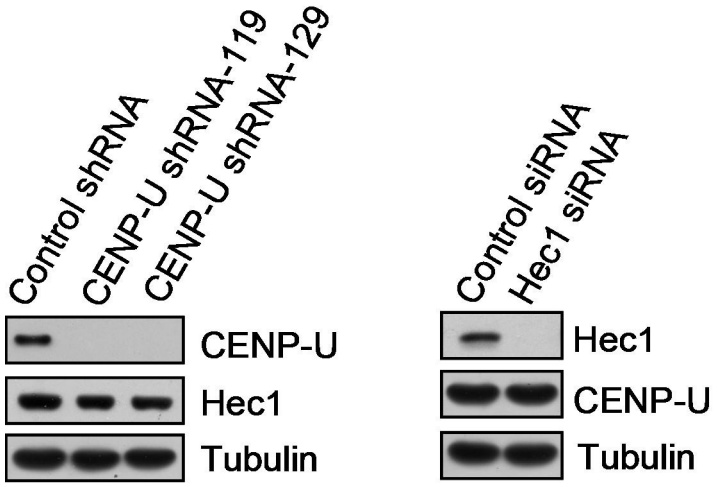

Figure S1. HeLa cells treated with indicated shRNA or siRNA oligonucleotides were collected for analyses of specificity and efficiency. CENP-U shRNA positive cells were selected with puromycin.

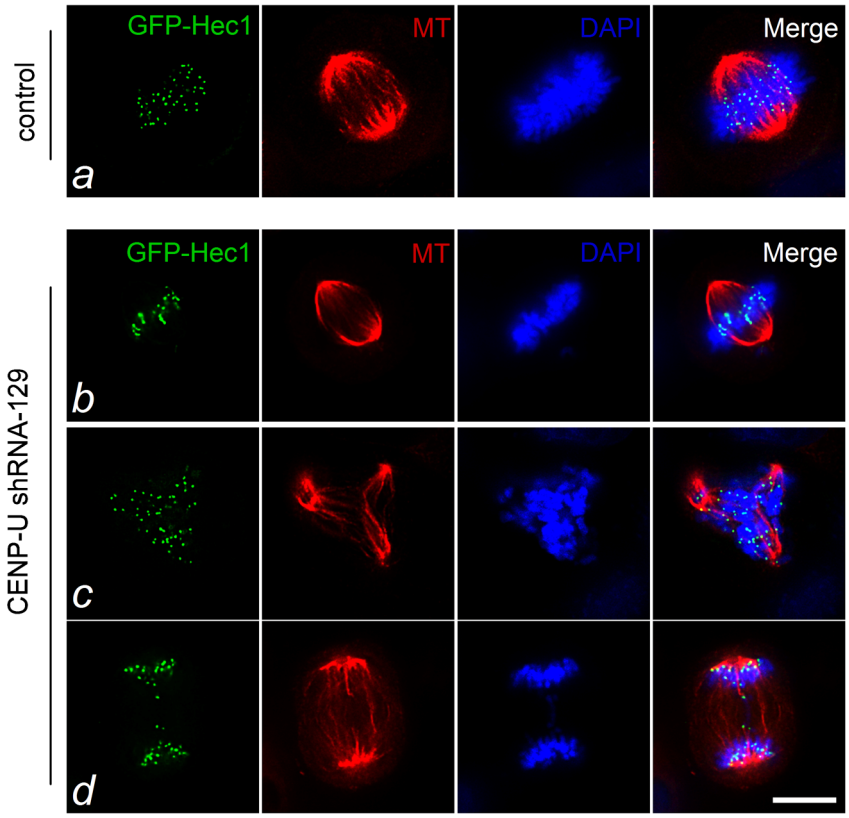

Figure S2. Suppression of CENP-U causes multiple mitotic defects. HeLa cells were transfected with CENP-U shRNA-129 along with GFP-Hec1 as a marker of transfection and examined for spindle and DNA morphology. After 48 hrs transfection, cells were fixed and stained with tubulin (*red*) and DAPI (*blue*). The following phenotypes were observed in the absence of CENP-U: chromosome misalignment (*b*), multipolar spindle (*c*), chromosome bridge in anaphase (*d*). Bar: 10 μm.

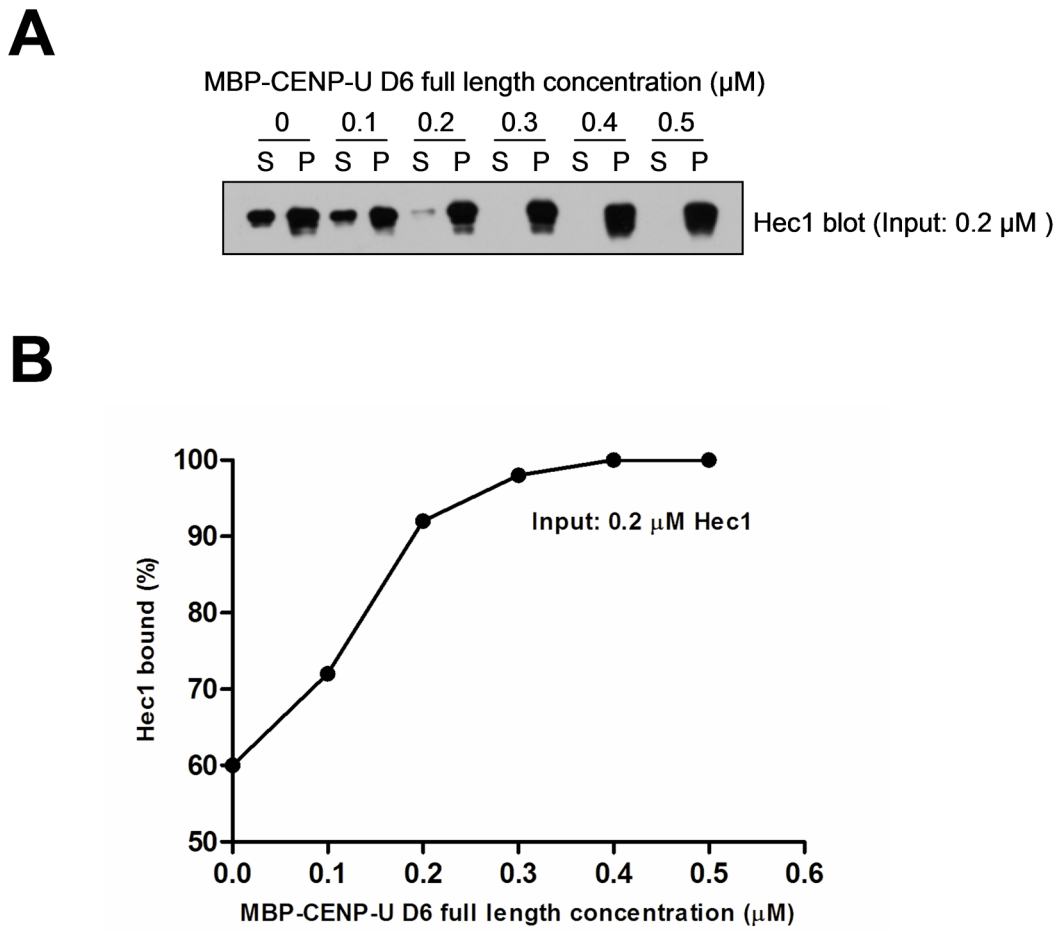

Figure S3. Hec1 microtubule binding affinity was enhanced in the presence of increasing concentrations of CENP-U.

A. A constant concentration of Hec1 (0.2 μM) and microtubules (1 μM) were sedimented with increasing concentrations of MBP-CENP-U D6 (0-0.5μM), and the supernatant and pellet samples were analysed by anti-Hec1 blot.

B. Plot of quantifications of the aforementioned MT cosedimentation assay.

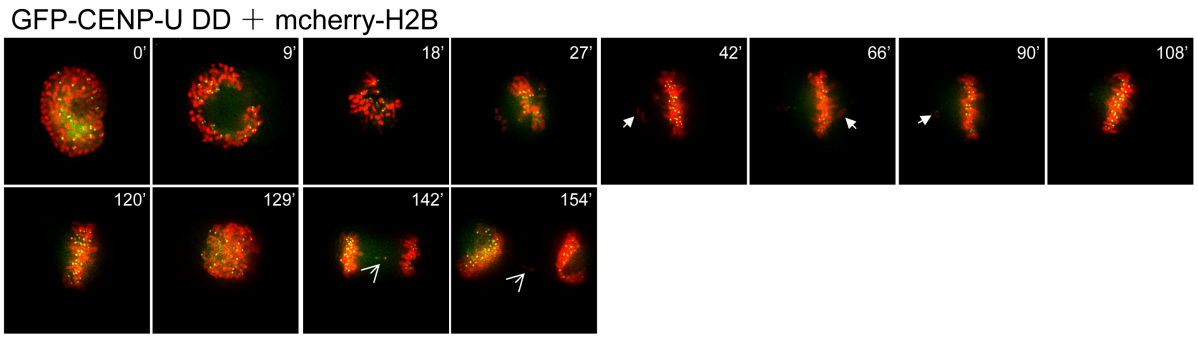

Figure S4. Real-time imaging of chromosome movements in HeLa cells co-transfected with mcherry-H2B and GFP-CENP-U DD. Chromosomes were marked by mcherry-H2B. Closed arrows indicate lagging chromosomes during alignment; open arrows indicate chromosome bridge in anaphase. Bar: 10 μm.