

1092 **Supplementary Figure Legends**

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1094 **Supplementary Figure 1. Cell-impermeable chelators effectively reduce Zn²⁺ levels**
1095 **in external media but do prevent initiation or continuation of Ca²⁺ oscillations.**

1096 (A) A representative trace of FluoZin3 fluorescence in replete monitoring media (TL-HEPES). The
1097 media was supplemented with cell-impermeable FluoZin-3, and after initiation of monitoring, the
1098 addition of EDTA (100 μM) occurred at the designated point (triangle). (B) The left black trace
1099 represents Ca²⁺ oscillations initiation by injection of *mPlcζ* mRNA (0.01 μg/μl). The oscillations were
1100 monitored in Ca²⁺ and Mg²⁺-free media and in the presence of EDTA (110 μM) to chelate residual
1101 divalent cations derived from the water source or reagents used to make the media. The right red trace
1102 represents the initiation of oscillations as above, but after a period indicated by the black and green
1103 bars, Ca²⁺ and Mg²⁺ were sequentially added back.

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1105 **Supplementary Figure 2. Overexpression of ER accessory protein ERp44 did not**
1106 **change the Ca²⁺ responses initiated by *mPlcζ* mRNA microinjection, Acetylcholine,**
1107 **or SrCl₂.**

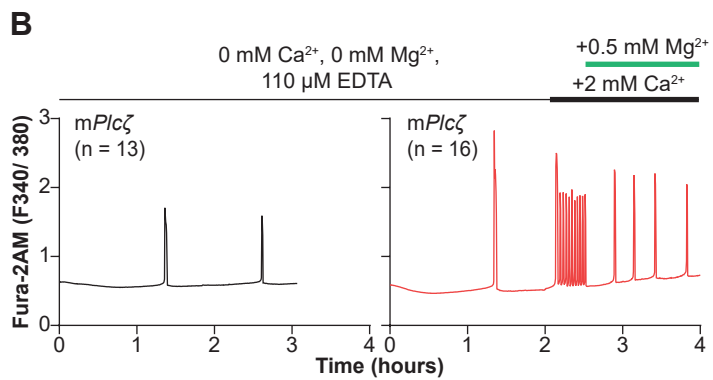
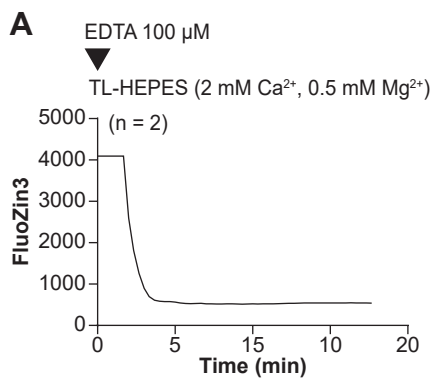
1108 (A) Representative immunofluorescent images of MII eggs with overexpression of
1109 ERp44. At 5 hr. post microinjection, eggs were treated with 10 or 50 μM of TPEN and
1110 incubated for 1 hr, after which they were fixed and stained. An anti-HA antibody was
1111 used. Scale bar: 10 μm. (B) Representative Ca²⁺ responses induced by *mPlcζ* mRNA
1112 microinjection (0.01 μg/ μl-left column), SrCl₂ (10 mM-center column), and
1113 acetylcholine (50 μM-right column) in eggs with (top panels) or without (bottom panels)
1114 ERp44 overexpression.

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1116 **Supplementary Figure 3. Elevated Zn²⁺ impairs egg activation and the subsequent**
1117 **embryo development.**

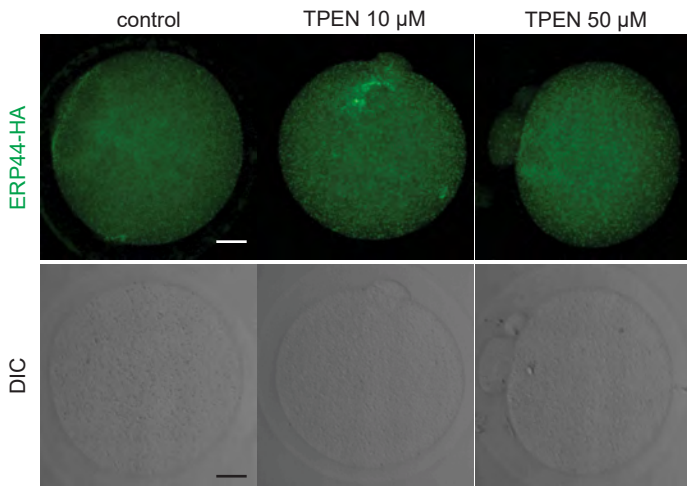
1118 (A) MII eggs were incubated in TL-HEPES containing 0, 0.1, or 1.0 μM ZnPT at room
1119 temperature for 10 min and washed several times with fresh TL-HEPES and injected with
1120 *mPlcζ* mRNA. After it, eggs and zygotes were cultured in KSOM for 24h. PN formation
1121 and 2-cell development were checked at 7 and 24h post-microinjection. Bars represent
1122 the percentages of injected eggs that reached the PN and the 2-cell stage. Scale bar: 50
1123 μm. (B) MII eggs injected with *mPlcζ* mRNA were incubated in KSOM without ZnPT
1124 for an hr. and then incubated in KSOM with 0 or 0.1 μM ZnPT for 24h. The second polar
1125 body extrusion, PN formation, and 2-cell development were checked at 2.5-, 7- and 24h.
1126 post-microinjection. Bars represent the percentages of injected eggs that reached the PN
1127 and the 2-cell stage. Scale bar: 50 μm.

Supplementary Figure 1.

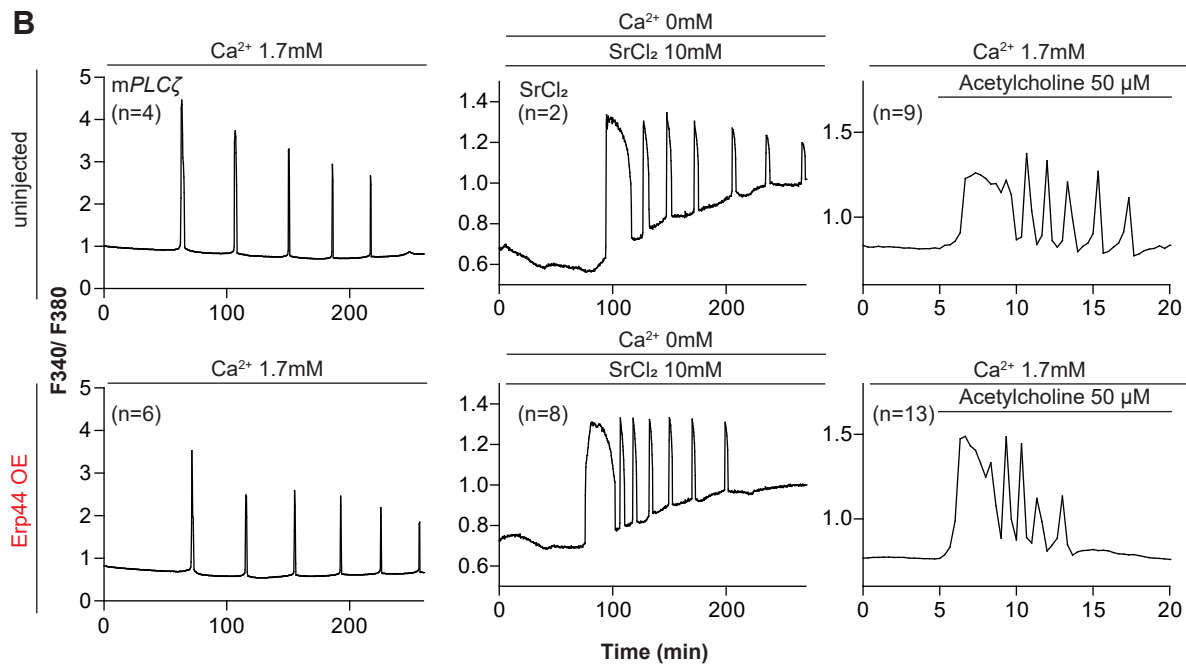


Supplementary Figure 2.

A

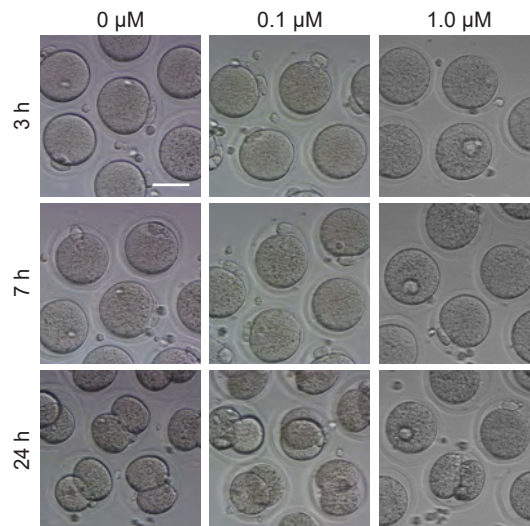
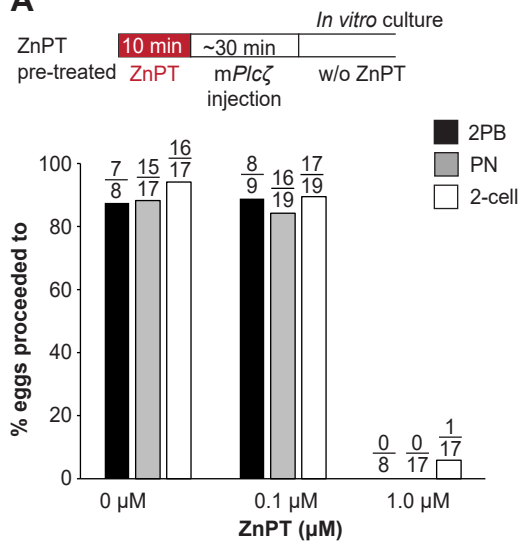


B



Supplementary Figure 3.

A



B

