Supplemental Materials

Molecular Biology of the Cell

Zhen et al.

Cbx2 stably associates with mitotic chromosomes via a PRC2 or PRC1-independent mechanism and is needed for recruiting PRC1 complex to mitotic chromosomes

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Supporting Information

Table of Contents

Supporting Figures

Reference

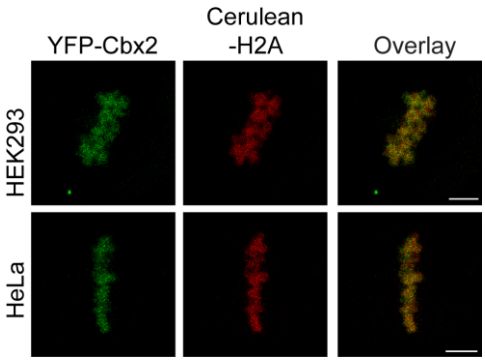

Figure S1. Mitotic chromosomal association of YFP-Cbx2 fusion protein in HEK293 and HeLa cells

YFP-Cbx2 and Cerulean-H2A fusion proteins were expressed in HEK293 and HeLa cells. The Cerulean-H2A was used to mark mitotic chromosomes. The confocal images of metaphase are presented. Scale bar is 5 μm.

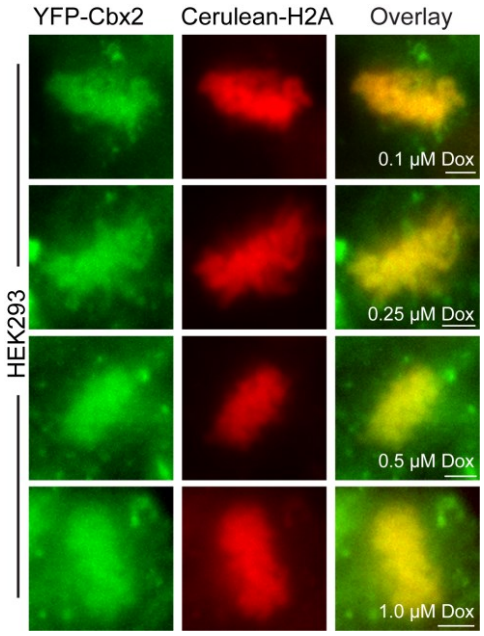

Figure S2. Effects of doxycycline concentration on level of YFP-Cbx2 association with mitotic chromosome

The expression of YFP-Cbx2 fusion protein in HEK293 cells was induced by using a range of doxycycline concentrations. Cerulean-H2A was used to mark mitotic chromosomes. The epifluorescence images of metaphase are presented. Scale bar is 5 μm.

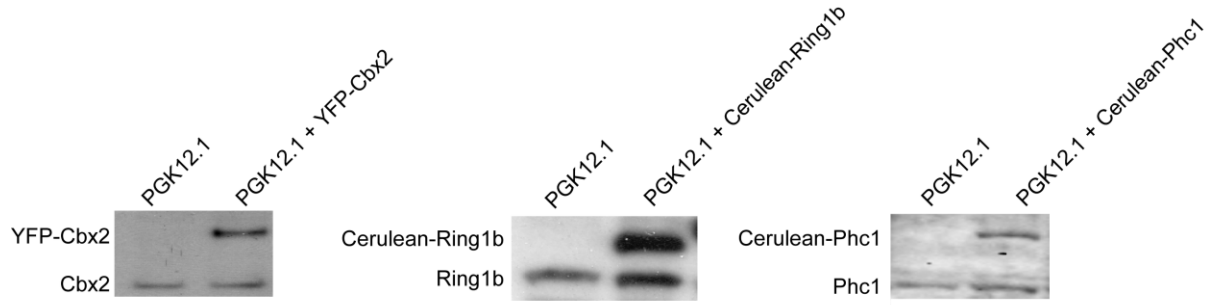

Figure S3. Western blot analysis of levels of endogenous and fusion proteins

Western blots were performed using antibodies against endogenous proteins. The cell extracts were prepared from PGK12.1 ES cells and PGK12.1 ES cells expressing either YFP-Cbx2 (PGK12.1 + YFP-Cbx2), Cerulean-Ring1b (PGK12.1 + Cerulean-Ring1b), or Cerulean-Phc1 (PGK12.1 + Cerulean-Phc1). The fusion proteins were induced to express by 0.5 μM of doxycycline for 2 days.

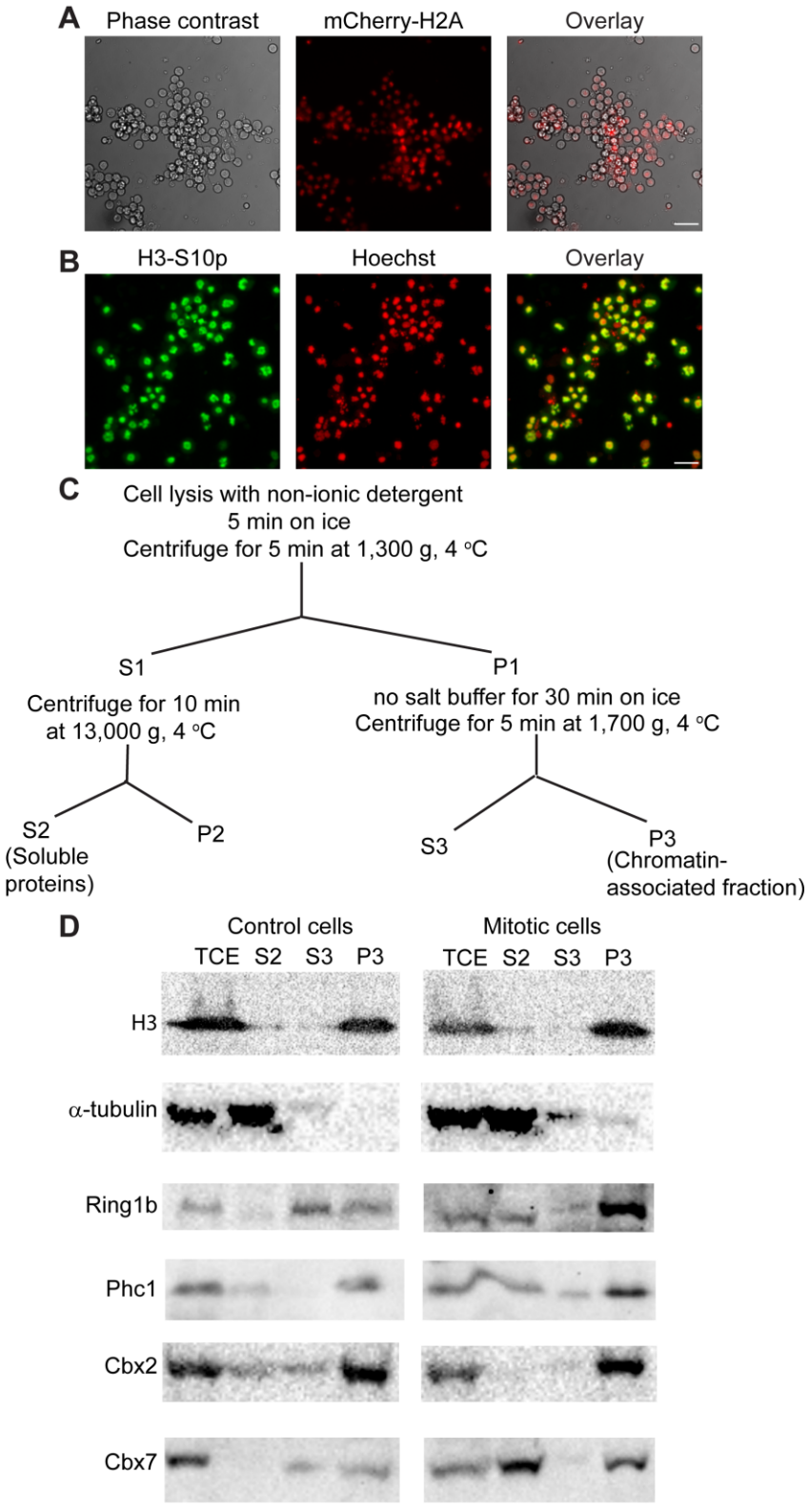

Figure S4. Analysis of mitotic chromatin binding of PRC1 proteins by chromatin fractionation

(A) Representative images of synchronized PGK12.1 ES cells stably expressing mCherry-H2A. The circular morphology of cells in phase contrast image indicates cell at mitotic stages. The mCherry-H2A was used to mark chromatin. Scale bar is 20 μm. (B) Epifluorescence images of synchronized PGK12.1 ES cells immunostained by anti-Histone H3 phospho S10 (H3PS10) antibody. DNAs were stained by Hoechst. Over 85% of cells show positive-H3PS10 staining. Scale bar is 20 μm. (C) Scheme of PRC1 protein fractionation, adapted from (Mendez and Stillman, 2000; Follmer *et al.*, 2012). (D) Western blot analysis of fractions from control and mitotic cells. H3 and α-tubulin are present in the expected fractions. Western blots showed that Ring1b, Phc1, Cbx2, and Cbx7 fractionate with mitotic chromatin.

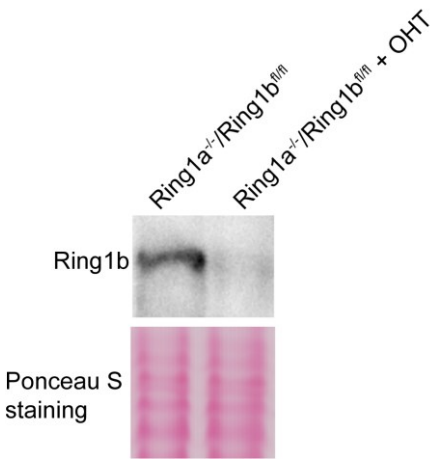

Figure S5. Western blot analysis of Ring1b in *Ring1a*$^{-/-}$/*Ring1b*$^{fl/fl}$ ES cells treated with or without OHT

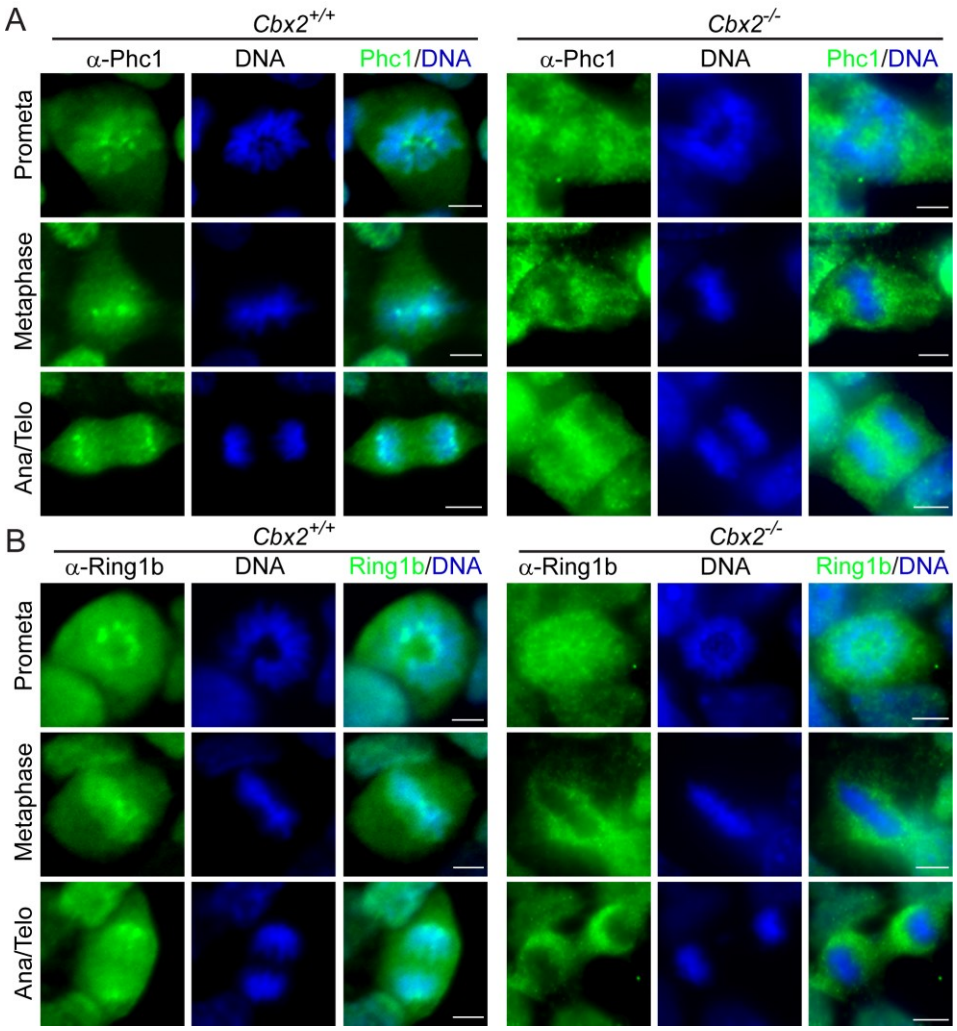

Figure S6. Immunostaining of Phc1 and Ring1b proteins in *Cbx2*$^{+/+}$ and *Cbx2*$^{-/-}$ ES cells

Cbx2$^{+/+}$ and *Cbx2*$^{-/-}$ ES cells were fixed and immunostained with antibodies that detect Phc1 and Ring1b (green) at different phases of mitosis. DNAs were stained with Hoechst (blue). Overlay images were shown. Scale bar is 5 μm

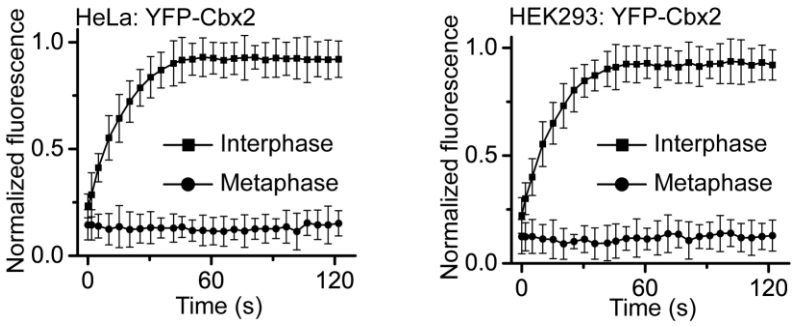

Figure S7. FRAP analysis of YFP-Cbx2 fusion protein binding to interphasic and mitotic chromatins in HeLa and HEK293 cells

The FRAP curves are the normalized fluorescence intensities of the bleached areas as a function of time after photobleaching and are average of at least 8 cells. Error bars indicate the standard deviations of means.

References

Follmer, N.E., Wani, A.H., and Francis, N.J. (2012). A polycomb group protein is retained at specific sites on chromatin in mitosis. PLoS Genet *8*, e1003135.
Mendez, J., and Stillman, B. (2000). Chromatin association of human origin recognition complex, cdc6, and minichromosome maintenance proteins during the cell cycle: assembly of prereplication complexes in late mitosis. Mol Cell Biol *20*, 8602-8612.