Supplementary Materials

The zinc transporter ZIP9 (Slc39a9) regulates zinc dynamics essential to egg activation in zebrafish

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Supplementary Table 1

Primers used for qPCR.

Primer	Forward	Reverse
zip9	AGCAGCTCCACGCTTACATT	CATGGACGACCAATCCGAGT
18s	AGAAACGGCTACCACATCCA	TCCCGAGATCCAACTACGAG

Supplementary Table 2

Oligonucleotide sequences used in gRNA generation and stop codon cassette.

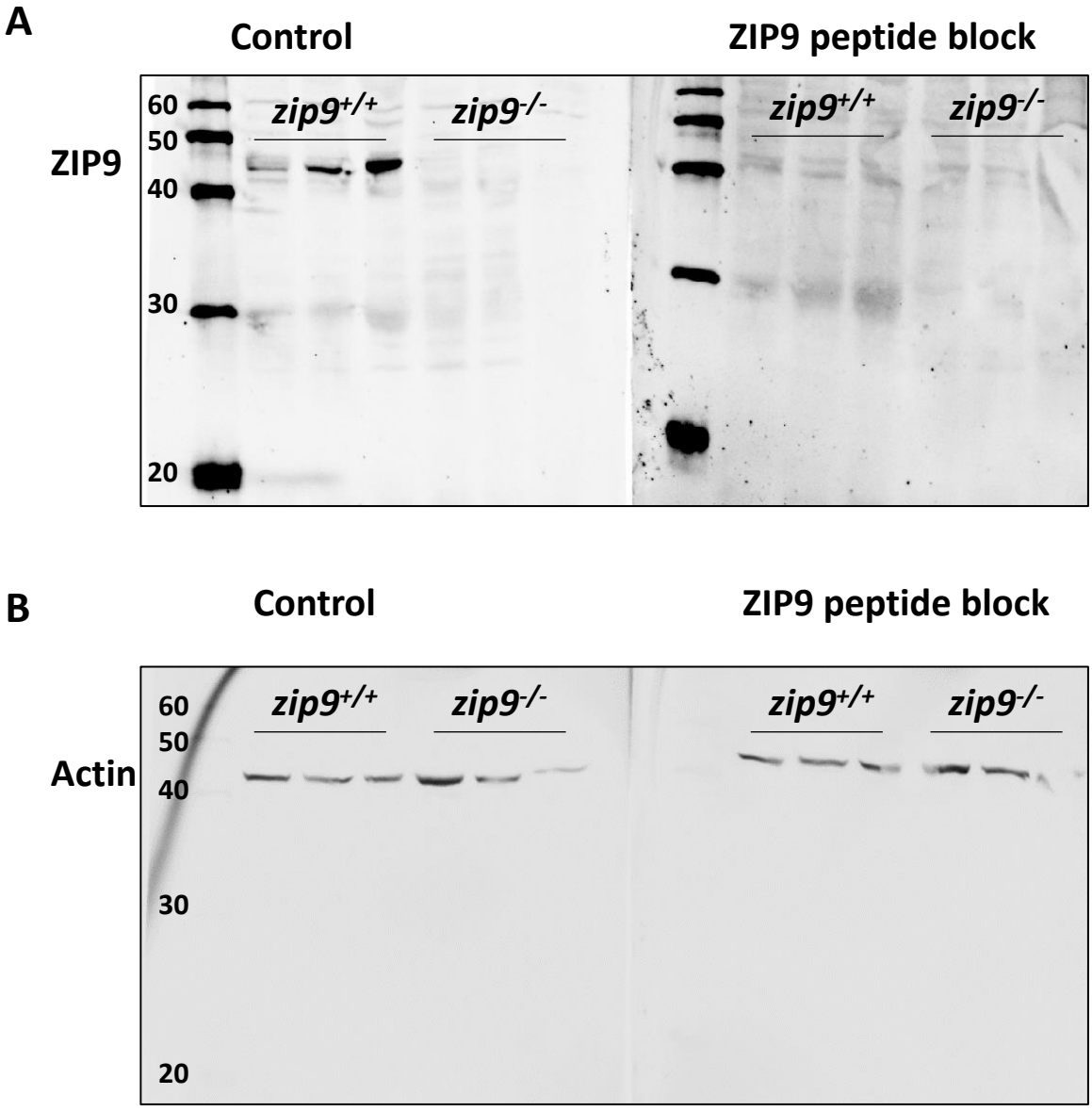
Primer	**(5'-3')**
Exon 3 specific	TAATACGACTCACTATAGAAGGAGTGGTGAGACCCAGGTTTTAGA GCTAGAAATAGCAAG
tracrRNA tail	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTA GCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC
Exon 3 specific stop codon cassette	GCAGAAGGAGTGGTGAGACCGTCATGGCGTTTAAACCTTAATTAA GCTGTTGTAGCAGCGGTGAACACGGCCACG

Supplementary Table 3

Primers used in screening assays.

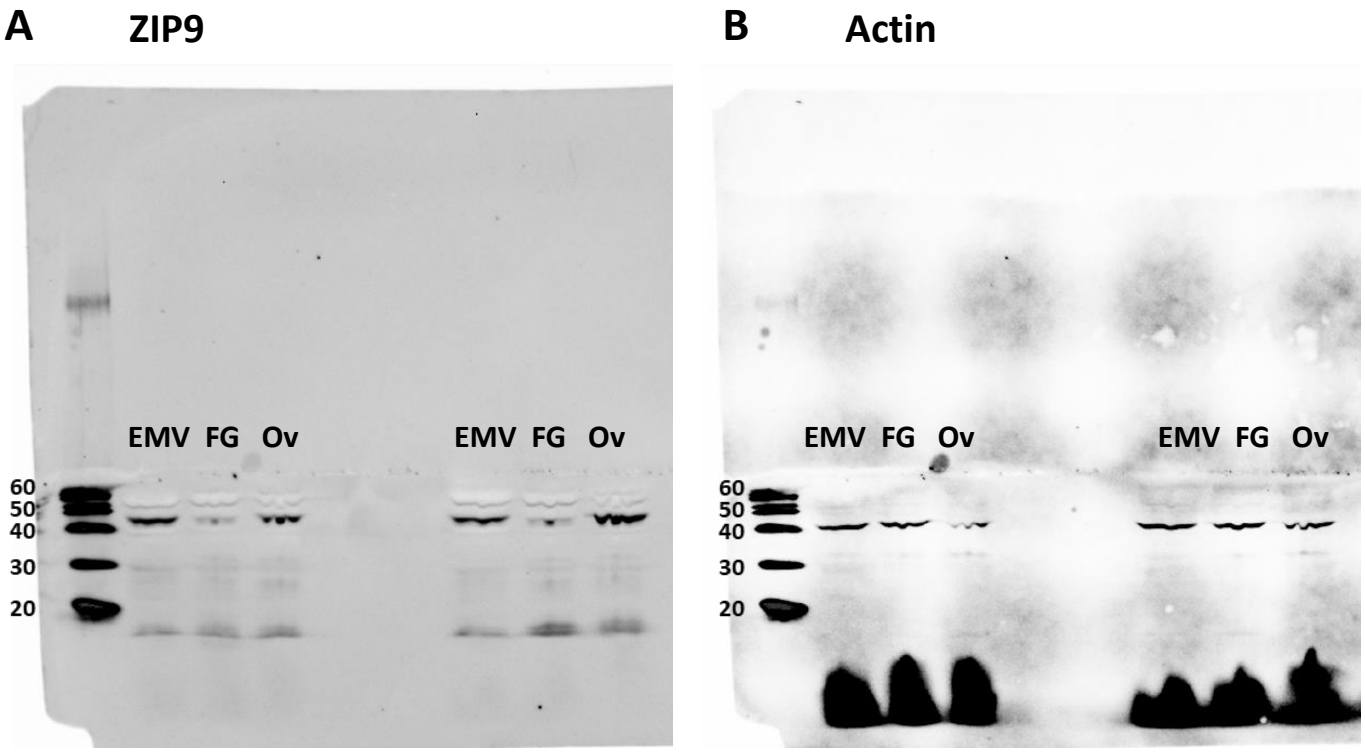
Primer	Forward	Reverse
Heteroduplex mobility assay	ATGCATTGTGCTGCTAGTTGTT	CCAGAGAAATCCCAATGTAAGC
Stop cassette specific	GGTGAGACCGTCATGGCGTTTA	CGCAGTGTGAGCCAATCCAAATA
Homo/heterozygous screening	ATGCATTGTGCTGCTAGTTGTT	CGCAGTGTGAGCCAATCCAAATA

Supplementary Figure 1



Supplementary Figure 1. Zebrafish ZIP9 antibody validation. A-B, Western blots of plasma membrane fractions (5 µg) from *zip9*$^{+/+}$ and *zip9*$^{-/-}$ ovaries incubated with control or peptide blocked ZIP9 antibody (A), or actin housekeeping control (B).

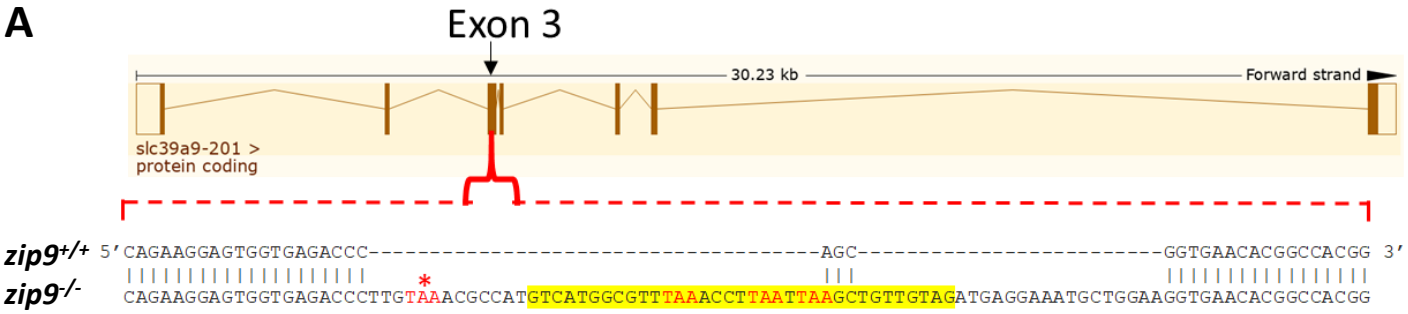
Supplementary Figure 2



Supplementary Figure 2. Full blots of Figure 1B; ZIP9 protein expression on plasma membrane of different stage oocytes/eggs. A, Detection of ZIP9 antibody. B, Detection of Actin antibody. Two representative samples of each stage shown. EMV, early-mid vitellogenic oocytes; FG, full grown oocytes; Ov, ovulated eggs.

Supplementary Figure 3

A



B

ZIP9$^{+/+}$

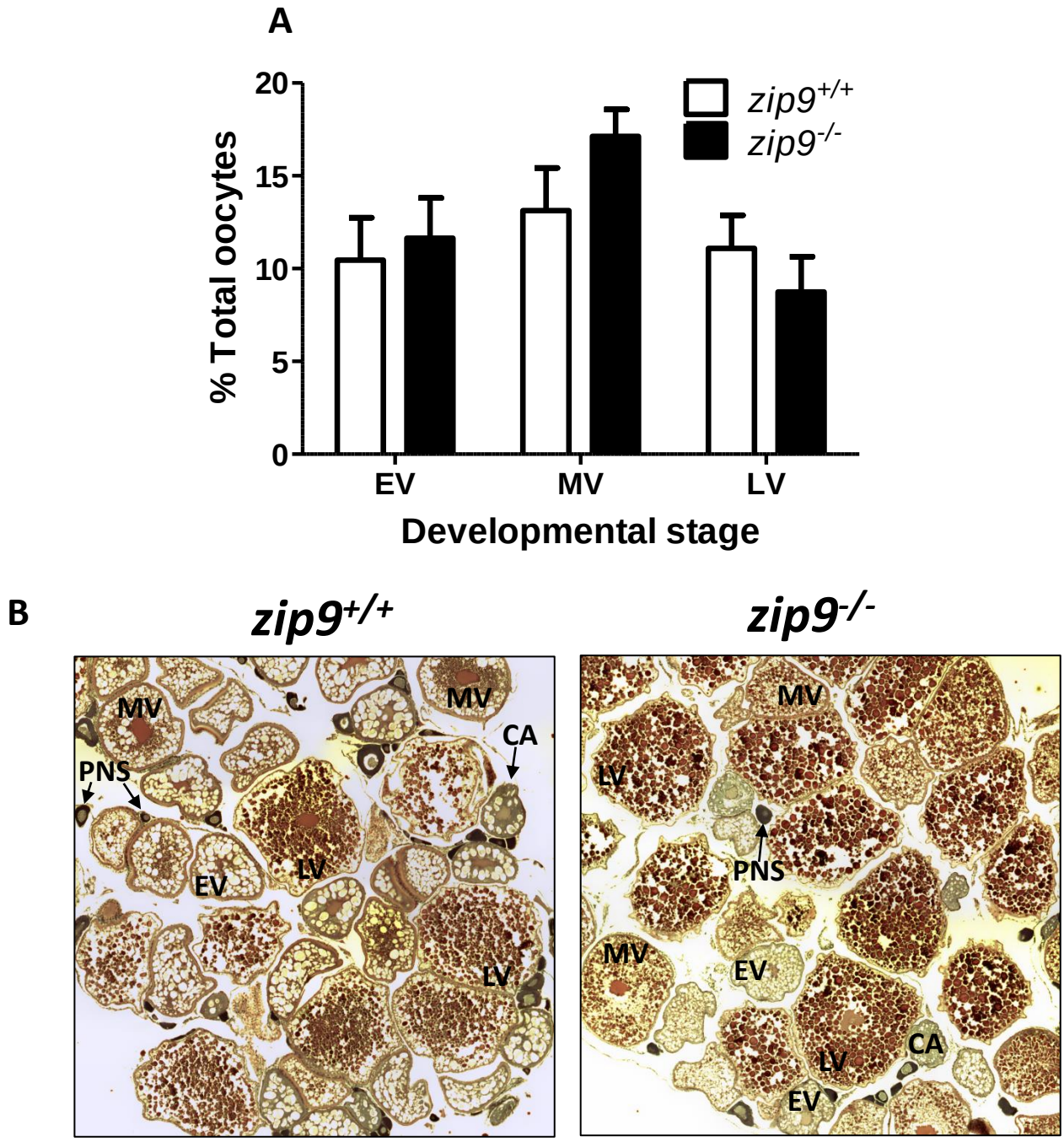
MDDFSSISLLSLSMLIGCYVAGTIPLAVN
FSEEKLKLVTVLGAGLLCGTALAVIIPEG
VHALYEEMLEGVHNHGHGQVEAEVSEQ
KVAEGVVRPSGEHGHGHEQLHAYIGISL
VLGFVFMLLVDQIGSAHMHSSDDPEAA
RAASSKITTTLGLVVHAAADGVALGAAAS
TSQTSVQLIVFVAIMLHKAPAAFGLVSF
LMHAGLERNRIRKHLLVFALAAPVLAMVT
YVGLSQSSKEALSDVNATGVAMLFSAGT
FLYVATVHVLPEVGGMGGHSHSPGGSA
GKGLSKLEVGALVLGCLIPLVLSIGHQH

ZIP9$^{-/-}$

MDDFSSISLLSLSMLIGCYVAGTIPLAVN
FSEEKLKLVTVLGAGLLCGTALAVIIPEG
VHALYEEMLEGVHNHGHGQVEAEVSEQ
KVAEGVVRPL **Stop**

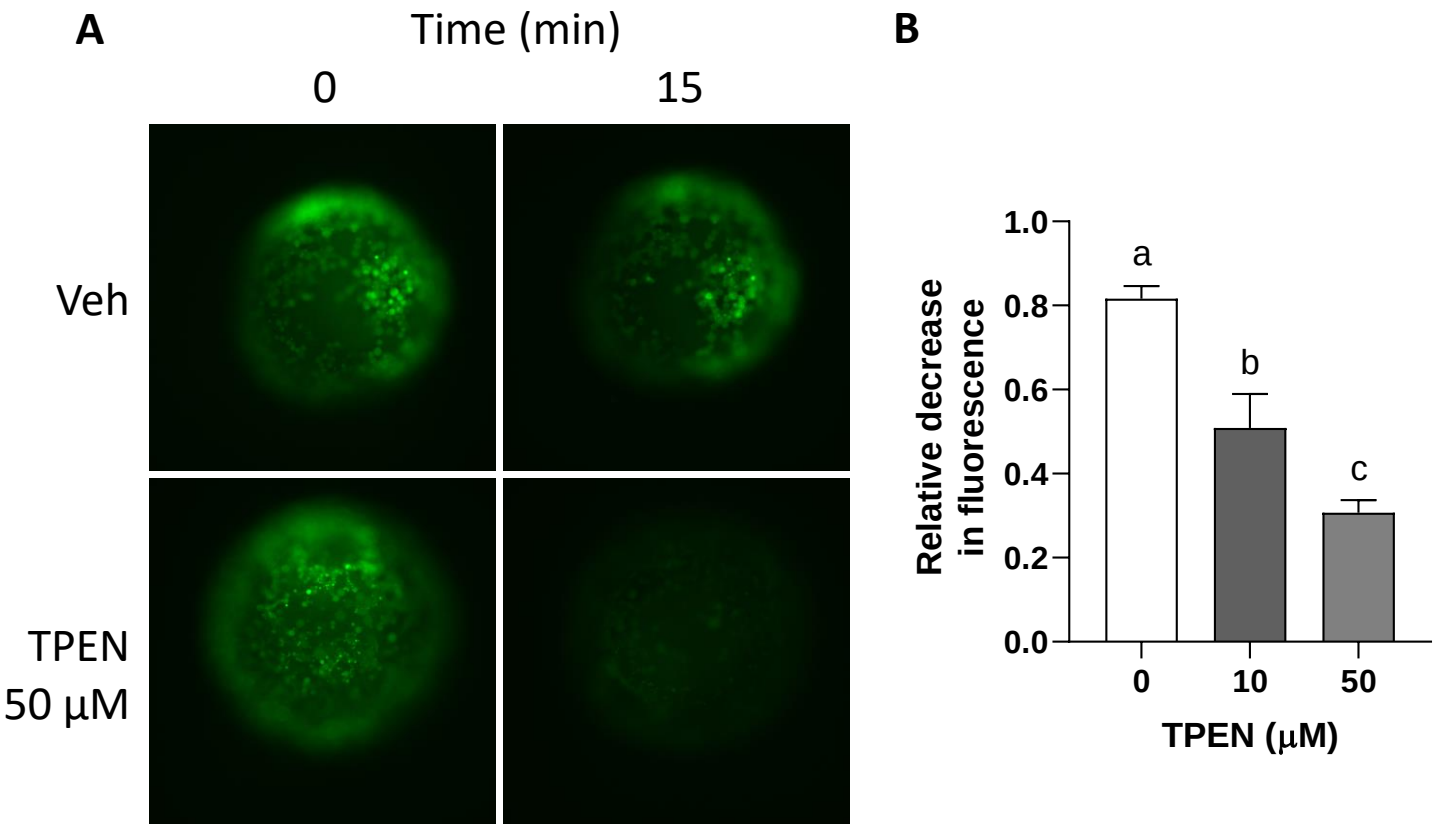
Supplementary Figure 3. *zip9* mutation generation. A, Schematic representation of *zip9*, and the comparison of mutant *zip9*$^{-/-}$ and *zip9*$^{+/+}$ exon 3 nucleotide sequence. B, Comparison of predicted ZIP9$^{-/-}$ and ZIP9$^{+/+}$ protein sequence. Highlighted nucleotide sequence designates the stop codon cassette, red-text nucleotides indicate stop codons, asterisk indicates stop codon resulting in truncation, red boxed-amino acids are adjacent to stop codon in ZIP9$^{-/-}$.

Supplementary Figure 4



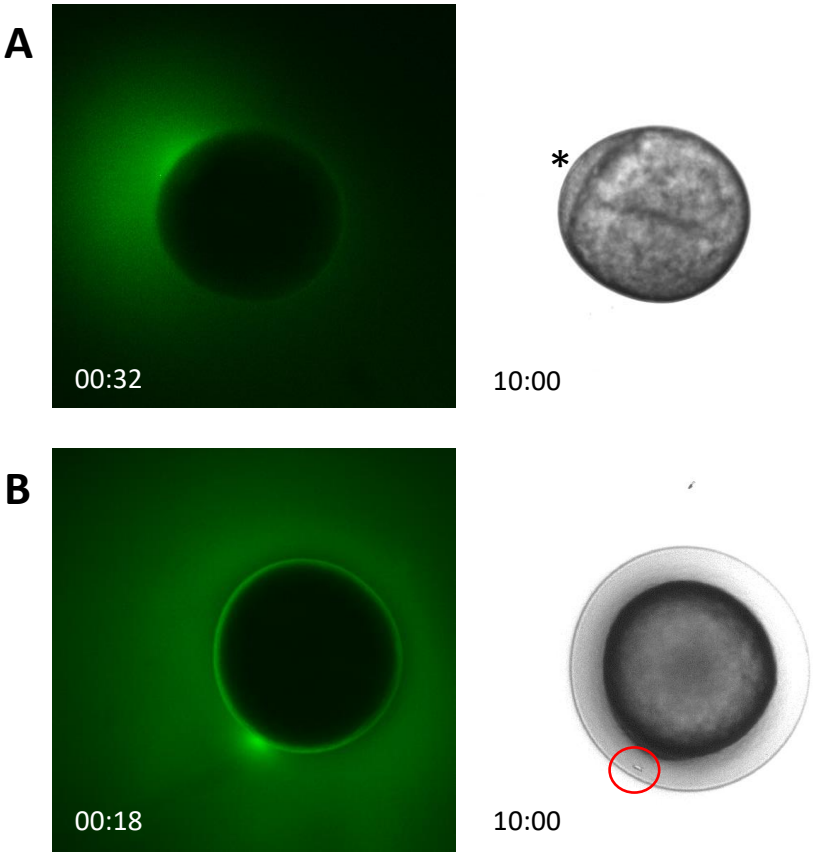
Supplementary Figure 4. Ovarian morphology of *zip9*$^{-/-}$ and *zip9*$^{+/+}$ fish. A, The relative proportion of ovarian follicles at different stages of development in *zip9*$^{-/-}$ and *zip9*$^{+/+}$ ovaries. B, Representative image of ovarian histology of *zip9*$^{-/-}$ and *zip9*$^{+/+}$ fish. All data represents means ± SEM, n=5. PNS, perinuclear stage; CA, cortical alveoli stage; EV, early vitellogenic stage; MV, mid vitellogenic stage; LV, late vitellogenic stage.

Supplementary Figure 5



Supplementary Figure 5. Specificity of FluoZin-3-AM. A, Representative images of pre-activated WT eggs loaded with FluoZin-3-AM and treated with vehicle or TPEN. B, Quantification of relative decrease of FluoZin-3-AM fluorescence in TPEN treated eggs. Data represents means ± SEM, n=6 (2 replicate egg from 3 fish). Significance was determined by one-way ANOVA with Bonferroni multiple comparison post-test. Different letters indicate significant differences between treatment groups in the post hoc test ($P < 0.05$).

Supplementary Figure 6



Supplementary Figure 6. Zinc exocytosis begins at the animal pole. A-B, FluoZin-3 fluorescence from WT eggs during zinc exocytosis (left) and brightfield images (right) 10 min post activation showing the blastodisc (*) and micropyle (red circle) respectively.

Description of supplemental movies

Supplemental Movie 1. Loss of zinc containing cortical vesicles upon activation of a representative wildtype egg. Scale bar: 100 µm. Images taken every 20 s for a total of 10 min.

Supplemental Movie 2. Loss of zinc containing cortical vesicles upon activation of a representative *zip9*$^{-/-}$ egg. Scale bar: 100 µm. Images taken every 20 s for a total of 10 min.

Supplemental Movie 3. Increase in extracellular zinc upon activation of a representative wildtype egg. Scale bar: 200 µm. Images taken every 2 s for a total of 10 min.

Supplemental Movie 4. Increase in extracellular zinc upon activation of a representative *zip9*$^{-/-}$ egg. Scale bar: 200 µm. Images taken every 2 s for a total of 10 min.

Supplemental Movie 5. A rise in intracellular calcium proceeds zinc exocytosis in a representative WT egg. Images taken every ~3.4 seconds for 5 min. green, extracellular zinc; purple, intracellular calcium.