Aurora B kinase activity-dependent and independent functions of the chromosomal passenger complex in regulating sister chromatid cohesion

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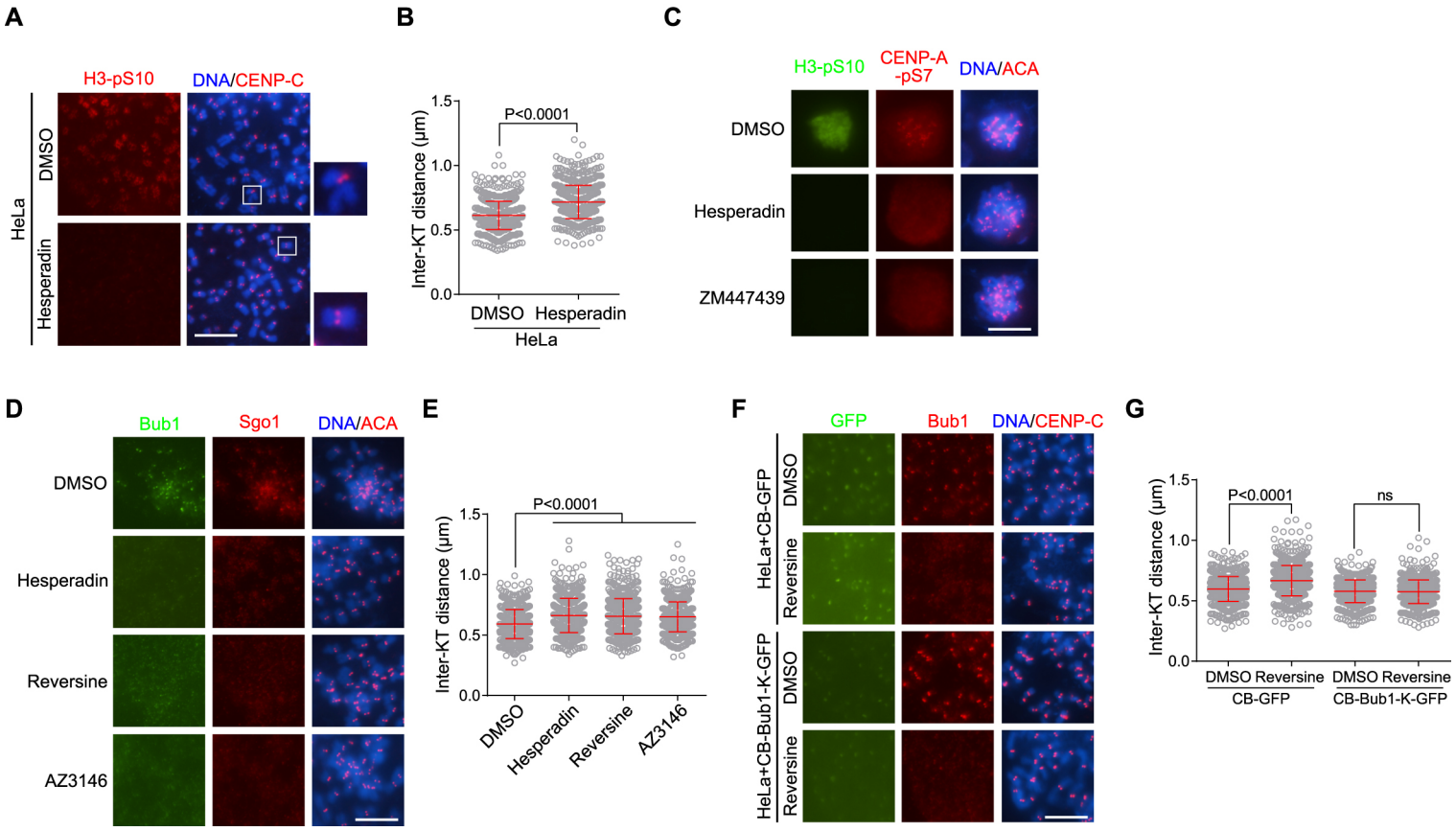
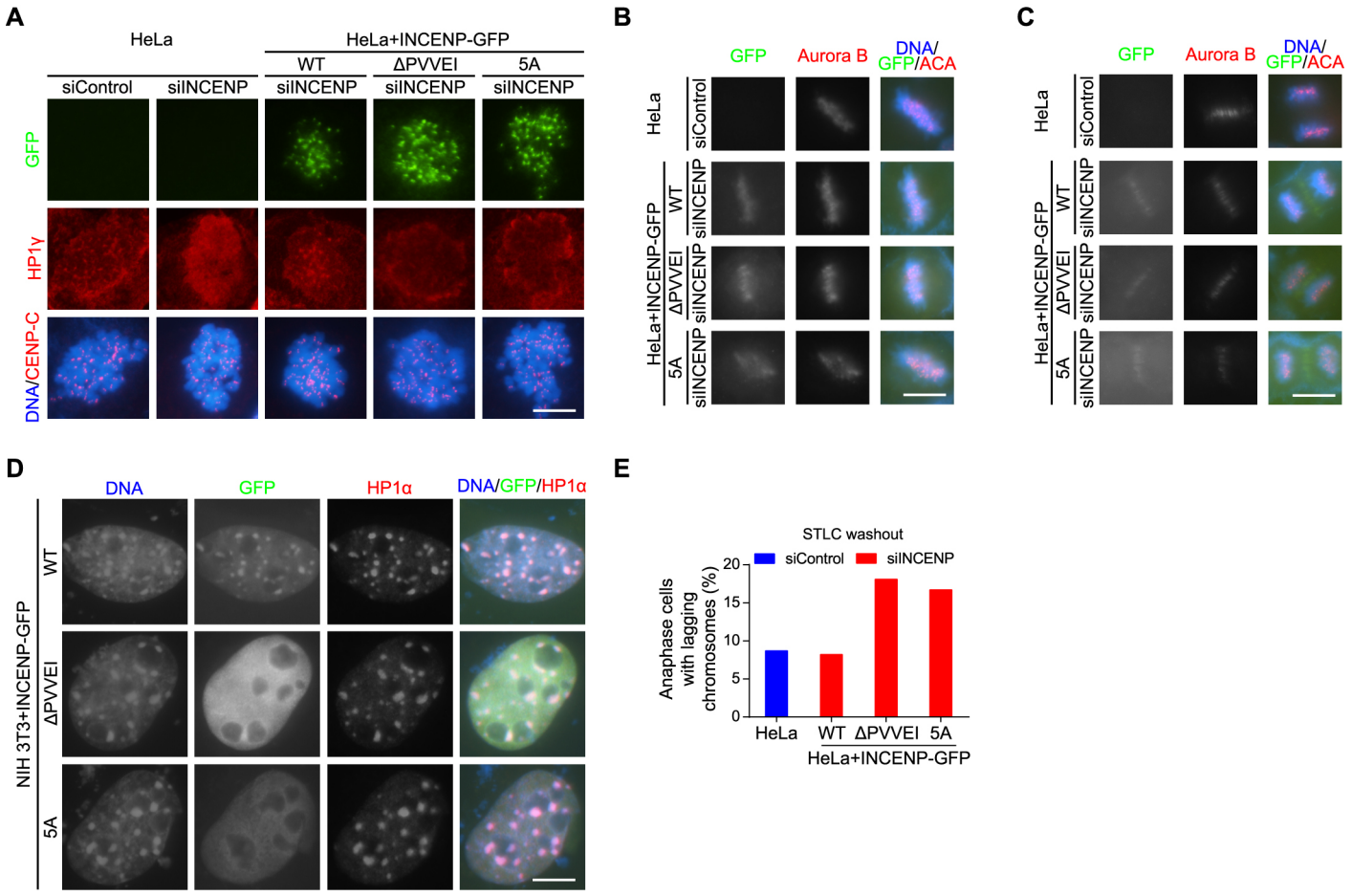
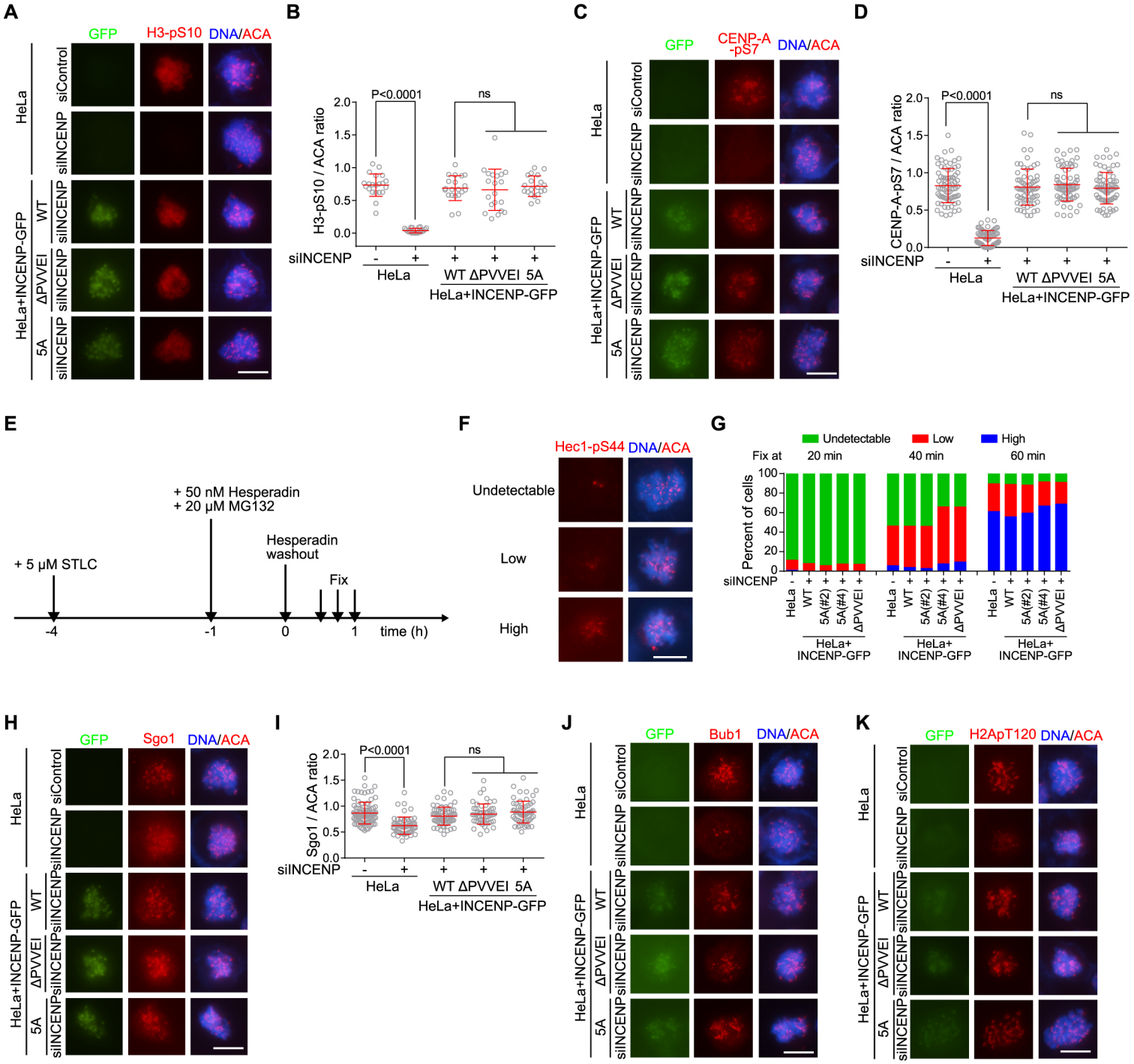
Supporting Information



Supplemental Figure S1. Inhibiting Aurora B kinase activity causes centromeric cohesion defects which can be rescued by centromere-tethered Bub1 (Related to Figure 1). (A and B) HeLa cells were treated for 1 h with nocodazole and DMSO or Hesperadin. Mitotic chromosome spreads were stained with DAPI and antibodies for H3-pS10 and CENP-C (A). The inter-KT distance was measured on 676-733 chromosomes in 20 cells (B). Means and SDs are shown (unpaired t-test). (C) HeLa cells were treated for 1 h with nocodazole and MG132, together with DMSO or the indicated inhibitors. Cells were then stained with DAPI, ACA and antibodies for H3-pS10 and CENP-A-pS7. Example images are shown. (D and E) HeLa cells were treated for 1 h with nocodazole and MG132, together with DMSO, Hesperadin, Reversine or AZ3146. Mitotic chromosome spreads were stained with DAPI and antibodies for Bub1, Sgo1 and ACA. Example images are shown (D). The inter-KT distance was measured on 641-654 chromosomes in 20 cells (E). (F and G) HeLa cells stably expressing CB-GFP or CB-Bub1-K-GFP were treated for 1 h with nocodazole and MG132, together with DMSO or Reversine. Mitotic chromosome spreads were stained with DAPI and antibodies for Bub1 and CENP-C. Example images are shown (F). The inter-KT distance was measured on around 800 chromosomes in 30 cells (G). Note that the Bub1 antibody does not recognize the kinase domain of Bub1. Scale bars, 10 μm.

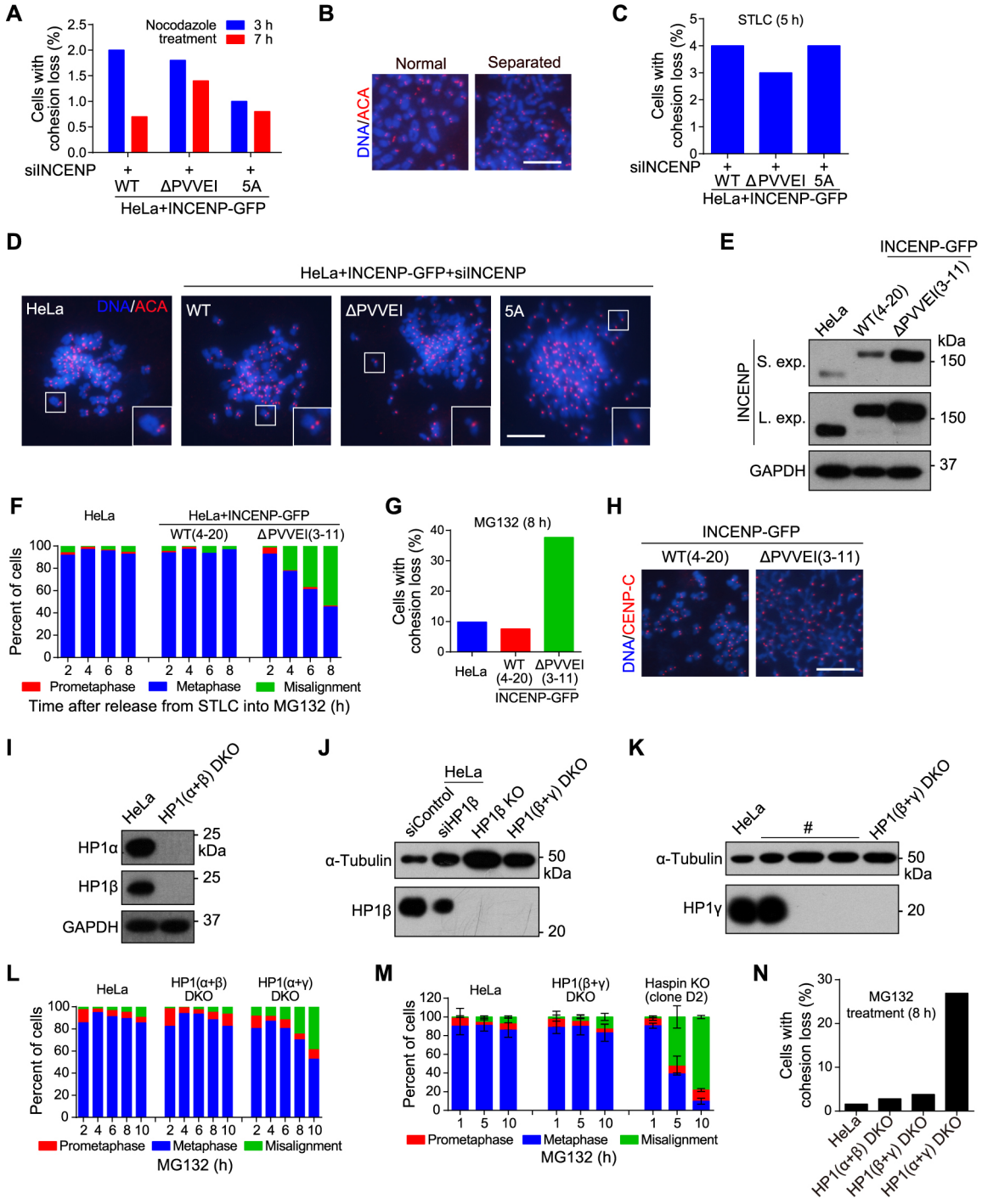


Supplemental Figure S2. Disrupting the INCENP-HP1 interaction delocalizes HP1 from mitotic centromeres and increases chromosome missegregation (Related to Figure 3). (A) HeLa cells and the indicated stable cell lines transfected with control or INCENP siRNA were treated with nocodazole for 3 h. Mitotic cells collected by shake-off were spun on slides and stained with DAPI and antibodies for GFP, HP1γ and CENP-C. (B and C) Asynchronous HeLa cells and the indicated stable cell lines transfected with control or INCENP siRNA were stained with DAPI and antibodies for Aurora B and ACA. The example metaphase (B) and anaphase (C) cells are shown. (D) NIH 3T3 cells transiently expressing the indicated INCENP-GFP proteins (wild-type or mutants) were fixed and stained with DAPI, and the anti-HP1α antibody. Example interphase cells are shown. (E) HeLa cells and the indicated stable cell lines transfected with control or INCENP siRNA were released from 5-h treatment with STLC, and then fixed and stained with ACA and DAPI. The percentage of cells with lagging chromosomes was determined in 100 anaphase cells. Scale bars, 10 μm.



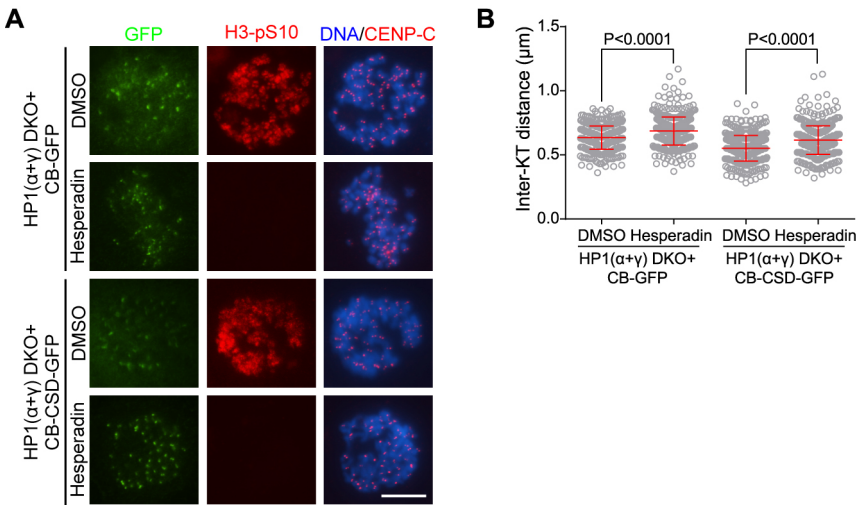
Supplemental Figure S3. Disrupting the INCENP-HP1 interaction does not compromise Aurora B kinase activity (Related to Figure 4). (A and B) HeLa cells and the indicated stable cell lines transfected with control or INCENP siRNA were treated with nocodazole for 1 h, and then, were stained with DAPI, ACA and the anti-H3-pS10 antibody (A). The immunofluorescence intensity ratio of H3-pS10/DNA was determined in 20 cells (B). (C and D) Cells treated as in (A) were stained with DAPI, ACA and the anti-CENP-A-pS7 antibody (C). The immunofluorescence intensity ratio of CENP-A-pS7 was determined on 71-91 chromosomes in 20 cells. (E-G) Treatment scheme for Aurora B reactivation assays in cells with treatment as described in (A) (E). Immunofluorescence microscopy of Hec1-pS44, ACA and DAPI in cells treated as in (A). Example images of cells with various levels of Hec1-pS44 are shown (F). Note that the Hec1-pS44 antibodies show spindle pole background staining that is not eliminated by Hesperadin treatment. Approximately 100 mitotic cells in each condition from one experiment were classified according to the intensity of Hec1-pS44 staining (G). (H-K) HeLa cells and the indicated stable cell lines transfected with control

or INCENP siRNA were treated with nocodazole for 1 h, and then, were stained with DAPI, ACA and antibodies for Sgo1 (H), Bub1 (J) or H2A-pT120 (K). The immunofluorescence intensity ratio of centromeric Sgo1/ACA was determined on 76-84 chromosomes in 20 cells. Means and SDs are shown (unpaired t-test). Scale bars, 10 μm.



Supplemental Figure S4. Disrupting the INCENP-HP1 interaction weakens centromeric cohesion (Related to Figure 5). (A and B) HeLa cells stably expressing INCENP-GFP (wild-type or the indicated mutants) were transfected with control or INCENP siRNA, and then treated with nocodazole for 3 h or 7 h. Mitotic chromosome spreads were stained with DAPI and ACA. The percentage of cells with cohesion loss was determined in around 100 cells (A). Example images are shown (B). (C) Cells transfected as described in (A) were treated with STLC for 5 h. Mitotic chromosome spreads were stained and quantified in around 100 cells. (D) HeLa cells and the indicated stable cell lines transfected with control or INCENP siRNA were exposed to MG132 for 7 h, as described in Figure 5E. Example images of the mitotic chromosome spreads are shown. (E) Lysates prepared from nocodazole-arrested mitotic HeLa cells in which endogenous INCENP was stably replaced by exogenous INCENP-GFP (wild-type or mutant) were immunoblotted. (F) Cells described in (E) were exposed to MG132, and then fixed at the indicated time points for DNA staining. The percentage of mitotic cells in prometaphase, metaphase, and metaphase with some misaligned

chromosomes, was determined in around 100 cells. (G and H) Cells described in (E) were exposed to MG132 for 8 h. Using mitotic chromosome spreads, the percentage of cells with cohesion loss was determined in around 100 cells (G). Example images are shown (H). (I-K) Asynchronous HeLa cells and the indicated HP1 KO clones were immunoblotted with the indicated antibodies. # in (K) represents irrelevant lanes. (L and M) HeLa cells and the indicated HP1 or Haspin KO clones were exposed to MG132, then fixed at the indicated time points for DNA staining, and quantified as described in (F) in around 100 cells (L) or 200 cells (M; n = 2). (N) HeLa cells and the indicated HP1 DKO clones were exposed to MG132 for 8 h. Using mitotic chromosome spreads, the percentage of cells with cohesion loss was determined in around 100 cells. Scale bars, 10 μm.



Supplemental Figure S5. Centromere-tethered HP1 cannot bypass the requirement for Aurora B kinase activity in centromeric cohesion protection (Related to Figure 6). (A and B) HP1α and HP1γ DKO cells stably expressing CB-GFP or CB-CSD-GFP were treated for 1 h with nocodazole and DMSO or Hesperadin. Mitotic chromosome spreads were stained with DAPI and antibodies for GFP, H3-pS10 and CENP-C (A). The inter-KT distance was measured on 647-676 chromosomes in 20 cells (B). Means and SDs are shown (unpaired t-test). Scale bar, 10 μm.