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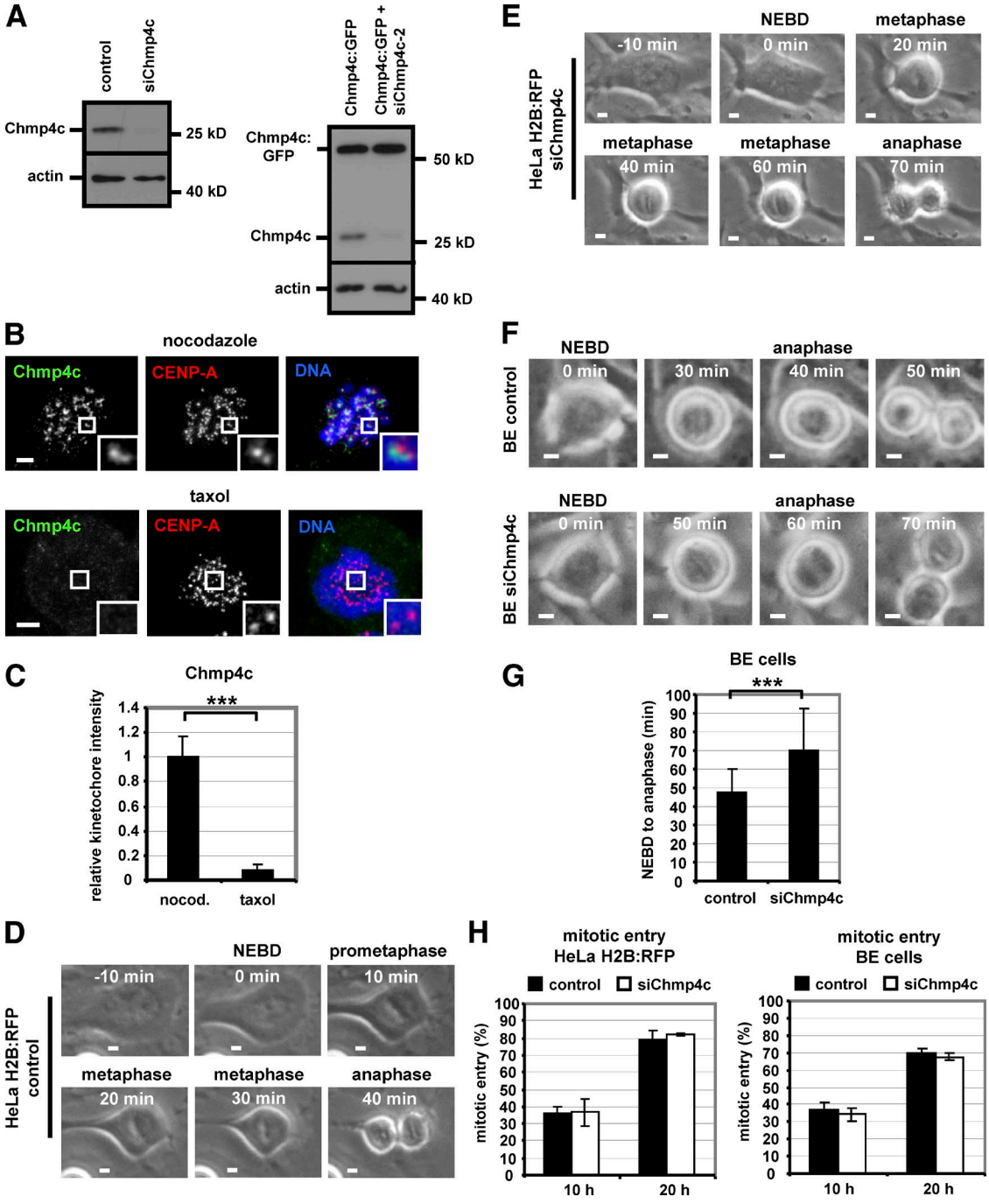


Figure S1. **Chmp4c-depletion delays anaphase onset. (A)** Western blot analysis of total Chmp4c or actin in cells transfected with negative siRNA (control), two different Chmp4c siRNAs (siChmp4c, siChmp4c-2), or Chmp4c:GFP. **(B and C)** Chmp4c localization in cells treated with nocodazole (nocod) or taxol for 4 h. Relative green/red fluorescence intensity is shown, and values in nocodazole were set to one. $n > 200$ kinetochores, 20 cells from three independent experiments. Insets in B show 1.7× magnification of kinetochores. **(D–F)** Phase-contrast images of HeLa H2B:RFP (D and E) or BE cells (F) transfected as in A and monitored by time-lapse microscopy. Time is from NEBD. Related to Videos 3–6. Bars, 5 µm. **(G)** Time from NEBD to anaphase, calculated from time-lapse movies as in F. Control: $n = 52$; siChmp4c: $n = 53$. Error bars show the SD. ***$P < 0.001$ compared with the control. Student's *t* test was used. **(H)** Percentage of cells entering mitosis calculated from time-lapse movies as in D–F. Time is from the start of filming. HeLa H2BRFP–control: $n = 264$ and siChmp4c: $n = 272$; BE cells–control $n = 292$ and siChmp4c: $n = 335$, from two independent experiments.

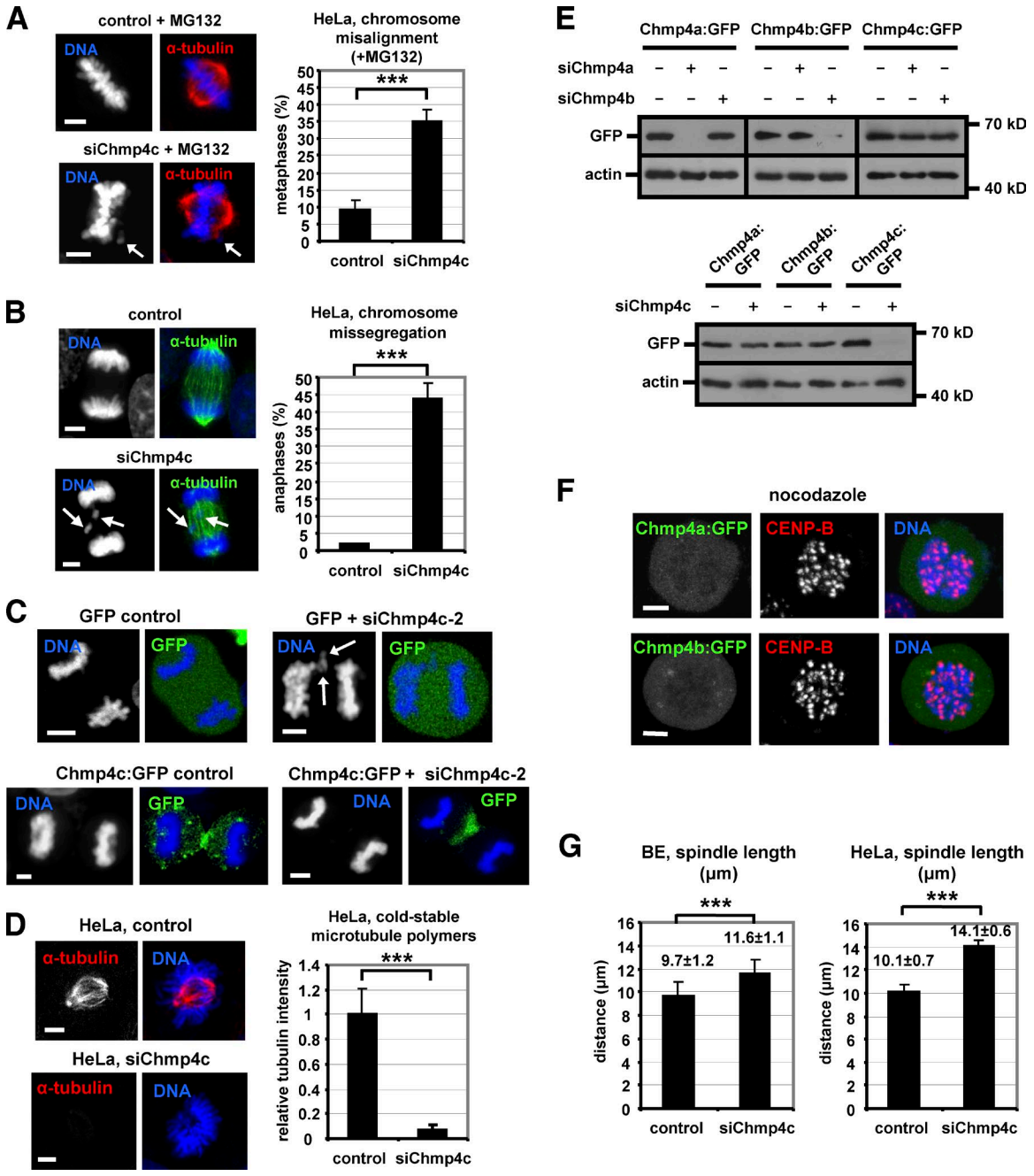


Figure S2. **Chmp4a and Chmp4b do not localize to kinetochores. (A and B)** Chromosome misalignment (A) or missegregation (B) in HeLa cells transfected with negative siRNA (control) or Chmp4c siRNA (siChmp4c) in the presence of MG132 for 1 h (A) or without MG132 (B). *n* = 300 cells from three independent experiments. **(C)** Cells expressing GFP or Chmp4c:GFP resistant to degradation by siChmp4c-2 were transfected with negative siRNA (control) or Chmp4c-2 siRNA (siChmp4c-2). Arrows indicate misaligned or missegregated chromosomes. **(D)** Cold-stable microtubule fibers in cells transfected as in A and treated with ice-cold medium for 15 min. *n* = 90 cells from three independent experiments. **(E)** Chmp4a, Chmp4b, and Chmp4c siRNAs do not cross-react with the other Chmp4 isoforms. Western blot analysis of total GFP and actin in cells transfected with Chmp4a:GFP, Chmp4b:GFP, or Chmp4c:GFP in the absence or presence of siChmp4a, siChmp4b, or siChmp4c. **(F)** Localization of Chmp4a:GFP and Chmp4b:GFP in cells treated with nocodazole for 4 h. Bars, 5 µm. **(G)** Metaphase-like spindle length in cells transfected as in A. Average values ± SD are shown. *n* = 30 cells from three independent experiments. Error bars show the SD. ***, P < 0.001 compared with control. Student's *t* test was used.

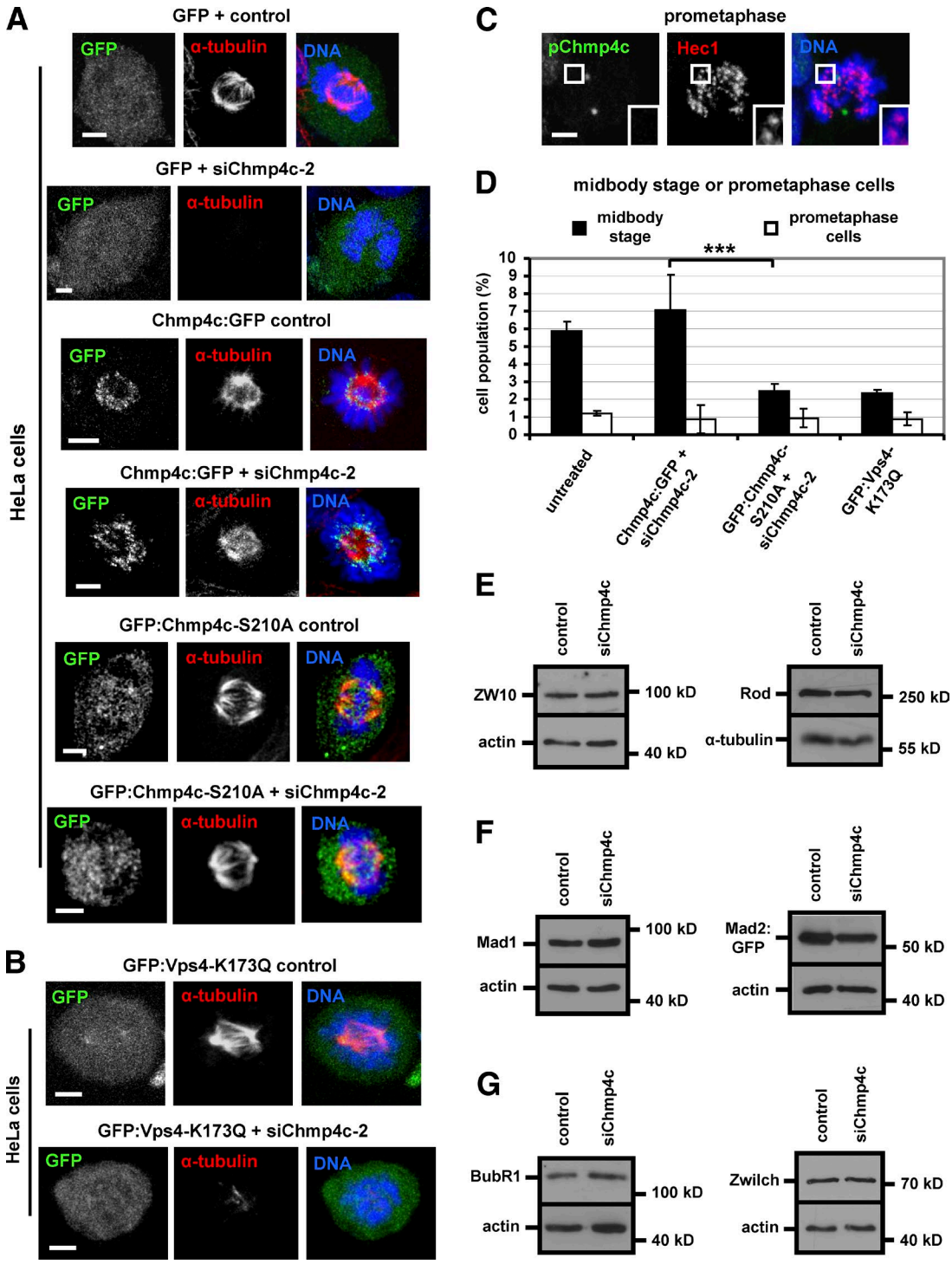


Figure S3. **Expression of Chmp4c-S210A or Vps4-K173Q mutant proteins reduces the frequency of midbody-stage cells. (A and B)** Cold-stable microtubule polymers. Cells expressing GFP, GFP:Chmp4c-S210A resistant to degradation by Chmp4c-2 siRNA (siChmp4c-2), or GFP:Vps4-K173Q were transfected with negative siRNA (control) or siChmp4c-2 and treated with ice-cold medium for 15 min. **(C)** Phosphorylated Chmp4c-S210, S214, and S215 (pChmp4c) were not detectable at kinetochores in prometaphase. 20 cells from two independent experiments were examined. Insets in C show 1.7× magnification of kinetochores. Bars, 5 µm. **(D)** Frequency of prometaphase or midbody stage cells. Cells were untreated or transfected as in A and B. Error bars show the SD from the mean from three independent experiments. A minimum of 300 cells was analyzed per experiment. ***, $P < 0.001$ compared with the control. Statistical significant differences were determined by ANOVA and Student's *t* test. **(E–G)** Western blot analysis of total ZW10, Rod, Mad1, Mad2:GFP, BubR1, Zwilch, or actin.

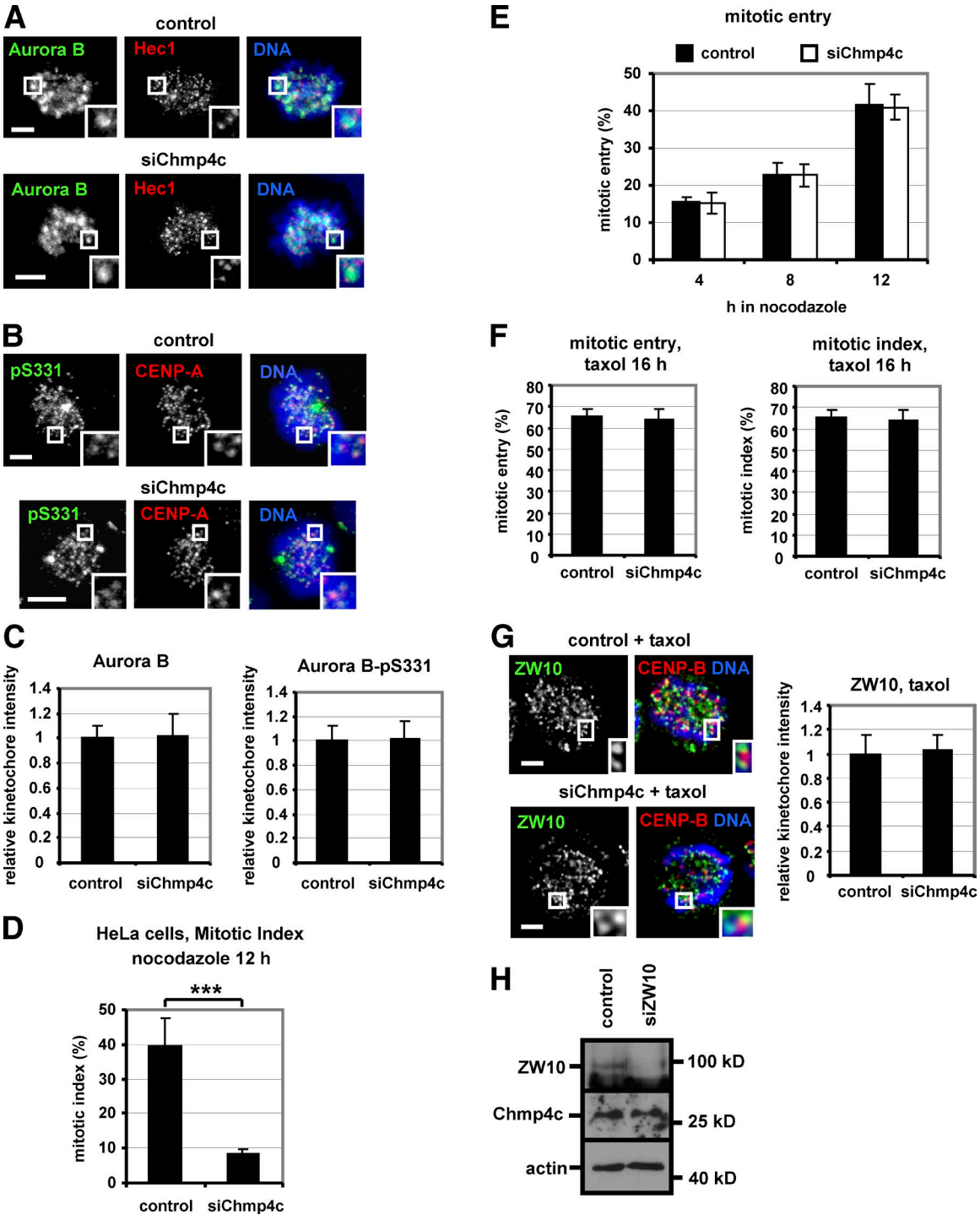


Figure S4. **Chmp4c is not required for taxol-induced mitotic arrest. (A–C)** Localization of total Aurora B or phosphorylated Aurora B-S331 (pS331) in cells transfected with negative siRNA (control) or Chmp4c siRNA (siChmp4c). **(D)** Mitotic index analysis of HeLa cells transfected as in A and treated with nocodazole. n = 300 from three independent experiments. ***, $P < 0.001$ compared with the control. Student's t test was used. **(E)** Percentage of BE cells entering mitosis in nocodazole as determined by phase-contrast time-lapse microscopy. Control: n = 367; siChmp4c: n = 413. **(F)** Mitotic entry and mitotic index analysis of BE cells treated with taxol, determined by phase-contrast time-lapse microscopy. Control: n = 301; siChmp4c: n = 374 from two independent experiments. Error bars show the SD. **(G)** Localization of ZW10. Cells were transfected as in A and treated with taxol for 4 h. Relative green/red fluorescence intensity is shown, and values in control were set to one. $n > 200$ kinetochores, 20 cells (C and G) from three independent experiments. Insets show 1.7× magnification of kinetochores. Bars, 5 µm. **(H)** Western blot analysis of total ZW10, Chmp4c, and actin in cells transfected with negative siRNA (control) or ZW10 siRNA (siZW10).

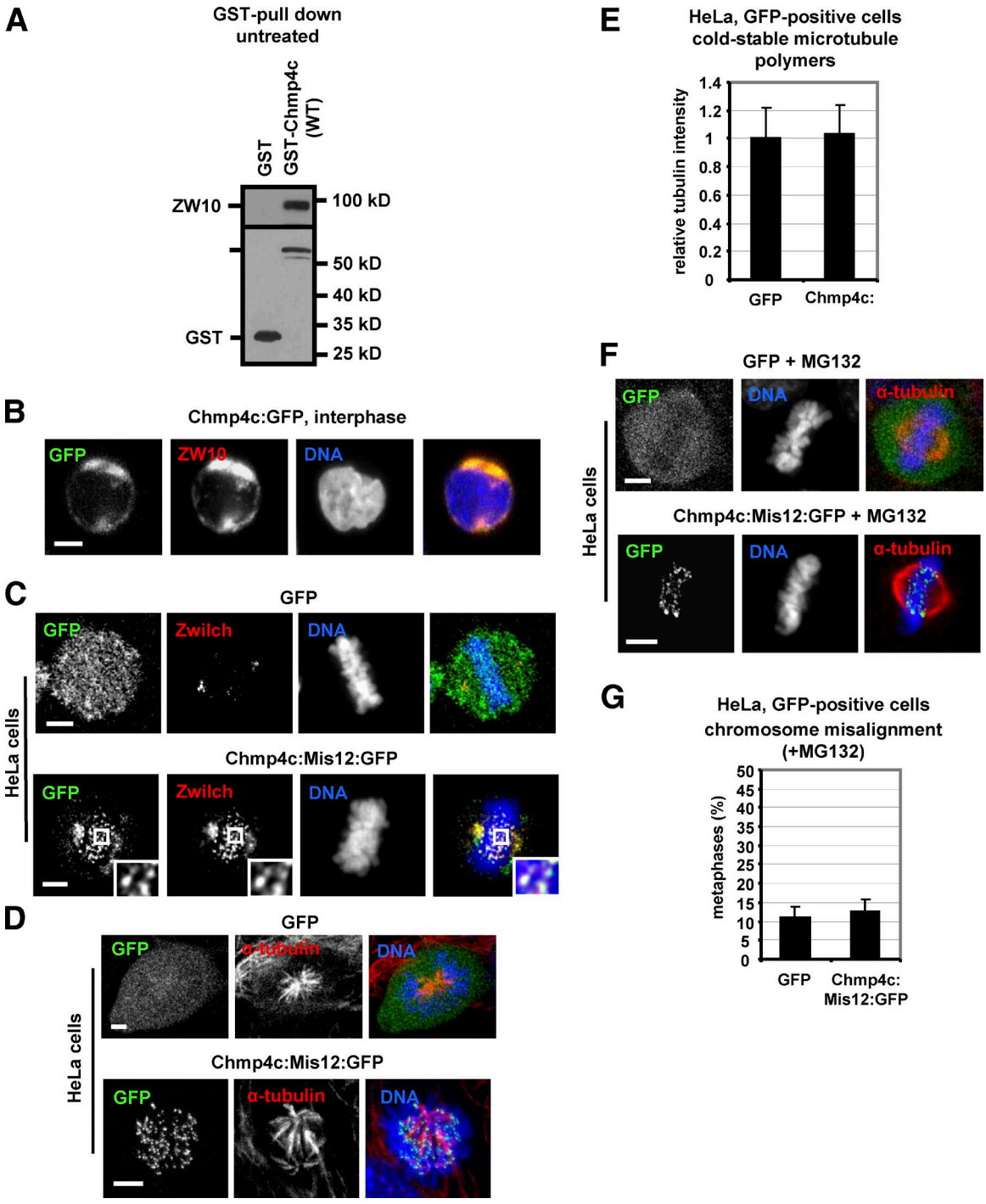
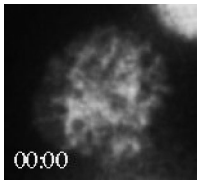
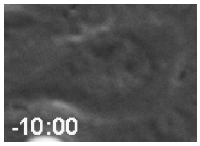


Figure S5. **Expression of Chmp4c:Mis12:GFP promotes localization of Zwilch to metaphase kinetochores. (A)** GST pull-down assay from untreated cells. Precipitated ZW10 or GST was detected by Western blotting. **(B)** Localization of Chmp4c:GFP and ZW10 in an interphase cell. 20 GFP-positive cells from two independent experiments were analyzed. **(C)** Localization of Zwilch in the metaphase plate in cells expressing GFP or Chmp4c:Mis12:GFP. Insets in C show 1.7× magnification of kinetochores. **(D and E)** Cold-stable microtubule polymers. Cells transfected as in C were treated with ice-cold medium for 15 min. Mean tubulin intensity is shown, and values in GFP were set to one. **(F and G)** Chromosome misalignment. Cells transfected as in C were treated with MG132 for 1 h. *n* = 90 cells from three independent experiments. Error bars show the SD. Bars, 5 µm.

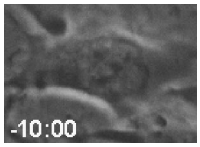


Video 1. **Control cells exhibit successful chromatin alignment and segregation.** HeLa cells stably expressing H2B:RFP (white) were transfected with negative siRNA and analyzed by fluorescence time-lapse microscopy. Time = 0, start of chromatin condensation. Frames were taken every 2.5 min for 37.5 min. Time counter shows minute:second. Display rate: one frame per second. Related image stills are shown in Fig. 2 A.

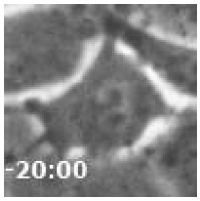
Video 2. **Chmp4c-deficient cells enter anaphase with misaligned chromatin.** HeLa cells stably expressing H2B:RFP (white) were transfected with Chmp4c siRNA and analyzed by fluorescence time-lapse microscopy. Time = 0, start of chromatin condensation. Frames were taken every 2.5 min for 65 min. Time counter shows minute:second. Display rate: one frame per second. Related image stills are shown in Fig. 2 B.



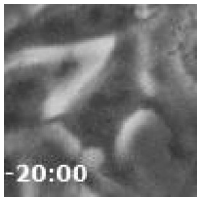
Video 3. **Progression from NEBD to anaphase in control HeLa H2B:RFP cells.** HeLa cells stably expressing H2B:RFP were transfected with negative siRNA and analyzed by phase-contrast time-lapse microscopy. Time = 0, NEBD. Frames were taken every 10 min for 60 min. Time counter shows minute:second. Display rate: one frame per second. Related image stills are shown in Fig. S1 D.



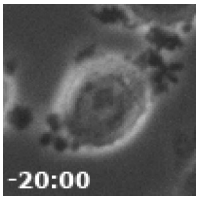
Video 4. **Progression from NEBD to anaphase in Chmp4c-depleted HeLa H2B:RFP cells.** HeLa cells stably expressing H2B:RFP were transfected with Chmp4c siRNA and analyzed by phase-contrast time-lapse microscopy. Time = 0, NEBD. Frames were taken every 10 min for 90 min. Time counter shows minute:second. Display rate: one frame per second. Related image stills are shown in Fig. S1 E.



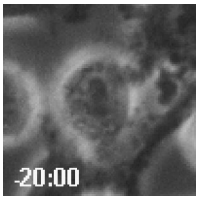
Video 5. **Progression from NEBD to anaphase in control BE cells.** BE cells transfected with negative siRNA were analyzed by phase-contrast time-lapse microscopy. Time = 0, NEBD. Frames were taken every 10 min for 80 min. Time counter shows minute :second. Display rate: one frame per second. Related image stills are shown in Fig. S1 F.



Video 6. **Progression from NEBD to anaphase in Chmp4c-depleted BE cells.** BE cells transfected with Chmp4c siRNA were analyzed by phase-contrast time-lapse microscopy. Time = 0, NEBD. Frames were taken every 10 min for 100 min. Time counter shows minute:second. Display rate: one frame per second. Related image stills are shown in Fig. S1 F.



Video 7. **Control cells treated with nocodazole arrest in mitosis with condensed chromatin.** BE cells transfected with negative siRNA were treated with 3.32 μM nocodazole and analyzed by phase-contrast time-lapse microscopy. Time = 0, NEBD. Frames were taken every 20 min for 800 min. Time counter shows minute:second. Display rate: one frame per second. Related image stills are shown in Fig. 5 B.



Video 8. **Chmp4c-deficient cells treated with nocodazole enter mitosis but decondense their chromatin and become multinucleated.** BE cells transfected with Chmp4c siRNA were treated with 3.32 μM nocodazole and analyzed by phase-contrast time-lapse microscopy. Time = 0, NEBD. Frames were taken every 10 min for 190 min. Time counter shows minute:second. Display rate: one frame per second. Related image stills are shown in Fig. 5 B.