Supplemental Figure S1. Cohesin localizes to mitotic spindle poles. Scale bar=5μm.

A. hSMC1-GFP localizes to spindle poles in mitotic HeLa cells. HeLa cells stably expressing GFP alone or hSMC1-GFP were pre-extracted with CSK buffer, fixed and co-stained with antibody specific for γ−tubulin to indicate the spindle pole (red). DNA was visualized by DAPI staining. Ku, an abundant DNA repair factor, was also detected with antibody specific for Ku86 (Santa Cruz, goat polyclonal) as a negative control. Mitotic spindle poles are indicated by white arrowheads. The merged images of the γ-tubulin-positive spindle pole regions are shown at higher magnification in the upper right-hand corners. The cellular localization of hSMC1-GFP and its incorporation into a cohesin complex were comparable to the endogenous protein (Hou *et al.*, 2007).

B. hSMC1, but not SA, localizes to the centrosomes during interphase. Interphase HeLa cells were CSK-extracted and stained with the indicated antibodies. The merged images of the γ-tubulin-positive centrosome regions are shown at higher magnification in the upper right-hand corners.

C. Cohesin localizes to the spindle poles in mitotic chicken DT40 cells. The wild type chicken DT40 cells were pre-extracted, fixed and stained with antibodies specific for γ−tubulin, SMC1, and SA as shown. Rad21 was detected in Rad21 conditional knockout DT40 cells treated with Dox for 24 hrs. Mitotic spindle poles are indicated by white arrowheads. The merged images of the γ-tubulin-positive spindle pole regions are shown at higher magnification in the upper right-hand corners.

Supplemental Figure S2. Analysis of Rad21(-) and CENP-H(-) chicken DT40 cells.

A. DAPI staining of Rad21(-) and CENP-H(-) cells. Rad21(-) cells were fixed at 27 hrs of Dox treatment. CENP-H(-) cells were fixed at 27 hrs and 35 hrs of Dox treatment. Cells were stained with DAPI to distinguish interphase and mitosis. Scale bar=10μm.
B. Immunofluorescent staining of CENP-H(-) cells. Dox-treated CENP-H(-) mitotic cells were fixed at 27 hrs or 35 hrs and stained with antibody specific for α-tubulin (green) and NuMA (red) as well as DAPI (blue). The merged images are shown. Compare to the image in Figure 3F. Scale bar=5μm.

Supplemental Figure S3. Intact spindles in cohesin-depleted mitotic cells. Following hSMC1 depletion by siRNA, mitotic cells were CSK-extracted, fixed by paraformaldehyde and stained with antibodies specific for α-tubulin and hSMC1. Scale bar=5μm.

Supplemental Figure S4. Microtubule reassembly following nocodazole treatment in control and hRAD21 siRNA-depleted interphase HeLa cells. Nocodazole treatment and recovery were as described for mitotic spindle reassembly (Fig. 4B). Nocodazole-treated cells without recovery and with 2 min of recovery time are shown. Scale bar=5μm.

Supplemental Figure S5. The effect of overexpression of the N-terminal fragment of NuMA on spindle reassembly. Mitotic cells overexpressing GFP or GFP-NuMA-N (Fig. 2) are subjected to the spindle reassembly assay following transient nocodazole treatment as in Figure 4B. α-tubulin was shown in red; DAPI in blue. Scale bar=5μm.

Supplemental Figure S6. Comparison of the effect of cohesin and SUV39H1 depletion on centromere positioning. (**A**) hRad21-depleted and (**B**) SUV39H1-depleted mitotic cells by siRNAs were CSK-extracted and fixed by paraformaldehyde. The centromere regions are visualized by antibody specific for Tripin/hSgo2. Depletion is confirmed by the corresponding antibody staining as indicated.

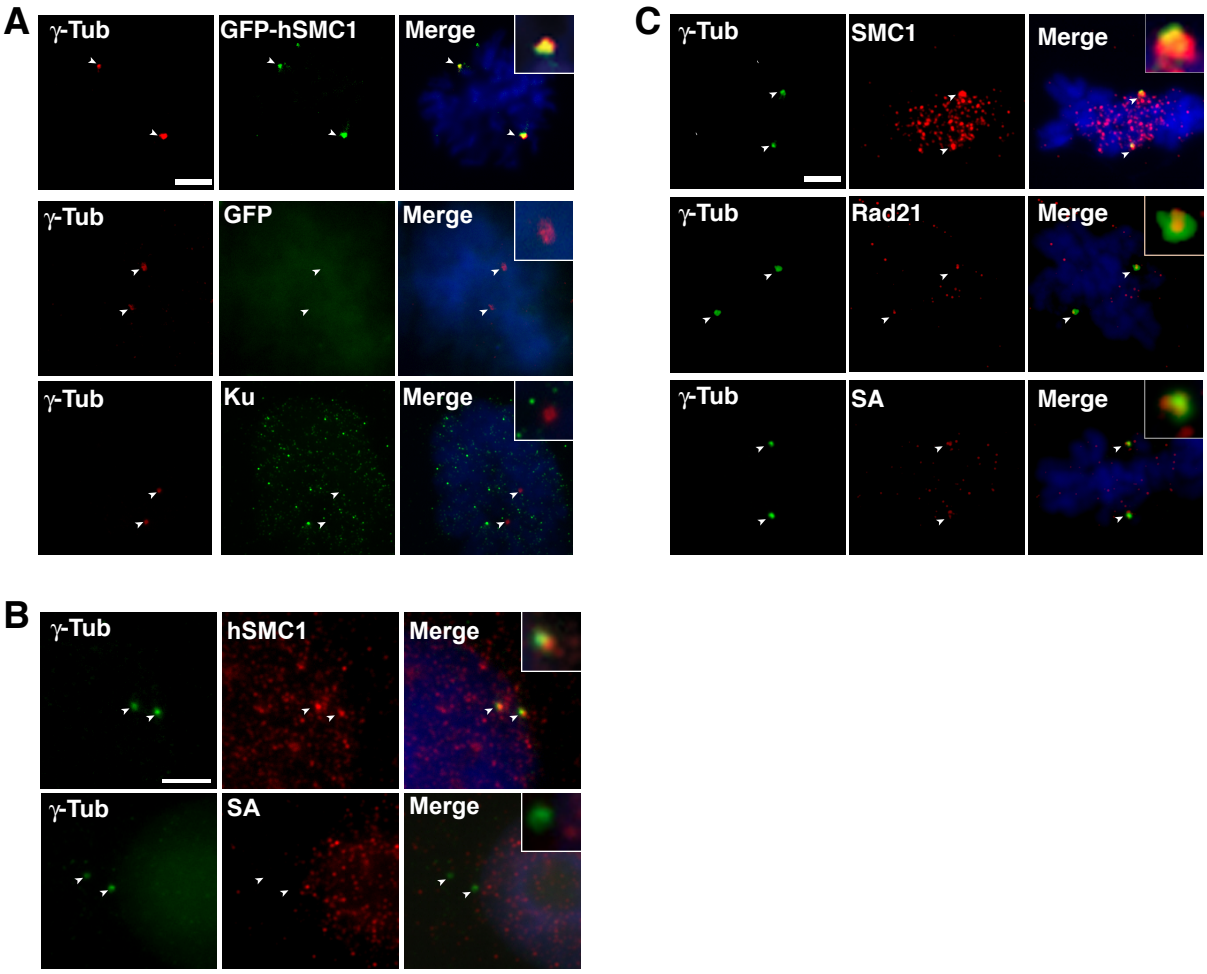
C. SUV39H1- and hRad21-depleted mitotic cells were subjected to the spindle reassembly following transient nocodazole treatment as in Figure 4B and stained with the indicated antibodies. Scale bar=5μm.

Supplemental Figure S7. Residual cohesin persists at the spindle poles in siRNA-treated cells.

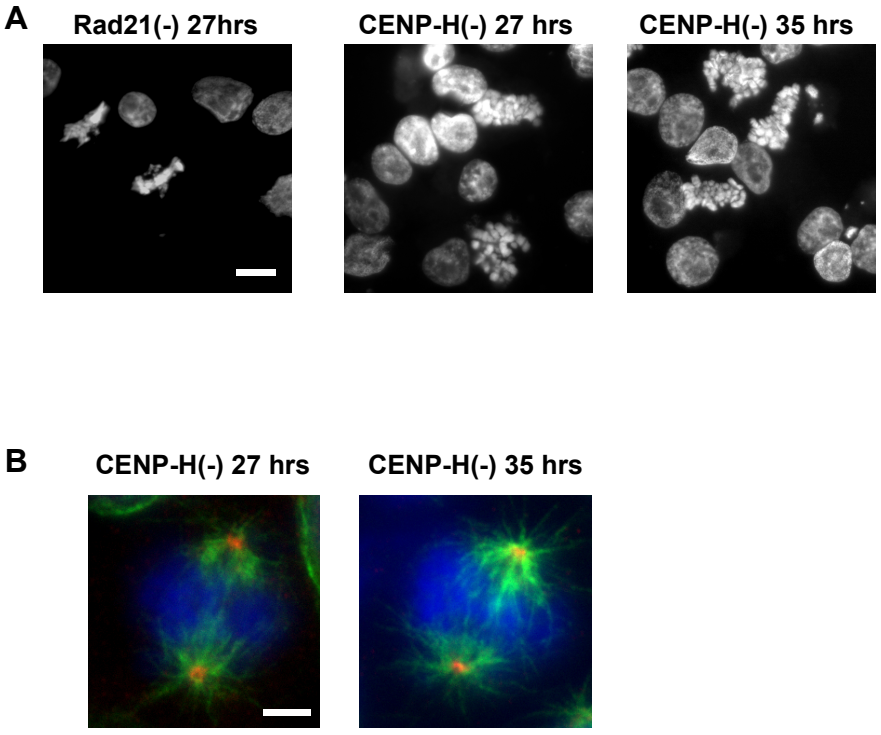
Cells were fixed 24 hrs after the second transfection of either control siRNA or siRNA against hSMC1, and stained with anti-hSMC1 antibody without extraction. Scale bar=5μm.

Hou, F., Chu, C.W., Kong, X., Yokomori, K., and Zou, H. (2007). The acetyltransferase activity of San stabilizes the mitotic cohesin at the centromeres in a shugoshin-independent manner. J. Cell Biol. *177*, 587-597.

Supplemental Figure S1



Supplemental Figure S2



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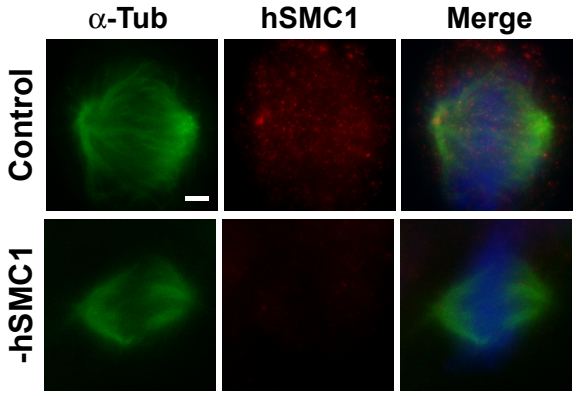

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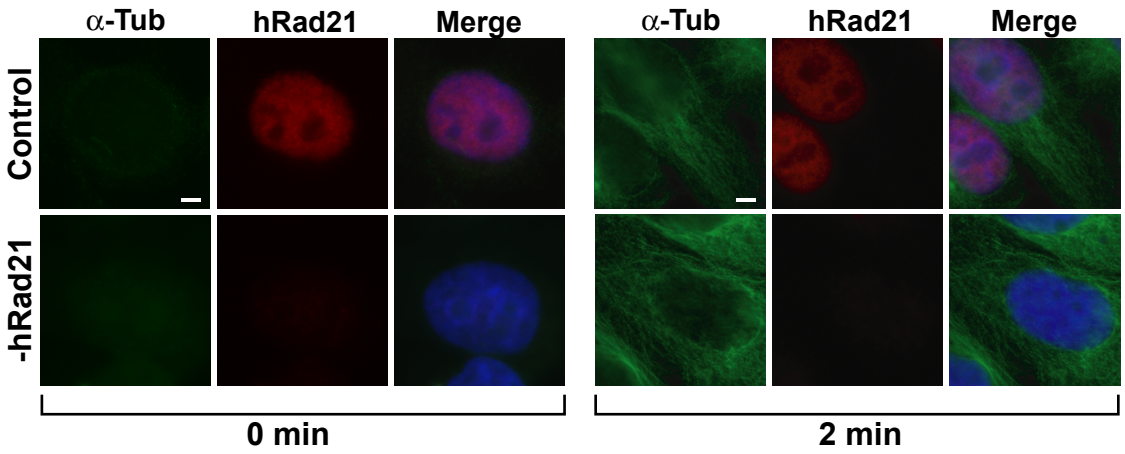
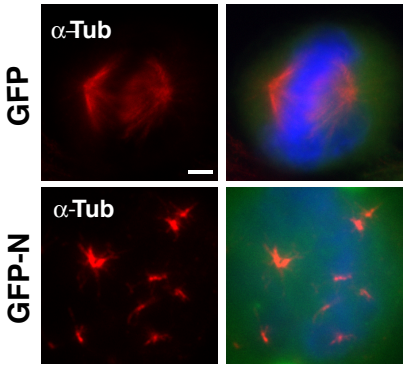
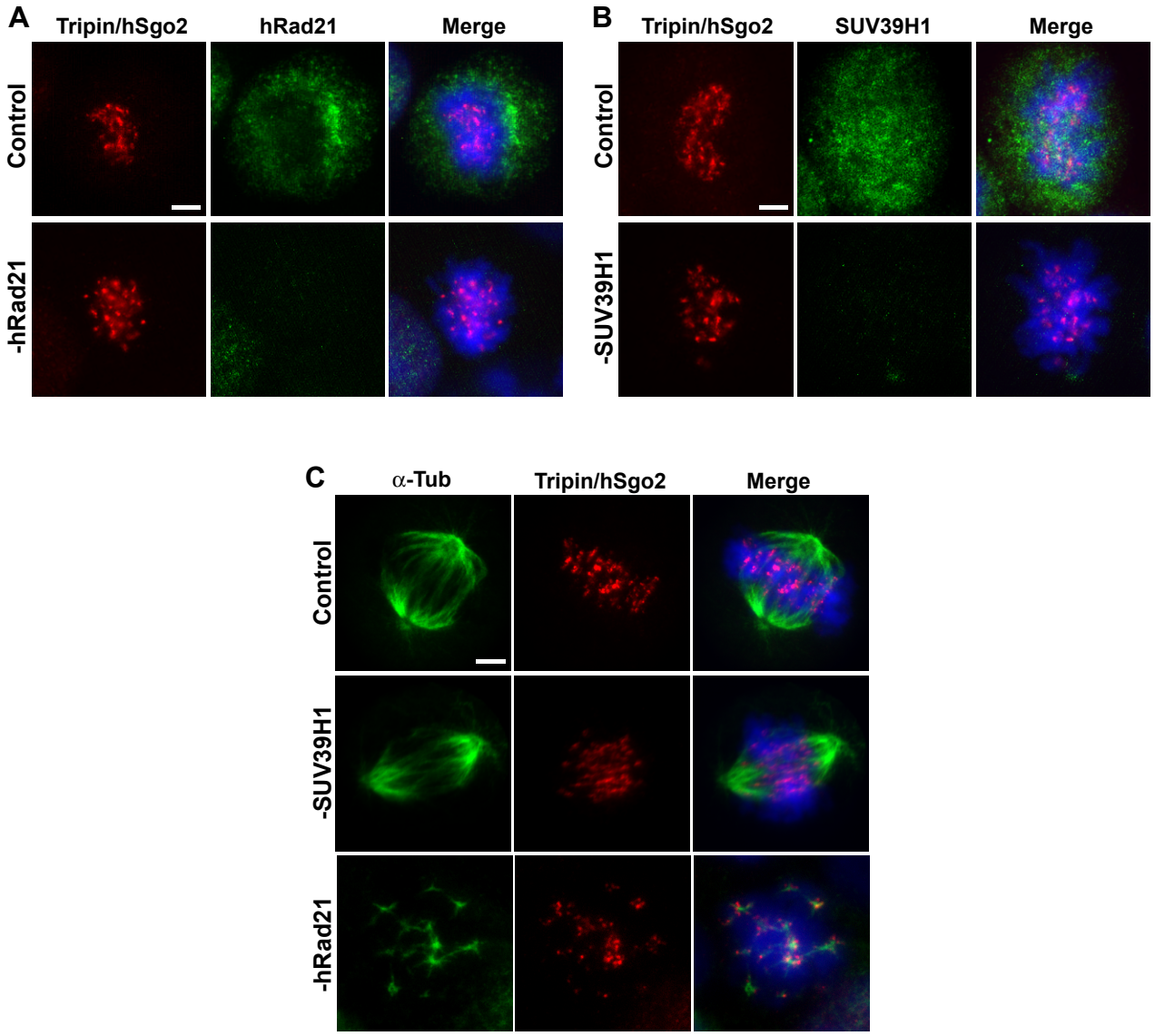


Supplemental Figure S5



Supplemental Figure S6



Supplemental Figure S7