Fig S1. Plk1 inhibition or depletion decreases phosphorylation of Haspin and H3T3 in HeLa cells.

(A) Pre-existing mitotic HeLa cells were removed by “shake-off,” then 3 µM nocodazole and the indicated inhibitors were added for 3 h. Lysates of mitotic cells isolated by shake-off were then prepared and subjected to immunoblotting.

(B) Uninduced HeLa Tet-On/myc-Haspin cells were transfected with control, Aurora B or Plk1 siRNA and, after 36 h, were incubated in the presence of nocodazole for 12 h. Lysates of mitotic cells collected by shake-off were analyzed by immunoblotting. Note that the first two lanes of this experiment, which serve as controls here, were previously published [S3].

(C) From the data in Figure 1D, the ratio of the intensity of H3T3ph staining in inhibitor-treated cells versus DMSO-treated cells was calculated as RPE1 cells progressed through prophase and prometaphase (as determined by DNA condensation). Plk1 inhibitors have their strongest relative effect in early mitosis, while Aurora B inhibitors have their strongest effect later in prometaphase.