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Supplemental Information

Chromosome Segregation Is Biased by Kinetochore Size

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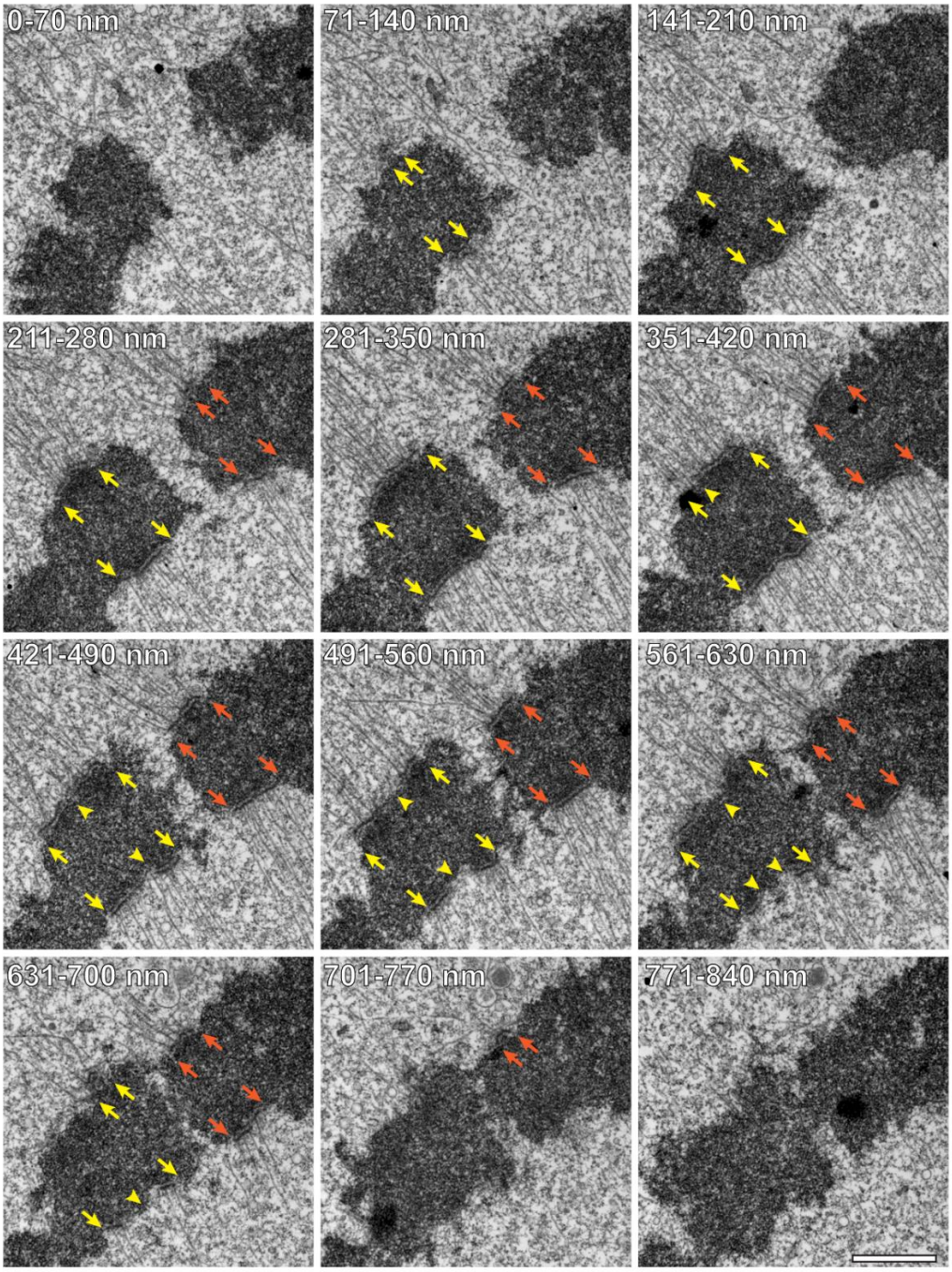

Figure S1. Serial electron microscope sections spanning two adjacent centromeres in a metaphase Indian muntjac fibroblast, related to Figure 2. Plates of C3X kinetochores are marked with yellow arrows. Smaller kinetochores plates on an adjacent non-compound centromere are marked with orange arrows. Notice multiple discontinuities within the plate of C3X kinetochore (arrowheads) likely reflecting their bent state in response to spindle forces and their modular organization. A surface-rendered model of the kinetochore plates and a Z-projection depicting manually-segmented chromatin, kinetochore plates and microtubules are shown in Figure 2. Depth occupied by each section within the volume is shown in nanometers. Scale bar = 1 μm.

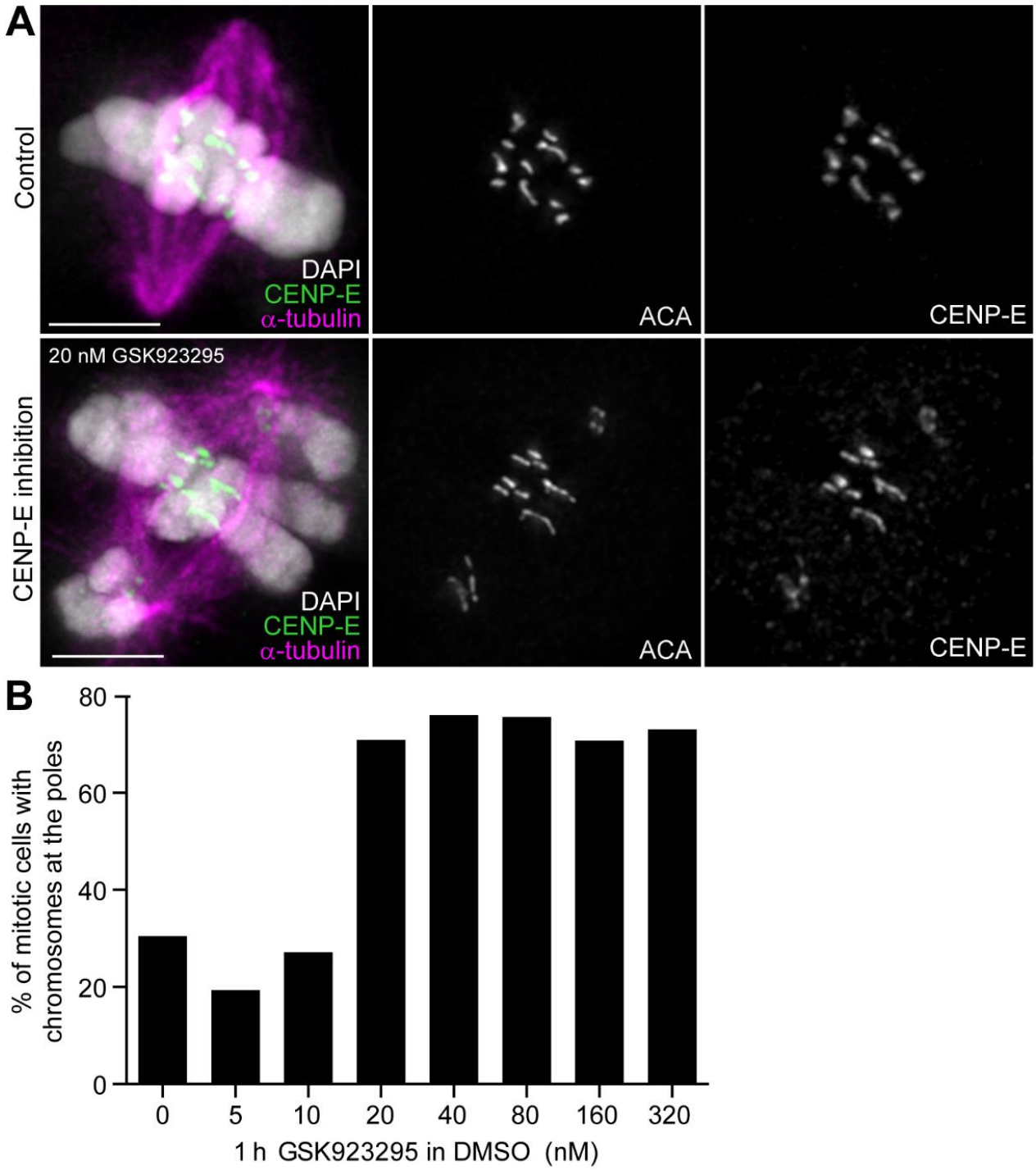

Figure S2. CENP- E inhibition does not affect its kinetochore localization, related to Figure 4. (**A**) Immunofluorescence of control Indian muntjac fibroblasts and after CENP-E inhibition, showing chromosomes (DAPI, white in merged image), CENP-E motor protein (green in merged image) and microtubules (α-tubulin, magenta in merged image). Scale bar = 5 μm. (**B**) GSK923295 titration. Percentage of mitotic cells with chromosomes at the pole, after 1h incubation with increasing concentrations of CENP-E inhibitor. Control cells (0) were treated with DMSO only.

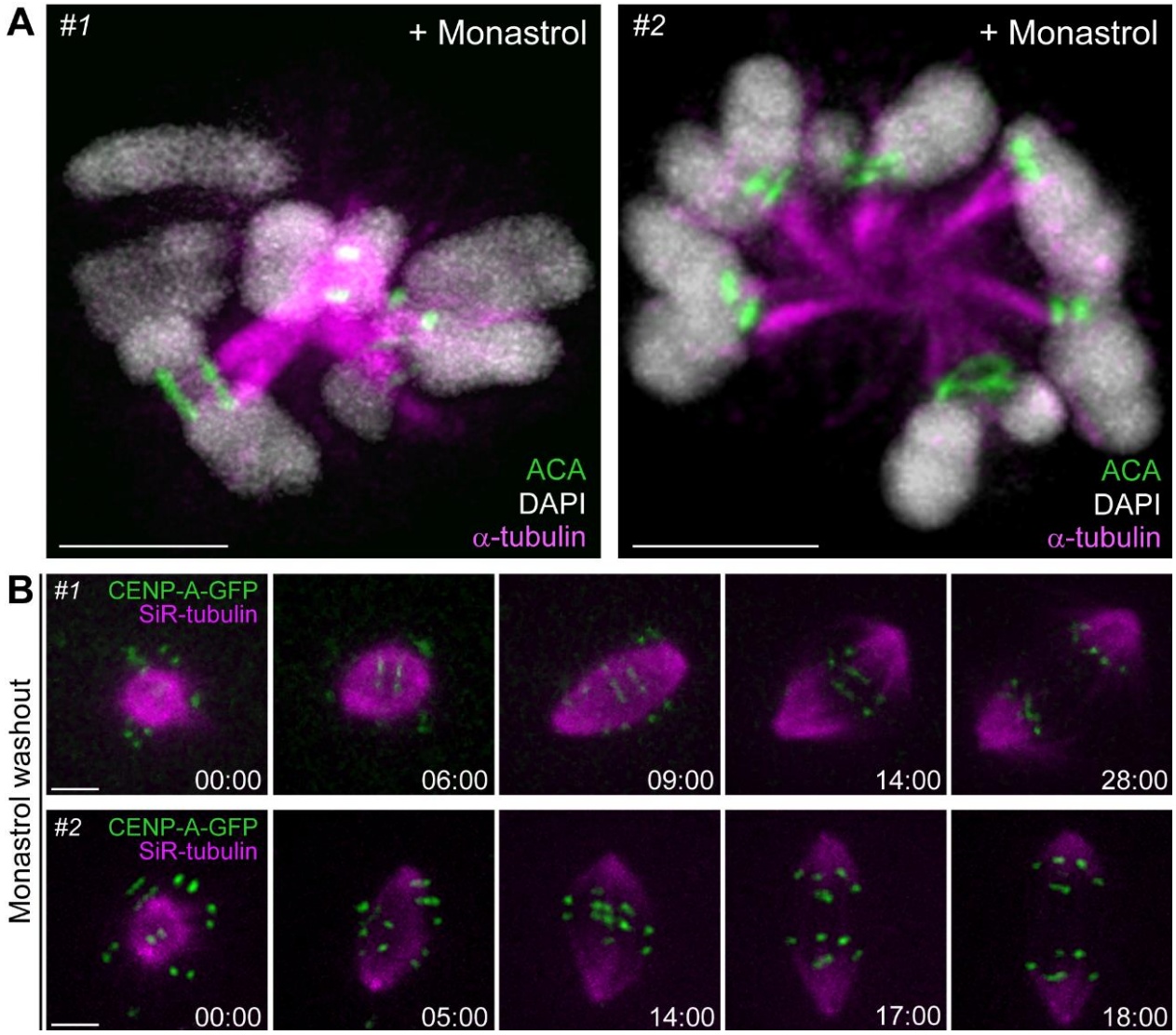

Figure S3. Monastrol treatment/washout in Indian muntjac fibroblasts, related to Figure 6. (**A**) Immunofluorescence of two Indian muntjac fibroblasts with monopolar spindles upon monastrol treatment, showing chromosomes (DAPI, white), kinetochores (ACA, green) and microtubules (α-tubulin, magenta). Scale bars = 5 μm. (**B**) Live-cell imaging of two Indian muntjac fibroblasts stably expressing CENP-A-GFP and treated with 20 nM SiR-tubulin (magenta) after monastrol washout. Scale bar = 5 μm. Time = min:sec.

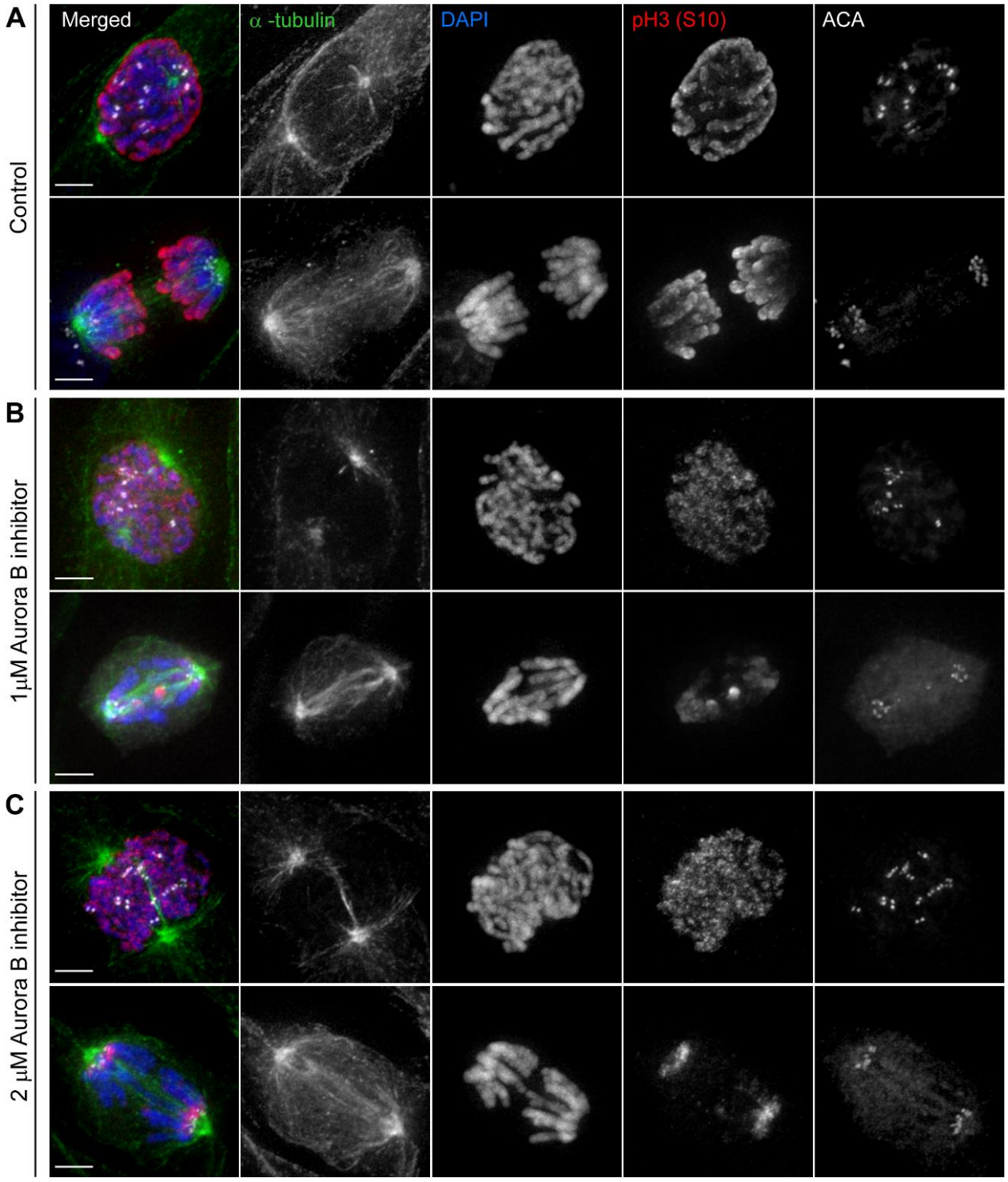

Figure S4. Titration of the Aurora B inhibitor in Indian muntjac fibroblasts, related to Figure 6. (**A-C**) Immunofluorescence images of Indian muntjac fibroblasts in control and when treated with 1 or 2 μM of the Aurora B inhibitor ZM 447439. Cells were incubated for 40 min before fixation. Kinetochores are in white, pH3(S10) in red, α-tubulin in green, chromosomes in blue. Scale bar = 5 μm.

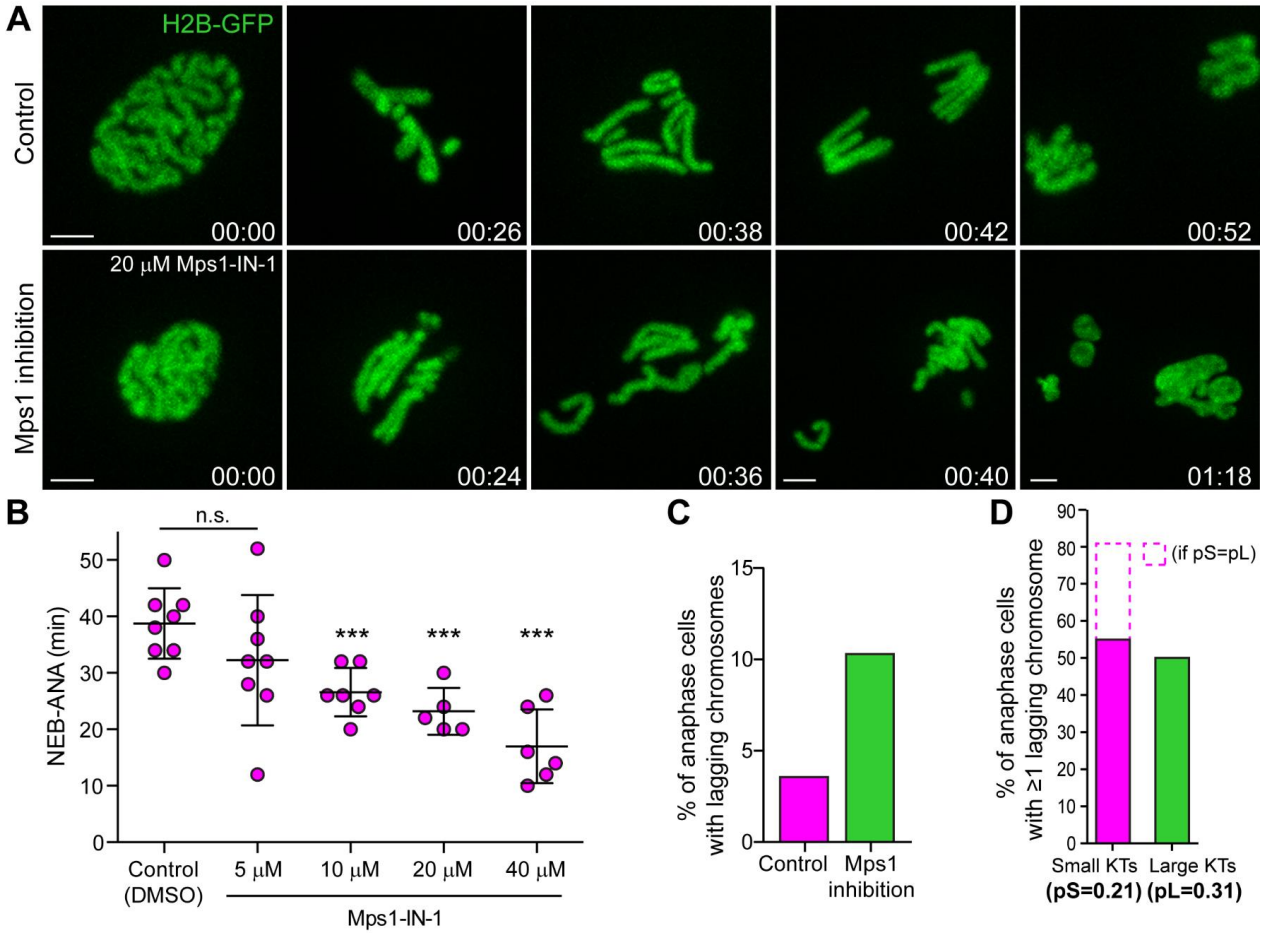

Figure S5. Preventing error correction also generates a missegregation bias towards chromosomes with large kinetochores, related to Figure 6. (**A**) Live-cell imaging of Indian muntjac fibroblasts stably expressing H2B-GFP (green) in controls and after treatment with 20 μM Mps1-IN-1. Scale bar = 5 μm. Time = h:min. (**B**) Quantification of mitotic duration from NEB to anaphase onset (ANA) in control (DMSO) or Mps1-IN-1-treated Indian muntjac fibroblasts with increasing concentrations. ***p<0.001 relative to controls (t-test, each data point represents one cell). (**C**) Frequency of anaphase cells with lagging chromosomes in control or Mps1-IN-1-treated (20 μM for 15 min prior to fixation) Indian muntjac fibroblasts (n= 696 cells, pool of 6 independent experiments). (**D**) Frequency of anaphase cells with at least 1 lagging chromosome with small or large kinetochores (KTs) after Mps1 inhibition (n=18 cells from 3 independent experiments). Dashed bar represents theoretical values for frequencies of lagging chromosomes with small kinetochores if the probability to lag was equal for chromosomes with small or large kinetochores.