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Supplemental information

Identification of embryonic RNA granules that act as sites of mRNA translation after changing their physical properties

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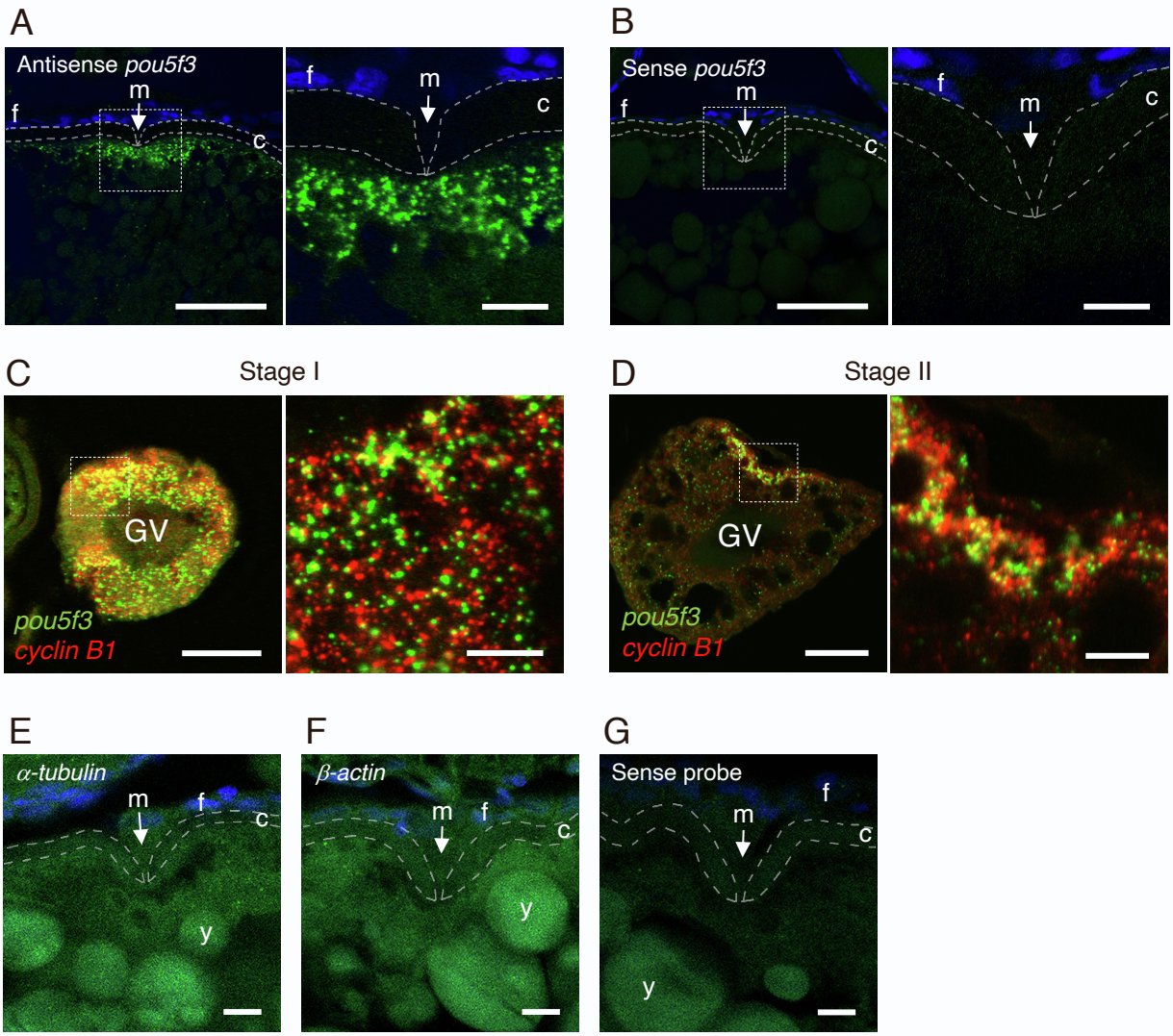

Figure S1. Growing and fully grown oocytes store *pou5f3* mRNA as RNA granules that differ from those of *cyclin B1* mRNA, related to Figure 1.

(A and B) FISH analysis of *pou5f3* mRNA in fully grown oocytes with antisense (A) and sense (B) RNA probes. The images on the right show enlarged views of the boxed regions. The chorion is outlined by broken lines. DNA is shown in blue. m, micropyle; f, follicle cells; c, chorion.

(C and D) Double FISH analyses of *pou5f3* (green) and *cyclin B1* (red) mRNAs in stage I and stage II growing oocytes. The images on the right show enlarged views of the boxed regions.

(E-G) FISH analysis of fully grown oocytes with *α-tubulin* antisense RNA probe (E) and with *β-actin* antisense (F) and sense (G) RNA probes. y, yolk granules. Note that yolk granules show auto-fluorescence in these images due to exposure to high-level laser. The signals of *α-tubulin* and *β-actin* antisense probes were found to be diffusely distributed in the oocyte cortical cytoplasm. No signal was detected with sense probes. Bars, (A-D) 50 μm in images on the left; 10 μm in images on the right, (E-G) 10 μm.

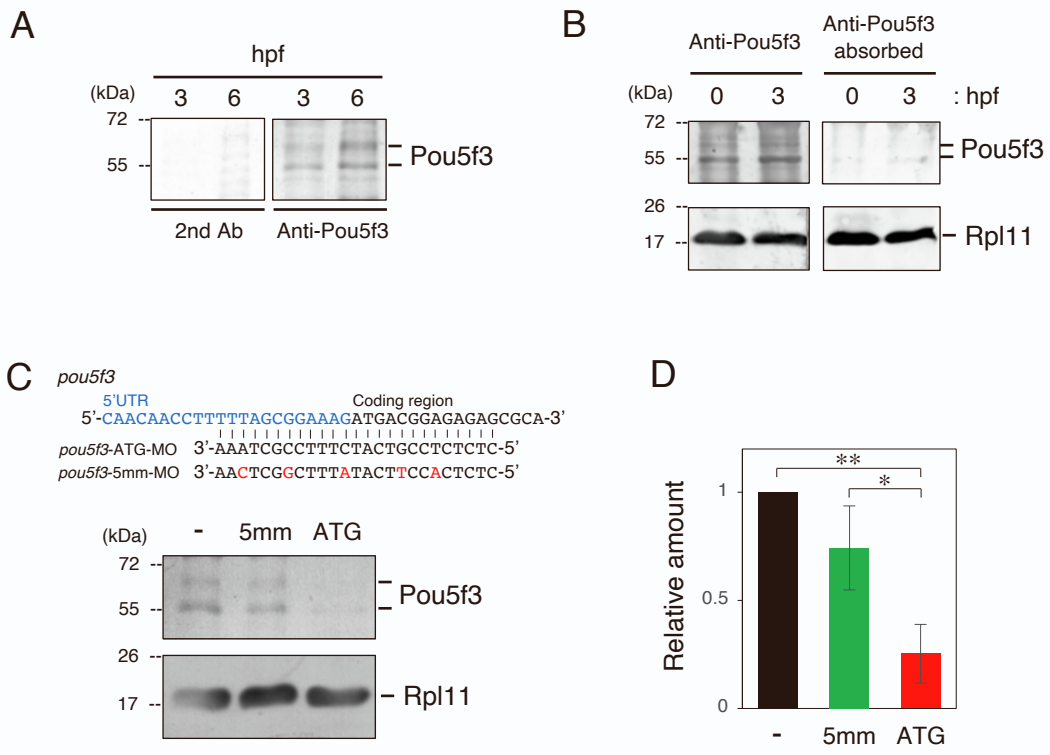

Figure S2. Detection of Pou5f3 with anti-Pou5f3 antibodies, related to Figure 2.

(A) Extracts of zebrafish embryos at 3 and 6 hpf were probed with the anti-Pou5f3 antibody (Anti-Pou5f3) or without the primary antibody (2nd Ab). Similar results were obtained with other anti-Pou5f3 antibodies produced in this study.

(B) Immunoblotting was performed with the anti-Pou5f3 antibody incubated with (Anti-Pou5f3 absorbed) or without (Anti-Pou5f3) Pou5f3-His recombinant proteins.

(C) Upper, the 5'UTR and coding region sequences of *pou5f3* mRNA that are targeted by *pou5f3*-ATG-MO. *pou5f3*-5mm-MO contains 5 mismatches (red). Lower, immunoblotting of Pou5f3 and Rpl11 in embryos not injected (-) and injected with *pou5f3*-5mm-MO (5mm) and *pou5f3*-ATG-MO (ATG) at 6 hpf.

(D) Quantitative analysis of immunoblotting (means ± SD; n = 3). *P < 0.05; **P < 0.01 (Tukey-Kramer test).

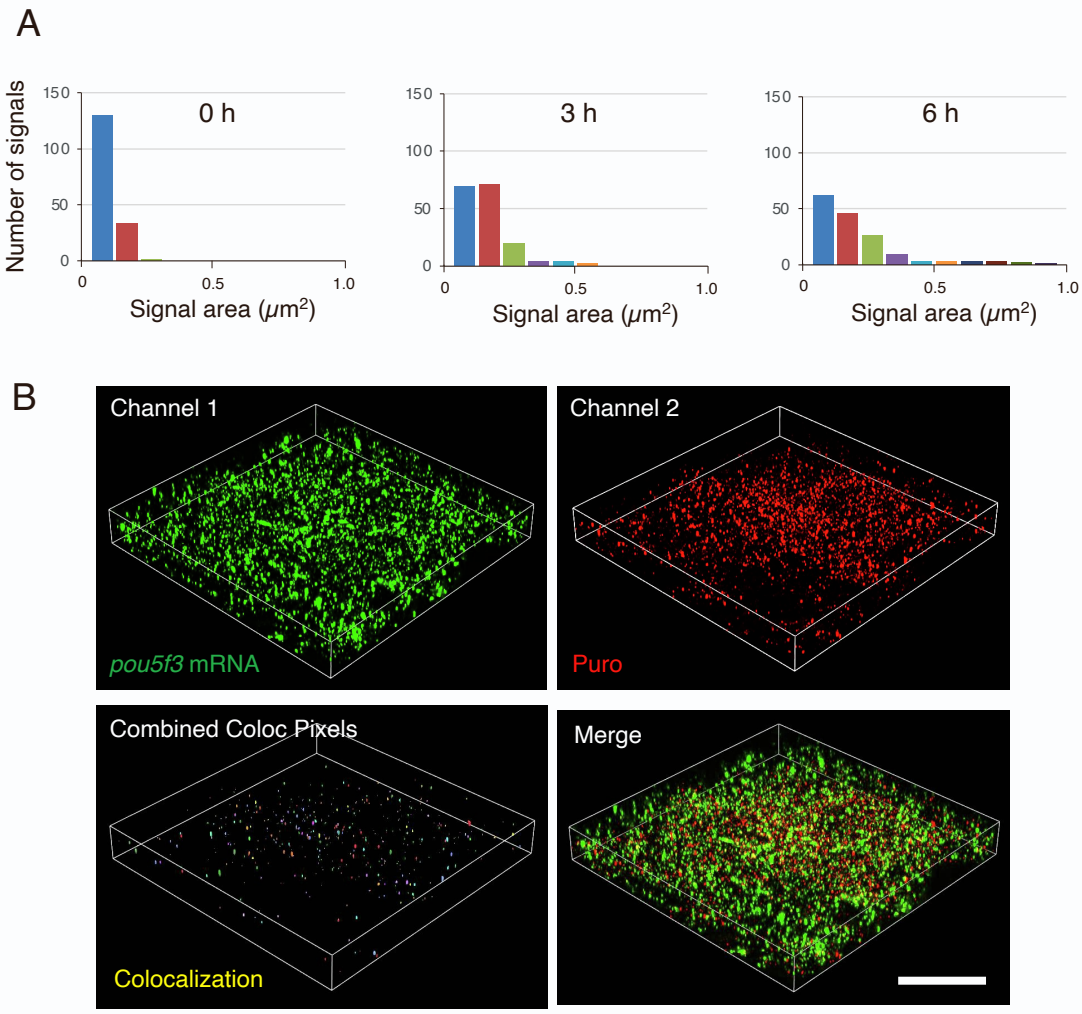

Figure S3. Quantitative analyses of signals of newly synthesized peptides, related to Figure 3.
(A) Quantitative analysis of the areas of newly synthesized peptides in embryos at 0, 3 and 6 hours (h) post fertilization. The size of signals started to increase after fertilization.
(B) Quantitative analysis of the colocalization of *pou5f3* RNA granules and newly synthesized peptides in embryos at 3 hpf using the Pixel Colocalization tool in AIVIA. Z-stack images of *pou5f3* mRNA were applied in Input Coloc Channel 1 and those of puromycylated peptides (Puro) were applied in Input Coloc Channel 2. After performing image analysis of these two inputted channels, the colocalized spaces were shown in Combined Coloc Pixels. Bar, 20 μm.

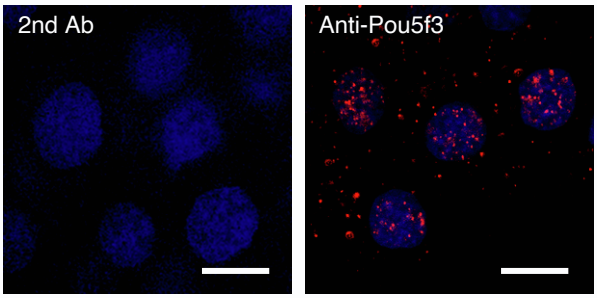

Figure S4. Pou5f3 is localized in the nucleus in embryonic cells, related to Figure 4.
Immunofluorescence (red) of embryos at 3 hpf without (2nd Ab) and with (Anti-Pou5f3) the anti-Pou5f3 antibody. DNA is shown in blue. Bars, 10 μm.

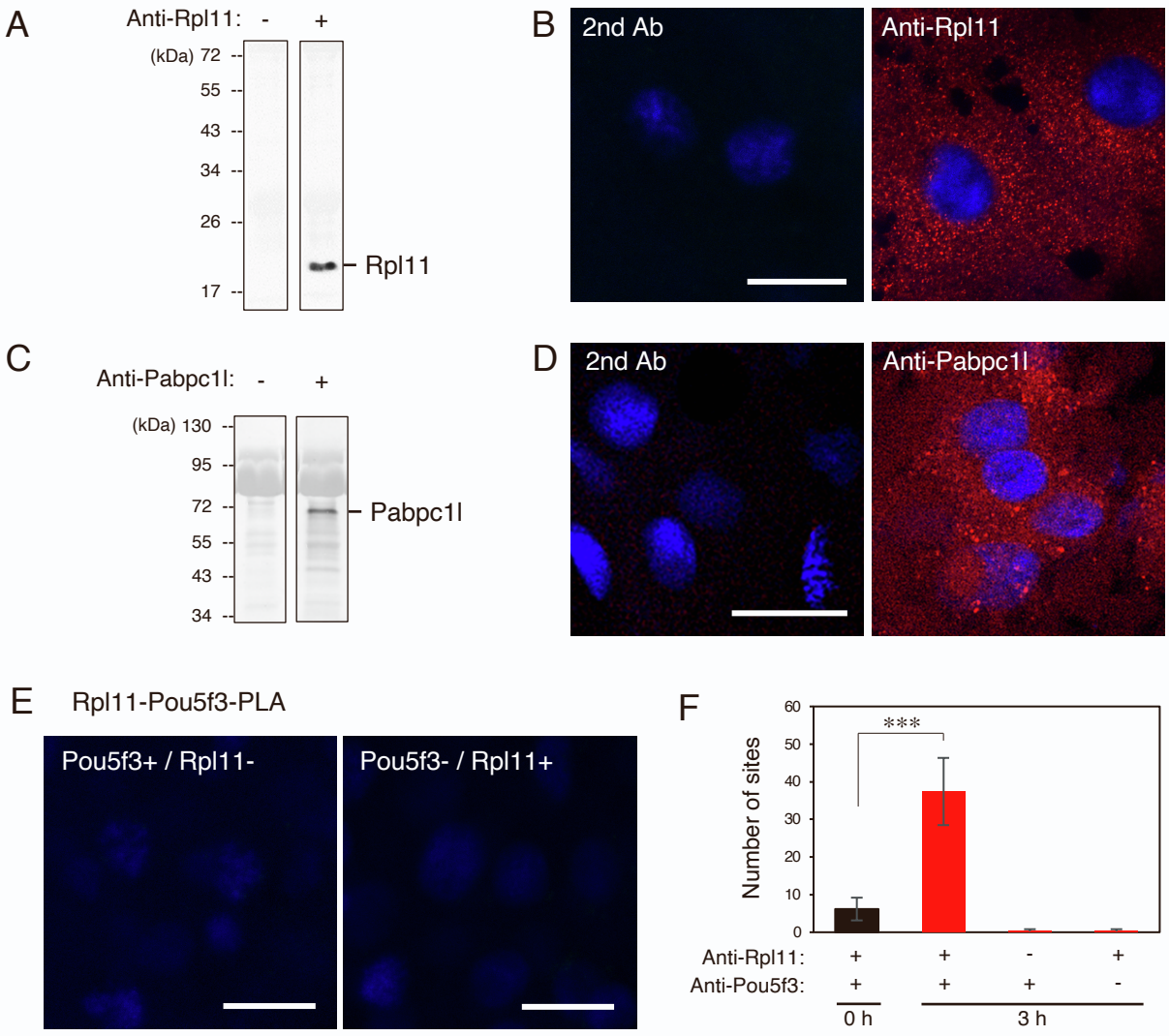

Figure S5. Detection of Rpl11, Pabpc1l and Rpl11-Pou5f3-PLA in embryos, related to Figure 5.

(A) Extracts of embryos at 3 hpf were probed with (+) or without (-) the anti-Rpl11 antibody.

(B) Fixed embryos at 3 hpf were probed with anti-Rpl11 antibody (Anti-Rpl11) or without the primary antibody (2nd Ab). Similar results were obtained from four independent experiments.

(C) Extracts of embryos at 3 hpf were probed with (+) or without (-) the anti-Pabpc1l antibody.

(D) Fixed embryos at 3 hpf were probed with anti- Pabpc1l antibody (Anti- Pabpc1l) or without the primary antibody (2nd Ab). Similar results were obtained from four independent experiments.

(E) Single confocal optical images of Rpl11-Pou5f3 PLA in embryos at 3 hpf. After fixation, embryos were incubated with (+) and without (-) anti-Pou5f3 antibody (Pou5f3) and anti-Rpl11 antibody (Rpl11). DNA is shown in blue. Bars, 20 μm.

(F) The number of Rpl11-Pou5f3 PLA sites per 360 μm^2 was counted (means ± standard deviations; n = 10). ***P < 0.001 (Tukey-Kramer test).

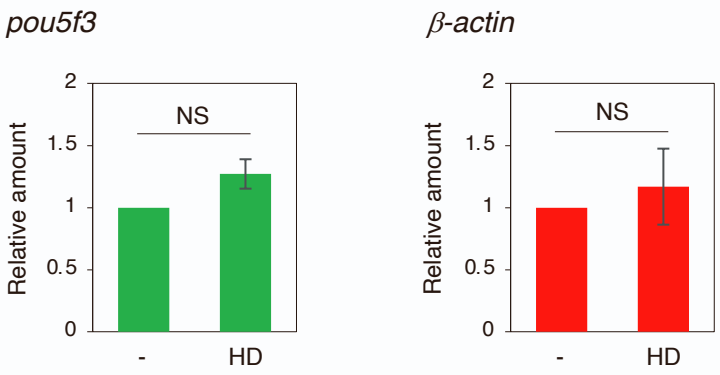

Figure S6. Quantitative analysis of mRNAs in embryos treated with hexanediol, related to Figure 6.

Quantitative PCR for *pou5f3* (left) and *β-actin* (right) mRNAs in embryos at 3 hpf treated without (-) and with hexanediol (HD) (means ± standard deviations; n = 3). NS, not significant (Student's *t*-test). The amount of *pou5f3* mRNA was not changed after hexanediol treatment.

Table S1 : List of primers used in current study, Related to STAR Methods

Oligonucleotides	**Source**	**Identifyer**
Morpholino: Pou5f3-ATG-MO 5'-CTCTCTCCGTCATCTTTCCGCTAAA-3'	This paper	N/A
Morpholino: Pou5f3-5mm-MO 5'-CTCACTCCTTCATATTTCGGCTCAA-3'	This paper	N/A
Primers for poly(A) test assay P1 anchor primer 5'-P-GGTCACCTTGATCTGAAGC-NH_2-3' P1' primer 5'-GCTTCAGATCAAGGTGACCTTTTT-3' z*pou5f3*-PAT-f1 primer 5'-TAGATGTACTCTTTGTCAGGGTGG-3' z*cyclin B1*-PAT-f1 primer 5'-GAGGGCCTTTCTAAGCATCTGGCTGTG-3' z*pou5f3*-PAT-f2 primer 5'-TGGAGGTCATGTGCCTTATCTTTC-3' z*cyclin B1*-3'UTR-f primer 5'-TACGGATTTCTTCACTGCCATG-3'	This paper	N/A
Primers for quantitative RT-PCR z*pou5f3*-qPCR-f1 primer 5'-AACACAAGCGCATCACTCTG-3' z*pou5f3*-qPCR-r1 primer 5'-ACAACGGCTTCAGTTTGCAC-3' z*β-actin*-qPCR-f1 primer 5'-AAATCGCTGCCCTGGTCGTT-3' z*β-actin*-qPCR-r1 primer 5'-CTGTCCCATGCCAACCATCA-3'	This paper	N/A