SUPPLEMENTARY FIGURES

Figure S1. HP1α localizes to centromeres in mitosis and interacts with INCENP. (A&B) HeLa tet-on cells that stably express HP1α-CFP, HP1β-CFP, or HP1γ-CFP were monitored with live-cell imaging. Arrow denotes the midbody localization of HP1α-CFP at telophase. (C) Metaphase spread of HeLa tet-on cells was stained with DAPI (blue in overlay), α-HP1α (green in overlay), and CREST (red in overlay). (D) Lysates and Myc IP of HeLa tet-on cells transfected with the indicated plasmids were blotted with α-Myc and α-HA.

Figure S2. (A) The endogenous INCENP and HP1α interact in mitosis. HeLa tet-on cells were arrested in G1 or mitosis with thymidine or nocodazole, respectively. Cell lysates, IgG control, or α-INCENP IP were blotted with α-INCENP, α-Aurora B, and α-HP1α. (B) Quantification of the mitotic chromosome signals of GFP-HP1α of cells in Figure 2A. The average intensity of GFP-HP1α at the centromeres of five chromosomes was divided by its average intensity at chromosome arms, and these ratios were plotted (N = 8 cells).

Figure S3. Expression of INCENP Δ125 rescues the cell-cycle and spindle-checkpoint defects of INCENP RNAi Cells. (A) HeLa tet-on cells were first transfected with vector, mCherry-INCENP WT or Δ125 plasmids 6 hr and then with INCENP siRNA for 24 hr, and were treated with Taxol (100 nM) for another 18 hr. Cell lysates were blotted α-INCENP and α-tubulin (loading control). (B) FACS analysis of HeLa tet-on cells transfected as described (A) except that the cells were not treated with Taxol. The populations of G1 (2N), G2 (4N), and mitotic (4N and MPM2+) cells are indicated by blue, brown, and red arrowheads, respectively. 10,000

events were counted in each sample. (C) Cells in (A) were analyzed by FACS as described in (B). The mitotic index of these cells is plotted (mean ± SD of two independent experiments).

Figure S4. INCENP depletion causes diffusive chromosome localization of Sgo1. (A) HeLa tet-on cells were transfected with INCENP siRNA for 48 hr. Metaphase chromosome spread was prepared from these cells and stained with DAPI, CREST and α-Sgo1. DAPI, Sgo1 staining, and CREST staining were colored blue, green, and red, respectively, in the overlay. (B) The average intensity of Sgo1 at the centromeres of five chromosomes was divided by its average intensity at chromosome arms, and these ratios were plotted (N = 10 cells).

Figure S5. The INCENP–HP1 interaction is dispensable for mitotic progression. (A) HeLa tet-on cells that stably express mCherry-INCENP WT (clone #4) or Δ125 (clone #15) were transfected with INCENP siRNA for 24 hr and then arrested at the G1/S boundary by thymidine treatment for 24 hr. Live-cell imaging was performed after the cells were released into fresh media. The cumulative percentage of mitotic cells was plotted against the mitotic duration (as defined by the time from nuclear envelope breakdown to the appearance of INCENP midbody staining at telophase) (N = 25 cells). (B) The mitotic duration of cells in (A) were blotted in a scatter plot. (C) Representative cells in (A) showing the dynamic localization patterns of mCherry-INCENP or Δ125.

Figure S6. The Sgo1–HP1 interaction is dispensable for sister-chromatid cohesion. (A) HeLa tet-on cells that stably express Myc-Sgo1 WT (clone #8) or P1A (clones #3 and #5) under the control of doxycycline were cultured in the absence (–) or presence (+) doxycycline (Dox) and

transfected with Sgo1 siRNA for 24 hr. Cell lysates were blotted with α-Myc and α-tubulin. (B) The mitotic index of cells in (A). Cells were stained with propidium iodide and α-H3-pS10 and analyzed by FACS. 10,000 events were counted for each sample. Mitotic cells have 4N DNA content and are H3-pS10-positive. (C) The extent of sister-chromatid separation of cells in (A) as determined by metaphase spread with Giemsa staining (N = 50 cells).

Figure S7. The Sgo1–HP1 interaction does not regulate mitotic progression or the timing of Sgo1 localization to mitotic centromeres. HeLa tet-on cells were first transfected with GFP-Sgo1 WT or P1A for 6 hr and then with Sgo1 siRNA for 24 hr. After that, cells were arrested at G2 with an 18-hr treatment of the Cdk1 inhibitor RO-3306 and then released into fresh media for live-cell imaging.

Table S1. Crystallographic and Refinement Data and Statistics of HP1β CSD–Sgo1P1

Parameter	Value
Space Group	$P2_12_12$
Unit cell dimensions a, b, c (Å)	76.8, 108.9, 41.9
α, β, γ (°)	90, 90, 90
Resolution (Å)	38.4-1.93 (1.96-1.93)*
Completeness (%)	99.9 (100)
Multiplicity	8.4 (8.3)
Unique reflections	26,940 (1,314)
$R_{sym}^{\dagger}$ (%)	9.7 (96.7)
I/σ(I)	16.5 (2.2)
Wilson B (Å^2)	28.5
Refinement Resolution (Å)	38.4-1.93
No. non-solvent atoms	2,520
No. solvent atoms	162
Cutoff F_o/σ_{Fo}	0
Avg. *B*-factors non-solvent (Å^2)	37.0
solvent	40.3
***R*-values** R_{work} (%)	18.9
R_{free} (%)	23.0
Ramachandran statistics[§] outliers (%)	0.0
most favored region (%)	99.3
r.m.s. deviations Bond lengths (Å)	0.011
Bond angles (°)	1.2

*Values in parentheses are for the highest resolution shell.

[†] $R_{sym} = \sum_h \sum_i \left| I_{h,i} - \langle I_h \rangle \right| / \sum_h \sum_i I_{h,i}$ where the outer sum (h) is over the unique reflections and the inner sum (i) is over the set of independent observations of each unique reflection.

[§]From MolProbity (Chen et al., 2010).

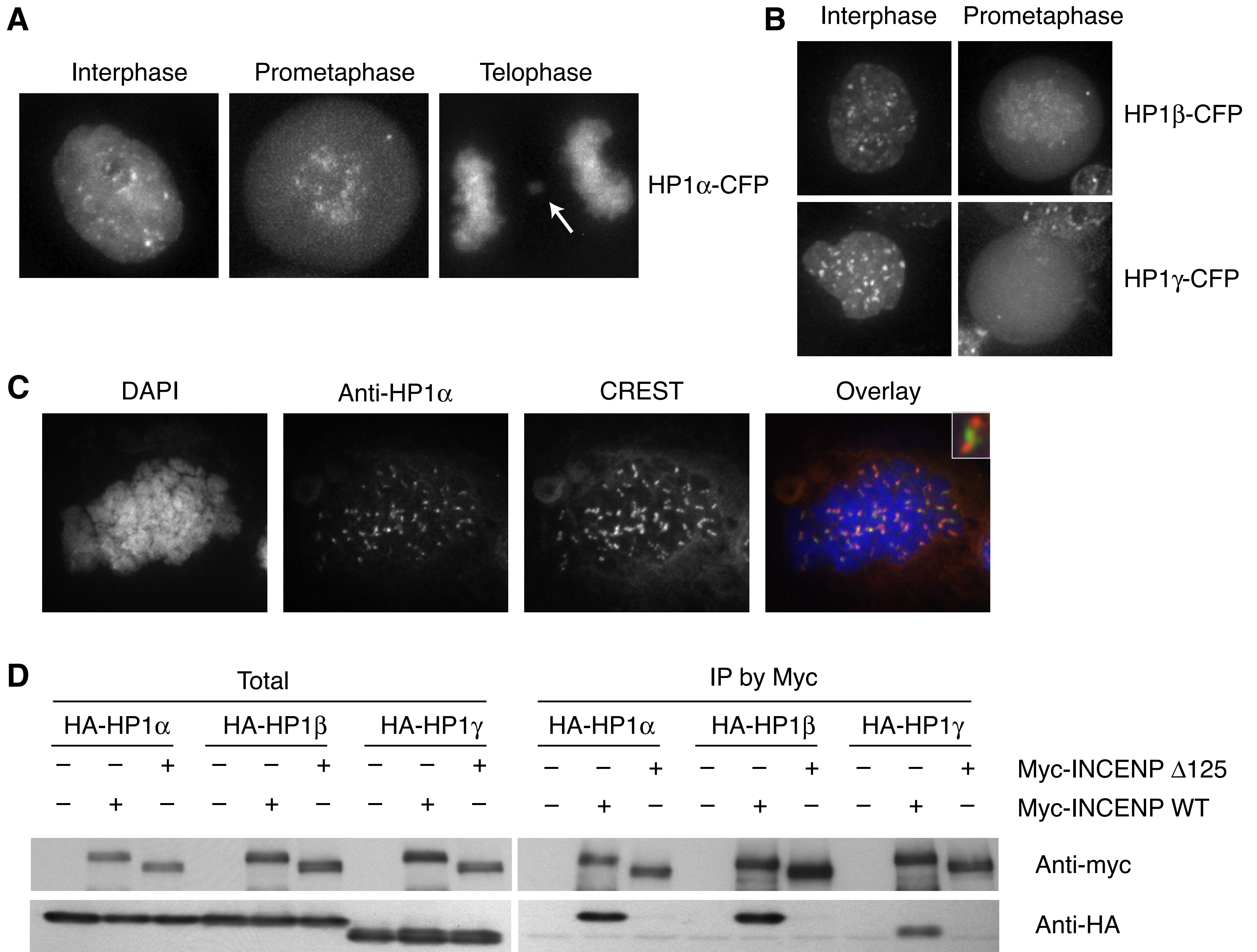
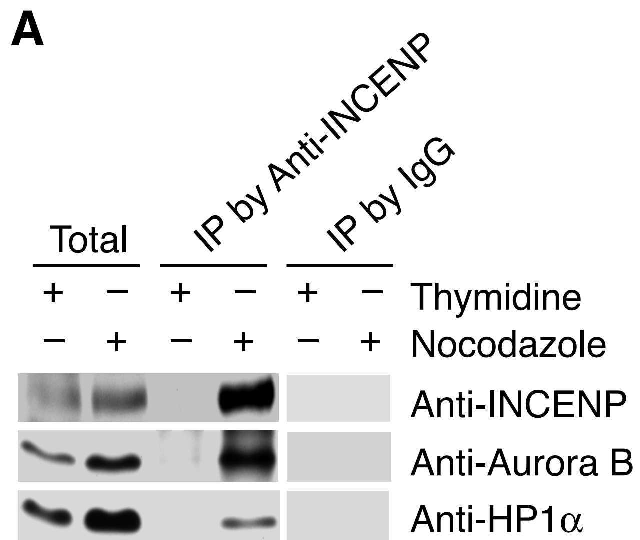
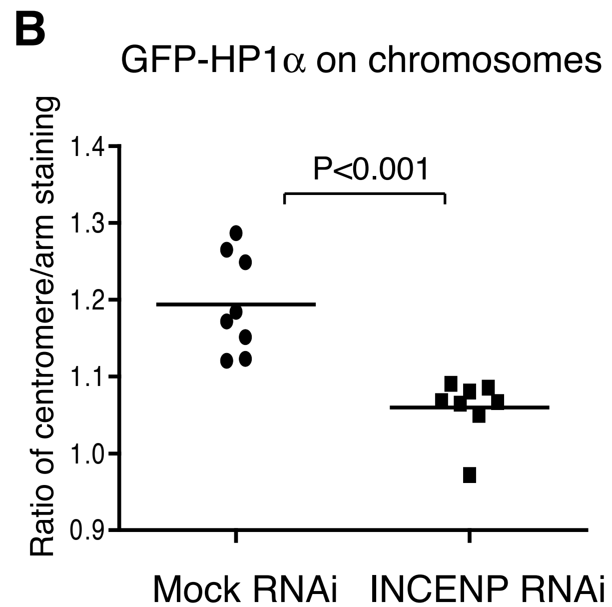


Figure S1





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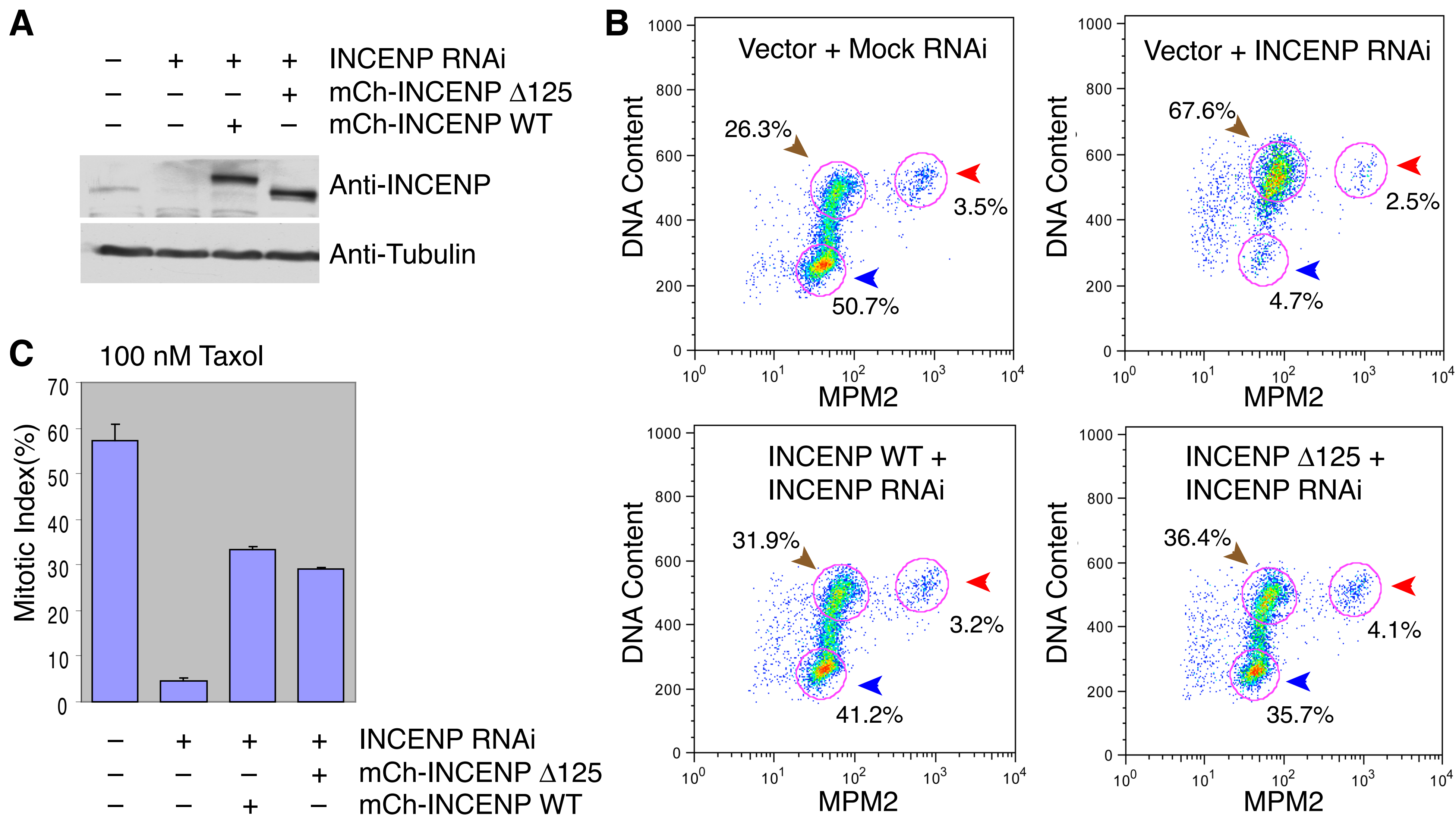


Figure S3

A

DAPI Sgo1 CREST Overlay

Mock RNAi

INCENP RNAi

B

Sgo1 on chromosomes

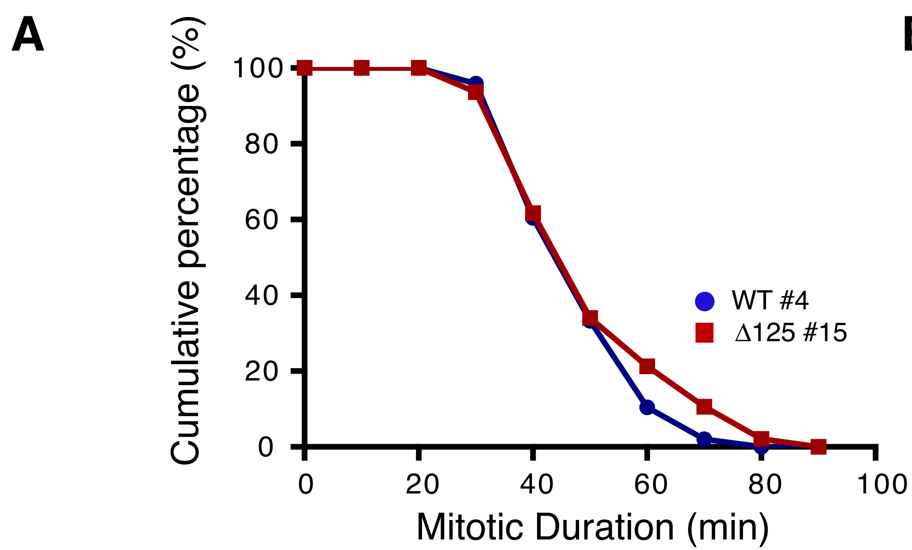
$P<0.001$

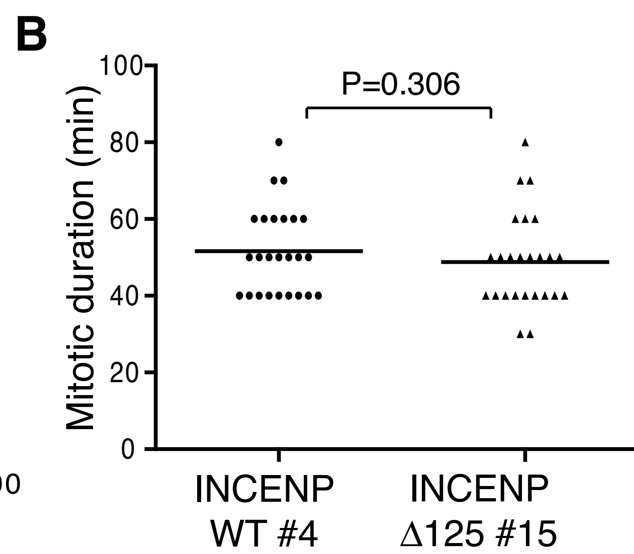
Ratio of centromere/arm staining

2.5
2.0
1.5
1.0
0.5
0.0

Mock RNAi INCENP RNAi

Figure S4





C

0 min 10 min 20 min 30 min 40 min 50 min

INCENP
WT #4

mCherry

DIC

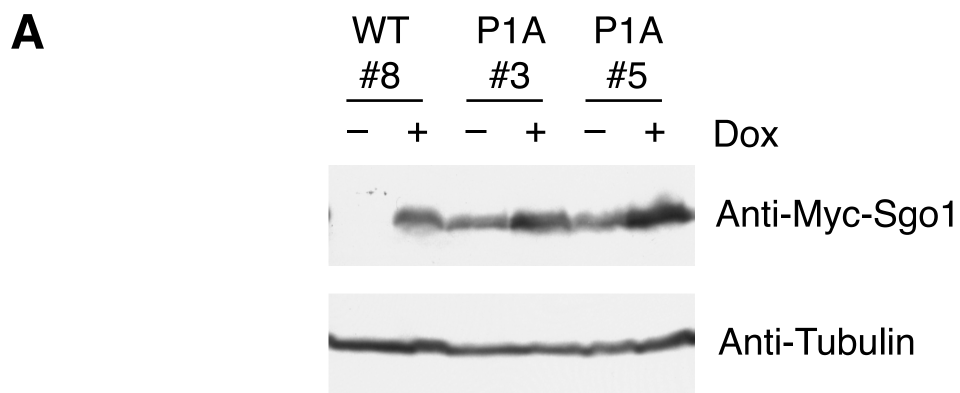
0 min 10 min 20 min 30 min 40 min 50 min

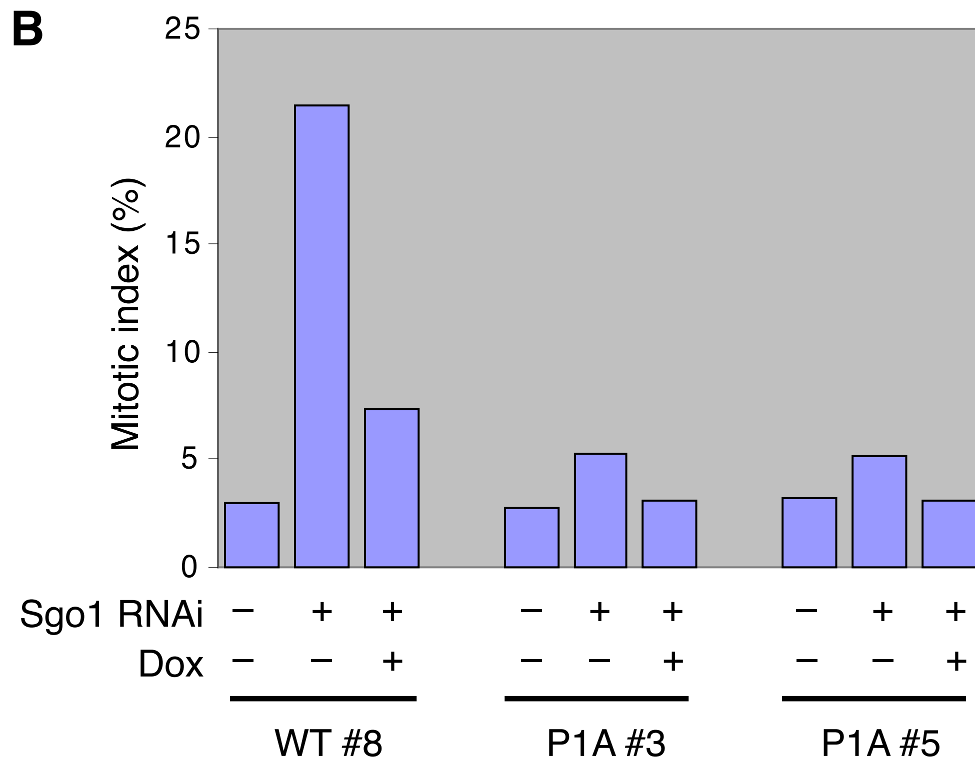
INCENP
Δ125 #15

mCherry

DIC

Figure S5





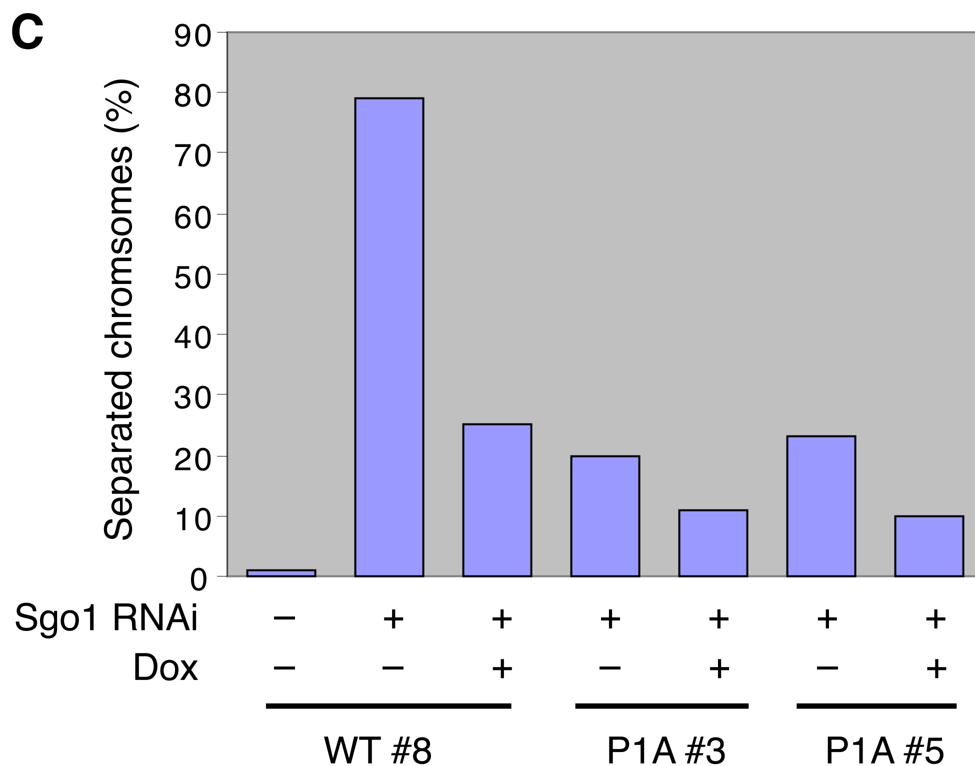


Figure S6

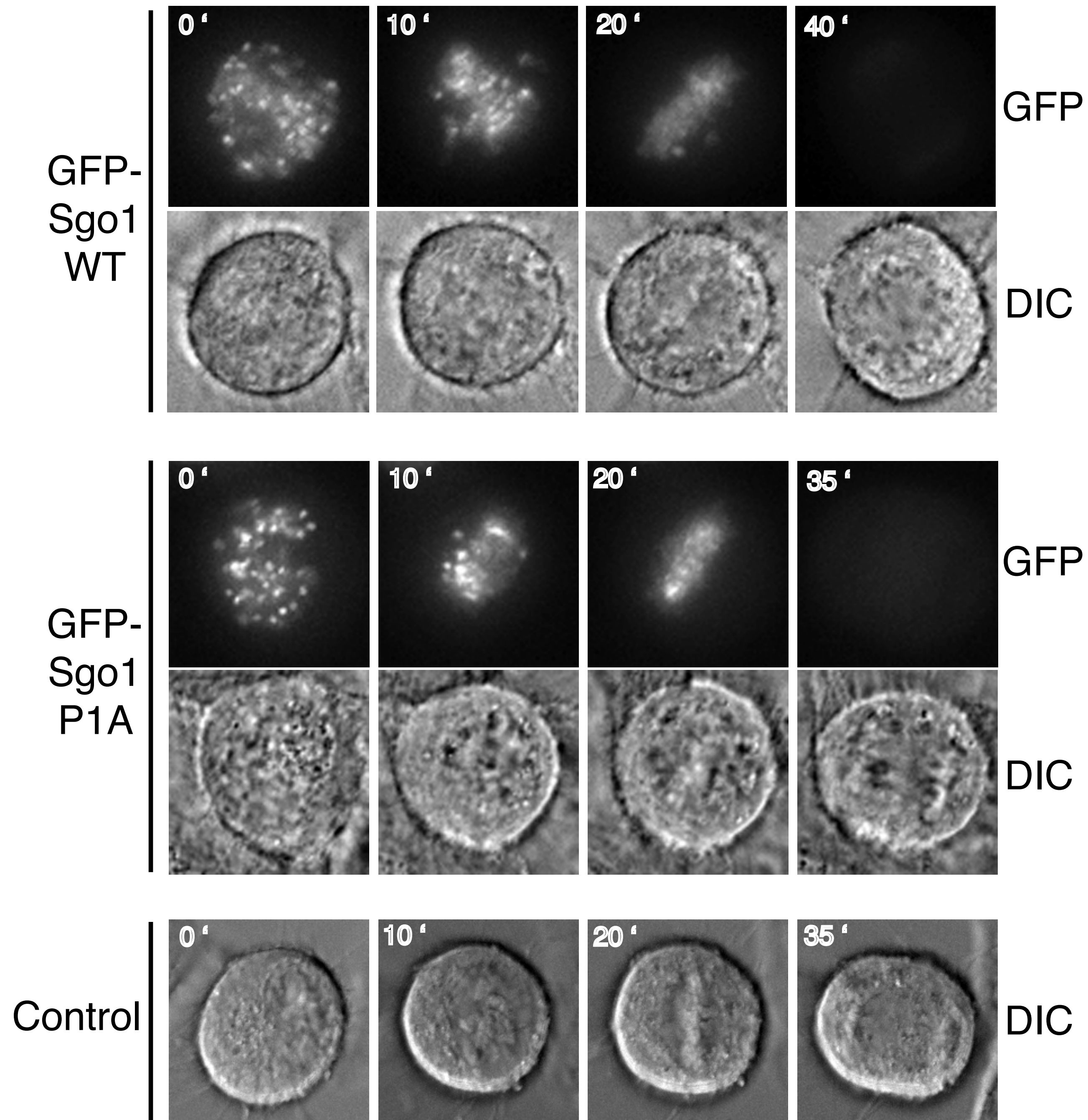


Figure S7