Edgerton et al., http://www.jcb.org/cgi/content/full/jcb.201511080/DC1

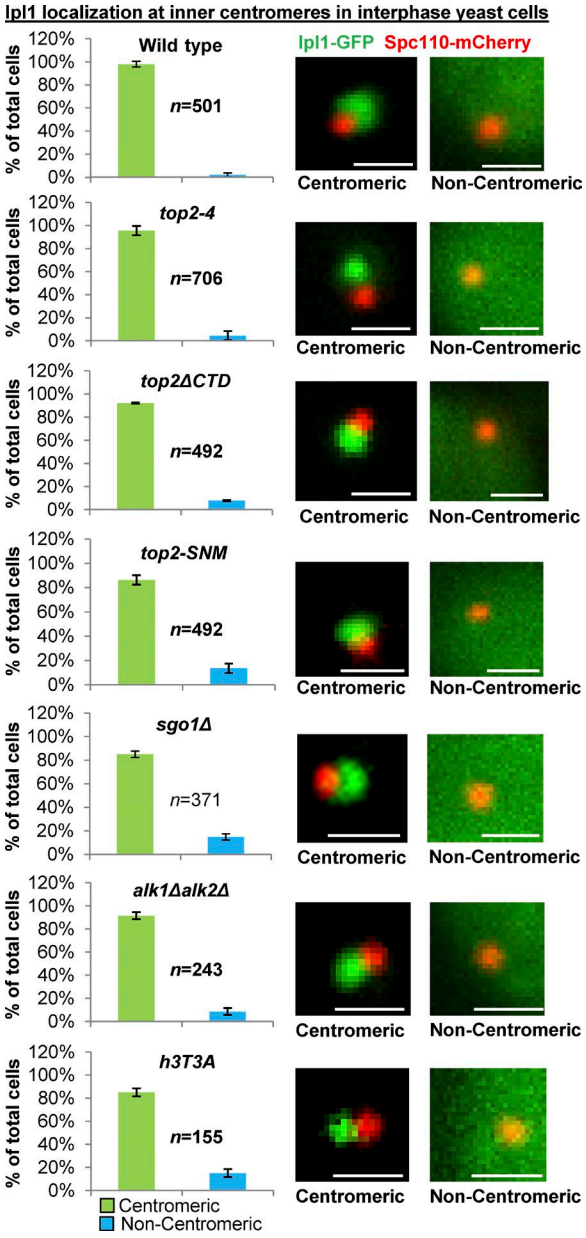

Figure S1. **Top2 is not required for Ipl1 recruitment to inner centromeres in interphase cells.** Representative images and quantification of Ipl1-GFP localization to inner centromeres in interphase yeast strains (grown at 30°C for 1 h; the nonpermissive temperature for *top2-4*). Here, interphase was defined as cells with a single spindle pole body, which more accurately corresponds to G1 cells and cells in early S phase. Spc110-mCherry indicates spindle poles. Bars, 1 µm. *n* is total number of cells scored from three independent experimental repeats. Error bars, standard deviation.

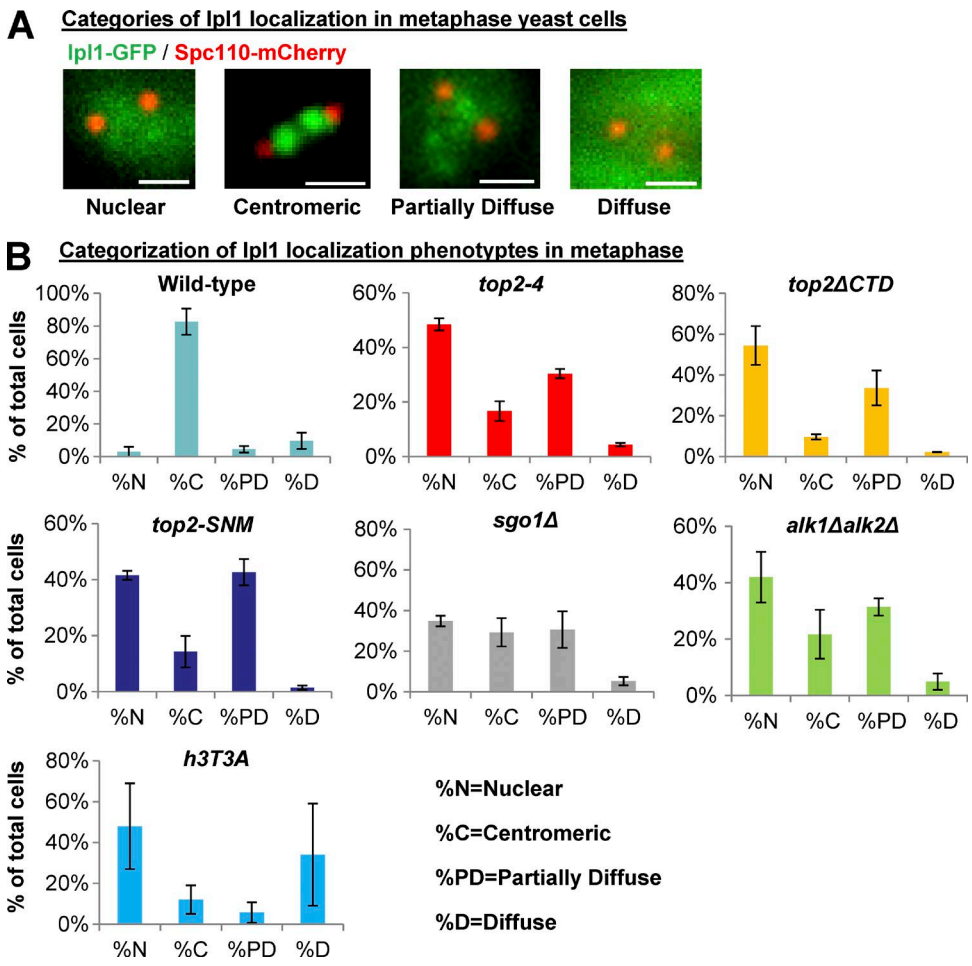

Figure S2. **Top2 is required for Ipl1 recruitment to inner centromeres in mitosis.** Representative images (A) and quantification (B) of Ipl1-GFP localization to inner centromeres in prometaphase/metaphase cells (0.5–2.0-µm spindle lengths) grown at 30°C for 1 h, the nonpermissive temperature for *top2-4*. Here, prometaphase/metaphase was defined as cells with two spindle pole bodies. Images show representative examples of the criteria used to classify cells based on the localization of Ipl1: Nuclear, homogenous nucleoplasmic localization; Inner Centromeres (Centromeric), localization restricted to two discrete clusters of centromeres in line and within the spindle axis defined by the spindle poles; Partially Diffuse, primarily homogenous nucleoplasmic localization, with a degree of heterogeneous intensity; Diffuse, homogenous nucleoplasmic and cytosolic localization. Plots show a breakdown of the data from the main figures of the manuscript to classify cells into these categories. Spc110-mCherry indicates spindle poles. Bars, 1 µm. *n* is total number of cells scored from three independent experimental repeats. Error bars, standard deviation.

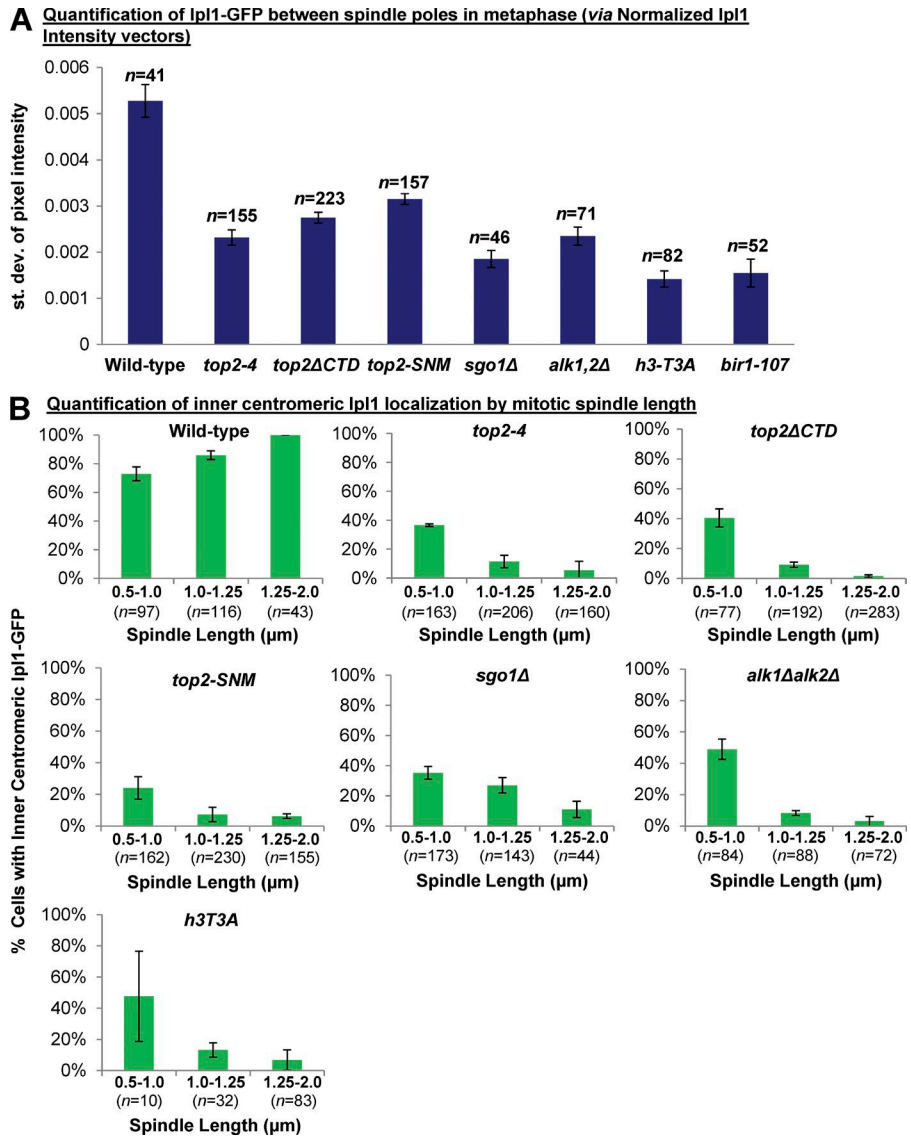

Figure S3. **Analysis of Ipl1 distribution between spindle poles and in cells binned into spindle length categories.** (A) Computational analysis. Quantification of Ipl1-GFP pixel intensities between spindle poles in mitotic yeast cells (grown at 30°C for 1 h; the nonpermissive temperature for *top2-4*), as previously described (Chacón and Gardner, 2013; see Materials and methods). In brief, GFP intensities were measured on 15-pixel-wide line scans with 50 equally spaced locations along each line between the spindle poles (defined by Spc110-mCherry). Cells analyzed had 1.25–2.0-µm spindles. The standard deviation along the line scans was calculated for each image, and over many images, a mean standard deviation was determined. This standard deviation is plotted as a histogram. Error bars show standard deviation of the means. Thus, higher standard deviation corresponds to Ipl1 at kinetochores. Lower standard deviation corresponds to homogeneous disorganization. *n* = total number of cells scored from three independent experimental repeats. Error bars, standard deviation. P values for each mutant versus wild-type are 0.000629857 (*top2-4*), 0.001663 (*top2ΔCTD*), 0.000183 (*sgo1Δ*), 0.000448143 (*alk1Δ alk2Δ*), 0.000252479 (*h3-T3A*), 0.00009 (*bir1-107*), and 0.000116 (*top2-SNM*), Student's *t* test. (B) Binned cells. Quantification of Ipl1-GFP localization to inner centromeres in cells that were binned according to spindle length (determined by measuring the distance between the centers of each Spc110-mCherry marked spindle pole body; see Materials and methods). Spindles 0.5–1.25 µm long correspond to stages from spindle pole separation to spindles in the process of assembly. Spindles 1.25–2.0 µm long correspond to prometaphase/metaphase. Yeast strains were grown at 30°C for 1 h (the nonpermissive temperature for *top2-4*). *n* is the total number of cells scored, pooled from three independent experimental repeats. Error bars, SEM.

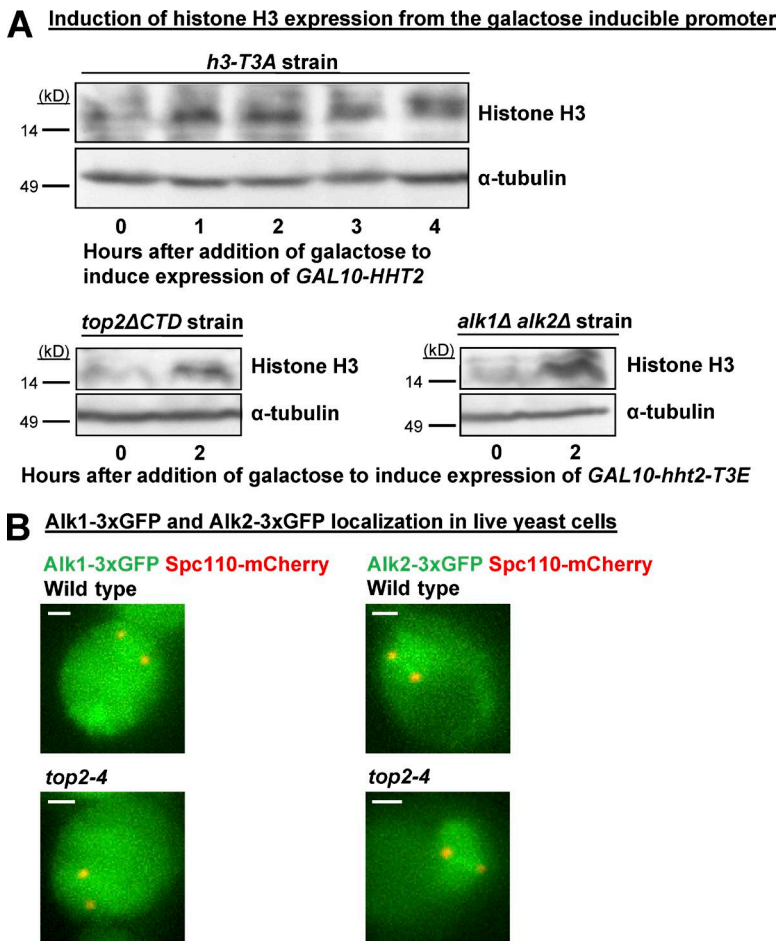

Figure S4. **Induction of histone H3 and localization of Alk1 and Alk2 in live cells.** (A) Induction of histone H3 and phosphomimetic mutant *h3-T3E* assayed by Western blotting. Wild-type histone H3 (*HHT2*) or a phosphomimetic threonine 3-to-glutamic acid mutant (*hht2-T3E*) was induced from the *GAL10* promoter by addition of 2% galactose at time 0. Wild-type H3 was induced in the *h3-T3A* mutant, and *hht2-T3E* was induced in the *top2ΔCTD* and *alk1Δ alk2Δ* mutants. Whole-cell extracts were immunoblotted to detect H3 and α-tubulin (loading control). Note that at time 0, endogenous H3 is detected, and thereafter, the additional signal corresponds to the exogenously induced H3 proteins. (B) Analysis of Alk1-3xGFP and Alk2-3xGFP localization in live cells. Visualization of endogenous Alk1 and Alk2 proteins in live cells by TIRF microscopy (endogenous *ALK1* and *ALK2* genes C-terminally fused to 3xGFP). Wild type and the *top2-4* mutant were analyzed after growth at the nonpermissive temperature for *top2-4* (30°C) for 1 h. Spc110-mCherry defines the spindle poles. Bars, 1 µm.

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

Strain no.	Genotype	Reference
MMWY72-22D	*MATα NUF2-GFP(HIS3) SPC110-mCherry(HYG^R) ade2-1oc ade3Δ*	Wargacki et al., 2010
4588	*MATa NUF2-GFP(HIS3) SPC110-mCherry(HYG^R) top2-4 ade2-1oc ade3Δ*	This study
4698	*MATa MAD2-GFP3x(KAN^R) SPC110-mCherry(HYG^R)*	This study
4678	*MATα MAD2-GFP3x(KAN^R) SPC110-mCherry(HYG^R) top2-4 his3 HIS2*	This study
PWY334-1B	*MATα IPL1-GFP3x(HIS3) SPC110-mCherry(HYG^R)*	Shimogawa et al., 2009
4638	*MATa IPL1-GFP3x(HIS3) SPC110-mCherry(HYG^R) top2-4*	This study
	MATa IPL1-GFP3x(HIS3) SPC110-mCherry(HYG^R) top2-4 YCp50-top2-Y782F(URA3)	This study
4671	*MATa IPL1-GFP3x(HIS3) SPC110-mCherry(HYG^R) top2::KAN^R leu2::top2-degron(LEU2),top2ΔCTD(TRP1)*	This study
	MATa IPL1-GFP3x(HIS3) SPC110-mCherry(HYG^R) top2::KAN^R leu2::top2-degron(LEU2),top2ΔCTD(TRP1) YCp50-top2-Y782F(URA3)	This study
4815	*MATa IPL1-GFP3x(HIS3) SPC110-mCherry(HYG^R) top2-SNM-HA-KAN^R Scc1-18-myc-TRP1*	This study
4841	*MATa IPL1-GFP3x(HIS3) SPC110-mCherry(HYG^R) sgo1::KAN^R*	This study
4665	*MATa IPL1-GFP3x(HIS3) SPC110-mCherry(HYG^R) alk1::KAN^R alk2::KAN^R*	This study
4838	*MATa IPL1-GFP3x(HIS3) SPC110-mCherry(HYG^R) bir1-107*	This study
4849	*MATa bar1::LEU2 SGO1-GFP3x(HIS3) SPC110-mCherry(HYG^R)*	This study
4851	*MATa bar1::LEU2 SGO1-GFP3x(HIS3) SPC110-mCherry(HYG^R) top2-4*	This study
4850	*MATa bar1::LEU2 SGO1-GFP3x(HIS3) SPC110-mCherry(HYG^R) top2::KAN^R leu2::top2-degron(LEU2),top2ΔCTD(TRP1)*	This study
4784	*MATα bar1::LEU2 IPL1-GFP3x(HIS3) SPC110-mCherry(HYG^R) top2-4 alk1::KAN^R alk2::KAN^R*	This study
4802	*MATα IPL1-GFP3x(HIS3) SPC110-mCherry(HYG^R) top2::KAN^R leu2::top2-degron(LEU2),top2ΔCTD(TRP1) alk1::KAN^R alk2::KAN^R*	This study
4599	*MATa IPL1-GFP3x(HIS3) SPC110-mCherry(HYG^R) hht1,hhf1::HYG^R HHT2::hht2-T3A(URA3)*	This study
4646	*MATa IPL1-GFP3x(HIS3) SPC110-mCherry(HYG^R) hht1,hhf1::HYG^R HHT2::hht2-T3A(URA3) pGAL1-HHF2-GAL10-HHT2(TRP1)*	This study
4708	*MATa bar1::LEU2 IPL1-GFP3x(HIS3) SPC110-mCherry(HYG^R) top2::KAN^R leu2::top2-degron(LEU2),top2ΔCTD(TRP1) pGAL1-HHF2-GAL10-HHT2-T3E(URA3)*	This study
4814	*MATa IPL1-GFP3x(HIS3) SPC110-mCherry(HYG^R) alk1::KAN^R alk2::KAN^R pGAL1-HHF2-GAL10-HHT2-T3E(URA3)*	This study
SBY2668	*MATa mtw1-1*	Pinsky et al., 2003
4762	*MATα bar1::LEU2 mtw1-1 hht1,hhf1::HYG^R HHT2::hht2-T3A(URA3) Spc42-GFP (TRP1)*	This study
4766	*MATa bar1Δ mtw1-1 alk1::KAN^R alk2::KAN^R Spc42-GFP (TRP1)*	This study
4764	*MATa bar1Δ mtw1-1 top2::KAN^R leu2::top2 degron(LEU2), top2ΔCTD(TRP1) Spc42-GFP (TRP1)*	This study
4852	*MATa bar1::LEU2 ALK1-3xGFP(URA) SPC110-mCherry(HYG^R)*	This study
4854	*MATa bar1::LEU2 ALK1-3xGFP(URA) SPC110-mCherry(HYG^R) top2-4*	This study
4855	*MATa bar1::LEU2 ALK2-3xGFP(URA) SPC110-mCherry(HYG^R)*	This study
4857	*MATa bar1::LEU2 ALK2-3xGFP(URA) SPC110-mCherry(HYG^R) top2-4*	This study
CRY1	*MATa his3-11,15 leu2-3112 trp1-1 ura3-1 ade2-1 can1-100*	Warsi et al., 2008
GSY001	*MATa TOP2-GFP*	This study
GSY003	*MATa TOP2-SNM-GFP*	This study
JBY2265	*MATa TOP2-ΔCTD-GFP*	This study
JBY2266	*MATa TOP2-ΔCTD-GFP*	This study
JBY746	*MATa TetR-GFP*	This study

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