**Table S1: Population studies examining fertility variance (litter attributes) in dKO females.** Seven mating pairs for WT and dKO mice, and three pairs for V3KO and tKO mice were used for analysis. (Litter size ***p < 0.001; Pups/female **p ≤ 0.005; Total pups **p < 0.005, chi-square).

| Male | x | Female | Litters Born (to date) | Litter Size (Mean ± S.D.) | Pups/Female (36 weeks) | Total Pups (36 weeks) |
|---|---|---|---|---|---|---|
| WT (n = 7) | x | WT (n = 7) | 41 | 8.28 ± 0.41 | 48.4 ± 9.3 | 339 |
| V3KO (n = 3) | x | V3KO (n = 3) | 18 | 7.94 ± 1.7 | 47.7 ± 9.45 | 143 |
| tKO (n = 3) | x | tKO (n = 3) | 18 | 7.44 ± 0.98 | 44.7 ± 1.53 | 134 |
| dKO (n = 7) | x | dKO (n = 7) | 40 | 5.49 ± 1.1*** | 31.6 ± 9.4** | 221** |

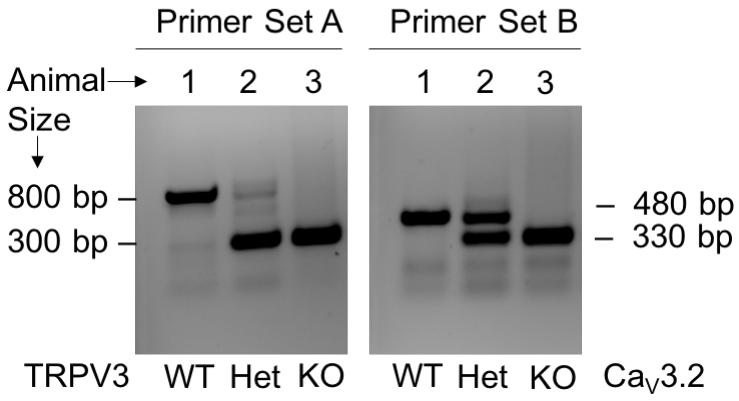


**Figure S1. PCR confirmation of targeted deletions of *Trpv3* and *Cacna1h* genes.** PCR genotyping of wild-type (WT), heterozygous (Het), and knockout (KO) mice. Two sets of primers were used to amplify *Trpv3* and *Cacna1h* (see Methods). Products yielded from Primer Set A: WT 800 bp, Het 800 & 300 bp, KO 300 bp. Products yielded from Primer Set B: WT 480 bp, Het 480 & 330 bp, KO 330 bp.

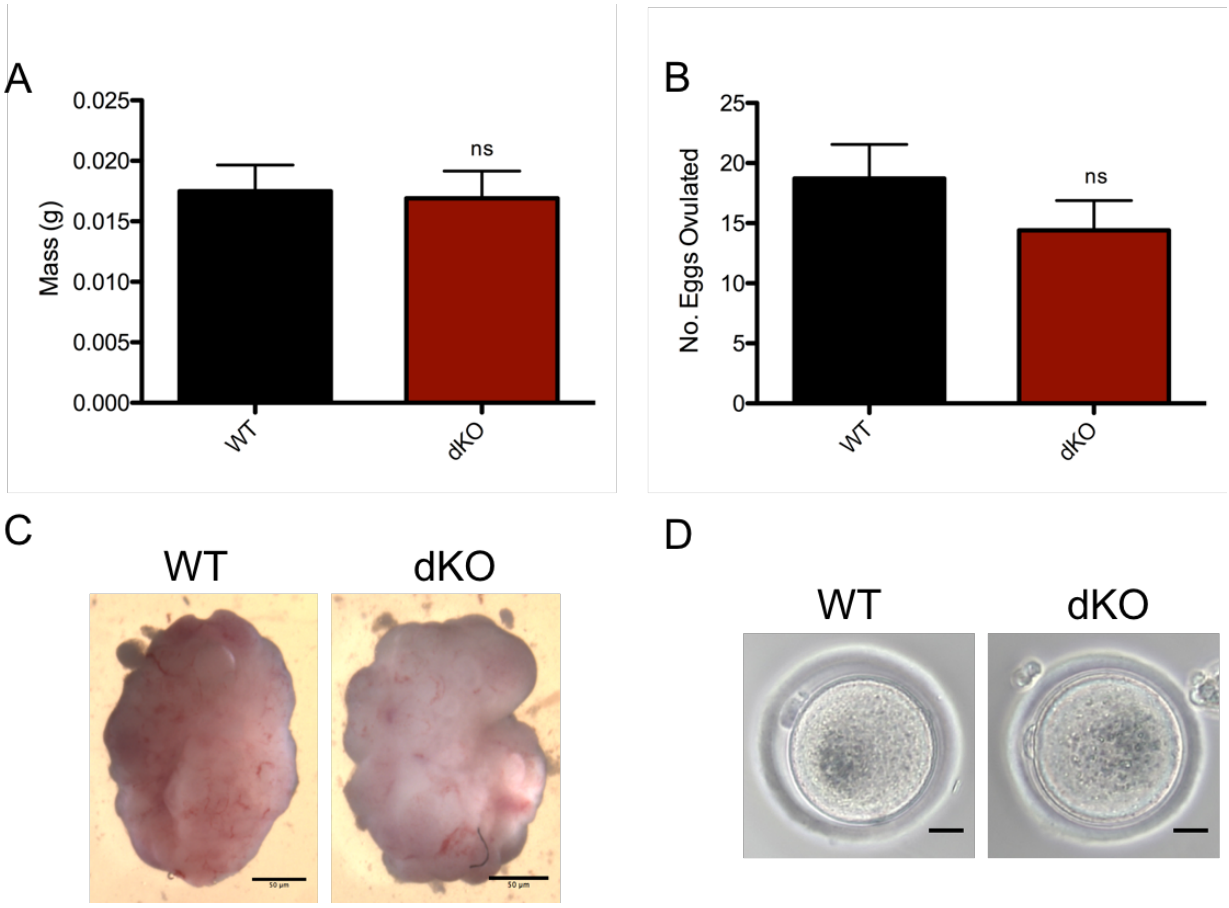


**Figure S2. Double knockout females display no morphological differences.**

A: Mean mass of superovulated ovaries. Mean ± S.E.M. for each genotype was calculated from n = 28 ovaries. B: Number of MII eggs ovulated 14 hours post-hCG injection. Mean ± S.E.M. for each genotype was calculated from n = 14 females. C: Ovaries were prepared for imaging by removing excess fat tissue and oviduct. Scale bar represents 50 μm. D: DIC image of MII egg. Scale bars represent 10 μm.

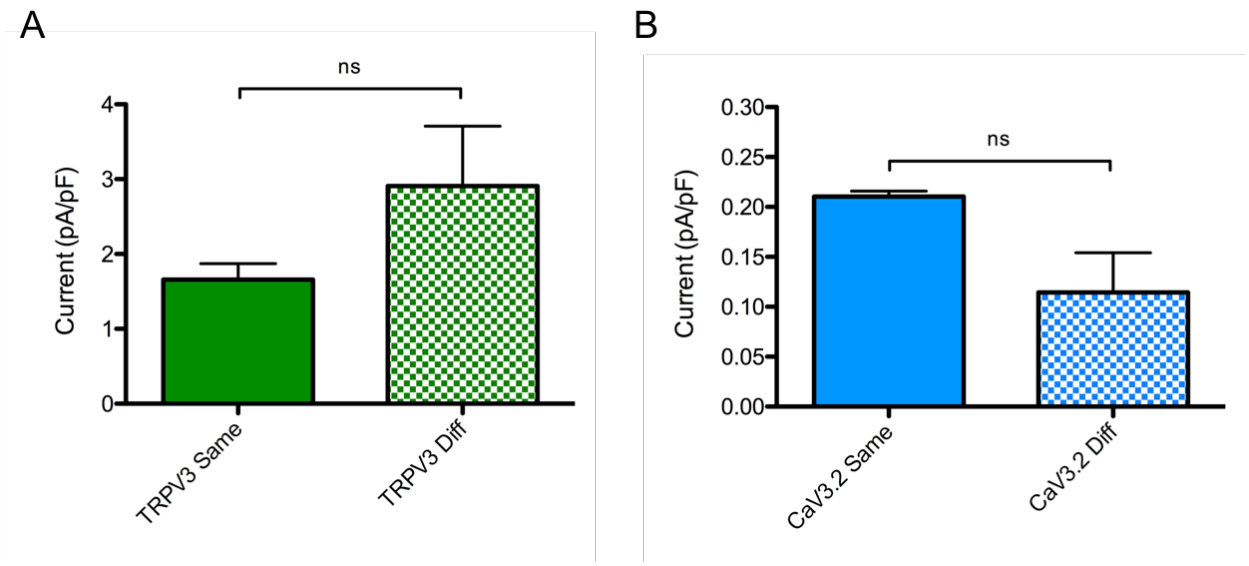


**Figure S3: $Ca_V3.2$ and subsequent TRPV3 patch clamp on the same egg confirms true dKO egg.**

The same dKO egg was subjected to a whole cell voltage clamp step protocol from -100 mV to +50 mV (Δ 10 mV) to measure $Ca_V$ current and subsequently a ramp protocol from -100 mV to +100 mV to measure TRPV3 current in presence of the agonist 2-APB (200 μM). A: TRPV3 measurements in a dKO egg immediately after measuring $Ca_V3.2$ (solid green bar, left). Mean ± S.E.M. current = 1.66 ± 0.21 pA/pF at +80 mV, n = 2 versus TRPV3 measurements in different dKO eggs (hashed green bar, right); mean ± S.E.M. current = 2.91 ± 0.78 pA/pF at +80 mV, n = 3 ($p > 0.05$). B: $Ca_V3.2$ measurements in dKO eggs that remained patched after the step protocol to subsequently measure TRPV3 current (solid blue bar, left), mean ± S.E.M. current at -20 mV = 0.21 ± 0.0055 pA/pF, n = 2 versus $Ca_V3.2$ current measurements made in different dKO eggs (hashed blue bar, right), mean ± S.E.M. current = 0.11 ± 0.04 pA/pF, n = 2 ($p > 0.05$).

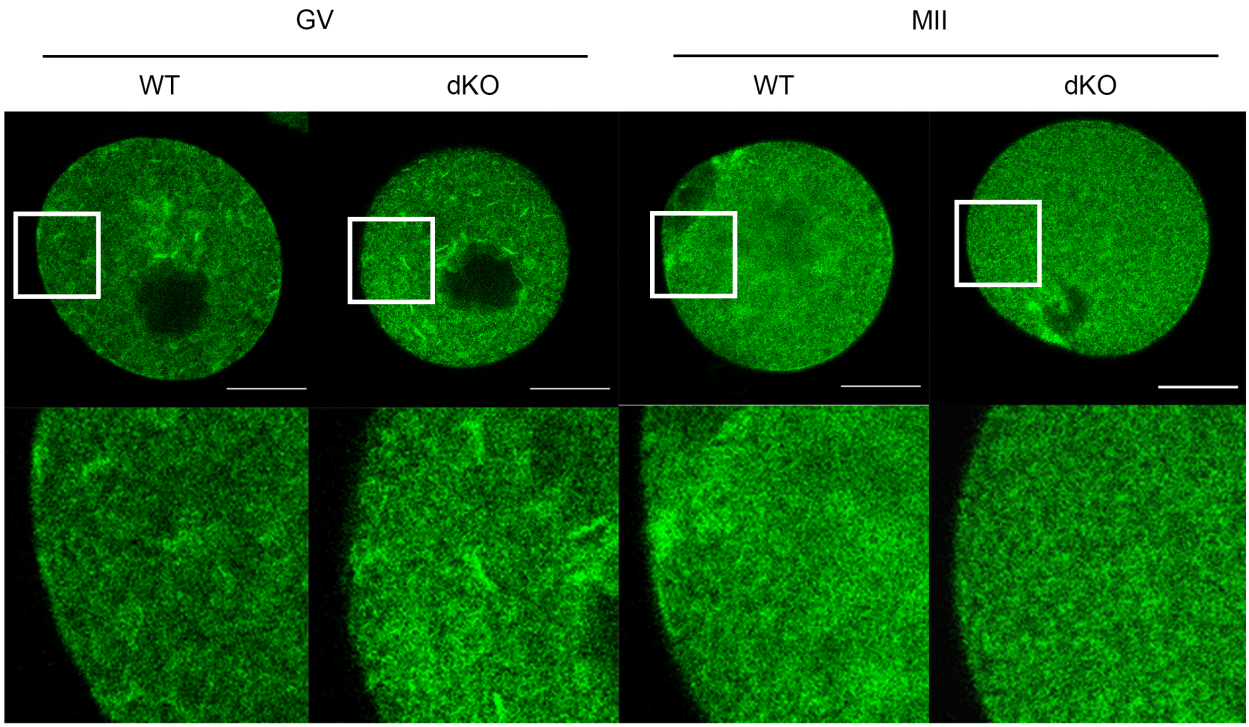


**Fig S4. Absence of TRPV3 and $Ca_V3.2$ in dKO oocytes/eggs does not alter ER organization.**
Confocal images of WT and dKO oocytes (left) and eggs (right) expressing the ER $Ca^{2+}$ sensor, D1ER. Lower panels represent zoom inset corresponding to white square. Scale bars represent 25 µm.

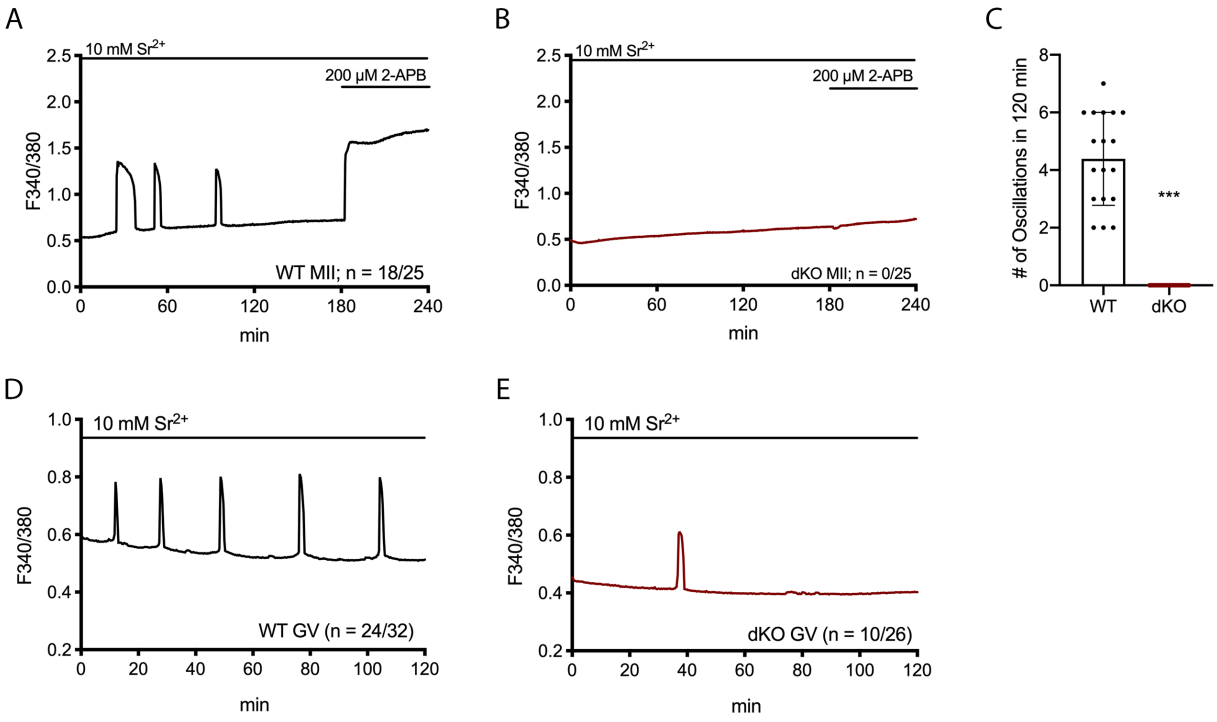


**Figure S5. dKO oocytes and eggs lacking TRPV3 and $Ca_V3.2$ channels do not support $Sr^{2+}$-induced oscillations or 2-APB stimulated influx.**

A-B: Oscillations induced in MII eggs by exposure to 10 mM $SrCl_2$. A: WT, n = 25/29. B: dKO, n = 0/24. 200 µM 2-APB was applied to media at 180 min. C: Number of oscillations in 120 min; WT:dKO: $p < 0.001$. Spontaneous oscillations induced by exposure to 10 mM $SrCl_2$. D: WT GV oocytes (n = 6/8). E: dKO GV oocytes. Representative dKO oocytes (n = 3/8).

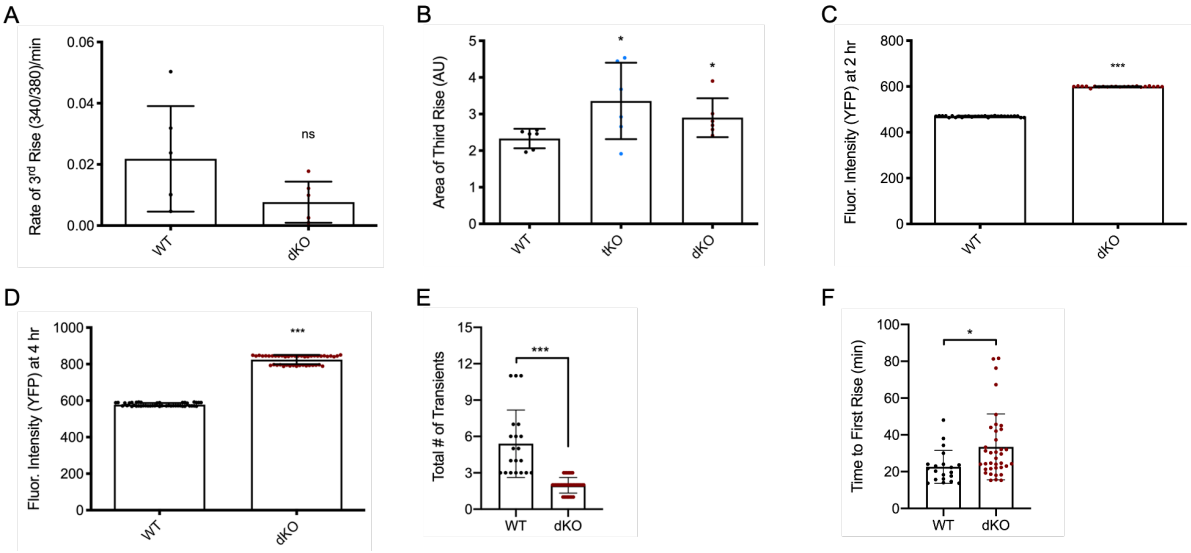


**Figure S6. Slower $Ca^{2+}$ influx underlies slower oscillations after PLCζ cRNA-initiated oscillations or IVF in dKO eggs.**

A: Slope analysis of the rise of the third $Ca^{2+}$ transient evaluated and compared between WT (black dots) and dKO (red dots) eggs; WT: 0.0218 ± 0.01; dKO: 0.00765 ± 0.003 ($p > 0.05$), n = 6 in both groups. B: Area under third $Ca^{2+}$ transient; WT (black dots): 2.33 ± 0.27 AU (n = 6) vs. tKO (blue dots): 3.36 ± 1.04 AU (n = 6), $p < 0.05$ vs. dKO (red trace): 2.9 ± 0.53 AU (n = 6), $p < 0.05$. C-D: Rate of protein translation measured by fluorescence intensity via injection of fluorescently tagged CaMKII construct after 2h (C) and 4h (D). Intensity was measured at 360 nm. B: WT (black dots): 469.4 ± 0.52 nm, n = 28 vs. dKO (red dots): 599.5 ± 0.51 nm, n = 22 ($p < 0.001$). E: WT (black dots): 578.1 ± 1.3 nm, n = 45 vs. dKO (red dots): 825 ± 3.8 nm, n = 45. F: Total number of $Ca^{2+}$ transients in response to IVF; WT (black dots): 5.4 ± 2.8 oscillations (n = 20) vs. dKO (red dots): 1.97 ± 0.64 oscillations (n = 37), $p < 0.001$. E: Time to first $Ca^{2+}$ transient in response to IVF; WT (black dots): 22.6 ± 9.0 min (n = 20) vs. dKO (red dots): 33.4 ± 17.9 min (n = 37), $p < 0.05$.

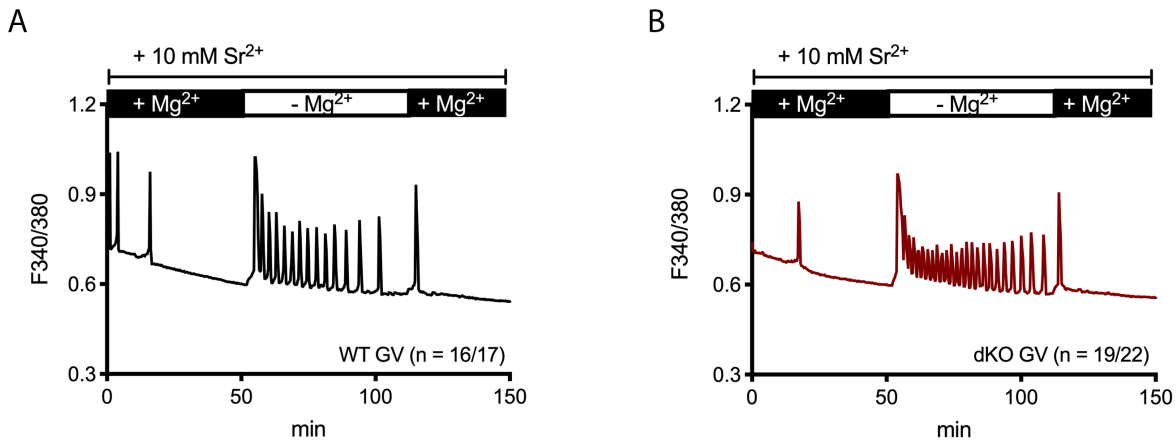


**Figure S7: Changes in $[Mg^{2+}]_o$ greatly affect $Sr^{2+}$ induced oscillations in GV oocytes.**

A-B: Spontaneous oscillations induced in GV oocytes by 10 mM $SrCl_2$. A: WT mean ± S.E.M. $Mg^{2+}$-free = 9.41 ± 1.1 oscillations, n = 9/9. B: dKO mean, $Mg^{2+}$-free = 11.3 ± 1.6 oscillations, n = 7/9, $p > 0.05$. $[Mg^{2+}]_o$ was changed via perfusion of media throughout continuous imaging.