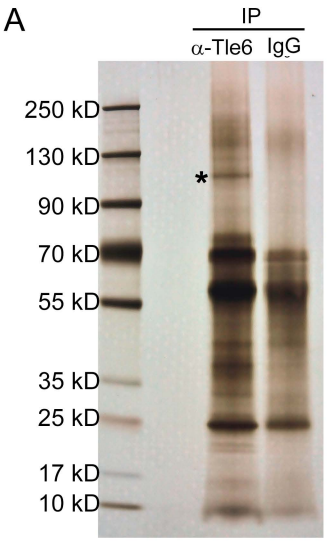
Figure S1

A



B

Proteins	Score	Coverage	Peptides	MV(kDa)
Nlrp5	80.69	39.06	36	125.4
Tle6	51.80	44.92	20	65.0
Ooep	5.44	25.00	4	18.4
Khdc3	22.77	42.49	12	37.9
Zbed3	11.76	34.21	9	25.6
Nlrp4f	22.54	18.89	14	107.8

Figure S1. Identification of Nlrp4f as a potential component of the SCMC by mass spectrometry (Modified from our previous report (Gao et al., 2018)). (A) Normal GV oocytes were precipitated with anti-Tle6 antibody, and IgG (negative control). The precipitated produces were separated by SDS-PAGE, examined by silver staining and analyzed by mass spectrometry. The asterisk indicated the possible band of Nlrp4f estimated by its molecular weight. (B) The information of mass spectrometry was shown for the known SCMC components and Nlrp4f.

Figure S2

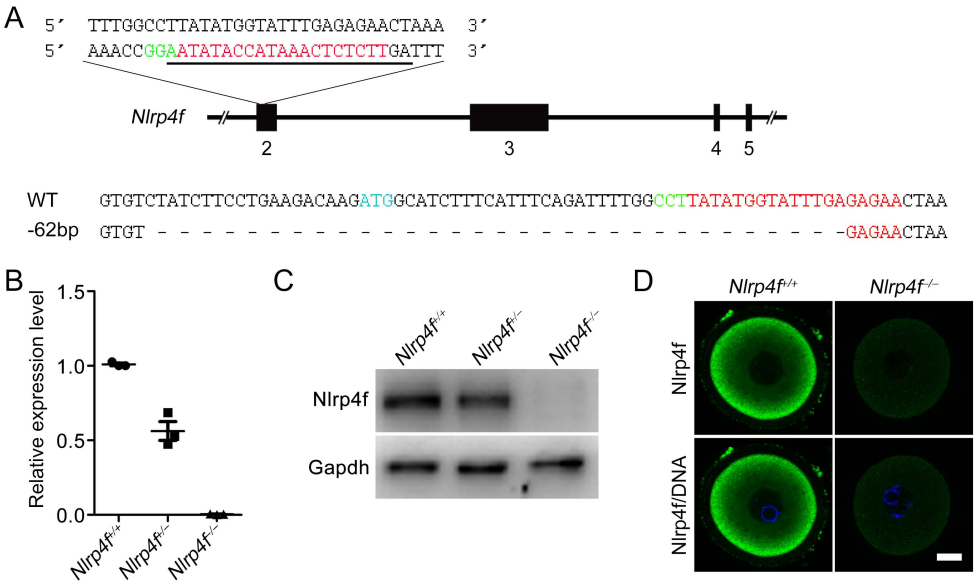


Figure S2. Generation of *Nlrp4f* knockout mice. (A) A schematic of the generation of *Nlrp4f* knockout mice using CRISPR/Cas9. The black rectangles are exons. The sgRNA-targeting sequence is underlined and shown in red, and the protospacer-adjacent motif (PAM) sequence is in green. The initiation codon ATG was labeled in blue. The sequences of normal (WT) and mutant alleles of the founder mice were also shown. (B) qRT-PCR analysis of *Nlrp4f* mRNA expression in *Nlrp4f*$^{+/+}$, *Nlrp4f*$^{-/+}$ and *Nlrp4f*$^{-/-}$ oocytes. The data was normalized to the abundance of *Gapdh* mRNA. The results were from three independent experiments. (C) Immunoblot analysis of Nlrp4f expression in *Nlrp4f*$^{+/+}$, *Nlrp4f*$^{-/+}$ and *Nlrp4f*$^{-/-}$ MII oocytes. Gapdh was used as an internal reference. (D) Immunofluorescent staining of GV oocytes from *Nlrp4f*$^{+/+}$ and *Nlrp4f*$^{-/-}$ mice with Nlrp4f antibody and Hochest 33342 (DNA). Scale bar: 20 μm.

Figure S3

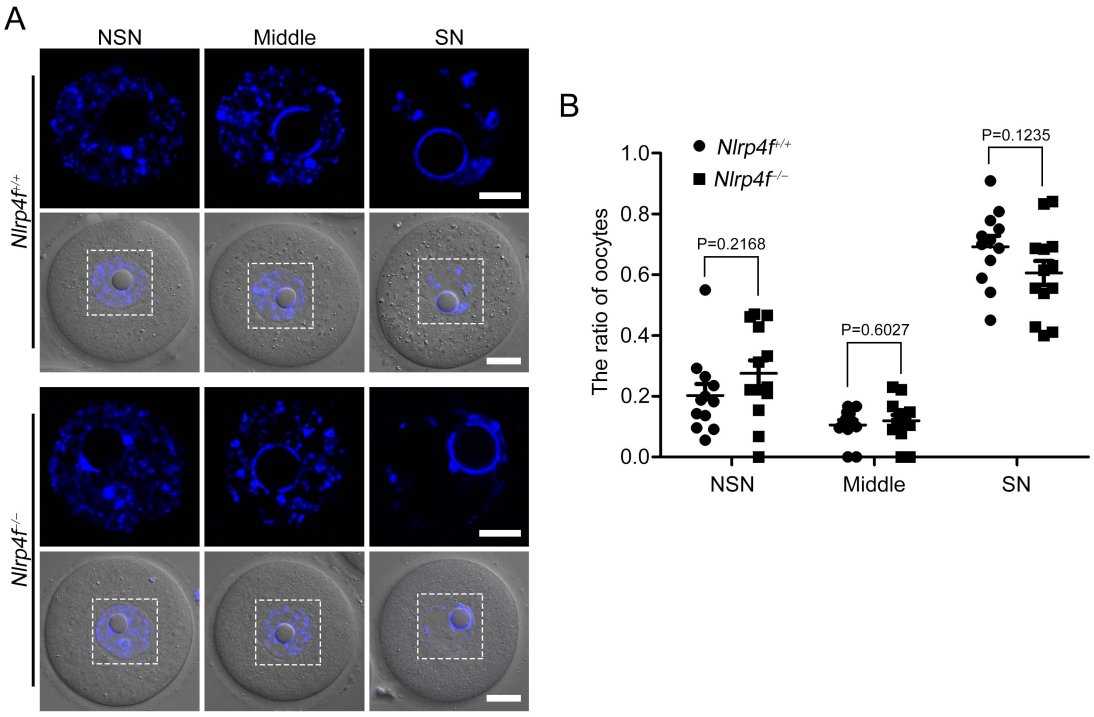


Figure S3. Chromatin configurations in fully grown oocytes. (A) Fully grown oocytes from *Nlrp4f*$^{+/+}$ and *Nlrp4f*$^{-/-}$ females were stained with Hochest 33342 for DNA. According to their chromatin configuration of DNA, the oocytes were classified into three types, NSN, Middle and SN type. The nucleus was dotted with white color and magnified. Scale bar in the upper panel: 10 μm. Scale bar in the down panel: 20 μm. (B) GV oocytes from *Nlrp4f*$^{+/+}$ (n = 12) and *Nlrp4f*$^{-/-}$ (n = 13) female mice were classified into three groups, and the ratio was calculated by the number of NSN, Middle and SN dividing the number of total oocytes. Error bars, s.e.m.

Figure S4

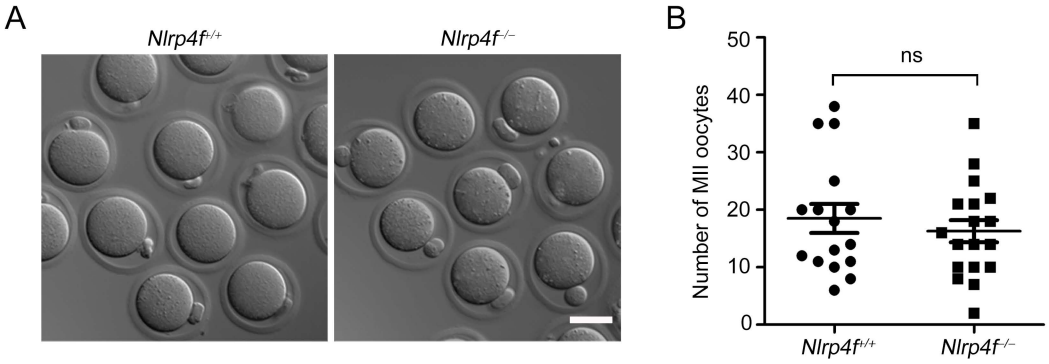


Figure S4. Ovulation in *Nlrp4f* null mice. (A) Representative images of bright field of *Nlrp4f*$^{+/+}$ and *Nlrp4f*$^{-/-}$ MII oocytes after superovulation. Scale bar: 50 μm. (B) The number of MII oocytes from *Nlrp4f*$^{+/+}$ and *Nlrp4f*$^{-/-}$ mice after superovulation. Error bars, s.e.m. ns, no significant.

Figure S5

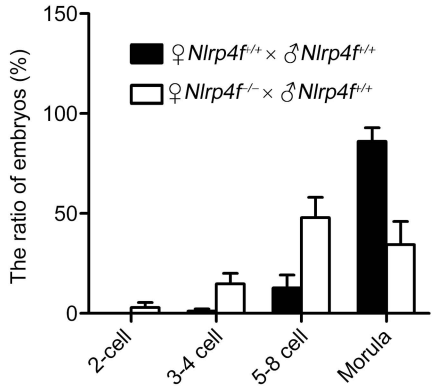


Figure S5. Abnormal development in embryos with depletion of maternal Nlrp4f. The ratio of embryos at different development stages from *Nlrp4f*$^{+/+}$ and *Nlrp4f*$^{-/-}$ females at E2.5.

Figure S6

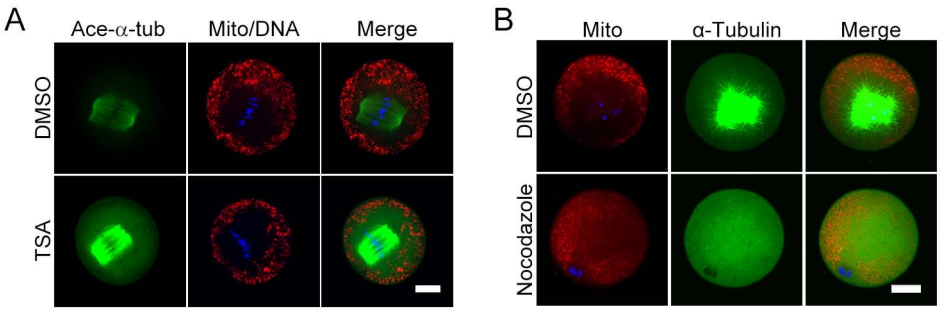


Figure S6. Drug treatment of *Nlrp4f*$^{-/-}$ oocytes. (A) *Nlrp4f*$^{-/-}$ oocytes were labeled with MitoTracker (red) after the treatment with DMSO or TSA at GVBD 1-2 h, then fixed and stained with anti-acetylated-α-tubulin antibody (Ace-α-tubulin, green) and Hochest 33342 (DNA, blue). Scale bar: 20 μm. (B) *Nlrp4f*$^{-/-}$ oocytes were labeled with MitoTracker (red) the treatment with DMSO or Nocodazole at GVBD 1-2 h, then fixed and stained with anti-α-tubulin antibody (green) and Hochest33342 (DNA, blue). Scale bar: 20 μm.

Figure S7

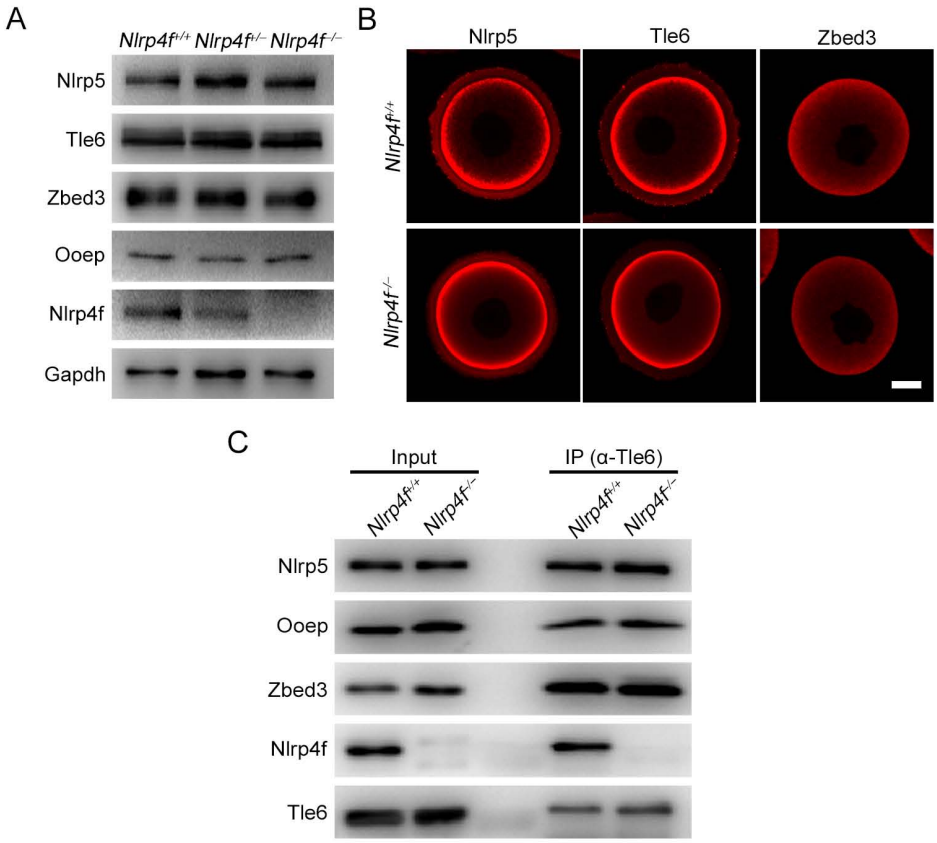


Figure S7. The expression patterns of the known SCMC components in *Nlrp4f*$^{-/-}$ oocytes. (A) Immunoblot of *Nlrp4f*$^{+/+}$, *Nlrp4f*$^{-/+}$ and *Nlrp4f*$^{-/-}$ GV oocytes with anti-Nlrp5, -Tle6, -Zbed3, -Ooep and -Nlrp4f antibodies. Gapdh was used as a loading control. (B) Immunofluorescent staining of GV oocytes from *Nlrp4f*$^{+/+}$ and *Nlrp4f*$^{-/-}$ females with anti-Nlrp5, -Tle6 and -Zbed3 antibodies. Scale bar: 20 μm. (C) Co-immunoprecipitation of GV oocytes (200) from *Nlrp4f*$^{+/+}$ and *Nlrp4f*$^{-/-}$ females with anti-Tle6 antibody, followed by immunoblot with specific antibodies for the SCMC proteins.

Figure S8

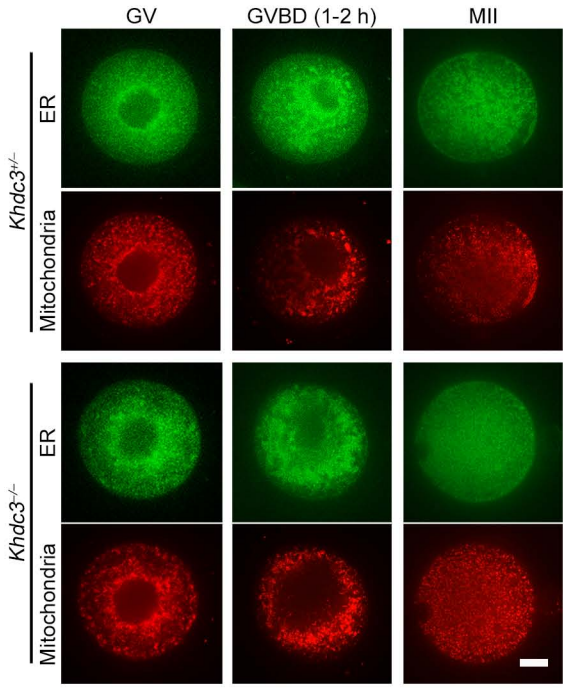
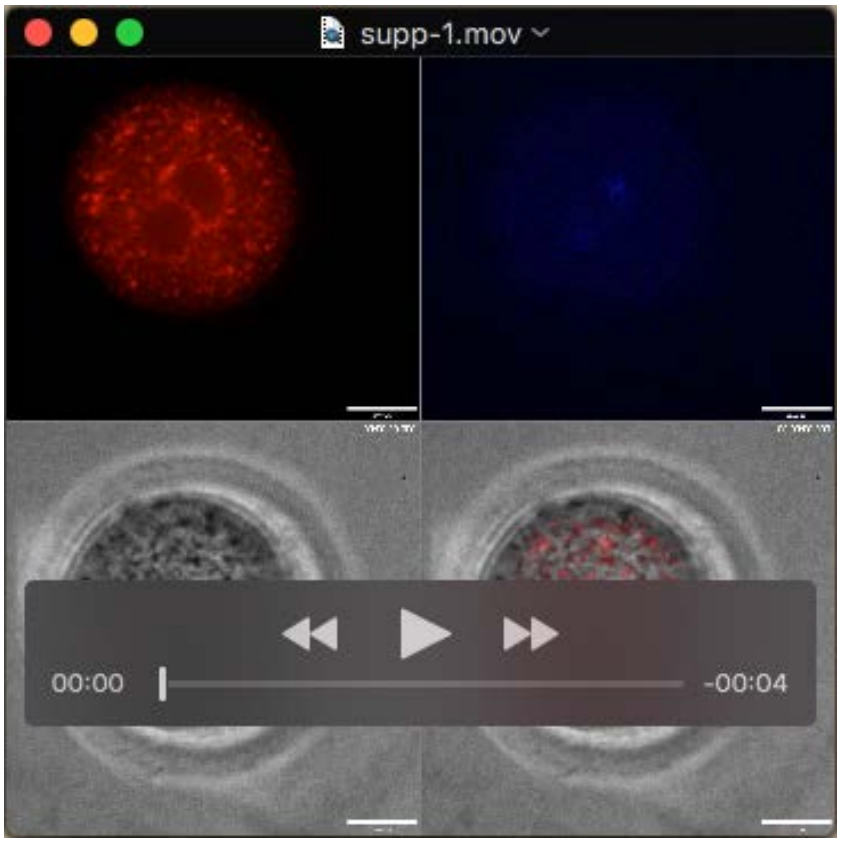


Figure S8. Disordered organelle distribution in *Khdc3*$^{-/-}$ oocytes. The oocytes were isolated from *Khdc3*$^{+/-}$ and *Khdc3*$^{-/-}$ females and were labeled with ER-Tracker (green) and MitoTracker (red) for ERs and mitochondria. *Khdc3*$^{-/+}$ (GV, n = 18; GVBD 1-2 h, n = 19; MII, n = 22) and *Khdc3*$^{-/-}$ (GV, n = 24; GVBD 1-2 h, n = 31; MII, n = 34) oocytes were investigated in three independent experiments. Scale bar: 20 μm.

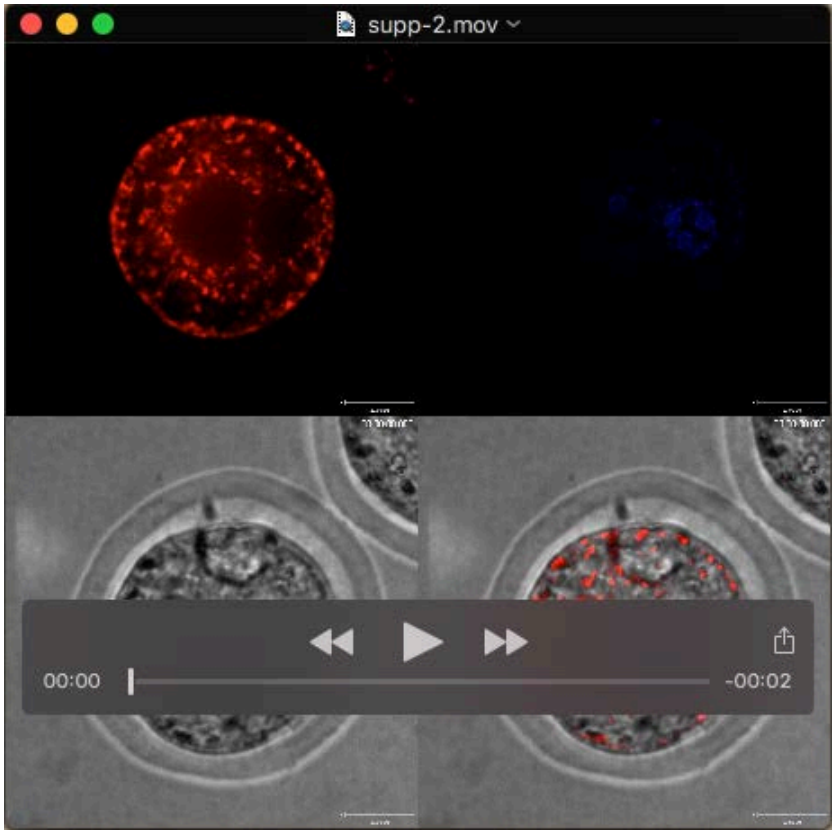
Table 1 The list of antibodies

Primary antibodies			
Antibody	For immunoblot	For immunofluorescence	Source
Mouse anti-Nlrp5	1:1000	1:200	
Mouse anti-Tle6	1:1000	1:200	
Rabbit anti-Ooep	1:2000	-	
Rabbit anti-Zbed3	1:2000	1:200	
Sheep anti-Khdc3	1:500	-	
Rabbit anti-Nlrp4f	1:2000	1:200	Produced by Abmart
Mouse anti-Gapdh	1:5000	-	Sungene biotech, KM9002T
Mouse anti-β-actin	1:5000	-	Sungene biotech, KM9001T
Mouse anti-Acetylated-α-tubulin	1:1000	-	Abcam, ab24610
Rabbit anti-α-tubulin	1:1000	-	Cell signaling, 2144
Mouse anti-α-tubulin-FITC	-	1:200	Sigma, F2168

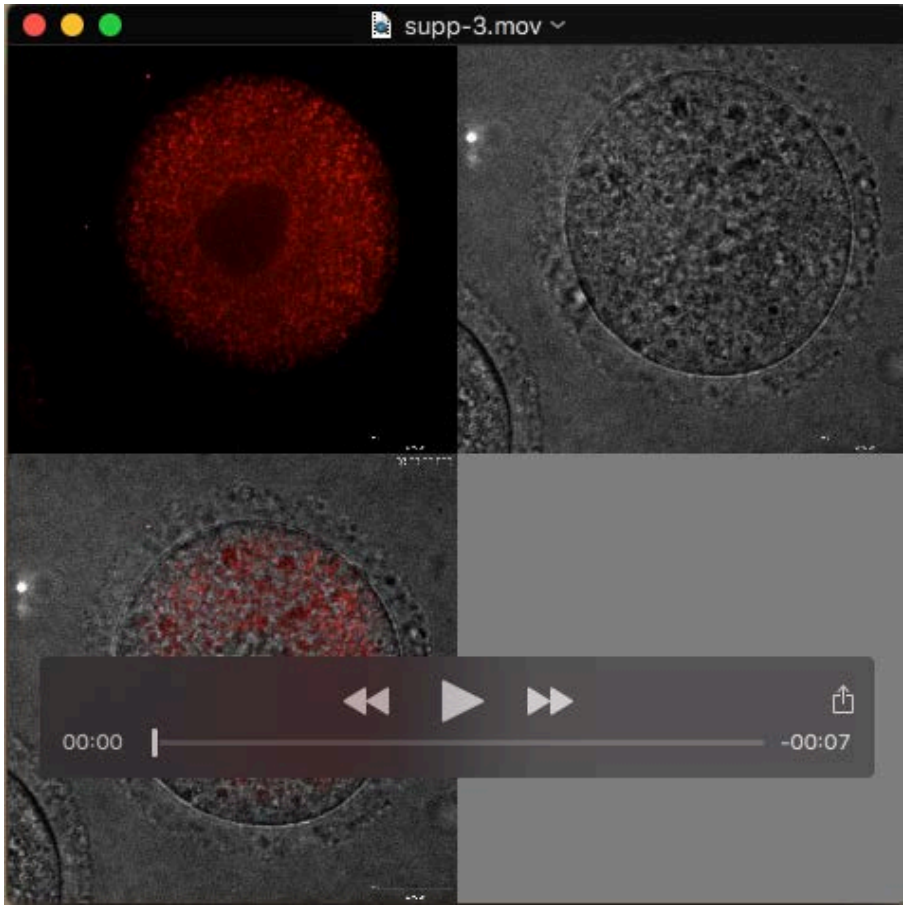
Secondary antibodies			
Antibody	For immunoblot	For immunofluorescence	Source
Alexa Fluor® 488 AffiniPure Donkey Anti-Rabbit IgG	-	1:500	Jackson Immuno Research, 711-545-152
Alexa Fluor® 594 AffiniPure Donkey Anti-Mouse IgG	-	1:500	Jackson Immuno Research, 715-585-150
Peroxidase AffiniPure Goat Anti-Rabbit IgG	1:5000	-	Jackson Immuno Research, 111-035-003
Peroxidase AffiniPure Goat Anti-Mouse IgG	1:5000	-	Jackson Immuno Research, 115-035-003
Peroxidase AffiniPure Donkey Anti-Sheep IgG	1:2000	-	Jackson Immuno Research, 713-035-003



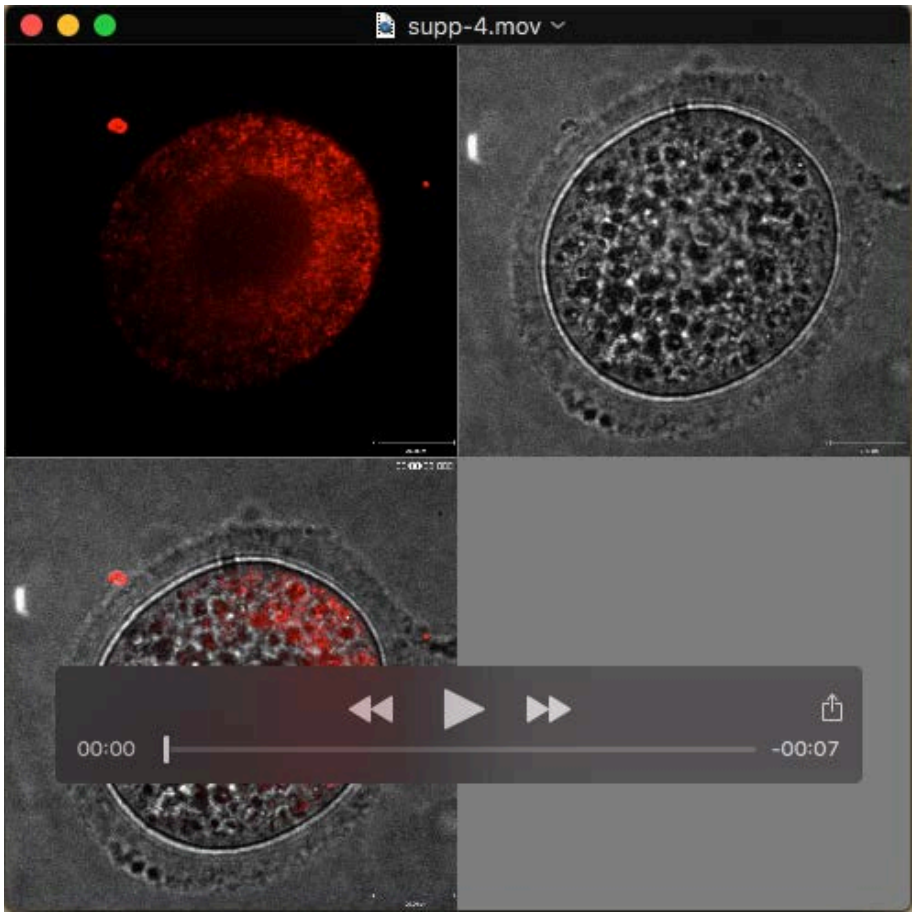
Movie 1. Mitochondria dynamics in the embryos from *Nlrp4f*$^{+/+}$ females during 1-cell to 2-cell development. Zygotes were isolated from *Nlrp4f*$^{+/+}$ females at 24 h after hCG stimulation, labeled with MitoTracker for mitochondria and Hochest 33342 for DNA and cultured to 2-cell stage. Time-lapse images were captured every 30 mins with UltraVIEW-VoX. Related to Fig. 4B.



Movie 2. Mitochondria dynamics in the embryos from *Nlrp4f*$^{-/-}$ females during 1-cell to 2-cell development. Zygotes were isolated from *Nlrp4f*$^{-/-}$ females at 26 hrs after hCG stimulation, labeled with MitoTracker for mitochondria and Hochest 33342 for DNA and cultured to 2-cell stage. Time-lapse images were captured every 30 mins with UltraVIEW-VoX. Related to Fig. 4B.



Movie 3. Mitochondria dynamics in the oocytes from *Nlrp4f*$^{+/+}$ females during GV to MII maturation. GV oocytes from *Nlrp4f*$^{+/+}$ females were labeled with MitoTracker for mitochondria and cultured to MII stage. Time-lapse images were captured every 30 mins with UltraVIEW-VoX. Related to Fig. 4D.



Movie 4. Mitochondria dynamics in the oocytes from *Nlrp4f*$^{-/-}$ females during GV to MII maturation. GV oocytes from *Nlrp4f*$^{-/-}$ females were labeled with MitoTracker for mitochondria and cultured to MII stage. Time-lapse images were captured every 30 mins with UltraVIEW-VoX. Related to Fig. 4D.