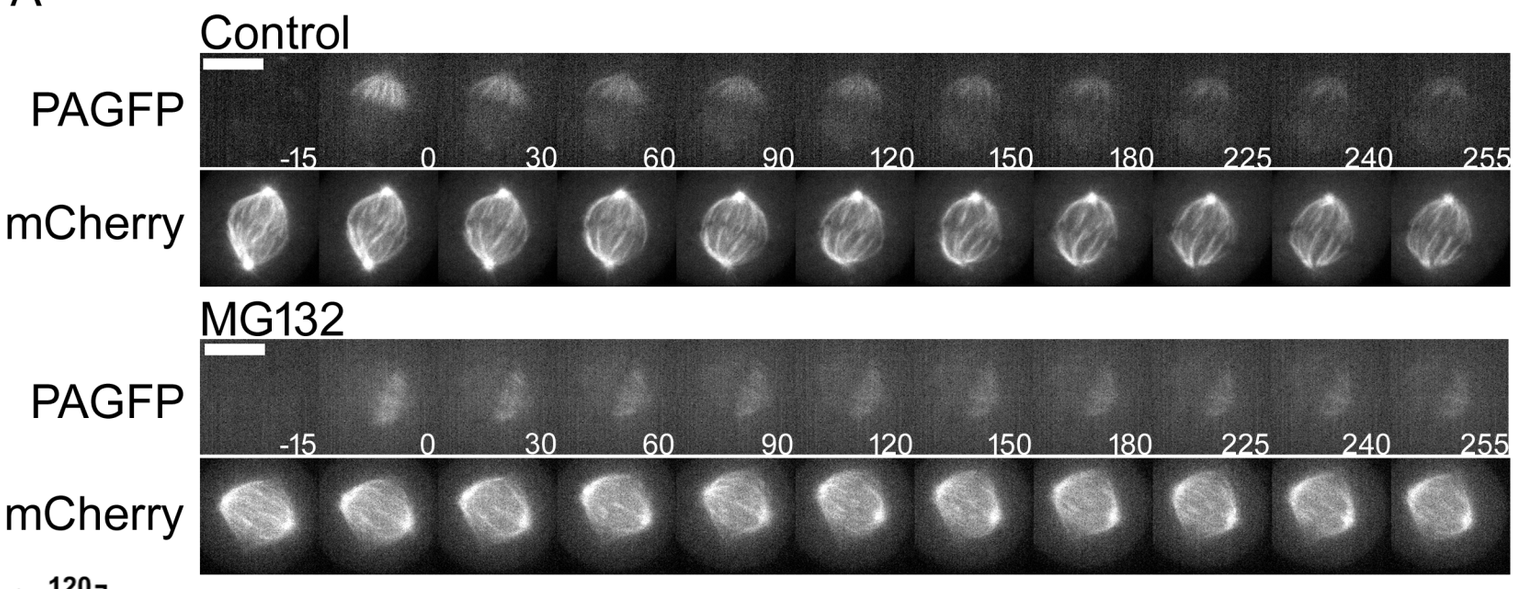
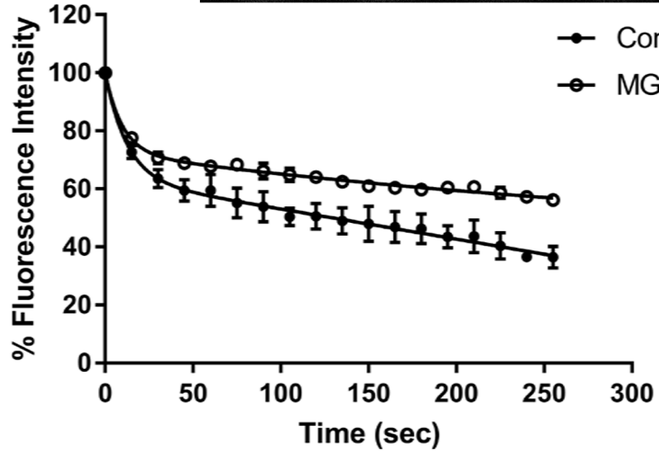
Figure S1

A





	Fast MT Population		Slow MT Population			
	%	$T_{1/2}$ (sec)	%	$T_{1/2}$ (min)	R^2	N
Control	34	7.6	65	5.8	0.994	9
MG132	28	7.3	72	14.5	0.994	11

Fig. S1. Loss of end-on kinetochore-microtubule attachments caused by Hec1 depletion induces scattering of chromosomes on the mitotic spindle. (Top) Select mid-volume frames of DNA labeled with SiR-DNA from cells treated as in Fig. 1 demonstrating Hec1 depletion phenotype. Time, sec. Bar, 10 μm. (Bottom, Left) Lysates from mitotic HeLa cells transfected with control or Hec1 siRNA and analyzed by Western blot probing for the indicated proteins. (Bottom, Right) Quantification of control and Hec1 depleted cells normalized, Actin levels were used as a loading control. The population of cells treated with siRNA show depletion of Hec1 protein of approximately 95%.

Figure S2

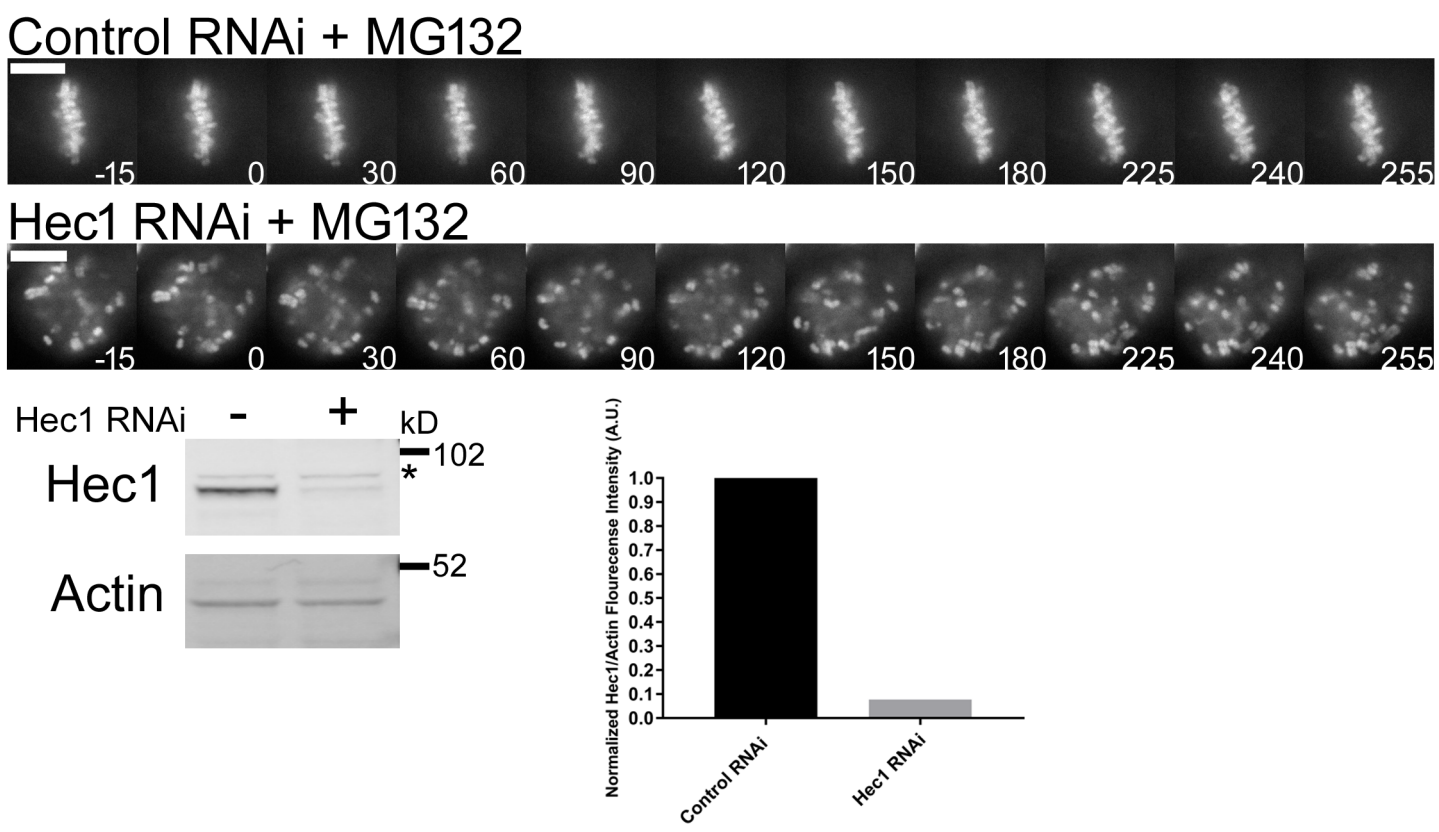
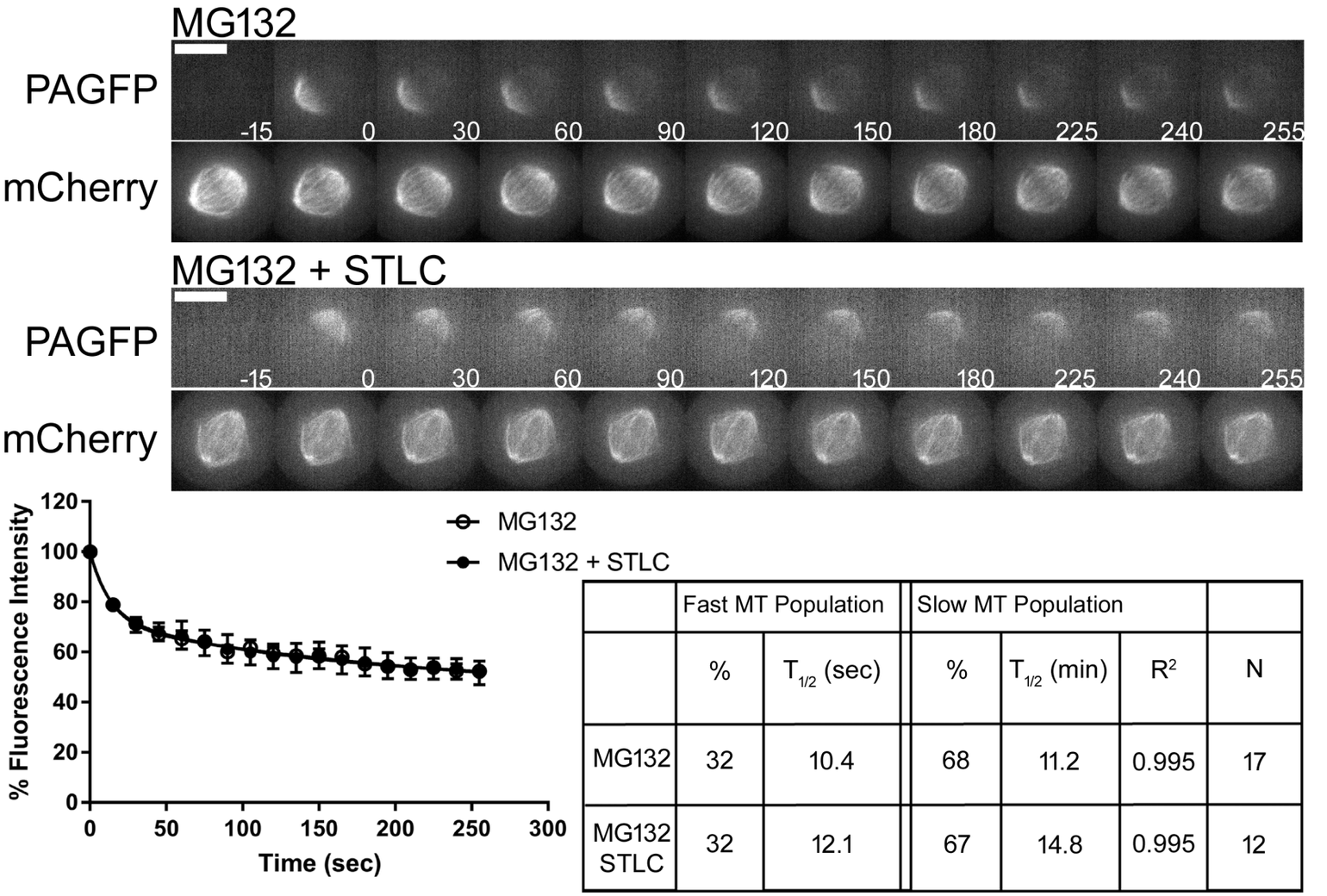


Fig. S2. Loss of end-on kinetochore-microtubule attachments caused by Hec1 depletion induces scattering of chromosomes on the mitotic spindle. (Top) Select mid-volume frames of DNA labeled with SiR-DNA from cells treated as in Fig. 1 demonstrating Hec1 depletion phenotype. Time, sec. Bar, 10 μm. (Bottom, Left) Lysates from mitotic HeLa cells transfected with control or Hec1 siRNA and analyzed by Western blot probing for the indicated proteins. (Bottom, Right) Quantification of control and Hec1 depleted cells normalized, Actin levels were used as a loading control. The population of cells treated with siRNA show depletion of Hec1 protein of approximately 95%.

Figure S3



	Fast MT Population		Slow MT Population			
	%	$T_{1/2}$ (sec)	%	$T_{1/2}$ (min)	R^2	N
MG132	32	10.4	68	11.2	0.995	17
MG132 STLC	32	12.1	67	14.8	0.995	12

Fig. S3. Eg5 activity is not required for maintenance of spindle bipolarity following prolonged mitotic arrest. (Top) Select frames from live cell imaging of Tubulin photoactivation in metaphase U2OS cells stably expressing photoactivatable GFP-Tubulin and mCherry-Tubulin following treatment with 10 μm MG132 for 3 h. Cells were then treated with either control DMSO or 10 μm STLC to induce monopolar spindles. Cells were incubated 30 min prior to imaging. mCherry-Tubulin frames are from the mid-volume plane. Time, sec. Bar, 10 μm. (Bottom) Fluorescence dissipation after photoactivation. The filled and unfilled circles represent the average values recorded at each time point after photoactivation. The bars represent SEM. MG132 n = 17 cells, MG132 + STLC n = 12 cells from four independent experiments. Lines indicate fitted curves (Control R^2 = 0.995; MG132 + STLC R^2 = 0.995).

Figure S4

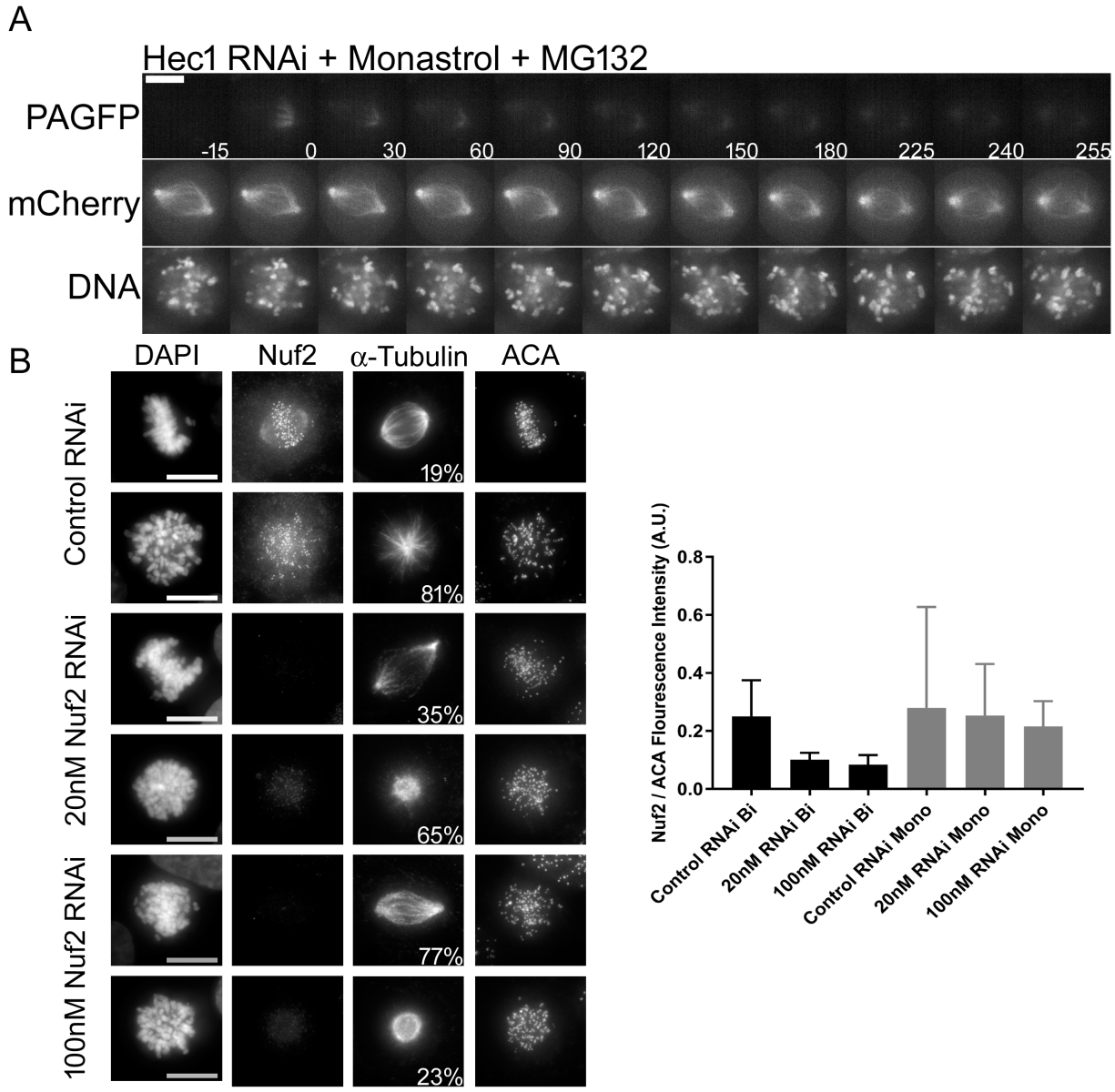
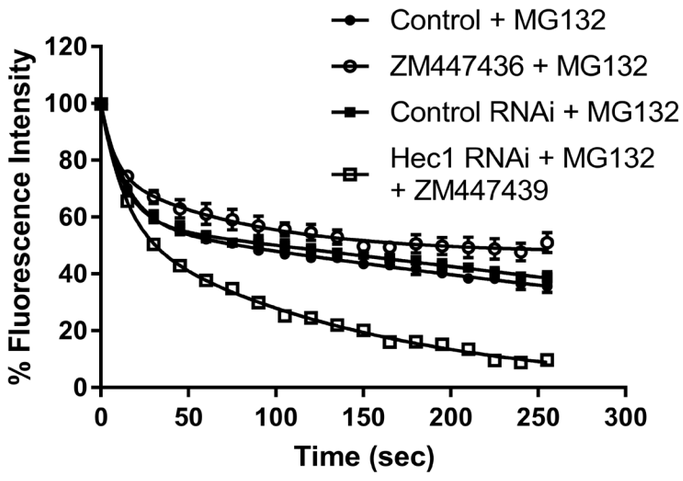


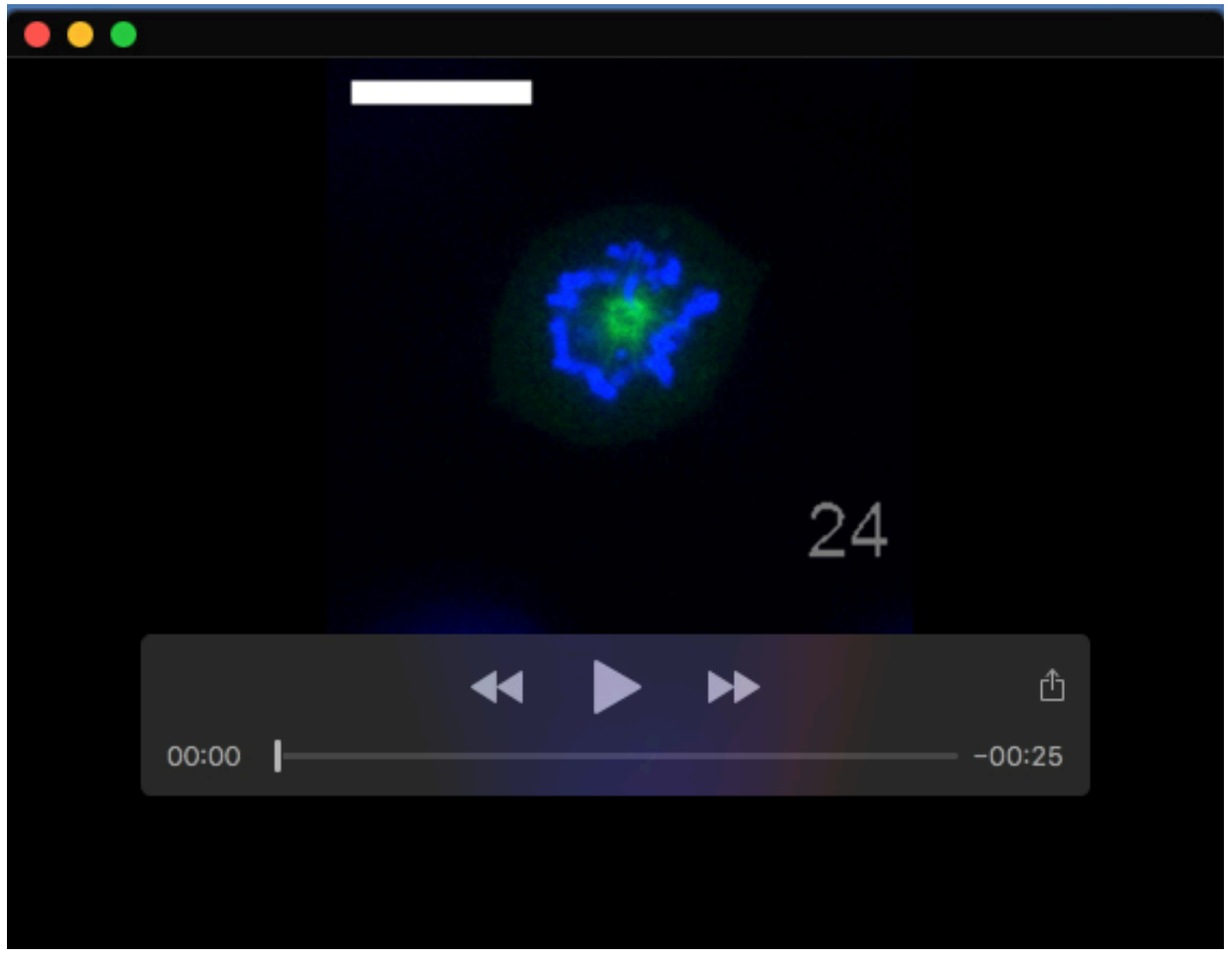
Fig. S4. Strong depletion of Ndc80 complex components allows bipolar spindle formation in cells treated with Eg5 inhibitor and causes loss of spindle checkpoint signals at kinetochores. (A) Select frames from live cell imaging of Tubulin photoactivation in U2OS cells stably expressing photoactivatable GFP-Tubulin and mCherry-Tubulin following transfection with Hec1 siRNA along with 100 μm Monastrol and 10 μm MG132 treatment to prevent mitotic exit. mCherry-Tubulin and DNA labeled with SiR-DNA frames are from the mid-volume plane. Time, sec. Bar, 10 μm. (B, left) Immunofluorescence images of U2OS cells stably expressing photoactivatable GFP-Tubulin and mCherry-Tubulin immunostained for DNA (DAPI), Nuf2, α-Tubulin, and ACA following transfection of either control, 20 nM Nuf2, or 100 nM Nuf2 siRNA. Cells were treated with 100μm Monastrol for 1 h prior to fixation. n = 150 cells for each condition. Images represent maximum-intensity projections. Nuf2 images are scaled equivalently. Bar, 10 μm. (B, right) Fluorescence intensity quantification of Nuf2 levels from the indicated conditions in the left panel. n = 5-8 cells for each condition. FI, Fluorescence Intensity. (C) Quantification of immunofluorescence images of U2OS cells stably expressing photoactivatable GFP-Tubulin and mCherry-Tubulin immunostained for DNA (DAPI), ACA, and Mad2 following transfection of either control or 50 nM Hec1 siRNA. Cells were treated with 10μm MG132 and/or 3.3μM Nocodazole for ~2 h prior to fixation as indicated. n = 3-11 cells for each condition. Images represent maximum-intensity projections. Mad2 images are scaled equivalently. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001. Statistical significance was determined by comparison to the appropriate experimental control.

Figure S5

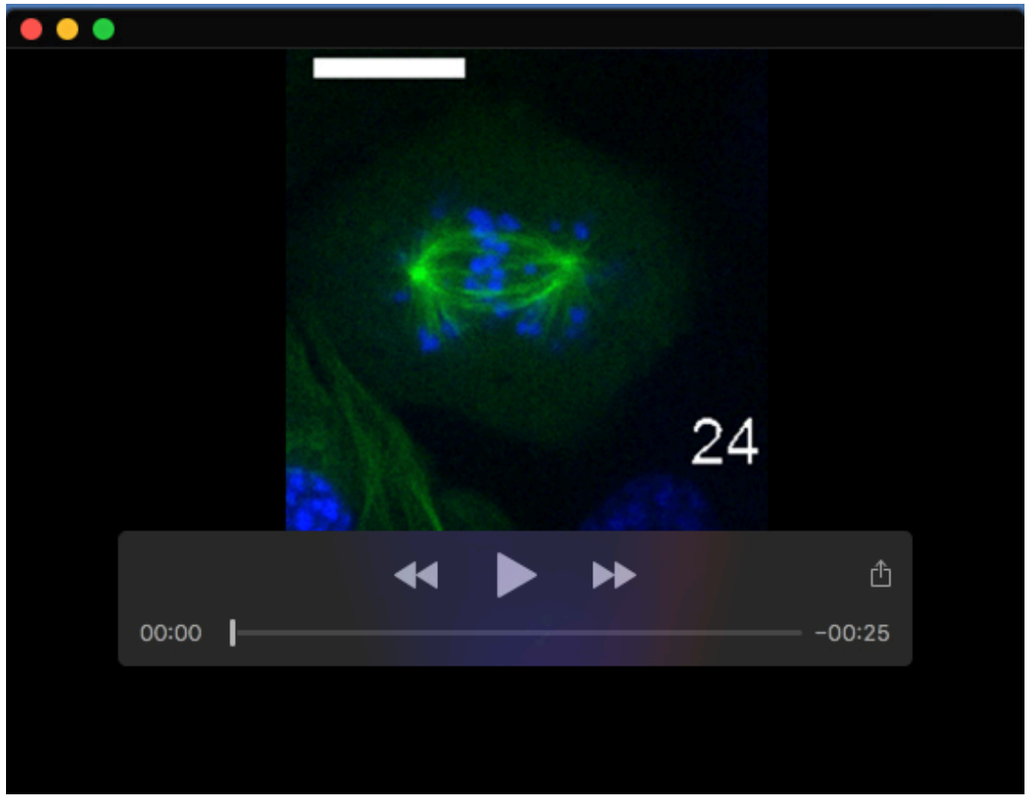


	Fast MT Population		Slow MT Population			
	%	$T_{1/2}$ (sec)	%	$T_{1/2}$ (min)	R^2	N
Control	40	10	60	8.5	0.999	19
ZM	37	11.8	61	13.9	0.984	18
RNAi	%	$T_{1/2}$ (sec)	%	$T_{1/2}$ (min)	R^2	N
Control	43	8.1	57	7.8	0.999	16
Hec1	46	9.1	54	2.2	0.998	18

Fig. S5. Compilation of Aurora kinase inhibition data from Figure 4 and Figure 5. Fluorescence dissipation after photoactivation data combined from figure 4 and figure 5.



Movie 1. Monopolar spindle formation in U2OS cell treated with 20 nM Nuf2 siRNA, monastrol and MG132. Example of a cell that forms a monopolar spindle. The cell expresses photoactivatable GFP-Tubulin and mCherry-Tubulin and is labeled with Sir-DNA. For each time point, DNA and mCherry-Tubulin images were acquired. Total time is 42 min. Bar, 10 μm.



Movie 2. Bipolar spindle formation in U2OS cell treated with 100 nM Nuf2 siRNA, monastrol and MG132. Example of a cell that forms a bipolar spindle. The cell expresses photoactivatable GFP-Tubulin and mCherry-Tubulin and is labeled with Sir-DNA. For each time point, DNA and mCherry-Tubulin images were acquired. Total time is 42 min. Bar, 10 µm.