Supplemental material

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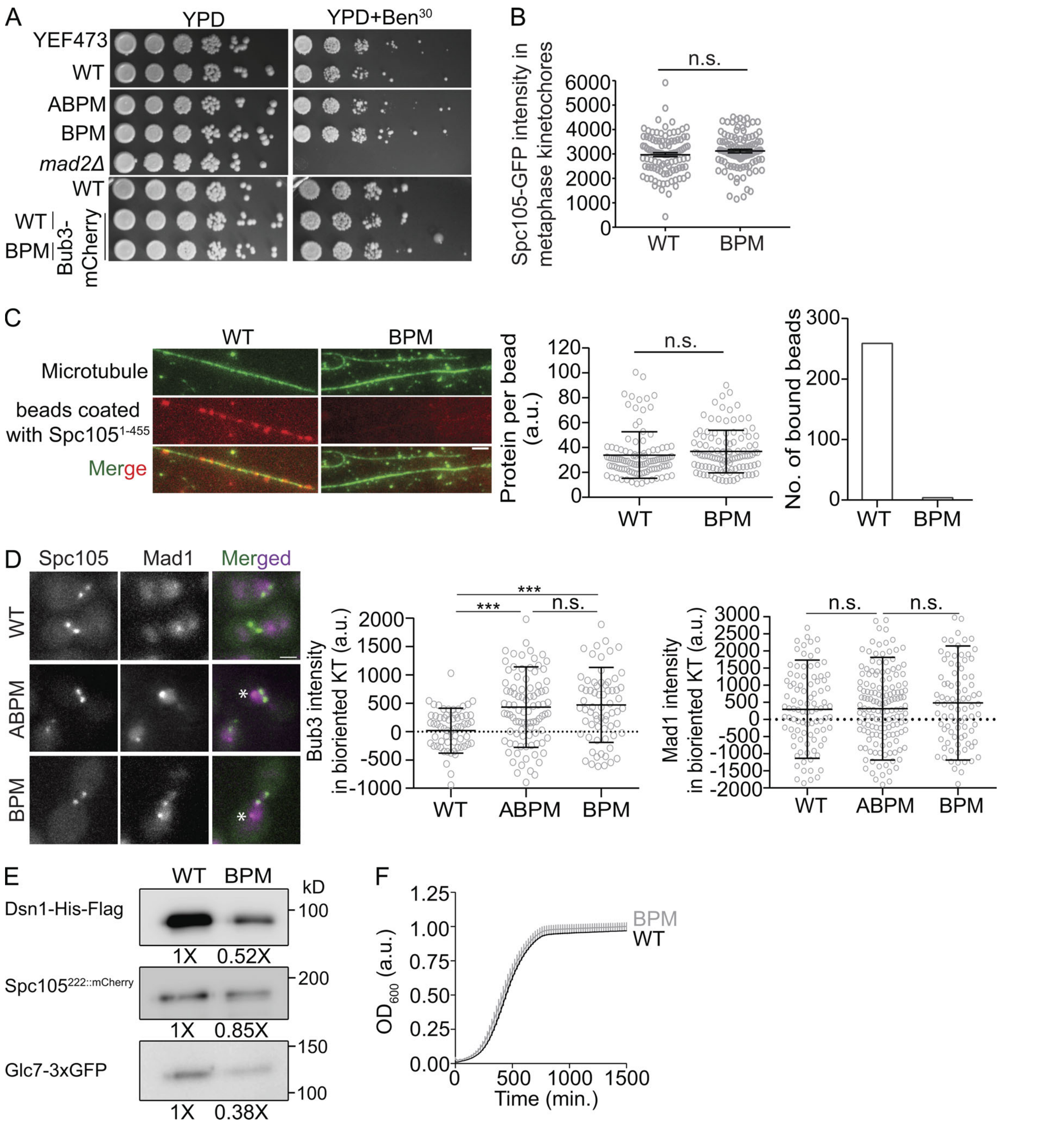

Figure S1. **Effect of the basic patch mutation on Glc7 recruitment, SAC silencing, and cell cycle kinetics.** Related to Fig. 1 and 2. **(A)** Serial dilutions of yeast cells of the indicated genotype on rich medium (YPD) and medium containing benomyl. YEF473 is the parent strain for nearly all the strains constructed in this study. WT refers to a WT strain expressing Spc105$^{222:GFP}$ (GFP inserted at amino acid 222 in Spc105). **(B)** Quantification of the fluorescence due to Spc105$^{222:GFP}$ and Spc105$^{222:GFP,BPM}$ in metaphase kinetochore clusters (data are shown as mean ± SEM; n = 102 for WT and 104 for BPM, pooled from two technical replicates; P = 0.1605 using unpaired t test). **(C)** Left: TIRF micrographs showing the interaction of polystyrene beads coated with recombinant Spc105 phosphodomain (red, amino acid residues 1–455, containing either WT or mutant basic patches) with X-rhodamine labeled, taxol-stabilized microtubules (green). Scale bar: ∼3.2 µm. Middle: Quantification of the average amount of protein conjugated per bead (data are shown as mean ± SD; n = 108 from two experiments; P = 0.24 using unpaired t test). Right: Number of interactions observed for the respective phosphodomain (n = 259 and 4 for WT and BPM, respectively, from two experiments). **(D)** Representative images of metaphase cells expressing the indicated protein. The asterisk in the merged image denotes a nuclear Mad1-mCherry aggregate that forms in the absence of the nuclear pore protein Nup60 (Scott et al., 2005). Scatter plots: Quantification of Bub3-mCherry and Mad1-mCherry recruitment by bioriented kinetochores in metaphase-arrested cells of the indicated genotype. Data are presented as mean + SD (n = 62, 95, and 68 for WT, ABPM, and BPM, respectively, pooled from two experiments in Bub3-mCherry intensity measurement, and n = 103, 165 and 100 for WT, ABPM, and BPM, respectively, pooled from two assays in Mad1-mCherry intensity measurement; P < 0.0001 [***] and 0.1622 for Bub3 and Mad1, respectively, using t test). Scale bar: ∼3.2 µm. **(E)** Replicate data related to Fig. 2 A. Here, the loading volumes were adjusted to equalize the amount of Spc105 coprecipitating with Dsn1-Flag to make it possible to visually assess the amount of Glc7-3xGFP coprecipitation. **(F)** Graph depicting quantification of the evolution of optical density at 600 nm (OD_{600}) for strains expressing Spc105^{WT} and Spc105^{BPM} as measured by a 96-well plate reader (three technical replicates were used; data for each time point are presented as mean + SEM). n.s., not significant.

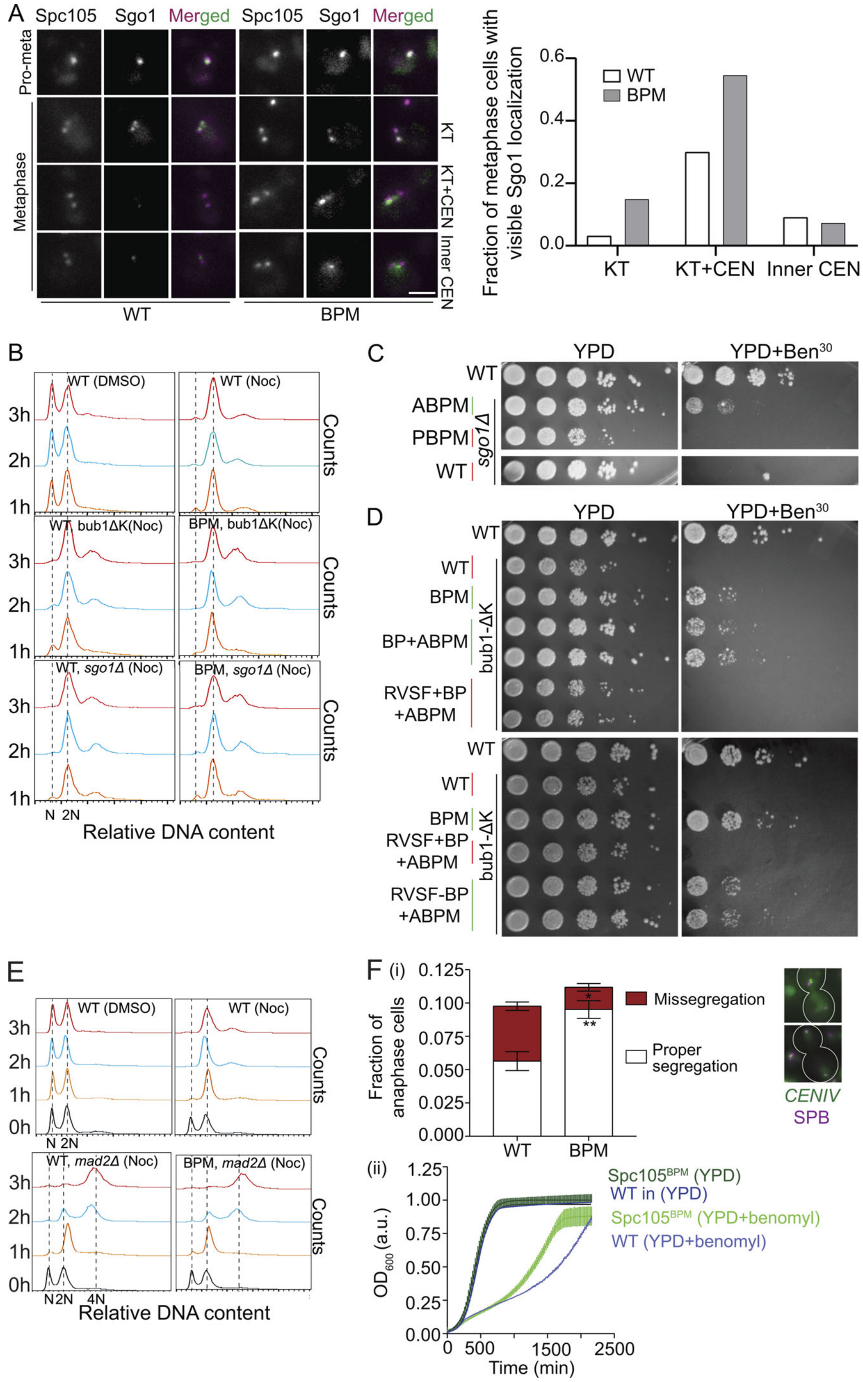

Figure S2. **The basic patch mutation improves chromosome segregation in benomyl-containing medium independently of Sgo1.** Related to Figs. 3 and 4. **(A)** Left: Representative micrographs showing the localization of Sgo1-GFP and Spc105$^{222:mCherry}$ in prometaphase (single kinetochore cluster) and metaphase (two distinct kinetochore clusters) cells. The designations used for scoring cell populations is indicated on the righthand side (KT, kinetochore-colocalized; KT+CEN, Sgo1 colocalizes with both kinetochores and the centromeric region in between the two kinetochore clusters; inner CEN, Sgo1 localized mainly in the centromeric region, positioned between two sister kinetochore foci). Scale bar: ~3.2 µm. Right: Scoring shown in the bar graph on the right. The numbers above represent the number of cells we examined for this assay (n = 67 and 209 for WT and BPM, respectively, pooled from two experiments; $P <$ 0.0084 for KT and KT+CEN and 0.6037 for inner CEN by Fisher's exact test). **(B)** Flow cytometry–based evaluation of SAC signaling activity in strains that lack Sgo1 or express Bub1$^{\Delta kinase}$. **(C and D)** Benomyl spotting assay of the indicated strains. **(E)** Quantification of DNA content using flow cytometry after activation of the SAC by nocodazole treatment (0 h). The first peak in each graph corresponds to cells with 1n DNA content (G1), whereas the second peak corresponds to cells with 2n DNA content (G2/M). In cells with an inactive SAC (*mad2Δ*), cells fail to arrest in M and shift to the 4n peak (tetraploid). **(F, i)** Right: Grouped bar graph shows the scoring of anaphase cells based on segregation of *CENIV* in benomyl. Data are shown as mean + SEM, where n = 1,424 and 1,173 for WT and BPM, respectively, pooled from at least three technical repeats. Two-way ANOVA revealed that P = 0.001 (**) and 0.0224 (*) for anaphase cells with proper segregation and missegregation, respectively. Left: Representative images of cells showing proper and improper segregation of chromosome IV. Scale bar: ~3.2 µm. **(F, ii)** Graph depicting the OD measurements of strains expressing Spc105^{WT} and Spc105^{BPM} at the indicated time points as measured by a 96-well plate reader. For each time point, three technical replicates were used. The data for each time point are presented as mean + SEM.

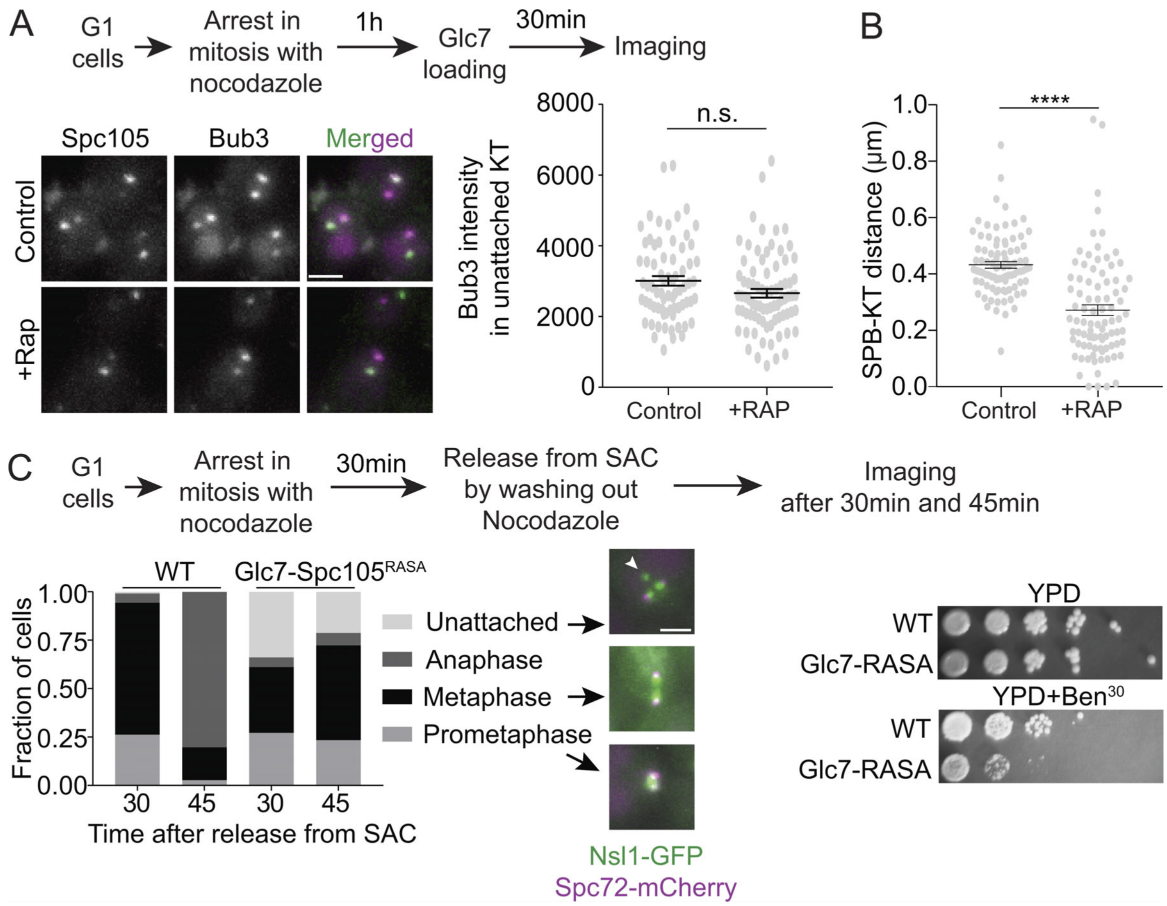

Figure S3. **Fusion of Glc7 to the N-terminus of Spc105 impairs chromosome biorientation.** Related to Fig. 5. **(A)** Top: Flow chart describes the workflow to tether Glc7 to the N-terminus of Spc105 in the presence of nocodazole. Bottom, left: Representative images of FRB-GFP-Spc105, Bub3-mCherry and merged in unattached kinetochores of control and rapamycin-treated cells. Scale bar: ∼3.2 µm. Bottom, right: Scatter plot showing Bub3-mCherry intensities in unattached kinetochores of rapamycin-treated and untreated control cells (mean ± SEM; P = 0.0585 by unpaired *t* test). Kinetochores analyzed for this assay: *n* = 71 and 78 for control and rapamycin-treated cells, respectively, pooled from two experiments performed using two biological replicates. **(B)** Scatter plot showing spindle pole body (SPB)–KT distances in untreated control and rapamycin-treated cells. Data are presented as mean ± SEM (*n* = 87 for both control and rapamycin-treated cells; data were pooled from three experimental repeats; ****, P < 0.0001 with unpaired *t* test). **(C)** Comparative characterization of the kinetics of kinetochore biorientation in the strain expressing the chimeric molecule Glc7-Spc105^{RASA} (constructed by Rosenberg et al. (2011); workflow shown at the top). Bottom, left: Bar graph displays the frequency of the indicated phenotypes. Kinetochores analyzed for this assay: *n* = 122 and 118 for WT and Glc7-RASA, respectively, at 30 min, and 118 and 94 for WT and Glc7-RASA at 45 min, pooled from two experiments performed using two biological replicates. Scale bar: ∼3.2 µm. Bottom, right: Spotting assay indicates that the *GLC7-spc105^{RASA}* strain is sensitive to benomyl. n.s., not significant.

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Table S1. **Strains used in this study, which were constructed in the Joglekar laboratory**

Strain (AJY#)	Genotype	Used in figure
5199	*MATa, trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, TetR-GFP (LEU2); SPC29-mCherry-HIS3, TetO-CENIV (URA3), spc105Δ::TRP1::*Spc105 $^{222::mCherry}$ *(KAN)*	1 C and S2 F, i
5201	*MATa, trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, TetR-GFP (LEU2); SPC29-mCherry-HIS3, TetO-CENIV (URA3), spc105Δ::TRP1::*Spc105 $^{222::mCherry,101-104,340-340::AAAA}$ *(KAN)*	1 C and S2 F, i
5078	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP}$ *(LEU2), SPC97-*mcherry*-HYG, prMET3-CDC20 (URA3)*	1 D
5080	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-343::AAAA}$ *(LEU2), SPC97-*mcherry*-HYG, prMET3-CDC20 (URA3)*	1 D
3635	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP}$ *(LEU2), BUB3-*mcherry*-HYG, prMET3-CDC20-URA3*	1 E, S1 B, S1 D
3637	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* spc105$^{222::GFP,101-104::AAAA}$ *(LEU2), BUB3-*mcherry*-HYG, prMET3-CDC20 (URA3)*	1 E, S1 D
3638	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-343::AAAA}$ *(LEU2), BUB3-*mcherry*-HYG, prMET3-CDC20 (URA3)*	1 E, S1 B, S1 D
3797	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP}$ *(LEU2), MAD1-*mCherry*-HYG, nup60Δ::TRP1, prMET3-CDC20 (URA3)*	1 E and S1 D
3798	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-343::AAAA}$ *(LEU2), MAD1-*mCherry*-HYG, nup60Δ::TRP1, prMET3-CDC20 (URA3)*	1 E and S1 D
3939	*MatA, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105 222::GFP (LEU2) 101-104::AAAA, nup60Δ:: Trp1, Mad1-mcherry:hyg, prMET3-Cdc20 (URA3)*	1 E and S1 D
5176	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* BP(Spc105$^{79-145}$)-Spc105$^{222::GFP, 101-104::AAAA}$ *(LEU2), BUB3-mCherry-HYG*	1 F, i
5177	*MATα trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* BP(Spc105$^{79-145}$)-Spc105$^{222::GFP, 101-104::AAAA}$ *(LEU2), BUB3-mCherry-HYG*	1 F, i
5384	*MATα trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* EBP2(Spc105$^{55-145}$)-Spc105$^{222::GFP, 101-104::AAAA}$ *(LEU2), BUB3-mCherry-HYG*	1 F, i
3606	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105 $^{222::GFP}$ *(LEU2), BUB3-mCherry-HYG*	1 F, i; 2 B; S1 A
3627	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105 $^{222::GFP,101-104,340-340::AAAA}$ *(LEU2), BUB3-mCherry-HYG*	1 F, i; 2 B; S1 A
4650	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105$^{222::GFP}$ (LEU2), Bub1-ymCherry-Hyg*	1 F, ii; 4 B; 5 C; S1 F; S2 F, ii
4651	*MATα trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105 $^{222::GFP}$ (LEU2), Bub1-ymCherry-Hyg*	1 F, ii; 4 B; 5 C
4652	*MATα trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105$^{101-104,340-343::AAAA}$ 222::GFP (LEU2), Bub1-ymCherry-Hyg*	1 F, ii; 4 B; 5 C; S1 F; S2 F, ii
4653	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105$^{101-104,340-343::AAAA}$ 222::GFP (LEU2), Bub1-ymCherry-Hyg*	1 F, ii; 4 B; 5 C
5732	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, RVSF+BP (Spc105$^{55-145}$)-Spc105$^{222::GFP\ 101-104}$ (LEU2), BUB1-ymCherry-HYG*	1 F, ii
5733	*MATα, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, RVSF+BP (Spc105$^{55-145}$)-Spc105$^{222::GFP\ 101-104}$ (LEU2), BUB1-ymCherry-HYG*	1 F, ii
4214	*MatA, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, TOG2-Spc105$^{222::GFP}$ (LEU2), Bub3-mCherry-Hyg, prMET3-Cdc20 (URA3)*	1 G
4215	*Matα, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, TOG2-Spc105 222::GFP 101-104::AAAA (LEU2), Bub3-mCherry-Hyg, prMET3-Cdc20 (URA3)*	1 G
5523	*MATa, trp1Δ63 ura3-52 his3Δ200 leu2Δ-1, can1, spc105Δ::NAT, SPC10$^{222::mCherry}$ (LEU2), Glc7-3XGFP-HIS3, Dsn1-HIS-FLAG (URA3)*	2 A and S1 E
5524	*MATa, trp1Δ63 ura3-52 his3Δ200 leu2Δ-1, can1, spc105Δ::NAT, SPC105$^{101-104,340-343::AAAA\ 222::mCherry}$ (LEU2), Glc7-3XGFP-HIS3, Dsn1-HIS-FLAG (URA3)*	2 A and S1 E
3626	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,\ 101-104::AAAA}$ *(LEU2), BUB3-mCherry-HYG*	2 B
3133	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1:: Spc105$^{222::GFP}$ (LEU2)*	S1 A; 2, C and D; 3, i–vi; 4, C, E, and F; 5 D; S2, B-E

Table S1. **Strains used in this study, which were constructed in the Joglekar laboratory (*Continued*)**

Strain (AJY#)	Genotype	Used in figure
3448	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,\ 101-104,340-340::AAAA}$ *(LEU2)*	S1 A; 2, C and D; 3, i–iii, v, and vi
4384	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-340::AAAA}$ *(LEU2),* bub1ΔK-*mCherry-HYG*	3 i, S2 D, 4 C, S2 B
4385	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-340::AAAA}$ *(LEU2),* bub1ΔK-*mCherry-HYG*	3 i
4415	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP}$ *(LEU2),* bub1ΔK-*mCherry-HYG*	3 i; 4, C and F; S2, B and D
4416	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP}$ *(LEU2),* bub1ΔK-*mCherry-HYG*	3 i
4485	*MATa, trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, BUB1*-mCherry-*HYG*	3 i
4203	*MATα, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP}$ *(LEU2), sgo1Δ::Kan*	3 ii, 4 F, 5 D, S2 B
4204	*MATα, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP}$ *(LEU2), sgo1Δ::Kan*	3 ii
4205	*MATa, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-340::AAAA}$ *(LEU2), sgo1Δ::Kan*	3 ii and S2 B
4206	*MATa, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-340::AAAA}$ *(LEU2), sgo1Δ::Kan*	3 ii
4233	*MATa, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP}$ *(LEU2), rts1Δ::Kan*	3 iii
4234	*MATa, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP}$ *(LEU2), rts1Δ::Kan*	3 iii
4235	*MATa, trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-340::AAAA}$ *(LEU2), rts1Δ::Kan*	3 iii
4236	*MATa, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-340::AAAA}$ *(LEU2), trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, rts1Δ::Kan*	3 iii
4022	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105-6A *(LEU2)*	3, ii, iii, and iv
4338	*trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP}$ *(LEU2), ipl1Δ::TRP1, ipl1-2 (H352Y, CEN, URA3)*	3 iv
4339	*trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP}$ *(LEU2), ipl1Δ::TRP1, ipl1-2 (H352Y, CEN, URA3)*	3 iv
4340	*trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-340::AAAA}$ *(LEU2), ipl1Δ::TRP1, ipl1-2 (H352Y, CEN, URA3)*	3 iv
4341	*trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-340::AAAA}$ *(LEU2), ipl1Δ::TRP1, ipl1-2 (H352Y, CEN, URA3)*	3 iv
4357	*trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP}$ *(LEU2), ipl1Δ::TRP1, IPL1 (CEN, URA3)*	3 iv
4362	*trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-340::AAAA}$ *(LEU2), ipl1Δ::TRP1, IPL1 (CEN, URA3)*	3 iv
4712	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP}$ *(LEU2), ndc80Δ:: TRP1::* ndc80-6A-*12MYC (KAN)*	3 v
4713	*Matα trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP}$ *(LEU2), ndc80Δ:: TRP1::* ndc80-6A-*12MYC (KAN)*	3 v
4714	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-340::AAAA}$ *(LEU2), ndc80Δ:: TRP1::* ndc80-6A-*12MYC (KAN)*	3 v
4715	*MATα trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-340::AAAA}$ *(LEU2), ndc80Δ:: TRP1::* ndc80-6A-*12MYC (KAN)*	3 v
4920	*MATα trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, dam1Δ::TRP1::*dam1(S257A S265A S292A) *(KAN)*	3 vi and 5 D
4921	*MATα trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, dam1Δ::TRP1::*dam1(S257A S265A S292A) *(KAN)*	3 vi
4904	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-340::AAAA}$ *(LEU2), dam1Δ::TRP1::*dam1(S257A S265A S292A) *(KAN)*	3 vi
4905	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-340::AAAA}$ *(LEU2), dam1Δ::TRP1::*dam1(S257A S265A S292A) *(KAN)*	3 vi
4964	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, dam1Δ::TRP1::*dam1(S257A S265A S292A) *(KAN), ndc80Δ:: TRP1::* ndc80-6A-*12MYC (KAN), NSL1-GFP-HIS3*	3 vi

Table S1. **Strains used in this study, which were constructed in the Joglekar laboratory (*Continued*)**

Strain (AJY#)	Genotype	Used in figure
4965	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, dam1Δ::TRP1::*dam1(S257A S265A S292A) *(KAN), ndc80Δ:: TRP1::* ndc80-6A-*12MYC (KAN), NSL1-GFP-HIS3*	3 vi
5000	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, dam1Δ::TRP1::*dam1 (S257A S265A S292A) *(KAN), ndc80Δ:: TRP1::* ndc80-6A-*12MYC (KAN), NSL1-GFP-HIS3, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::mCherry,101-104,340-340::AAAA}$ *(LEU2)*	3 vi
5273	*MATa, trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, TetR-GFP (LEU2); SPC29-mCherry-HIS3, TetO-CENIV (URA3), spc105Δ::TRP1::*Spc105$^{222::mCherry}$ *(KAN), sgo1Δ::TRP1*	4 A
5267	*MATa, trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, TetR-GFP (LEU2); SPC29-mCherry-HIS3, TetO-CENIV (URA3), spc105Δ::TRP1::*Spc105$^{222::mCherry,101-104,340-340::AAAA}$ *(KAN), sgo1Δ::TRP1*	4 A
5756	*MATa, trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105*$^{222::GFP,21-24\ (GILK)::AAAA}$ *(LEU2), BUB1-mCherry-HYG*	4 B
5757	*MATa, trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105*$^{222::GFP,21-24\ (GILK)::AAAA}$ *(LEU2), BUB1-mCherry-HYG*	4 B
5778	*MATα, trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2:Spc105*$^{222::GFP\ GILK:AAAA,101-104::AAAA}$ *(LEU2), BUB1-mCherry-HYG*	4 B
5825	*MATa, trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2:Spc105* $^{222::GFP\ 21-24\ (GILK)::AAAA,101-104::AAAA}$ *(LEU2), BUB1-mCherry-HYG*	4 B
5764	*MATα, trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2:Spc105*$^{222::GFP,21-24\ (GILK)::AAAA,101-104::AAAA}$ *(LEU2), bub1-ΔK-mCherry-HYG*	4 C
5765	*MATα, trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105*$^{222::GFP,21-24\ (GILK)::AAAA}$ *(LEU2), bub1ΔK-mCherry-HYG*	4 C
5840	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::Spc105*$^{222::GFP}$ *(LEU2), Spc97-mCherry-HYG, mad3Δ::KAN*	4 D
5841	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::Spc105*$^{222::GFP}$ *(LEU2), Spc97-mCherry-HYG, mad3Δ::KAN*	4 D
5842	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::Spc105*$^{222::GFP,RASA\ (V76,F78::A)}$ *(LEU2), Spc72-mCherry-HYG, mad3Δ::URA3*	4 D
5843	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::Spc105*$^{222::GFP,RASA\ (V76,F78::A)}$ *(LEU2), Spc72-mCherry-HYG, mad3Δ::URA3*	4 D
4555	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP}$ *(LEU2), mad2Δ::TRP1*	4 E, S1 A, S2 E
4556	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP}$ *(LEU2), mad2Δ::TRP1*	4 E
4557	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::*Spc105$^{222::GFP,101-104,340-340::AAAA}$ *(LEU2), mad2Δ::TRP1*	4 E and S2 E
4558	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104,340-340::AAAA}$ *(LEU2), mad2Δ::TRP1*	4 E
4949	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT1, leu2Δ-1::* Spc105$^{222::GFP,RASA\ (V76,F78::A)}$ *(LEU2), mad2Δ::TRP1*	4 E
4950	*MATα, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT1, leu2Δ-1::* Spc105$^{222::GFP,RASA\ (V76,F78::A)}$ *(LEU2), mad2Δ::TRP1*	4 E
4951	*leu2Δ-1 trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, mad2Δ::TRP1*	4 E
5007	*MATα, leu2Δ-1 trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, bub3Δ::KAN*	4 E
5009	*MATa, trp1Δ63 leu2Δ-1 his3Δ200 lys2-8Δ1, spc105Δ::NAT1, ura3-52::* Spc105$^{222::GFP,RASA\ (V76,F78::A)}$ *(URA3), bub3Δ::KAN*	4 E
5010	*MATa, trp1Δ63 leu2Δ-1 his3Δ200 lys2-8Δ1, spc105Δ::NAT1, ura3-52::* Spc105$^{222::GFP,RASA\ (V76,F78::A)}$ *(URA3), bub3Δ::KAN*	4 E
4682	*MATa, trp1Δ63 leu2Δ-1 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105*$^{222::GFP}$ *(LEU2), bub3Δ::KAN*	4 E
4683	*MATa, trp1Δ63 leu2Δ-1 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105*$^{222::GFP}$ *(LEU2), bub3Δ::KAN*	4 E
4737	*trp1Δ63 leu2Δ-1 his3Δ200 lys2-8Δ1, spc105Δ::NAT,Spc105*$^{101-104,340-343::AAAA\ 222::GFP}$ *(LEU2), bub3Δ::KAN*	4 E
4738	*trp1Δ63 leu2Δ-1 his3Δ200 lys2-8Δ1, spc105Δ::NAT,Spc105*$^{101-104,340-343::AAAA\ 222::GFP}$ *(LEU2), bub3Δ::KAN*	4 E
5699	*MATα, trp1Δ63 leu2Δ-1 his3Δ200 lys2-8Δ1, bub1ΔK-mCherry-HYG, mad2Δ::TRP1*	4 F

Table S1. **Strains used in this study, which were constructed in the Joglekar laboratory (*Continued*)**

Strain (AJY#)	Genotype	Used in figure
5635	*MATa, trp1Δ63 leu2Δ-1 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105*$^{101-104,340-343::AAAA\ 222::GFP}$ *(LEU2), mad2Δ::TRP1, bub1ΔK-mCherry-HYG*	4 F
5636	*MATa, trp1Δ63 leu2Δ-1 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105*$^{101-104,340-343::AAAA\ 222::GFP}$ *(LEU2), mad2Δ::TRP1, bub1ΔK-mCherry-HYG*	4 F
5637	*MATa, trp1Δ63 leu2Δ-1 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105*$^{RASA\ (V76,F78::A)\ 222::GFP}$ *(LEU2), mad2Δ::TRP1, bub1ΔK-mCherry-HYG*	4 F
5638	*MATa, trp1Δ63 leu2Δ-1 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105*$^{RASA\ (V76,F78::A)\ 222::GFP}$ *(LEU2), mad2Δ::TRP1, bub1ΔK-mCherry-HYG*	4 F
4979	*trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, mad2Δ::TRP1, sgo1Δ::KAN*	4 F
4980	*MATα, trp1Δ63 leu2Δ-1 ura3-52 his3Δ200 lys2-8Δ1, mad2Δ::TRP1, sgo1Δ::KAN*	4 F
4981	*MATα, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT1, leu2Δ-1::* Spc105$^{222::GFP,RASA\ (V76,F78::A)}$ *(LEU2), mad2Δ::TRP1, sgo1Δ::KAN*	4 F
4982	*MATα, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT1, leu2Δ-1::* Spc105$^{222::GFP,RASA\ (V76,F78::A)}$ *(LEU2), mad2Δ::TRP1, sgo1Δ::KAN*	4 F
4978	*MATα, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT1, leu2Δ-1::* Spc105$^{222::GFP,RASA\ (V76,F78::A)}$ *(LEU2), mad2Δ::TRP1, sgo1Δ::KAN*	4 F
5698	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, fpr1Δ, FRB-GFP-Spc105 (LEU2), Spc72-mCherry-HYG, Glc7-1XFKBP12-HIS3, prMet3-Cdc20 (URA3)*	5 A and S3 B
5495	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, leu2Δ-1::* FRB-GFP-Spc105 *(LEU2), Glc7-1XFKBP-HIS3, SPC72*-mCherry-*HYG, prMET3-CDC20 (URA3)*	5 B
4733	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105*$^{222::GFP\ 105-107::EEE,S109E}$ *(LEU2), Bub1-ymCherry-Hyg*	5 C
4734	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105*$^{222::GFP\ 105-107::EEE,S109E}$ *(LEU2), Bub1-ymCherry-Hyg*	5 C
4778	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,101-104::AAAA}$ *(LEU2), sgo1Δ::Kan*	5 D
5087	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT1, leu2Δ-1::* Spc105$^{222::GFP,105-107::EEE,S109E}$ *(LEU2), sgo1Δ::KAN*	5 D
5088	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT1, leu2Δ-1::* Spc105$^{222::GFP,105-107::EEE,S109E}$ *(LEU2), sgo1Δ::KAN*	5 D
5548	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105*$^{222::GFP\ 105-107::EEE,S109E}$ *(LEU2), dam1Δ::TRP1::dam1(S257A S265A S292A)::KAN-MX*	5 D
5549	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, Spc105*$^{222::GFP\ 105-107::EEE,S109E}$ *(LEU2), dam1Δ::TRP1::dam1(S257A S265A S292A)::KAN-MX*	5 D
5186	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT1, leu2Δ-1::* Spc105$^{222::GFP,101-104::AAAA}$ *(LEU2), sgo1Δ::KAN*	5 D and S2 C
5187	*MATα, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT1, leu2Δ-1::* Spc105$^{222::GFP,101-104::AAAA}$ *(LEU2), sgo1Δ::KAN*	5 D
5188	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT1, leu2Δ-1::* Spc105$^{222::GFP,S77D}$ *(LEU2), sgo1Δ::KAN*	5 D
5162	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT1, leu2Δ-1::* Spc105$^{222::GFP,S77D,105-107::EEE,S109E}$ *(LEU2), sgo1Δ::KAN*	5 D
5163	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT1, leu2Δ-1::* Spc105$^{222::GFP,S77D,105-107::EEE,S109E}$ *(LEU2), sgo1Δ::KAN*	5 D
3442	*MATa trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::GFP,\ 101-104::AAAA}$ *(LEU2)*	S1 A
4526	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* spc105$^{222::mCherry,101-104,340-340::AAAA}$ *(LEU2), SGO1-GFP-HIS3*	S2 A
4527	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1,spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::mCherry}$*(LEU2), SGO1-GFP-HIS3*	S2 A
4528	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1::* Spc105$^{222::mCherry}$*(LEU2), SGO1-GFP-HIS3*	S2 A
5086	*MATa, spc105Δ::NAT, leu2Δ-1::* Spc105 $^{222::GFP,340-343::AAAA}$ *(LEU2), sgo1Δ::KAN*	S2 C

Table S1. **Strains used in this study, which were constructed in the Joglekar laboratory (*Continued*)**

Strain (AJY#)	Genotype	Used in figure
5381	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1:: BP* (Spc105$^{79–145}$)-Spc105$^{222::GFP,101–104,340–340::AAAA}$ *(LEU2),* bub1ΔK-*mCherry-HYG*	S2 D
5382	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1:: BP* (Spc105$^{79–145}$)-Spc105$^{222::GFP,101–104,340–340::AAAA}$ *(LEU2),* bub1ΔK-*mCherry-HYG*	S2 D
5389	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1:: RVSF+BP* (Spc105$^{55–145}$)-Spc105$^{222::GFP,101–104,340–340::AAAA}$ *(LEU2),* bub1ΔK-*mCherry-HYG*	S2 D
5390	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, leu2Δ-1:: RVSF+BP* (Spc105$^{55–145}$)-Spc105$^{222::GFP,101–104,340–340::AAAA}$ *(LEU2),* bub1ΔK-*mCherry-HYG*	S2 D
5749	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, RVSF* (Spc105$^{55–145,101–104::AAAA}$)*+Spc105 222:: GFP 101-104::AAAA (LEU2),bub1ΔK-mCherry-HYG*	S2 D
5750	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, spc105Δ::NAT, RVSF* (Spc105$^{55–145,101–104::AAAA}$)*+Spc105 222:: GFP 101-104::AAAA (LEU2),bub1ΔK-mCherry-HYG*	S2 D
5505	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, fpr1Δ, FRB-GFP-Spc105 (LEU2), Glc7-1XFKBP12-HIS3, Bub3-ymCherry-HYG*	S3 A
5506	*MATa, trp1Δ63 ura3-52 his3Δ200 lys2-8Δ1, fpr1Δ, FRB-GFP-Spc105 (LEU2), Glc7-1XFKBP12-HIS3, Bub3-ymCherry-HYG*	S3 A
5817	*MATa, trp1Δ63 ura3-52 his3Δ200 leu2Δ-1, can1, Nsl1-GFP-NAT, Spc72-mCherry-HYG*	S3 C
5818	*MATa, trp1Δ63 ura3-52 his3Δ200 leu2Δ-1, can1, Nsl1-GFP-NAT, Spc72-mCherry-HYG*	S3 C
5819	*MATa, trp1Δ63 ura3-52 his3Δ200 leu2Δ-1, can1, Nsl1-GFP-NAT, Spc72-mCherry-HYG, lys2::LYS2-GAL-HO Glc7-spc105* $^{RASA\ (V76,F78::A)}$	S3 C
5820	*MATa, trp1Δ63 ura3-52 his3Δ200 leu2Δ-1, can1, Nsl1-GFP-NAT, Spc72-mCherry-HYG, lys2::LYS2-GAL-HO Glc7-spc105* $^{RASA\ (V76,F78::A)}$	S3 C
3380	*MATa, trp1Δ63 ura3-52 his3Δ200 leu2Δ-1, can1*	S3 C
2675	*MATa, trp1Δ63 ura3-52 his3Δ200 leu2Δ-1, can1, lys2::LYS2-GAL-HO Glc7-spc105*$^{RASA\ (V76,F78::A)}$	S3 C

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

Plasmid	Origin	Parent	Description
pAJ108	Joglekar laboratory	pSK954	ndc80-6A (T21A, S37A, T54A, T71A, S95A, S100A)-12Myc (*KAN*)
pSB148	Biggins laboratory	pRS316	*prIPL1+IPL1+trIPL1 (CEN, URA3)*
pSB617	Biggins laboratory		dam1(S257A S265A S292A), (*KAN)*
pAJ449	This study	pRS305	*prSPC105*+Spc105$^{222::GFP}$+*trSPC105 (LEU2)*
pAJ492	This study	pRS305	*prSPC105*+Spc105$^{222::GFP,340-343::AAAA}$+*trSPC105 (LEU2)*
pAJ521	This study	pET28a+MBP	HIS(X6)-MBP-Spc105$^{222::GFP}$ (1-455)
pAJ525	This study	pRS305	*prSPC105*+Spc105$^{222::GFP,101-104::AAAA}$+*trSPC105 (LEU2)*
pAJ526	This study	pRS305	*prSPC105*+Spc105$^{222::GFP,101-104,340-343::AAAA}$+*trSPC105 (LEU2)*
pAJ528	This study	pET28a+MBP	HIS(X6)-MBP-Spc105$^{222::GFP,101-104,340-343::AAAA}$ (1-455)
pAJ603	This study	pRS305	*prSPC105*+FRB-GFP-Spc105+*trSPC105* (*LEU2*)
pAJ632	This study	pRS305	*prSPC105*+TOG2 (Stu2$^{311-550}$)+ Spc105$^{222::GFP}$+*trSPC105 (LEU2)*
pAJ633	This study	pRS305	*prSPC105*+TOG2 (Stu2$^{311-550}$)+ Spc105$^{222::GFP,101-104::AAAA}$ +*trSPC105 (LEU2)*
pAJ671	This study	pRS316	*prIPL1+ipl1-2* (H352Y)+*trIPL1 (CEN, URA3)*
pAJ689	This study	pRS305	*prSPC105*+Spc105$^{79-145}$(BP)+Spc105$^{222::GFP,101-104::AAAA}$ +*trSPC105 (LEU2)*
pAJ695	This study	pRS305	*prSPC105*+Spc105$^{222::mCherry}$ +*trSPC105 (LEU2)*
pAJ696	This study	pRS305	*prSPC105*+Spc105$^{222::mCherry,101,104,340-343::AAAA}$+*trSPC105 (LEU2)*
pAJ743	This study	pRS305	*prSPC105*+Spc105$^{222::GFP,105-107::EEE,S109E}$+*trSPC105 (LEU2)*
pAJ775	This study	pRS305	*prSPC105*+Spc105$^{222::GFP,RASA\ (V76,F78::A)}$+*trSPC105 (LEU2)*
pAJ805	This study	pRS305	*prSPC105*+Spc105$^{222::GFP,S77D}$+*trSPC105 (LEU2)*
pAJ806	This study	pRS305	*prSPC105*+Spc105$^{222::GFP,S77D,105-107::EEE,S109E}$ +*trSPC105 (LEU2)*
pAJ817	This study	pSK954	*prSPC105*+Spc105$^{222::mCherry,101-104::AAAA,340-343::AAAA}$+*trSPC105 (KAN)*
pAJ818	This study	pSK954	*prSPC105*+Spc105$^{222::mCherry}$+*trSPC105 (KAN)*
pAJ840	This study	pRS305	*prSPC105*+Spc105$^{56-145}$(RVSF+BP)+Spc105$^{222::GFP,101-104::AAAA}$ +*trSPC105 (LEU2)*
pAJ885	This study	pRS305	*prSPC105*+Spc105$^{56-145,101-104::AAAA}$ (RVSF)+Spc105$^{222::GFP,101-104::AAAA}$ +*trSPC105 (LEU2)*
pAJ887	This study	pRS305	*prSPC105*+Spc105$^{222::GFP,GILK::AAAA}$ +*trSPC105 (LEU2)*
pAJ890	This study	pRS305	*prSPC105*+Spc105$^{222::GFP,GILK::AAAA,101-104::AAAA}$ +*trSPC105 (LEU2)*

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