Supplemental Figure 1
(A) Unsynchronized U2OS cells were stained with anti-pS165 (green) and anti-Hec1, 9G3, antibodies (red), and DAPI (blue). (B) Dynamic changes of Hec1 pS165 staining during mitotic progression in Ptk2 cells. Cells were immunostained with anti-pS165 (green) and anti-Hec1 antibodies (red), and DAPI (blue). Scale bars, 5 μm.

Supplemental Figure 2
Western blot analysis of U2OS parental cells and U2OS cells expressing RNAi-resistant Hec1- WT-GFP. The expression of BubR1, Mad1, Mad2, Zw10, and Zwint1 between these two cells were examined with their specific antibodies. β-actin served as a loading control.

Supplemental Figure 3
The cold-stability of kinetochore-microtubules is reduced in cells expressing Hec1 S165E but not WT or S165A. Average MT intensity (mean ± s.e.m.; $n > 10$ transfected cells) was measured for each condition. Intensities are normalized against time 0 for each condition (MT intensity at time 0 is 100%). NS, nonsignificant ($p>0.05$).

Supplemental Figure 4
(A) Unsynchronized MCF10A cells were treated with DMSO, 5, 10, or 20 μM MPS1-IN-1 inhibitor for 1 hour and stained with anti-pS165 (red) and anti-Hec1, 9G3, antibodies (green), and DAPI (blue). (B) Unsynchronized Ptk2 cells were treated with DMSO, 5, or 10 μM MPS1-IN-1 inhibitor for 1 hour and stained with anti-pS165 (red) and anti-Hec1, 9G3, antibodies (green), and DAPI (blue). Scale bars, 5 μm.

Supplemental Figure 5
U2OS (A), or Ptk2 (B) cells were immunostained with anti-pS165 antibodies (red) and anti-Hec1, 9G3, antibodies (green), using pre-fixation extraction buffer containing either DMSO, 100 nM, or 500 nM of specific phosphatase inhibitor. Cells were counterstained with DAPI (blue). Scale bars, 5 μm.

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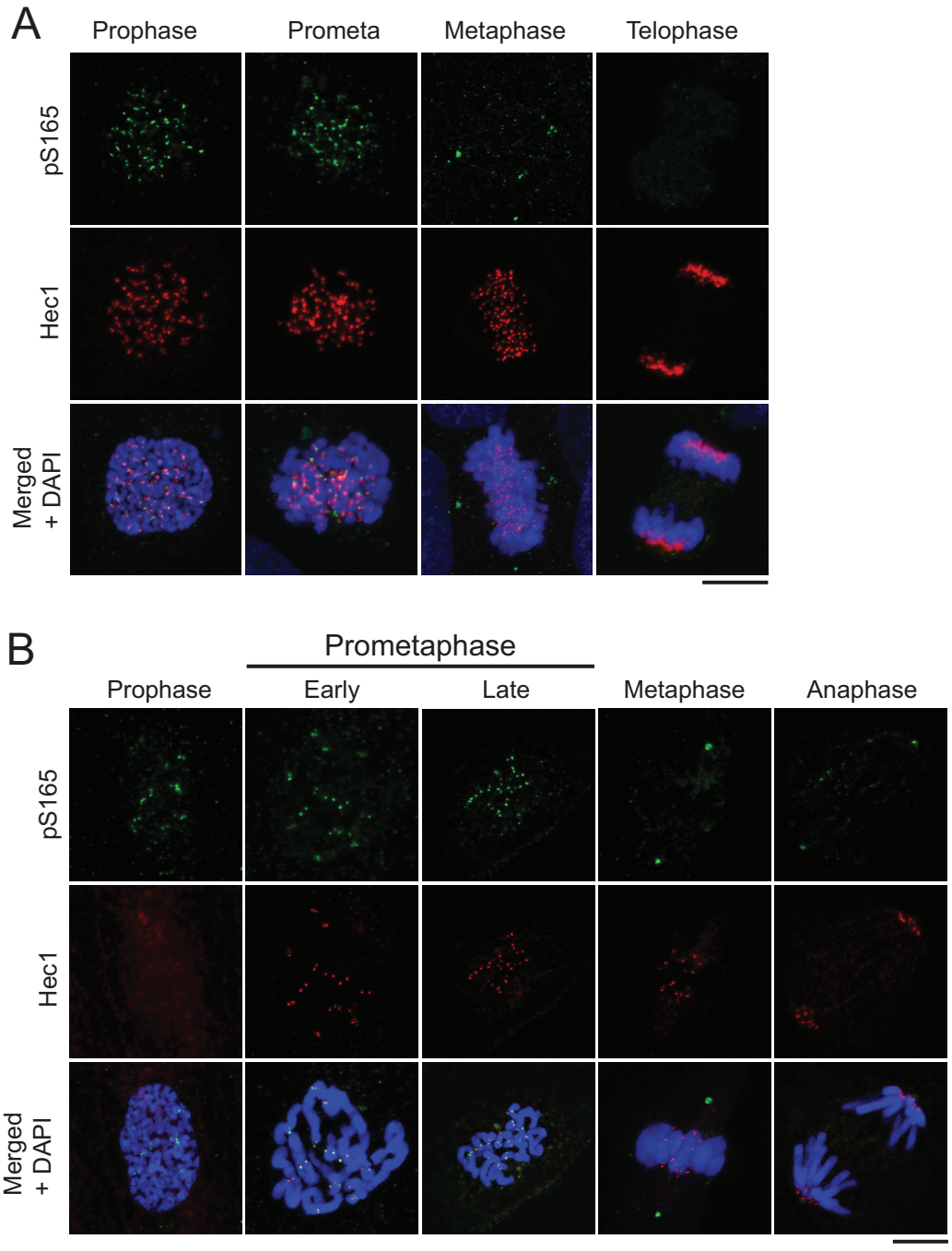

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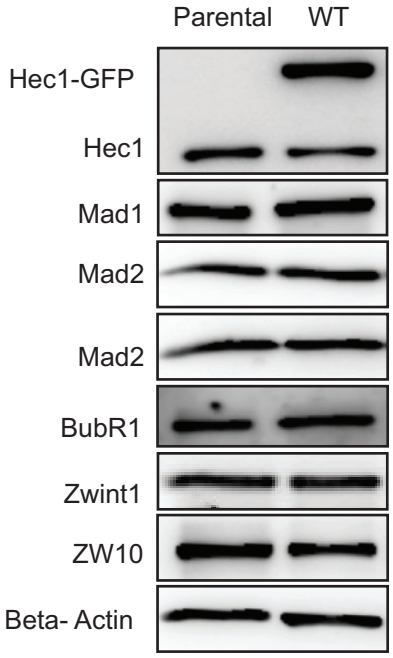

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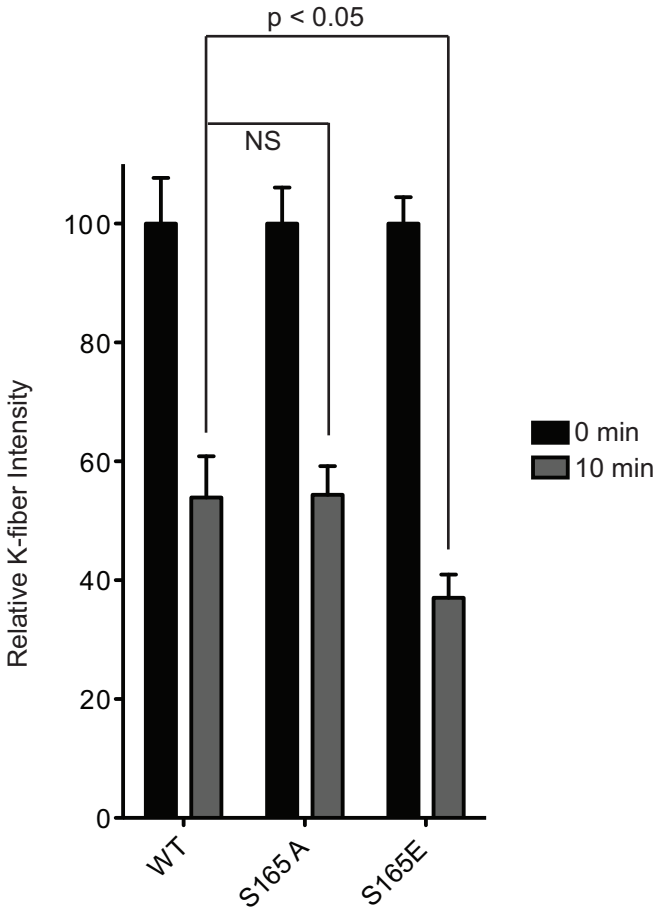

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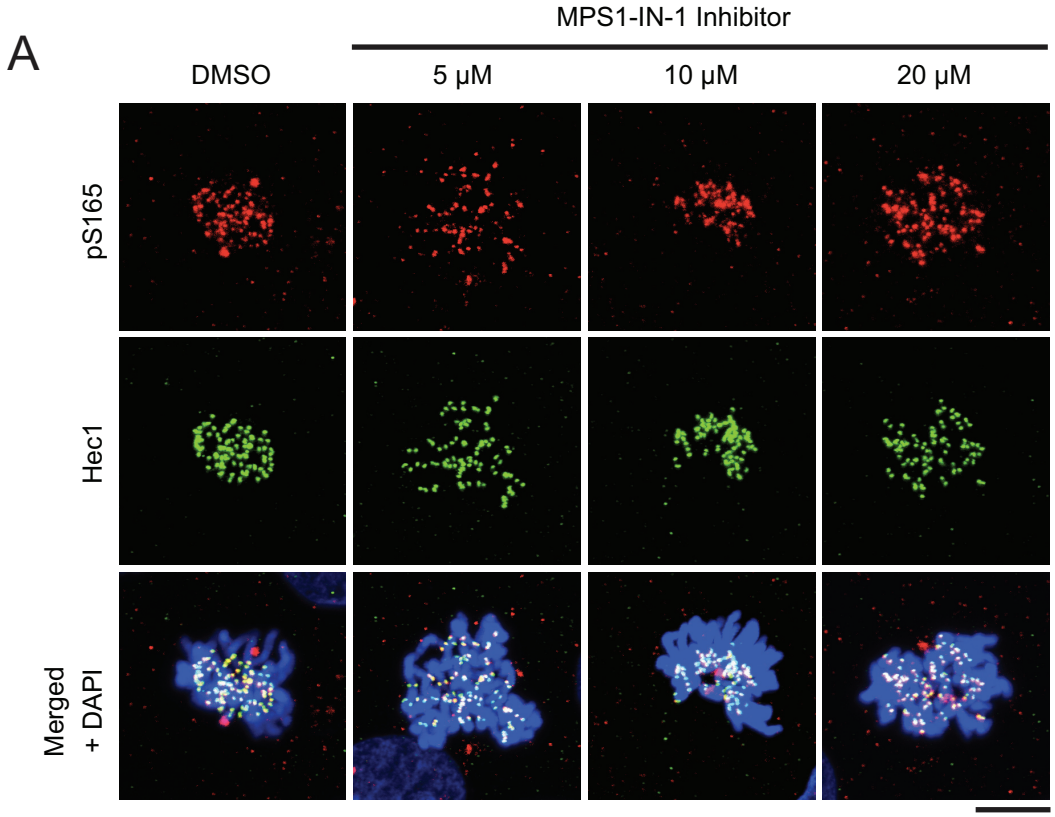

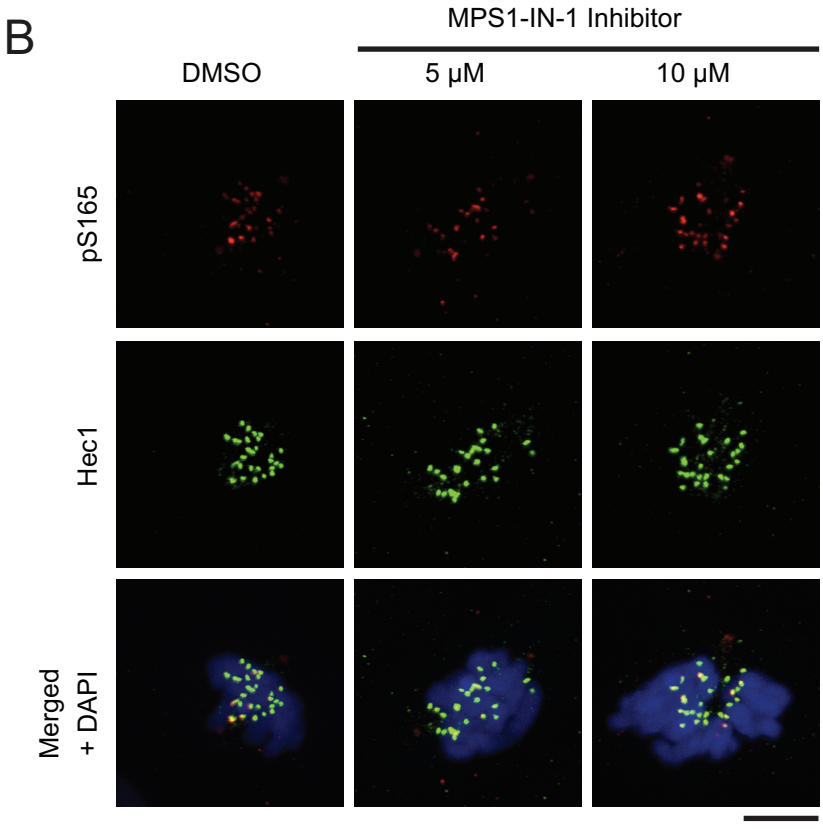

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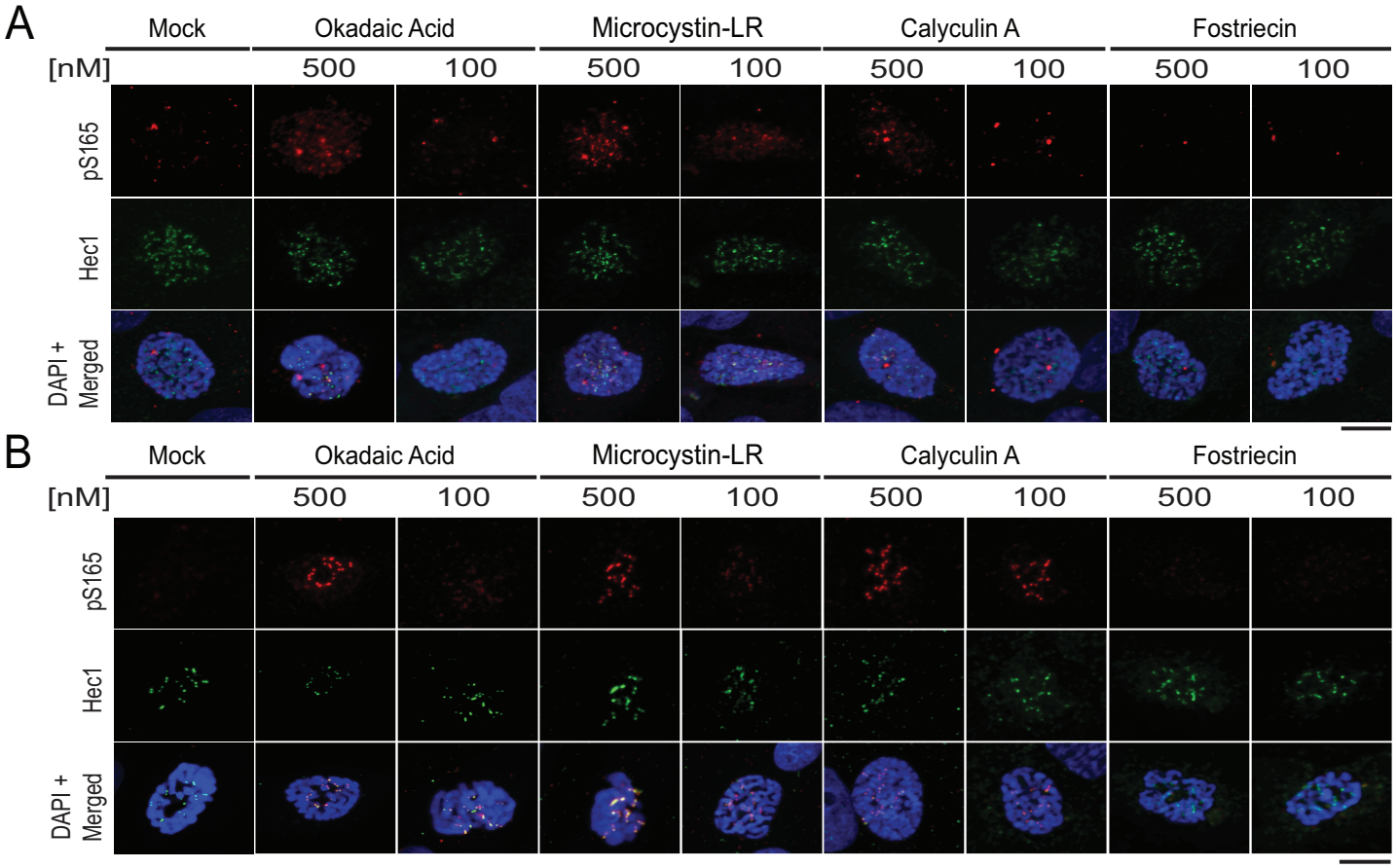
Supplemental Figure 5 - Wei et al.



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