A gradient of increasing Aurora B activity is required for cells to execute mitosis

Zhenjie Xu[1], Paola Vagnarelli, Hiromi Ogawa, Kumiko Samejima[*] and William C. Earnshaw[*]

Wellcome Trust Centre *for* Cell Biology, University of Edinburgh, Edinburgh, EH9 3JR, UK

[1]present address: Department of Molecular and Cellular Physiology, Stanford University School of Medicine, 279 Campus Drive, Stanford, CA 94305-5345, USA.

[*]authors for correspondence: bill.earnshaw@ed.ac.uk, kumiko.samejima@ed.ac.uk

KEYWORDS: INCENP, Aurora B, DT40

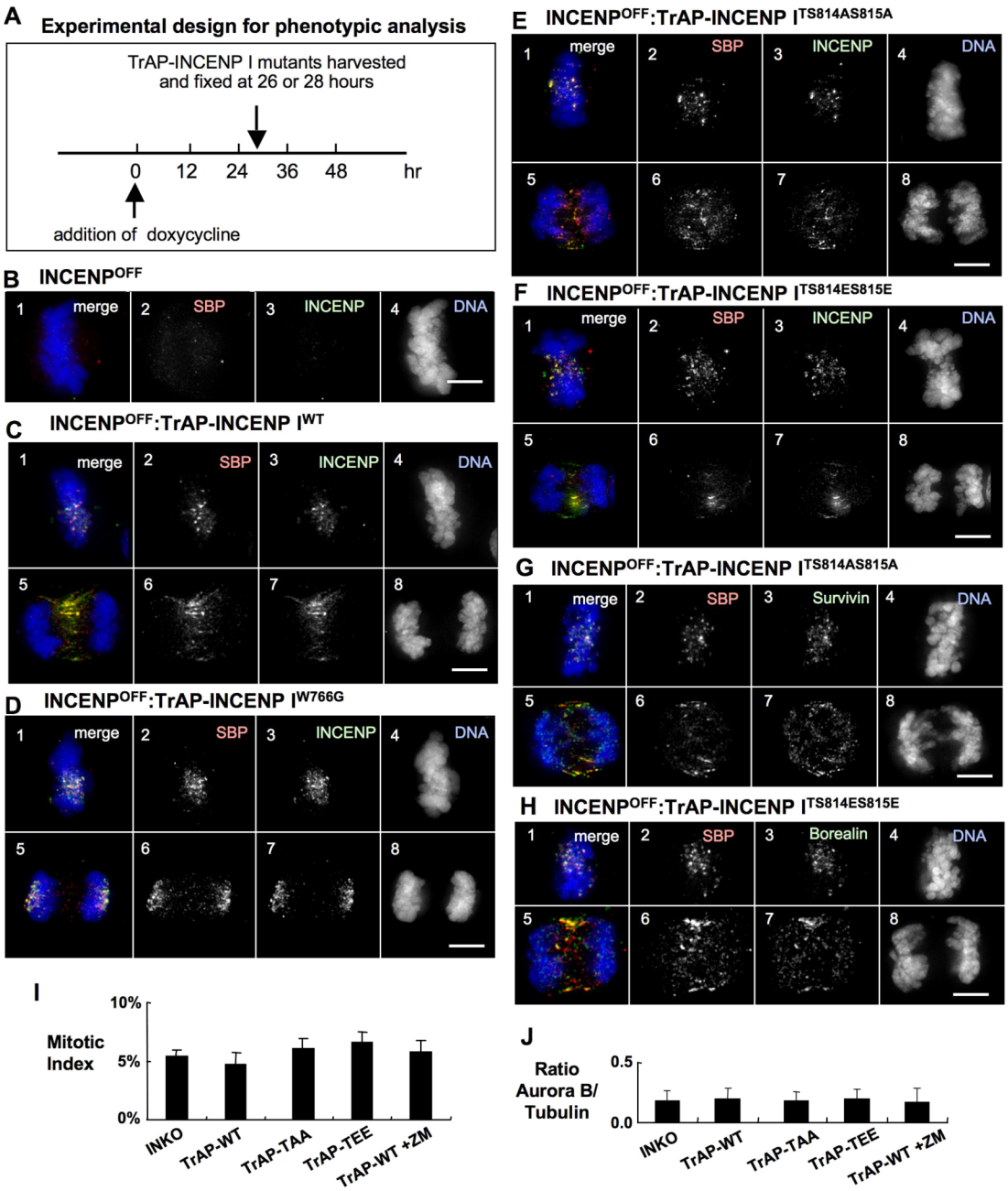
Supplementary Materials

Supplementary Figure Legends

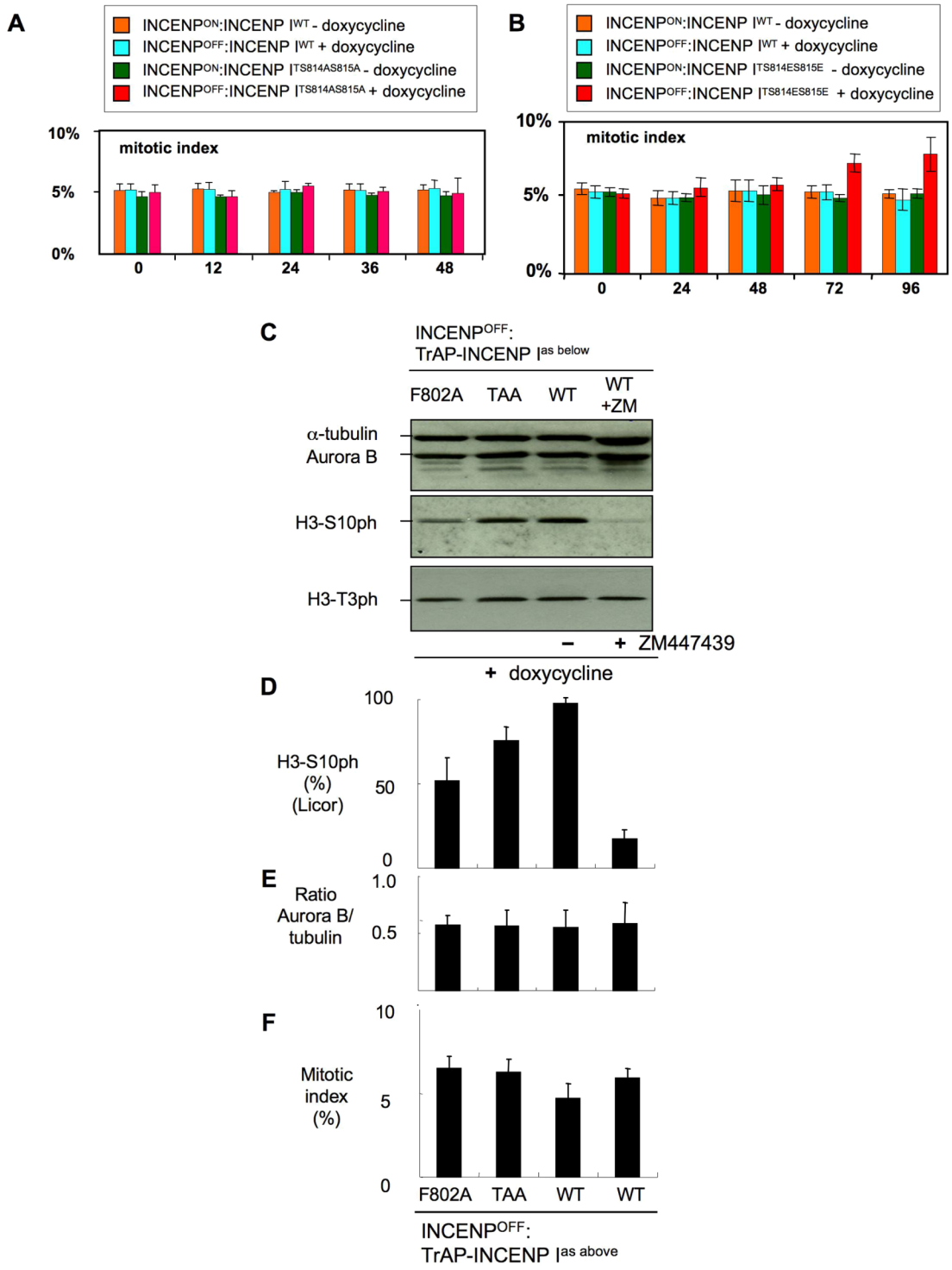
Supplementary Figure 1. Location of TrAP-INCENP Class I structural mutants in conditional INCENP knockout cells. (A) Experimental design for analysis of the INCENP class I mutant cell lines. INCENP^{OFF} cells expressing TrAP-INCENP^{WT} or TrAP-INCENP^{TSS} mutants were harvested after incubation for 26 or 28 hours in doxycycline. Experiments were repeated independently more than 3 times and no difference was observed between cell populations harvested at the two time points**. (B-F)** Immunofluorescence analysis of INCENP^{OFF} cells and INCENP^{OFF} cell lines expressing different INCENP^{TSS} mutants. **(B)** Endogenous INCENP is efficiently repressed in the presence of doxycycline. **(C)** TrAP-INCENP^{WT} localizes normally in INCENP^{OFF} cells. **(D)** TrAP-INCENP^{W766G} localizes normally at metaphase (panels 1-4), but fails to transfer to the central spindle at anaphase in INCENP^{OFF} cells (panels 5-8). **(E,F)** TrAP-INCENP^{TS814AS815A} and TrAP-INCENP^{TS814ES815E} both localize normally throughout mitosis in INCENP^{OFF} cells. SBP staining (panels 2,6, red) reveals the localization of the exogenous INCENP. Total INCENP staining (panels 3,7; green) and DAPI staining for DNA (panels 4,8, blue). **(G,H)** INCENP^{OFF} cells expressing the indicated TrAP-INCENP^{TSS} mutant were stained with anti-SBP to show the localization of the exogenous INCENP (panels 2,6; red) or with antibodies to Survivin (**G** panels 3,7:Green) or Borealin (**H**, panels 3,7; green). Scale bar - 5 μm. **(G,H)** Controls for the measurements of Aurora B activity shown in Figure 2. **(G)** Mitotic index of

each cell line at the time of harvesting. **(H)** Ratio of Aurora B protein levels versus the loading control alpha-tubulin for each sample, as measured on the LiCor Odyssey.

Supplementary Figure 2. Mitotic indices of INCENP^{OFF} cultures expressing exogenous INCENP^{WT}, INCENP^{TS814AS815A} and INCENP^{TS814ES815E} and Quantitative Comparison of Aurora B kinase activity in INCENP^{OFF} cells expressing INCENP^{F802A} and INCENP^{TS814AS815A} mutants. **(A,B)** Mitotic indices of INCENP^{OFF} cultures expressing exogenous INCENP^{WT}, INCENP^{TS814AS815A} and INCENP^{TS814ES815E} . **(C)** Estimation of Aurora B activity by immunoblotting. Asynchronous cells were harvested following treatment with doxycycline for 28 hours or with 2 μM ZM447439 for 5 hours and lysates subjected to immunoblotting with the antibodies indicated. α-tubulin and Haspin kinase substrate H3T3ph are shown as controls. **(D)** Measurement of H3S10ph levels in the same samples using the LiCor Odessy. **(E)** Ratio of Aurora B protein levels versus the loading control α-tubulin for each sample, as measured on the Li-Cor Odyssey. **(F)** Mitotic index of each cell line at the time of harvesting.



Xu et al. Suppl. Fig. 1



Xu et al. Suppl. Fig. 2