Wheelock et al., https://doi.org/10.1083/jcb.201609061

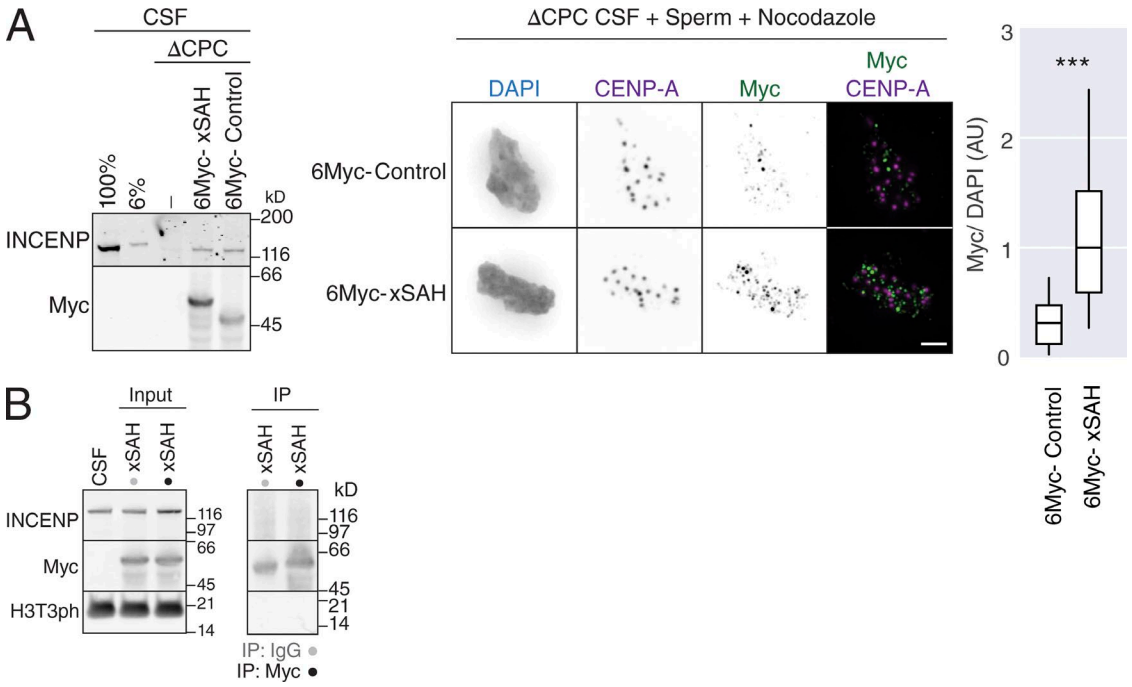

Figure S1. **The SAH domain of xINCENP can be localized to chromatin without binding to the endogenous CPC.** (A) Depletion efficiency (left), representative immunofluorescence images (middle), and quantification (right) of 6Myc-xSAH localized to sperm chromatin in nocodazole after depletion of endogenous INCENP. 6Myc-xSAH still shows weak localization along sperm chromatin relative to a 6Myc-tagged protein that does not bind sperm chromatin (6Myc-Control), even when 95% of endogenous INCENP is depleted. Representative images approximate the median. Median, IQR (box), and ±1.5 × IQR (error bars) are shown. $n = 15$ spreads per sample, two-tailed Mann–Whitney t test; ***, $P \leq 0.001$. Bar, 2 µM. (B) Total extract (Input) and immunoprecipitation (IP) of M-phase extract containing the indicated constructs tagged with 6Myc. Extract was immunoprecipitated with either anti-IgG (gray circles) or anti-Myc (black circles). Endogenous INCENP does not copurify with 6Myc-xSAH.

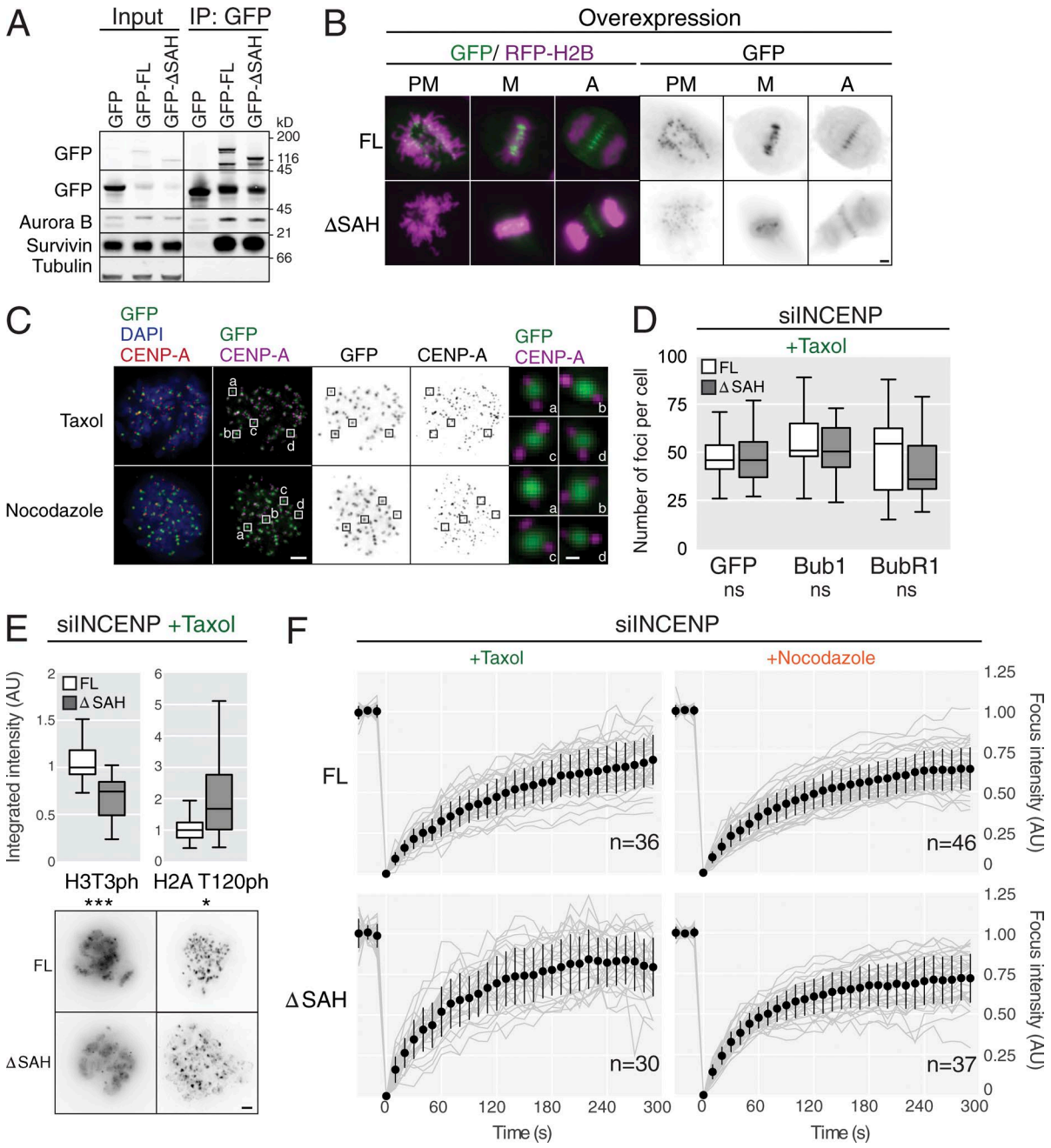

Figure S2. **The SAH domain is important for enrichment of hINCENP at the centromere.** (A) Western blot of total extract (Input) and anti-GFP immunoprecipitation (IP) from mitotic HeLa cell extracts containing the indicated construct. (B) Immunofluorescence visualizing hINCENP and hINCENP ΔSAH expressed over endogenous INCENP in asynchronous cells. Bar, 2 µM. (C) hINCENP localization in taxol- and nocodazole-treated cells. Cells expressing LAP-hINCENP FL were treated with INCENP siRNA, arrested in either taxol or nocodazole, and processed for immunofluorescence as in Fig. 2 B. hINCENP (green) primarily localizes as a single focus between pairs of CENP-A foci (purple), consistent with inner centromere localization. Whole cells are on the left and enlargements of representative centromere pairs are on the right. Bars: (whole cell) 2 µm; (single kinetochore pair) 0.25 µm. (D) Number of foci per cell for each epitope in taxol-treated cells expressing the indicated construct processed as in C. Foci were quantified using an automated watershed segmentation algorithm in Metamorph. Median, IQR (box), and ±1.5 × IQR (error bars) are shown. Two-tailed Mann–Whitney *t* test; $n \geq 20$ cells per sample; ns, not significant. (E) Quantification (top) and representative images (bottom) of the level of H3T3ph and H2A T120ph in cells expressing hINCENP FL or hINCENP ΔSAH after knockdown of endogenous INCENP and incubation with taxol (similar to Fig. 2 B). H3T3ph shows a reduction, whereas H2A T120p appears on chromosome arms. Median, IQR (box), and ±1.5 × IQR (error bars); representative images approximate the median are shown. Two-tailed Mann–Whitney *t* test; $n = 20$ cells per sample; *, $P \leq 0.05$; ***, $P \leq 0.001$. Bar, 2 µm. (F) All individual (gray) FRAP recovery curves for data presented in this article (Fig. 2, D and E). Mean curve (black) is shown with error bars as SD. Data compiled from $n = 2$ independent experiments for each condition, $n \geq 30$ cells total per condition.

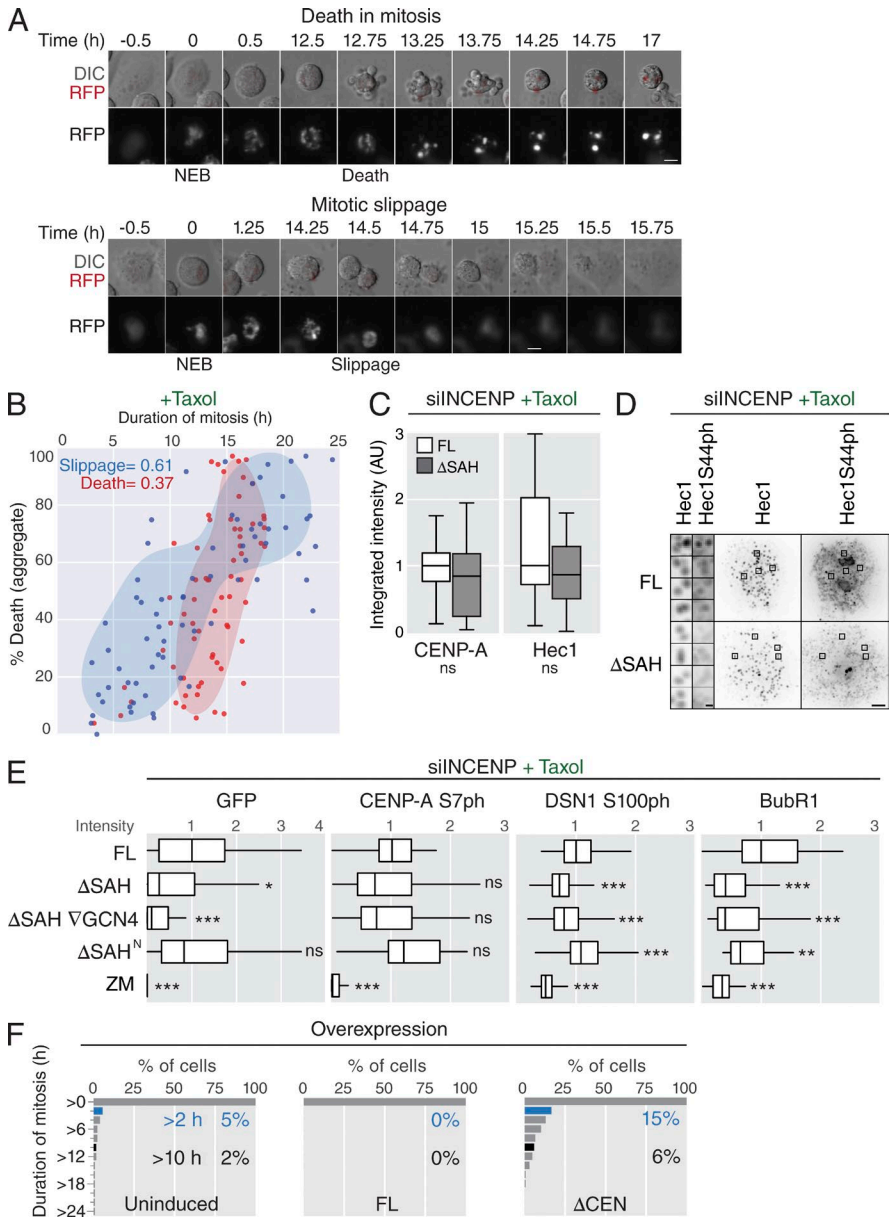

Figure S3. **Functional analysis of hINCENP in taxol-treated HeLa cells.** (A) Representative montages of mitotic cell fate in taxol for cells expressing LAP-hINCENP after treatment with INCENP siRNA. Each cell can exit mitosis by undergoing death in mitosis (top) or mitotic slippage (bottom). NEB and mitotic exit by death or slippage were determined by cell morphology (DIC, gray) and chromatin morphology (RFP-H2B, red). Death in mitosis is characterized by prolonged nuclear blebbing leading to the formation of a relatively immobile, crenulated sphere. Mitotic slippage is characterized by failed cytokinesis (sometimes leading to the appearance of two spheres, only one of which contains chromatin, as at 14.5 h), rapid cell flattening, chromatin decondensation, and formation of a single multinucleate cell. Bar, 10 µm. (B) Scatter plot of the median DoM versus percentage cell death. Each point represents a single sample from one experiment (including all experiments in this article). Values for cells undergoing death in mitosis (red) or mitotic slippage (blue) are plotted separately for each sample against the total percentage death for that sample. Shaded areas are kernel density estimates. r^2 values for linear regression are indicated at top left. (C) Immunofluorescence quantification of kinetochore proteins as in Fig. 3 D. Whole-cell integrated intensity was quantified for CENP-A and Hec1 with $n \geq 20$ cells per sample. Median, IQR (box), and ±1.5 × IQR (error bars) are shown. Two-tailed Mann–Whitney *t* test; ns, not significant. (D) Immunofluorescence images of Hec1 S44ph at individual kinetochores standardized to total Hec1 at that kinetochore (for Fig. 3 F). Images are zoom-ins of four representative kinetochore pairs (left) from a cell of representative intensity (right). Bars: (whole cell) 2 µm; (kinetochore pair) 0.25 µm. (E) Immunofluorescence quantification of GFP-INCENP (centromere), CENP-A S7ph (centromere), Dsn1 S100ph (individual kinetochores, using a marker epitope), and BubR1 (kinetochore) of cells expressing the indicated hINCENP construct in taxol after knockdown of endogenous INCENP or treatment with the Aurora B inhibitor ZM447439 (ZM). Expression of hINCENP ΔSAH ∇GCN4 stimulates Aurora B activity (Fig. 4 B) but does not rescue the defects in CPC localization, Aurora B–dependent phosphorylation, or SAC protein recruitment from deleting the SAH domain (ΔSAH). Conversely, a minimal deletion in the MT-binding region of INCENP (ΔSAH^N) does not affect GFP-INCENP localization but is still defective in the SAC (Fig. 6 C) and recruitment of the SAC protein BubR1. Median, IQR (box), and ±1.5 × IQR (error bars) are shown. Statistics are from a two-tailed Mann–Whitney *t* test; ns, not significant; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; $n \geq 25$ for centromere and kinetochore samples except ZM, where $n = 10$; $n \geq 1{,}292$ for individual kinetochore quantification. (F) Controls for RO-3306 synchronization and release experiment in Fig. 4 F. Cells were arrested at the G2/M transition by treatment with the Cdk1 inhibitor RO-3306. After inhibitor washout, cells synchronously entered mitosis, and the DoMs of untreated cells or those expressing the indicated construct over endogenous INCENP were quantified. The majority of untreated cells exit mitosis rapidly after RO-3306 washout. Plots show the cumulative minimum DoM for each construct. Blue bars, arrested for at least 2 h; black bars, arrested for at least 10 h; $n \geq 92$ cells per condition.

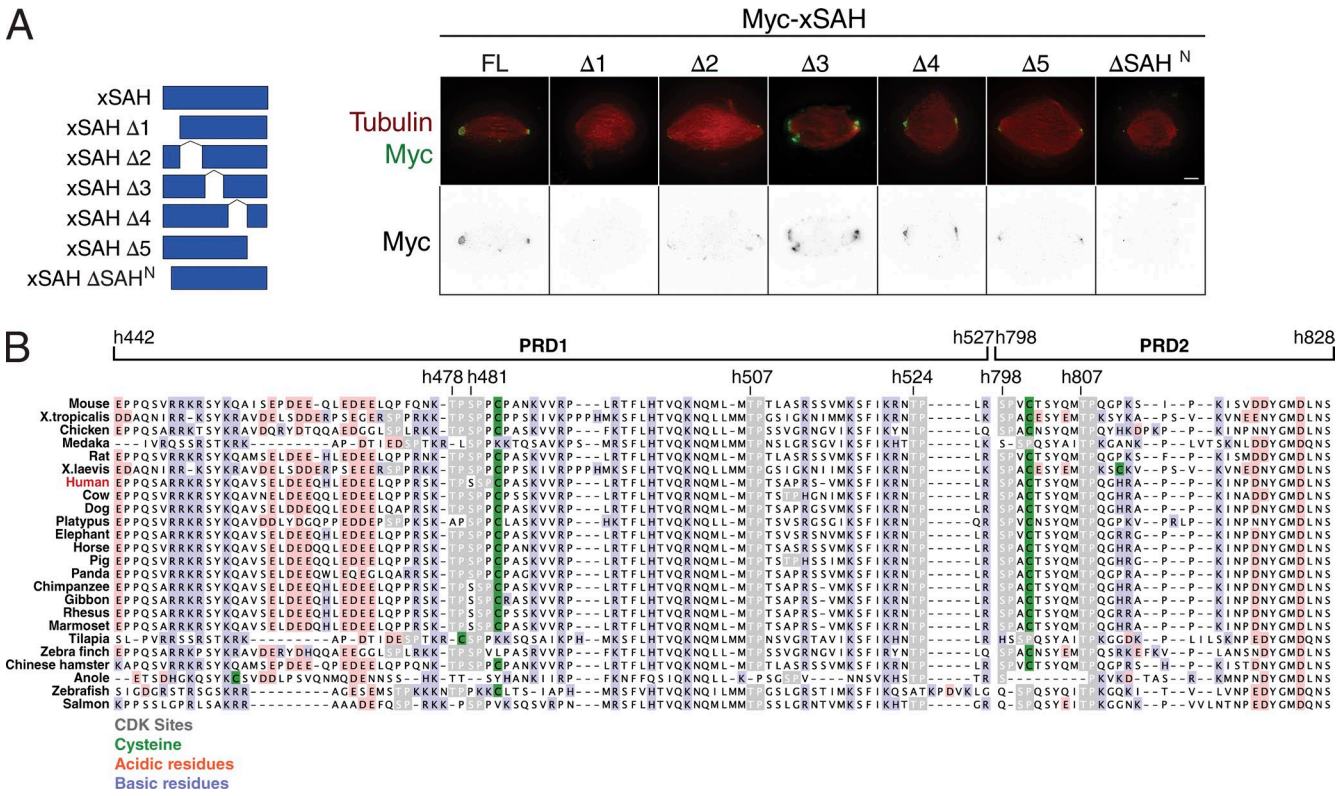

Figure S4. **Characterization of the SAH and PRD domains.** (A) Diagram of 6Myc-tagged xINCENP SAH domain constructs in this figure (left). Immunofluorescence visualizing the indicated Myc-tagged constructs on the spindle in *Xenopus* egg extract (right). Bar, 5 µM. (B) Alignment of PRD1 and PRD2 among 25 vertebrate INCENP species. Alignment made using JalView 2.8.2 Tcoffee with default settings. Putative Cdk sites (gray), cysteine (green), negatively charged residues (red), and positively charged residues (blue) are indicated.

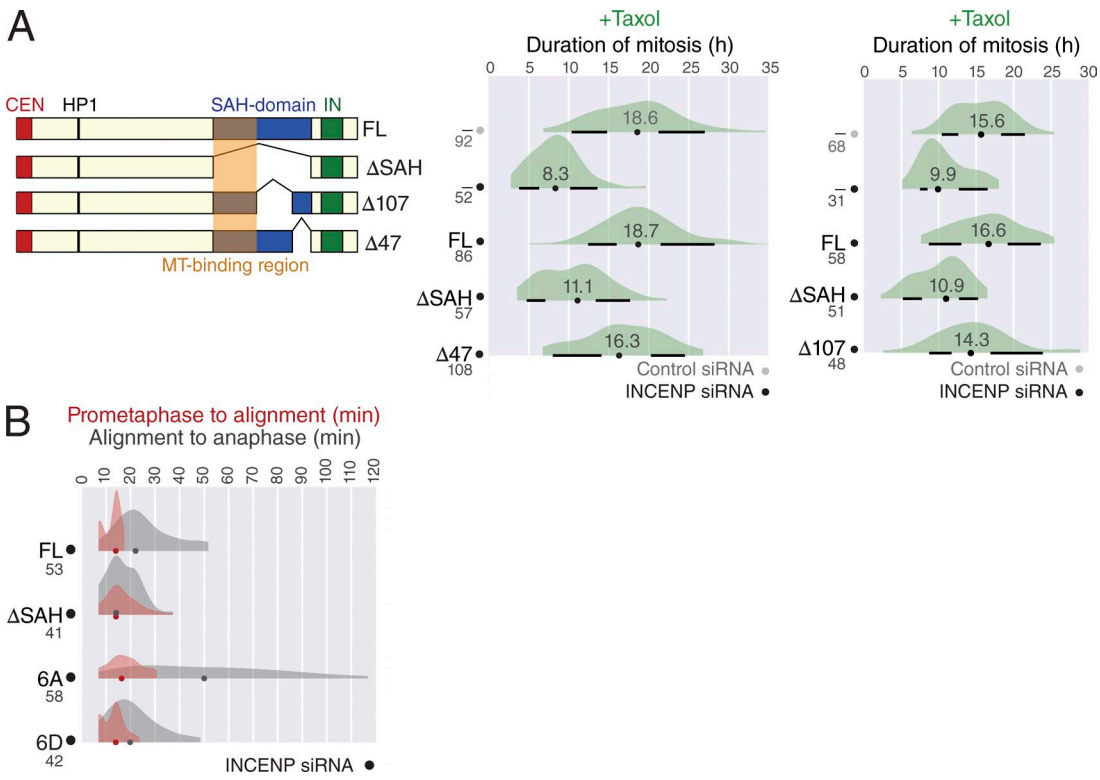

Figure S5. **Impacts of SAH deletion on kinetochore phosphorylation and mitotic arrest in taxol-treated HeLa cells.** (A) Two nonoverlapping deletions outside the MT-binding region of the SAH domain (light orange) have minor effects on the duration of mitosis in taxol. Compared with deletion of the entire SAH domain ($\Delta SAH^{528–795}$), deletion of 107 aa (right, $\Delta SAH^{641–747}$) or 47 aa (left $\Delta SAH^{748–795}$) shows minor defects in the DoM compared with hINCENP FL. Data for the 47-aa deletion are from the same experiment as Fig. 3 A. Data for the 107-aa deletion are from the same experiment as Fig. 6 C. Data from a single experiment. (B) Decomposition of duration of mitosis for cells in Fig. 7 D into the time from prometaphase to alignment (red) and the time from alignment to anaphase (gray). Although all cells align chromatin with similar kinetics, cells expressing hINCENP 6A take more than twice as long to initiate anaphase. Representative of $n = 3$ independent experiments.

Provided online are two tables in Excel. Table S1 is a list of plasmids and Table S2 is a list of antibodies.