

Fig. S1. (Complement to Figure 1). Chromosomes segregated in *bqt1Δ sad1.2* meicytes harbour kinetochore signal. (A) Frames from films of meicytes carrying Hht1-CFP (at one of the two endogenous *hht1⁺* loci), ectopically expressed mCherry-*atb2* (tubulin) and endogenously tagged Sad1.2-GFP. Numbering indicates meiotic progression in minutes; t = 0 is just before spindle formation. Sad1.2 localises at SPB-mediated spindle poles in 100% of *bqt1⁺ sad1.2* meicytes (20 cells were analysed from more than two independent experiments). Yellow arrowheads indicate the location of Sad1.2-GFP during SPB-mediated spindle formation. (B) In *bqt1Δ sad1.2* cells, without SPB separation, segregated chromosomes harbour kinetochores. Snapshots of wt and *bqt1Δ sad1.2* meicytes after meiosis: kinetochore (Mis6-GFP), chromatin (Hht1-CFP), and the SPB (Sid4-mCherry) are visualized. Unseparated SPBs are indicated by the yellow asterisks. Bars represent 5 μ m.

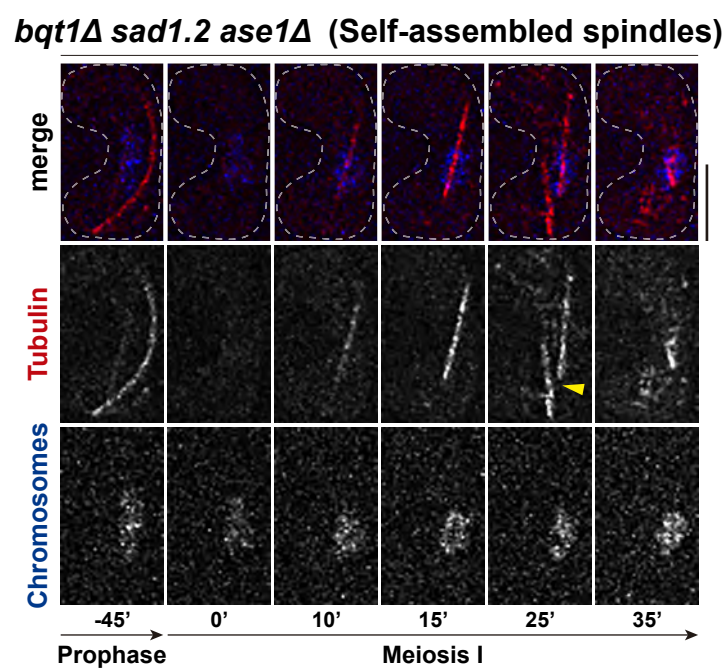


Fig. S2. (Complement to Figure 2). Ase1/PRC1 is an essential component of *bqt1Δ sad1.2* spindles. Frames from films of meicytes carrying chromosomes and spindles tagged as in Figure 2. Numbering indicates meiotic progression in minutes; t = 0 is just before spindle formation. Bars represent 5 μ m. Yellow arrowhead indicates discrete breakage of the spindle structure (25').

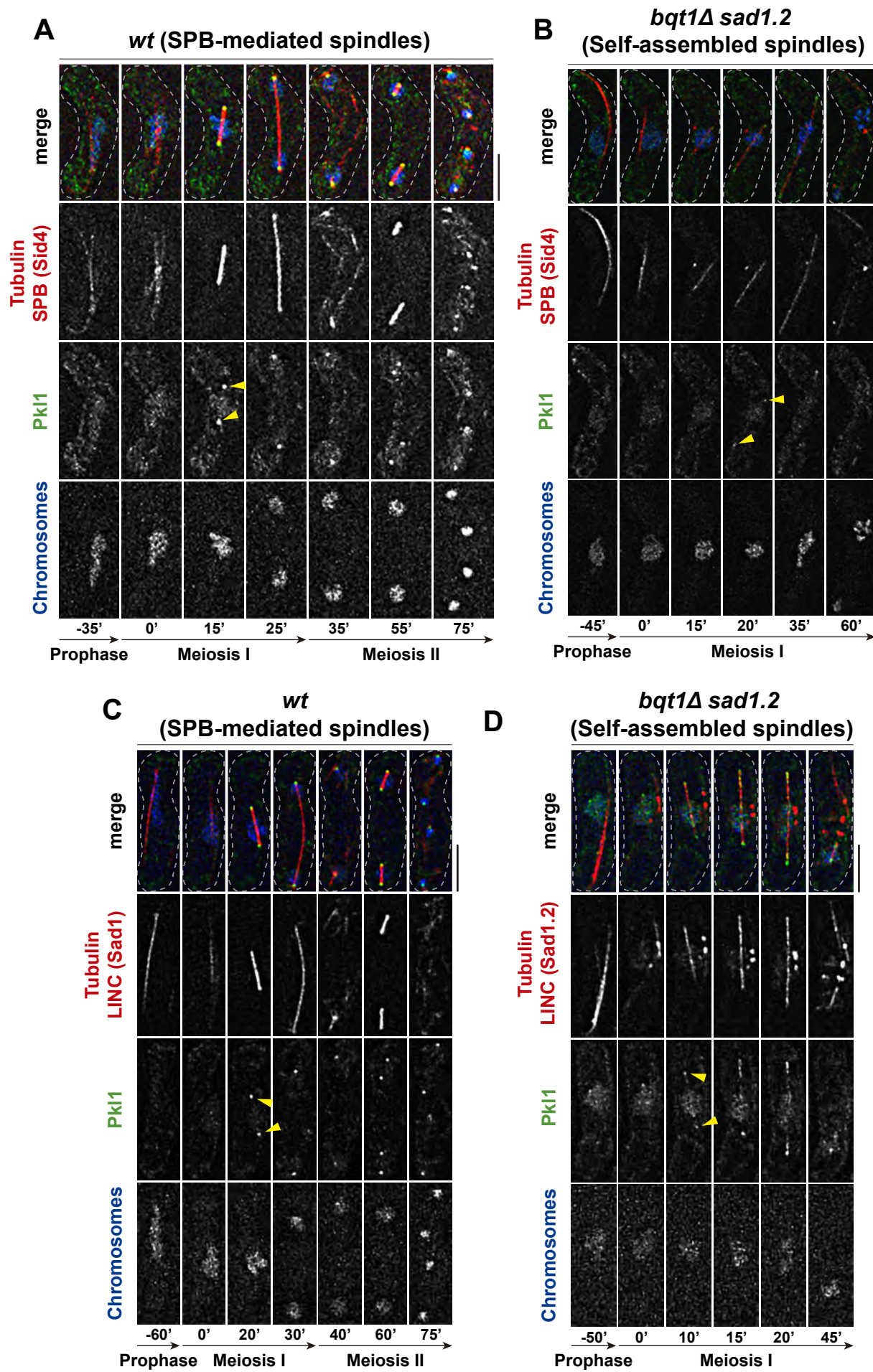


Fig. S3. (Complement to Figure 3). The location of Pki1 at the spindle poles in *bqt1Δ sad1.2* cells is SPB-independent. (A-B) Frames from films of meocytes with endogenously mCherry-tagged Sid4 and GFP-tagged Pki1, chromosomes and tubulin are visualized as in Figure 3. (C-D) To visualize the LINC complex, Sad1 and Sad1.2 were endogenously tagged with RFP and mCherry, respectively. Sid4 or Sad1.2 and Pki1 does not colocalise at self-assembled spindle poles in 100% of *bqt1Δ sad1.2* meocytes (n=20 cells scored in more than three independent experiments). Numbering indicates meiotic progression in minutes; t = 0 is just before spindle formation. Bars represent 5 μ m.

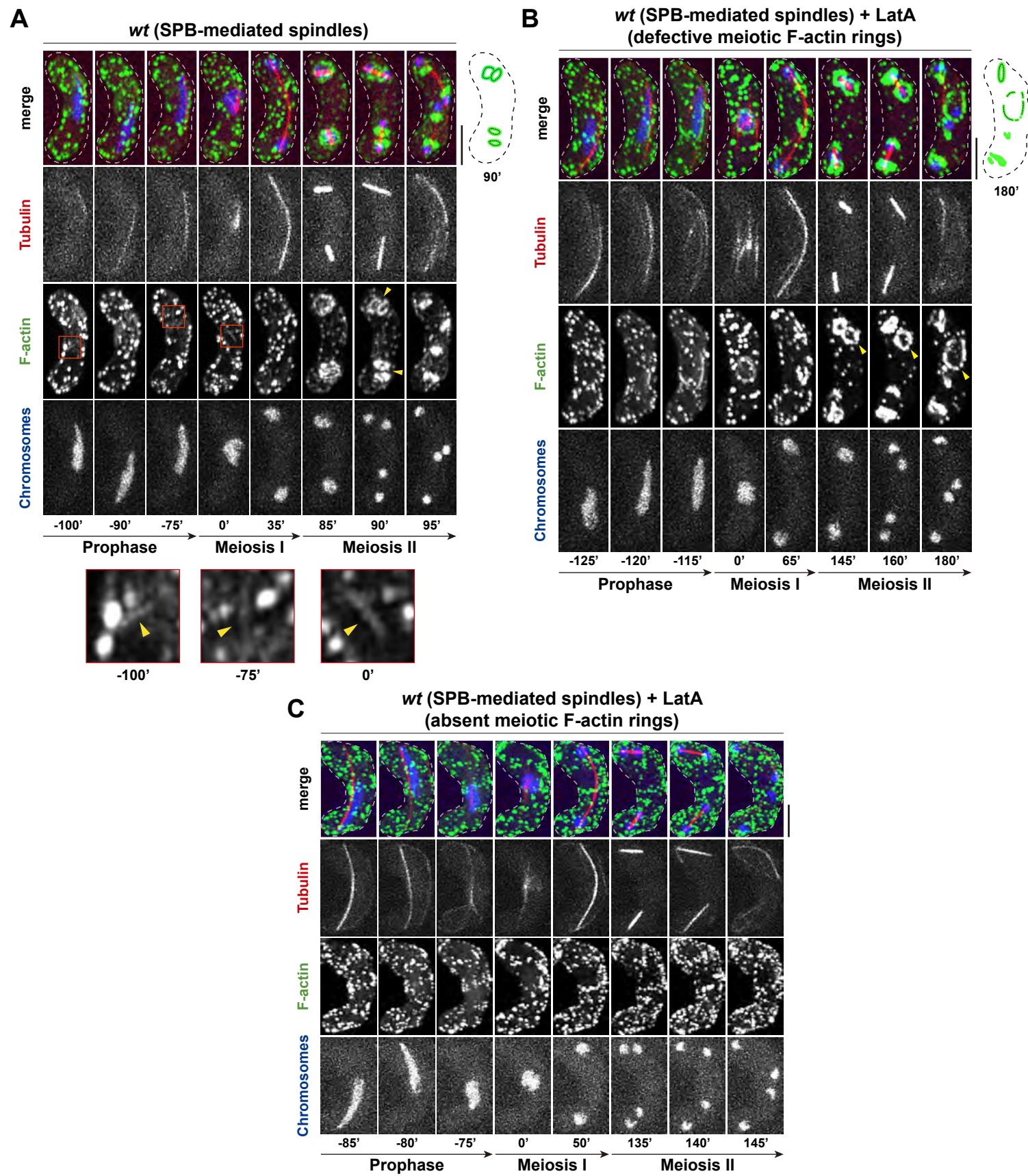


Fig. S4. (Complement to Figure 4). SPB-mediated meiotic spindle formation and behaviour are independent of the F-actin network in fission yeast meiosis. (A-C) Frames from films in meiosis. Numbers underneath represent time (in minutes) from MI onset. Scale bars represent 5 μ m. F-actin networks is viewed via Lifeact-GFP, chromatin via histone H3 tagged at one of the two endogenous *hht1+* loci, and tubulin via ectopically expressed mCherry-Atb2. (B-C) Behaviour of SPB-mediated and F-actin network upon addition of actin-depolymerizing drug Latrunculin A (LatA, 4 μ M). Various effects of LatA on meiotic actin rings morphology/dynamics are shown in (B, 145' to 180') and (C, 135' to 145').

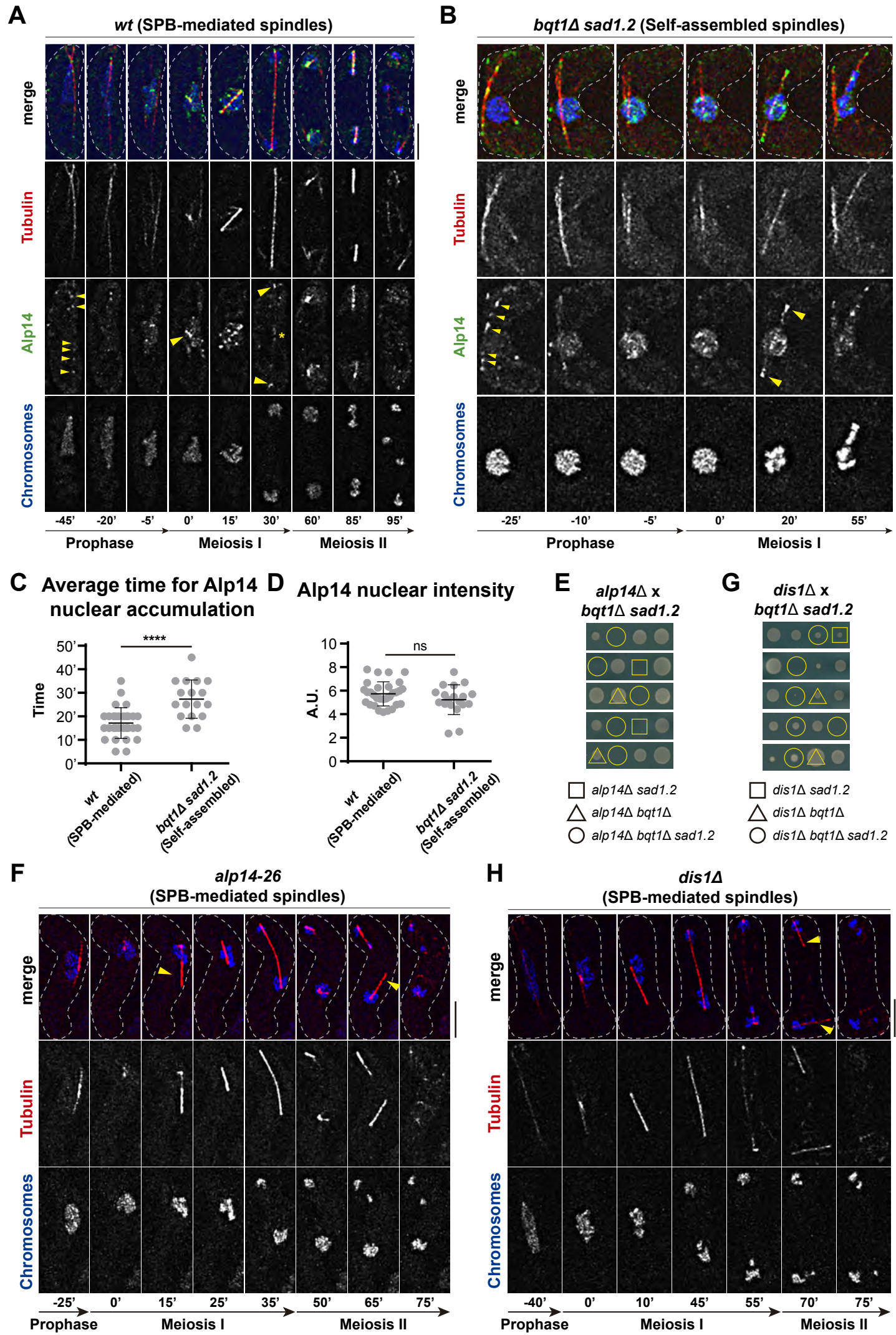


Fig. S5. (Complement to Figure 6). Alp14/XMAP215 microtubule polymerase localizes to self-assembled spindles. (A-B) Frames from films of meiocytes carrying chromosomes and spindles tagged as in Figure 6 with endogenously GFP-tagged Alp14. Numbering indicates meiotic progression in minutes; $t = 0$ is just before spindle formation. Bars represent 5 μm . Yellow arrowheads indicate localization of Alp14 at oscillating microtubules in prophase (A, -45'), at the tubulin focus from which the spindle forms (A, 0') and at MI SPB-mediated spindle poles (A, 30'). Yellow asterisk indicates localization of Alp14 at the MI SPB-mediated spindle midzone (A, 30'). (B) Localization of Alp14 during meiosis in the *bqt1 Δ sad1.2* background. Yellow arrowheads indicate localization of Alp14 at oscillating microtubules in prophase (-25') and at poles of the self-assembled spindle (20'). (C) Average time for Alp14 nuclear accumulation for SPB-mediated and self-assembled spindles in *wt* and *bqt1 Δ sad1.2* backgrounds, respectively. t-test: ****, $P < 0.0001$. $N = 30$ for *wt*, $N = 17$ for *bqt1 Δ sad1.2*. (D) Quantification of maximum nuclear fluorescence signal of Alp14. t-test: ns, $P > 0.05$. $N = 30$ for *wt*, $N = 19$ for *bqt1 Δ sad1.2*. (C-D) Bars represent mean and standard deviation. (E) *bqt1 Δ sad1.2 alp14 Δ* cells present a synthetic lethality when spores germinate after tetrads dissection analysis. Spores were grown at 25°C for 5 days. 52 asci were analysed with 94% of lethality for *bqt1 Δ sad1.2 alp14 Δ* . (F) Extra example to Figure 6B. (G) Spore analysis after tetrads dissection. Spores were grown at 25°C for 5 days. 66 asci were analysed showing *bqt1 Δ sad1.2 dis1 Δ* 37% of lethality. (H) Extra example to Figure 6G. (F-H) Yellow arrowheads depict spindle behavior defects.

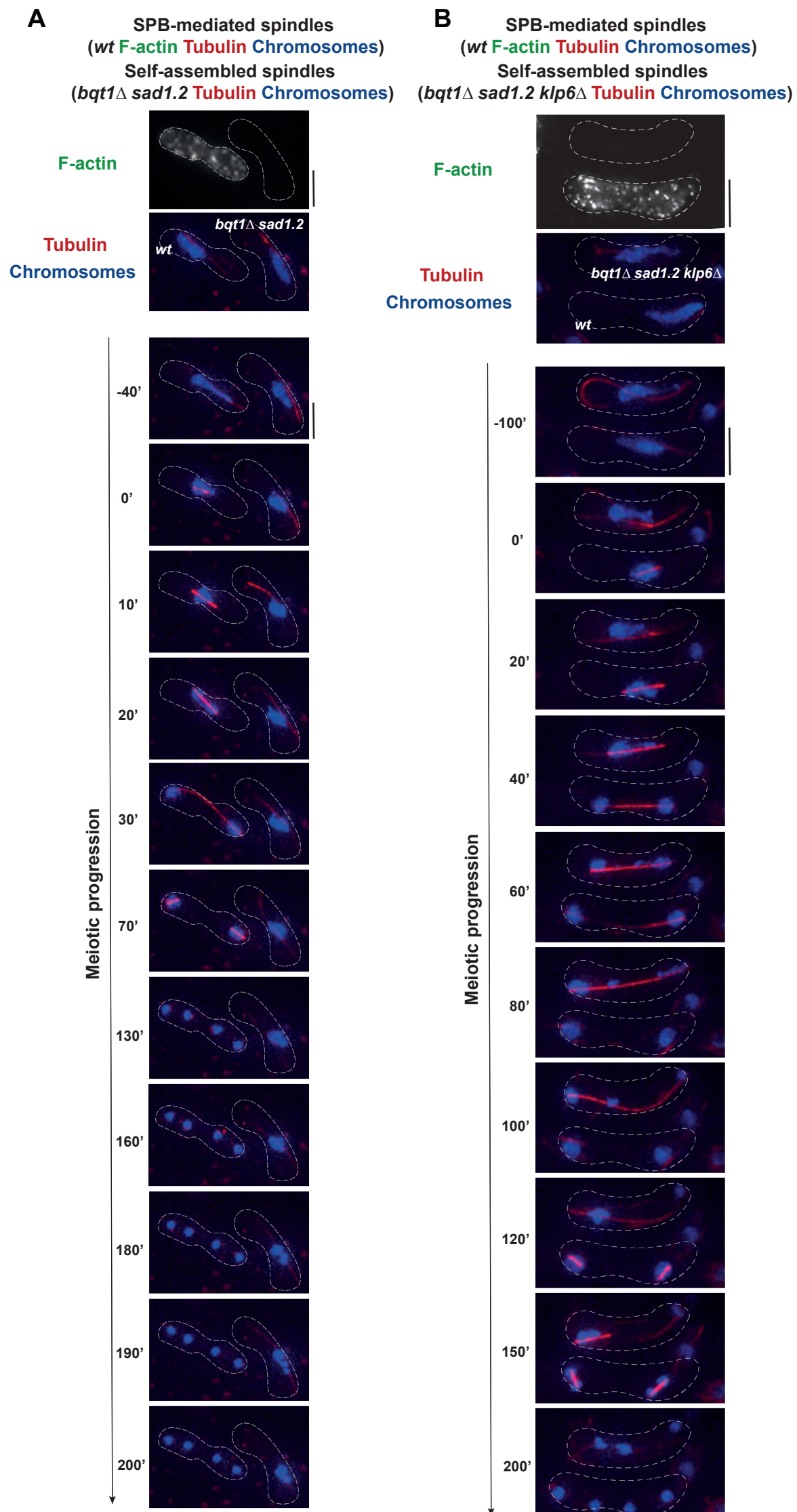


Fig. S6. (Complement to Figure 7). Elimination of Klp6 improves spindle self-assembly in *bqt1* Δ *sad1.2* cells. Frames from films of meicytes carrying chromosomes and spindles tagged as in Figure 7. *wt* cells harbour Lifeact-GFP as a genotype marker. *wt* and *bqt1* Δ *sad1.2* meicytes were patched on the same SPA plate and filmed in the same field. Numbering indicates meiotic progression in minutes; t = 0 is just before spindle formation of the *wt* cell. Bars represent 5 μ m.

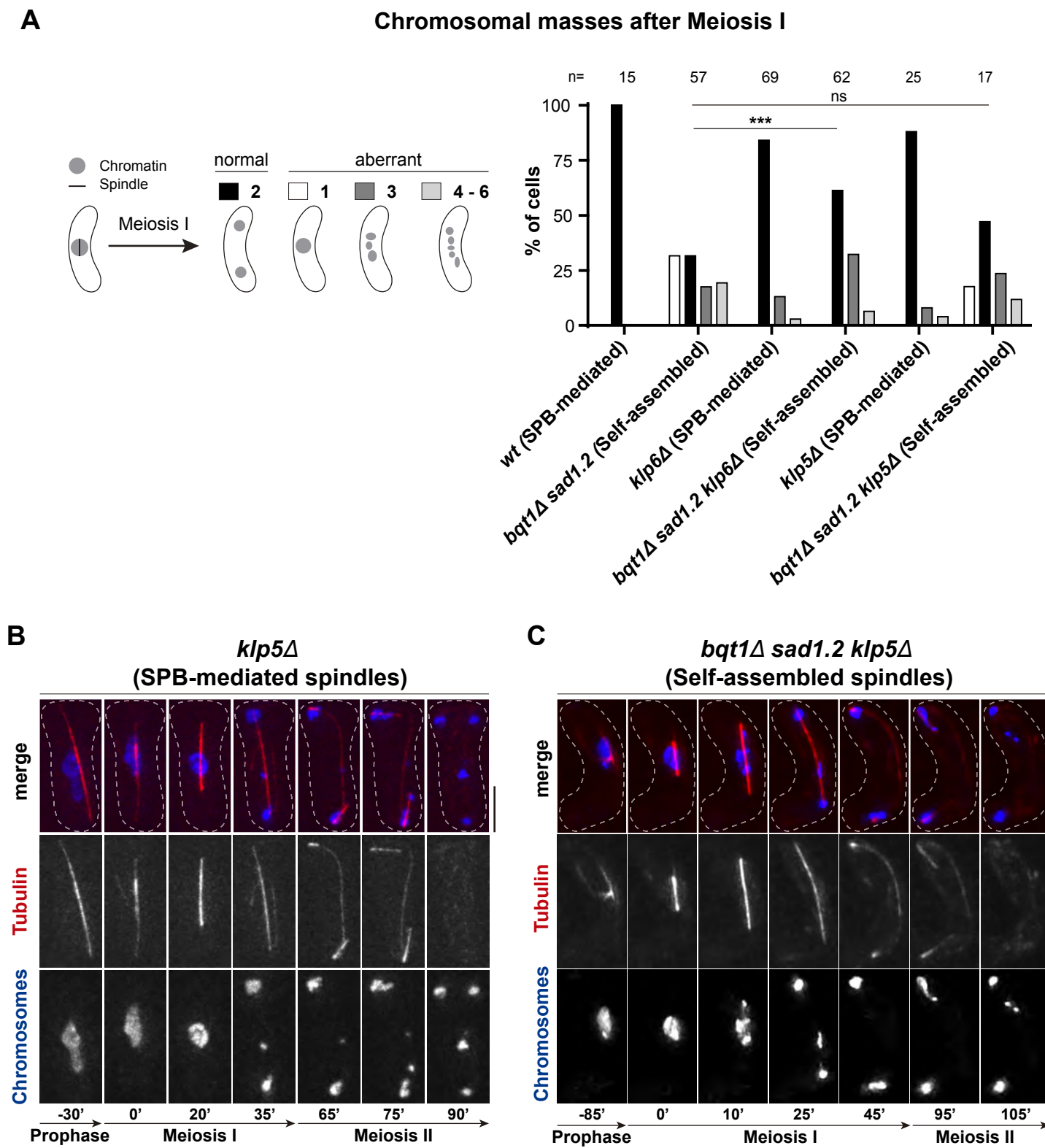
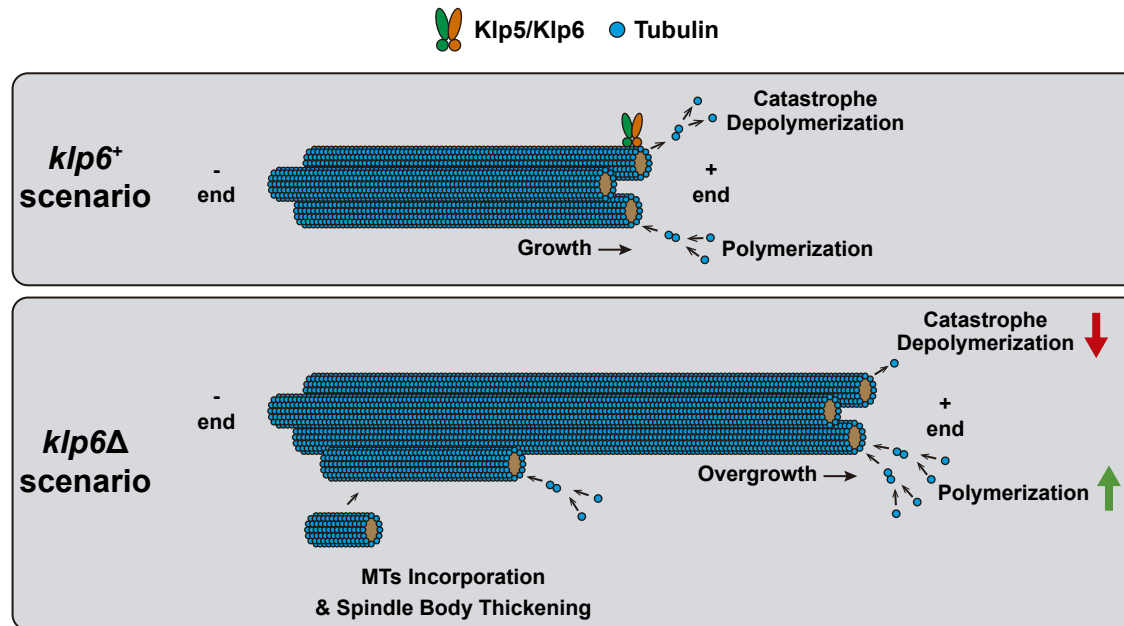
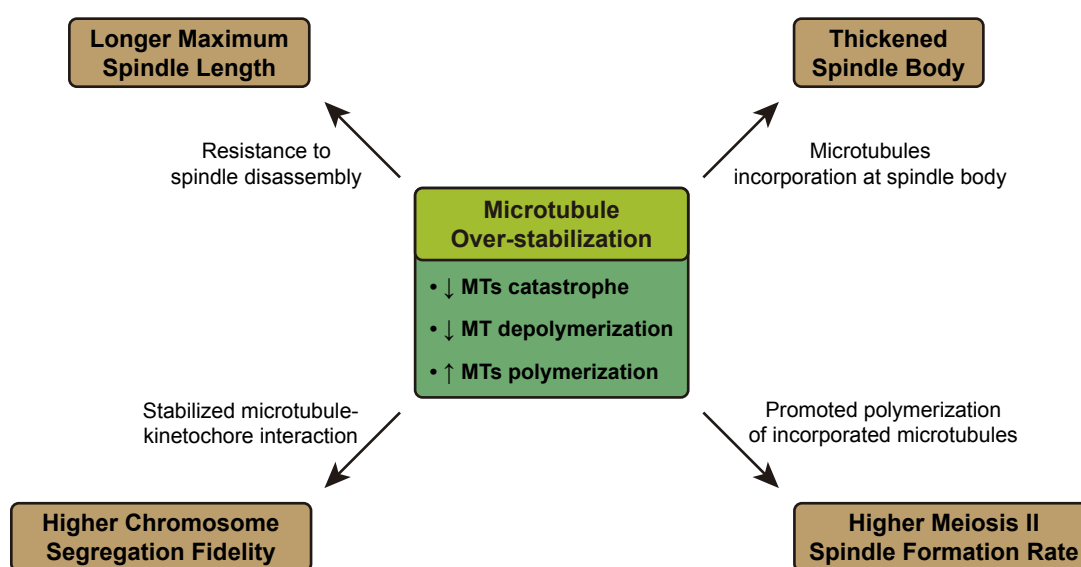


Fig. S7. (Complement to Figure 7). Elimination of Klp5 improves spindle self-assembly in *bqt1Δ sad1.2* cells. (A) Quantification of chromosome segregation fidelity of SPB-mediated and self-assembled spindles in presence and absence of *klp6*. Fisher's exact test for the category of two chromosomal masses after MI: *, $P < 0.05$; ***, $P < 0.001$; ****, $P < 0.0001$. n is the total number of cells scored from more than three independent experiments. (B-C) Frames from films of meicytes carrying chromosomes and spindles tagged as in Figure 7. Numbering indicates meiotic progression in minutes; t = 0 is just before spindle formation. Bars represent 5 μ m.

A Effect of Klp6 Elimination on Self-assembled Spindles Microtubules



B Self-assembled Spindles Microtubules Over-stabilization



C Summary of Self-assembled Spindle Improvement

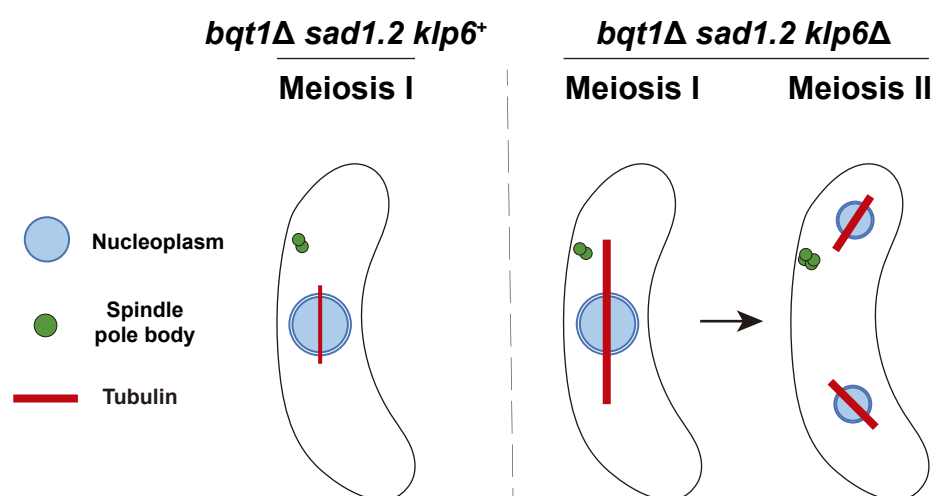


Fig. S8. (Complement to Figure 8) Schematic of proposed mechanisms of self-assembled spindle improvement by elimination of Klp6. (A) Speculated primary effects of elimination of microtubule-destabilizing activity of Klp5/Klp6 (*klp6Δ* setting) upon microtubule dynamics equilibrium. (B) Different pathways through which microtubule over-stabilization caused by Klp6 elimination could be responsible for self-assembled spindles improvement. (C) Representation of the global improvement of self-assembled spindle structure, formation and chromosome segregation fidelity by elimination of Klp6.

Table S1. Strains used in this study

Strain	Mating Type	Genotype	Origin
AFA51	<i>h⁹⁰</i>	<i>ade6-M210 ura4-D18 hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1</i>	Pineda-Santaella et al., 2019
AFA54	<i>h⁹⁰</i>	<i>ade6-M210 ura4-D18 hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 sad1.T3S.S52P-13Myc-hphMX6 bqt1::hygMX6</i>	Pineda-Santaella et al., 2019
AFA119	<i>h⁹⁰</i>	<i>ade6-M210 hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 Pact1-LA-GFP:leu1+</i>	This study
AFA206	<i>h⁺</i>	<i>his3-D1 hht1-CFP:his3+ Pnda3-mCherry-atb2:aur1 alp4-GFP:kanMX6</i>	This study
AFA210	<i>h⁹⁰</i>	<i>his3-D1 hht1-CFP:his3+ Pnda3-mCherry-atb2:aur1 alp4-GFP:kanMX6</i>	This study
AFA219	<i>h⁹⁰</i>	<i>hht1-CFP:his3+ Pnda3-mCherry-atb2:aur1 alp4-GFP:kanMX6 sad1.T3S.S52P-13Myc-hphMX6 bqt1::hygMX6</i>	This study
AFA306	<i>h⁹⁰</i>	<i>ade6-M210 ura4-D18 hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 ase1Δ::KanMX6</i>	This study
AFA316	<i>h⁹⁰</i>	<i>ade6-M210 ura4-D18 hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 sad1.T3S.S52P-13Myc-hphMX6 bqt1::hygMX6 ase1Δ::KanMX6</i>	This study
AFA319	<i>h⁹⁰</i>	<i>hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 sad1.T3S.S52P-13Myc-hphMX6 bqt1::hygMX6 Pact1-LA-GFP:leu1+</i>	This study
AFA336	<i>h⁹⁰</i>	<i>hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 alp14-GFP:Kan</i>	This study
AFA339	<i>h⁹⁰</i>	<i>his3-D1 hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 sad1.T3S.S52P-13Myc-hphMX6 bqt1::hygMX6 alp14-GFP:Kan</i>	This study
AFA350	<i>h⁹⁰</i>	<i>kanR-Palp4-GFP-pkl1 hht1-CFP:his3 aur1R-Pnda3-mCherry-atb2</i>	This study
AFA406	<i>h⁹⁰</i>	<i>kanR-Palp4-GFP-pkl1 hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 sad1.T3S.S52P-13Myc-hphMX6 bqt1::hygMX6</i>	This study
AFA416	<i>h⁹⁰</i>	<i>ade6-M210 hht1-CFP:his3+ Pnda3-mCherry-Atb2:aur1 sad1-GFP:HygMX6</i>	This study
AFA437	<i>h⁹⁰</i>	<i>ade6-M210 hht1-CFP:his3+ Pnda3-mCherry-atb2:aur1 bqt1::hygMX6 sad1.2-GFP:KanMX6</i>	This study
AFA438	<i>h⁹⁰</i>	<i>hht1-CFP:his3+ Pnda3-mCherry-Atb2:aur1 sad1.2-GFP:HygMX6</i>	This study
AFA450	<i>h⁹⁰</i>	<i>hht1-CFP:his3+ Pnda3-mCherry-atb2:aur1 klp9-GFP:KanMX6</i>	This study
AFA467	<i>h⁹⁰</i>	<i>hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 sad1.T3S.S52P-13Myc:hphMX6 bqt1::hygMX6 klp9-GFP</i>	This study
AFA498	<i>h⁺</i>	<i>his3-D1 hht1-CFP:his3+ Pnda3-mCherry-atb2:aur1 alp4-GFP:kanMX6</i>	This study
AFA503	<i>h⁺</i>	<i>kanR-Palp4-GFP-pkl1 hht1-CFP:his3 aur1R-Pnda3-mCherry-atb2</i>	This study
AFA507	<i>h⁻</i>	<i>hht1-CFP:his3+ Pnda3-mCherry-atb2:aur1 sad1-mRFP:kan</i>	This study
AFA509	<i>h⁻</i>	<i>hht1-CFP:his3+ Pnda3-mCherry-atb2:aur1 sid4-mCherry:NatMX6</i>	This study
AFA528	<i>h⁻</i>	<i>sad1.2-mCherry:hygMX6 bqt1::NatMX6 alp4-GFP:kanMX6 hht1-CFP:his3 mCherry-atb2:aur1</i>	This study
AFA530	<i>h⁺</i>	<i>sad1.2-mCherry:hygMX6 bqt1::NatMX6 alp4-GFP:kanMX6 hht1-CFP:his3 mCherry-atb2:aur1</i>	This study
AFA531	<i>h⁺</i>	<i>sad1.2-mCherry:hygMX6 bqt1::natMX6 GFP-Pkl1:kanMX6 hht1-CFP:his3 mCherry-atb2:aur1</i>	This study
AFA533	<i>h⁻</i>	<i>sad1.2-mCherry:hygMX6 bqt1::natMX6 GFP-Pkl1:kanMX6 hht1-CFP:his3 mCherry-atb2:aur1</i>	This study
AFA534	<i>h⁺</i>	<i>sad1.2:natMX6 bqt1::hygMX6 alp4-GFP:kanMX6 hht1-CFP:his3+ Pnda3-mCherry-atb2:aur1</i>	This study
AFA535	<i>h⁻</i>	<i>sad1.2:bleMX6 bqt1::hygMX6 sid4-mCherry:NatMX6 hht1-CFP:his3+ Pnda3-mCherry-atb2:aur1</i>	This study
AFA543	<i>h⁺</i>	<i>sad1.2:natMX6 bqt1::hygMX6 kanR-Palp4-GFP-pkl1 hht1-CFP:his3 aur1R-Pnda3-mCherry-atb2</i>	This study
AFA550	<i>h⁹⁰</i>	<i>dis1::ura4+ sad1.2:hygMX6 bqt1::natMX6 hht1-CFP:his3+ mCherry-atb2:aur1</i>	This study
AFA551	<i>h⁺</i>	<i>dis1::ura4+ hht1-CFP:his3+ mCherry-atb2:aur1</i>	This study
AFA552	<i>h⁻</i>	<i>dis1::ura4+ hht1-CFP:his3+ mCherry-atb2:aur1</i>	This study
AFA1431	<i>h⁹⁰</i>	<i>ade6- hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 klp6::kanMX6</i>	This study
AFA1437	<i>h⁹⁰</i>	<i>hht1-CFP:his3 Pnda3-mCherry-Atb2:aur1 sad1.T3S.S52P::natMX6 bqt1::hygMX6 klp6::kanMX6</i>	This study