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Supplemental Data

Myosin Phosphatase-Targeting Subunit 1 Regulates Mitosis by Antagonizing Polo-like Kinase 1

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Supplemental Experimental Procedures

Bacterial Expression Vectors

The following bacterial expression was performed: Full-length, rat1 isoform (Dirksen et al., 2000) of MYPT1 was cloned into a pGEX4T-1 vector (GE Healthcare Bio-Sciences, Piscataway, NJ) as a GST-tagged protein. Truncation mutants of MYPT1 were generated by PCR and cloned into a pQE30 vector (Qiagen , Valencia, CA) as His-tagged proteins. Mutations of MYPT1 at phosphorylation sites were performed using a QuickChange multi site-directed mutagenesis kit (Stratagene, La Jolla, CA). The entire DNA sequences were verified by DNA sequencing. The bacterial expression vectors for the PBD of human PLK1, as well as its double mutant (K540M/H538A, which cannot bind to a phosphopeptide) were kindly provided by Dr. M. Yaffe, (Elia et al., 2003), and expressed as GST-tagged proteins.

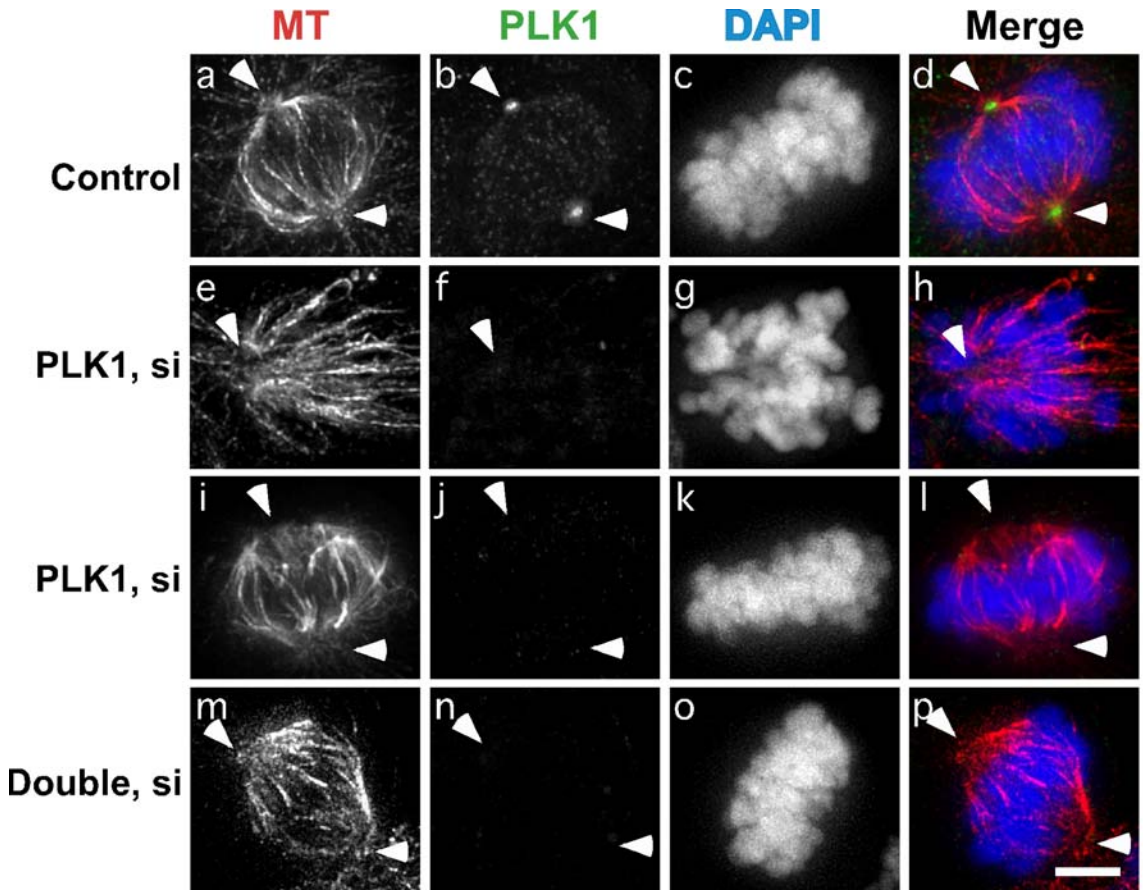

Figure S1. Double Depletion of MYPT1 and PLK1 Restores Bipolar Spindle Assembly

Localization of tubulin (red), PLK1 (green) and DNA (blue) in control (a-d), PLK1-depleted (e-l) and double-depleted SW 962 cells (m-p). Arrowheads, centrosomes. Bar, 5µm.

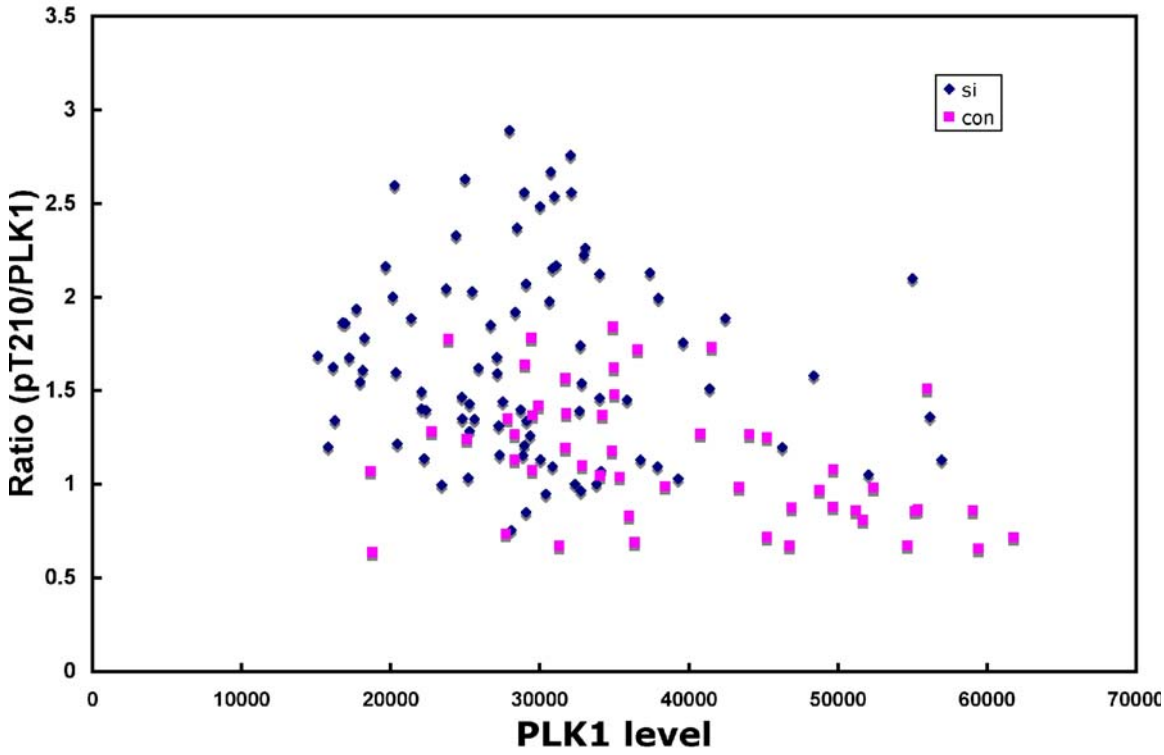

Figure S2. Fluorescent Intensity Ratio (pThr210/PLK1) versus PLK1 Expression Level

Note that ratios were higher in MYPT1 depleted cells while PLK1 expression levels are similar between control and MYPT1-depleted cells.

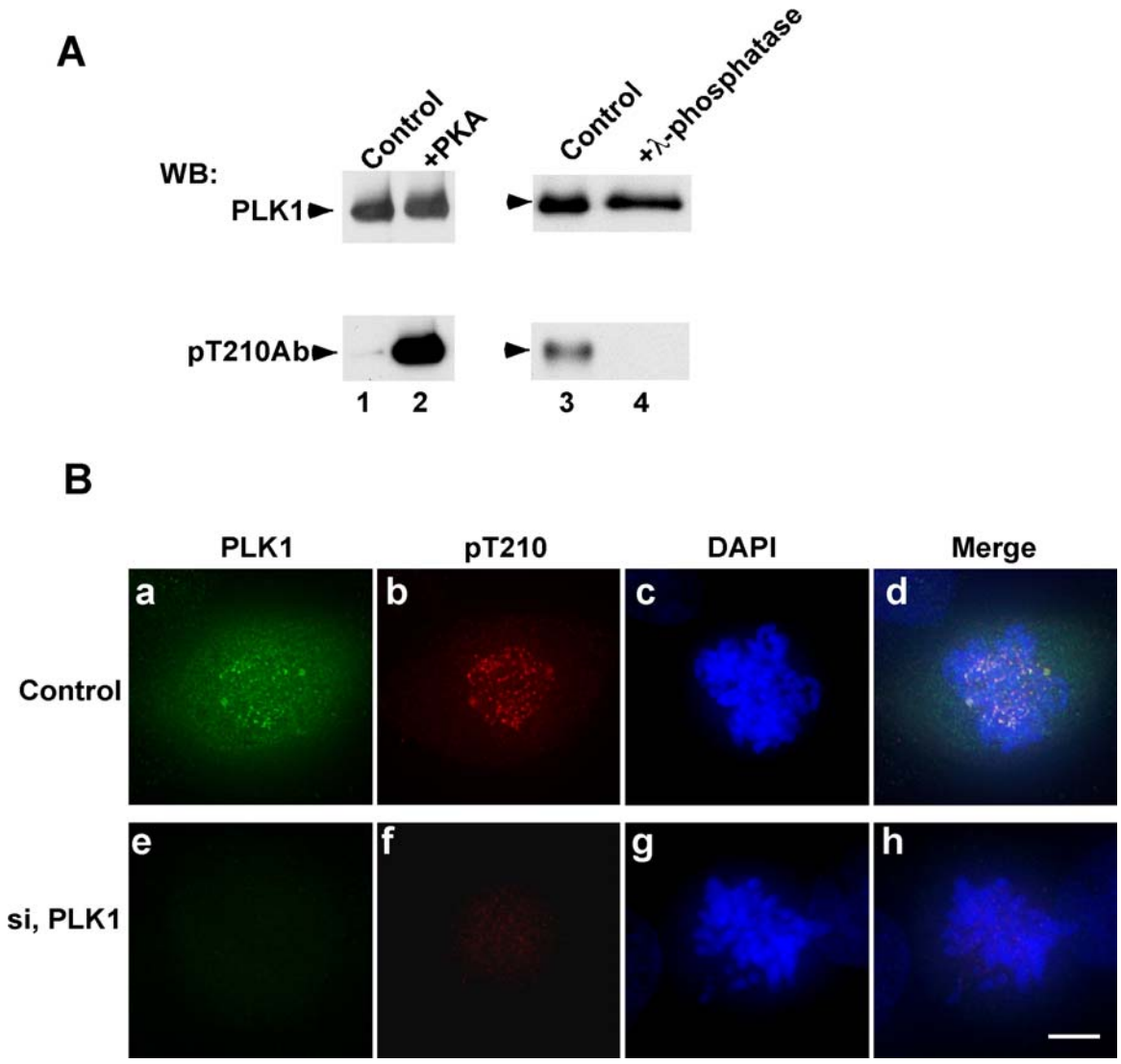

Figure S3. Specificity of an Anti-phosphoT210 Antibody

A. Phosphorylation-dependent immunoreaction of the antibody. Baculovirus expressed human GST-PLK1 (lanes 1 & 2) and His-tagged PLK1 (lanes 3 & 4) were phosphorylated with Protein kinase A (lane 2) or dephosphorylated by incubation with λ-phosphatase (lane 4). These samples were immunblotted with the anti-phosphoT210 antibody (lower panel). The same blots were stripped and re-blotted with a pan PLK1 antibody to confirm equal loading (upper panel). Note that phosphorylation with PKA (which is known to phosphorylate PLK1 at T210 (Kelm et al., 2002)) greatly increased the reactivity while λ-phoshatase treatment eliminated the antibody reactivity. Lanes, 1 and 3, control. B. Depletion of PLK1 by siRNA diminished phosphoT210 immunostaining. Control (a-d), as well as PLK1-depleted (e-h) HeLa cells were fixed with formaldehyde. After permeabilization with acetone at –20^{o}C, cells were double labeled with the pan PLK1 antibody (a & e) and the phosphoT210 antibody (b & f). DNA was stained with DAPI (c & g). Merged images (d & h) are also shown.

Supplemental References

Dirksen, W. P., Vladic, F., and Fisher, S. A. (2000). A myosin phosphatase targeting subunit isoform transition defines a smooth muscle developmental phenotypic switch. Am J Physiol Cell Physiol *278*, C589-600.

Elia, A. E., Rellos, P., Haire, L. F., Chao, J. W., Ivins, F. J., Hoepker, K., Mohammad, D., Cantley, L. C., Smerdon, S. J., and Yaffe, M. B. (2003). The molecular basis for phosphodependent substrate targeting and regulation of Plks by the Polo-box domain. Cell *115*, 83-95.

Kelm, O., Wind, M., Lehmann, W. D., and Nigg, E. A. (2002). Cell cycle-regulated phosphorylation of the Xenopus polo-like kinase Plx1. J Biol Chem *277*, 25247-25256.