

Supplementary Figure

Meiosis-specific cohesin complexes display essential and distinct roles in mitotic embryonic stem cell chromosomes

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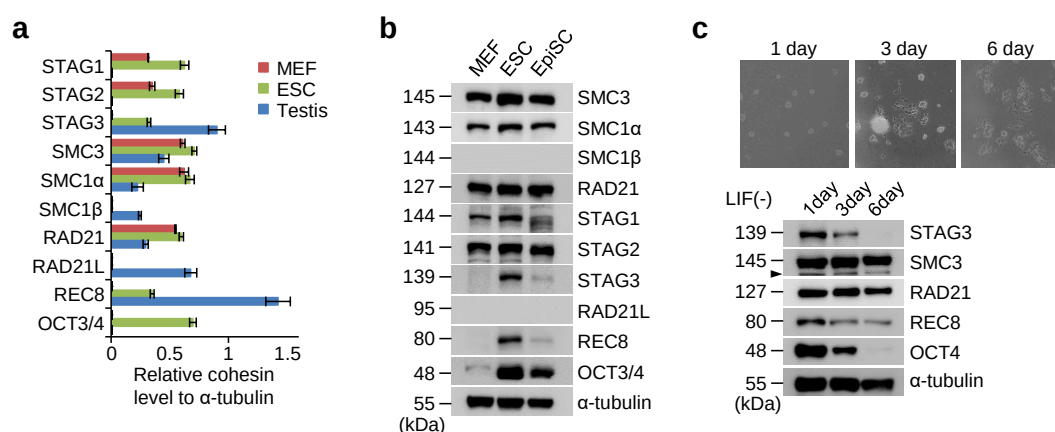


Fig. S1. Expression analysis of cohesin components.

a Quantification of expression level of cohesin factors in MEFs, ESCs, and mouse testis. The error bars are the mean $\pm$ SD from three independent experiments. **b** Expression analysis of cohesin components in MEFs, ESCs, and Epiblast stem cells (EpiSC). α -tubulin served as a protein loading control. **c** Expression of cohesin components during ESC differentiation. ESCs were differentiated by withdrawing leukemia inhibitory factor (LIF) from the culture medium. The arrowhead indicates non-specific bands.

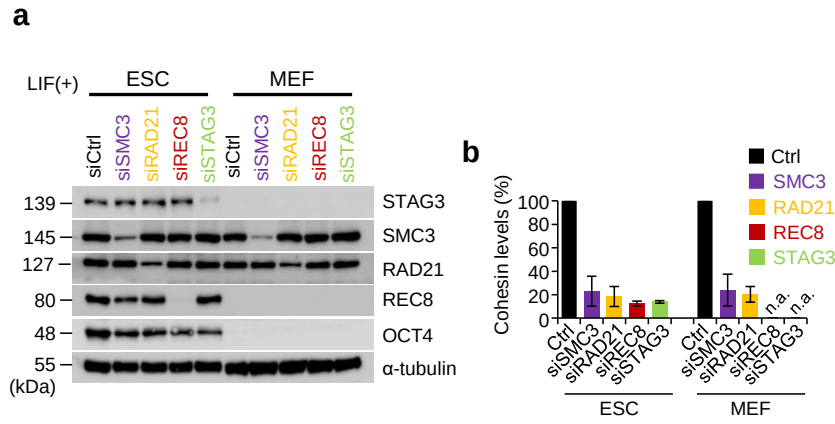


Fig. S2. Analysis of the knockdown efficiency of the cohesin components using siRNA pool.

a Knockdown of cohesin factors in ESCs and MEFs. The expression levels of SMC3, RAD21, REC8, and STAG3 were analyzed following transfection with a siRNA pool against SMC3, RAD21, REC8, and STAG3 (siSMC3, siRAD21, siREC8, and siSTAG3) by western blotting. siCtrl, the control cells with a nontargeting siRNA. α -tubulin served as a protein loading control. **b** Quantification of cohesin-knockdown efficiency. The error bars are the mean $\pm$ SD from three independent experiments.

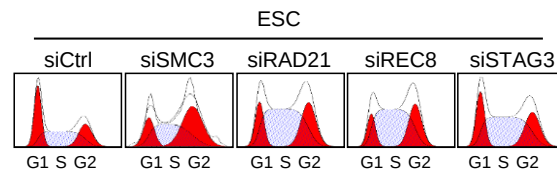


Fig. S3. Cell cycle analysis after knockdown of cohesin components.

Cell cycle profiles of ESCs treated with siSMC3, siRAD21, siREC8, and siSTAG3. Cell samples were stained with DAPI and characterized by FACS analysis.

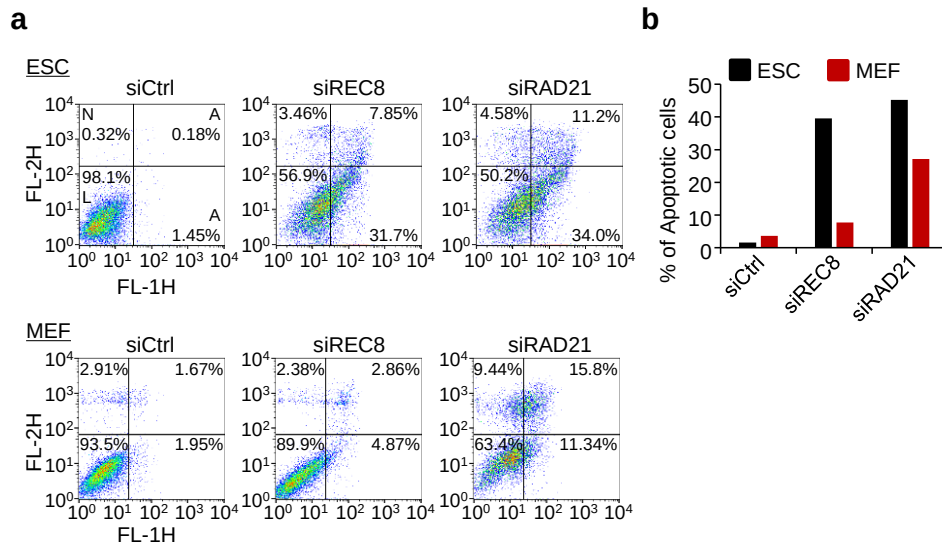


Fig. S4. Analysis of apoptosis after knockdown of RAD21 and REC8.

a Analysis of apoptosis in ESCs and MEF. The cells were incubated with siRNA in serum-free medium. The proportion of apoptotic cells was quantified with FITC-conjugated annexin V (2 μ g/ml) and PI (15 μ g/ml). Scatter plots indicate the distribution of FITC-conjugated annexin V and PI staining. The cells are classified as “live-cell” (bottom left), “early apoptotic-cell” (bottom right), “late apoptotic-cell” (top right) and “necrotic-cell” (top left).

b Quantification of apoptotic cells in ESCs and MEFs.

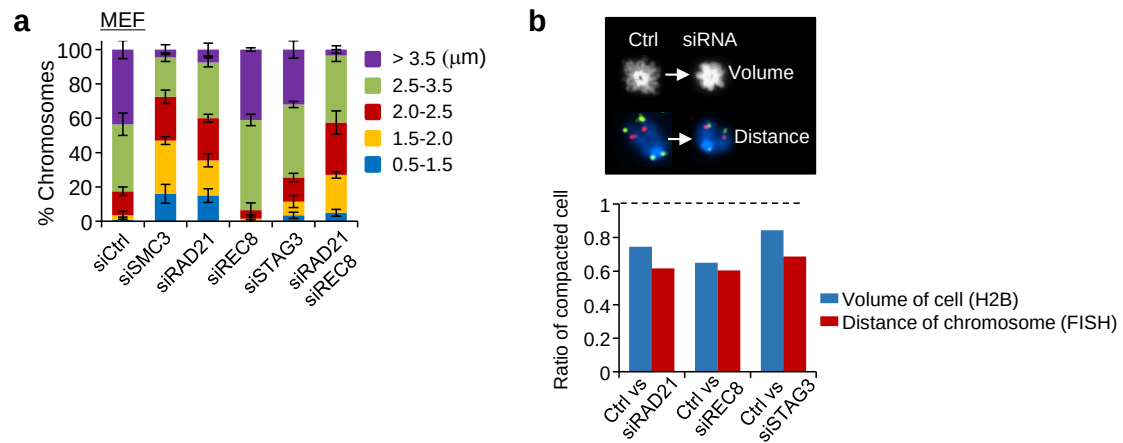


Fig. S5. Chromosome compaction in spread samples and fixed whole nuclei during metaphase.

a Quantification of chromosome lengths. The length of the chromosomes in MEF was measured by calculating the distance between both sides of the telomere probes. The error bars are the mean $\pm$ SD from three independent experiments. **b** Quantification of chromosome volume in the fixed whole nuclei (H2B) and distance in spread samples hybridized with telomeric probes and locus-specific probes. The volume and distance from metaphase chromosomes in cells depleted of mitotic/meiotic cohesin components showed similar reductions in proportion.

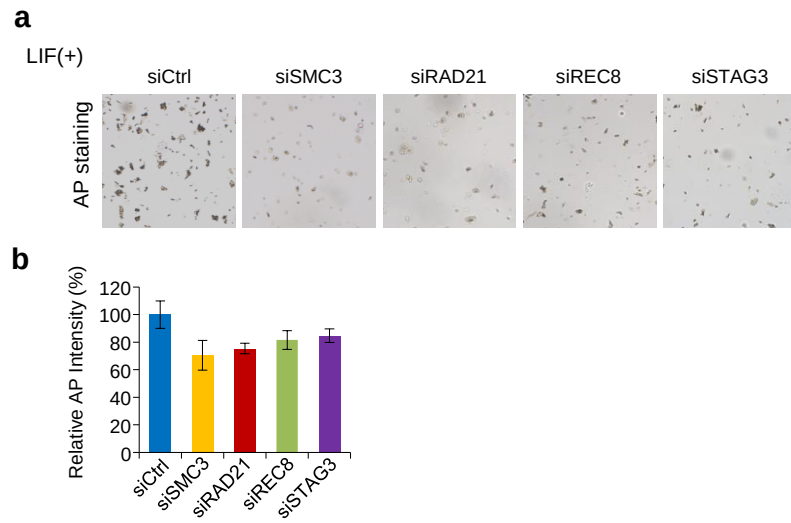


Fig. S6. Depletion of cohesin components leads to ESC differentiation.

a Analysis of alkaline phosphatase staining following transfection with a siRNA pool against SMC3, REC8, RAD21, and STAG3 in ESCs. **b** Quantification of alkaline phosphatase staining. The percentage of AP-positive colonies is shown on bar graphs. The error bars are the mean $\pm$ SD from three independent experiments.

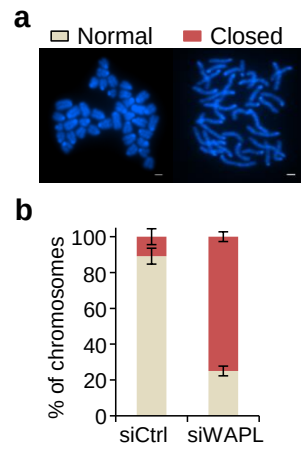


Fig. S7. Change in chromosome structure after WAPL knockdown.

a Representative images showing normal and closed chromosomes in ESCs. Scale bars are 2.5 μm . **b** Chromosome spreads of ESCs, including normal and closed chromosomes. The error bars are the mean $\pm$ SD from three independent experiments.

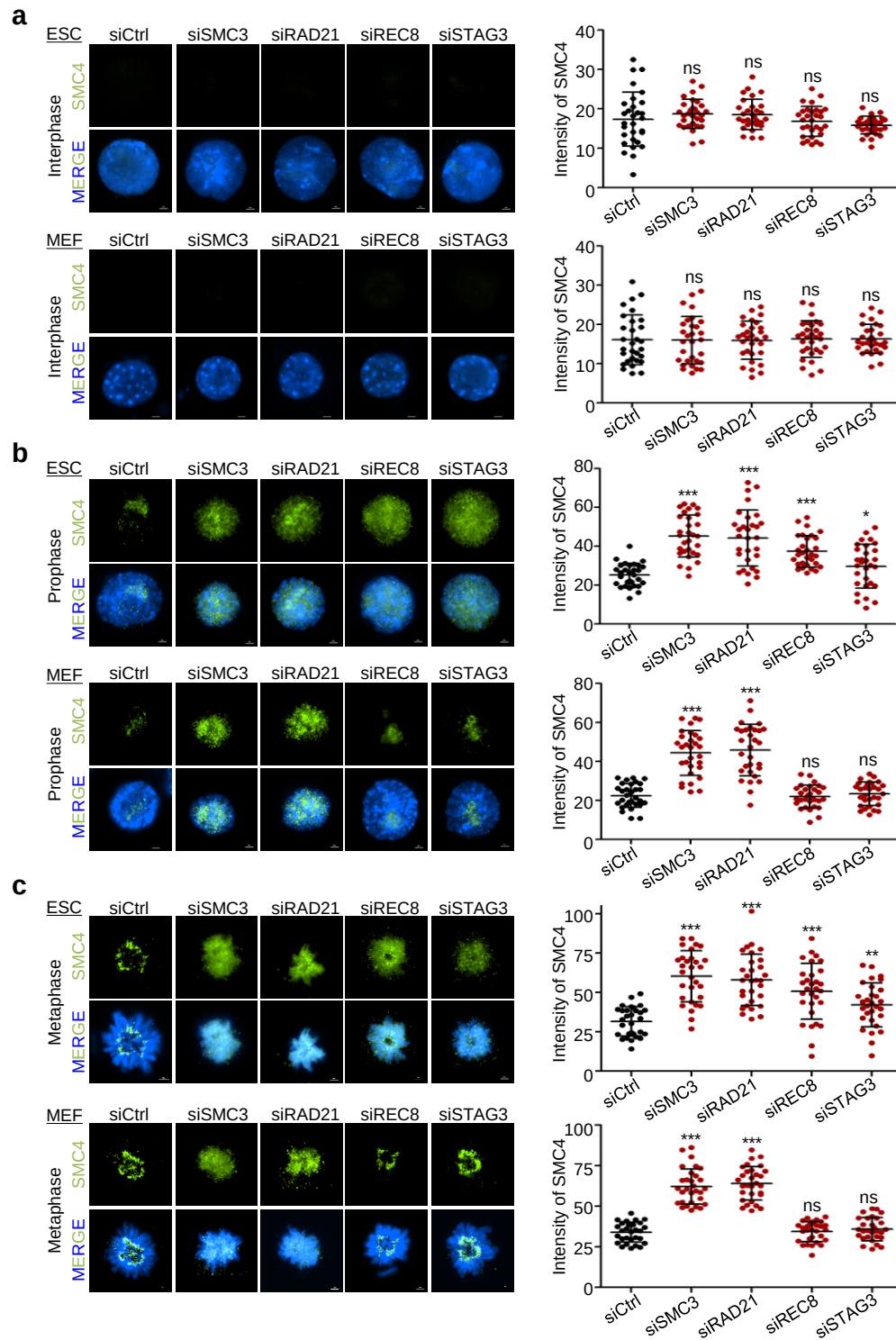


Fig. S8. Localization pattern of condensin from interphase to metaphase in ESC and MEF.

a–c Analysis of condensin intensity from interphase to metaphase in ESCs and MEF. Cells were immunostained with an antibody against SMC4 and counterstained them with DAPI. Scale bars are 2.5 μ m. Staining intensity was analyzed using the Nikon NIS software. P-values (paired two-tailed t-test) were calculated using GraphPad Prism 5 software. ns: not-significant, * $P < 0.5$, ** $P < 0.01$, and *** $P < 0.001$.

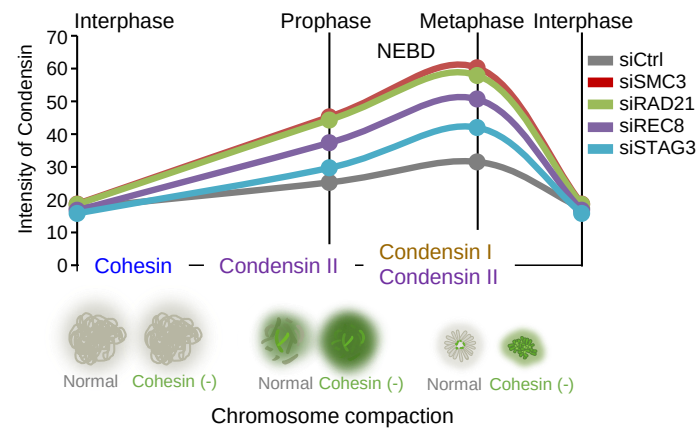
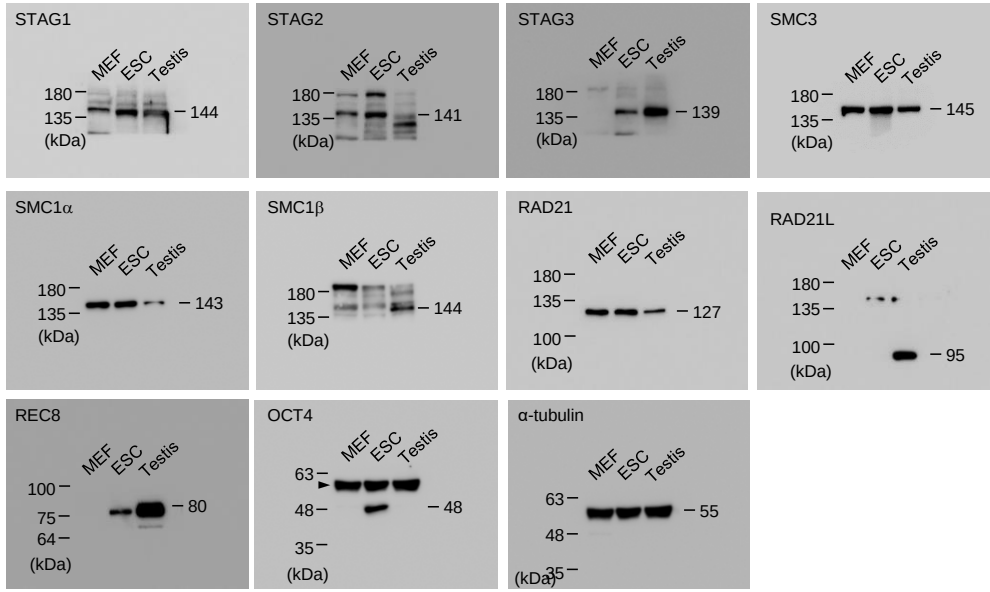
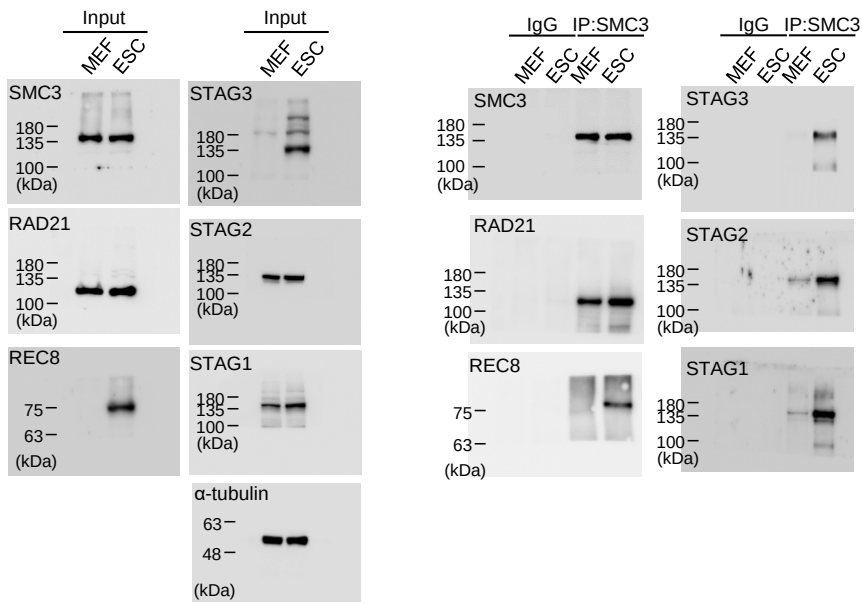
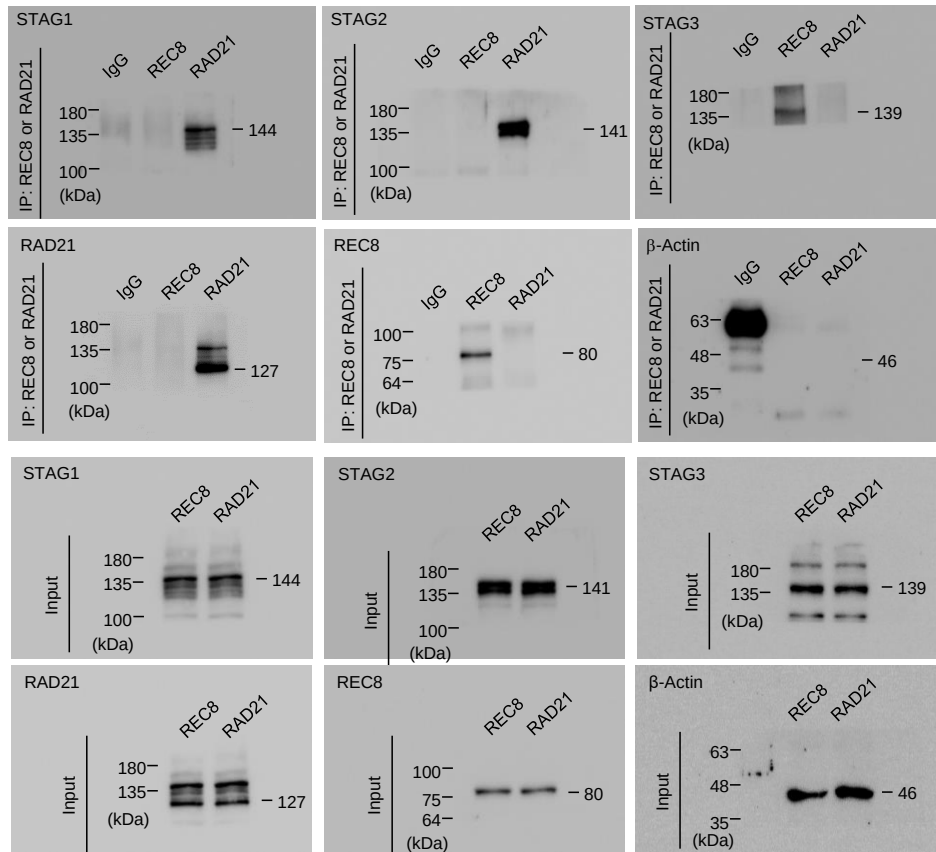
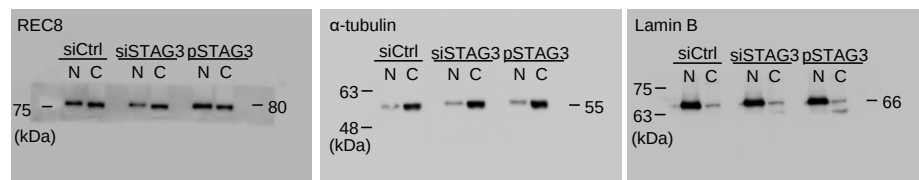


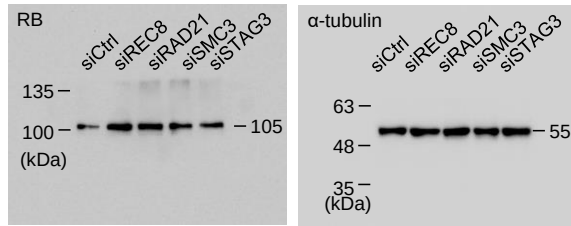
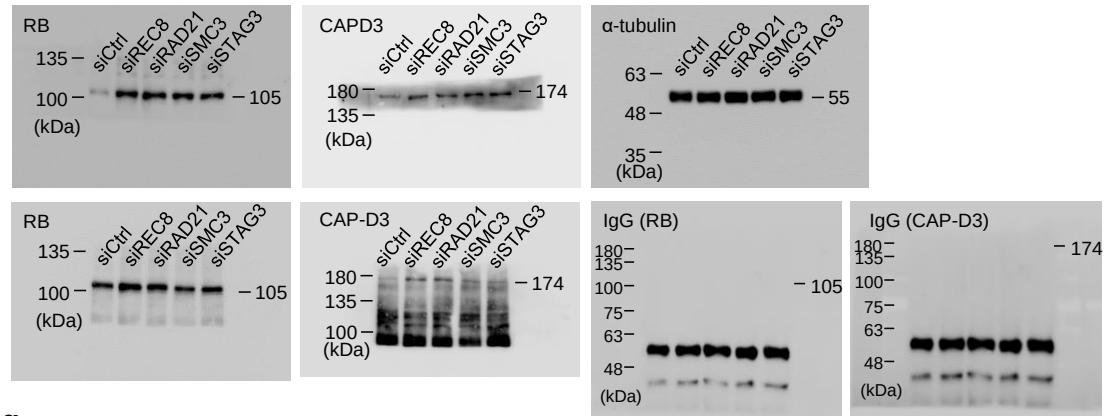
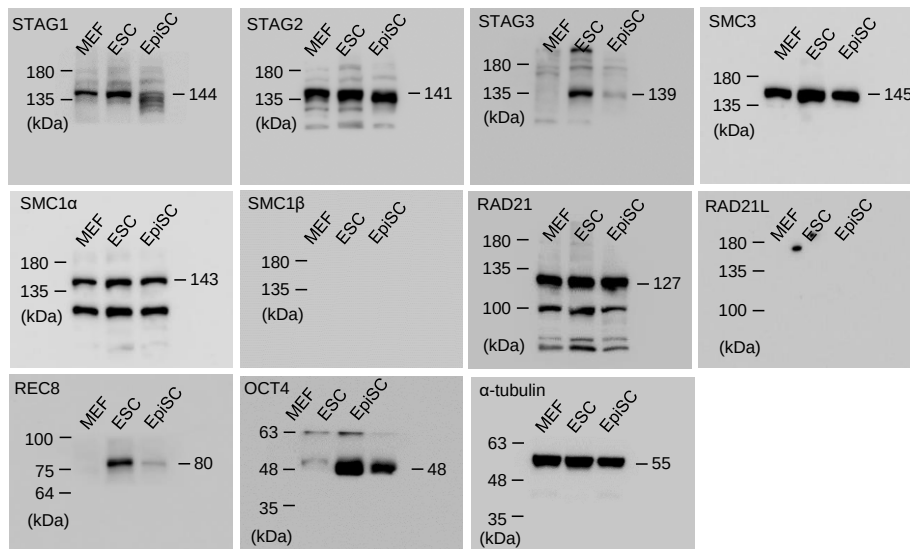
Fig. S9. Changes in condensin intensity during the cell cycle of ESC and MEF.

Analysis of condensin intensity at interphase, prophase, and metaphase in ESC. Intensity values of condensin were quantified from supplementary figure 8. NEBD, Nuclear envelope breakdown.

a

**b**

c**d**

e**f****g**

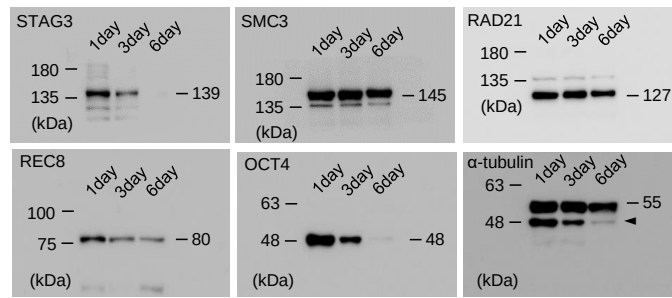
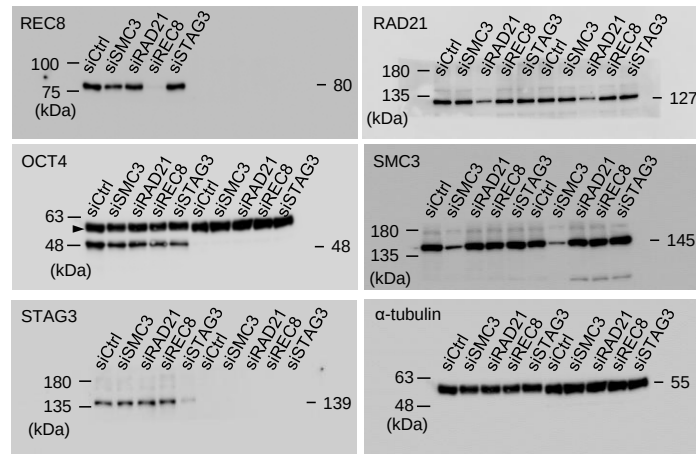
h**i**

Fig. S10. Uncropped western blot gel images. **a** Corresponds to Fig. 1b; **b** Corresponds to Fig. 1d; **c** Corresponds to Fig. 1i; **d** Corresponds to Fig. 1k; **e** Corresponds to Fig. 4c; **f** Corresponds to Fig. 4e; **g** Corresponds to Fig. S1b; **h** Corresponds to Fig. S1c; **i** Corresponds to Fig. S2a. The arrowhead indicates non-specific bands.