Supplementary Fig. S1

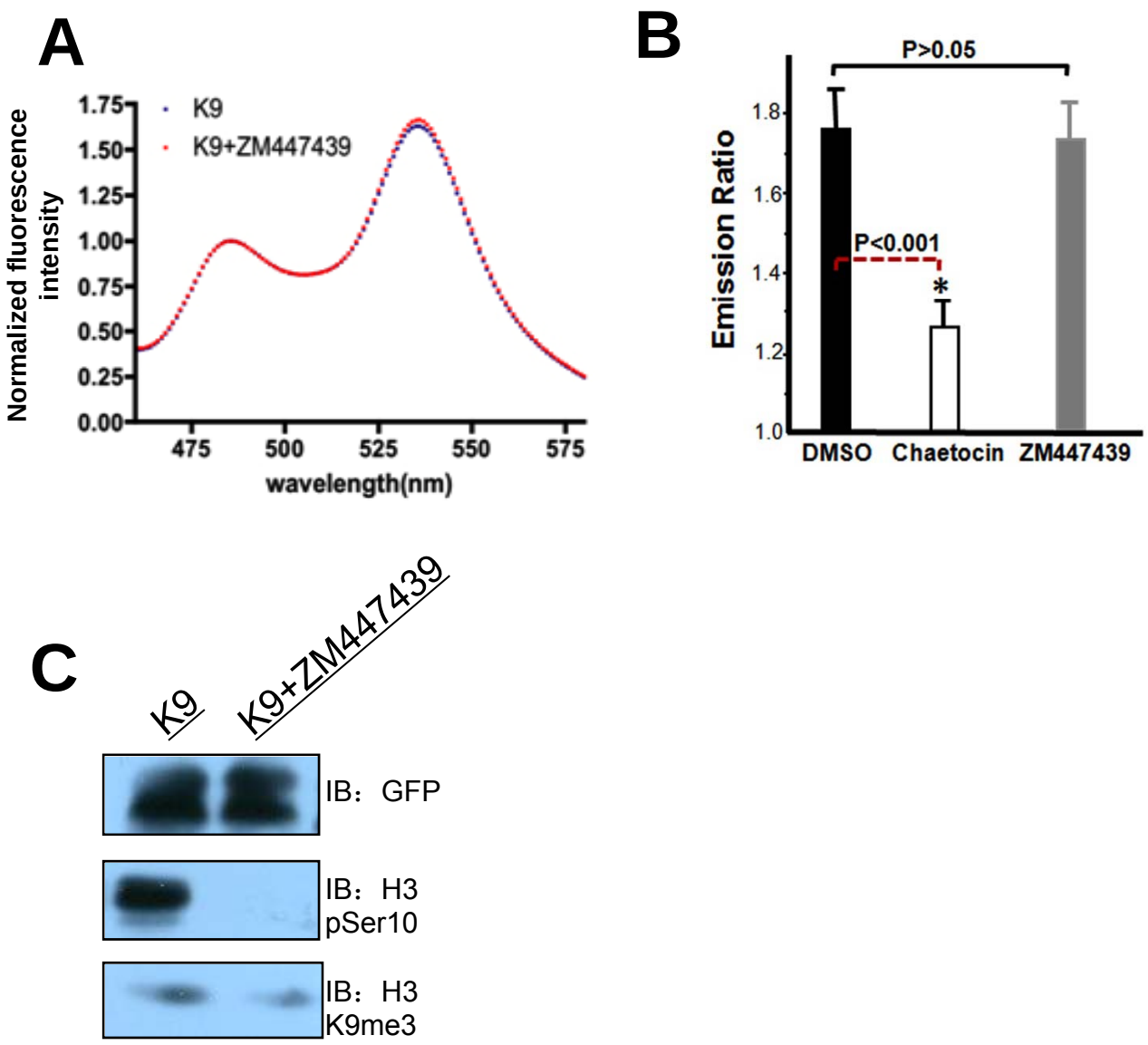

Figure S1. Characterization of MARC activity related to H3S10 phosphorylation

A. Aliquots of HeLa cells expressing MARC and synchronized with 200 ng/ml nocodazole. Before cells were lysed for spectrometric measurement of FRET, Aurora B inhibitor ZM447439 was added into one aliquot to suppress the phosphorylation of H3S10. FRET measurement and normalization were done as described by Chu et al., (2011). Note the FRET was readily apparent as the emission peak of YFP at 527 nm and a drop of CFP emission. Addition of ZM447439 did not cause a dramatic change of FRET.

B. Statistic analyses of MARC activity in the presence of chaetocin and Aurora B inhibitor ZM447439. Note that chaetocin, but not ZM447439, abolished the MARC activity, suggesting that phosphorylation of S10 does not affect MARC reporting of H3K9 tri-methylation.

C. Cell lysates from the above measurement were used for western blotting analyses to validate the H3S10 dephosphorylation by ZM447439.

Fig. S2

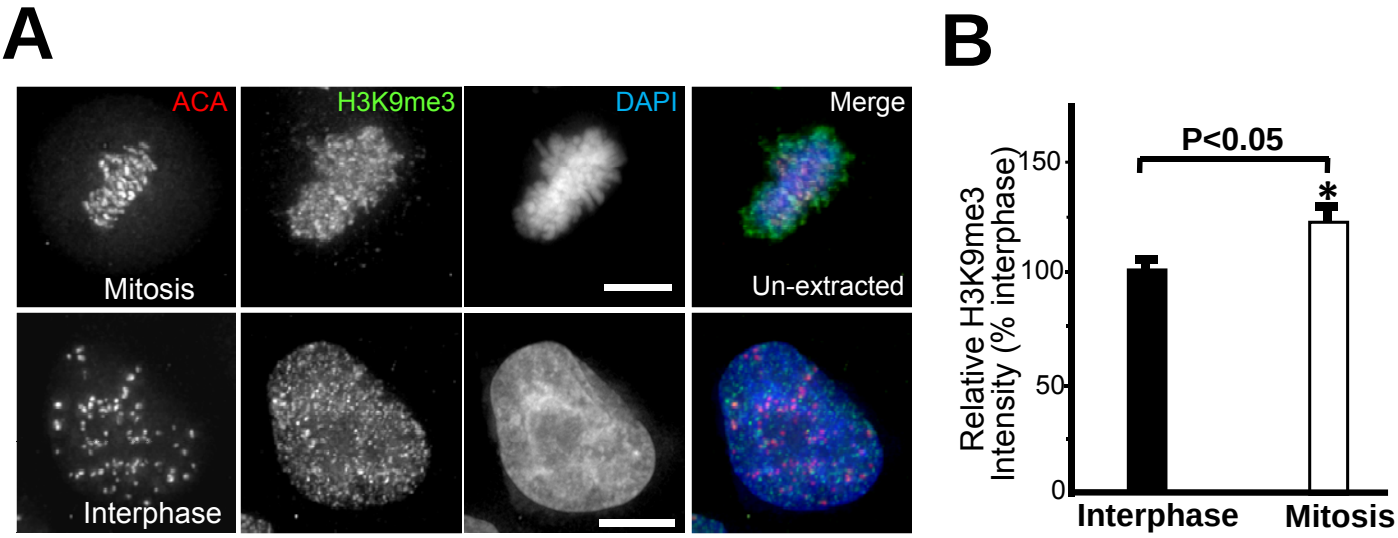

Figure S2. H3K9me3 distribution in interphase and mitotic cells

A. Localization of SUV39H1 to centromere of mitotic cells. HeLa cells grown on a coverslip were fixed with 4% formaldehyde without a pre-extraction procedure. The fixed cells were then stained with antibodies against ACA and SUV39H1, chromosomes was stained with DAPI. Note that both interphase and mitotic cells display distinct localization related to ACA labeling. Without the pre-extraction, the centromere localization is less apparent due to an intensive heterochromatin staining. Bar, 10 μm.

B. Statistic analyses of H3K9me3 labeling in interphase and mitotic cells. The pixel intensities at each centromere or heterochromatin site were normalized against adjacent ACA pixel values to account for any variations in staining or image acquisition. Note that the H3K9me3 level is relative higher in mitotic cells compared to that of interphase cells (mean±SE; 10 cells from each categories).

Fig. S3

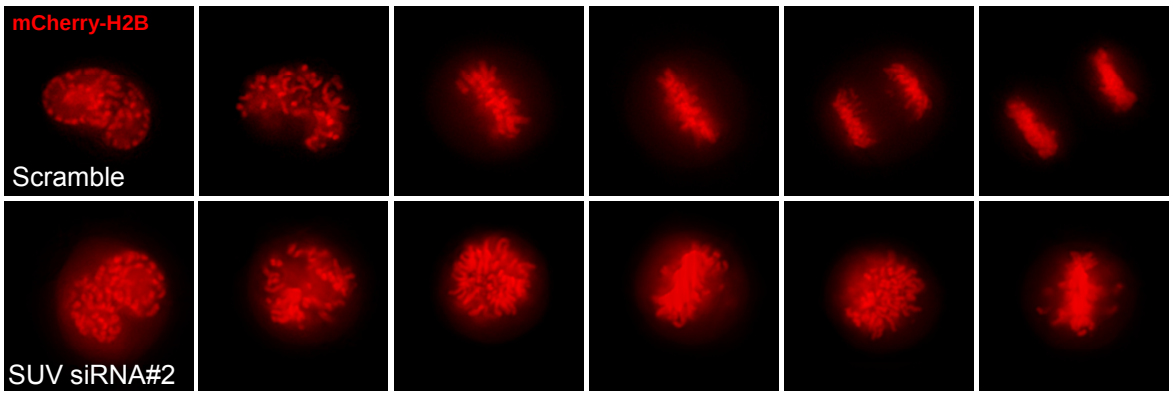

Figure S3. Suppression of SUV39H1 perturbs mitotic progression.

HeLa cells were transiently co-transfected with mCherry-H2B and SUV39H1 siRNA (#2). Twenty hours after the transfection, cells were imaged for mitosis.

Fig. S4

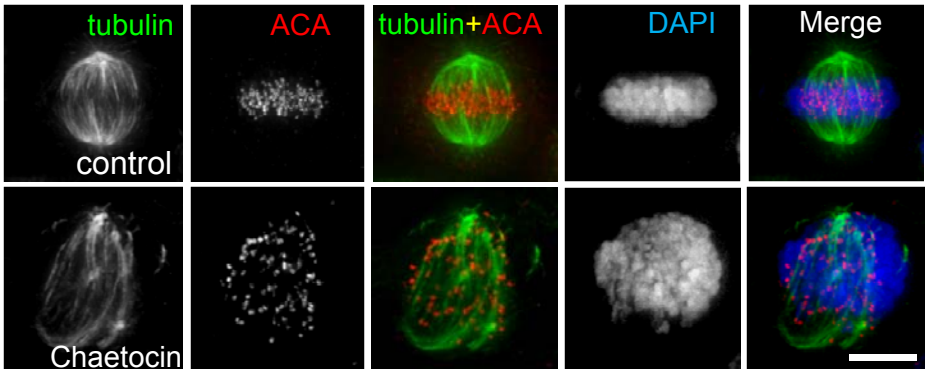

Figure S4. Chaetocin treatment perturbs microtubule dynamics but not bipolar spindle
Immunofluorescence images from control and chaetocin-treated cells were collected HeLa cells after treatment with chaetocin or DMSO (control). Cells were stained with ACA (*red*), DAPI (*blue*), and anti-tubulin antibody (*green*). It is readily apparent that bipolar spindle is not perturbed by chaetocin treatment. However, spindle microtubules are less stable in chaetocin-treated cells. *Bar,* 10 μm.

Fig. S5

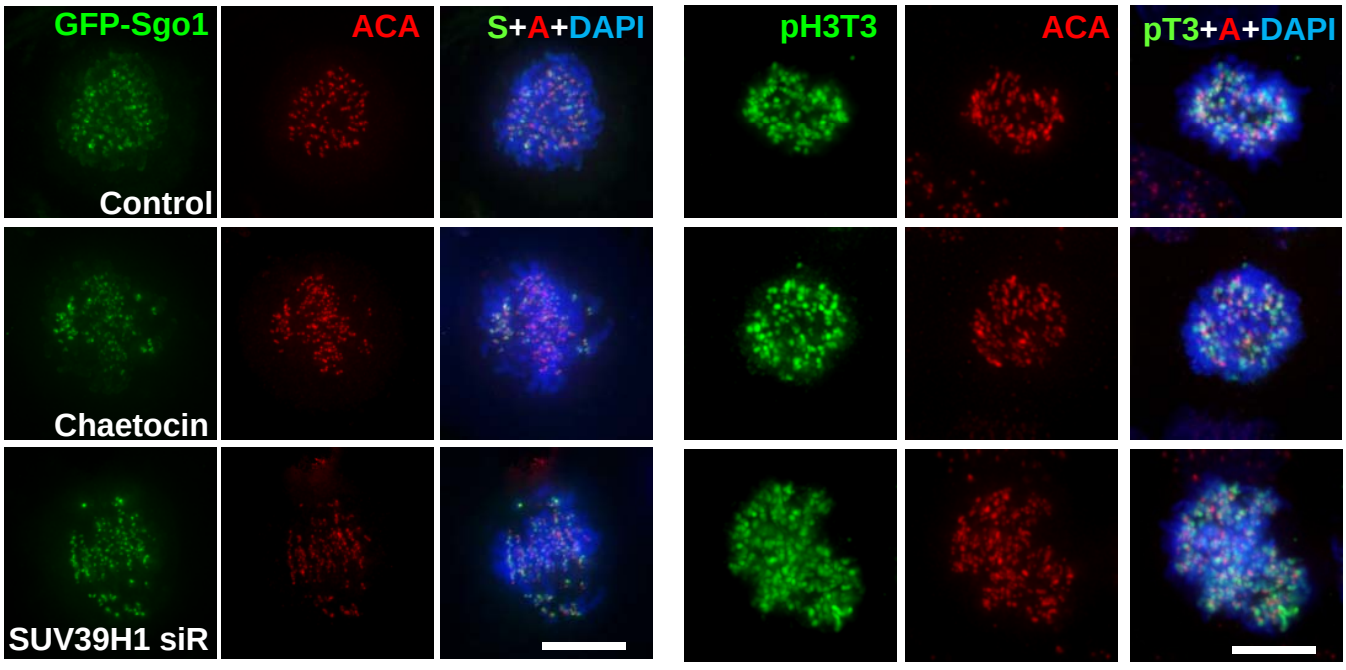

Figure S5. Suppression of SUV39H1 does not perturb Sgo1 and pH3T3 localization.
HeLa cells were suppressed SUV39H1 either by chaetocin or siRNA as described in Figure 5. Treated cells were then stained for Sgo1 or pH3T3 with counter-stain of ACA. No apparent changes of Sgo1 and pH3T3 staining were noticed in SUV39H1-suppressed cells. *Bar,* 10 μm.

Fig. S6

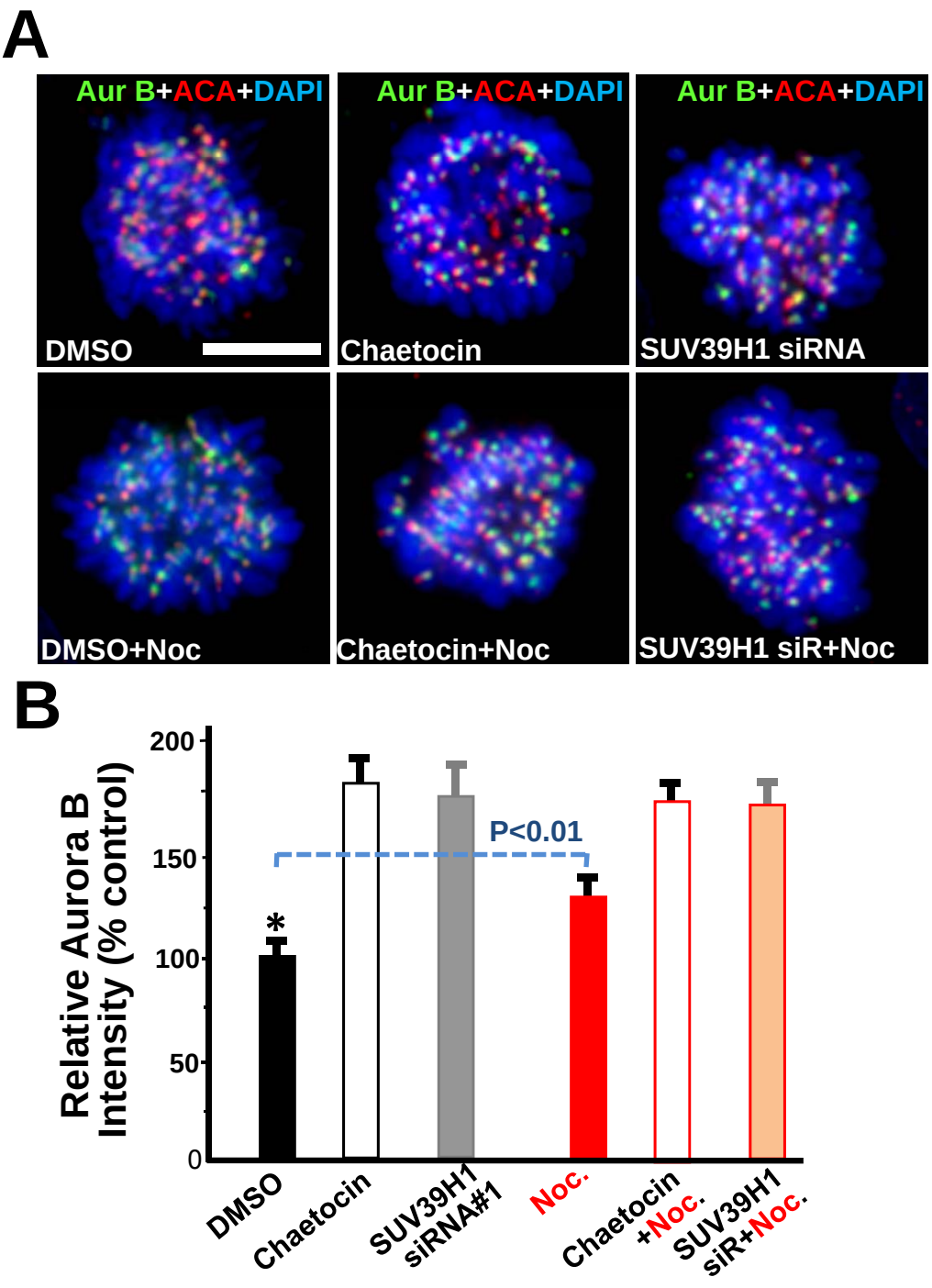

Figure S6. An enhanced Aurora B localization to the centromere in SUV39H1-suppressed cells is not a function of kinetochore microtubule attachment .

A. Aliquots of HeLa cells were treated to suppress SUV39H1 either by chaetocin or siRNA as described in Figure 5. Aliquots of treated cells were exposed to 20 μM nocodazole to depolymerize kinetochore microtubules before fixation. The fixed cells were then stained for Aurora B (green) with counter-stain of ACA (red). Nocodazole treatment results in a dramatic enrichment of Aurora B to kinetochore of control but not SUV39H1-suppressed cells. *Bar,* 10 μm.

B. Statistic analyses of Aurora B immunofluorescence intensity at kinetochore indicate that enrichment of Aurora B to SUV39H1-suppressed kinetochore is not due to misaligned chromosomes (mean±SE; 10 cells from each categories).

Fig. S7

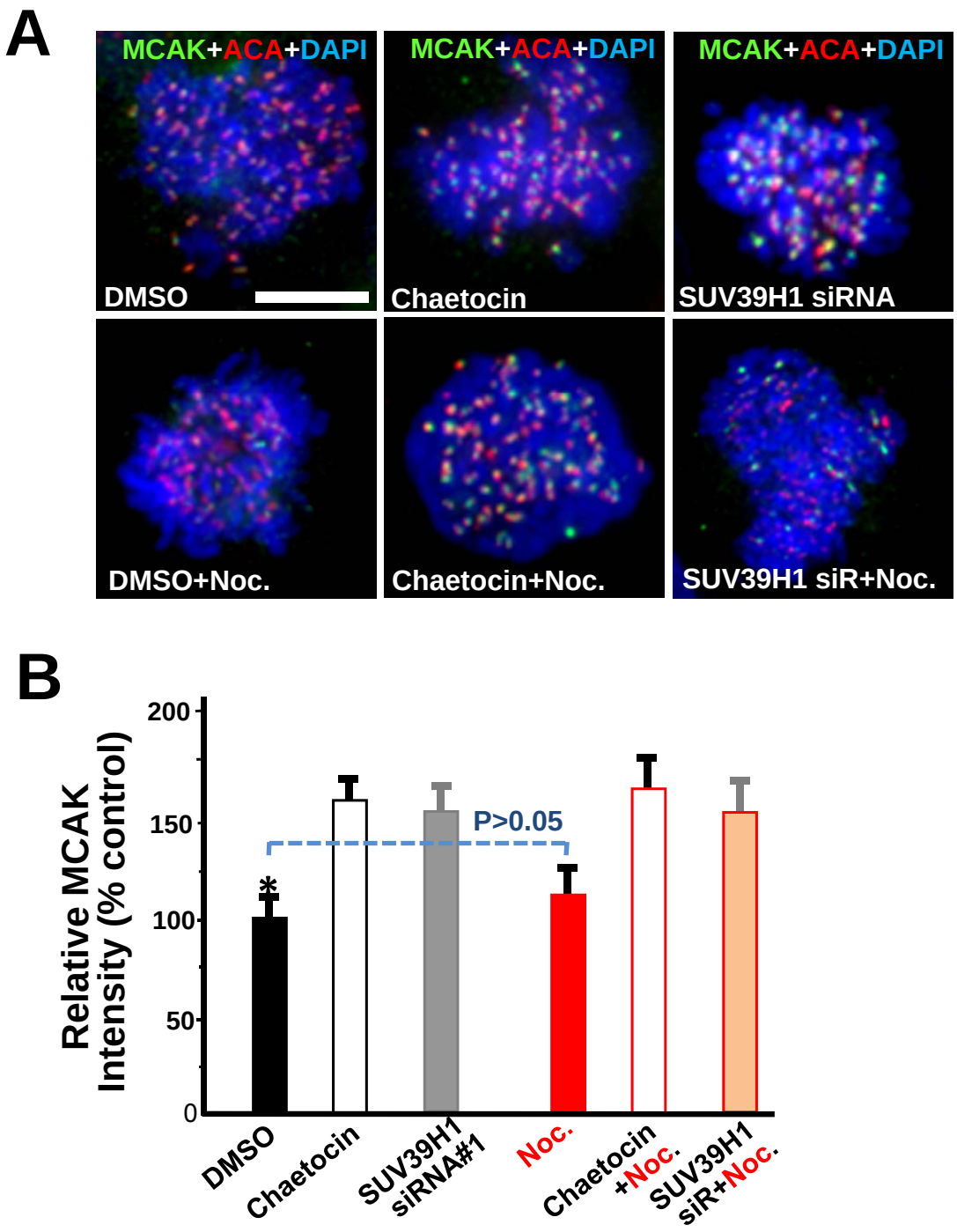

Figure S7. An enhanced MCAK localization to the centromere in SUV39H1-suppressed cells is not a function of kinetochore microtubule attachment .

A. Aliquots of HeLa cells were treated to suppress SUV39H1 either by chaetocin or siRNA as described in Figure 5. Aliquots of treated cells were exposed to 20 μM nocodazole to depolymerize kinetochore microtubules before fixation. The fixed cells were then stained for MCAK (green) with counter-stain of ACA (red). Nocodazole treatment results in a brief enrichment of MCAK to kinetochore of control but not SUV39H1-suppressed cells. *Bar*, 10 μm.

B. Statistic analyses of MCAK immunofluorescence intensity at kinetochore indicate that enrichment of MCAK to SUV39H1-suppressed kinetochore is not due to misaligned chromosome (mean±SE; 10 cells from each categories).