Petsalaki and Zachos, http://www.jcb.org/cgi/content/full/jcb.201310071/DC1

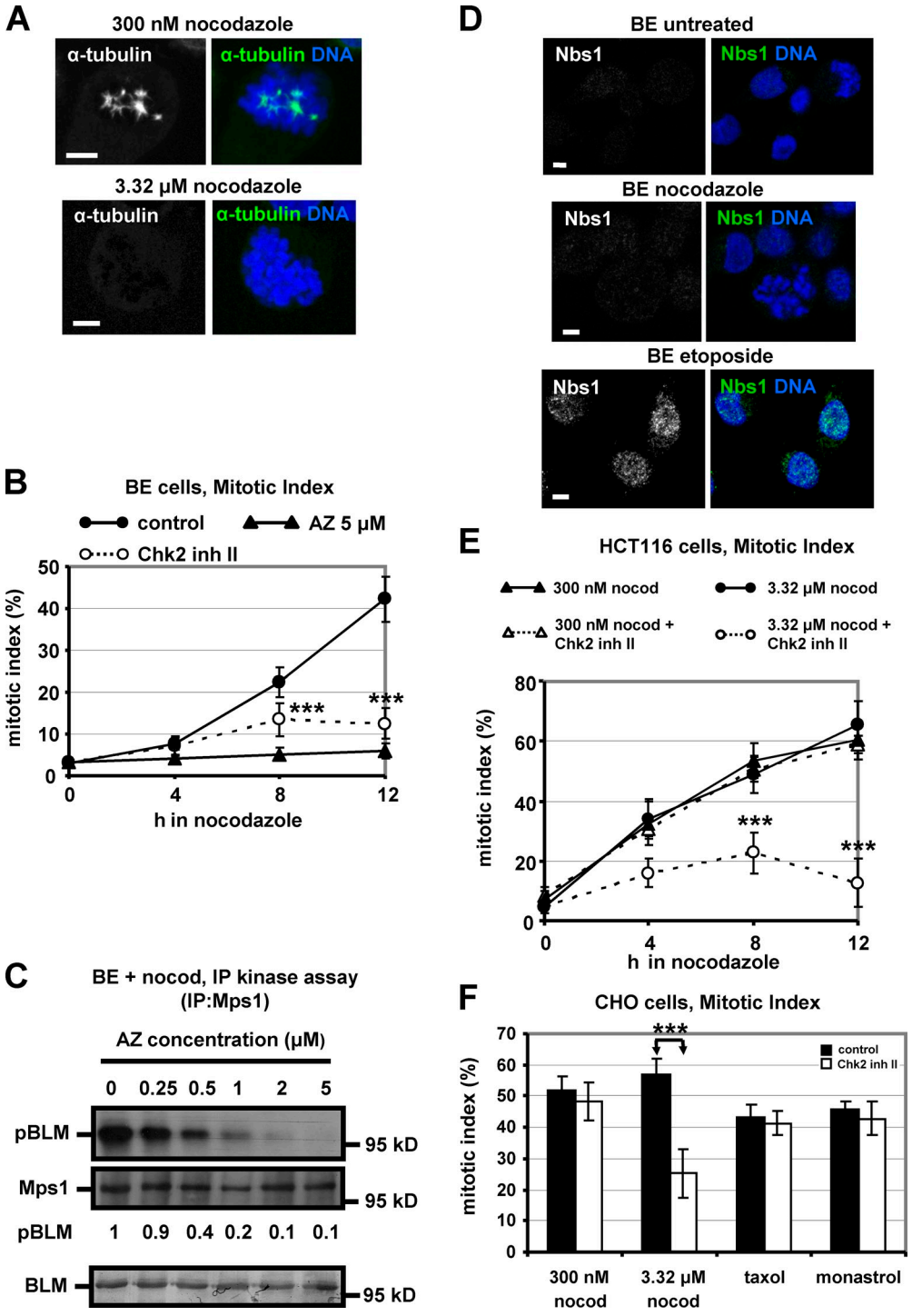


Figure S1. **Chk2 is dispensable for prometaphase accumulation in 300 nM nocodazole.** (A) Microtubule depolymerization in BE cells treated with nocodazole for 1 h. (B) Mitotic index analysis. BE cells were treated with 3.32 μM nocodazole in the absence (control) or presence of Chk2 inhibitor II (inh II) or AZ3146 (AZ). (C) Immunoprecipitation kinase assay. BE cells were treated with 3.32 μM nocodazole in the presence of AZ3146 and MG132 for 8 h. (top) Mps1-associated phosphorylation of GST-BLM (9–479) substrate (pBLM) and Western blot analysis of immunoprecipitated (IP) Mps1. pBLM values at 0 μM AZ3146 were taken as 1. (bottom) Ponceau staining of total GST-BLM (9–479) levels. (D) Localization of Nbs1. Cells were untreated or treated with 3.32 μM nocodazole or etoposide for 4 h. Nbs1 foci on the DNA were readily observed in cells treated with etoposide but not nocodazole. (E) Mitotic index analysis. HCT116 cells were treated with 300 nM or 3.32 μM nocodazole (nocod) in the absence or presence of Chk2 inhibitor. (F) CHO cells were treated as in E for 12 h. Error bars show the SD from the means of three independent experiments. ***, $P < 0.001$ compared with controls. Bars, 5 μm.

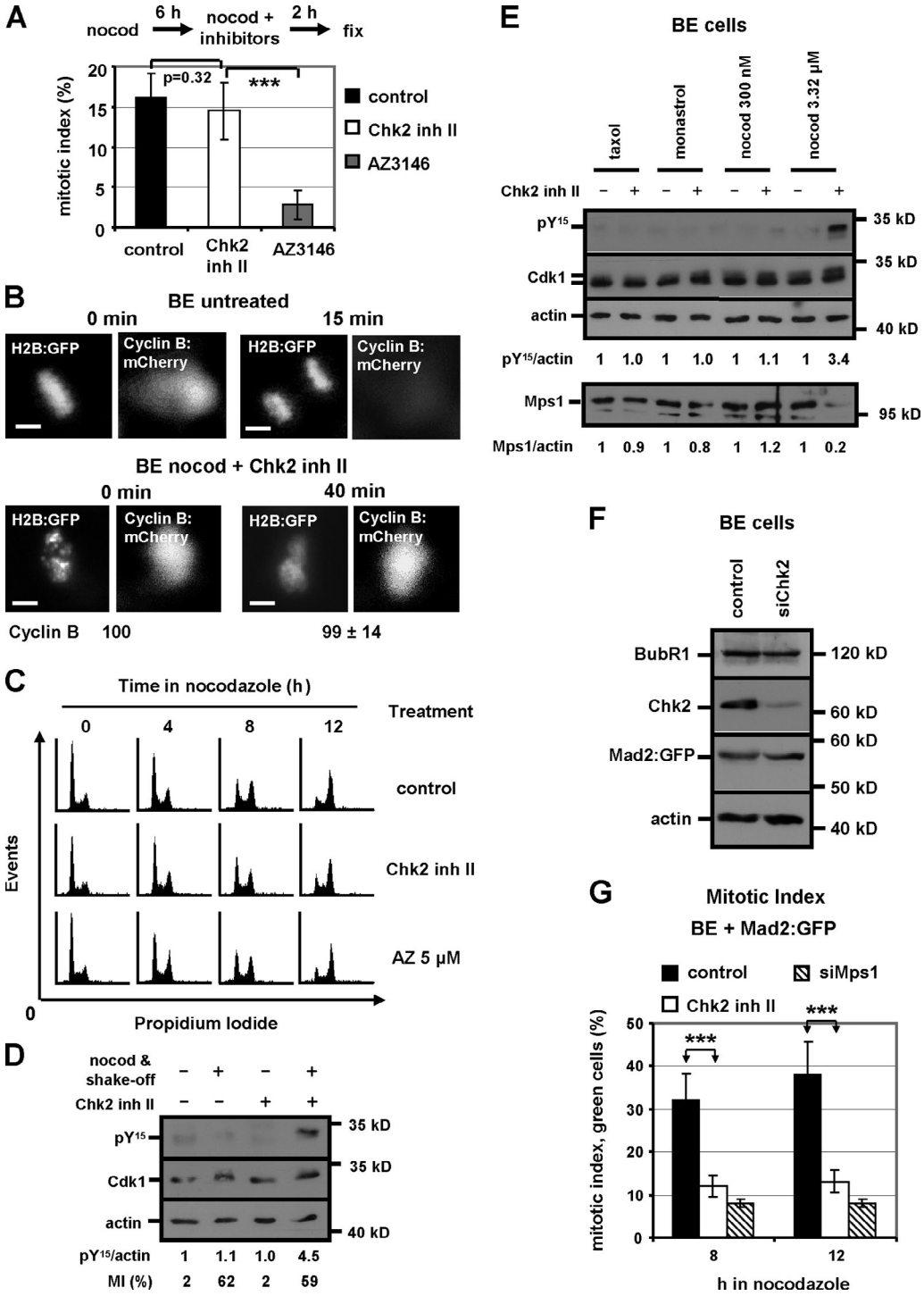


Figure S2. **Chk2-deficient cells exhibit Cdk1-Y15 phosphorylation and Mps1 degradation in high nocodazole.** (A) Mitotic index analysis. BE cells were treated with 3.32 µM nocodazole (nocod) for 8 h, and Chk2 inhibitor II, AZ3146, or no inhibitor (control) was added for the last 2 h. (B) Cyclin B–mCherry fluorescence. Cells expressing H2B-GFP and Cyclin B–mCherry were untreated or treated with 3.32 µM nocodazole (nocod) and Chk2 inhibitor II (inh II) and analyzed by time-lapse microscopy. (top cell) Example of an untreated cell in metaphase (0 min) or in anaphase (15 min). (bottom cell) Example of a cell in prometaphase (0 min) or after mitotic exit (45 min). Values show mean Cyclin B fluorescence intensity ± SDs. Cyclin B fluorescence levels in prometaphase were taken as 100. *n* = 5 cells. Bars, 5 µm. (C) DNA content flow cytometry analysis of BE cells treated with 3.32 µM nocodazole in the absence (control) or presence of Chk2 inhibitor II or AZ3146 (AZ). (D) Western blot analysis of total phosphorylated Y15 (pY15), Cdk1, and actin in the absence or presence of Chk2 inhibitor II. Where indicated, BE cells were enriched in mitosis by treatment with 3.32 µM nocodazole for 8 h and shake-off. Values at the first lane were taken as 1. MI, mitotic indices. (E) Western blot analysis of total pY15, Cdk1, Mps1, and actin. Cells were treated with the indicated drugs for 8 h in the absence or presence of Chk2 inhibitor II. Values in the absence of Chk2 inhibitor were taken as 1. (F) Western blot analysis of total BubR1, Chk2, Mad2-GFP, and actin in cells transfected with Mad2-GFP in combination with negative siRNA (control) or Chk2 siRNA (siChk2). (G) Mitotic index analysis. Cells expressing Mad2-GFP were treated with 3.32 µM nocodazole in the absence (control) or presence of Chk2 inhibitor II or in combination with Mps1 siRNA (siMps1). Mitotic index shows the percentage of mitotic green cells/total green cells. Error bars show the SD from the means of three independent experiments. ***, P < 0.001. Lines in A and G show which samples were compared with each other to calculate p-value.

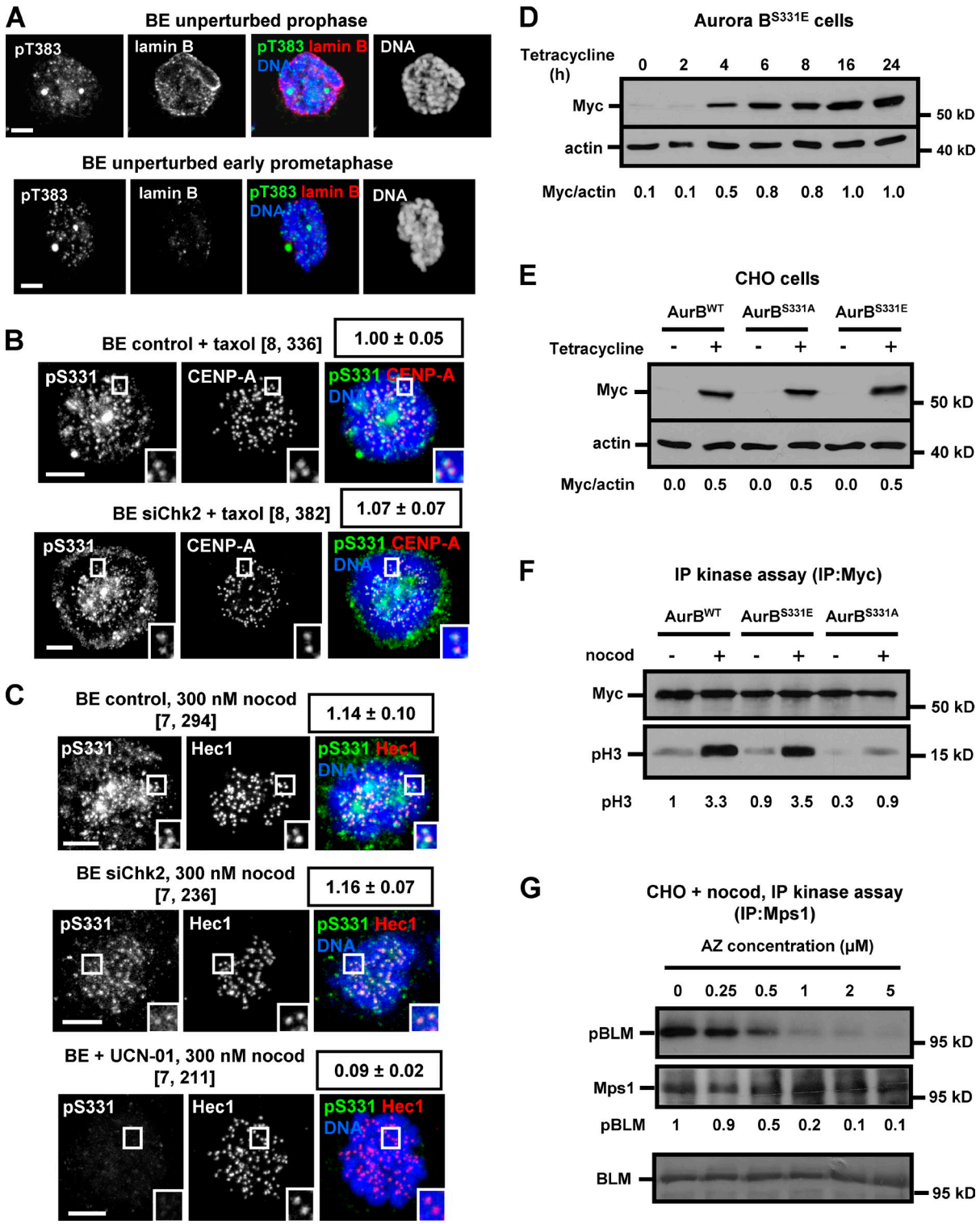


Figure S3. **Chk2 is dispensable for Aurora B–S331 phosphorylation in the presence of taxol or 300 nM nocodazole.** (A) Localization of phosphorylated Chk2-T383 (pT383) in unperturbed prophase and early prometaphase. (B and C) Aurora B–phospho-S331 (pS331) staining. (B) Cells transfected with negative siRNA (control) or Chk2 siRNA (siChk2) were treated with taxol for 4 h. (C) Cells transfected as in B or treated with UCN-01 were treated with 300 nM nocodazole (nocod) for 4 h. Boxed values show mean green/red fluorescence intensity ± SDs. Values in square brackets show kinetochore pairs and number of cells analyzed. Insets show 1.7× magnification of kinetochores. (D) Western blot analysis of 6×Myc–Aurora B (Myc) and actin in CHO S331E Aurora B cells after induction with tetracycline. (E) Western blot analysis of total Myc and actin in WT, S331A, or S331E Aurora B (AurB) cells in the absence or presence of tetracycline for 16 h. (F) Immunoprecipitation kinase assay. Tetracycline-induced WT, S331A, or S331E Aurora B cells were untreated or treated with 3.32 μM nocodazole for 8 h. (top) Western blot analysis of immunoprecipitated (IP) Myc. (bottom) Western blot analysis of Myc-associated phosphorylation of Ser10 of histone H3 (pH3). Values at untreated WT were taken as 1. (G) Immunoprecipitation kinase assay. CHO cells were treated with 3.32 μM nocodazole in the presence of AZ3146 (AZ) and MG132 for 8 h. (top) Mps1-associated phosphorylation of GST-BLM (9–479) substrate (pBLM) and Western blot analysis of immunoprecipitated Mps1. Values at 0 μM AZ3146 were taken as 1. (bottom) Ponceau staining of total GST-BLM (9–479) levels. Bars, 5 μm.

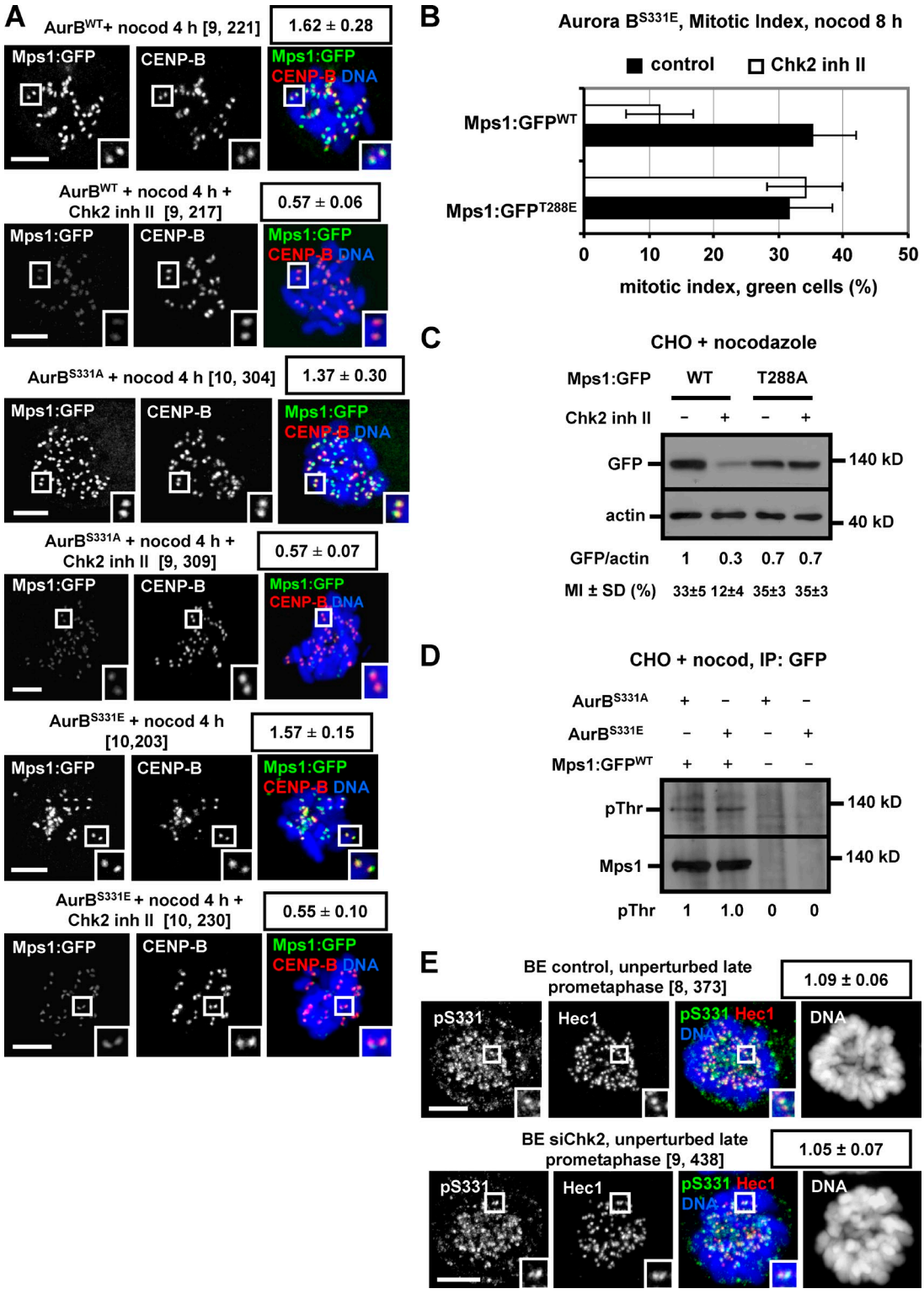


Figure S4. **Chk2 inhibition reduces Mps1 localization to kinetochores in high nocodazole.** (A) Tetracycline-induced CHO WT, S331A, or S331E Aurora B (AurB) cells expressing Mps1-GFP were treated with 3.32 µM nocodazole (nocod), MG132, and 2 µM AZ3146 for 4 h in the absence or presence of Chk2 inhibitor II. (B) Mitotic index analysis. Tetracycline-induced S331E Aurora B cells expressing WT or T288E Mps1-GFP were treated with 3.32 µM nocodazole in the absence (control) or presence of Chk2 inhibitor II (inh II) for 8 h. Mitotic index shows the percentage of mitotic green cells/total green cells. Error bars show the SD from the means of three independent experiments. (C) Western blot analysis of total GFP and actin in CHO cells transfected with WT or T288A Mps1-GFP and treated as in B. Mitotic index (MI) of green cells ± SD from the means of three independent experiments. (D) Western blot analysis of GFP-associated phosphothreonine (pThr) and Mps1 after GFP immunoprecipitation (IP). Tetracycline-induced S331A or S331E Aurora B cells expressing WT Mps1-GFP or untransfected were treated with 3.32 µM nocodazole for 8 h. Values in the first lane were taken as 1. (E) Aurora B phospho-S331 (pS331) staining. Cells were transfected with negative (control) or Chk2 siRNA (siChk2). (A and E) Boxed values show mean green/red fluorescence intensity ± SDs. Values in square brackets show kinetochore pairs and number of cells analyzed. Bars, 5 µm. The insets show 1.7 × magnification of kinetochores.

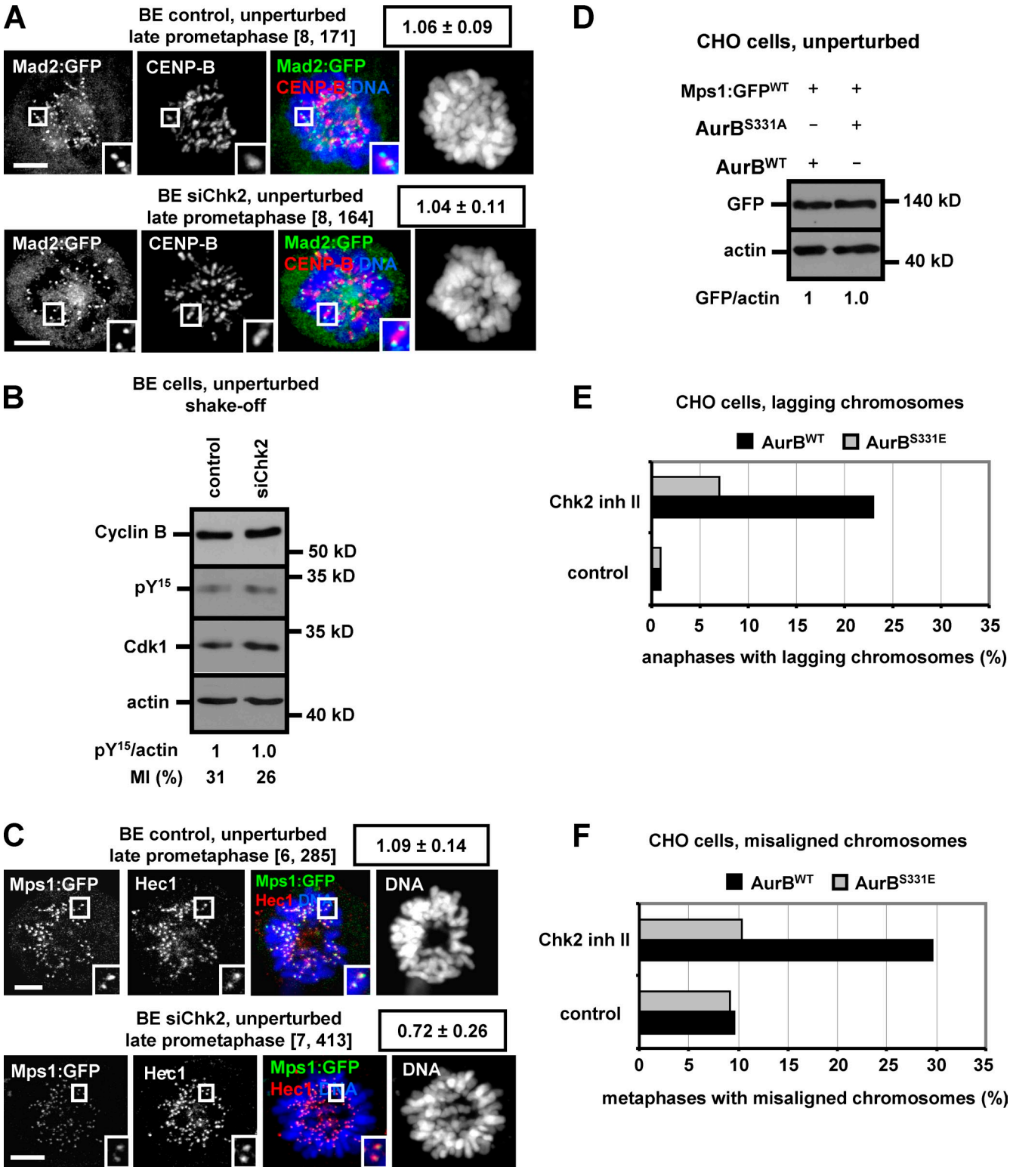
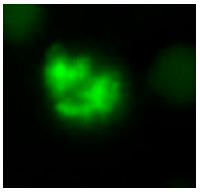
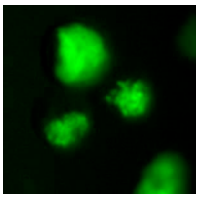


Figure S5. **Chk2 is dispensable for Mad2 localization to kinetochores in unperturbed late prometaphase.** (A) Cells expressing Mad2-GFP were transfected with negative (control) or Chk2 siRNA (siChk2). (B) Western blot analysis of total Cyclin B, phospho-Tyr15 (pY^{15}), Cdk1, and actin in cells transfected as in A and isolated by shake-off. Values at controls were taken as 1. MI, mitotic index. (C) Mps1 localization. Cells expressing Mps1-GFP were transfected as in A and treated with 2 µM AZ3146 for 2 h. (A and C) Boxed values show mean green/red fluorescence intensity ± SDs. Values in square brackets show kinetochore pairs and number of cells analyzed. Bars, 5 µm. The insets show 1.7× magnification of kinetochores. (D) Western blot analysis of total GFP and actin. CHO WT or S331A Aurora B (AurB) cells expressing WT Mps1-GFP were induced with tetracycline. Values at CHO^{WT} were taken as 1. (E) Frequency of anaphases with lagging chromosomes. Tetracycline-induced WT or S331E Aurora B cells were untreated (control) or treated with Chk2 inhibitor II (inh II) for 2 h. $n > 100$ per treatment from a single experiment. (F) Frequency of metaphases with misaligned chromosomes. Tetracycline-induced WT or S331E Aurora B cells were treated with MG132 in the absence (control) or presence of Chk2 inhibitor II for 2 h. $n > 100$ per treatment from a single experiment.



Video 1. **Control cells arrest in mitosis in high nocodazole.** BE cells stably expressing H2B-GFP (green) were treated with 3.32 μM nocodazole. Images were analyzed by time-lapse fluorescence microscopy using a fluorescence microscope (DMIRE2; Leica). Frames were taken every 20 min for 4 h.



Video 2. **Chk2-deficient cells exit mitosis in high nocodazole.** BE cells stably expressing H2B-GFP (green) were treated with 3.32 μM nocodazole in the presence of Chk2 inhibitor II and analyzed by time-lapse fluorescence microscopy using a fluorescence microscope (DMIRE2; Leica). Frames were taken every 5 min for 40 min.