Xu et al., http://www.jcb.org/cgi/content/full/jcb.200906053/DC1

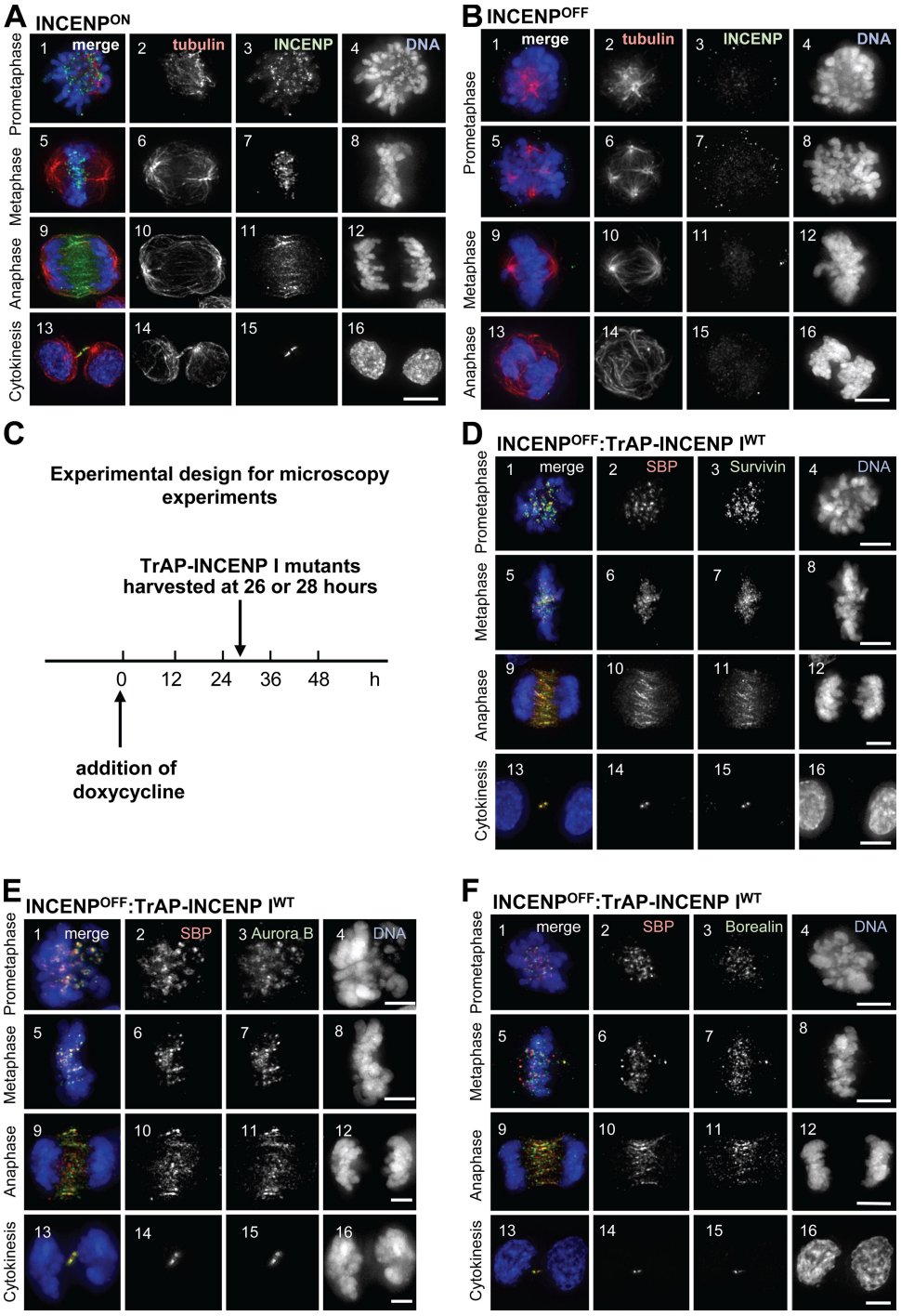

Figure S1. **INCENP class I is sufficient to keep DT40 cells alive.** (A and B) Immunofluorescence analysis of an INCENP$^{ON/OFF}$ cell line. INCENP (green; panels 3, 7, 11, and 15) shows a typical chromosomal passenger dynamic localization throughout mitosis in INCENP^{ON} cells and is not detectable in the INCENP^{OFF} cells (doxycycline incubation, 26 h). The figure also shows α-tubulin (red; panels 2, 6, 10, and 14) and DAPI staining for DNA (blue; panels 4, 8, 12, and 16). (C) Experimental design to characterize the INCENP class I mutant cell lines is shown. INCENP^{OFF} cells expressing TrAP-INCENP^{WT} or TrAP-INCENP^{mutants} were harvested after incubation for 26 or 28 h in doxycycline. Experiments were repeated independently more than three times, and no difference was observed between cell populations harvested at the two time points. (D–F) In INCENP^{OFF} cells, exogenous TrAP-INCENP^{WT} (red; panels 2, 6, 10, and 14) colocalized with endogenous Survivin (D), aurora B (E), and Borealin (F; green; panels 3, 7, 11, and 15). Cells were also stained with DAPI to label DNA (blue; panels 4, 8, 12, and 16). Bars, 5 µm.

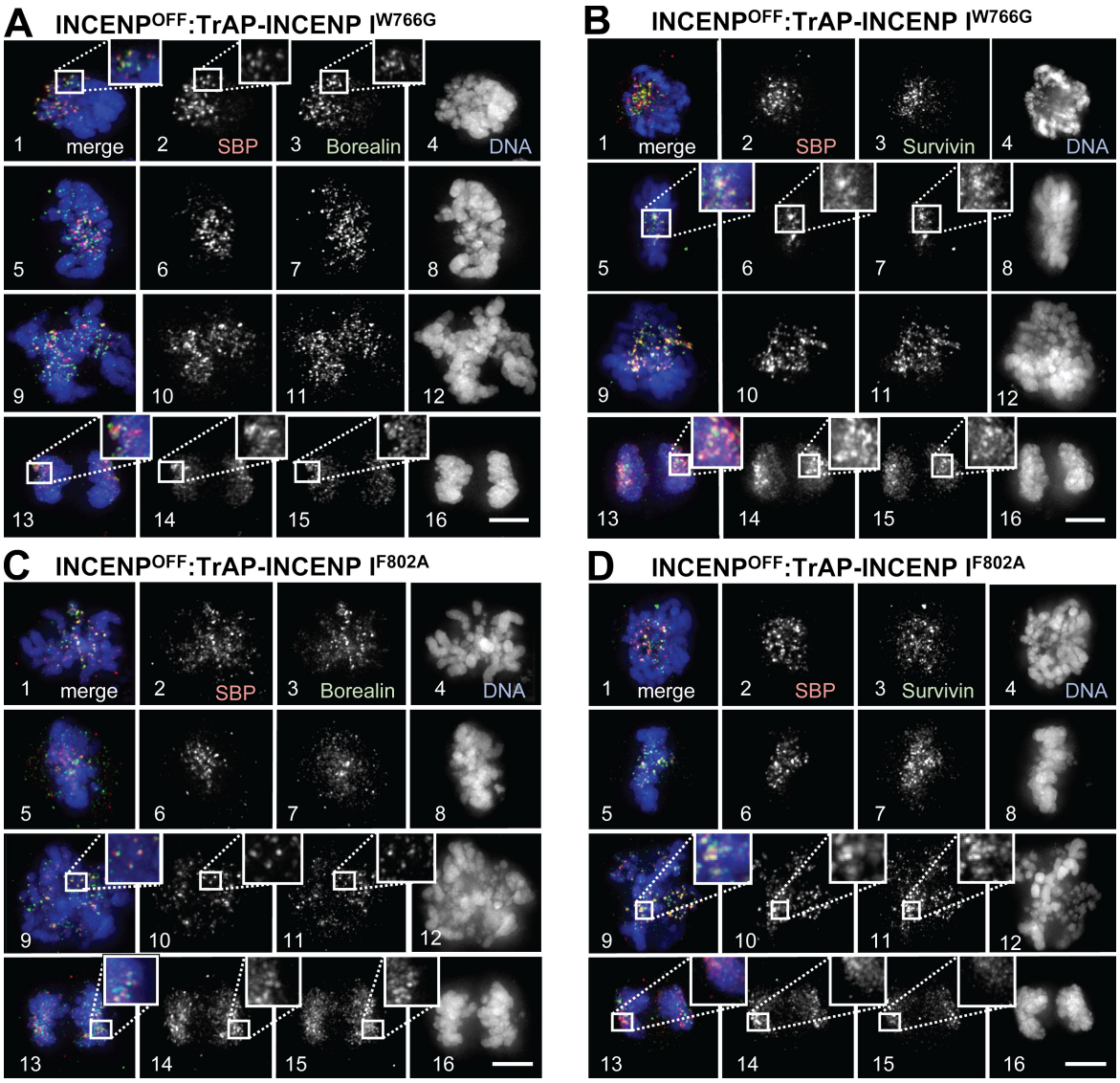

Figure S2. **Distribution of Survivin and Borealin in cells expressing only mutant INCENP.** (A–D) INCENP^{OFF} cells expressing TrAP-INCENP^{W766G} (A and B) or TrAP-INCENP^{F802A} (C and D) were stained with anti-SBP to show the localization of the exogenous INCENP (red; panels 2, 6, 10, and 14) or with antibodies to Borealin (A and C; green; panels 3, 7, 11, and 15) or Survivin (B and D; green; panels 3, 7, 11, and 15) plus DAPI for DNA (blue; panels 4, 8, 12, and 16). Bars, 5 µm.

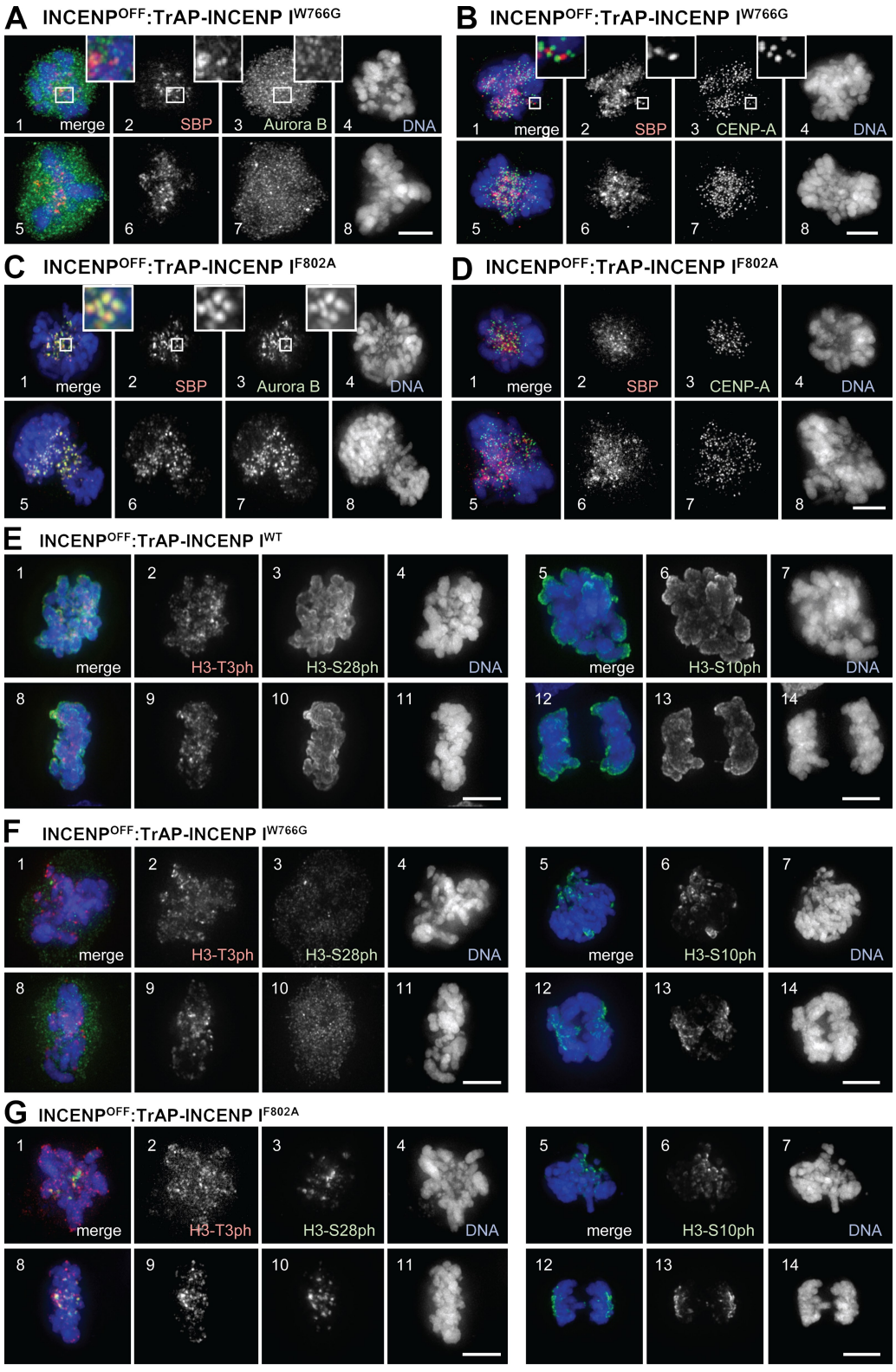

Figure S3. **Distribution of aurora B and CENP-A and levels of histone H3 phosphorylation in INCENP^{OFF} cells expressing various INCENP mutants.** (A–D) INCENP^{OFF} cells expressing TrAP-INCENP^{W766G} (A and B) or TrAP-INCENP^{F802A} (C and D) were stained with anti-SBP to show the localization of the exogenous INCENP (red; panels 2 and 6) or with antibodies to aurora B (green; A and C; panels 3 and 7) or CENP-A (B and D; green; panels 3 and 7) plus DAPI for DNA (blue; panels 4 and 8). These images are from the same experiment shown in Fig. 5. (E–G) Staining for H3T3ph, a marker of haspin kinase activity (red; panels 2 and 9), H3S28ph (green; panels 3 and 10), H3S10ph (green; panels 6 and 13), plus DNA (blue; panels 4, 7, 11, and 14) in INCENP^{OFF} cells expressing TrAP-INCENP^{WT} (E), TrAP-INCENP^{W766G} (F), or TrAP-INCENP^{F802A} (G). In INCENP^{OFF} cells expressing TrAP-INCENP^{W766G} and TrAP-INCENP^{F802A}, levels of H3S28ph and H3S10ph are significantly reduced relative to cells expressing TrAP-INCENP^{WT}, whereas levels of H3T3ph are unchanged. Insets show magnified views of boxed regions. Bars, 5 μm.

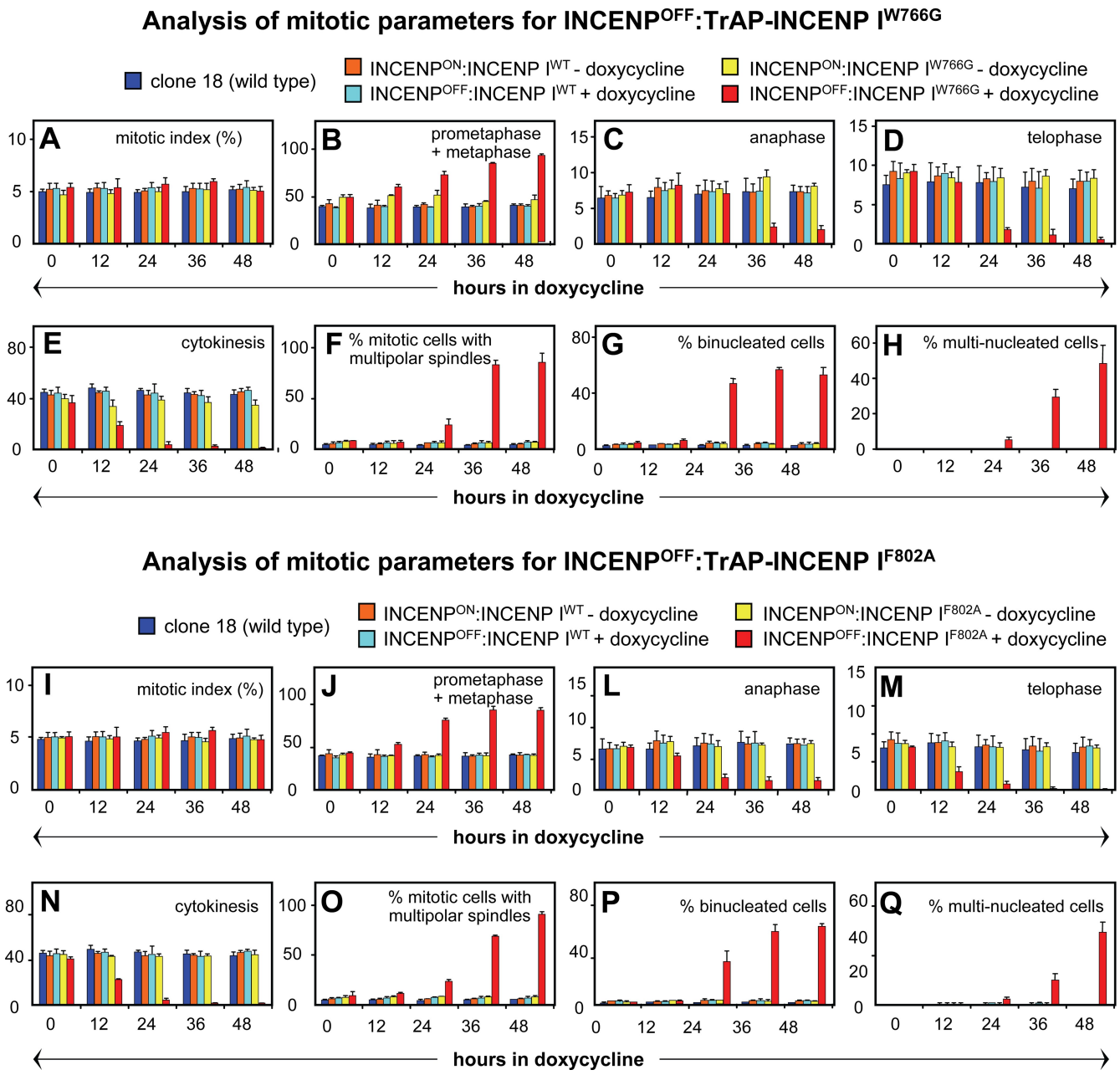

Figure S4. **Analysis of mitotic parameters in INCENP^{OFF} cells expressing INCENP^{W766G} and INCENP^{F802A}.** (A–Q) DT40 cells were plated on coverslips, stained with antibodies to aurora B, microtubules, or DAPI for DNA, and scored for the following parameters as described in Materials and methods: mitotic index (A and I), percentage of mitotic cells in prometaphase or metaphase (B and J), anaphase (C and L), telophase (D and M), or cytokinesis (E and N), percentage of mitotic cells with multipolar spindles (F and O), percentage of binucleated cells (G and P), and percentage of multinucleated cells (H and Q). Genotypes were as follows: wild-type DT40, INCENP^{ON}/TrAP-INCENP^{WT}, INCENP^{OFF}/TrAP-INCENP^{WT}, INCENP^{ON}/TrAP-INCENP^{W766G} or INCENP^{ON}/TrAP-INCENP^{F802A}, and INCENP^{OFF}/TrAP-INCENP^{W766G} or INCENP^{OFF}/TrAP-INCENP^{F802A}. Either 200 (B–E, H, J–N, and Q) or 500 (A, F, G, I, O, and P) cells were scored at each time point in each of three independent experiments. INCENP^{OFF}/TrAP-INCENP^{W766G} cells and INCENP^{OFF}/TrAP-INCENP^{F802A} exhibit characteristics of a classical passenger-null phenotype. Error bars indicate SD.

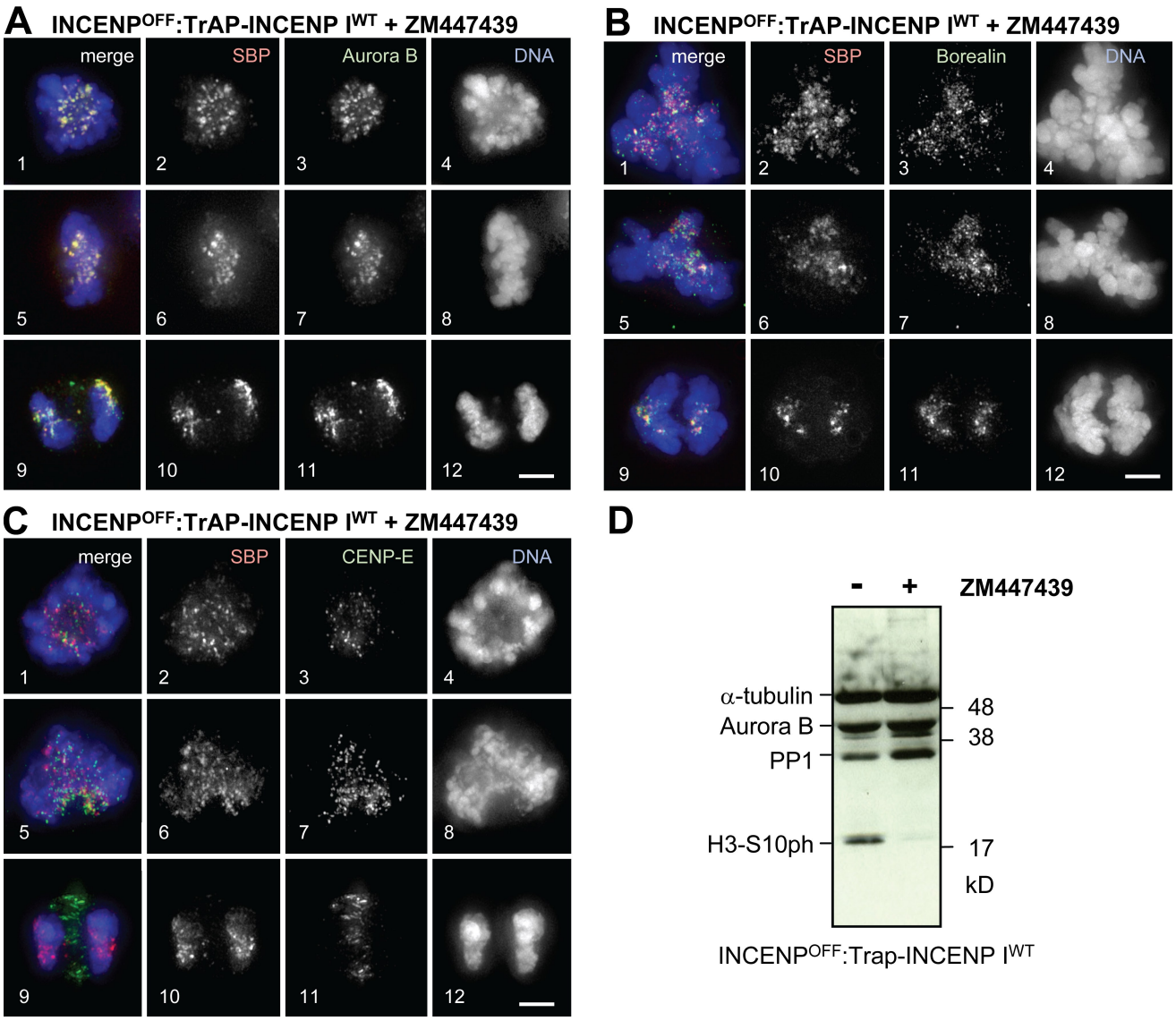

Figure S5. **Effect of aurora B kinase inhibitor ZM447439 on CPC and CENP-E behavior in INCENP^{OFF}/TrAP-INCENP^{WT} cells.** (A–C) INCENP^{OFF} cells expressing TrAP-INCENP^{WT} and incubated with ZM447439 for 3 h were stained with anti-SBP to show the localization of the exogenous INCENP (red; panels 2, 6, and 10) or with antibodies to aurora B (A; green; panels 3, 7, and 11), Borealin (B; green; panels 3, 7, and 11) or CENP-E (C; green; panels 3, 7, and 11) plus DAPI for DNA (blue; panels 4, 8, and 12). In the presence of ZM447439, aurora B and Borealin localize to centromeres but fail to transfer to the central spindle during anaphase. In contrast, CENP-E transfers normally to the central spindle during anaphase. (D) Immunoblots confirm that the level of H3S10ph is reduced significantly in the presence of ZM447439 as a result of aurora B inhibition. Levels of aurora B and PP1 remained the same. α-Tubulin was used as a loading control. Bars, 5 μm.