

Figure S1. Relatively weak ubiquitin staining is observed in oocytes.

(A) Stylized drawing of one gonad arm connected to the spermatheca and the uterus. The landmark events before and after fertilization and their typical time course are indicated. In *C. elegans*, oocytes of the hermaphrodite gonad are arrested in diakinesis of meiotic prophase I. The mature oocyte ovulates and enters the spermatheca containing sperms, which is followed by immediate fertilization (1 oocyte ovulates every 23 min, on average). After fertilization, embryos move to the uterus, complete meiosis I and meiosis II, and start zygotic development. *emb-27(RNAi)* blocks the metaphase to anaphase transition of meiosis I. (B) The wild-type N2 gonads were dissected from adult hermaphrodites and stained with an anti-ubiquitin antibody (red), an anti-K63-linked ubiquitin antibody (green), and DAPI (blue). The upper images were acquired under the same condition used in Fig. 1 A–C. Images of the same oocytes were also obtained using a higher laser power (middle and lower panels). Embryos are numbered by the position from the spermatheca. SP, spermatheca. Enlarged images ($\times 4$) are shown as insets. Bar, 10 μm .

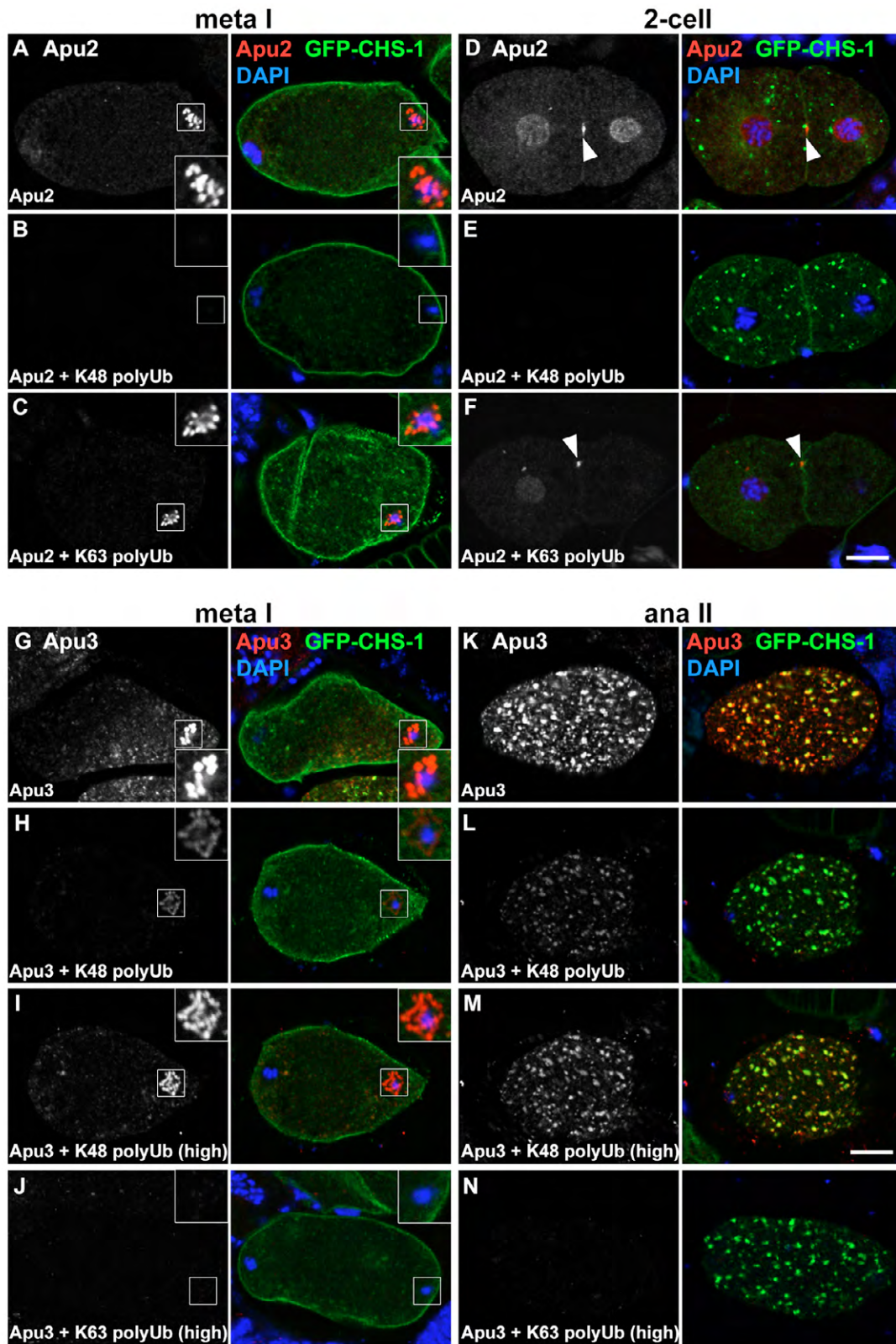


Figure S2. Specificity of anti-K48-ubiquitin and anti-K63-linked ubiquitin antibodies.

(A-F) Embryos expressing GFP-CHS-1 were stained with the anti-K48-linked ubiquitin antibody (Apu2) or the antibody preincubated with purified K48- or K63-linked polyubiquitin. Embryos in metaphase I (A-C) and the 2-cell stage (J-N) are shown. Insets show the MO staining ($\times 2$), and arrowheads in F indicate the cleavage furrow. All images were acquired under the same conditions. (G-N) Embryos expressing GFP-CHS-1 were stained with the anti-K63-linked ubiquitin antibody (Apu3) or the antibody preincubated with purified K48- or K63-linked polyubiquitin. Embryos in metaphase I (G-J) and anaphase II (K-N) are shown. Images in I, J, M, and N were acquired using a higher laser power. Insets show the MO staining ($\times 2$). Merged images of antibody staining, GFP-CHS-1, and DAPI are shown for reference. Bar, 10 μm .

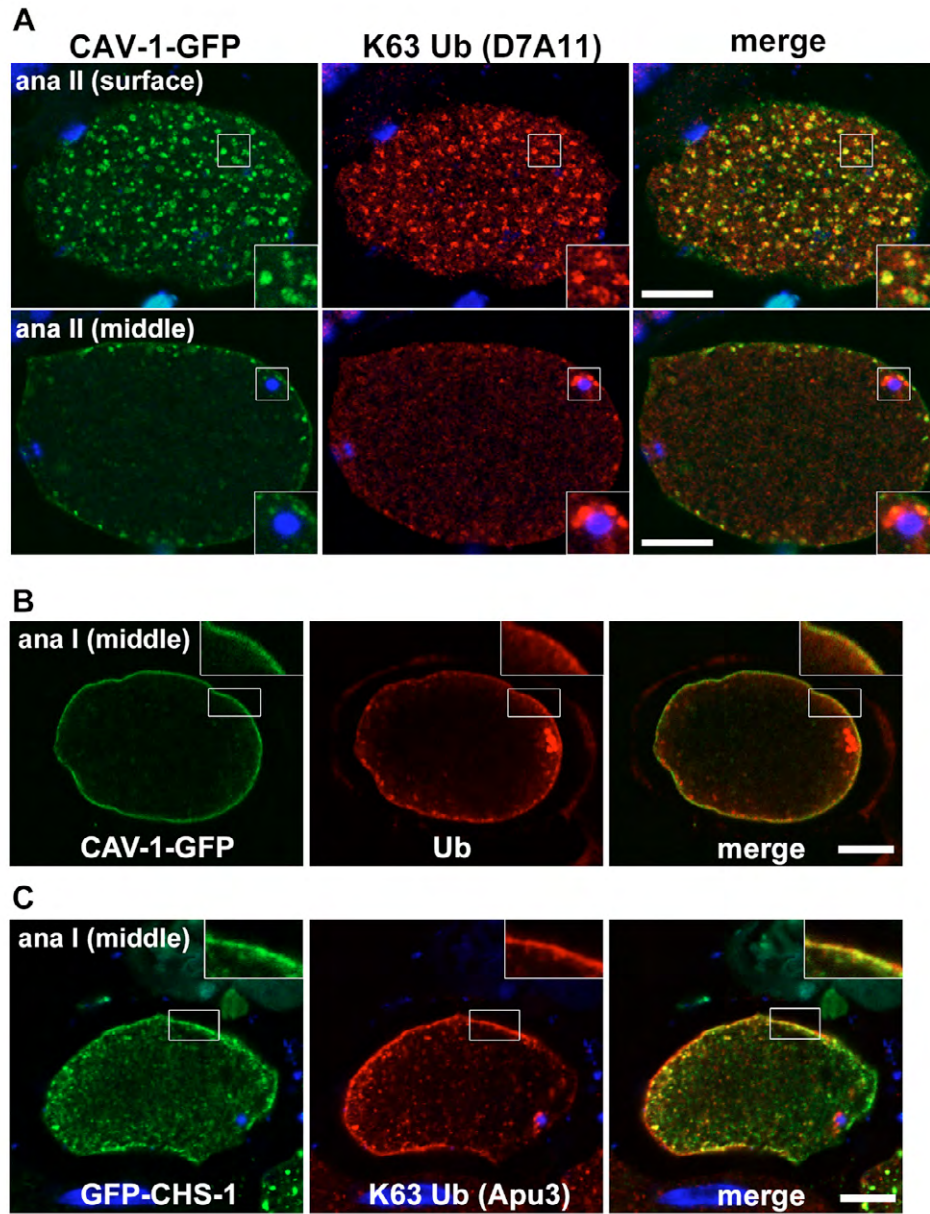


Figure S3. Ubiquitination is detectable on the PM in late anaphase I.

(A) Embryos expressing CAV-1-GFP were stained with an anti-K63-linked ubiquitin antibody (D7A11). Surface and middle sections of an embryo in anaphase II are shown. Insets ($\times 2$) show endosomes (upper panel) and the MOs (lower panel). (B and C) Embryos expressing CAV-1-GFP or GFP-CHS-1 were stained with an anti-ubiquitin antibody (FK2; B) or an anti-K63-linked ubiquitin antibody (Apu3; C), respectively. Embryos in late anaphase I are shown. Ubiquitin staining was detected on the PM in this stage. Insets show enlarged images of the boxed area. Bars, 10 μm .

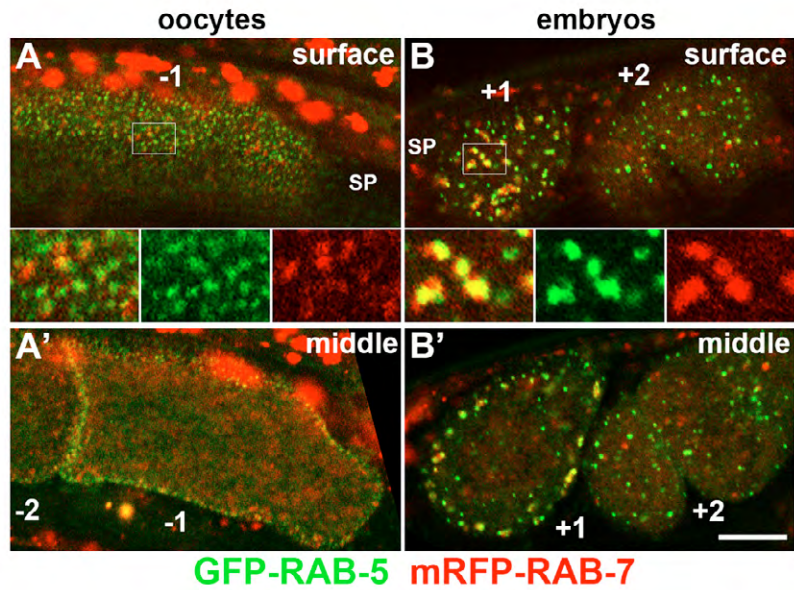


Figure S4. RAB-5- and RAB-7-positive endosomes are remodeled after fertilization.

Subcellular localization of GFP-RAB-5 (green) and mRFP-RAB-7 (red) in oocytes and embryos of a single hermaphrodite. The surface (A and B) and middle (A' and B') planes of oocytes (A and A') and embryos (B and B') are shown. All images were acquired under the same conditions. In A and B, enlarged images ($\times 3$) of the boxed areas are also shown. SP, spermatheca. Bar, 10 μm .

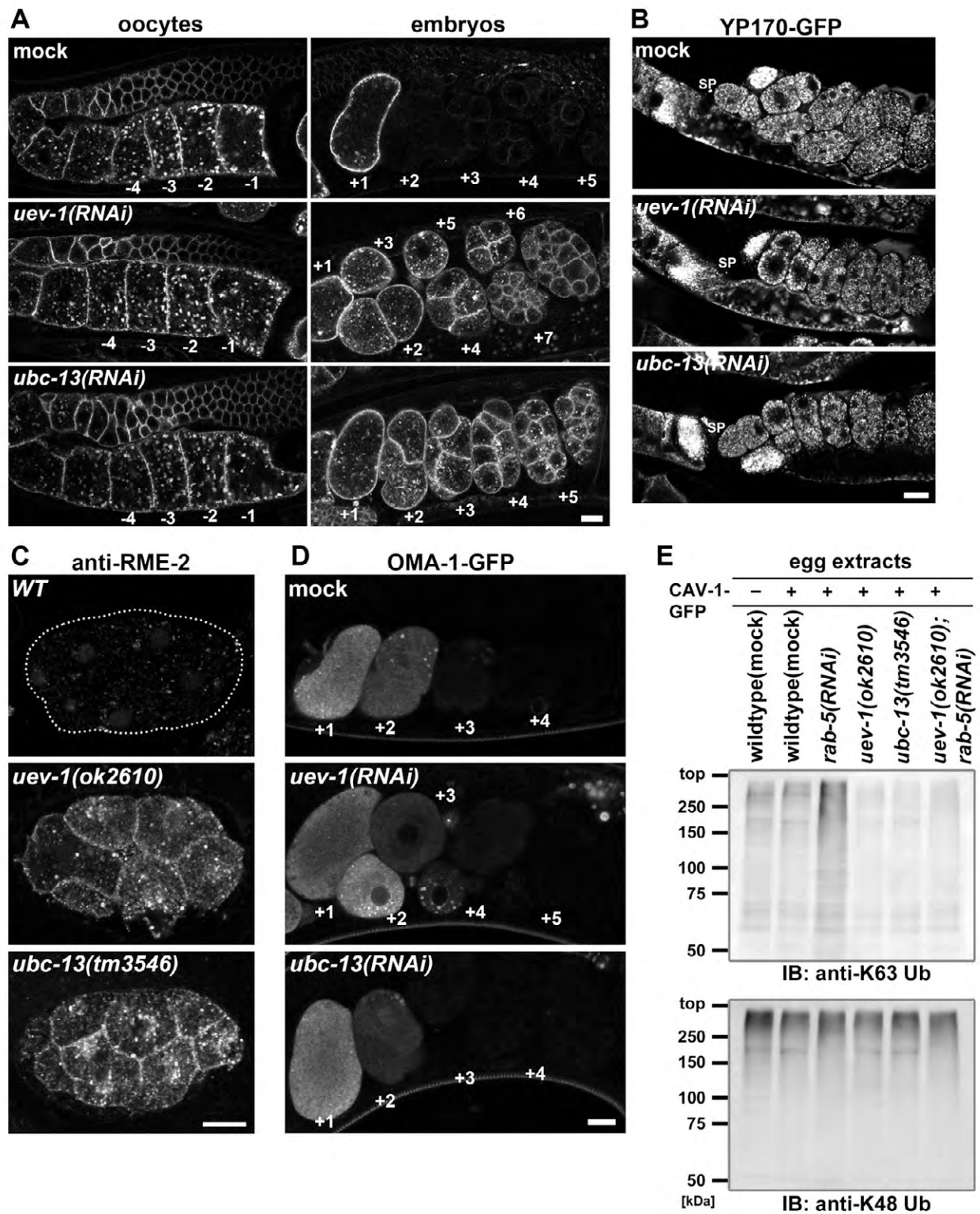


Figure S5. Phenotypes of *ubc-13* and *uev-1* mutants.

(A) RNAi of *ubc-13* or *uev-1* inhibits clearance of CAV-1-GFP in embryos. Oocytes and embryos were observed in wild-type worms and in *ubc-13(RNAi)* or *uev-1(RNAi)* mutants expressing CAV-1-GFP. (B) Yolk uptake was normal in *ubc-13* and *uev-1* mutants. Worms expressing YIT-2-GFP were treated with *ubc-13* or *uev-1* RNAi. (C) Degradation of endogenous RME-2 was inhibited in *ubc-13* and *uev-1* mutants. Wild-type embryos and *ubc-13(tm3546)* or *uev-1(ok2610)* mutant embryos were stained with an anti-RME-2 antibody. The 8-cell stage embryos are shown. (D) Degradation of OMA-1-GFP was normal in *ubc-13* and *uev-1* mutants. Worms expressing OMA-1-GFP were treated with *ubc-13* or *uev-1* RNAi. Embryos are numbered by the position from the spermatheca. Bars, 10 μ m. (E) Embryo lysates (10 μ g) were prepared from the indicated strains and probed with anti-K63- and anti-K48-linked ubiquitin antibodies.