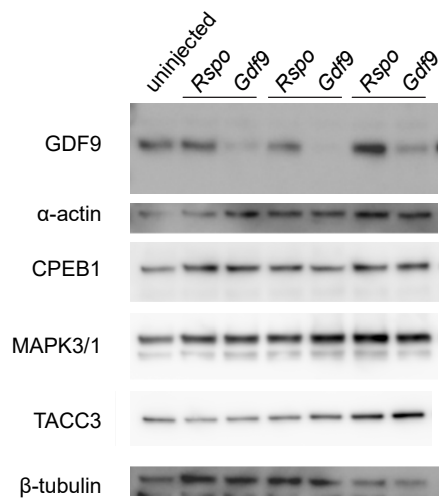


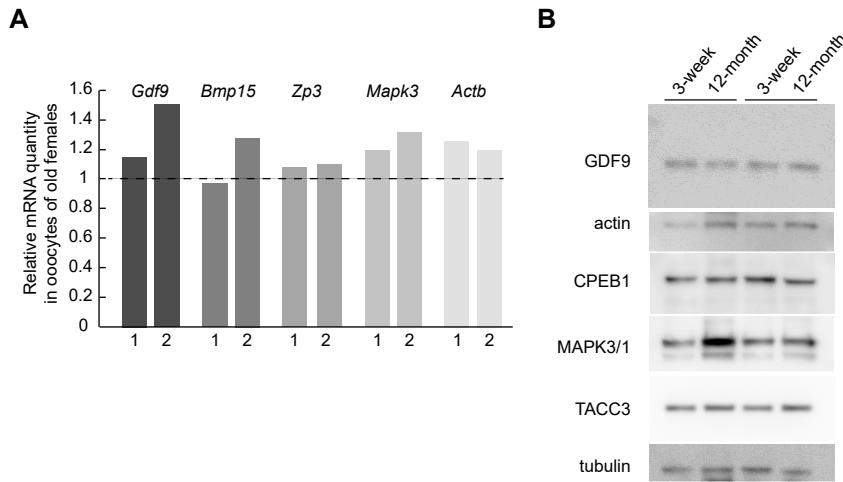
**Figure S1. The number of TZPs increases during oocyte growth (related to Figure 2).**

**(A)** Living oocytes at different stages of growth were stained using FM1-43 and imaged using confocal microscopy. Using a confocal optical section at the equatorial plane of the oocyte, the diameter was measured and the number of TZPs was quantified. Left: representative images of early- and late-stage growth oocytes. Bar = 20  $\mu\text{m}$ . Right: Number of TZPs plotted as a function of oocyte diameter. **(B)** Model for elaboration of new TZPs. As the surface area of the growing oocyte expands, mitotic proliferation of the granulosa cells (light brown) produces new cells (dark brown) that initially lack TZPs. These elaborate new TZPs that penetrate through the zona pellucida (blue) to reach the oocyte surface (pink).



**Figure S2. Injected RNAi selectively depletes GDF9 from oocytes (related to Figure 3).**

Oocytes within GOCs were injected with siRNA targeting either Rspo (control) or Gdf9. Following incubation for 5 days, the oocytes were separated from the granulosa cells and subjected to immunoblotting using the indicated antibodies. Three independent biological replicates were performed. The left-most lane shows uninfected oocytes. All samples were transferred onto a single membrane for analysis. Thirty oocytes per lane.



**Figure S3. GDF9 and other factors are not depleted in oocytes of aged females (related to Figure 4).**

**(A)** Fully grown oocytes in antral follicles were collected from 3-month and 13-month old females on the same day and used to quantify the indicated mRNAs. Rabbit globin mRNA was added before RNA purification as a standard. The experiment was performed twice, using 22 oocytes per group in replicate 1 and 27 oocytes per group in replicate 2. The amount of each mRNA in the oocytes from 13-month mice is expressed relative to the amount in the 3-month mice, which has been set to a value of 1. **(B)** Fully grown oocytes were collected from 3-week and 12-month old females on the same day and used for immunoblotting. The experiment was performed twice. Thirty oocytes per lane. The partially visible band at the bottom of the second lane in the tubulin blot is MAPK3, which was previously assayed on the membrane.