Supplementary Materials and Methods

Mice and breeding

The following mouse strains were used: CF-1 females (6-10 weeks, Harlan Laboratories), C57BL/6J females (6-10 weeks, Jackson Laboratories), and B6SJLF1/J males (2-6 months, Jackson Laboratories). Methods for the generation and genotyping of *Rgs2*$^{-/-}$ mice were reported previously (Oliveira-Dos-Santos et al., 2000). *Rgs2*$^{-/-}$ females, 6-10 weeks of age, were generously provided by Dr. Yaping Tu (Creighton Univ. School of Medicine). C57BL/6J females were used to obtain *Rgs2*$^{+/+}$ control eggs for ACh and ZP2 conversion experiments. For analysis of litter sizes, *Rgs2*$^{+/+}$ and *Rgs2*$^{-/-}$ mice were housed in breeding cages of one male with 1-3 females. Pregnant females were separated into individual cages prior to delivery, and pups were counted at birth. Litter data was collected for a period of 32 months from 8 (*Rgs2*$^{-/-}$ male x *Rgs2*$^{-/-}$ female) and 11 (*Rgs2*$^{+/+}$ male x *Rgs2*$^{+/+}$ female) breeding cages and for 10 months from 2 (*Rgs2*$^{-/-}$ male x *Rgs2*$^{+/+}$ female) breeding cages. All mice were maintained under approved protocols at each institution and complied with the Institute of Laboratory Animal Research Guide for the Care and Use of Laboratory Animals.

Culture media

In vitro maturation and post-injection culture were performed in minimal essential medium alpha (MEMα; Life Technologies) containing 5% calf serum (Atlanta Biologicals). For in vitro fertilization experiments, ZP-free eggs were loaded with 10 μM fura-2 AM (Life Technologies) in potassium simplex optimized medium (KSOM; EMD Millipore) containing 0.04% pluronic F-127 (Life Technologies) for 30 minutes. Eggs were adhered to Cell-Tak-coated (EMD Millipore) glass-bottom dishes in 90 μL BSA-free KSOM covered with mineral oil. Sperm were prepared in human tubal fluid (HTF; EMD Millipore) and were added to the imaging dish at a final concentration of 10^5 sperm/mL along with 4 μL HTF containing 30

mg/mL BSA to bring the final BSA concentration to 1.25 mg/mL. Acid and ACh addition experiments were performed in Leibovitz L-15 medium (L-15; Life Technologies). Ca^{2+}- and Mg^{2+}-free CZB medium without BSA or polyvinyl alcohol was prepared in-house (Chatot et al., 1989).

Ca^{2+} store measurements

Eggs were loaded with 10 μM fura-2 AM for 30 min in MEMα containing 5% calf serum and 0.04% pluronic F-127. The eggs were then washed and adhered to glass-bottom dishes in 2 mL of Ca^{2+}- and Mg^{2+}-free CZB without BSA or polyvinyl alcohol. In all experiments, control and experimental eggs were assayed at the same time in the same treatment dish. Baseline ratiometric imaging was performed for at least 5 min, followed by addition of thapsigargin to a final concentration of 10 μM. Imaging of F340 and F380 was performed as described previously (Miao et al., 2012). Area under the curve was measured for the first 10 min and was calculated using trapezoidal area for baseline-subtracted curves. Maximum amplitude was also determined relative to baseline measurements prior to thapsigargin addition. Both measurements were expressed relative to the mean value of control eggs from the same experiment.

Antibodies and DNA construct

Mouse monoclonal anti-RGS2 antibody (M01, clone 4C4; Abnova) was diluted 1:100. Rat monoclonal anti-ZP2 antibody (M2c.2) was kindly provided by Jurrien Dean (Rankin et al., 2003) and was diluted 1:500. Secondary antibody was peroxidase-conjugated anti-mouse or anti-rat IgG (Santa Cruz). FITC-conjugated anti-alpha-tubulin antibody (Sigma) was diluted 1:100 and rhodamine-phalloidin (Life Technologies) was used at a final concentration of 2 U/ml. The *HA-Rgs2* cRNA expression construct, in pcDNA3.1+, was obtained from Missouri S&T cDNA Resource Center (Rolla, MO).

Primers used

Rgs2 forward	5'-TTCTGGTTGGCTTGTGAAGA-3'
Rgs2 reverse	5'-CTTCTGAGCTGTGGTGAAGC-3'
Gpr68 forward	5'-CTCCTCCTCACCAGCTTCAA-3'
Gpr68 reverse	5'-CAGGTAAGGACAGCTAGGCA-3'
EGFP forward	5'-AGAACGGCATCAAGGTGAAC-3'
EGFP reverse	5'-TGCTCAGGTAGTGGTTGTCG-3'

Supplemental References

Chatot, C. L., Ziomek, C. A., Bavister, B. D., Lewis, J. L. and Torres, I. (1989). An improved culture medium supports development of random-bred 1-cell mouse embryos in vitro. *J. Reprod. Fertil.* **86**, 679-688.

Miao, Y. L., Stein, P., Jefferson, W. N., Padilla-Banks, E. and Williams, C. J. (2012). Calcium influx-mediated signaling is required for complete mouse egg activation. *Proc. Natl. Acad. Sci. USA* **109**, 4169-4174.

Oliveira-Dos-Santos, A. J., Matsumoto, G., Snow, B. E., Bai, D., Houston, F. P., Whishaw, I. Q., Mariathasan, S., Sasaki, T., Wakeham, A., Ohashi, P. S., et al. (2000). Regulation of T cell activation, anxiety, and male aggression by RGS2. *Proc. Natl. Acad. Sci. USA* **97**, 12272-12277.

Rankin, T. L., Coleman, J. S., Epifano, O., Hoodbhoy, T., Turner, S. G., Castle, P. E., Lee, E., Gore-Langton, R. and Dean, J. (2003). Fertility and taxon-specific sperm binding persist after replacement of mouse sperm receptors with human homologs. *Dev. Cell* **5**, 33-43.

SUPPLEMENTARY FIGURES

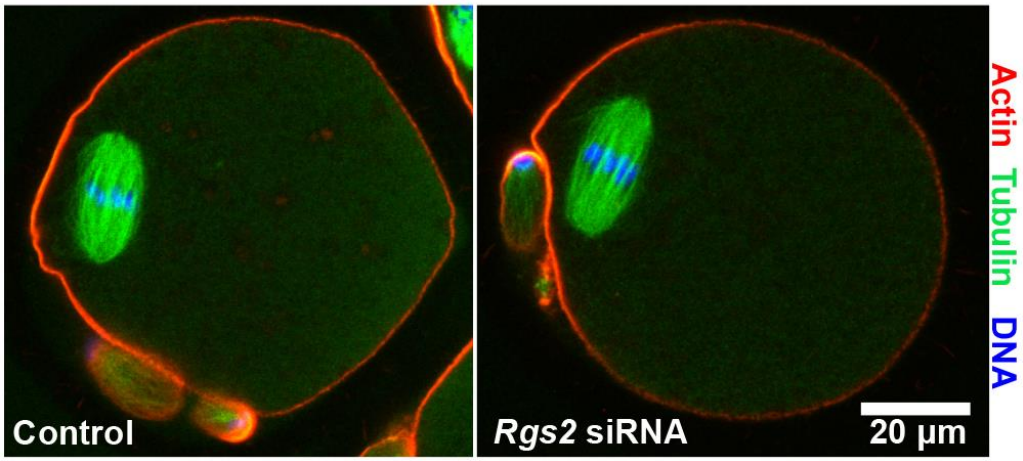

Figure S1. Normal oocyte maturation in eggs lacking RGS2. Oocytes were microinjected at the GV stage with scrambled siRNA (control) or *Rgs2* siRNA, then matured in vitro. A. Success of maturation of control and *Rgs2* siRNA-injected oocytes. Number of oocytes to reach the indicated stage out of the total maturing is shown. GVBD, germinal vesicle breakdown; MII, metaphase II. B. MII eggs were immunostained for actin (red), tubulin (green), and DNA (blue). Shown are representative images of 14-15 eggs per group. Scale bar: 20 µm.

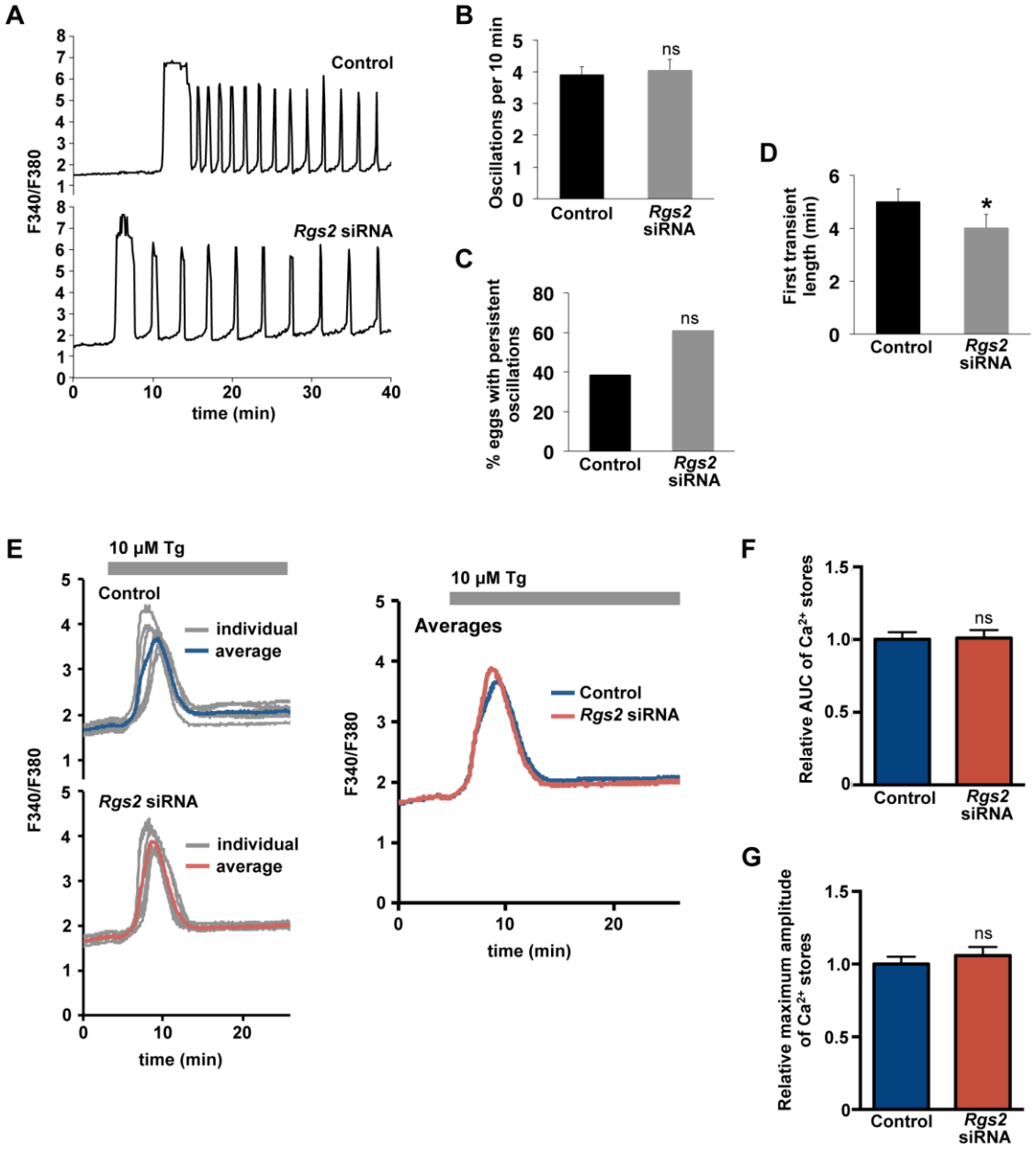

Figure S2. Ca^{2+} oscillatory patterns following fertilization and Ca^{2+} stores in eggs lacking RGS2. A. Representative Ca^{2+} tracings from eggs matured *in vitro* from control GV oocytes or oocytes microinjected with *Rgs2* siRNA. B. Oscillation frequency. Graph shows mean±s.e.m. of 31-36 eggs from 5 independent experiments. ns, no significant difference, Mann–Whitney *U*-test. C. Percentage of eggs with Ca^{2+} oscillations persisting for at least 60 min. Each column represents total of 31-36 eggs. ns, no significant difference, Chi square analysis. D. Duration of first Ca^{2+}

transient. Graph shows mean±s.e.m. of 34-41 eggs from 5 independent experiments. **P*<0.05, Mann–Whitney *U*-test. E. Representative traces of thapsigargin (Tg)-induced Ca^{2+} release for individual ZP-intact MII eggs (gray lines) and averages for 6 eggs (colored lines) are shown, along with the average traces shown together on one graph. F-G. Relative Ca^{2+} stores in ZP-intact control and *Rgs2* siRNA eggs. Graphs show mean±s.e.m. of area under the curve (AUC) (F) and maximum amplitude relative to baseline (G) for *N*=24-25 eggs from 4 independent experiments.

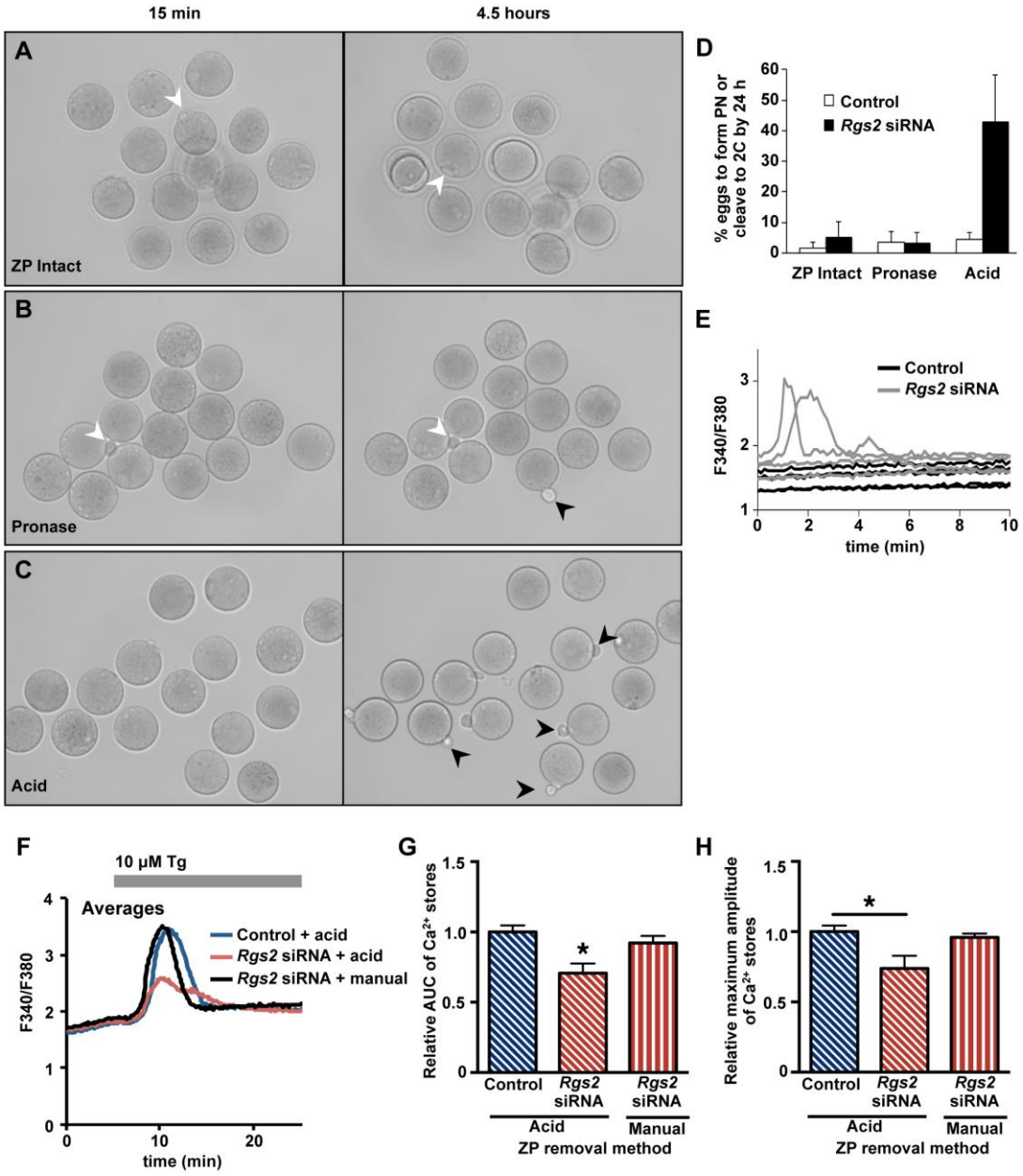

Figure S3. Effect of different methods of ZP removal on cell cycle resumption, Ca^{2+} release, and ER Ca^{2+} stores in eggs lacking RGS2. A-C. Photomicrographs of RGS2-depleted eggs at the indicated times following ZP removal. White arrowheads, first polar body; Black arrowheads, second polar body A. ZP not removed. B. ZP removed by pronase treatment. C. ZP removed using acid treatment. D. Percentage of pronuclear (PN) stage or 2-cell (2C) stage embryos following the indicated ZP removal method. Graph shows mean±s.e.m. of 8-19 eggs from 2-3

independent experiments. E. Ca^{2+} traces from control eggs or eggs lacking RGS2 (*Rgs2* siRNA) >1 hour following ZP removal using acid. Five representative traces are shown for each group. F. Thapsigargin (Tg)-induced Ca^{2+} release in ZP-free control and RGS2-depleted eggs following ZP removal using the indicated technique. Compiled average tracings of *N*=3-5 eggs/group are shown. G-H. Relative Ca^{2+} stores in control and *Rgs2* siRNA eggs. Graphs show mean±s.e.m. of 8-12 eggs/group from 2 independent experiments of area under the curve (AUC) (G) and maximum amplitude relative to baseline (H). **P*<0.05, one-way ANOVA with Tukey's multiple comparison test.

Table S1. Percentages of RGS2-depleted oocytes that underwent GVBD and developed to the MII stage.

	Control	*Rgs2* siRNA-injected
GVBD	64/64 (100%)	71/71 (100%)
MII	52/64 (81%)	56/71 (79%)