leu2-Δ1/leu2-Δ1 his3-Δ200/his3-Δ200 trp1-Δ63/trp1-Δ63	Bi and Pringle, 1996
yJC2477	MATa arp1Δ::kanR hsl1Δ::TRP1 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63 ura3-52::URA3-GFP-TUB1	This study
yJC2480	MATa arp1Δ::kanR elm1Δ::TRP1 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63 ura3-52::URA3-GFP-TUB1	This study
yJC2738	MATa arp1Δ::kanR elm1Δ::TRP1 swe1Δ::LEU2 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63 ura3-52::URA3-GFP-TUB1	This study
yJC3464	MATa arp1Δ::KanR ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63	This study
yJC3677	MATa ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200	This study
yJC3806	MATa arp1Δ::kanR elm1Δ::TRP1 lte1Δ::TRP1 ura3-52::URA3-metP-GFP-TUB1 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63	This study
yJC3807	MATa arp1Δ::kanR swe1Δ::LEU2 ura3-52::URA3-metP-GFP-TUB1 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63	This study
yJC3808	MATa arp1Δ::kanR elm1Δ::TRP1 lte1Δ::TRP1 ura3-52::URA3-metP-GFP-TUB1 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63	This study
yJC3809	MATa arp1Δ::kanR swe1Δ::LEU2 ura3-52::URA3-metP-GFP-TUB1 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63	This study
yJC3810	MATa arp1Δ::kanR swe1Δ::LEU2 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63	This study
yJC3871	MATα dyn1Δ::His3 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63 ura3-52::URA3-GFP-TUB1	This study
yJC3943	MATa arp1Δ::KanR snf1Δ::TRP1 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63	This study
yJC3944	MATa arp1Δ::KanR snf1Δ::TRP1 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63	This study
yJC4078	MATa dyn1Δ::TRP1 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJC4168	MATa dyn1Δ::HIS3 elm1Δ::kanmx4 ura3::URA3-GFP-TUB1 lys2-801 leu2 his3 trp1-Δ63 met15Δ0	This study
yJC4170	MATa dyn1Δ::HIS3 elm1Δ::kanmx4 ura3::URA3-GFP-TUB1 lys2-801 leu2 his3 trp1-Δ63	This study
yJC4171	MATα dyn1Δ::HIS3 sak1Δ::kanmx4 ura3::URA3-GFP-TUB1 lys2-801 leu2 his3	This study
yJC4174	MATα dyn1Δ::HIS3 tos3Δ::kanmx4 ura3 leu2 his3 trp1-Δ63 met15Δ0	This study
yJC4176	MATa dyn1Δ::HIS3 tos3Δ::kanmx4 ura3::URA3-GFP-TUB1 lys2-801 leu2 his3 met15Δ0	This study
yJC4484	MATa dyn1Δ::HIS3 sak1Δ::kanmx3 tos3Δ::kanmx4 ura3::URA3-GFP-TUB1 lys2-801 his3 leu2 met15Δ0	This study
yJC5251	MATa bub2Δ::HIS3MX6 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJC5254	MATa elm1Δ::hygB ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJC5363	MATα dyn1Δ::HIS3 elm1Δ::kanmx4 ura3::URA3-GFP-TUB1 lys2-801 leu2Δ0 his3 trp1-Δ63	This study
yJC5603	MATa dyn1Δ::TRP1 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63	Moore et al., 2009a
yJC5920	MATa ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63	Moore et al., 2009a
yJC6380	MATa dyn1Δ::KanMX6 cdc15-2 leu2-3::LEU2-GFP-TUB1 ura3 trp1-1 ade1 omns	Moore et al., 2009b
yJC6382	MATα KIN4-13myc::KanMX6 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJC6389	MATa LTE1-3GFP::TRP1 elm1Δ::hygB ura3-52::URA3-mCherry-TUB1 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJC6390	MATa LTE1-3GFP::TRP1 ura3-52::URA3-mCherry-TUB1 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJC6396	MATa KIN4-13myc::KanMX6 elm1Δ::hygB ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJC6419	MATa tos3Δ::KanMX6 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJC6447	MATa bfa1Δ::NatMX ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJC6448	MATa kin4Δ::KanMX6 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJC6474	MATα elm1Δ::hygB sak1Δ::natR tos3Δ::KanMX6 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJC6492	MATa sak1Δ::natMX6 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJC6493	MATa sak1Δ::natMX dyn1Δ::KanMX6 cdc15-2 leu2-3::LEU2-GFP-TUB1 ura3 trp1-1 ade1 omns	This study
yJC6497	MATa bub2Δ::TRP1MX dyn1Δ::KanMX6 cdc15-2 leu2-3::LEU2-GFP-TUB1 ura3 trp1-1 ade1 omns	This study
yJC6498	MATa arp1Δ::hygB KIN4-tdimer2::KanMX6 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63	This study
yJC6499	MATa elm1Δ::hygB arp1Δ::hygB KIN4-tdimer2::KanMX6 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63	This study
yJC6573	MATa kin4Δ::KanMX6 elm1Δ::hygB ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJC6848	MATa ELM1-tdimer2::KanMX6 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJC6849	MATα elm1-420Δ-tdimer2::KanMX6 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63	This study
yJC6852	MATa ELM1-tdimer2::KanMX6 arp1Δ::hygB ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63	This study
yJC6854	MATα elm1-420Δ-tdimer2::KanMX6 arp1Δ::hygB ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63	This study

Table S1. **Strains and plasmids used in this study** (Continued)

Strains	Genotype	Source
yJC6926	<i>MATa elm1Δ::hygB dyn1Δ::KanMX6 cdc15-2 leu2-3::LEU2-GFP-TUB1 ura3 trp1-1 ade1 omns</i>	This study
yJC7003	<i>MATα SAK1-tdimer2::URA3 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJC7066	<i>MATa lte1Δ::TRP1 dyn1Δ::HIS3 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJC7067	<i>MATa elm1Δ::hygB dyn1Δ::HIS3 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJC7068	<i>MATa elm1Δ::hygB lte1Δ::TRP1 dyn1Δ::HIS3 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJC7069	<i>MATa tos3Δ::KanMX6 dyn1Δ::HIS3 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJC7070	<i>MATa tos3Δ::KanMX6 lte1Δ::TRP1 dyn1Δ::HIS3 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJC7071	<i>MATa sak1Δ::NatR dyn1Δ::HIS3 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJC7072	<i>MATa sak1Δ::NatR lte1Δ::TRP1 dyn1Δ::HIS3 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJC7073	<i>MATa TOS3-mCitrine::spHIS5 ura3-52::URA3-CFP-TUB1 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJC7079	<i>MATa elm1Δ::hygB dyn1Δ::HIS3 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJC7080	<i>MATa kin4-T209D-13myc::KanMX6 dyn1Δ::HIS3 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJC7081	<i>MATa kin4-T209D-13myc::KanMX6 elm1Δ::hygB dyn1Δ::HIS3 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJC7083	<i>MATa tos3Δ::hygB dyn1Δ::KanMX6 cdc15-2 leu2-3::LEU2-GFP-TUB1 ura3 trp1-1 ade1 omns</i>	This study
yJC7084	<i>MATa kin4-T209D-13myc::KanMX6 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJC7085	<i>MATa kin4Δ::KanMX6 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJC7251	<i>MATa ELM1-tdimer2::KanMX6 BNI4-GFP::KanMX6 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJC7252	<i>MATa ELM1-tdimer2::KanMX6 KCC4-GFP::KanMX6 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJC7285	<i>MATa kin4Δ::KanMX6 dyn1Δ::HIS3 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJC7286	<i>MATa TRP1-pGAL-elm1-420Δ-tdimer2::KanMX6 ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJC7292	<i>MATα BNI4-elm1Δ420-tdimer2::KanMX6 elm1Δ::hygB ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJC7293	<i>MATa KCC4-elm1Δ420-tdimer2::KanMX6 elm1Δ::hygB ura3-52 lys2-801 leu2-Δ1 his3-Δ200 trp1-Δ63</i>	This study
yJC7296	<i>MATa BNI4-elm1Δ420-tdimer2::KanMX6 elm1Δ::hygB dyn1Δ::HIS3 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJC7297	<i>MATa BNI4-elm1Δ420-tdimer2::KanMX6 elm1Δ::hygB dyn1Δ::HIS3 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJC7298	<i>MATa KCC4-elm1Δ420-tdimer2::KanMX6 elm1Δ::hygB dyn1Δ::HIS3 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJC7299	<i>MATa TRP1-pGAL-elm1Δ420-tdimer2::KanMX6 dyn1Δ::HIS3 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
yJC7300	<i>MATa TRP1-pGAL-elm1Δ420-tdimer2::KanMX6 dyn1Δ::HIS3 ura3-52 lys2-801 leu2-Δ1::LEU2-GFP-TUB1 his3-Δ200 trp1-Δ63</i>	This study
Plasmids		
pBJ197/pRS306	URA3, Amp ^r	Sikorski and Heiter, 1989
pBJ216/pRS426	2μ, URA3, Amp ^r	Christianson et al., 1992
pBJ1351/pSK1050	pUC19-TUB1-GFP::LEU2 integration plasmid. Derived from pAFS125.	Song and Lee, 2001
pBJ1488	pRS316-CDC3-GFP, CEN, URA3, Amp ^r	Caviston et al., 2003
pBJ1492/pJM1139	swe1Δ1- HA-12myc in pRS316 (CEN, URA3, Amp ^r)	McMillan et al., 2002
pBJ1606	elm1-K117R in pRS416 (CEN, URA3, Amp ^r)	This study
pBJ1649	pGAL-TOS3-tap, 2μ, URA3, Amp ^r	Gelperin et al., 2005
pBJ1650	pGAL-SAK1-tap, 2μ, URA3, Amp ^r	Gelperin et al., 2005
pBJ1651	pGAL-KIN4-tap, 2μ, URA3, Amp ^r	Gelperin et al., 2005
pBJ1652	pGAL-BFA1-tap, 2μ, URA3, Amp ^r	Gelperin et al., 2005
pBJ1689	pGAL-tos3-D189A, 2μ, LEU2d, Amp ^r	This study
pBJ1691	pADH1-sak1-D277A-3HA, 2μ, URA3	This study
pBJ1695	pGAL-GST-HisX6-ELM1, 2μ, URA3, Amp ^r	Zhu et al., 2001
pBJ1840	pGAL-kin4-T209A-tap, 2μ, URA3, Amp ^r	This study
pBJ1896	pGAL-kin4-T209D-tap, 2μ, URA3, Amp ^r	This study

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