Figure S1

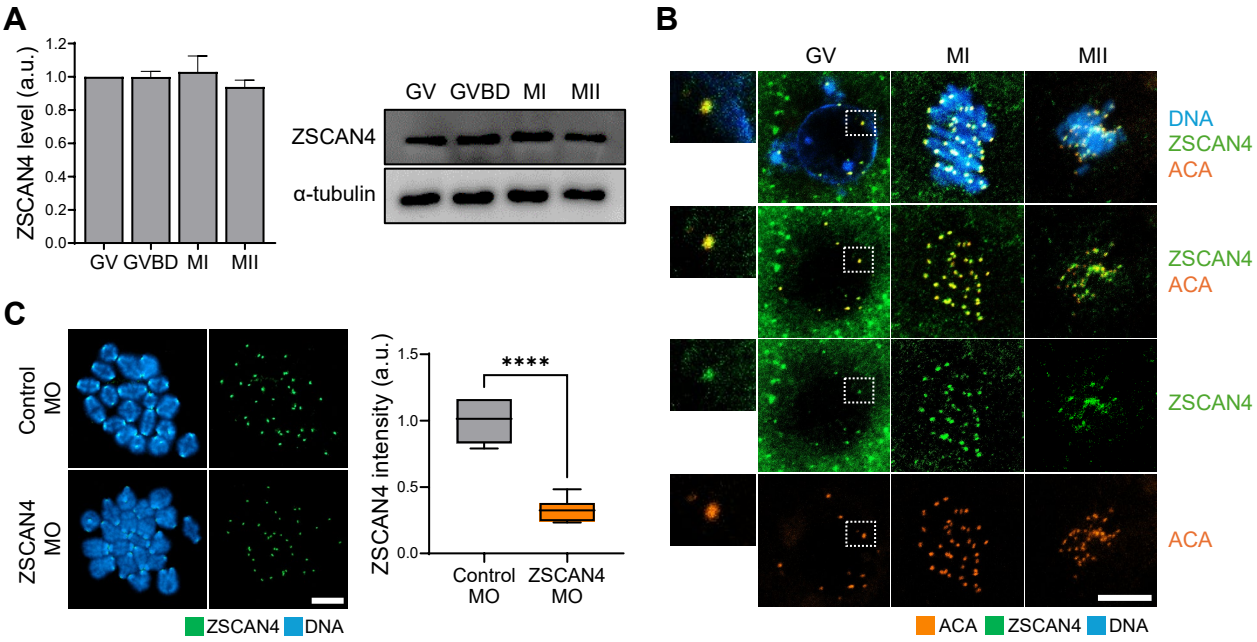

Figure S1. Expression and localization of ZSCAN4 in mouse oocytes. (A) Immunoblot analysis of ZSCAN4 expression during meiotic maturation. Oocytes were collected after 0, 2, 8, and 16 h of culture, corresponding to the GV, GVBD, MI, and MII stages, respectively, and subjected to immunoblot analysis with ZSCAN4 antibody. Each lane includes 50 oocytes, and α-tubulin was used as a loading control. Data are presented as the mean ± SEM of two independent experiments. (B) Subcellular localization of ZSCAN4 during mouse oocyte meiotic maturation. Immunofluorescence staining was performed using ZSCAN4 and ACA antibodies. Scale bar, 10 μm. (C) Representative images of chromosome spreads at the MI stage, stained with ZSCAN4 antibody. Oocytes were injected with control MO or ZSCAN4 MO for ZSCAN4 depletion. Scale bar, 10 μm. Quantification of ZSCAN4 intensity. Data are presented as the mean ± SEM of three independent experiments. ****p<0.00001.

Figure S2

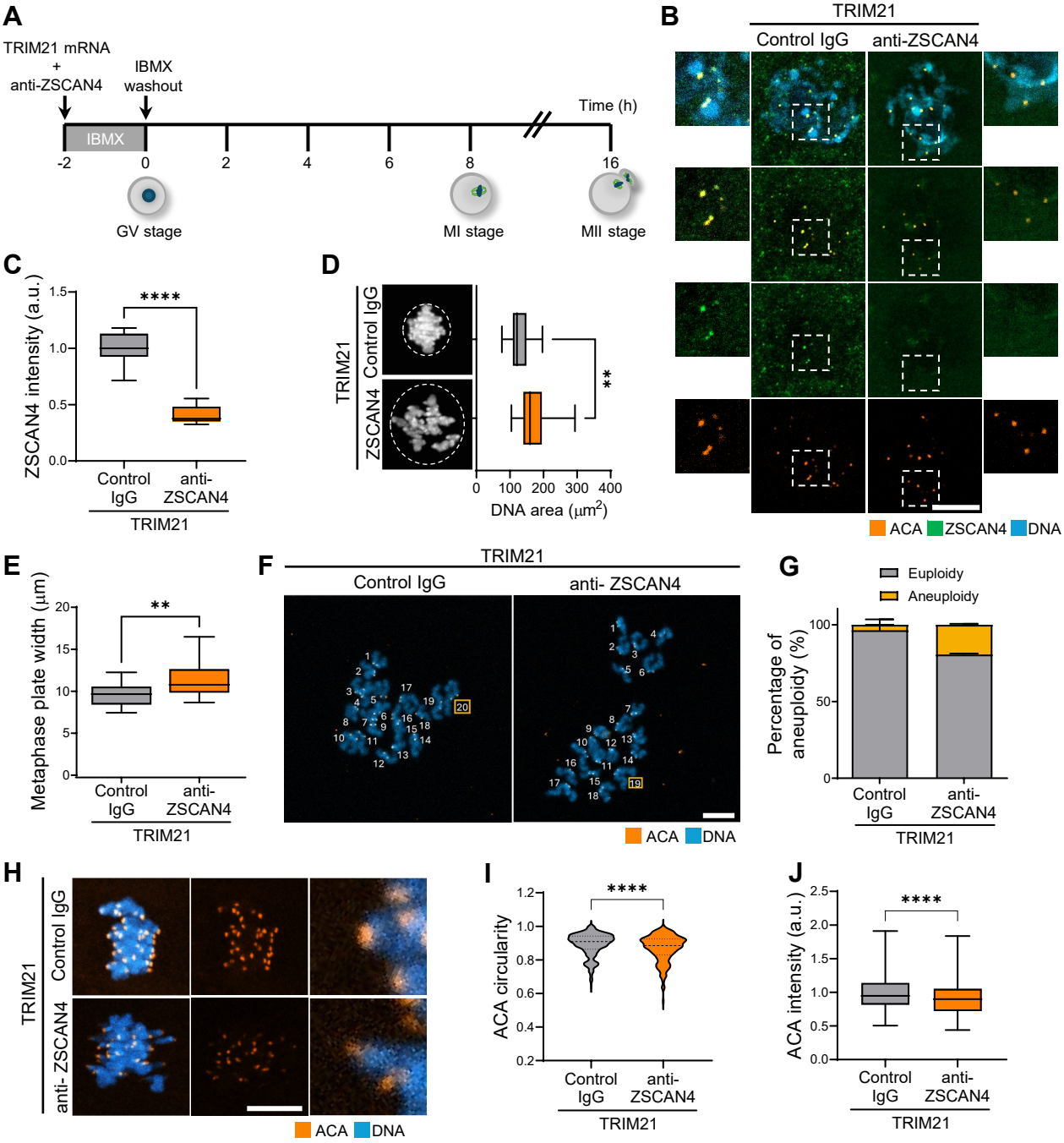

Figure S2. Trim-Away-mediated depletion of ZSCAN4 in mouse oocytes. (A) Schematic diagram depicting the Trim-Away approach for depleting ZSCAN4. GV oocytes were injected with TRIM21 mRNA and ZSCAN4 antibody; control oocytes were injected TRIM21 mRNA and IgG. After 2 h recovery in IBMX-supplemented medium, oocytes were released from IBMX and cultured for 8 h and 16 h to reach the MI and the MII stage, respectively. (B) Representative images of control and ZSCAN4-depleted GV oocytes stained with ZSCAN4 and ACA antibodies. Scale bar, 10 μm. (C) Quantification of ZSCAN4 intensity. Data are presented as the mean ± SEM of two independent experiments. ****p<0.00001. (D) Representative images and quantification of DNA area at the MI stage. DNA area was measured as the area of a circle that includes all the aligned chromosomes. Data are presented as the mean ± SEM of two independent experiments. **p<0.001. (E) Quantification of metaphase plate width. Data are presented as the mean ± SEM of two independent experiments. **p<0.001. (F) Representative images of chromosome spread stained with ACA antibody in control and ZSCAN4-depleted oocytes. (G) Quantification of percentage of aneuploidy. Data are presented as the mean ± SEM of two independent experiments. (H) Representative images of control and ZSCAN4-depleted oocytes stained with ACA antibody. (I-J) Quantification of ACA circularity and ACA intensity. Data are presented as the mean ± SEM of three independent experiments. ****p<0.00001.

Figure S3

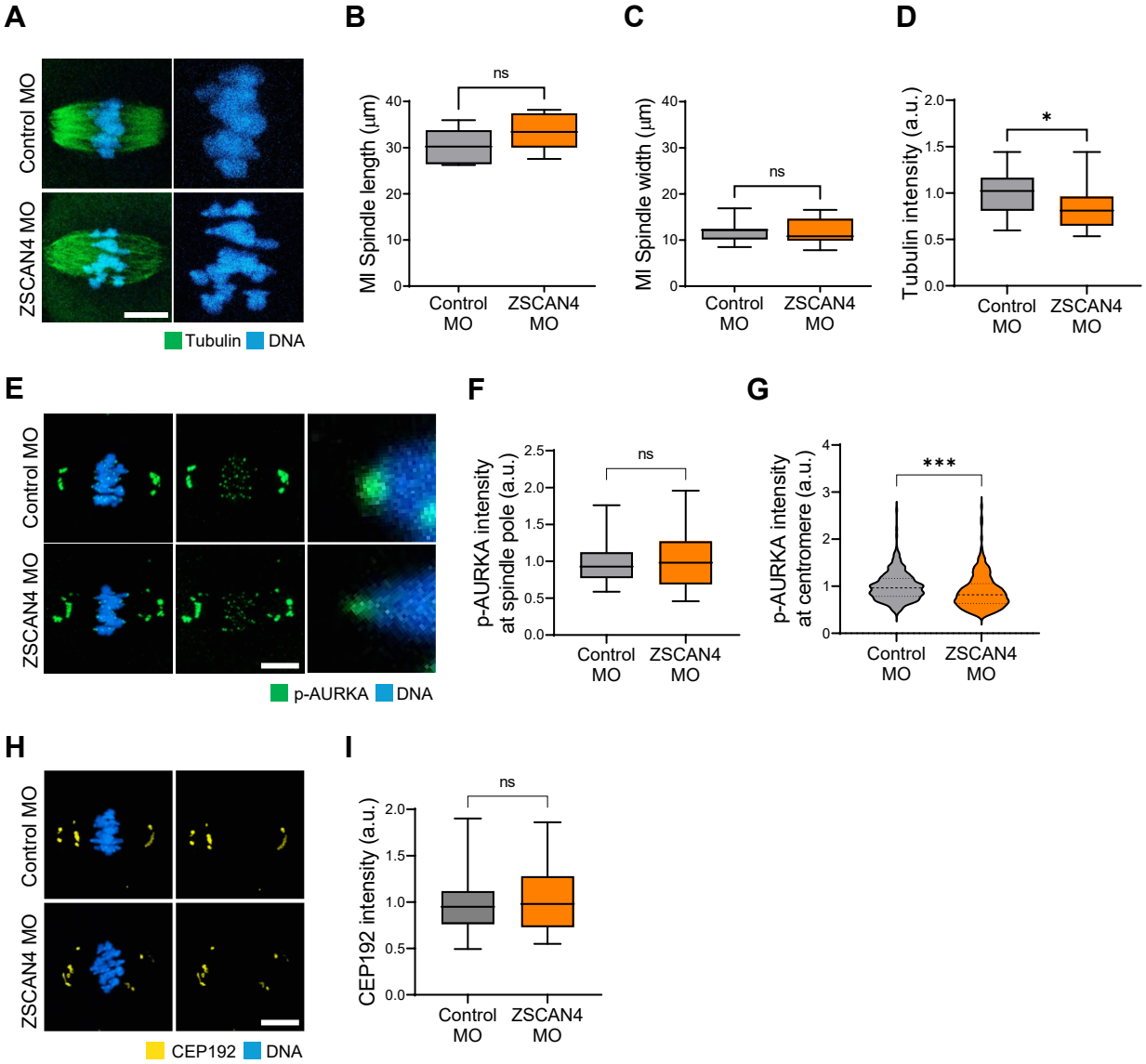

Figure S3. No discernible difference in spindle morphology and pole integrity after ZSCAN4 depletion. (A) Representative images of control and ZSCAN4-depleted oocytes stained with acetylated alpha-tubulin antibody. Scale bar, 10 μm. (B–D) Quantification of spindle length, width, and intensity of control and ZSCAN4-depleted oocytes at the MI stage. Spindle length was measured as the distance between the two spindle poles, and spindle width was measured as the widest part of the barrel-shaped spindle. Data are presented as the mean ± SEM of three independent experiments. ns, not significant. *p<0.05. (E) Representative images of control and ZSCAN4-depleted oocytes stained with phosphorylated aurora kinase A (p-AURKA) antibody. Scale bar, 10 μm. (F–G) Quantification of p-AURKA intensity at the spindle pole and centromere, respectively. Data are presented as the mean ± SEM of two independent experiments. ns, not significant. ***p<0.0001. (H) Representative images of control and ZSCAN4-depleted oocytes stained with CEP192 antibody. Scale bar, 10 μm. (I) Quantification of CEP192 intensity at the spindle pole. Data are presented as the mean ± SEM of two independent experiments. ns, not significant.

Figure S4

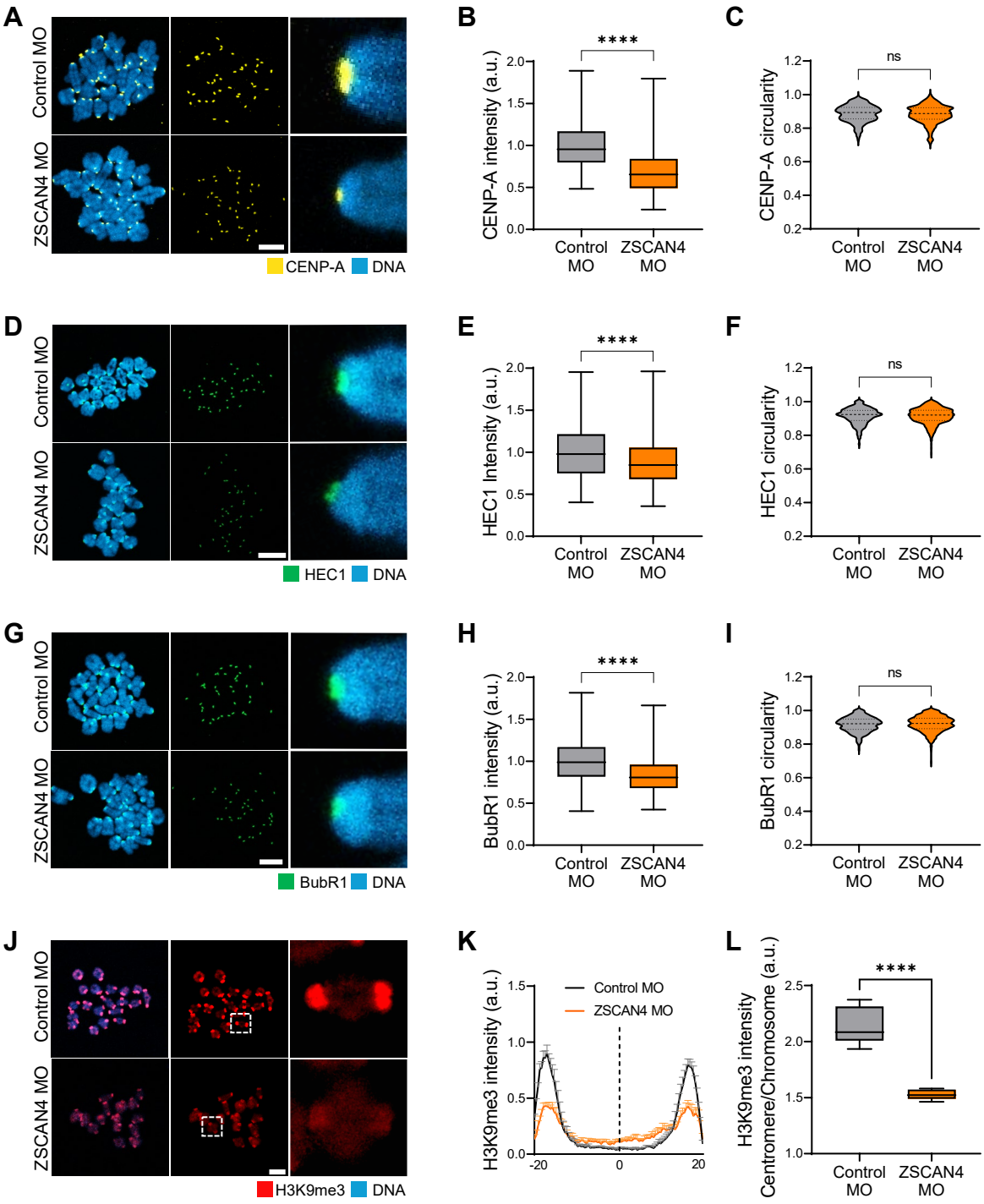

Figure S4. Decreases in CENP-A, HEC1, BubR1, and H3K9me3 after ZSCAN4 depletion. (A, D, G) Representative chromosome spread images of control and ZSCAN4-depleted oocytes stained with CENP-A (or HEC1 or BubR1) at the MI stage. Scale bar, 10 μm. (B, E, H) Quantification of CENP-A (or HEC1 or BubR1) intensity. Data are presented as the mean ± SEM of two independent experiments. ****p<0.00001. (C, F, I) Quantification of CENP-A (or HEC1 or BubR1) circularity. Data are presented as the mean ± SEM of two independent experiments. ns, not significant. (J) Representative chromosome spread images of control and ZSCAN4-depleted oocytes stained with H3k9me3 antibody. Scale bar, 10 μm. (K) Plot graph depicting H3K9me3 intensity from one chromosome end (-20) to the opposite end (20). Ten representative chromosomes from control and ZSCAN4-depleted oocytes were selected to depict the plot. (L) Quantification of the ratio intensity of H3k9me3 at centromeres over each chromosome (centromere/chromosome). Data are presented as the mean ± SEM of two independent experiments. ****p<0.00001.

Figure S5

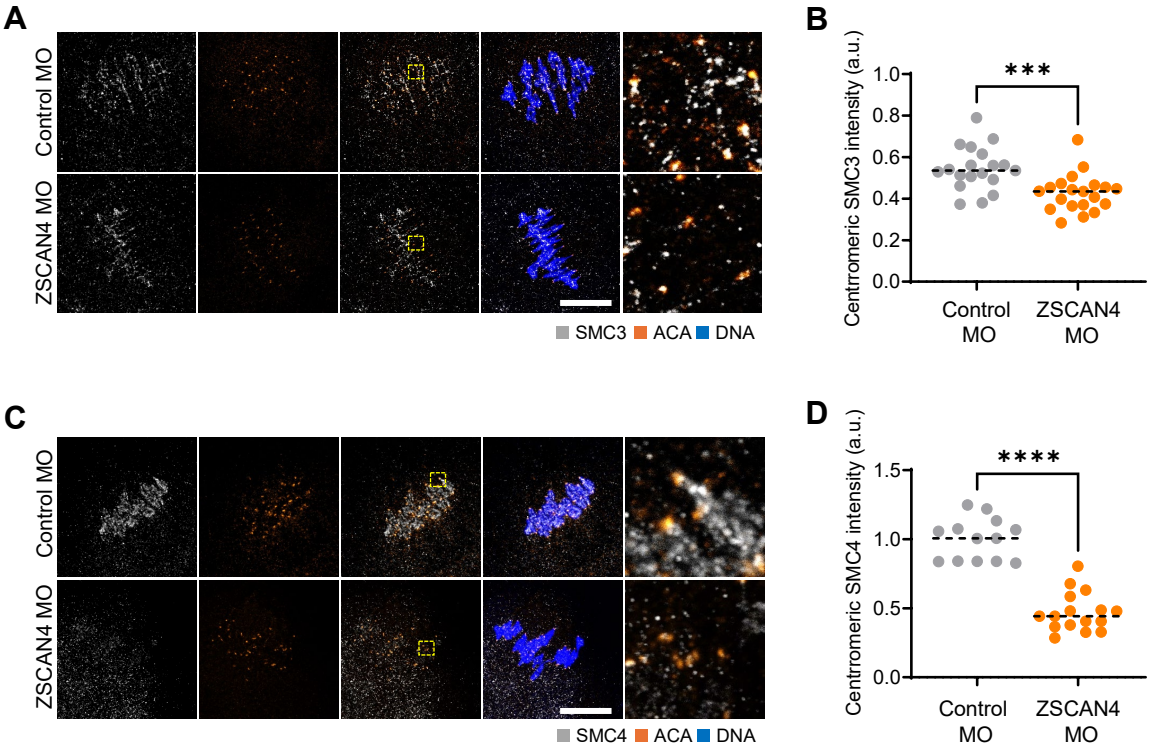

Figure S5. Decreases in SMC3 and SMC4 levels after ZSCAN4 depletion. (A, C) Representative images of control and ZSCAN4-depleted oocytes stained with SMC3 or SMC4 antibody. Regions outlined by yellow box are enlarged and shown in right panel. Scale bar, 10 μm. (B, D) Quantification of SMC3 or SMC4 intensity. Data are presented as the mean ± SEM of two independent experiments. ***p<0.0001, ****p<0.00001.

Figure S6

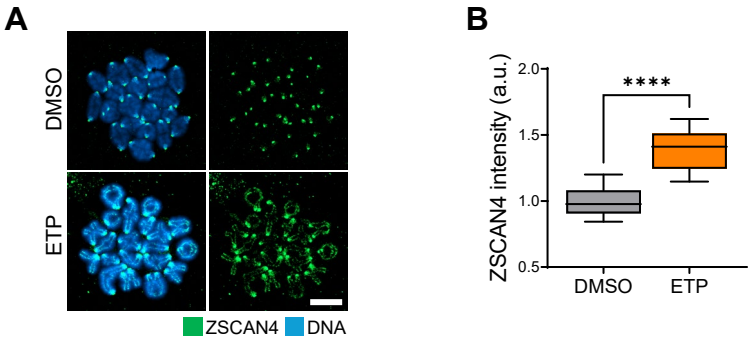

Figure S6. Increases in ZSCAN4 levels after DNA damage induction. (A) Representative images of chromosome spread stained with ZSCAN4 and ACA antibodies in DMSO- or ETP-treated oocytes. Scale bar, 10 μm. (B) Quantification of ZSCAN4 intensity. Data are presented as the mean ± SEM of three independent experiments. ****p<0.00001.

Figure S7

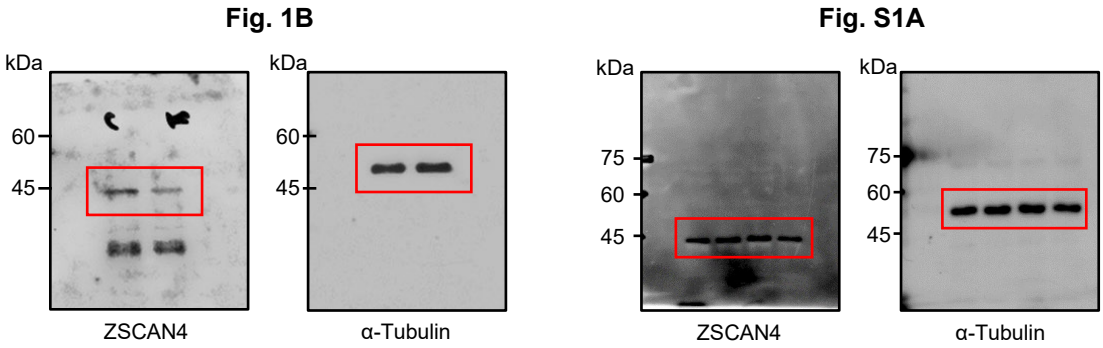

Figure S7. Full-length immunoblot images. Uncropped full-length images of Western blotting membranes presented in Figure 1B and the Supplementary Figure S1A.