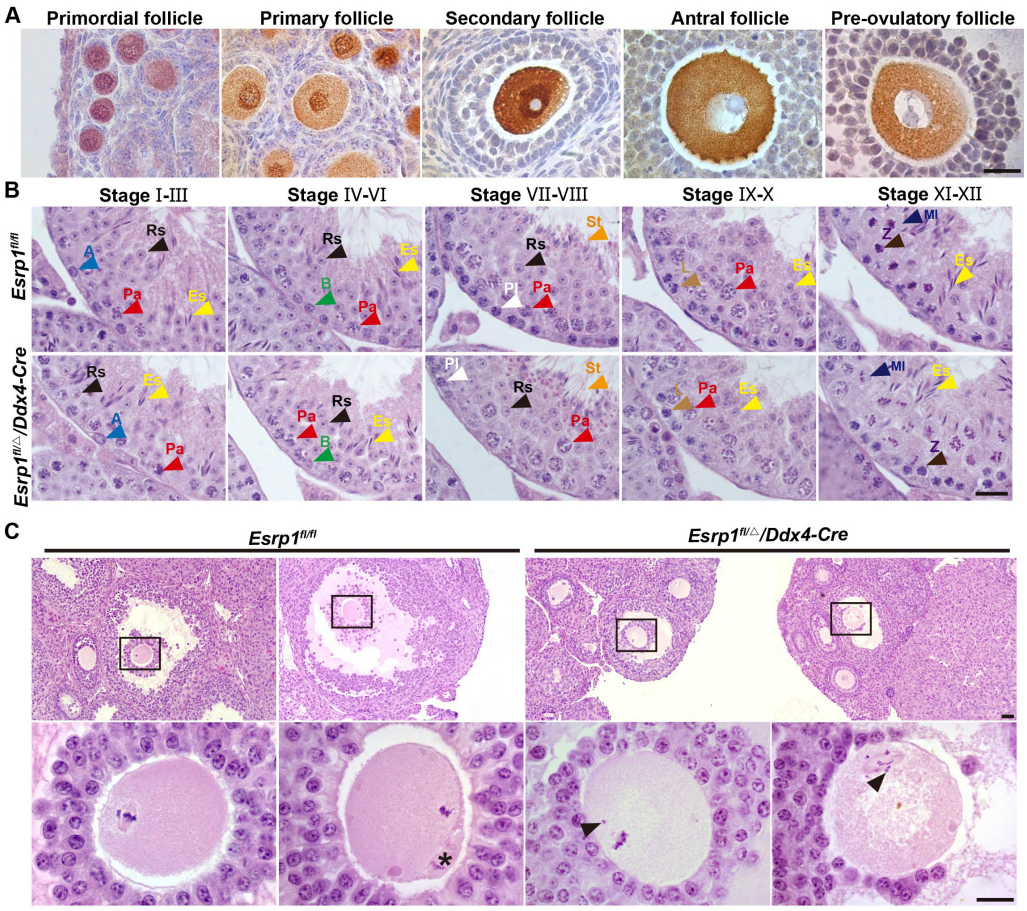

Figure S1. Expression and subcellular localization of ESRP1 during follicular development. (A) An immunohistochemistry (IHC) of ESRP1 in ovarian sections showing ESRP1 expression in follicles at indicated developmental stages. Scale bars, 20 μm. (B) HE staining of adult *Esrp1*$^{fl/fl}$ and *Esrp1*$^{fl/\triangle}$*/Ddx4-Cre* testicular sections. Scale bars, 20 μm. Abbreviations: A: Type A spermatogonia; B: Type B spermatogonia; PI: Preleptotene spermatocyte; L: Leptotene spermatocyte; Z: Zygotene spermatocyte; Pa: Pachytene spermatocyte; MI: Post-prophase I meiotic phase; Rs: Round spermatid; Es: Elongated spermatid; St: spermatozoa. (C) HE staining of six-week-old *Esrp1*$^{fl/fl}$ and *Esrp1*$^{fl/\triangle}$*/Ddx4-Cre* ovary sections. The magnified areas are shown in low panels. Asterisks indicate the PB1, and black arrowheads indicate scattered chromosomes. Scale bars, 20 μm.

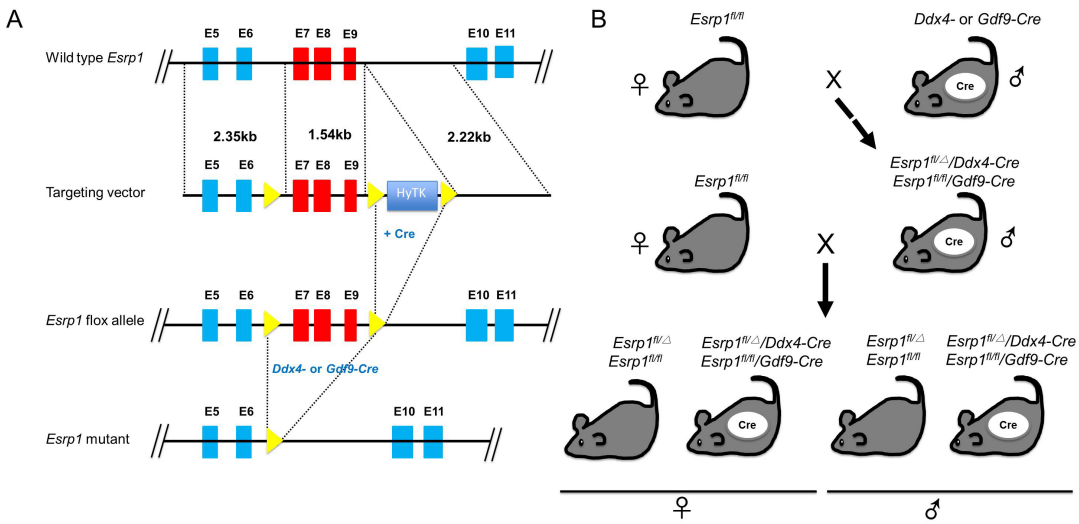

Figure S2. Generation of *Esrp1* conditional-knockout mice. (A) Construction of *Esrp1*$^{Flox/Flox}$ and generation of *Esrp1*$^{fl/\triangle}$*/Ddx4-Cre* and *Esrp1*$^{fl/fl}$*/Gdf9-Cre* mice. Exons 7–9 of *Esrp1* were deleted by *Ddx4-Cre*-mediated or *Gdf9-Cre*-mediated recombination. (B) Schematic of the breeding scheme to produce germline-specific *Esrp1* knockout in *Esrp1*$^{fl/\triangle}$*/Ddx4-Cre* mice and oocyte-specific *Esrp1* knockout in *Esrp1*$^{fl/fl}$*/Gdf9-Cre* mice.

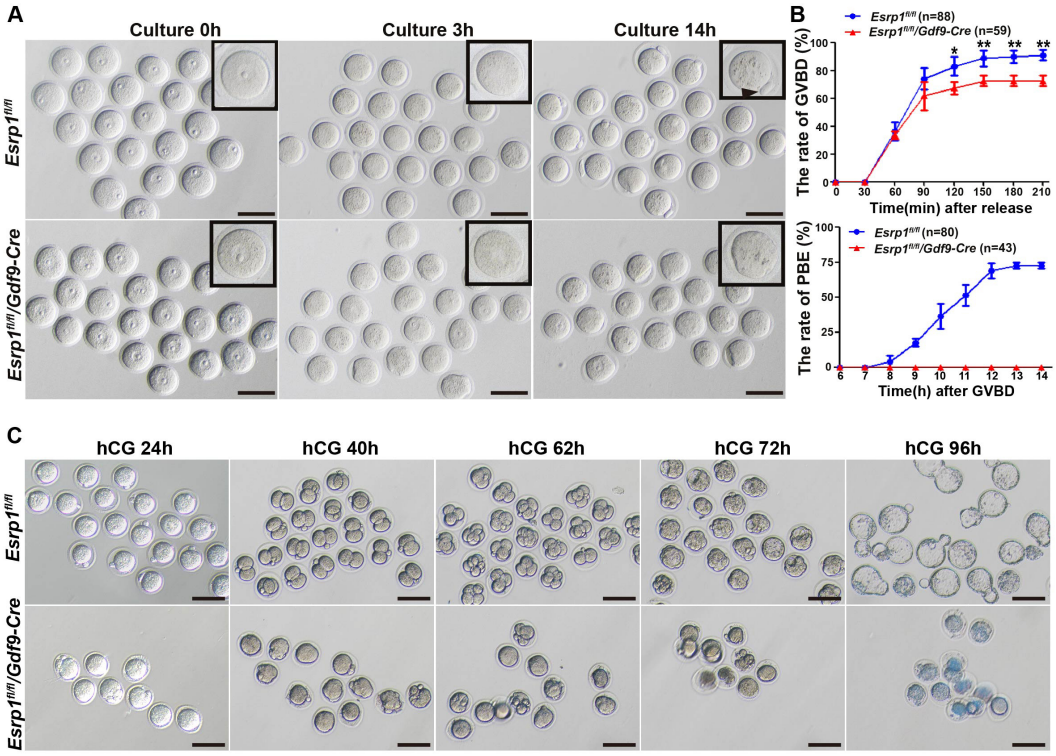

Figure S3. Compromised meiosis and embryonic development in *Esrp1*-knockout oocytes. (A) DIC imaging of the progression of meiosis in *Esrp1*$^{fl/fl}$ and *Esrp1*$^{fl/fl}$*/Gdf9-Cre* oocytes in culture. Time indicates hours after oocytes were released from meiotic arrest. Scale bar, 100 mm. (B) GVBD and PBE rates of cultured oocytes shown in (A). n: number of used oocytes. (C) DIC imaging of *Esrp1*$^{fl/fl}$ and *Esrp1*$^{fl/fl}$*/Gdf9-Cre* female embryos cultured *in vitro*. Embryonic development was monitored at the indicated time points after hCG administration. Scale bar, 100 μm.

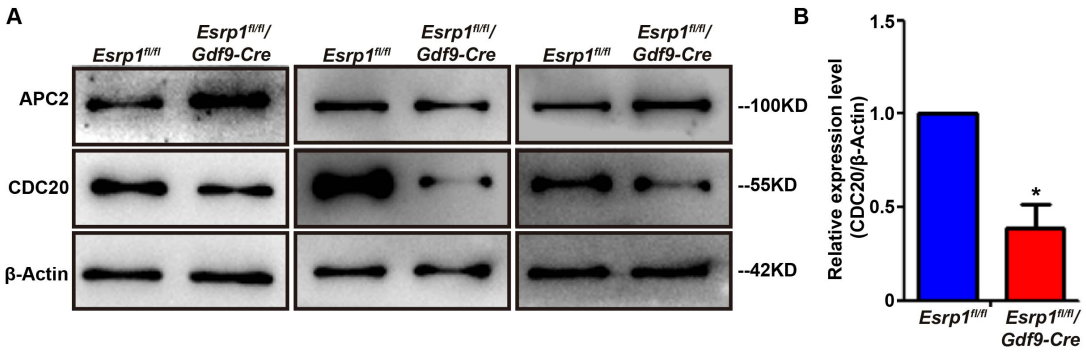

Figure S4. Insufficient APC/C activity in *Esrp1*- knockout oocytes.

(A) Western-blot analysis of APC2 and CDC20 in oocytes collected after 10 h in culture from *Esrp1*$^{fl/fl}$ and *Esrp1*$^{fl/fl}$*/Gdf9-Cre* mice. β-actin served as a loading control. (B) Relative abundances of CDC20 in oocytes from *Esrp1*$^{fl/fl}$ and *Esrp1*$^{fl/fl}$*/Gdf9-Cre* mice were measured by Western-blot analyses shown in (A) (*P < 0.05).

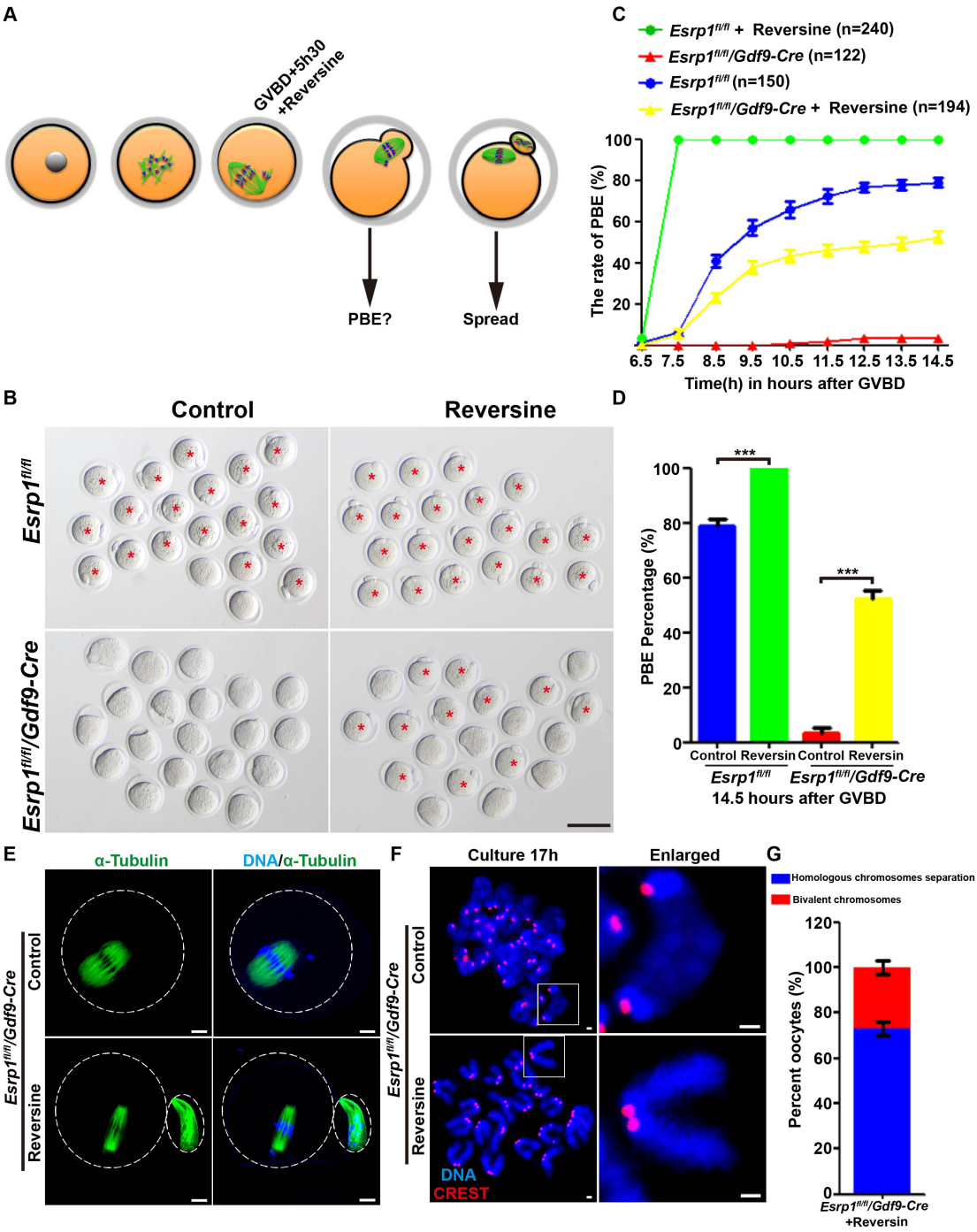

Figure S5. Metaphase I arrest in *Esrp1*-knockout oocytes is rescued via inhibition of SAC activity. (A) Oocytes were treated with reversine (0.5 μM) at the indicated time to override a potential SAC arrest. (B) DIC imaging of *Esrp1*$^{fl/fl}$ and *Esrp1*-knockout oocytes treated with reversine shows that they completed the first meiosis, as determined by PBE cultured for 16.5 h. The asterisk indicates the PB1. Scale bar, 100 μm. At least three independent experiments involving a total of at least six mice

were performed. (C) Time of PBE of *Esrp1*$^{fl/fl}$ and *Esrp1*$^{fl/fl}$*/Gdf9-Cre* oocytes. Reversine was added at GVBD+5.5 h, where indicated. n: number of used oocytes. (D) Statistical analysis of the PBE rates of oocytes shown in (B). Data from *Esrp1*$^{fl/fl}$ oocytes (control: 78.96% ± 2.31%) and (reversine: 100%) are shown. Data from *Esrp1*$^{fl/fl}$ */Gdf9-Cre* oocytes (control: 3.49% ± 1.78%) and (reversine: 52.58%± 2.51%) are shown (***P < 0.001). (E) Immunofluorescent staining with α-tubulin to visualize spindles (green) and co-stained with DAPI to visualize chromosomes (blue) show *Esrp1*-knockout oocytes had extruded PB1 after reversine treatment. Scale bar, 10 μm. (F) Oocytes were spread 17 h after culture. Kinetochores were stained with CREST (red) and chromosomes with DAPI (blue). *Esrp1*-knockout oocytes had entered to metaphase II with univalent sister chromatids after reversine treatment. Insets show typical chromosome figures observed. Scale bar, 1 μm. (G) The proportions of homologous chromosomes separation and bivalent chromosomes in *Esrp1*- knockout oocytes that failed to extrude the PB1 after reversine treatment.

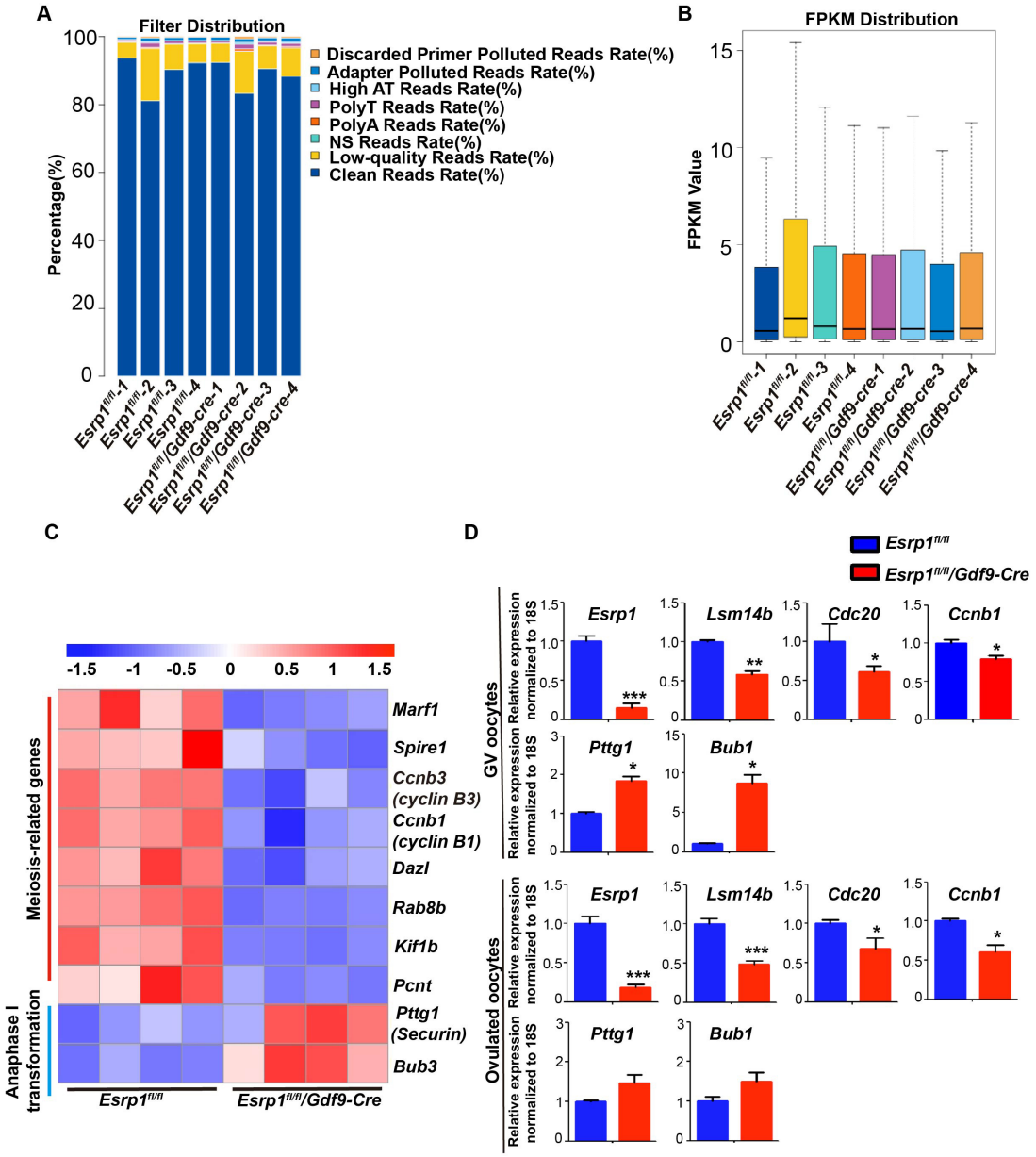

Figure S6. ESRP1 regulates mRNA transcription involved in oocytes meiosis. (A) Filter distribution in each sample; the clean reads were filtered for analyses. (B) Distribution of global gene expression in each sample. (C) A heatmap showing the transcript level of key genes that were involved in meiosis in *Esrp1*$^{fl/fl}$ and *Esrp1*$^{fl/fl}$*/Gdf9-Cre* oocytes. The color indicates the expression level. (D) Real-time RT-PCR analysis of mRNA levels of a cohort of genes. The mRNA levels of *Esrp1, Lsm14b, cdc20,*and *Ccnb1* were decreased in both GV-stage and ovulated oocytes from *Esrp1*$^{fl/fl}$*/Gdf9-Cre* mice compared to those in *Esrp1*$^{fl/fl}$ mice. The mRNA levels of *Bub1*and *Pttg1* were increased in both GV-stage and ovulated oocytes from *Esrp1*$^{fl/fl}$*/Gdf9-Cre* mice compared to those in *Esrp1*$^{fl/fl}$ mice. (*$p < 0.05$, **$p < 0.01$ and***$p < 0.001$).

Table S1. Primers used in this study.

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Table S2. Primary antibodies and secondary antibodies used in this study.

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Table S3. List of significantly changed transcripts in *Esrp1*-knockout oocytes identified by RNAseq analysis.

Click here to Download Table S3

Table S4. List of significant alternative-splicing events in *Esrp1*-knockout oocytes identified by CASH.

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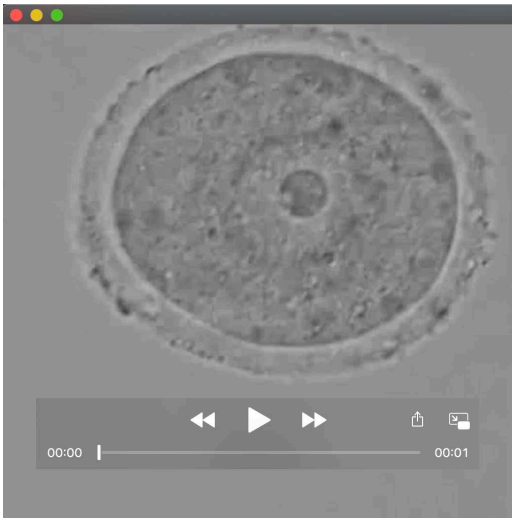
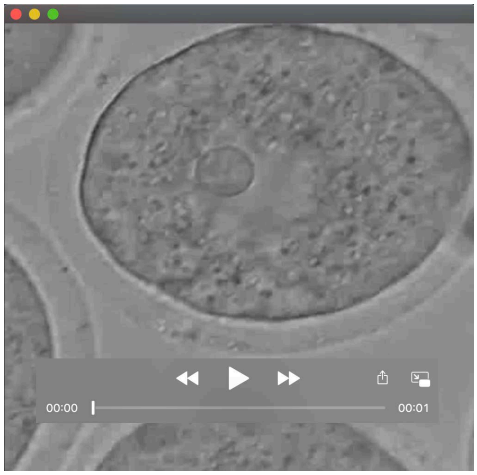
Movies 1. A representative movie showing normal meiosis in a control oocyte.



Movies 2. A representative movie showing no GVBD in an *Esrp1*-knockout oocyte.



Movies 3. A representative movie showing no GVBD in a different *Esrp1*-knockout oocyte.