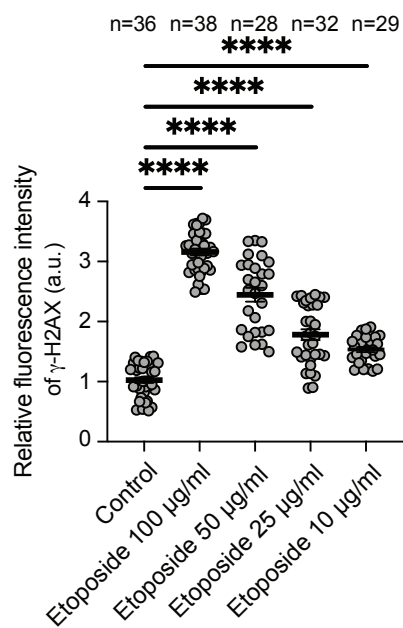
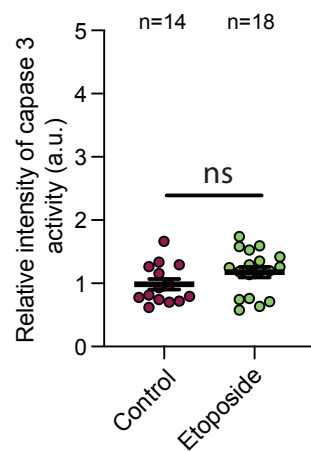


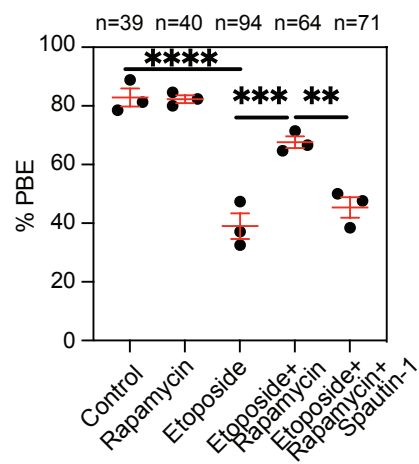
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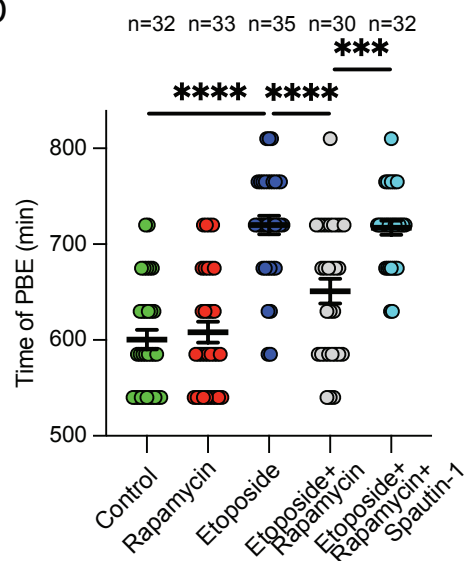
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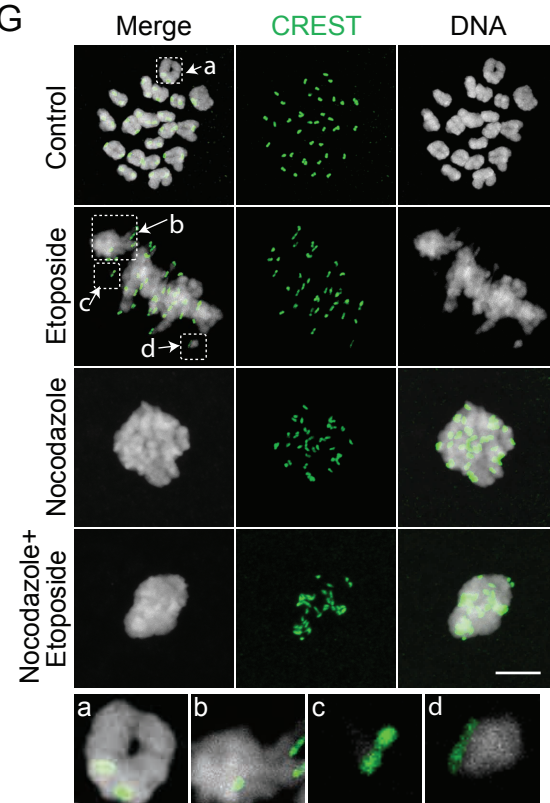
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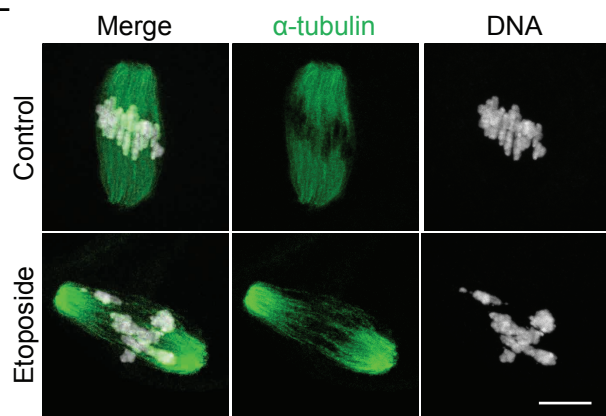
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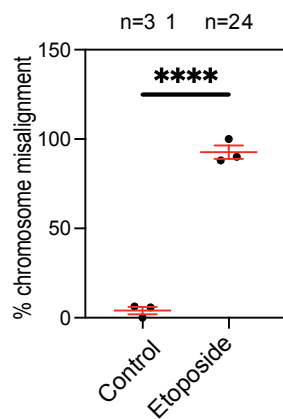
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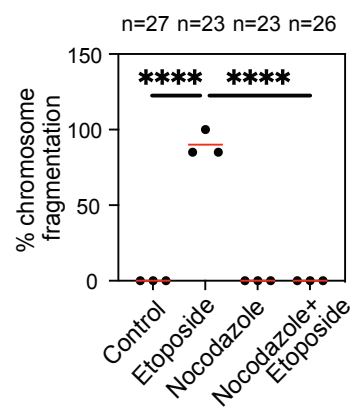
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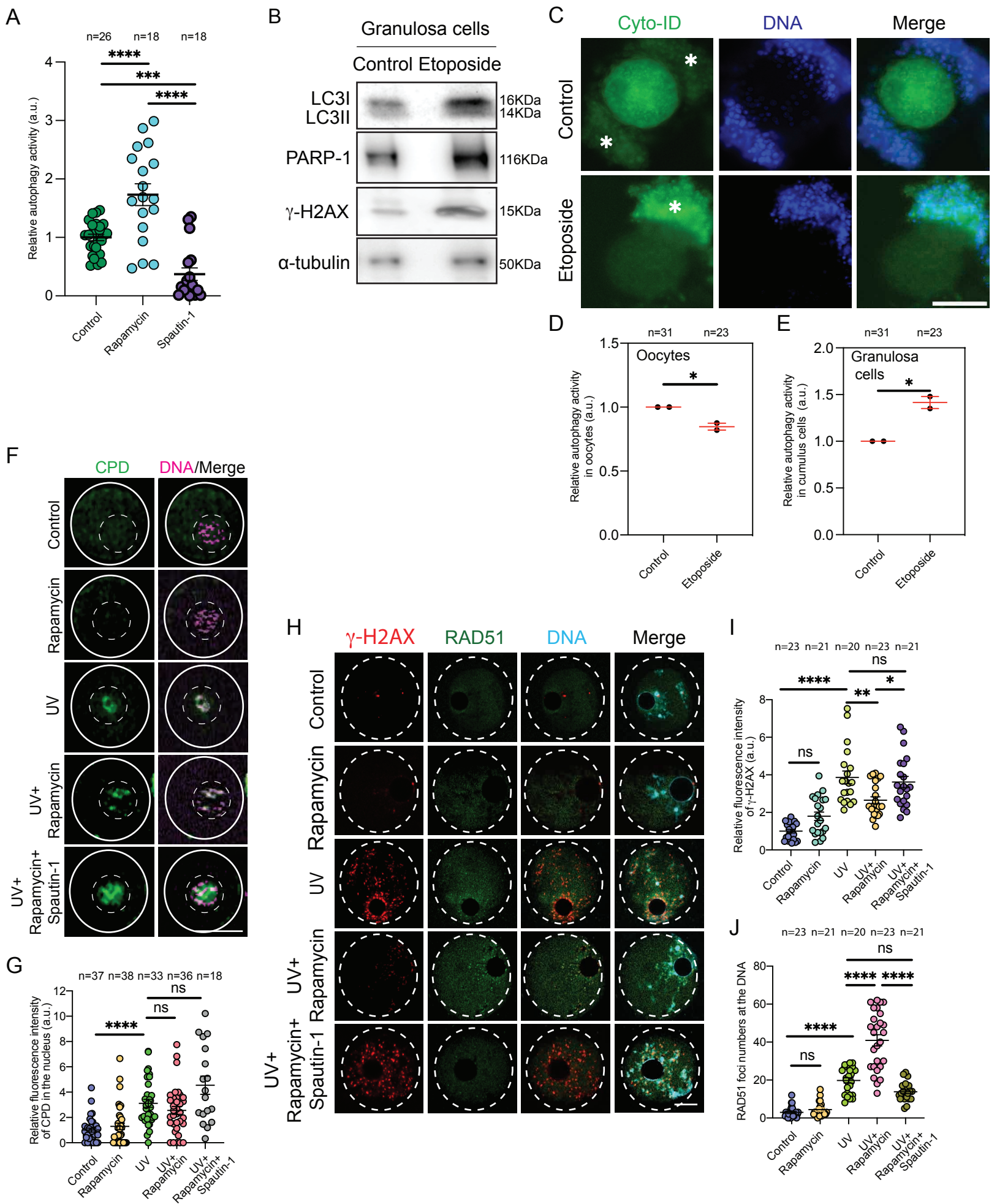
F



H

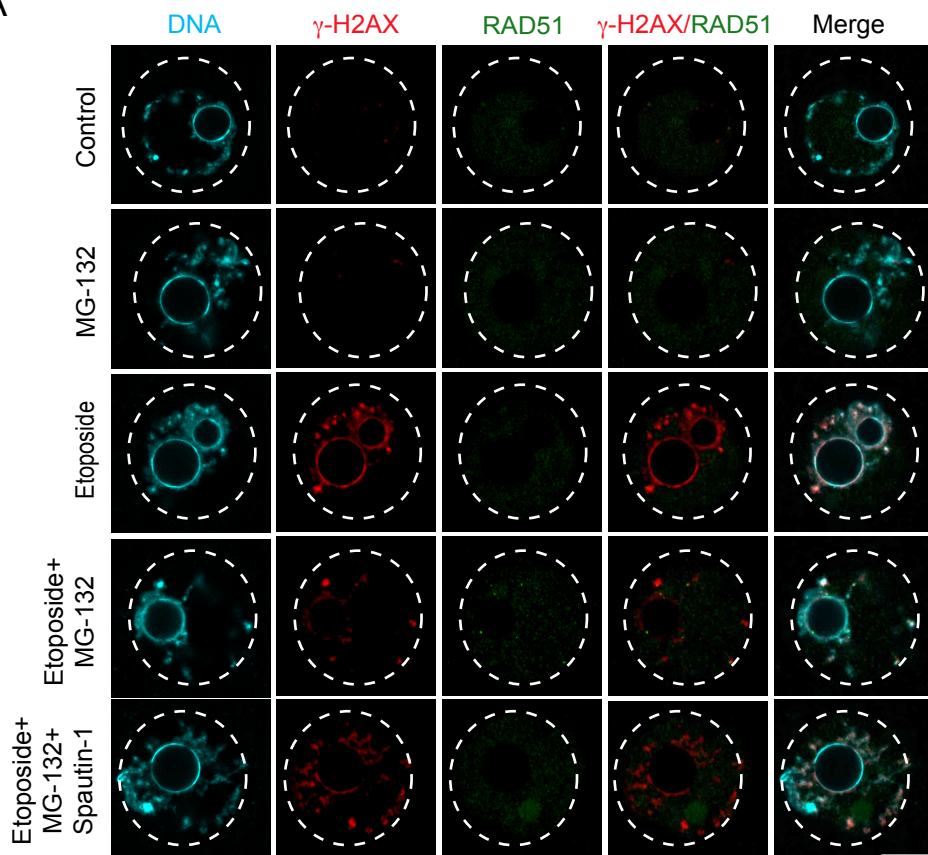


Supplementary Figure 1: Effect of DNA double-strand break induction on oocyte meiotic progression. (A) Full-grown germinal vesicle (GV) oocytes were incubated for 3 h in milrinone-containing CZB medium supplemented with etoposide at the indicated concentrations. Oocytes were fixed and immunostained with γ H2AX antibody. Quantification of γ H2AX fluorescence intensity is shown. (B) Metaphase I oocytes were stained with Caspase 3 (DEVD)₂ detection kit to detect caspase 3 activity. Quantification of caspase 3 activity fluorescence intensity is shown. (C,D) Full-grown GV oocytes were incubated with the indicated treatment in milrinone-containing CZB medium for 3 h followed by washing and time-lapse imaging during *in vitro* maturation in CZB medium. (C) Quantification of first polar body extrusion (PBE) percentage at 16 h after maturation. (D) Quantification of PBE timing. (E) Full-grown GV oocytes were incubated with etoposide for 3 h in milrinone-containing CZB medium followed by washing and *in vitro* maturation for 7 h (metaphase I) in CZB medium. Metaphase I oocytes were fixed and immunostained with anti- α -tubulin antibody. DNA was stained with DAPI. (F) Quantification of chromosome misalignment percentage in E. (G) Full-grown prophase I oocytes were incubated with DMSO (control) or etoposide for 3 h in milrinone containing CZB medium followed by washing and *in vitro* maturation for 7 h (metaphase I) in CZB medium with or without nocodazole. Metaphase I oocytes were fixed and immunostained with CREST antibody to label kinetochores. White arrows represent intact (a,b) or fragmented (c,d) chromosomes. DNA was stained with DAPI. (H) Quantification of chromosome fragmentation percentage in G. Scale bars represent 10 μ m. Data are expressed as mean $\pm$ SEM. Student-t-test (B and F) and One-way ANOVA was used to analyze the data. Values with asterisks differ significantly, **p < 0.01, ***p < 0.001, ****p < 0.0001. The total number of analyzed oocytes is specified above each graph. All experiments were replicated three times except G and H (four replicates).

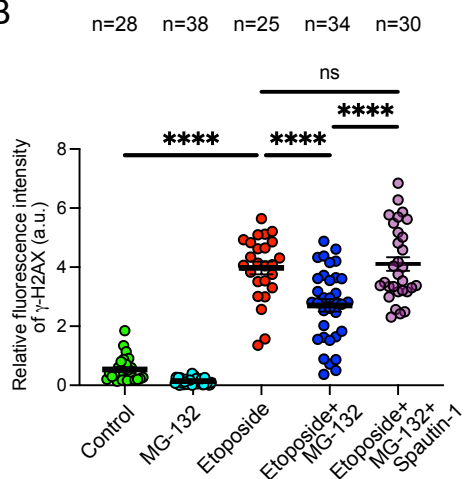


Supplementary Figure 2: Autophagy regulates DNA damage repair. (A) Full-grown germinal vesicle (GV) oocytes were incubated in milrinone-containing CZB medium supplemented with the indicated treatments for 3 h followed by assessing autophagic activity by using Cyto-ID detection kit. Relative autophagy activity quantification is shown. (B) Mouse cumulus oocyte complexes (COCs) were cultured in milrinone-containing CZB medium supplemented with DMSO (control) or etoposide for 3 h. Oocytes and cumulus granulosa cells were lysed prior to Western blot analysis by using LC3, PARP-1, γ H2AX and α -tubulin antibodies. Representative images are shown (same membrane relabeled). (C) Porcine cumulus oocyte complexes (COCs) were cultured in milrinone-containing CZB medium supplemented with DMSO (control) or etoposide for 3 h followed by assessing autophagic activity by using Cyto-ID detection kit. Quantification of relative autophagy activity in oocytes (D) and cumulus cells (E). (F) Full-grown GV oocytes were incubated with the indicated treatments for 3 h in milrinone-containing CZB medium followed by assessing cyclobutene Pyrimidine dimers (CPD). The white circle represents the oocyte, whereas the white dashed circle represents the nucleus. Scale bars represent 50 μ m (G) Quantification of CPD fluorescence intensity in F. (H) Full-grown GV oocytes within the indicated treatments were incubated in milrinone-containing CZB medium for 3 h. The oocytes were fixed at the GV stage and immunostained with γ H2AX and RAD51 antibodies. (I) Quantification of γ H2AX fluorescence intensity in H. (J) Quantification of RAD51 foci numbers at the DNA. DNA was stained with DAPI. Scale bars represent 10 μ m. Data are expressed as mean $\pm$ SEM. Student-t-test (D and E) and One-way ANOVA was used to analyze the data. Values with asterisks differ significantly, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. The total number of analyzed oocytes is specified above each graph. All experiments were replicated three times except D-E (two replicates).

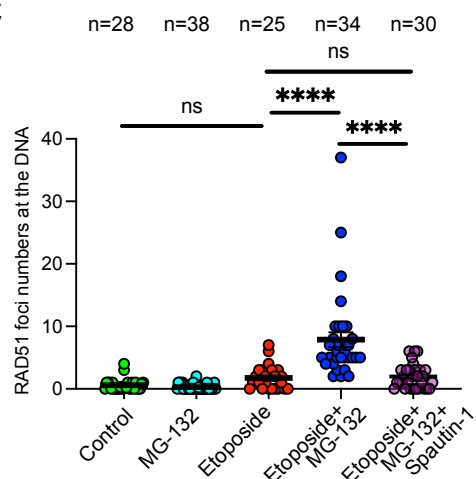
A



B

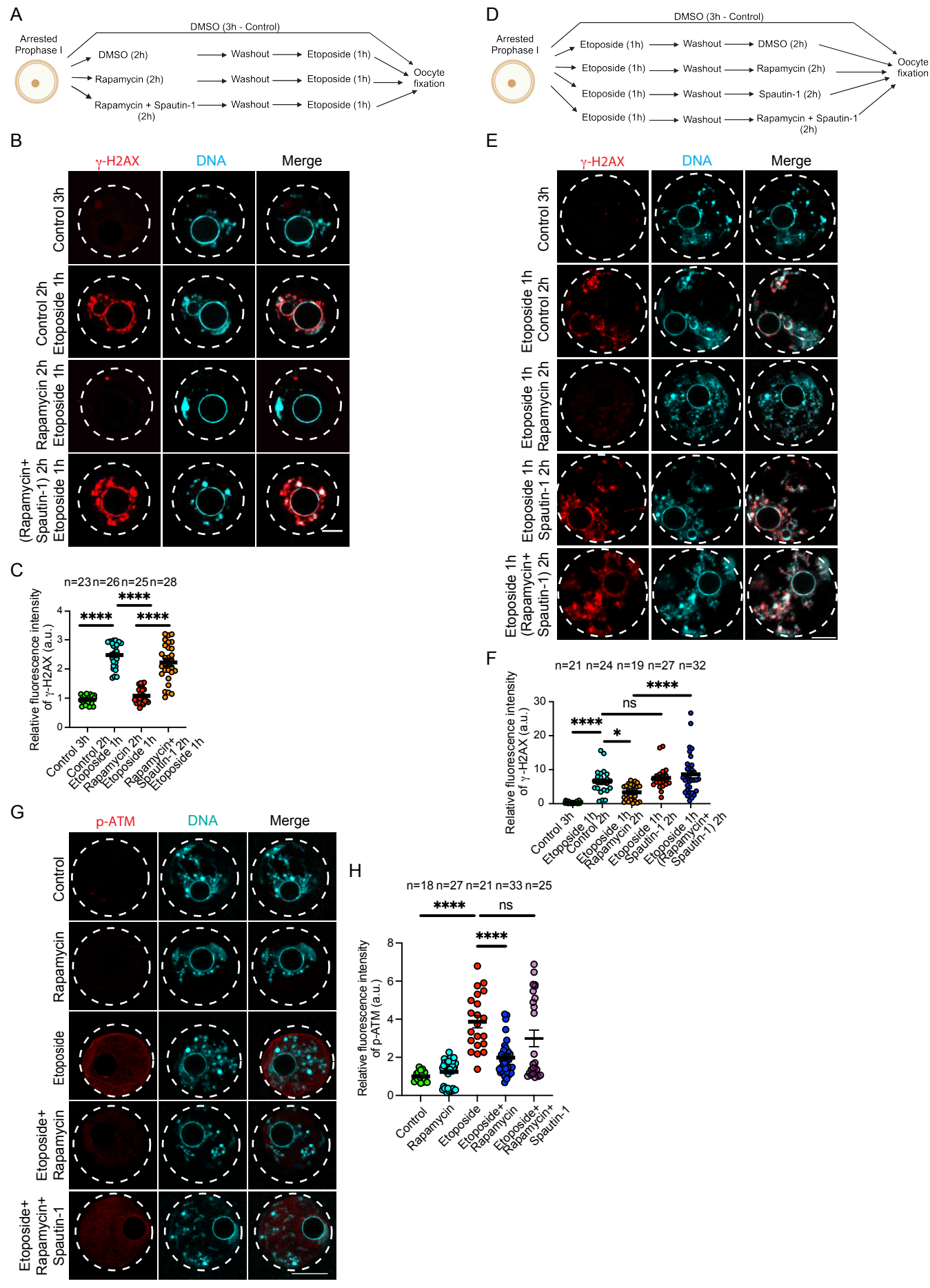


C



Supplementary Figure 3: Autophagy induction by MG-132 promotes DNA damage repair.

(A) Full-grown germinal vesicle oocytes were incubated in milrinone-containing CZB medium supplemented with the indicated treatments for 3 h. Oocytes were fixed and immunostained with γ H2AX and RAD51 antibodies. DNA was stained with DAPI. Representative images are shown. Scale bar represents 10 μ m. (B) Quantification of γ H2AX fluorescence intensity in A. (C) Quantification of RAD51 foci numbers in A. Data are expressed as mean $\pm$ SEM. One-way ANOVA was used to analyze the data. Values with asterisks differ significantly, **** $p < 0.0001$. The total number of analyzed oocytes (from three independent replicates) is specified above the graph.



Supplementary Figure 4: Autophagy has both DNA damage protective and DNA damage

repairing functions in oocytes. (A) Schematic depicting the treatment of oocytes for assessing

the protective effect of autophagy induction on DSBs. (B) Full-grown germinal vesicle (GV)

oocytes were incubated in milrinone-containing CZB medium supplemented with DMSO

(control), rapamycin or rapamycin+spautin-1. After 2 h the oocytes were incubated in etoposide-

containing medium for an additional 1 h. All oocytes were fixed and immunostained with

γ H2AX antibody. (C) Quantification of γ H2AX fluorescence intensity in B. (D) Schematic

depicting the treatment of oocytes for assessing the effect of autophagy induction on DNA

damage repair. (E) Full-grown GV oocytes were incubated in milrinone-containing CZB medium

supplemented with DMSO (control) for 3 h or etoposide for 1 h. The etoposide-treated oocytes

were then washed and cultured in DMSO, rapamycin, spautin-1 or rapamycin+spautin-1

containing medium for an additional 2 h. Oocytes were fixed and immunostained with γ H2AX

antibody. (F) Quantification of γ H2AX fluorescence intensity in E. (G) Full-grown GV oocytes

were incubated in milrinone-containing CZB medium supplemented with the indicated

treatments for 3 h. Oocytes were fixed and immunostained with p-ATM antibody. (H)

Quantification of p-ATM fluorescence intensity in G. DNA was stained with DAPI. Scale bar

represents 10 μ m. Representative images are shown. Data are expressed as mean $\pm$ SEM. One-

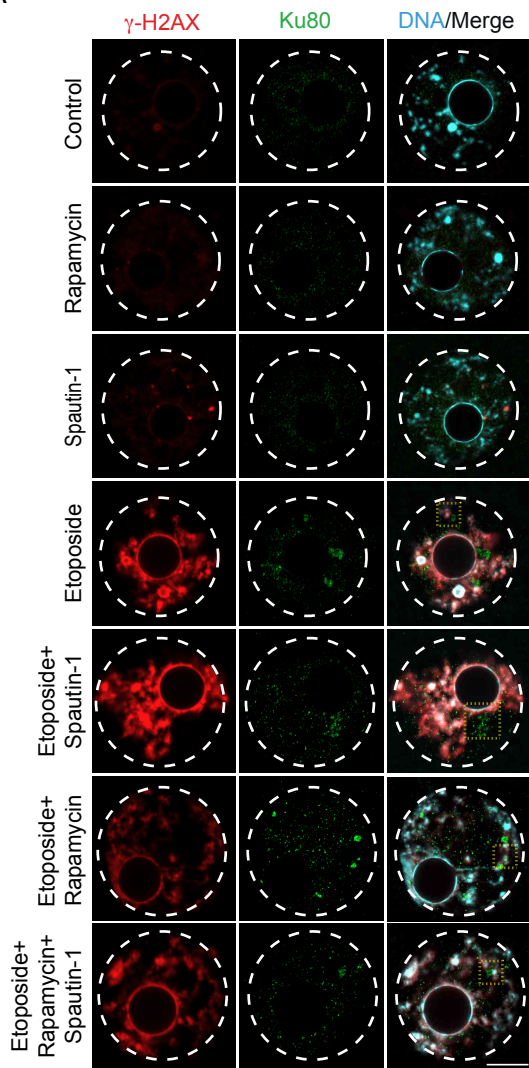
way ANOVA was used to analyze the data. Values with asterisks differ significantly, * $p < 0.05$,

**** $p < 0.0001$. The total number of analyzed oocytes (from three independent replicates) is

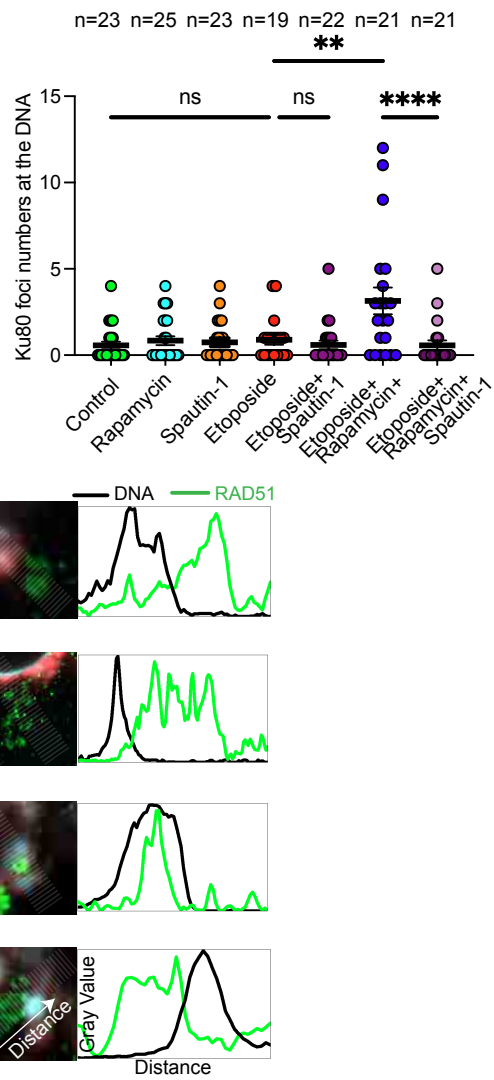
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BioRender.com/j741149.

A



B

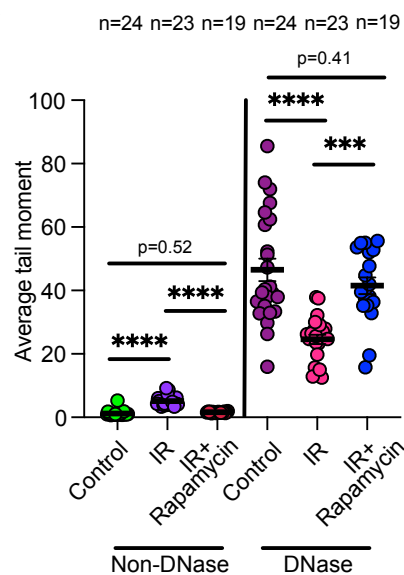


Supplementary Figure 5: Autophagy promotes Ku80 recruitment to the DNA in DNA-

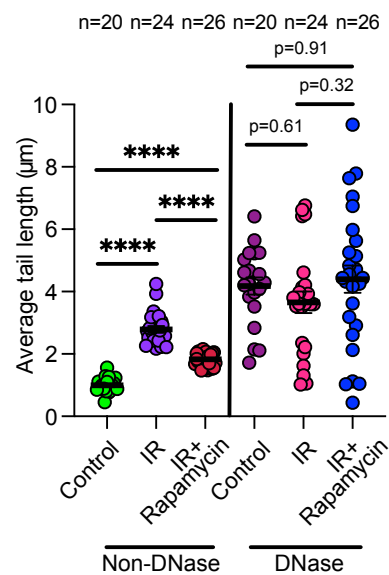
damaged oocytes. (A) Full-grown germinal vesicle (GV) oocytes were incubated in milrinone-containing CZB medium supplemented with the indicated treatments for 3 h. Oocytes were fixed and immunostained with Ku80 and γ H2AX antibodies. Right panels represent zoomed examples of fluorescence intensity of Ku80 at the DNA using the plot profiles function in Image J. Shown are representative images. DNA was stained with DAPI. Scale bars represent 10 μ m. (B)

Quantifications of the number of Ku80 foci localized at the DNA. Data are expressed as mean $\pm$ SEM. One-way ANOVA was used to analyze the data. Values with asterisks differ significantly, **p < 0.01, ****p < 0.0001. The total number of analyzed oocytes (from three independent replicates) is specified above each graph.

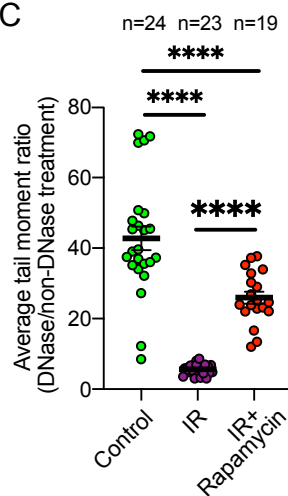
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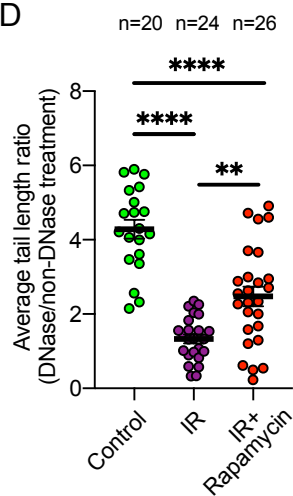
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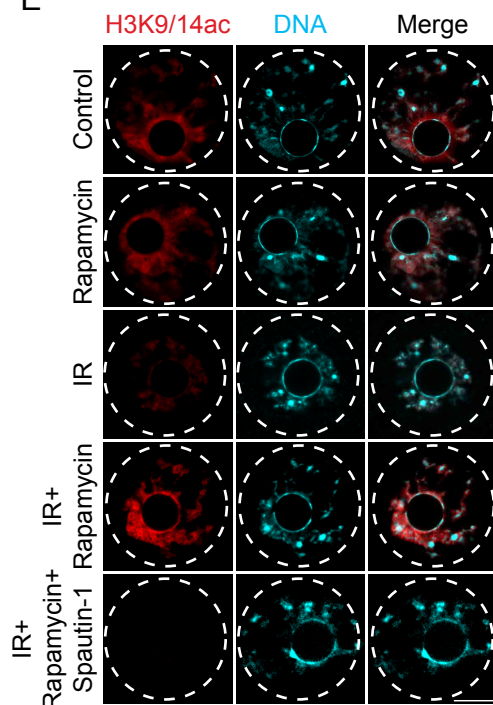
C



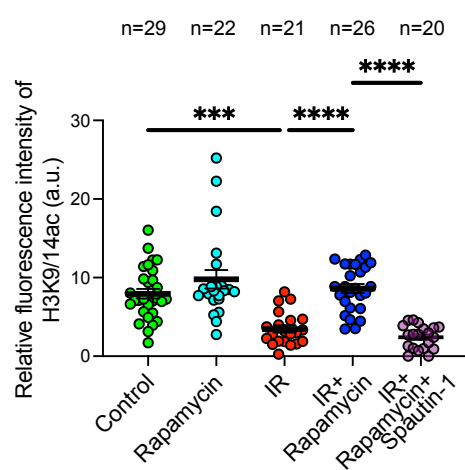
D



E

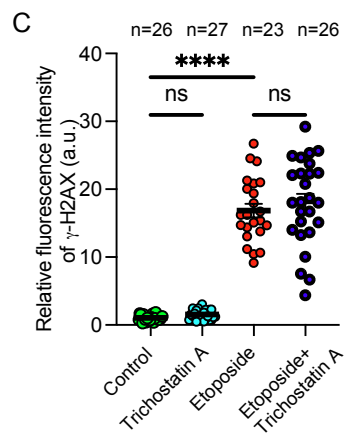
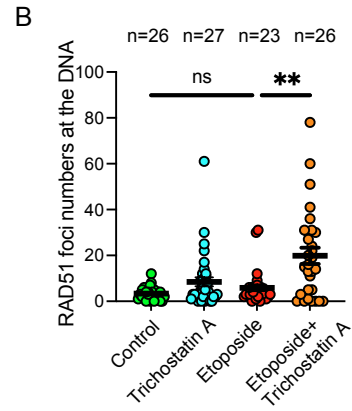
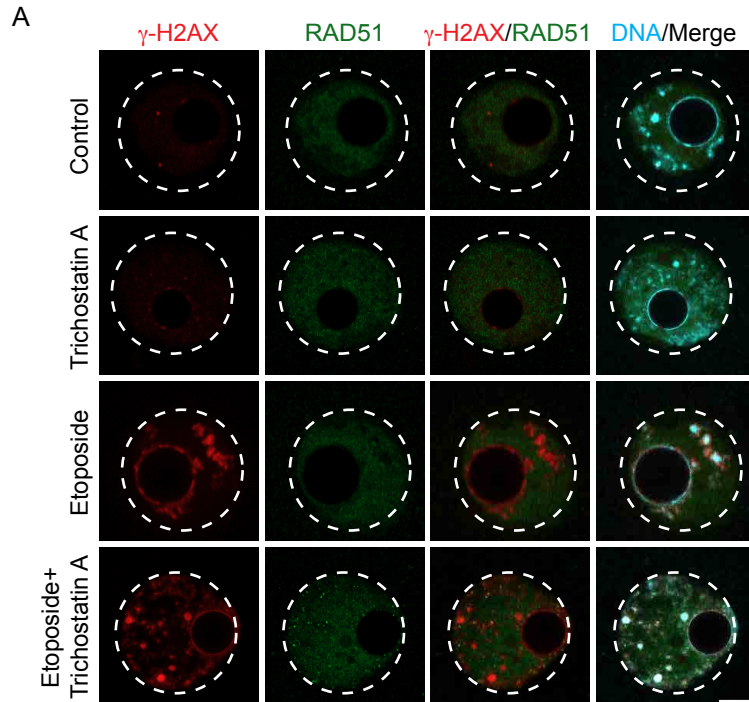


F



Supplementary Figure 6: Autophagy induction rescues DSBs in irradiated oocytes.

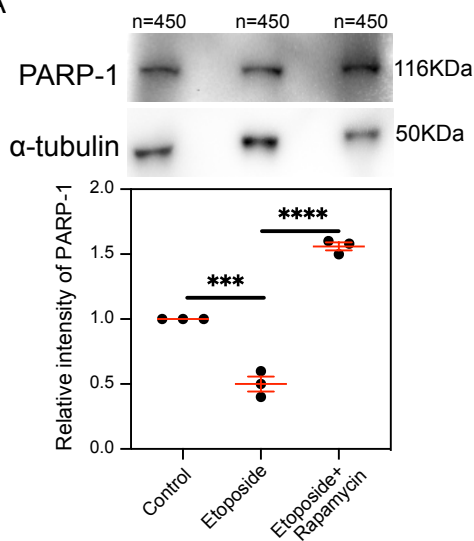
(A-D) Full-grown germinal vesicle (GV) oocytes were cultured in milrinone-containing CZB medium and exposed to ionizing radiation (IR) with or without rapamycin. The DNase-treated and non-DNase-treated oocytes were subjected to the alkaline comet assay. (A) Quantification of DNA tail moment. (B) Quantification of DNA tail length. (C) Quantification of average DNA tail moment ratio (DNase/non-DNase treatment). (D) Quantification of average DNA tail length ratio (DNase/non-DNase treatment). (E) Full-grown GV oocytes were incubated in milrinone-containing CZB medium and exposed to the indicated treatments. The oocytes were fixed and immunostained with H3K9/14ac antibody. Shown are representative images. DNA was stained with DAPI. Scale bar represents 10 μ m. (F) Quantification of H3K9/14ac fluorescence intensity in E. Data are expressed as mean $\pm$ SEM. One-way ANOVA was used to analyze the data. Values with asterisks differ significantly, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. The total number of analyzed oocytes (from three independent replicates) is specified above each graph.



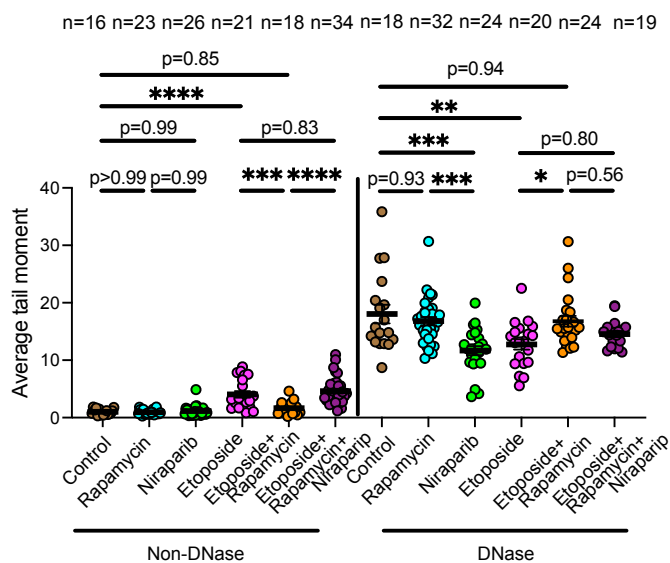
Supplementary Figure 7: RAD51 localization to the DNA requires open chromatin

structure. (A) Full-grown germinal vesicle (GV) oocytes were incubated in milrinone-containing CZB medium supplemented with the indicated treatments for 3 h. Oocytes were fixed and immunostained with RAD51 and γ H2AX antibodies. Shown are representative images. DNA was stained with DAPI. Scale bars represent 10 μ m. (B) Quantifications of the number of RAD51 foci numbers at the DNA. (C) Quantification of γ H2AX fluorescence intensity. Data are expressed as mean $\pm$ SEM. One-way ANOVA was used to analyze the data. Values with asterisks differ significantly, ** $p < 0.01$, **** $p < 0.0001$. The total number of analyzed oocytes (from three independent replicates) is specified above each graph.

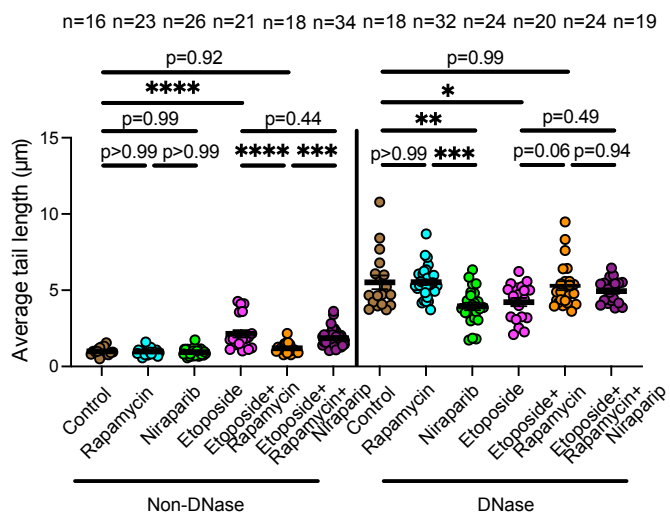
A



B

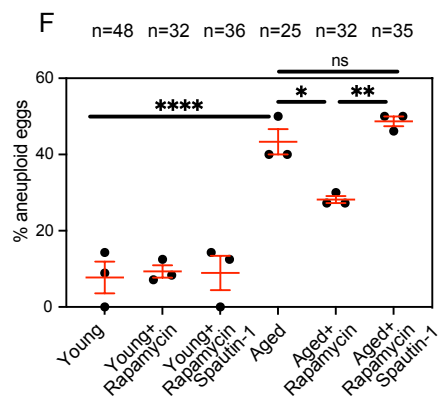
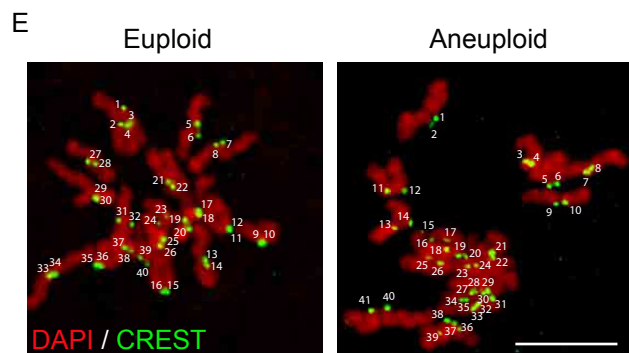
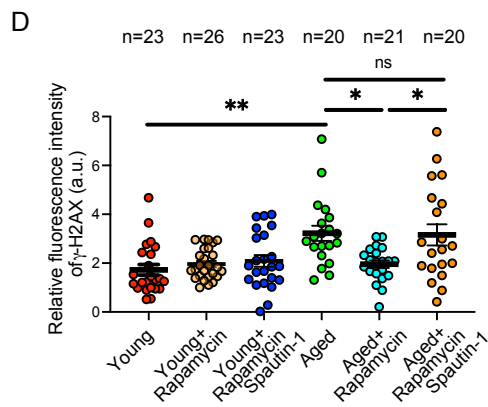
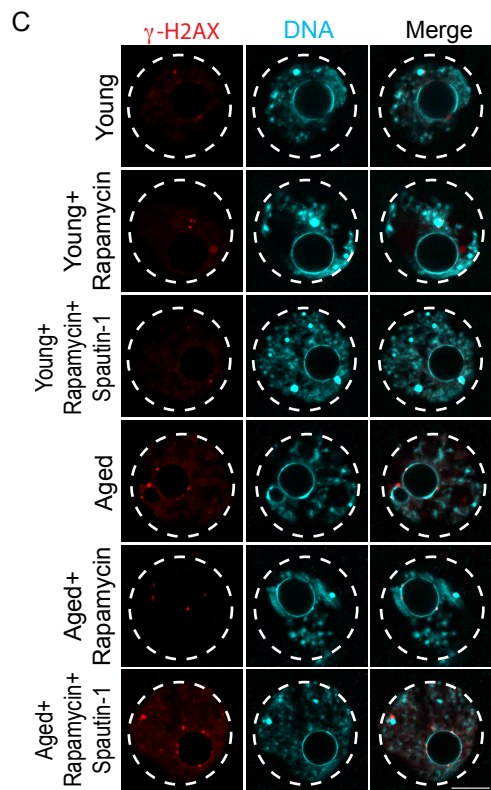
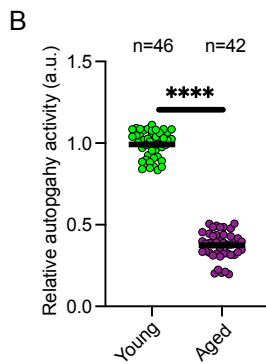
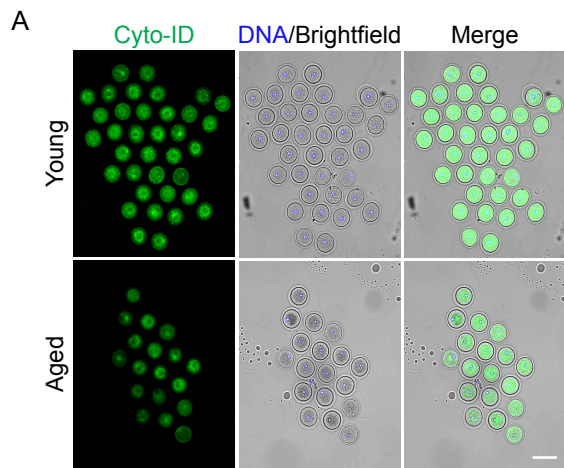


C



Supplementary Figure 8: PARP-1 promotes open chromatin structure in DNA-damaged

oocytes. (A) Full-grown germinal vesicle (GV) oocytes were incubated with the indicated treatments for 3 h in milrinone-containing CZB medium followed by Western blot analysis. Representative western blot images are shown (upper panel). Quantification of PARP-1 level (lower panel). (B,C) Full-grown GV oocytes were cultured in milrinone-containing CZB medium and exposed to the indicated treatments. The DNase-treated and non-DNase-treated oocytes were subjected to the alkaline comet assay. (B) Quantification of DNA tail moment in Fig. 6C. (C) Quantification of DNA tail length in Fig. 6C. Data are expressed as mean $\pm$ SEM. One-way ANOVA was used to analyze the data. Values with asterisks differ significantly, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. The total number of analyzed oocytes (from three independent replicates) is specified above each graph.



Supplementary Figure 9: Reduced autophagy is the likely cause of weakened DNA damage repair in oocytes from aged mice. (A) Full-grown GV oocytes from young mice (6-8-week-old) and aged mice (12-14-month-old) were assessed for autophagic activity by using Cyto-ID autophagy detection kit. Representative images are shown. Scale bar represents 100 μm . (B) Quantification of autophagy activity in A. (C) Full-grown GV oocytes from young mice (6-8-week-old) and aged mice (12-14-month-old) were incubated for 3 h with the indicated treatments in milrinone-containing CZB medium. Oocytes were fixed and immunostained with γH2AX antibody. DNA was stained with DAPI. Representative images are shown. Scale bar represents 10 μm . (D) Quantification of γH2AX fluorescence intensity in C. (E) Full-grown GV oocytes from young mice (6-8-week-old) and aged mice (12-14-month-old) were *in vitro* matured for 14 h (metaphase II). Oocytes were treated with monastrol for 2 h, fixed and immunostained with CREST antibody to label kinetochores. Oocytes were scored either as euploid (containing 40 kinetochores) or aneuploid (containing ± 40 kinetochores). Representative images are shown. (F) Quantification of aneuploidy percentage in E. Scale bars represent 10 μm . DNA was stained with DAPI. Data are expressed as mean $\pm$ SEM. Student-t-test (B) or One-way ANOVA (D and F) were used to analyze the data. Values with asterisks differ significantly, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$. The total number of analyzed oocytes (from three independent replicates) is specified above each graph.