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Supplemental Information

Zinc Dynamics during *Drosophila* Oocyte Maturation and Egg Activation

Qinan Hu, Francesca E. Duncan, Andrew B. Nowakowski, Olga A. Antipova, Teresa K. Woodruff, Thomas V. O'Halloran, and Mariana F. Wolfner

Transparent Methods

Fly strains and maintenance

All *Drosophila* strains and crosses were maintained or performed at 25 °C on a 12/12 light/dark cycle, on standard yeast-glucose-agar media (10% w/v yeast, 8.9% w/v agar (Genesee #66-111), 10% w/v glucose, 0.05% v/v phosphoric acid, 0.5% v/v propionic acid, 0.27% w/v p-hydroxybenzoic acid methyl ester, 2.5% v/v ethanol). When needed, 10 mM N,N,N′,N′-tetrakis(2-pyridiylmethyl)-1,2-ethylenediamine (TPEN) in ethanol, 10 mM neocuproine in ethanol, 10 mM ammonium tetrathiomolybdate (TM) in water or 10mM $ZnSO_4$ in water stock solution was added to melted food to the indicated final concentration and mixed well before the food solidified. An equal volume of solvent (ethanol or water) was added to control food. All chemicals were obtained from MilliporeSigma.

Egg-laying and egg hatchability assay

Oregon-R-P2 (ORP2) wildtype flies were reared on control yeast-glucose medium, or this medium containing the indicated concentrations of TPEN (TPEN food), or medium containing TPEN and $ZnSO_4$ (TPEN rescue food). To determine how TPEN affected fertility, 3- to 5-day-old virgin females and males were mated in single pairs on TPEN food. Matings were observed and the males were removed after a single mating had completed. Females were allowed to lay eggs in the mating vial for 24 hours and were then transferred to a new vial also containing TPEN food. Females were transferred twice to new vials of TPEN food before being discarded. The number of eggs and pupae in each vial were counted. Egg hatchability was calculated by dividing the number of pupae by the number of eggs. For 3 pairs of single matings from each group, we counted the number of unhatched eggs for two days after egg-laying to calculate the number of hatched ones. Those numbers were equal to the eventual number of pupae in the selected vials, confirming the reliability of our method of calculating egg hatchability. The same procedure was followed for assays with neocuproine and TM.

DNA constructs and transgenic flies

To create a null allele of *znt35C*, we generated pU6-chiRNA constructs following protocols described on the FlyCRISPR website (https://flycrispr.org/; Gratz et al., 2013). We generated two constructs to express sgRNAs with the following target sequences (PAM sequences are underlined): gRNA1, GGGCACGATGACAATGATCCCGG; gRNA2, GCAGATTTTCCAAGGCATCGAGG. These constructs were co-injected by Rainbow Transgenic Flies, Inc. into *nos-Cas9-attP2* embryos. We identified a chromosome carrying an edited *znt35C* gene, in which a 2 bp deletion (CCGGGATCATTGTCATCGTGCCC, deleted bases are underlined) in exon 3 caused by gRNA1 led to a frameshift in the coding sequence of Znt35C's transmembrane domains and a premature stop codon. Since this mutation is predicted to completely disrupt Znt35C's transmembrane domain, it is likely a null allele. We established a fly stock that carries the mutated allele, which we have named *znt35C*^{1}, over the CyO balancer chromosome (See crossing scheme in **Figure S10**).

X-ray fluorescence microscopy sample preparation

Oocytes were dissected in isolation buffer (IB) from the indicated female virgin flies aged on yeasted food for 3-5 days as described by (Page and Orr-Weaver, 1997). *In vitro* activated eggs were made by incubating dissected mature oocytes in Modified Robb's buffer (Hu and Wolfner, 2019) for 30 min. *In vivo* activated but unfertilized eggs were collected on a grape-juice agar plate from the indicated female flies after they had mated with spermless males (Boswell, 1985; Hu and Wolfner, 2019). We attempted to prepare oocytes for microscopy by standard methods of washing and mounting samples in standard protocol that used 100 mM ammonium acetate solution (Kim et al., 2010), but the oocytes lysed under these conditions. Alternatively, we washed oocytes and eggs with a high osmolarity buffer (400 mM sucrose) which prevents oocytes from activating (Horner and Wolfner, 2008b). Oocytes and eggs were then transferred by forceps to a 1µL drop of 400 mM

sucrose on an intact 5 mm × 5 mm silicon nitride window (Norcada). Samples were allowed to dry on a heated stage warmed to 37 °C and stored in a desiccating canister until used.

Synchrotron-based X-ray fluorescence microscopy

X-ray fluorescence microscopy (XFM) was performed at beamline 2-ID-E at the Advanced Photon Source (Argonne National Laboratory). With a single-bounce Si (111) monochromator, 10-keV X-rays were monochromatized and focused to a spot size of 1 × 1 μm using Fresnel-zone plate optics (X-radia). Raster scans were done in steps of 1 μm with 15° sample tilt to detector. Fluorescence spectra were collected with a 10 ms dwell time using a 4-element silicon drift detector (Vortex-EM). Quantification and image processing were performed with MAPS software (Vogt, 2003). The fluorescence signal was converted to a two-dimensional concentration in $\mu g/cm^2$ by fitting the spectra against the thin-film standard AXO 1X (AXO Dresden GmbH). We assumed that no elemental content was lost during sample preparation.

Statistics

One-way ANOVA tests were used to detect significant differences in levels of transition metals between wildtype and *znt35C*^{1} across oocyte stages and in egg number and egg hatchability upon TPEN, neocuproine, ammonium tetrathiomolybdate (TM) or $ZnSO_4$ treatment. Tukey's HSD test or Duncan's new multiple range test was used to identify groups that were significantly different from each other in the ANOVA tests.

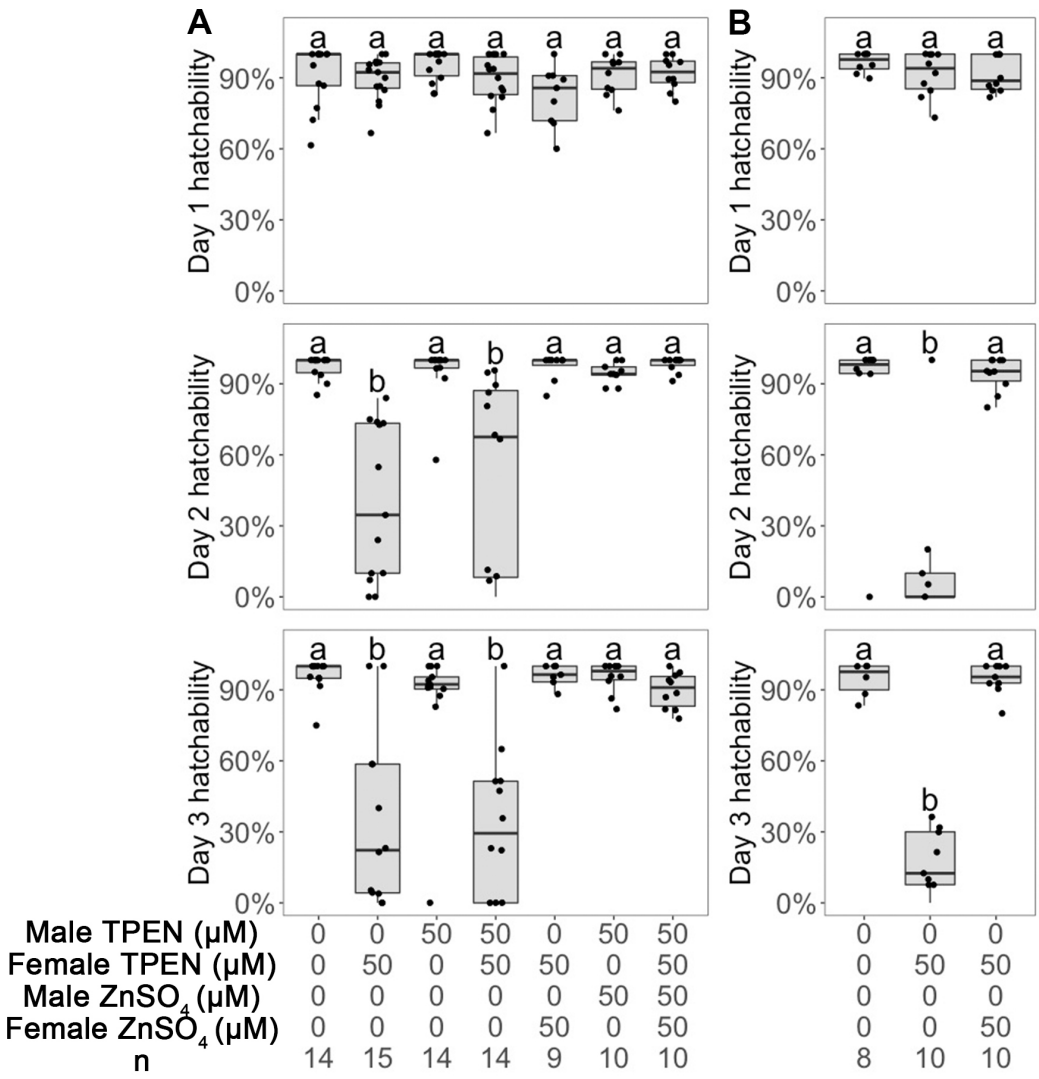

Figure S1 50 μM dietary TPEN reduces female *Drosophila* egg hatchability from day 2 post mating, related to Figure 1. (A) Egg hatchability breakdown by day of **Figure 1B**. 50 μM dietary TPEN significantly reduces females egg hatchability starting from day 2. **(B)** Egg hatchability of the second generation of females reared on TPEN mated with control males reared on normal food. Egg hatchability is still significantly reduced from day 2 post mating. Male TPEN: concentration of dietary TPEN on which the males were raised and tested. Female TPEN: concentration of TPEN added to food on which the females were raised and tested. Male $ZnSO_4$: concentration of $ZnSO_4$ added to food on which the males were raised. Female $ZnSO_4$: concentration of $ZnSO_4$ added to food on which the females were raised. n: sample size of each group. a, b: significance groups (significant difference of mean with $p<0.05$ between groups with different labels).

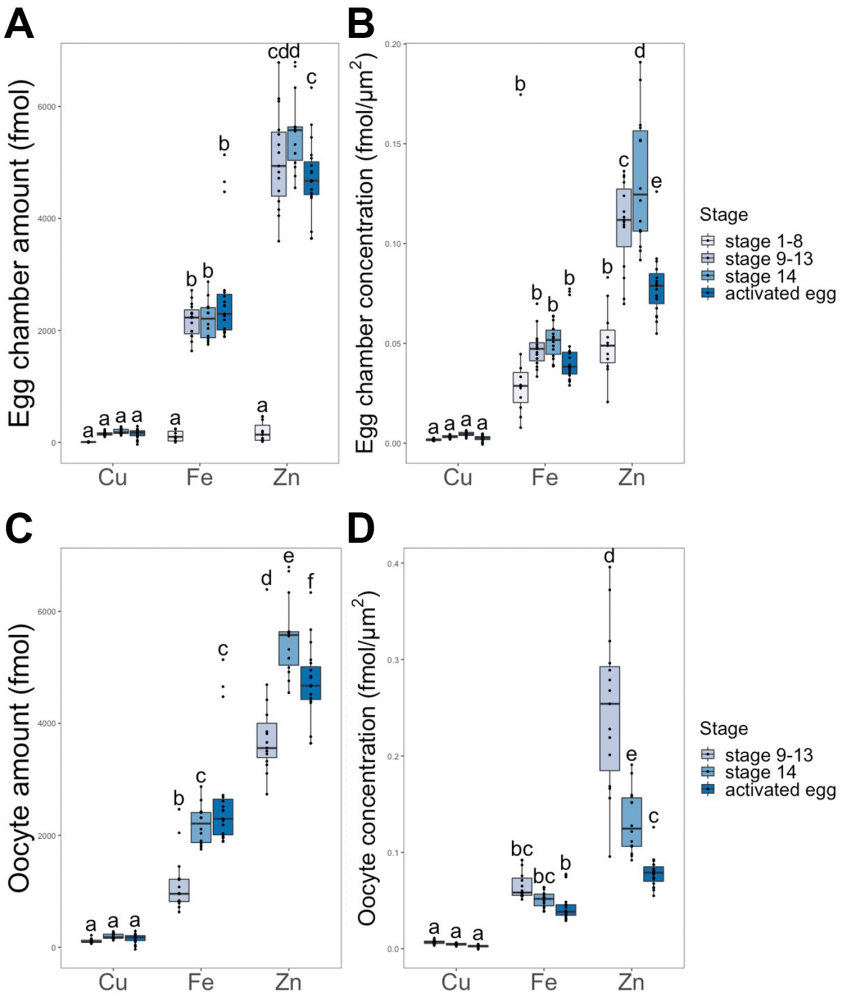

Figure S2 Levels of copper, iron and zinc in egg chambers and within oocytes during *Drosophila* oogenesis and egg activation, related to Figure 2. Copper, iron and zinc are the most abundant transition metals measured during oogenesis and egg activation. The total metal content **(A and C)** and metal concentration **(B and D)** in egg chambers **(A-B)** and oocytes **(C-D)** are plotted. Stage 1-8 oocytes n=11, stage 9-13 oocytes n=15, stage 14 oocytes n=14, activated eggs n=19. a, b, c, d, e, f: significance groups (significant difference of mean with $p<0.05$ between groups with different labels).

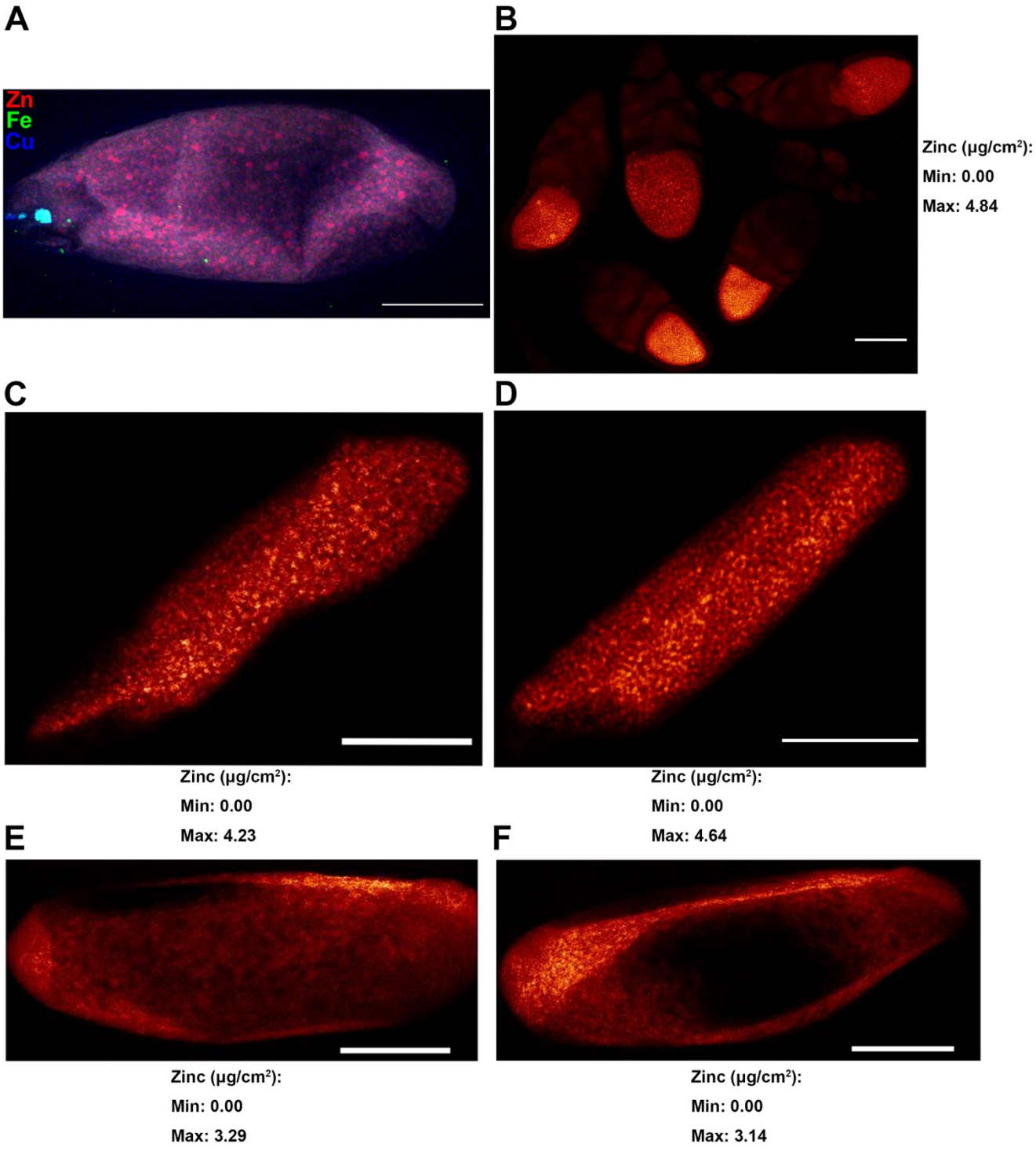

Figure S3 Additional examples of zinc distribution during oogenesis and after egg activation in wildtype *Drosophila*, related to Figure 3. (A) Overlay of iron, copper and zinc distribution in **Figure 3B**. Red: zinc, green: iron, blue: copper. **(B-F)**Representative XFM images showing zinc distribution in: **(B)** Egg chambers around stage 10; **(C-D)** Stage 14 mature oocytes; **(E-F)** Activated but unfertilized eggs. All scale bars = 100 µm.

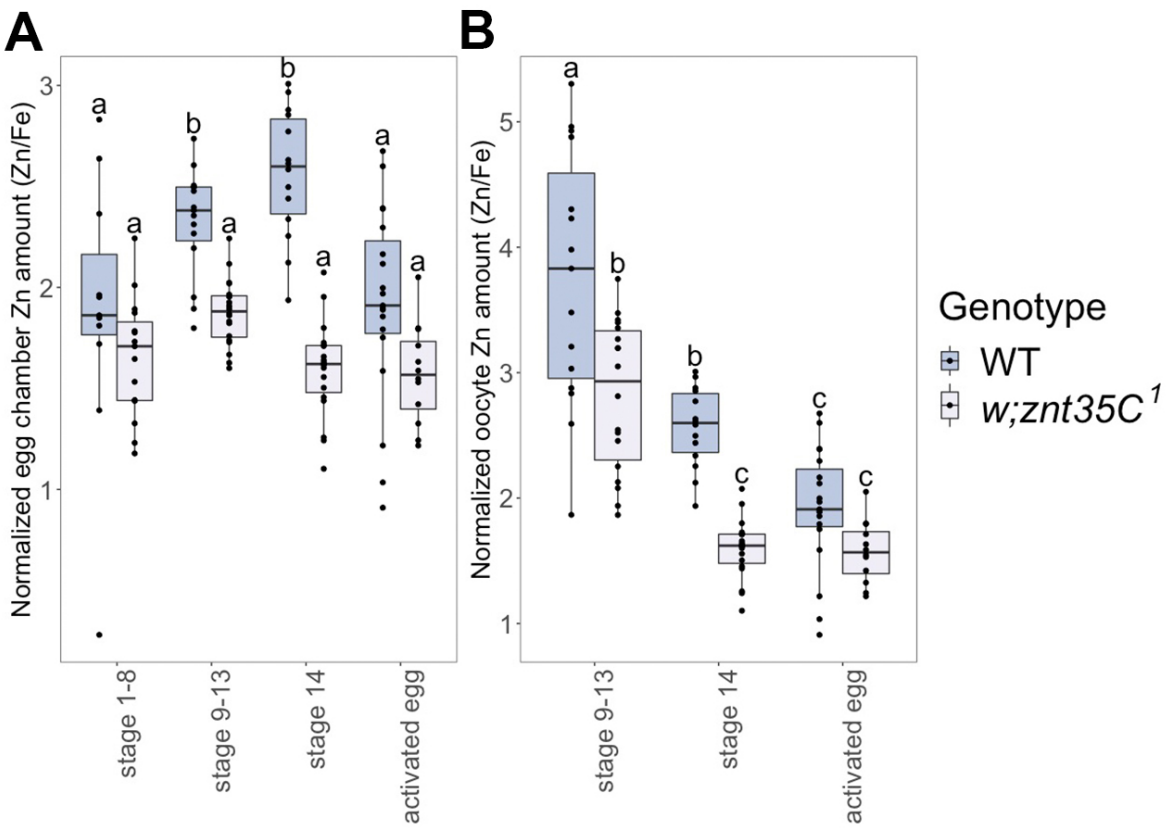

Figure S4 Normalized zinc quantification over oocyte maturation and activation in wildtype and *w; znt35C[1] Drosophila*, related to Figure 2. Zinc content normalized to iron content in ORP2 wildtype and *w; znt35C[1]* in each egg chamber **(A)** and **(B)** oocyte. WT: ORP2 wildtype. KO: *w; znt35C[1]* null mutant. Stage 1-8 oocytes WT n=11, KO n=15, stage 9-13 oocytes WT n=15, KO n =20, stage 14 oocytes WT n=14, KO n=19, activated eggs WT n=19, KO n=12. a, b, c: significance groups (significant difference of mean with $p<0.05$ between groups with different labels).

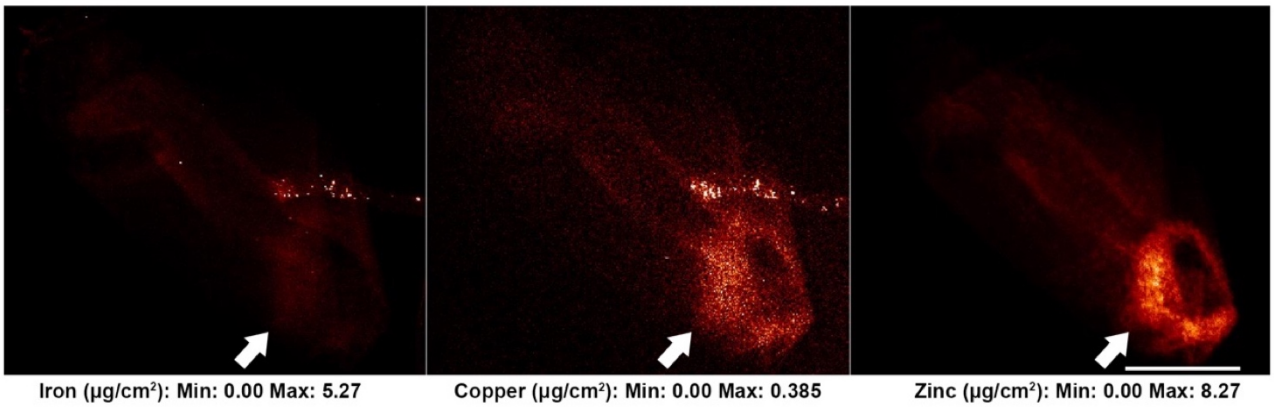

Figure S5 Distribution of iron, copper and zinc in centrifuged eggs activated *in vivo*, related to Figure 3. Iron, copper and zinc accumulated at the posterior end (indicated by arrows) of *in vivo* activated eggs after centrifugation (n=3). Scale bar = 100 µm.

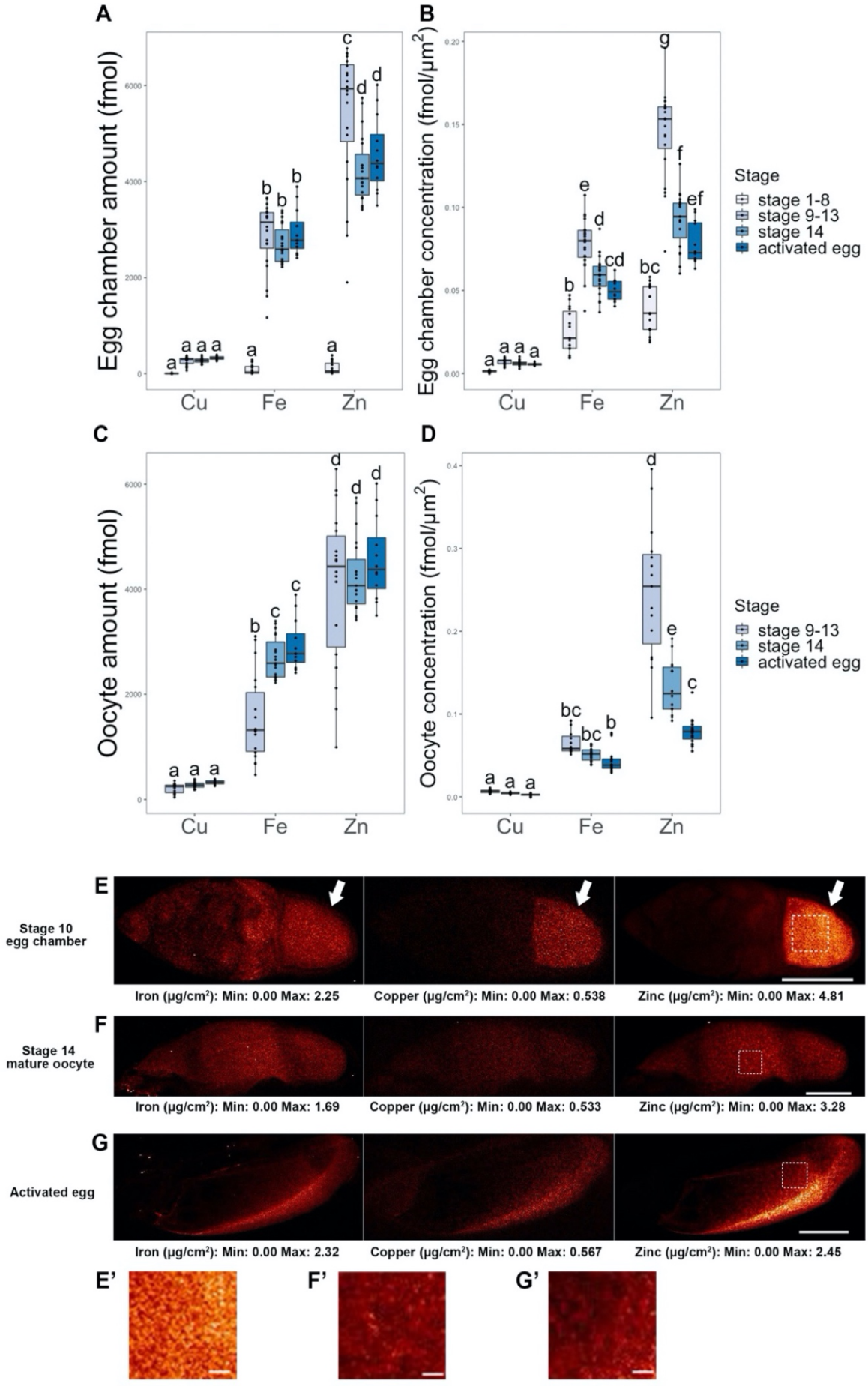
A
Egg chamber amount (fmol)
Cu
Fe
Zn
B
Egg chamber concentration (fmol/µm²)
Stage
stage 1-8
stage 9-13
stage 14
activated egg
C
Oocyte amount (fmol)
D
Oocyte concentration (fmol/µm²)
E
Stage 10
egg chamber
Iron (µg/cm²): Min: 0.00 Max: 2.25
Copper (µg/cm²): Min: 0.00 Max: 0.538
Zinc (µg/cm²): Min: 0.00 Max: 4.81
F
Stage 14
mature oocyte
Iron (µg/cm²): Min: 0.00 Max: 1.69
Copper (µg/cm²): Min: 0.00 Max: 0.533
Zinc (µg/cm²): Min: 0.00 Max: 3.28
G
Activated egg
Iron (µg/cm²): Min: 0.00 Max: 2.32
Copper (µg/cm²): Min: 0.00 Max: 0.567
Zinc (µg/cm²): Min: 0.00 Max: 2.45
E'
F'
G'

Figure S6 Levels and distributions of copper, iron and zinc in egg chambers and within oocytes during oogenesis and egg activation in *w; znt35C^{1}*, related to Figure 4. Copper, iron and zinc remain the most abundant transition metals measured during oogenesis and egg activation in *w; znt35C^{1}* mutants. The total metal content **(A and C)** and metal concentration **(B and D)** in egg chambers **(A-B)** and oocytes **(C-D)** are plotted. Stage 1-8 oocytes n=15, stage 9-13 oocytes n=20, stage 14 oocytes n=19, activated eggs n=12. a, b, c, d, e, f, g: significance groups (significant difference of mean with $p<0.05$ between groups with different labels). **(E-G)** In *w; znt35C^{1}* mutants, representative XFM images showing iron, copper and zinc distribution in: **(E)** an egg chamber around stage 10. Within an egg chamber, iron, copper and zinc are all still more concentrated in the oocyte (arrows); **(F)** a mature oocyte (stage 14). Zinc displays an even distribution.; **(G)** an activated but unfertilized egg. Zinc displays an even distribution. Scale bars = 100 μm. **(E'-G')** Enlarged view of zinc distribution in **(E-G)**. Dashed squares indicate enlarged regions. Scale bars = 10 μm.

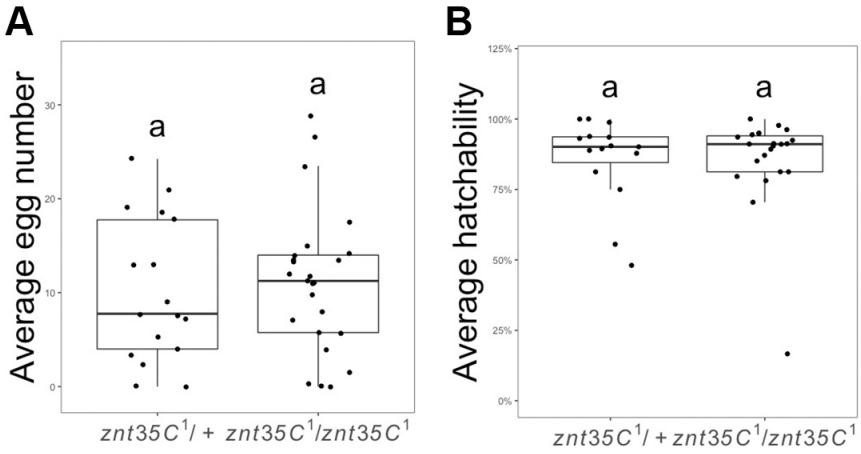

Figure S7 *znt35C[1]* does not affect female egg production and hatchability, related to Figure 4. Average **(A)** egg number and **(B)** hatchability over 4 days produced by control (*znt35C[1]*/+, n=17) and *znt35C* knockout (*znt35C[1]*/*znt35C[1]*, n=25) females mated with ORP2 wildtype males. a: significance groups (significant difference of mean with $p<0.05$ between groups with different labels).

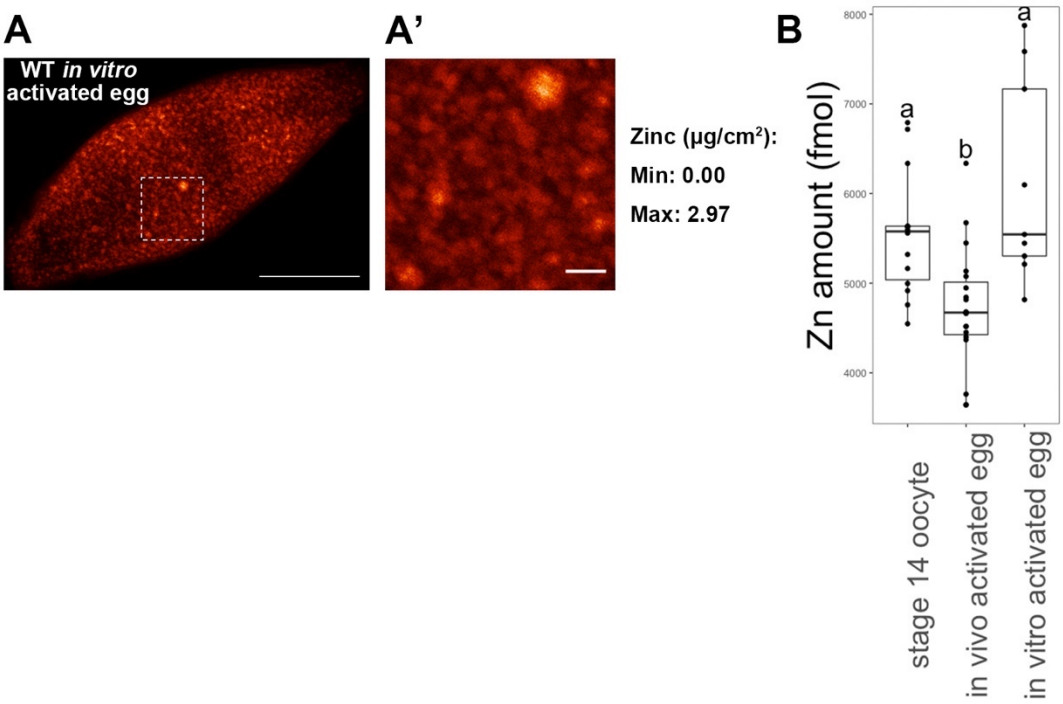

Figure S8 Zinc distribution and level in eggs activated *in* vitro, related to Figure 2 and 3. (A) Activation *in vitro* did not lead to a decrease in number of zinc-enriched granules in contrast to what was seen with *in vivo* egg activation. Scale bar = 100 µm; **(A')** Enlarged view of **(A)**. Dashed squares indicate enlarged regions. Scale bar = 10 µm; **(B)** Zinc levels in stage 14 mature oocyte, *in vivo* and *in vitro* activated eggs. Egg activation *in vitro* did not significantly reduce oocyte zinc levels in contrast to what was seen *in vivo* (stage 14 oocyte n=14, *in vivo* activated egg n=19, *in vitro* activated egg n=9). a, b: significance groups (significant difference of mean with $p<0.05$ between groups with different labels).

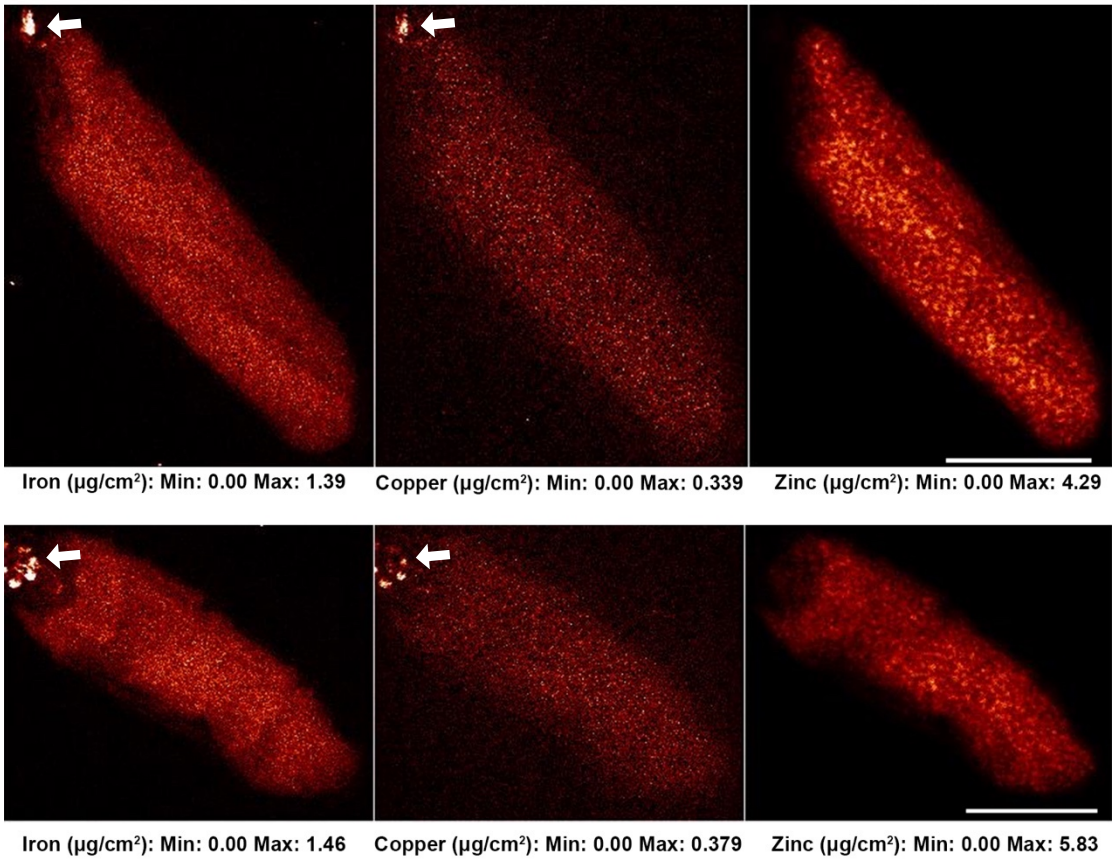

Figure S9 Additional examples showing granules enriched with iron and copper at the anterior end of wildtype mature oocytes, related to Figure 3. As seen in **Figure 3B**.

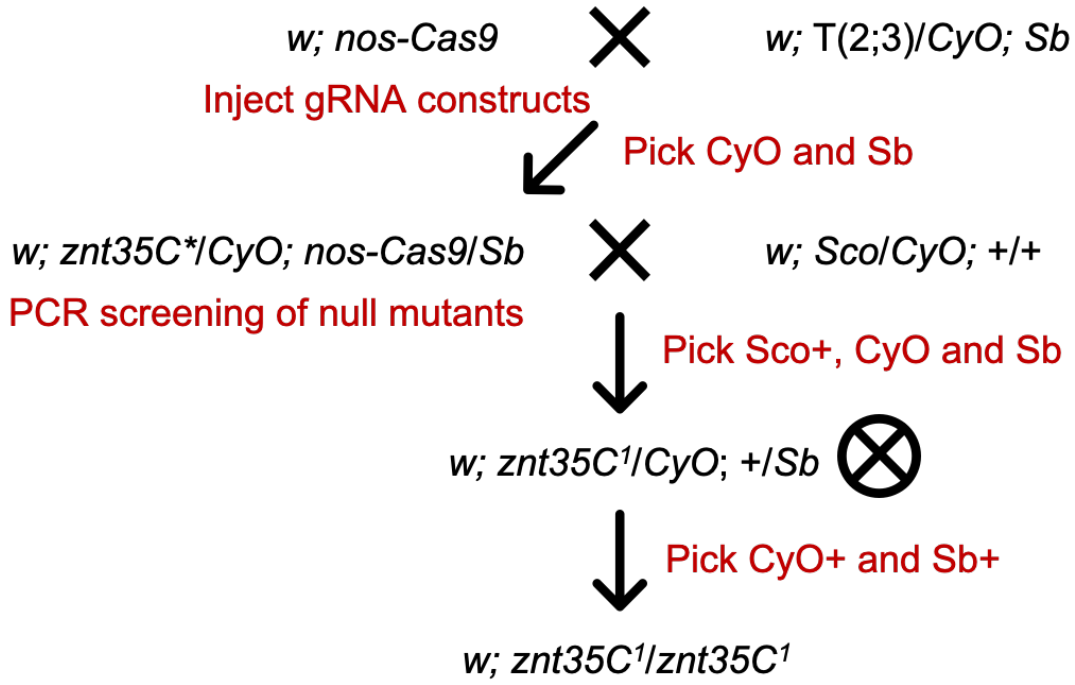

Figure S10 Crossing scheme to generate the *w; znt35C^{1}* mutant fly strain, related to Figure 4.