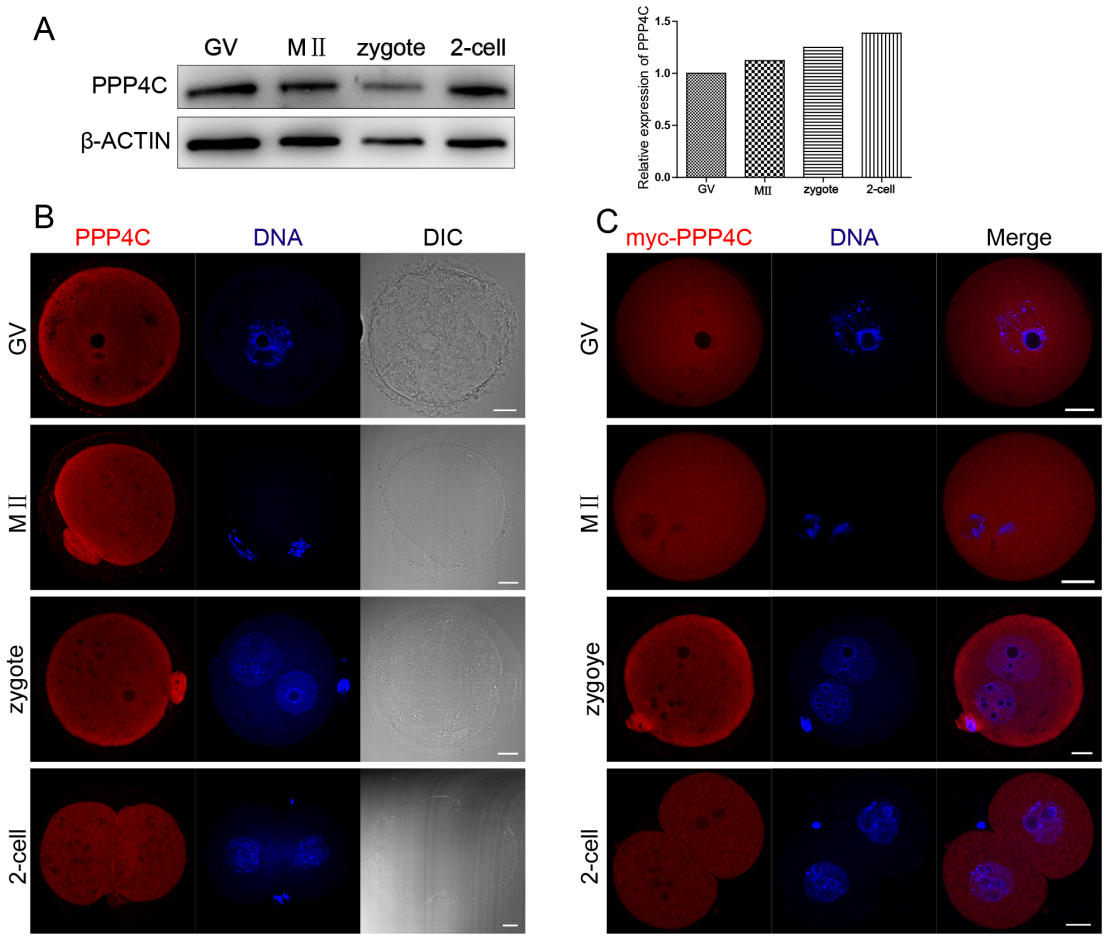


**Fig. S1. Characterization of PPP4C during mouse oocyte meiotic maturation and early embryo development.** (A) Western blot showing the expression pattern of PPP4C in oocytes, zygotes and 2-cell embryos. A total of 100 oocytes were collected after being cultured for 0 and 14 h, corresponding to the germinal vesicle (GV) and metaphase Ⅱ (MⅡ) stages, respectively. A total of 100 embryos were collected at 24 h and 48h after hCG treatment with successful mating, corresponding to 1-cell and 2-cell stages. Samples were immunoblotted using anti-PPP4C and anti-β-ACTIN antibodies. (B) Representative images of subcellular localization of PPP4C during oocyte meiotic maturation and early embryo development. Oocytes were double stained for PPP4C (red) and DNA (blue) at the GV, MⅡ, 1-cell and 2-cell stages. (C) Representative images of subcellular localization of myc-PPP4C during oocyte meiotic maturation and early embryo development. Cells were injected with *myc-Ppp4c* mRNA before staining. Scale bars: 20 μm.

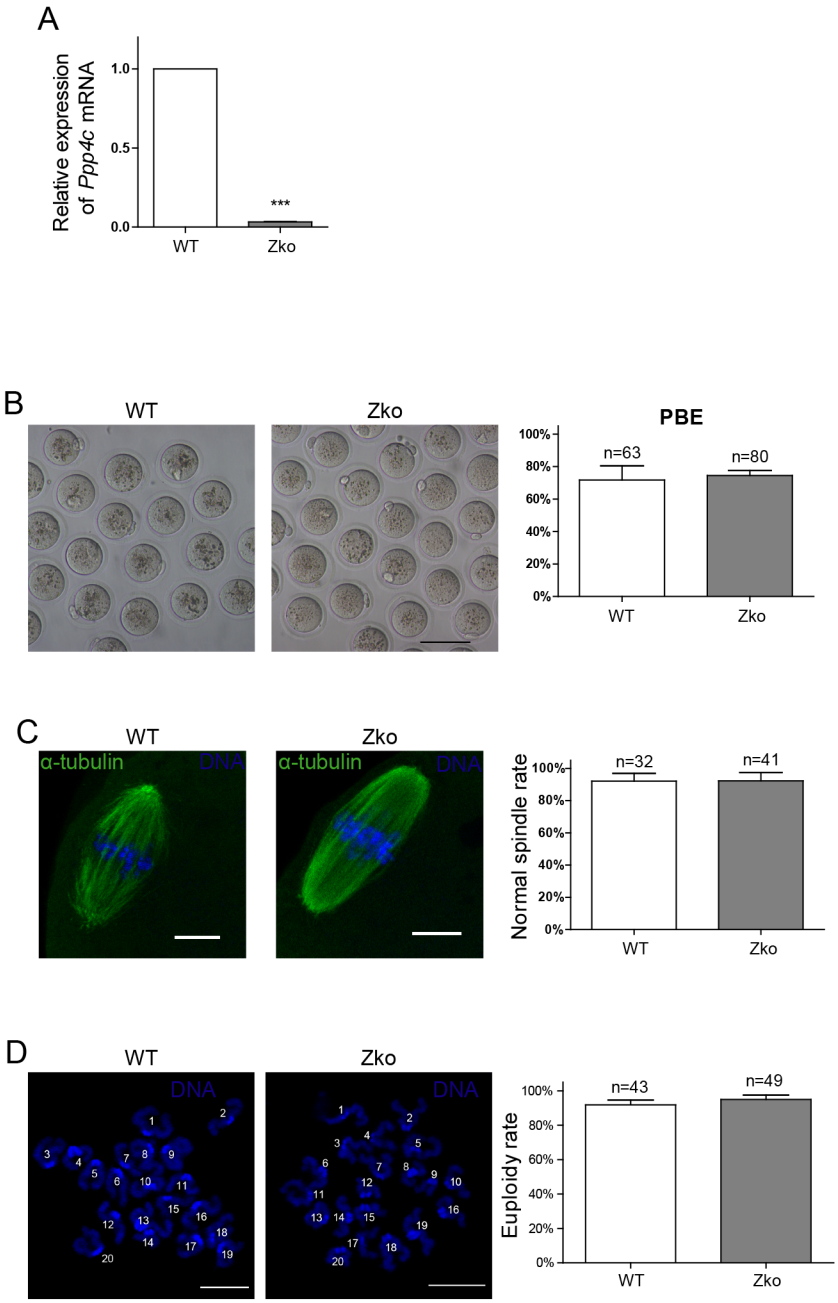


**Fig. S2. Depletion of PPP4C does not affect oocyte meiotic maturation.** (A) Total RNA samples prepared with ~50 WT and Zko oocytes and subjected to real-time RT-PCR showed no expression of *Ppp4c* mRNA in Zko oocytes. Results are mean ± SEM. * * *P < 0.001. (B) Comparable PBE rates of WT and Zko oocytes. Germinal vesicle (GV) oocytes were isolated and matured in vitro; oocytes that extruded the first polar body (PBE) were counted at 14 h. Representative DIC images are shown. Data are presented

as mean ± SEM. In vitro maturation (IVM) experiments were repeated at least three times. Scale bars: 100 μm. (C) Representative images of staining for DNA (blue) and immunostaining for α-tubulin (green) showing normal spindle assembly in Zko oocytes at the metaphase of MII stage. Scale bars: 10 μm. The percentages of oocytes with a normal spindle at the MII stage of each genotype are presented as mean ± SEM. The numbers of analyzed oocytes are indicated (n). (D) Chromosome spread of MII oocytes from WT and Zko mice, showing chromosomes stained with DAPI (blue). Representative images are shown. Scale bars: 10 μm. The number of chromosomes from each oocyte was counted and the percentages showing euploidy (i.e. 20 pairs of chromatids) MII oocytes of each genotype are presented as mean ± SEM. The total numbers of analyzed oocytes are indicated (n).

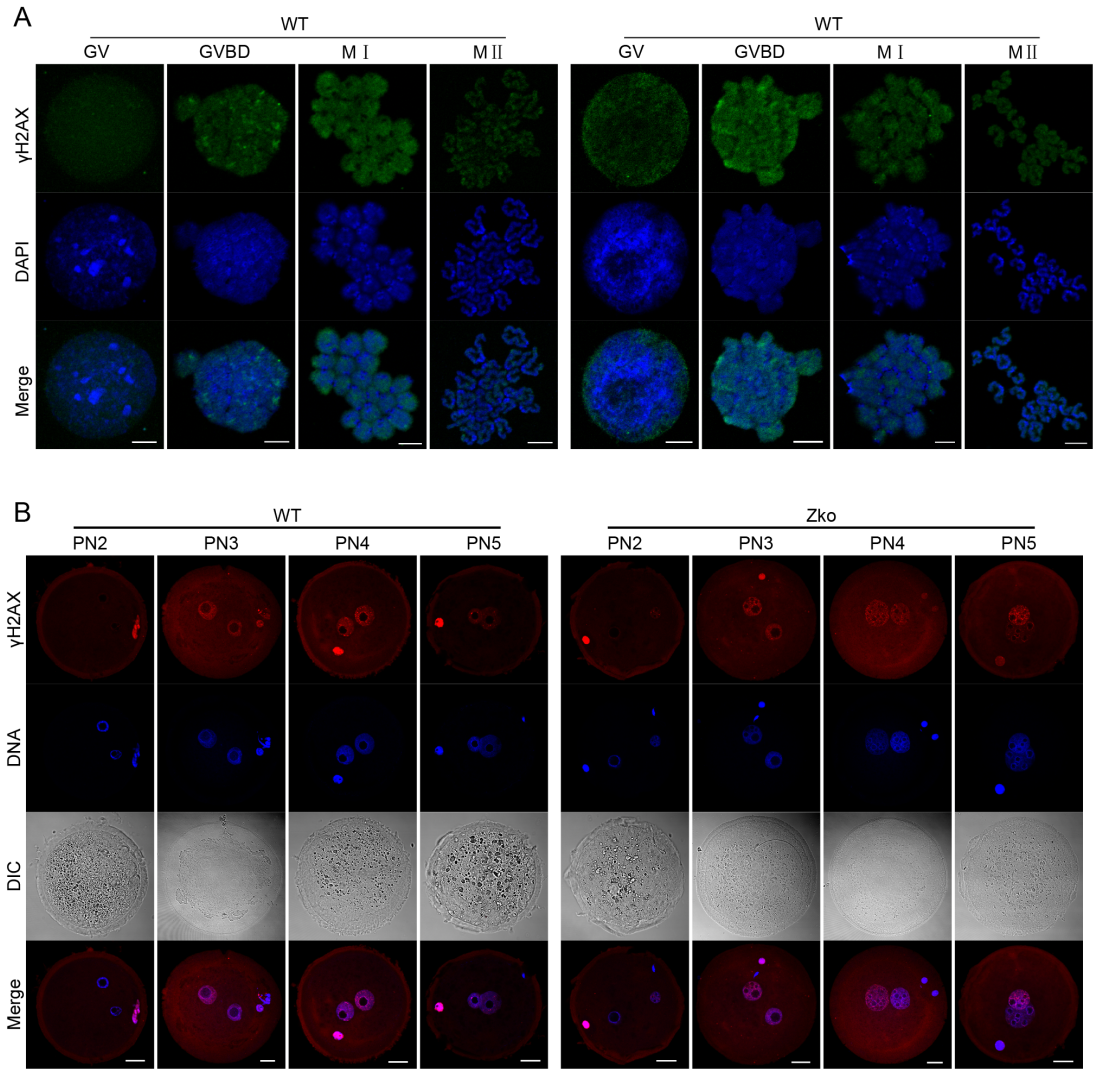


**Fig. S3. Depletion of PPP4C impairs genomic integrity of fertilized eggs.** (A) γ H2AX was assessed during oocyte maturation using chromatin spread preparations (B) Zygotes of WT and Zko females were obtained at 22, 24, 28 and 30 h post-hCG and stained with anti-γH2AX antibody. Scale bars: 20 μm.

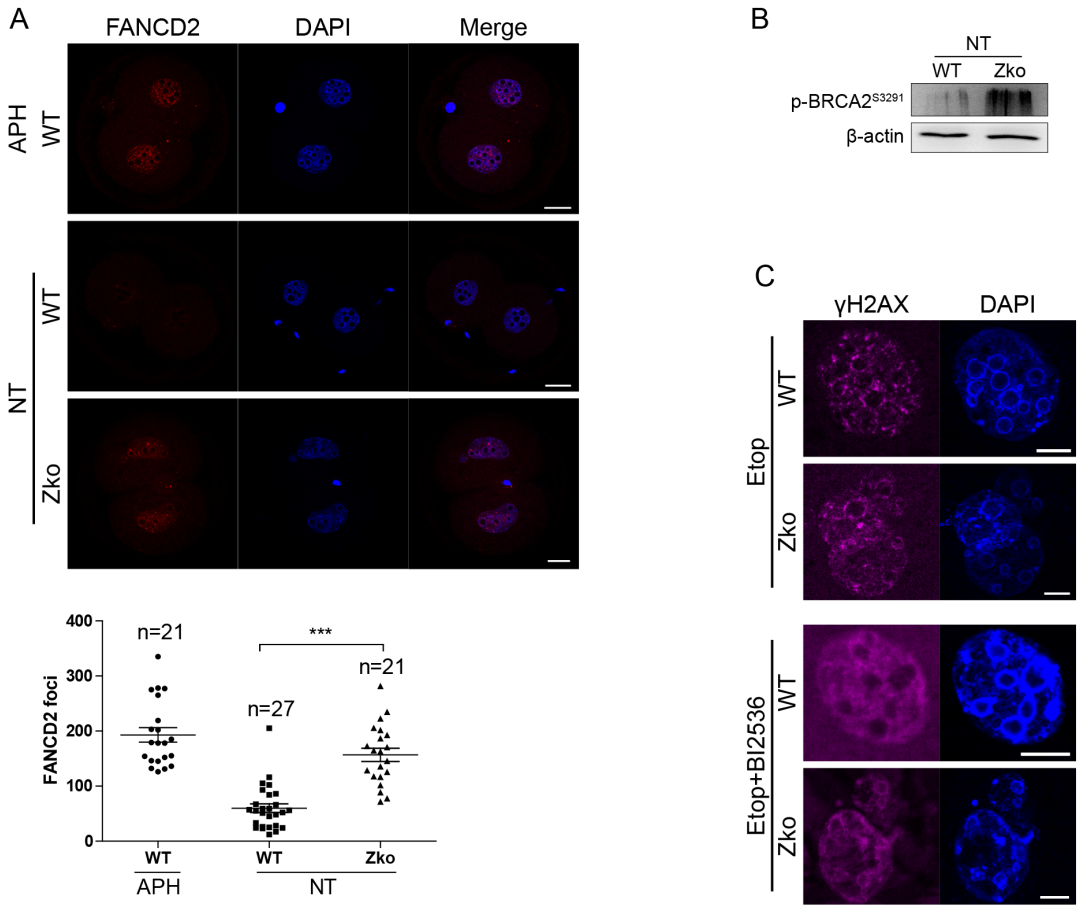


**Fig. S4.** (A) FANCD2 foci were assessed in G2 phase 2-cell WT and Zko embryos. Aphidicolin (an inhibitor of DNA synthesis, APH，0.1 μg/mL treated from G1 to G2 phase) treated group was used as a positive control. Foci were analyzed with imaris software. Results are mean ± SEM from three independent experiments. * * *P < 0.001. The total numbers of analyzed embryos are indicated as n. (B) BRCA2 S3291 was hyper-phosphorylation in PPP4C-deficiency 2-cell embryos. Levels of the indicated proteins in G2 phase of 2-cell embryos were analyzed by Western blot. (C) Inhibition of PLK1 since G1 phase affects γ H2AX foci. 2-cell embryos were treated with or without BI2536 since G1 phase, and DNA damage was induced by etoposide for 3h in G2 phase before staining.

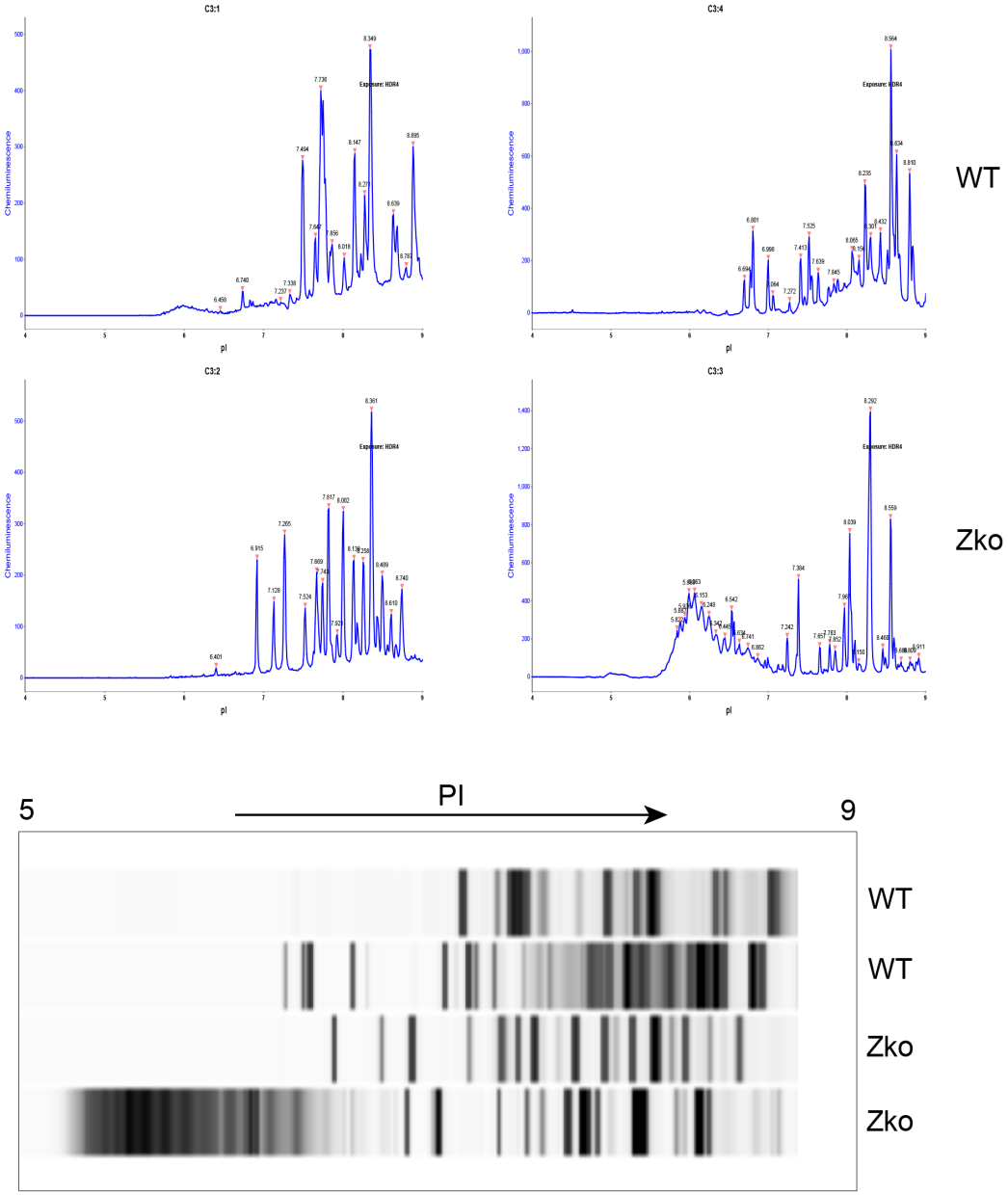


**Fig. S5. Quantification of posttranslational modification (PTM) of PLK1 in G2 phase of 2-cell embryos as revealed by NICIF**. Top, representative traces for PLK1. Bottom, NICIF pseudoblot representation.

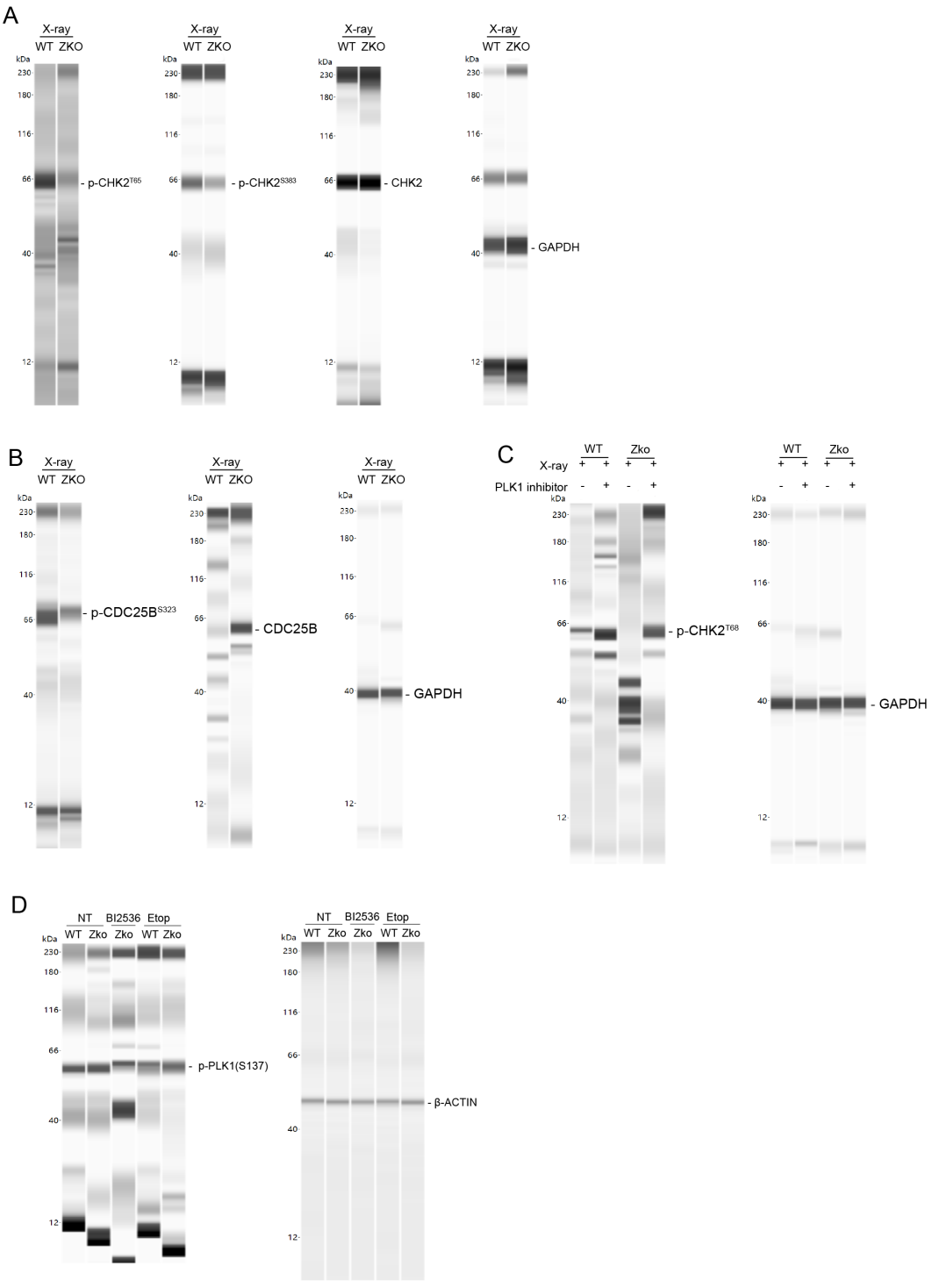


**Fig. S6. Entire original wes pictures.**

**Table S1. Original data of breeding assay.**

| | WT | | | | Zko | | | |
|---|---|---|---|---|---|---|---|---|
| | F2710 | F7253 | F7037 | F5386 | F2708 | F2797 | F7255 | F7040 |
| 1st mon | 4 | 9 | 5 | 7 | 0 | 0 | 3 | 0 |
| 2nd mon | 11 | 15 | 11 | 12 | 4 | 0 | 13 | 3 |
| 3rd mon | 19 | 15 | 16 | 21 | 4 | 4 | 20 | 6 |
| 4th mon | 25 | 27 | 22 | 28 | 4 | 4 | 20 | 6 |
| 5th mon | 30 | 31 | 35 | 30 | 8 | 7 | 25 | 10 |
| 6th mon | 36 | 40 | 45 | 30 | 8 | 7 | 25 | 17 |
| litters/mother | 7 | 5 | 6 | 5 | 2 | 2 | 4 | 4 |

**Table S2. Original data of embryos at E10.5.**

| | NO. embryos/female | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| WT | 8 | 9 | 9 | 7 | 4 | 8 | 8 | 10 |
| Zko | 2 | 5 | 4 | 5 | 1 | 5 | 7 | 6 |

**Table S3. Antibodies and reagents.**

| Name | Application | Dilution |
|---|---|---|
| anti-PPP4C(Abcam,ab16475) | IF | 1:200 |
| | WB | 1:1000 |
| anti-RAD51 (Abcam,ab133534) | IF | 1:200 |
| anti-BrdU (Abcam,ab6326) | IF | 1:200 |
| anti-α-tubulin (Sigma,DM1A) | IF | 1:1000 |
| PLK1 (Sigma,SAB1404220) | WB | 1:1000 |
| | NICIF | 1:50 |
| anti-MYC (Sigma,M4439) | IF | 1:1000 |
| anti-γH2AX (Cell Signaling,5438S) | IF | 1:200 |
| | WB | 1:1000 |
| anti-γH2AX (Cell Signaling,7631T) | IF | 1:200 |
| | WB | 1:1000 |
| anti-p-CHK1$^{S345}$ (Cell Signaling,2341S) | WB | 1:1000 |
| anti-RPA2 (Cell Signaling,2208) | IF | 1:200 |
| anti-GAPDH (Cell Signaling,5174) | WB | 1:1000 |
| | Wes | 1:100 |
| anti-α-tubulin(Cell Signaling,2144) | WB | 1:1000 |
| anti-p-CDK1 Y15 (Abclonal,AP0016) | WB | 1:1000 |
| anti-HA (Abclonal, AE008) | WB | 1:1000 |
| | IP | 1:25 |
| anti-CHK2 (Abclonal,A0466) | Wes | 1:25 |
| anti-p-BRCA2$^{S3291}$ (Immunoway,YP0512) | WB | 1:1000 |

| | | |
|---|---|---|
| anti-p-CHK2$^{T68}$ (Immunoway,YP0065) | Wes | 1:25 |
| anti-p-CHK2$^{S383}$ (Immunoway,YP0538) | Wes | 1:25 |
| anti-p-CDC25B$^{S323}$ (Immunoway,YP0057) | Wes | 1:25 |
| anti-p-PLK1$^{T210}$ (Immunoway,YP0964) | WB | 1:1000 |
| anti-p-PLK1$^{S137}$ (Immunoway,YP0434) | Wes | 1:25 |
| anti-FANCD2 (Abcam,ab108928) | IF | 1:200 |
| alexa 594 goat anti-rabbit (Invitrogen,A11012) | IF | 1:1000 |
| alexa FITC goat anti-rat (ZSGB-Bio,ZF-0315) | IF | 1:1000 |
| alexa 488 goat anti-mouse (Invitrogen,A11029) | IF | 1:1000 |
| alexa 647 goat anti-mouse (Invitrogen,A31571) | IF | 1:1000 |
| HRP-conjugated anti-mouse (ZSGB-BIO, 2304) | WB | 1:2000 |
| HRP-conjugated anti-rabbit (ZSGB-BIO, 2301) | WB | 1:2000 |
| Ro-3306 (selleck, s7747) | CDK1 inhibitor | 10μM |
| BI-2536 (Medchemexpress, HY-50698) | Plk1 inhibitor | 200nM |
| BML-277 (selleck,S8632) | CHK2 inhibitor | 10μM |
| Etoposide (Sigma, E1383) | lesion inducer | 50ug/ml |
| BrdU (Sigma, 858811) | DNA synthesis maker | 100uM |
| aphidicolin (Sigma, A-0781) | DNA synthesis inhibitor | 0.1ug/ml |

Abbreviation:
IF, immunofluorescence staining
NICIF, Nanimmuno capillary isoelectric focusing
WB, western blotting
Wes, Automated Western immunoblotting

**Table S4. Primer sequences.**

| | Name | 5'->3' |
|---|---|---|
| Guiding RNA targeting at Ppp4c | sgRNA-L | CACCGGTCAGGAATTGGGCTGGGC |
| | | AAACGCCCAGCCCAATTCCTGACC |
| | sgRNA-R | CACCGCCTCGATACAAGAATTAGA |
| | | AAACTCTAATTCTTGTATCGAGGC |
| PCR | Ppp4c-detF | GCATACTTCTGTCCCACG |
| | Ppp4c-detR | GTGTTTCCTGGGAGACTCCC |
| qRT-PCR | Ppp4c-qF | CTTGGTAGAAGAGAGCAACGTG |
| | Ppp4c-qR | CGCCACCTACTCTGAACAGC |
| | Gapdh-qF | TTGTCTCCTGCGACTTCAACA |
| | Gapdh-qR | ACCAGGAAATGAGCTTGACAAAG |
| Site-directed mutant | PLK1-S137D-F | GACCTCCTGGAGCTGCACA |
| | PLK1-S137A-F | GCCCTCCTGGAGCTGCAC |
| | PLK1-S137-R | CCTCCTGCGACAGAGCTC |
| | PLK1-T210D-F | GACTTGTGTGGCACTCCTA |
| | PLK1-T210A-F | GCCTTGTGTGGCACTCCT |
| | PLK1-T210-R | CTTCTTTCGTTCCCCTTCATAT |