Supplementary Materials

Molecular Biology of the Cell

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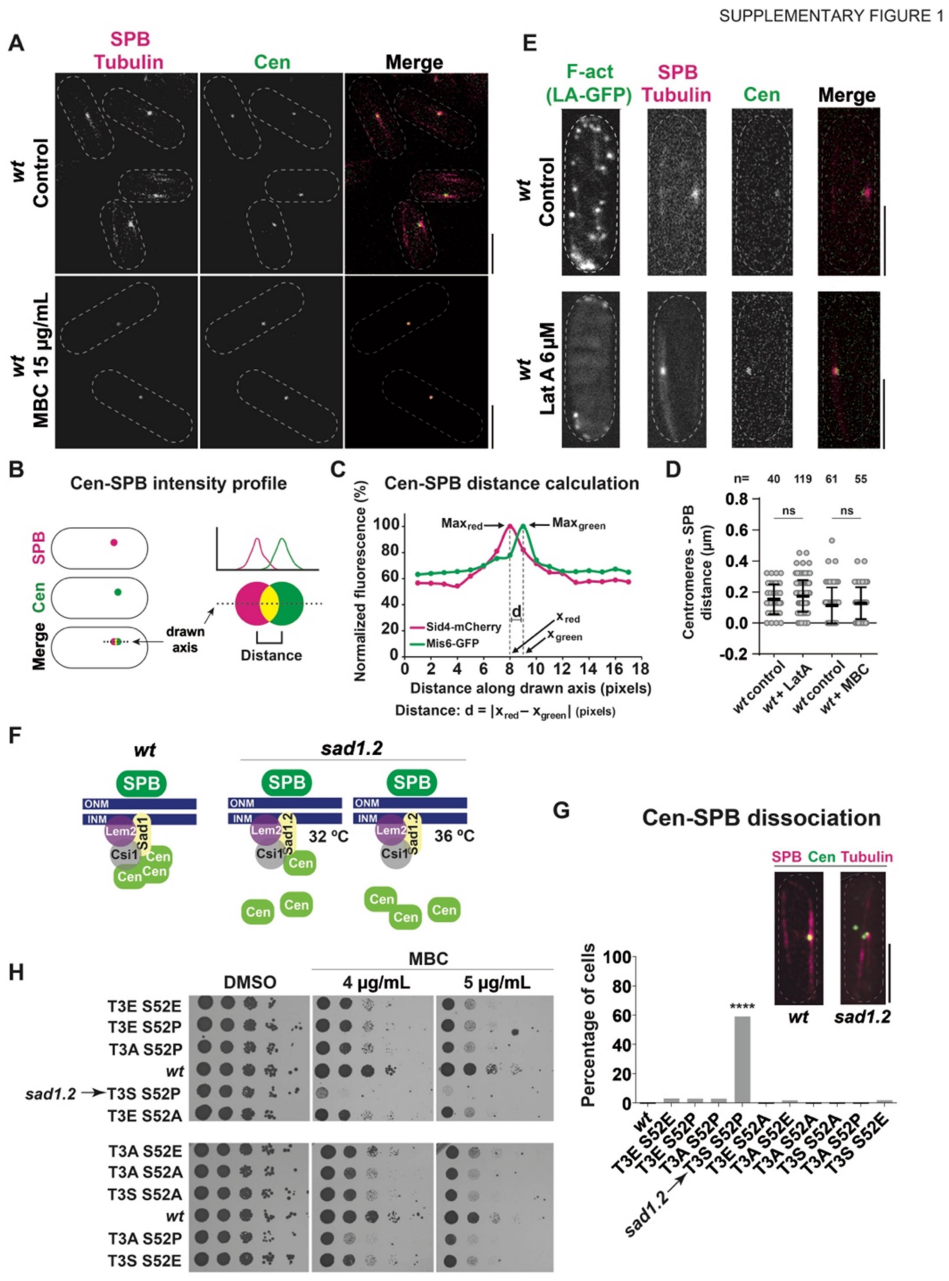
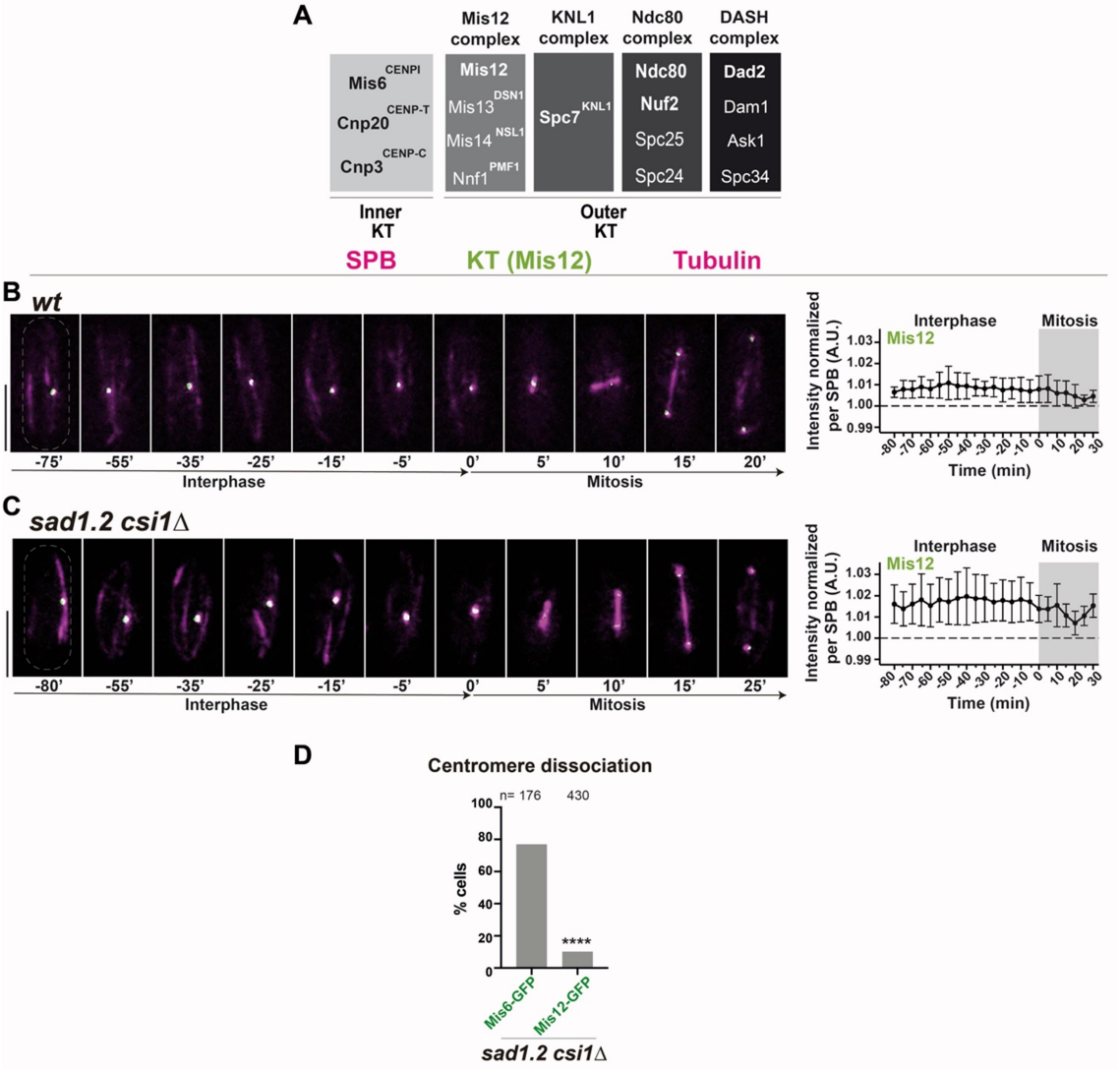


Supplementary Figure 1 (related to Figure 1). Probing requirements for the Rabl chromosome configuration in fission yeast.

(A) Centromere-SPB association patterns upon addition of the microtubule-depolymerizing drug carbendazim (MBC, 15 µg/mL). Centromere (Cen) imaged via endogenously tagged Mis6-GFP. Sid4-mCherry (endogenously tagged; SPB) and ectopically expressed mCherry-Atb2 (Tubulin). Scale bars

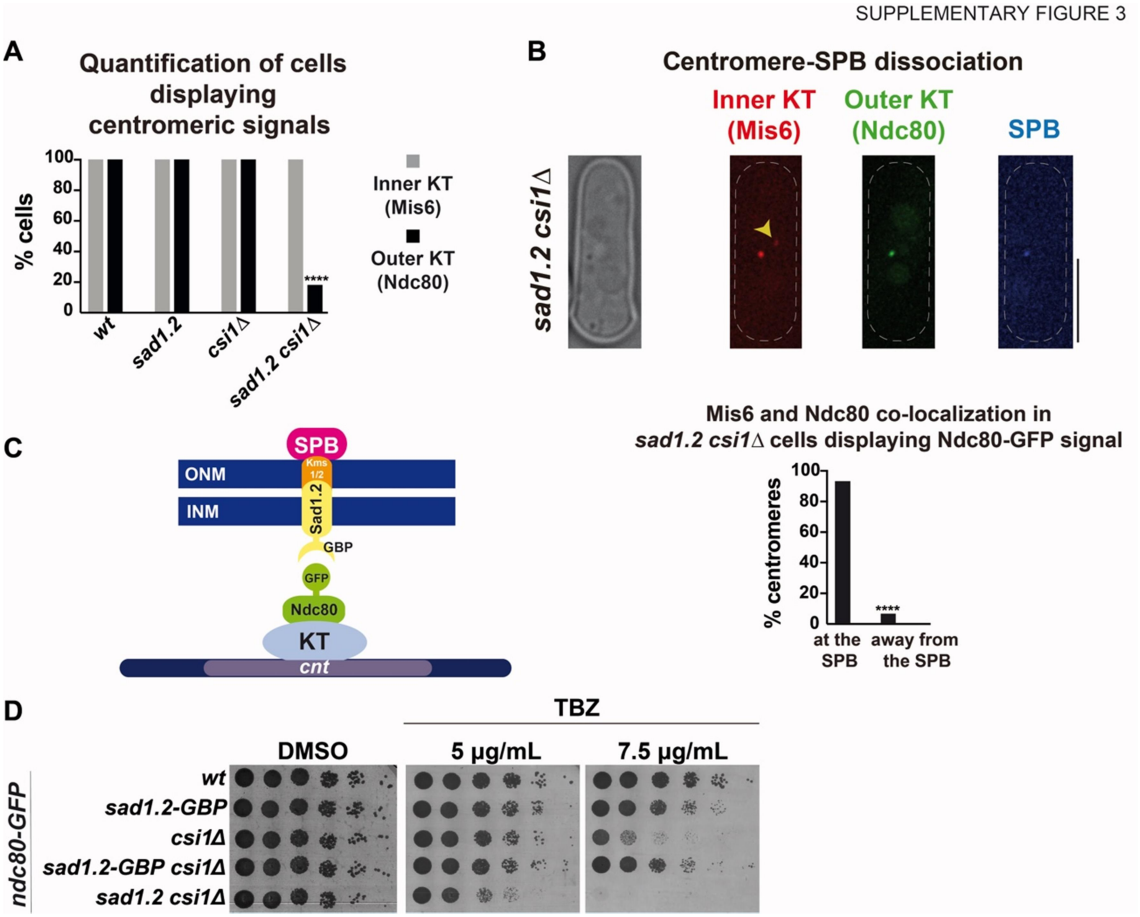
represent 5 μm. (B-C) Scheme of the quantification method followed to measure the distance between the centromere and the SPB. (D) The distance between the SPB and the centromeres is shown; *n* is the total number of cells scored from more than three independent experiments. Data were subject to Fisher's exact test: *ns* = not significant. (E) The centromere-SPB associations remains intact upon the addition of the actin-depolymerizing drug Latrunculin A (Lat A, 6 μM). F-Actin was visualized via the GFP-tagged Lifeact label (LA-GFP). (F) Schematic of the centromere-SPB organization during interphase in *wt* and *sad1.2* cells, at 32ºC and 36ºC. ONM, outer nuclear membrane; INM, inner nuclear membrane (Fernandez-Alvarez and Cooper, 2017b). (G) Quantification of centromere-SPB dissociations. Tags as in (A). Scale bar means 5 μm. 50 cells were scored for each genotype over more than three independent experiments. Fisher's exact test: ****, $p < 0.0001$. (H) Serial dilutions (5-fold) of log-phase cultures were spotted and grown on rich media with DMSO (control) and rich media containing MBC, and incubated at 32ºC during 48 h.



Supplementary Figure 2 (related to Figure 2). The endogenous GFP-tagging of Mis12 reduces the penetrance of centromere-SPB dissociation defects in *sad1.2 csi1Δ* cells.

(A) Main components of the *S. pombe* outer kinetochore, modified from (Hayashi et al., 2006). Uppercase fonts represent orthologs in humans when the name is different in yeast. In bold, the proteins explored in this study. (B-C) Frames from films of cells carrying Sid4-mCherry (SPB), ectopically expressed mCherry-Atb2 (Tubulin), and endogenously tagged Mis12-GFP. Scale bars represent 5 μm. Mis12-GFP intensity was quantified as in Figure 2. 10 cells during more than three independent experiments were monitored for focal

intensity of Mis12-GFP. Error bars represent standard deviations; *t = 0* min is just before SPB spindle formation. (D) Quantification of centromere-SPB dissociations. *n* is the total number of cells scored from more than two independent experiments. *p*-value was determined by Fisher's exact test, **** $p < 0.0001$.



Supplementary Figure 3 (controls for Figure 3). Outer kinetochore is dissociated from centromeres during interphase in Rabl configuration-deficient cells.

(A) Quantification of the phenotypes showed in Figures 2 and 3 for Mis6 and Ndc80 proteins, respectively. 50 cells for each genotype were scored during more than three independent experiments. *p*-value was determined by Fisher's exact test, **** $p < 0.0001$. (B) Ndc80 localization is abolished in centromeres detached from the SPB (pointed by yellow arrow). Quantification of this phenotype was performed during five independent experiments scoring 68

sad1.2 csi1Δ cells displaying Ndc80-GFP signal. *p*-value was determined by Fisher's exact test, **** $p < 0.0001$. *sad1.2 csi1Δ* cells were endogenously tagged Mis6-mCherry (inner kinetochore, KT), Ndc80-GFP (outer kinetochore, KT), and endogenously tagged Sad1.2-Turquoise2 (SPB). Bar represents 5 μm. (C) Schematic of GFP-GBP system used to force interaction between Sad1.2 and centromeres. (D) Serial dilution of log-phase cultures was spotted and analysed as in Figure 1C but using TBZ as microtubule depolymerizing drug.

Strain	Relevant genotype	Source	Figure
AFA394	*h+ ade6-M216 ura4-D18 leu1-32 lem2Δ::natMX6 csi1Δ::bleMX6*	This work	Fig. 1A, B, C
AFA1020	*h- ade6-M210 ura4-D18 leu1-32 sad1.T3S.S52P-13Myc:hphMX6 csi1Δ::natMX6*	This work	Fig. 1A, B, C
AFA10	*h+ ade6-M216*	Lab stock	Fig. 1B, C
AFA217	*h- ade6-M216 sad1.T3S.S52P-13Myc:natMX6*	Lab stock	Fig. 1B, C
AFA1536	*h- ade6-M216 leu1-32 csi1::natMX6*	Lab stock	Fig. 1B, C
AFA1540	*h+ ade6-M216 lem2::natMX6*	Lab stock	Fig. 1B, C
AFA1556	*h90 ade6-M216 sad1.T3S.S52P-13Myc:hphMX6 lem2::natMX6*	This work	Fig. 1B, C
AFA25	*h90 ade6-M210 leu1-32 Pnda3-mCherry-atb2:aur1 sid4-mCherry:natMX6 mis6-GFP:kanMX6*	This work	Fig. 1D, G, H, I; Fig. 2A; Fig. S1 A, D, E, G, H; Fig. S3 A
AFA166	*h90 ade6-M210 leu1-32 Pnda3-mCherry-atb2:aur1 sid4-mCherry:natMX6 mis6-GFP:kanMX6 sad1.T3S.S52P-13Myc:hphMX6 csi1Δ::bleMX6*	This work	Fig. 1E, F, G, H, I; Fig. 2B, C; Fig. S2 D; Fig. S3 A
AFA131	*h90 ade6-M216 Pnda3-mCherry-atb2:aur1 sid4-mCherry:natMX6 mis6-GFP:kanMX6 sad1.T3S.S52P-13Myc:hphMX6*	This work	Fig. 1G, H, I; Fig. S1 G, H; Fig. S3A
AFA1	*h- ade6-M210 leu1-32 Pnda3-mCherry-atb2:aur1 sid4-mCherry:natMX6 mis6-GFP:kanMX6 csi1Δ::bleMX6*	This work	Fig. 1G, H, I; Fig. S3 A
AFA1071	*h- ade6 ura4-D18 leu1-32 mis6-GFP:kanMX6 Pnda3-mCherry-atb2:aur1 sid4-mCherry:natMX6 lem2::natMX6*	This work	Fig. 1G, H, I
AFA1500	*h- ade6-M216 mis6-GFP:kanMX6 sad1.T3S.S52P-13Myc:hphMX6 lem2::natMX6*	This work	Fig. 1G, H, I
AFA1072	*h- ade6 ura4-D18 leu1-32 mis6-GFP:kanMX6 Pnda3-mCherry-atb2:aur1 sid4-mCherry:natMX6 lem2::natMX6 csi1::bleMX6*	This work	Fig. 1G, H, I
AFA21	*h+ ade6-M210 Pnda3-mCherry-atb2:aur1 sid4-mCherry:natMX6 cnp20-GFP:kanMx6*	This work	Fig. 2D
AFA1033	*h90 ade6-M210 Pnda3-mCherry-atb2:aur1 sid4-mCherry:NatMX6 cnp20-GFP:kanMx6 sad1.T3S.S52P-13Myc:hphMX6 csi1Δ::bleMX6*	This work	Fig. 2E, F
AFA1506	*h90 ade6-M210 leu1-32 Pnda3-mCherry-atb2:aur1 sid4-mCherry:natMX6 ndc80-GFP:kanMX6*	This work	Fig. 3A; Fig. S3 A, D
AFA459	*h90 ade6-M216 Pnda3-mCherry-atb2:aur1 sid4-mCherry:natMX6 ndc80-GFP:kanMx6 sad1.T3S.S52P-13Myc:hphMX6 csi1Δ::bleMX6*	This work	Fig. 3B; Fig. S3 A, D
AFA630	*h90 ade6-M216* leu1-32 *Pnda3-mCherry-atb2:aur1 sid4-mcherry:natMX6 ndc80-GFP:kanMX6 sad1.T3S.S52P-GBP:hphMX6 csi1Δ:natMX6*	This work	Fig. 3C; Fig. S3 D
AFA6	*h+ ade6-M216 leu1-32 ura4-D18 Pnda3-mCherry-atb2:aur1 sid4-mCherry:natMX6 nuf2-GFP-HA:kanMX*	This work	Fig. 3D
AFA1047	*h90 ade6-M216 Pnda3-mCherry-atb2:aur1 sid4-mCherry:natMX6 nuf2-HA-GFP:kanMX6 sad1.T3S.S52P-13Myc:hphMX6 csi1Δ::bleMX6*	This work	Fig. 3E
AFA1740	*h- cdc25-22*	Jiménez lab	Fig. 4A
AFA1944	*h- cdc25-22 ndc80-GFP-HA:kanMX6*	This work	Fig. 4A
AFA1945	*h- cdc25-22 ndc80-GFP-HA:kanMX6 sad1.T3S.S52P-GBP:hphMX6 csi1Δ:natMX6*	This work	Fig. 4A

Strain	Relevant genotype	Source	Figure
AFA456	*h- cdc10-129*	Moreno lab	Fig. 4A, B
AFA1951	*h- cdc10-129 ndc80-GFP-HA:kanMX6*	This work	Fig. 4A, B
AFA1938	*h- cdc10-129 ndc80-GFP-HA:kanMX6 sad1.T3S.S52P-GBP:hphMX6 csi1Δ:natMX6*	This work	Fig. 4A, B
AFA1662	*h- ade6-M210 leu1-32 Pnda3-mCherry-atb2:aur1 sid4-mCherry:natMX6 spc7-GFP:kanMX6*	This work	Fig. 4C
AFA1675	*h+ ade6-M210 leu1-32 Pnda3-mCherry-atb2:aur1 sid4-mCherry:natMX6 spc7-GFP:kanMx6 sad1.T3S.S52P-13Myc:hphMX6 csi1Δ::natMX6*	This work	Fig. 4D
AFA832	*h+ ade6-216 leu1-32 ura4-D18 Pnda3-mCherry-atb2:aur1 sid4-mCherry:natMX6 dad2::dad2-GFP-HA:kanMX6*	This work	Fig. 4E
AFA1059	*h+ ade6-M216 leu1-32 ura4-D18 Pnda3-mCherry-atb2:aur1 sid4-mCherry:natMX6 dad2::dad2-GFP-HA:kanMX6 sad1.T3S.S52P-13Myc:hphMX6 csi1::bleMX6*	This work	Fig. 4F
AFA90	*ade6-M216 sad1.T3E.S52E-GFP:kanMX6 mis6-mCherry:natMX6*	This work	Fig. S1 G, H
AFA103	*ade6-M216 sad1.T3E.S52P-GFP:kanMX6 mis6-mCherry:natMX6*	This work	Fig. S1 G, H
AFA106	*ade6-M210 sad1.T3A.S52P-GFP:kanMX6 mis6-mCherry:natMX6*	This work	Fig. S1 G, H
AFA181	*ade6-M210 sad1.T3E.S52A-GFP:kanMX6 mis6-mCherry:natMX6*	This work	Fig. S1 G, H
AFA73	*ade6 sad1.T3A.S52E-GFP:kanMX6 mis6-mCherry:natMX6*	This work	Fig. S1 G, H
AFA76	*ade6 sad1.T3A.S52A-GFP:kanMX6 mis6-mCherry:natMX6*	This work	Fig. S1 G, H
AFA79	*ade6 sad1.T3S.S52A-GFP:kanMX6 mis6-mCherry:natMX6*	This work	Fig. S1 G, H
AFA85	*ade6 sad1.T3S.S52E-GFP:kanMX6 mis6-mCherry:natMX6*	This work	Fig. S1 G, H
AFA7	*h- ade6-M216 leu1-32 ura4-D18 his2-D1 Pnda3-mCherry-atb2:aur1 sid4-mCherry:natMX6 mis12-GFP-HA:kanMX6*	This work	Fig. S2 B
AFA1052	*h- ade6-M216 Pnda3-mCherry-atb2::aur1 sid4-mCherry::natMX6 mis12-HA-GFP:kanMX6 sad1.T3S.S52P-13Myc:hphMX6 csi1Δ::bleMX6*	This work	Fig. S2 C, D
AFA1030	*h90 ade6-M216 Pnda3-mcherry-atb2:aur1 sid4-mCherry:natMX6 ndc80-GFP:kanMX6 sad1.T3S.S52P-13Myc:hphMX6*	This work	Fig. S3 A
AFA1568	*h90 ade6-M216 leu1-32 Pnda3-mCherry-atb2:aur1R sid4-mCherry:natMX6 ndc80-GFP:kanMX6 csi1Δ::bleMX6*	This work	Fig. S3 A, D
AFA1069	*h90 ade6+ leu1-32 mis6-mCherry:hphMX6 ndc80-GFP:kanMX6 sad1.T3S.S52P-turq2:natMX6 csi1::bleMX6*	This work	Fig. S3 B
AFA627	*h90 ade6-M216* leu1-32 *Pnda3-mCherry-atb2:aur1 sid4-mcherry:natMX6 ndc80-GFP:kanMX6 sad1.T3S.S52P-GBP:hphMX6*	This work	Fig. S3 D

Supplementary Table 1. Strains used in this study.

Primer	Name	Sequence	Figure
oAFA14	act1_FW	GATTCTCATGGAGCGTGGTT	Fig. 4B
oAFA15	act1_REV	CGCTCGTTTCCGATAGTGAT	Fig. 4B
oAFA119	cnt1/cnt3_FW	AGCGAAACCATGTGAGCATGT	Fig. 4B
oAFA120	cnt1/cnt3_REV	AGGCTGCTTGCTCTTTTCGT	Fig. 4B

Supplementary Table 2. Primers used in this study for ChIP-qPCR experiments.