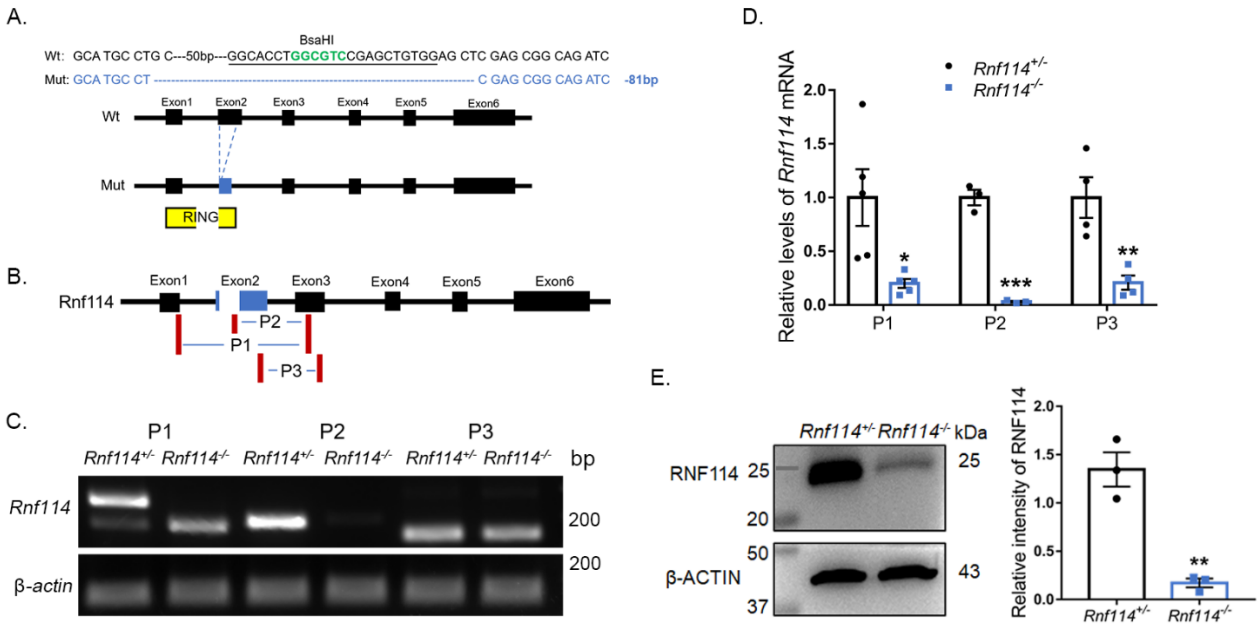

Figure S1.

(A, B) Diagrams showing the deletion of sequences from the mouse *Rnf114* gene locus, the destruction of the RNF114 protein domain (A) and the location of primers (B) designed to detect the knockout efficiency. (C) Representative images of *Rnf114* mRNA levels primed by P1, P2, and P3 in *Rnf114*$^{+/-}$ and *Rnf114*$^{-/-}$ metaphase II (MII) oocytes. β-actin was the loading control for the integrity of the RNA samples. N = 3 independent replicates. (D) Quantitative RT-PCR results showing *Rnf114* mRNA levels primed by P1, P2, and P3 in *Rnf114*$^{+/-}$ and *Rnf114*$^{-/-}$ MII oocytes. N = 3-5 independent replicates. (E) Immunoblot showing the RNF114 protein levels in *Rnf114*$^{+/-}$ and *Rnf114*$^{-/-}$ ovaries. Corresponding gray scale were measured. β-Actin was the loading control. N = 3, independent replicates. All graphs are presented as the means ± SEM. $^{**}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to the control group in the unpaired two-tailed t-test.

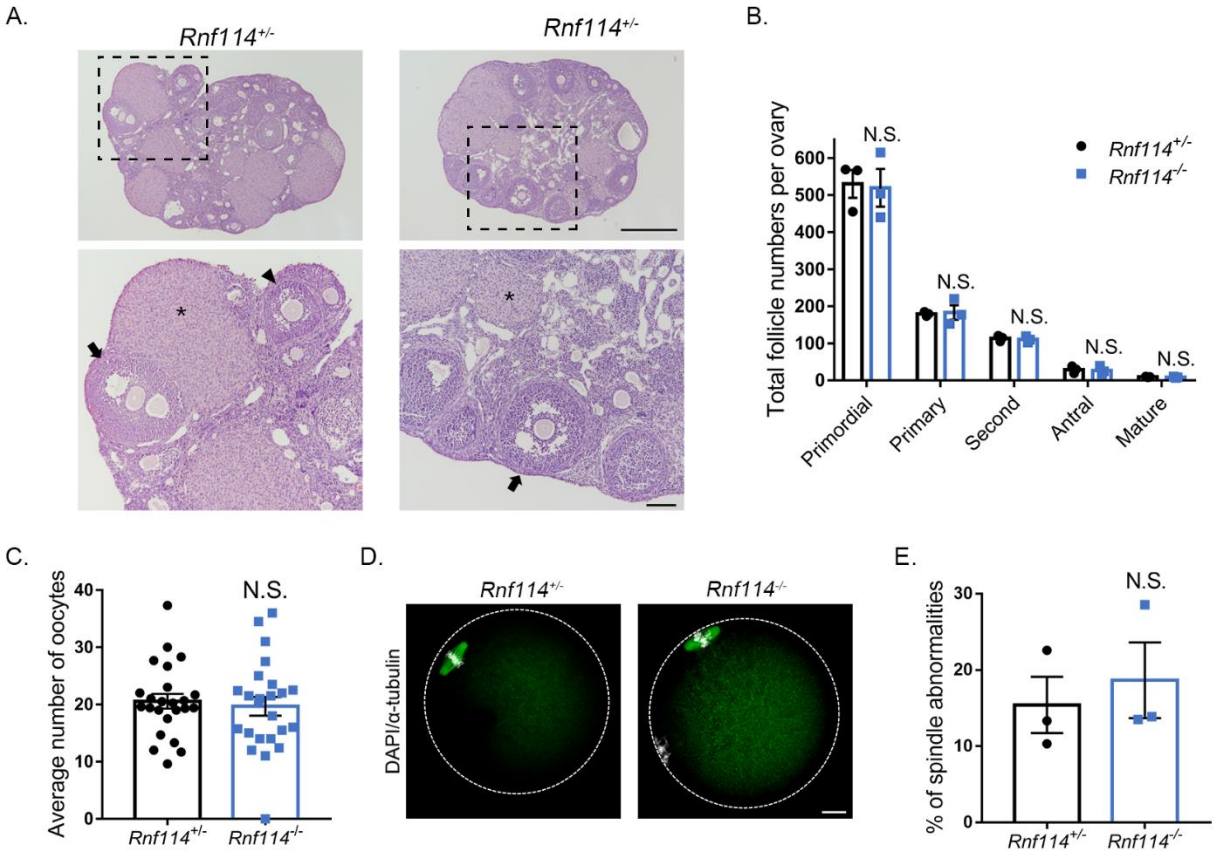

Figure S2.

(A) H- and E-stained images showing the ovarian histology of *Rnf114*$^{+/-}$ and *Rnf114*$^{-/-}$ females. The black dotted lines represent the enlarged areas. Antral follicles (arrowheads), secondary follicles (arrows) follicles, and corpora lutea (*) are indicated. Scale bar = 500 μm in the whole picture and scale bar = 100 μm in the greater zoom. (B) The average of follicle count at each stage in the control and mutant mice. N = 3, independent replicates. (C) Average number of MII oocytes from *Rnf114*$^{+/-}$ and *Rnf114*$^{-/-}$ females after superovulation. N = 24, total of 61-82 mice/group. (D) Microscopic images showing spindles in the *Rnf114*$^{+/-}$ and *Rnf114*$^{-/-}$ MII oocytes. Dashed lines indicate oocyte outlines. Scale bar = 20 μm. (E) Spindle abnormalities in the *Rnf114*$^{+/-}$ and *Rnf114*$^{-/-}$ MII oocytes. N = 3, total of 90-94 oocytes for each group. The above graphs are presented as the means ± SEM. N.S. = no significance in the unpaired two-tailed t-test.

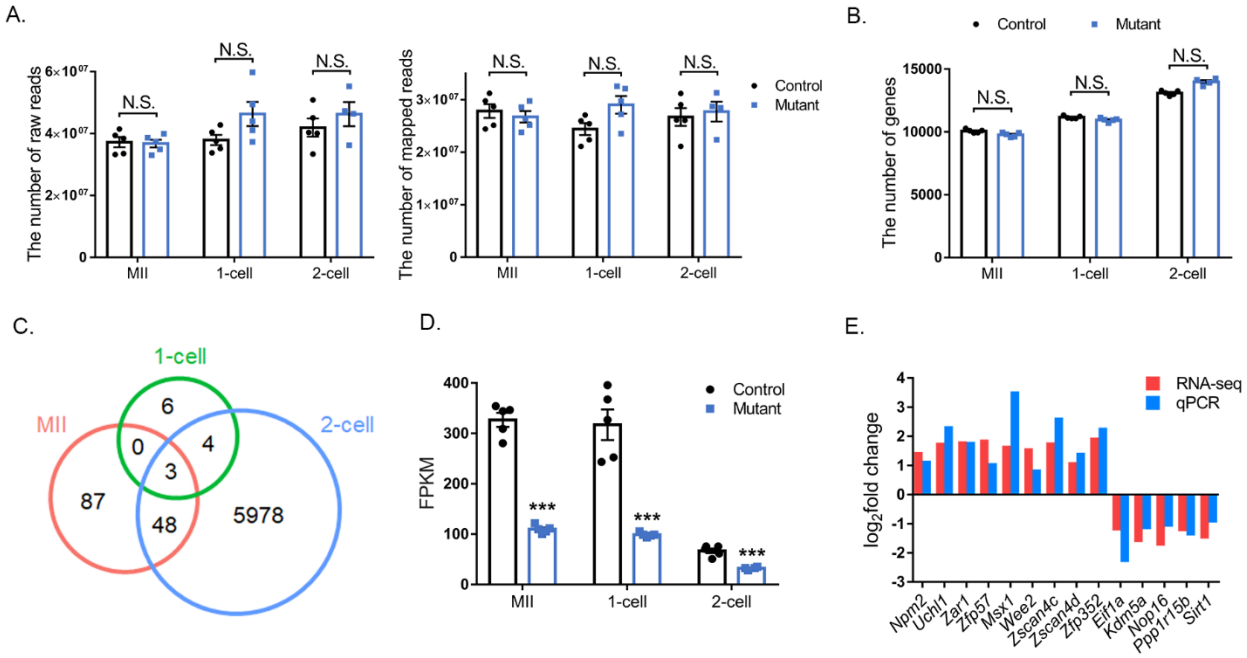

Figure S3.

(A) The average number of raw and mapped reads in control and mutant group. N = 4-5, independent replicates. (B)The average number of genes detected in control and mutant group, with FPKM >1 as the gene expression threshold. N = 4-5, independent replicates. (C) A Venn diagram illustrating the overlap of DEGs in MII oocytes and 1-cell and 2-cell embryos. (D) FPKM statistics for the RNF114 gene in the control and mutant groups at different time points. N = 4-5, independent replicates. Data are presented as the means ± SEM. N.S. = no significance, ***$P < 0.001$ in the unpaired two-tailed t-test. (E) The log_2-fold change of representative DEGs and corresponding qRT-PCR verification results. This qRT-PCR was performed on independent samples with 3 independent replicates.

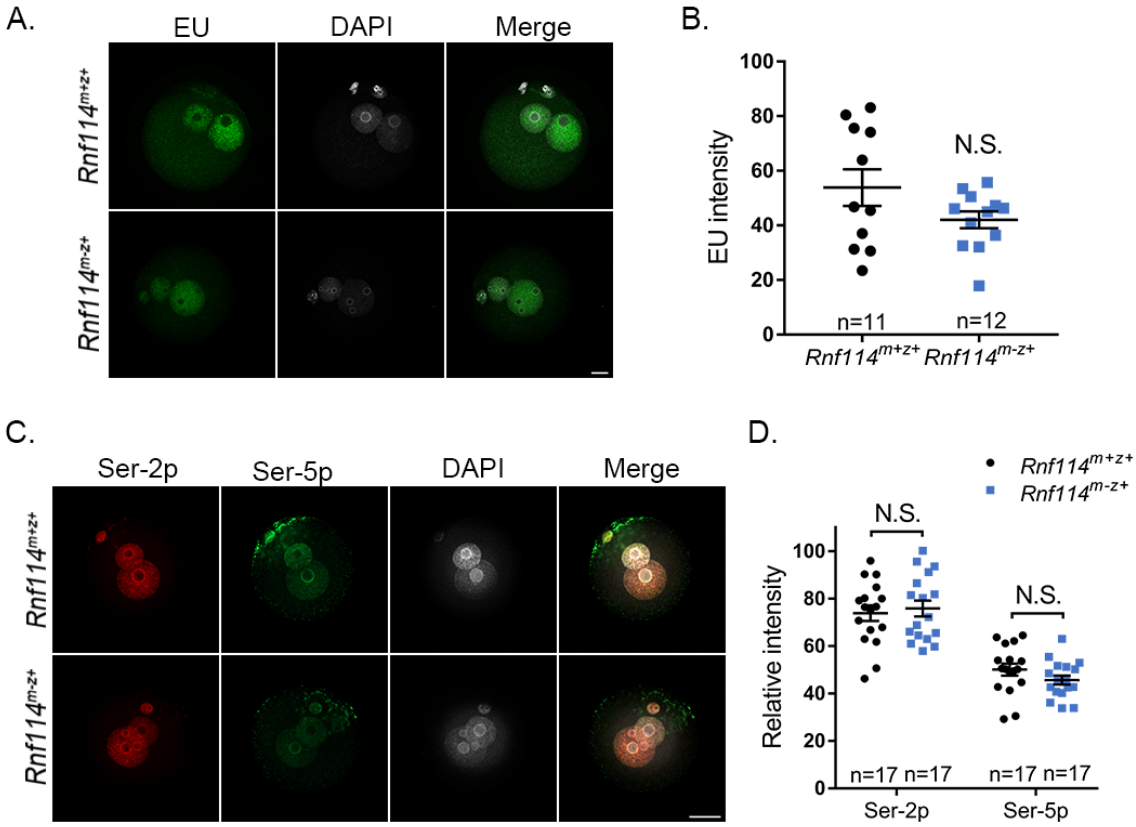

Figure S4.

(A, B) EU staining (A) and quantification of EU staining (B) in *Rnf114*$^{m+z+}$ and *Rnf114*$^{m-z+}$ 1-cell embryos. (C) Phosphorylation levels of serine moieties at positions 2 and 5 of Pol II for *Rnf114*$^{m+z+}$ and *Rnf114*$^{m-z+}$ 1-cell embryos. (D) Quantification of Ser-2p and Ser-5p staining. All error bars represent SEM. N.S. = no significance in the unpaired two-tailed t-test. All scale bar = 20 μm.

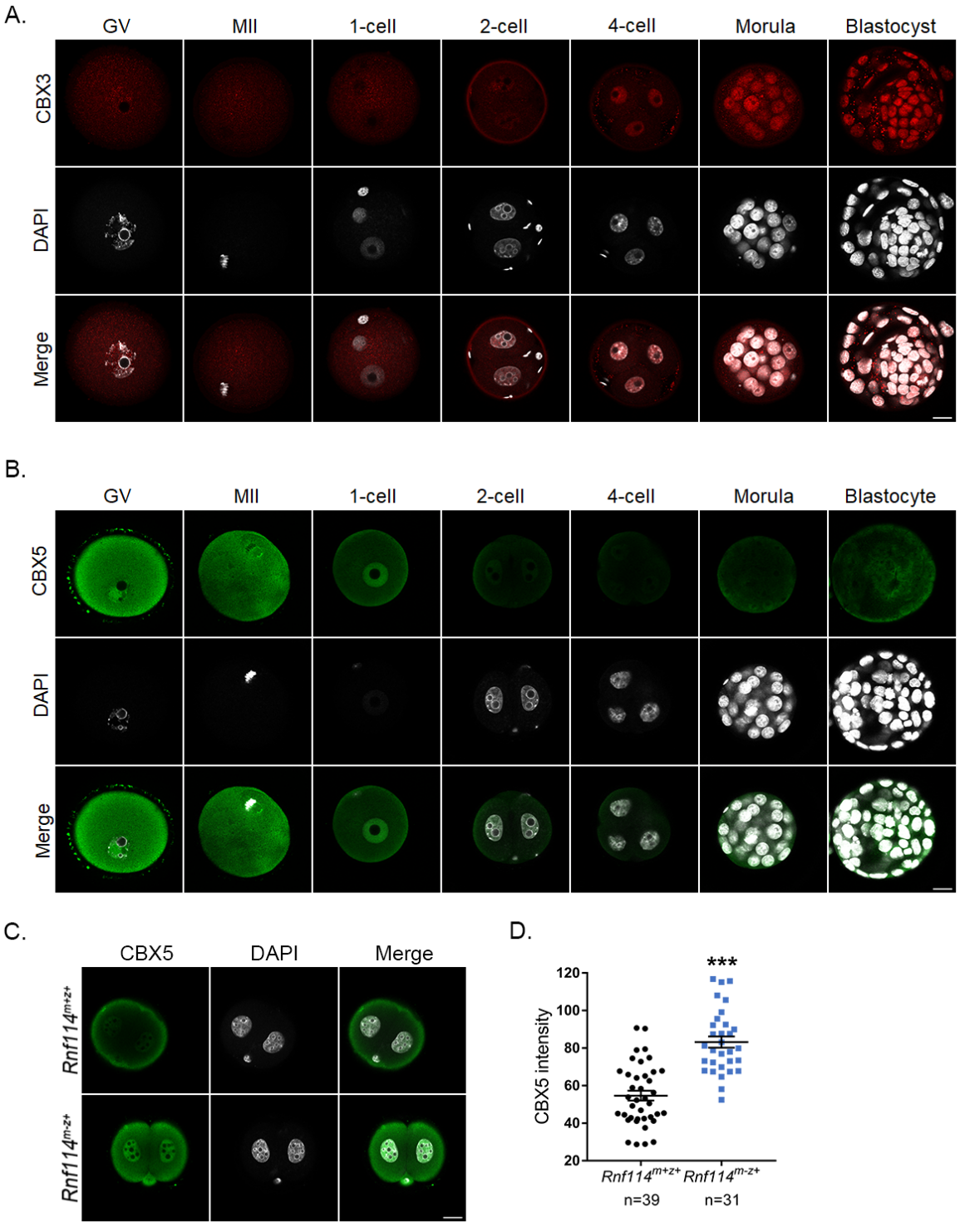

Figure S5.

(A, B) The expression of CBX3 (A) and CBX5 (B) in oocytes and preimplantation embryos. (C) Representative immunofluorescence images of CBX5 in the *Rnf114*$^{m+z+}$ and *Rnf114*$^{m-z+}$ 2-cell embryos. (D) Quantification of CBX5 staining in the *Rnf114*$^{m+z+}$ and *Rnf114*$^{m-z+}$ 2-cell embryos. Error bars represent SEM. ***P < 0.001 in the unpaired two-tailed t-test. All 90 scale bar = 20 μm.

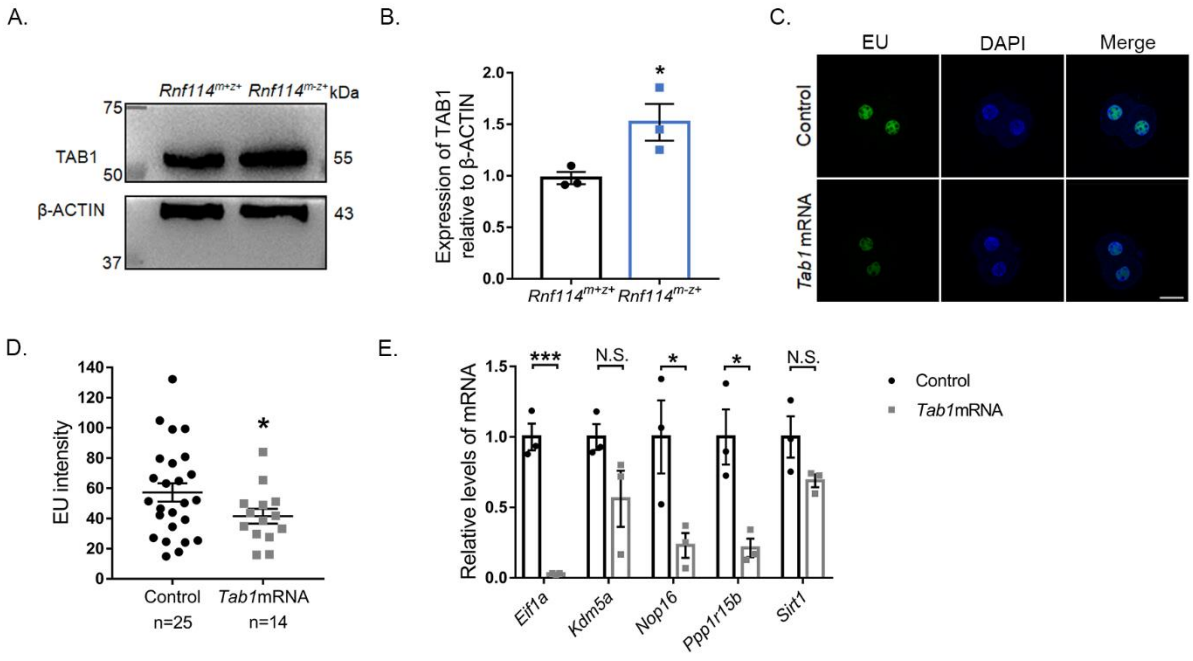

Figure S6.

(A) Representative western blot showing the levels of TAB1 in *Rnf114*$^{m+z+}$ and *Rnf114*$^{m-z+}$ 2-cell embryos. β-Actin was used as a loading control. (B) Quantitative analysis of the TAB1 protein from western blot. N = 3, independent replicates. Data are presented as the means ± SEM. (C) Representative images of EU staining after overexpressing *Tab1* in the WT 2-cell embryos. Scale bars = 20 μm. (D) Quantification of EU staining in WT 2-cell embryos with TAB1 overexpression. Error bars represent SEM. (E) Quantitative RT-PCR results showing the expression level of major ZGA genes in the WT 2-cell embryos with overexpressed TAB1 protein. N = 3, independent replicates. All graphs are presented as the means ± SEM. N.S. = no significance, **P* < 0.05, ****P* < 0.001 in the unpaired two-tailed t-test.

Table S1. Sample details for RNA sequencing

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Table S2. All DEGs between the control and mutant groups via RNA sequencing analysis at the MII oocyte, 1-cell and 2-cell embryo stages (adjusted P-value < 0.05).

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Table S3. Enriched GO terms for DEGs in the *Rnf114*$^{m-z+}$ 2-cell embryos compared with those in *Rnf114*$^{m+z+}$ (FDR < 0.05)

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Table S4. Differential expression of proteins in the *Rnf114*$^{-/-}$ oocytes identified by mass spectrometry (P-value < 0.05)

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Table S5. Top twenty significant GO catalogues of differentially expressed proteins in the *Rnf114*$^{-/-}$ oocytes

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Table S6. List of primer sequences and the sequences of siRNA in this study

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Table S7. Detailed information on the antibodies used for western blot and immunofluorescence staining

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