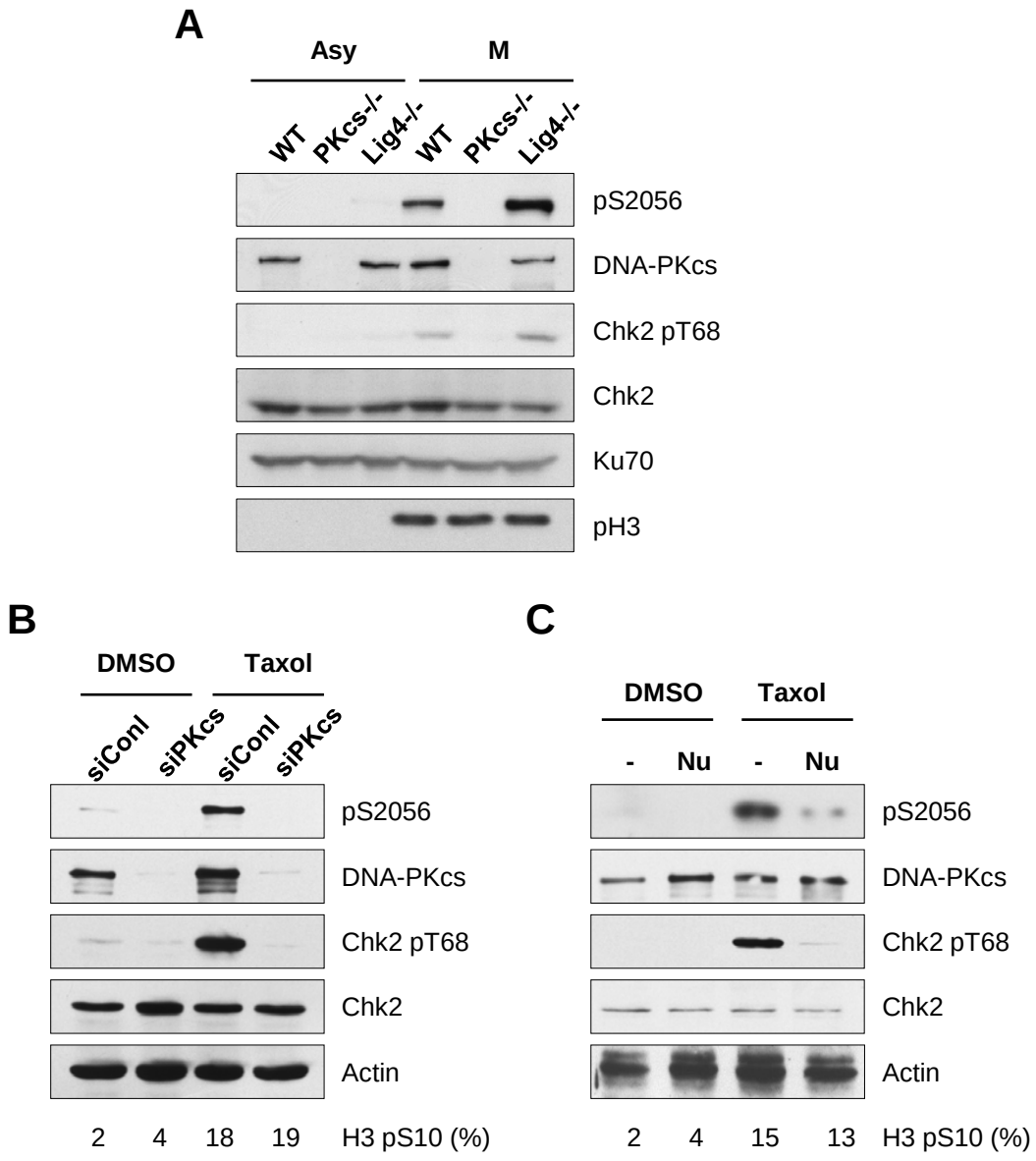
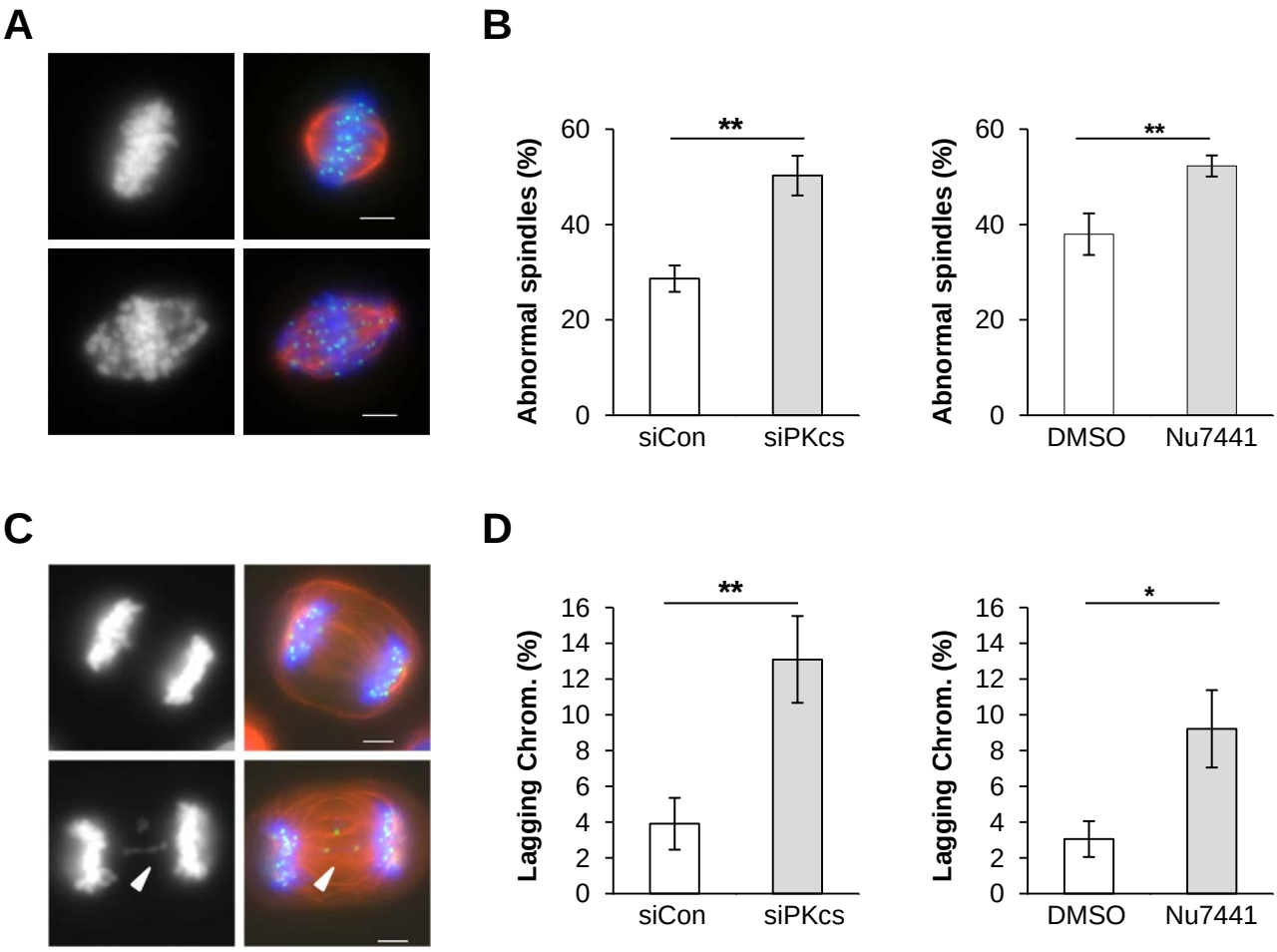
Supplemental Figure 1



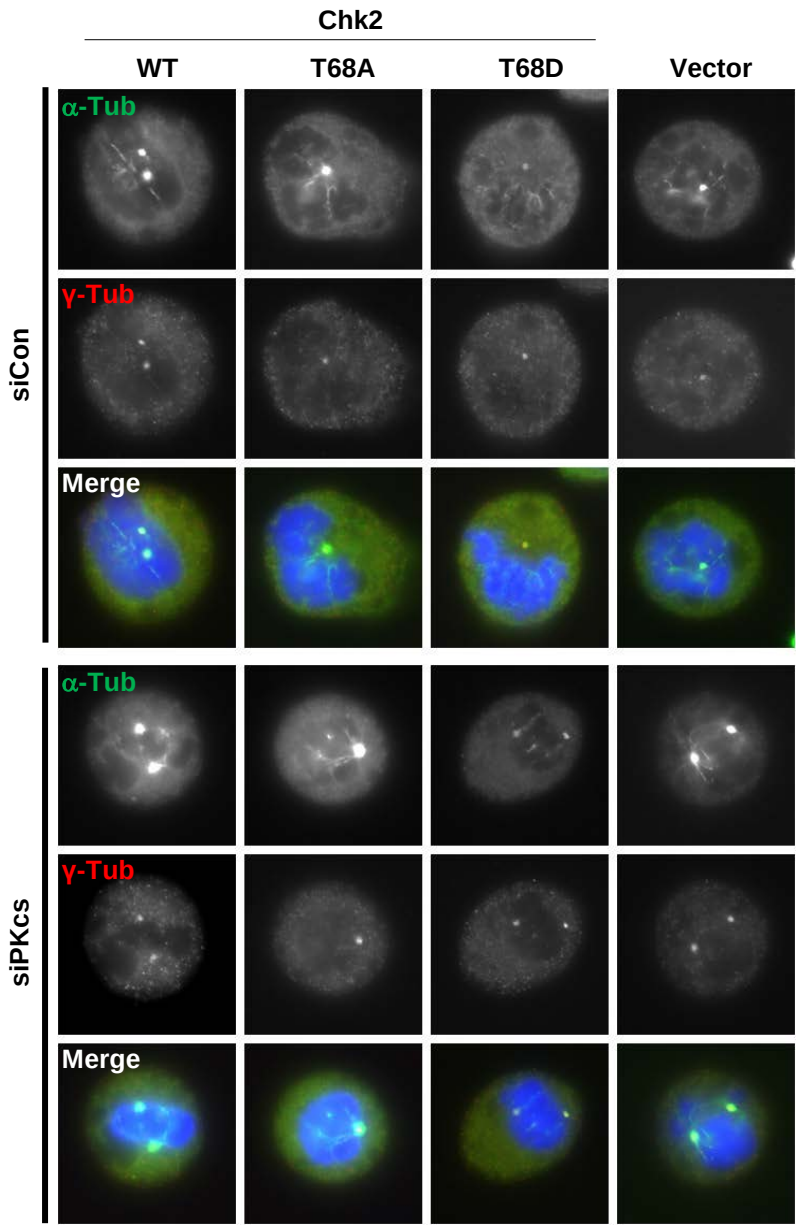
Supplemental Figure 1. DNA-PKcs is required for Chk2 Thr68 phosphorylation in mitosis. (A) HCT116 WT, DNA-PKcs-/-, and Ligase4-/- cells were synchronized with nocodazole and were analyzed by western blot. (B) HeLa cells transfected with siRNA against control or DNA-PKcs were treated with paclitaxel (100nM) for 6 hrs. Whole cell lysates were western blotted with the indicated antibodies. Mitotic index were assessed by pS10 H3 staining. (C) HeLa cells were treated with paclitaxel and Nu7441 for 6 hrs. Whole cell lysates were analyzed by western blot.

Supplemental Figure 2



Supplemental Figure 2. Loss of DNA-PKcs increases mitosis defect and chromosomal instability. (A) HeLa cells were transfected with siRNA against DNA-PKcs or control, 24 hours later cell were treated with 100 ng/ml nocodazole for 16 hours, and were released into fresh medium containing 10uM MG132 for 3 hours, or wild type HeLa cells were treated with 100 ng/ml nocodazole for 16 hours, and then were released into fresh medium or medium with 10uM Nu7441for 3 hours, both of which containing 10uM MG132. Cells were fixed and stained for DNA (blue), tubulin (red), and a centromere marker CREST (green). Bar, 5 um. (B) Percentage of mitotic cells showed misaligned chromosomes. The result was generated from three independent analyses . (C) Unperturbed DNA-PKcs knockdown or Nu7441 treated HeLa cells were fixed and stained for tubulin (red), CREST (green), and DNA (blue). (D) Percentages of mitotic cells showed lagging chromosome were counted from three independent analyses. Bar, 10 um. ** $p < 0.01$.

Supplemental Figure 3



Supplemental Figure 3. The DNA-PKcs-Chk2 signaling pathway modulates microtubule nucleation during mitosis. (A) HeLa cells stably expressing Chk2 wild type, T68A mutant, or T68D mutant were transfected with siRNA against control or DNA-PKcs. Forty-eight hours after transfection, cells were subjected to microtubule nucleation analysis as described. Cells were fixed 2min after microtubule re-nucleation. All images were reproduced at the same magnification.