Supporting Information

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SI Materials and Methods

Collection of Mouse Oocytes and Zygotes. The specific pathogen free (SPF)-grade hybrid mice B6D2F1 (C57BL/6×DBA/2) were housed in the animal facility of the National Institute of Biological Sciences. All studies adhered to procedures consistent with the National Institute of Biological Sciences Guide for the care and use of laboratory animals.

Fully grown germinal vesicle (GV) oocytes were collected from 4- to 8-wk-old B6D2F1 female mice 44–48 h after pregnant mare serum gonadotropin (PMSG) injection. The antral follicles were punctured by 30-gauge needles, and the GV oocytes enclosed by several layers of cumulus cells were selected. The cumulus cells were mechanically removed, and the denuded oocytes were collected. Metaphase II (MII) oocytes were collected from the oviducts of B6D2F1 mice after sequential injection of females 8–12 wk of age with PMSG and human chorionic gonadotrophin (hCG). Adherent cumulus cells were removed by hyaluronidase treatment, and the cumulus cell-free MII oocytes were collected. The zygotes were collected from the oviducts of female B6D2F1 mice that were mated with male mice.

Protein Extraction. Oocyte proteins were extracted with FastPrep. Briefly, all samples were resuspended with 50 μL PBS. Next, 170 μL lysis buffer (20 mM Hepes, pH 7.4, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM dithiothreitol DTT) and 7 μL 50× CPI (complete, EDTA-free; Roche) were added and transferred to a 1.5-mL screw-capped tube containing 300 μL prechilled 0.5-mm-diameter glass beads. The oocytes were lysed using a FastPrep-24 instrument (MP Biomedicals) on speed 6 for 20 s two times, and between spins, the samples were chilled on ice for 3 min. The lysates were collected into a 2-mL tube by piercing a hole at the bottom of the 1.5-mL screw-capped tube and spinning at 300 × *g* for 1 min. Next, the lysates were centrifuged at 13,400 × *g* for 20 min. Proteins in supernatants were precipitated with 25% trichloroacetic acid (TCA) for 30 min on ice and centrifuged at 13,400 × *g* for 30 min. The pellets from TCA precipitation were washed two times with cold acetone, air-dried, and dissolved in 70 μL 100 mM Tris (pH 8.5) and 8 M urea.

Digestion. The lysate supernatant protein solutions were reduced with 5 mM Tris-(2-carboxyethyl)-phosphine (Pierce) at room temperature for 20 min and then alkylated with 10 mM iodoacetamide at room temperature for 20 min. After a 4-fold dilution with 100 mM Tris (pH 8.5), methylamine was added to 20 mM to reduce carbamylation, and $CaCl_2$ was added to 1 mM. The samples were digested with trypsin (substrate to enzyme mass to mass ratio at 50:1) at 37 °C for 16 h. The reactions were quenched with the addition of formic acid to 5%, and insoluble particulates were removed by centrifugation (14,000 × *g* for 20 min). Digested supernatant fractions were stored at −80 °C without further treatment until MS analysis.

The pellet fractions of the oocyte lysates were resuspended in 10 μL 5× Invitrosol LC/MS surfactant blend, vortexed, and incubated for 5 min at 60 °C. Both samples were diluted with 10 μL H_2O, 64 μL 100% acetonitrile (ACN), and 16 μL 500 mM Tris (pH 8.5), and then, they were sealed with parafilm. After 20–30 min of sonication, the samples were digested with trypsin (substrate to enzyme mass to mass ratio is 50:1) at 37 °C for 16 h. The reactions were quenched with the addition of formic acid to 5%, and insoluble particulates were removed by centrifugation (14,000 × *g* for 20 min). The solutions were reduced to less than 50 μL in a speed vacuum dryer heated to 50 °C. Next, 200 μL buffer A (95% water, 5% acetonitrile, 0.1% formic acid) were added, and the digested pellet samples were stored at −80 °C without further treatment until MS analysis.

LC-MS/MS Analysis. Digested peptide mixtures were pressure-loaded onto a fused silica capillary column packed with 5-μm Partisphere strong cation exchanger (SCX; Whatman) and 5-μm Luna C18 material (RP; Phenomenex), with a Kasil frit at the SCX end. The SCX and RP sections were 2 cm and 3 cm long, respectively, and the column was washed with buffer A (95% water, 5% acetonitrile, 0.1% formic acid). After desalting, a 9-cm, 100-μm internal diameter capillary with a 5-μm pulled tip packed with 5 μm Luna C18 material was attached to the two-phase column with a union, and the entire assembly was placed inline with an Agilent 1100 quaternary HPLC and analyzed using a 12-step separation. Three buffer solutions were used: 5% acetonitrile/0.1% formic acid (buffer A), 80% acetonitrile/0.1% formic acid (buffer B), and 500 mM ammonium acetate/5% acetonitrile/0.1% formic acid (buffer D). The first step consisted of a 2-min gradient from 0% to 15% buffer B, followed by a 47-min gradient to 100% buffer B and 10 min of 100% buffer B. Next, the buffer was switched to 100% buffer A in 1 min and kept at 100% buffer A for another 9 min. Steps 2–10 had the following profile: 1 min of 100% buffer A, 2 min of x% buffer D, 5 min of 100% buffer A, a 2-min gradient from 0% to 15% buffer B, a 60-min gradient from 15% to 50% buffer B, a 40-min gradient from 50% to 100% buffer B, and a 15 min 100% buffer A after a 5-min gradient from 100% buffer B to 100% buffer A. The 2-min buffer D percentages (x) in steps 2–10 were 5%, 10%, 15%, 20%, 30%, 50%, 60%, 70%, and 80%, respectively. Steps 11 and 12 consisted of 1 min of 100% buffer A, 9 min of 100% buffer D, and 5 min of 100% buffer A, followed by a 5-min gradient to 15% buffer B, a 50-min gradient to 50% buffer B, a 45-min gradient to 100% buffer B, 15 min of 100% buffer B alone, and 15 min of 100% buffer A after a 5-min gradient from 100% buffer B to 100% buffer A. As peptides were eluted from the microcapillary column, they were electrosprayed directly into a LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific) with the application of a distal 2.5 kV spray voltage. The mass spectrometer was operated in the data-dependent mode. Survey MS scans were acquired in the orbitrap with the resolution set to a value of 60,000. Each survey scan (400–2,000 *m/z*) was followed by eight data-dependent tandem mass (MS/MS) scans at 35% normalized collision energy. Automatic gain control (AGC) target values were 200,000 for the survey scan and 10,000 for the MS/MS scan. Target ions already selected for MS/MS were dynamically excluded for 30 s.

Data Analysis. Tandem mass spectra were searched against European Bioinformatics Institute International Protein Index mouse protein database using the ProLuCID[1] protein database search algorithm. ProLuCID search results were then filtered with DTASelect 2.0 using a cutoff of 1% for peptide false identification rate (−fp 0.01). Peptides with Z score < 4 (i.e., 4 SDs away from the average) or DeltaMass > 7 ppm (−DM 7) were rejected. Furthermore, the minimum number of peptides to identify a protein was set to 1 (−p 1). Spectral counts for each protein were extracted from DTASelect files into Excel spreadsheets and normalized to the total spectral counts of the sample from which a protein was identified. Gene ontology analysis was performed using the GSEABase package of BioConductor (http://www.bioconductor.org/). Proteins were divided into biological process, molecular function, and cellular component categories and their subcategories. Expression Analysis Systematic Explorer (EASE) was used to analyze gene

ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Genes in a KEGG pathway are judged to be overrepresented if a larger fraction of genes within that pathway is expressed compared with all genes in the genome. GenMAPP was used to analyze gene expression changes, and Cytoscape software was used to draw the picture.

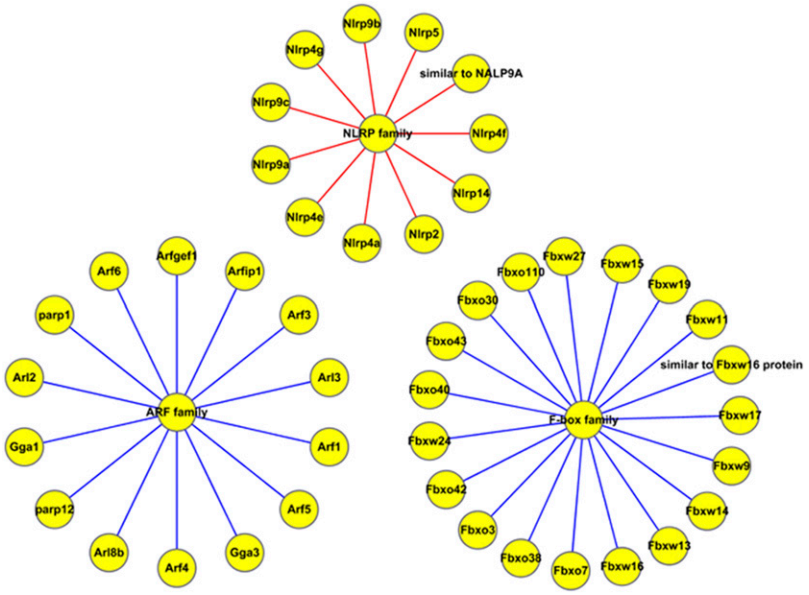

Fig. S1. The protein families expressed specifically in the mouse oocyte but not in ES cells, which include the ADP ribosylation factor (ARF) family, the NACHT, leucine rich repeat and PYD containing (NLRP) family, and F-box domain protein family.

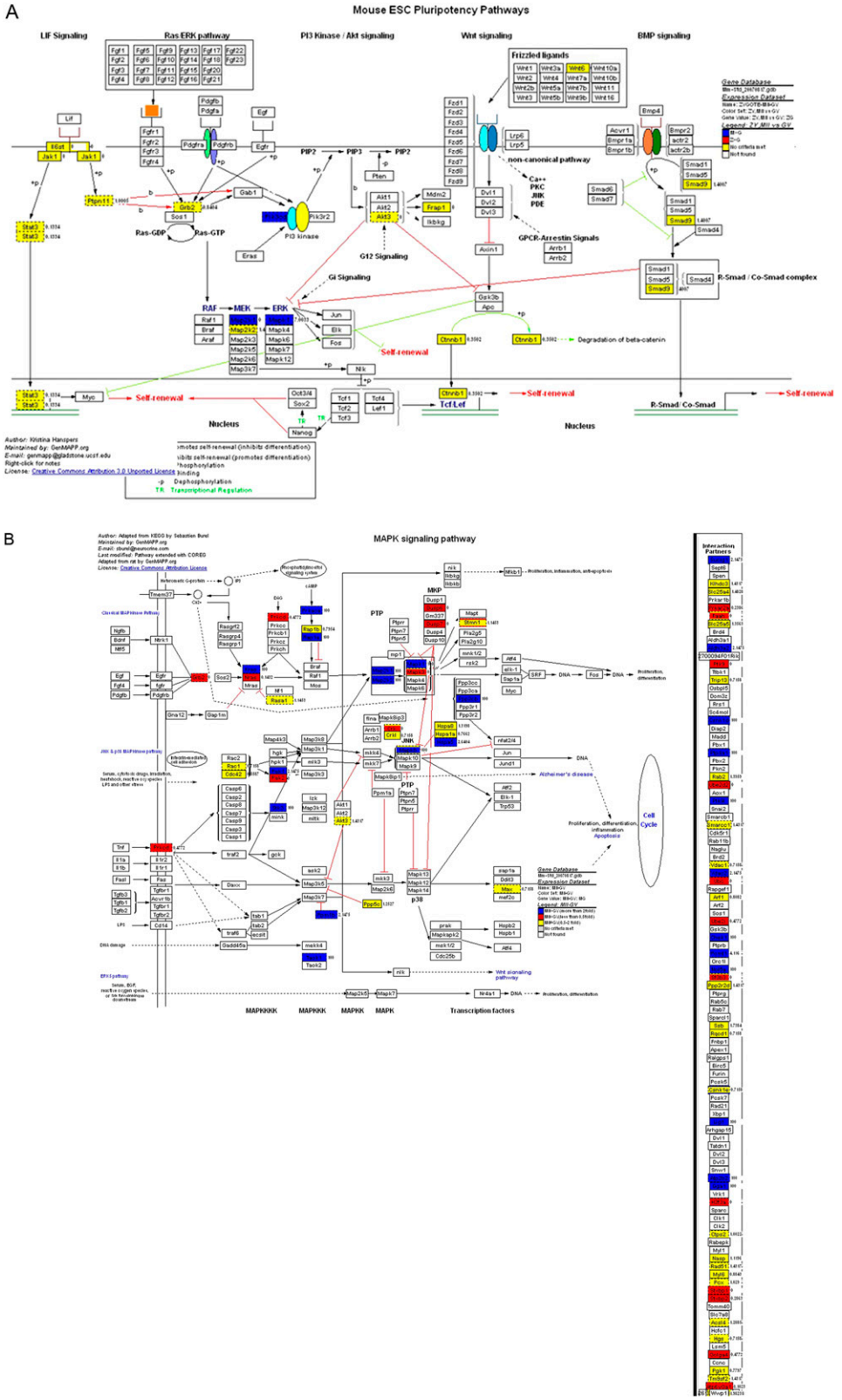

Fig. S2. (*A*) The proteins expressed in mouse oocyte, which are involved in the ES cells pluripotency pathway. The blue rectangles indicated that the proteins expressed more in the MII oocyte. The yellow rectangles indicated that the expression level of the proteins was similar among GV, MII oocyte, and zygote. (*B*) The MAPK pathway proteins expressed in the GV oocyte and MII oocyte. The blue rectangles indicated that the proteins expressed more in the MII oocyte. The yellow rectangles indicated that the expression level of the proteins was similar in both GV and MII.

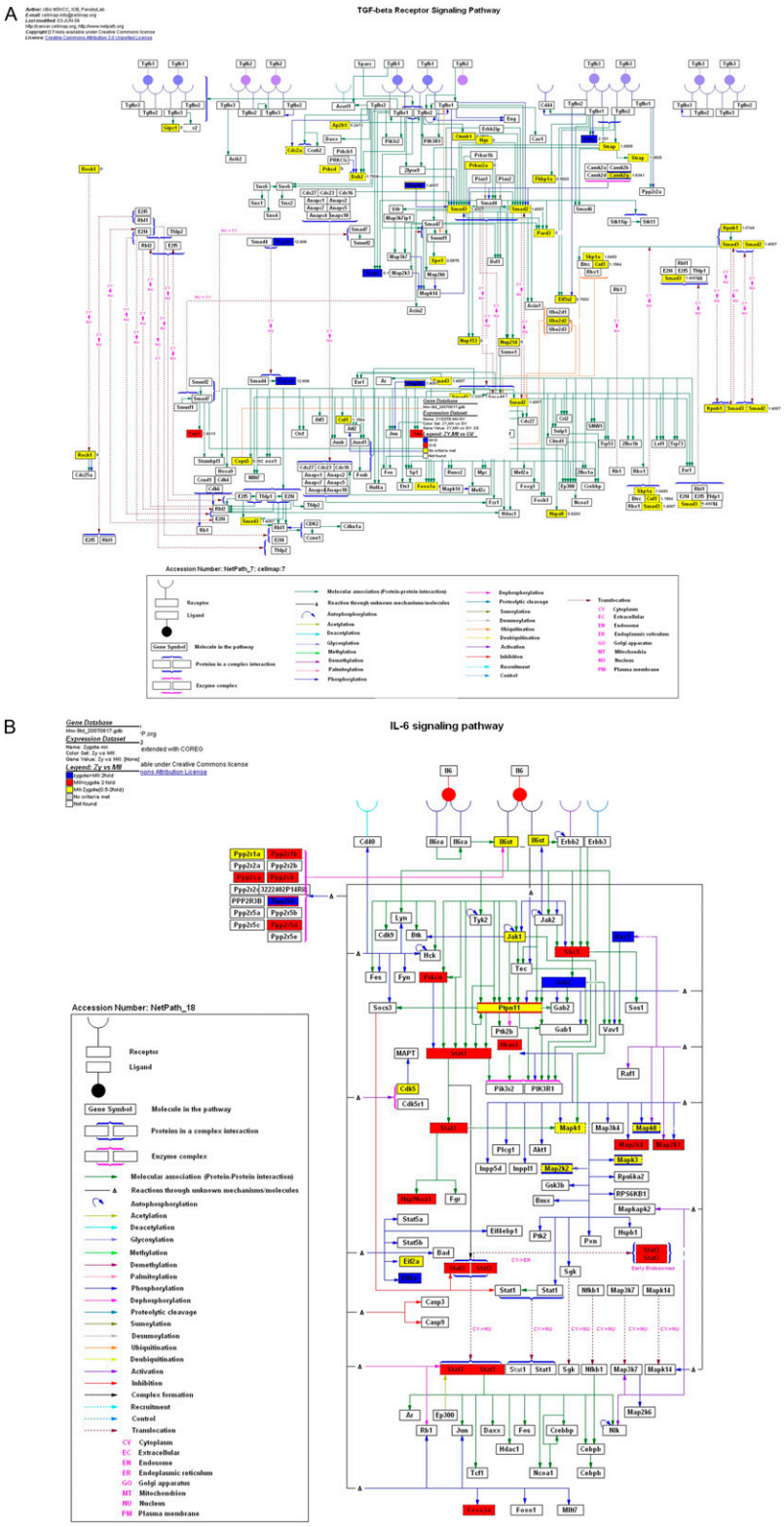

Fig. S3. (*A*) The TGF pathway proteins expressed in the GV oocyte and MII oocyte. The blue rectangles indicated that the proteins expressed more in the MII oocyte. The red rectangles indicated that the proteins expressed more in GV oocyte. The yellow rectangles indicated that the expression level of the proteins was similar in both GV and MII. (*B*) The LIF-STAT3 signaling pathway proteins expressed in oocyte and zygote. The blue rectangles indicated that the proteins expressed more in zygote. The red rectangles indicated that the proteins expressed more in MII oocyte. The yellow rectangles indicated that the expression level of the proteins was similar in both the zygote and MII oocyte.

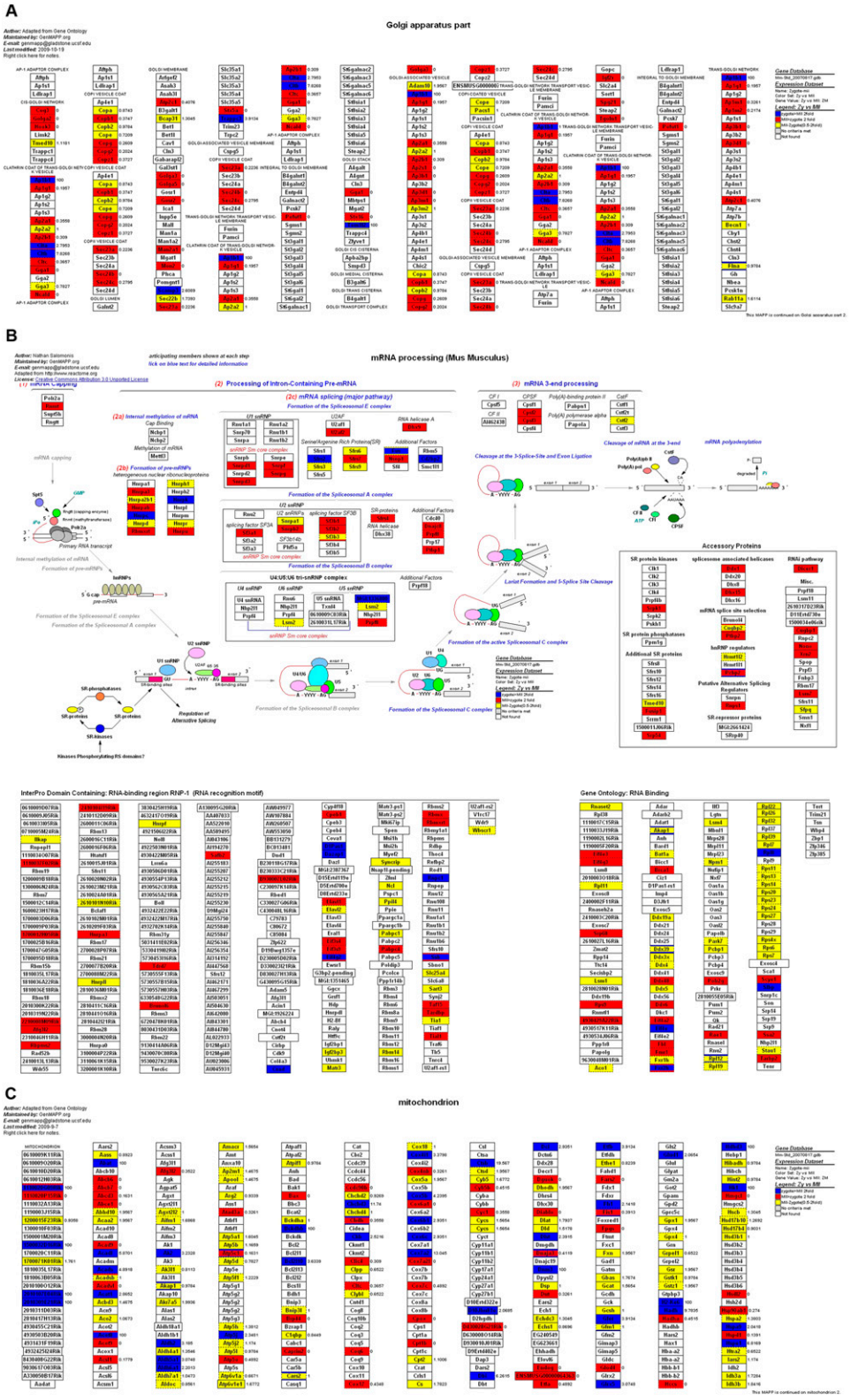

Fig. S4. (*A*) The Golgi proteins expressed in the GV oocyte and MII oocyte. The blue rectangles indicated that the proteins expressed more in the GV oocyte. The red rectangles indicated that the proteins expressed more in MII oocyte. The yellow rectangles indicated that the expression level of the proteins was similar in both GV and MII oocyte. (*B*) The mRNA processing proteins in oocyte and zygote. The blue rectangles indicated that the proteins expressed more in zygote. The red rectangles indicated that the proteins expressed more in MII oocyte. The yellow rectangles indicated that the expression level of the proteins was similar in both the zygote and MII oocyte. (*C*) Mitochondrion proteins expressed in oocyte and zygote. The blue rectangles indicated that the proteins expressed more in zygote. The red rectangles indicated that the proteins expressed more in MII oocyte. The yellow rectangles indicated that the expression level of the proteins was similar in both the zygote and MII oocyte.

Other Supporting Information Files

Dataset S1 (XLS)
Dataset S2 (XLS)
Dataset S3 (XLS)
Dataset S4 (XLS)