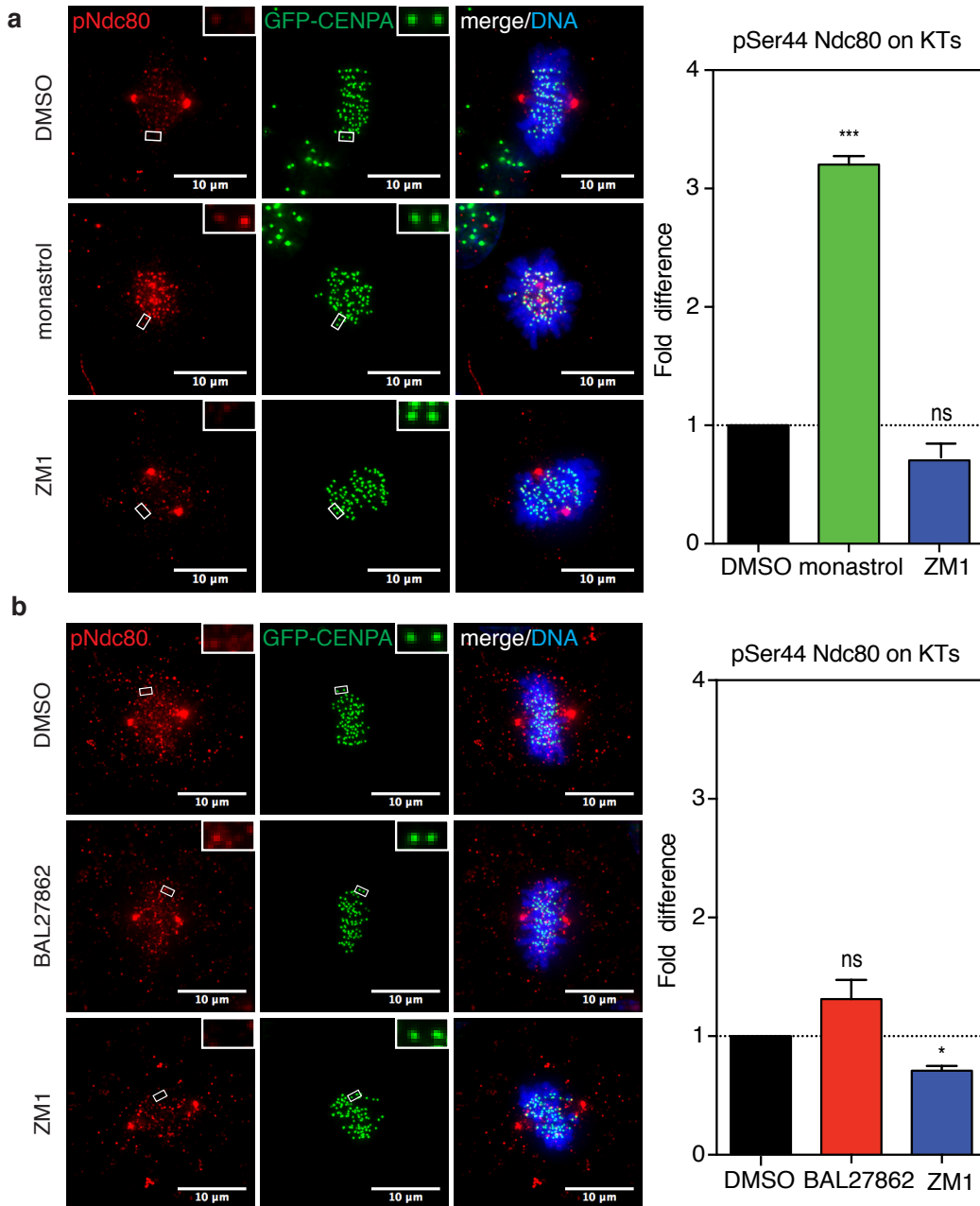


Supplementary Information

**Complete microtubule-kinetochore occupancy  
favours the segregation of merotelic attachments**

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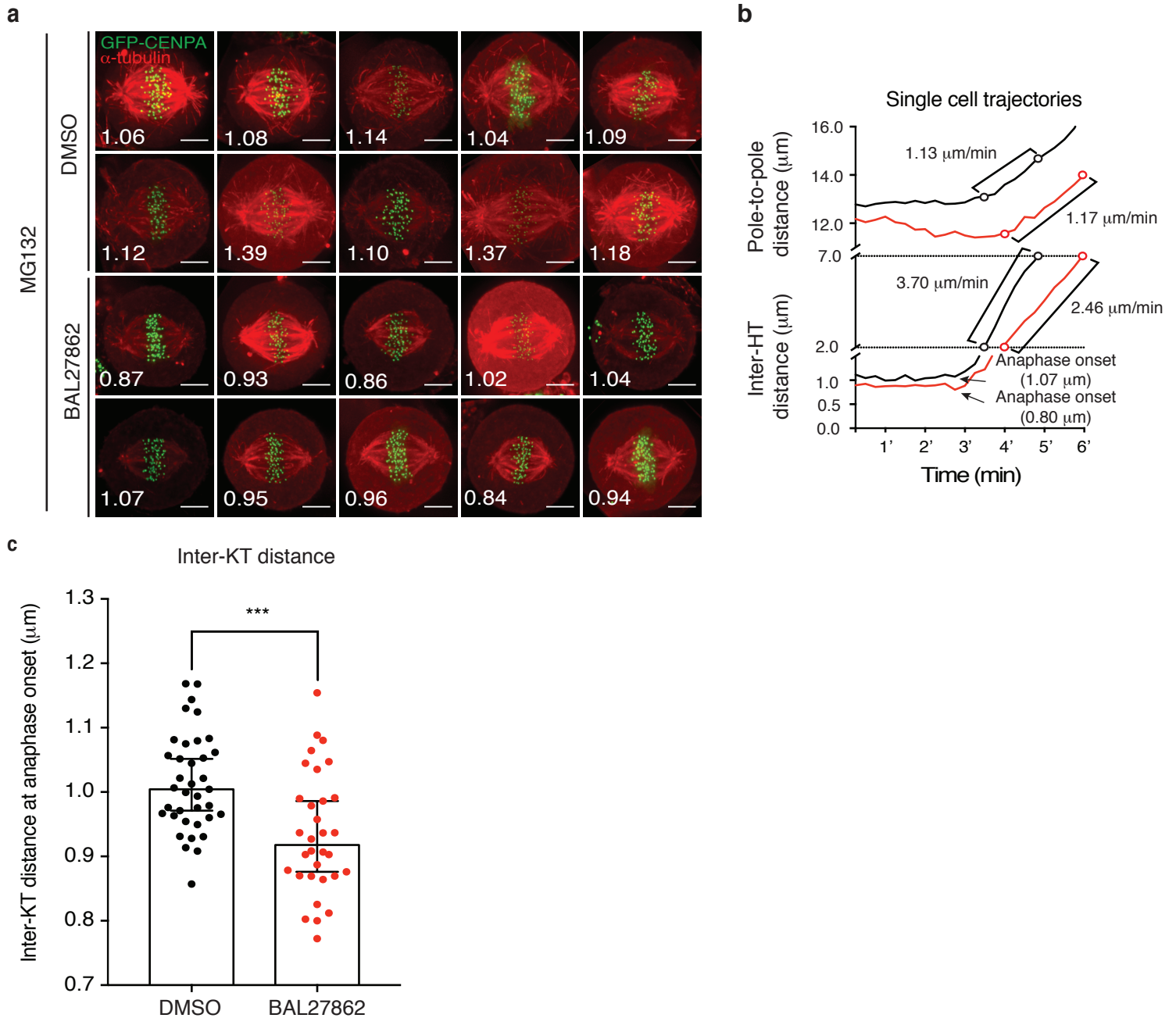
## Supplementary Figure 1



### Supplementary Figure 1. Low inter-KT distances barely increase Ndc80 phosphorylation at KT's (a)

Left panel shows immunofluorescence images of hTert-RPE1 GFP-CENPA cells treated with DMSO, 100  $\mu$  M monastrol or 2  $\mu$ M ZM1 and stained with pSer44-Ndc80 antibodies (red); insets show exemplary sister-KT pairs; scale bars = 10  $\mu$ m; right panel shows quantification of normalized ratio pSer44-Ndc80/GFP-CENPA after background subtraction, DMSO vs. monastrol  $p < 0.001$  in ratio paired two-tailed t-test, error bars are s.e.m..  $N = 3$ ,  $n = 300-400$  kinetochores. **(b)** Left panel shows immunofluorescence images of metaphase hTert-RPE1 GFP-CENPA cells treated with DMSO, 12 nM of BAL27862 or 2  $\mu$ M ZM1 and stained with pSer44-Ndc80 antibodies (red); insets show exemplary sister-KT pairs; scale bars = 10  $\mu$ m. Right panel shows quantification of normalized ratio pSer44-Ndc80/GFP-CENPA after background subtraction. Error bars are s.e.m..  $N = 3$ ,  $n = 280-600$  kinetochores, ratio paired two-tailed t-test, ns = non significant. \* represents  $p < 0.05$ .

## Supplementary Figure 2



**Supplementary Figure 2. Visualization of the effects of BAL27862 on astral MTs and anaphase spindle elongation** (a) Immunofluorescence images of ten randomly selected hTert-RPE1 GFP-CENPA cells from a single experiment. Cells were treated in parallel with DMSO or 12 nM BAL27862, 10  $\mu\text{M}$  MG132, and stained with  $\alpha$ -tubulin antibodies (red). Scale bars = 5  $\mu\text{m}$ . An average inter-KT distance per each cell is indicated in the left bottom corner. Note smaller asters in BAL27862-treated cells. (b) Single-cell trajectories of hTert-RPE1 Centrin1-GFP/GFP-CENPA cells treated with DMSO (black) or 12 nM of BAL27862 (red); average inter-KT distance before anaphase onset (bottom), the anaphase sister-KT velocities (centre) and spindle elongation velocities (top). T = 0 is arbitrary. (c) Quantification of inter-KT distances at anaphase onset of hTert-RPE1 Centrin1-GFP/GFP-CENPA cells; N = 4, n = 32-37 cells, error bars are 95% CI, p = 0.0002, two-tailed t-test.