Supplementary materials

File S1. CTAB-based total RNA isolation protocol

Solutions and reagents
1)CTAB buffer for homogenisation:
2% (w/v) CTAB
2 M NaCl
100 mM Tris-HCl
20 mM EDTA
pH = 8 (adjusted by 1 M NaOH).
10% (v/v) β-mercaptoethanol
Deionized water

2) Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v)
Water-saturated phenol (pH = 4.5)
Chloroform
Isoamyl alcohol.

3) 8 M LiCl
4) 80% Ethanol.
5) 96 - 100% Ethanol

The components in the extraction buffer, excluding β-mercaptoethanol, were mixed and heated for 30 min at 100 °C for sterilisation.

Protocol for isolation of RNA

1. Pre-heat 2 ml of CTAB buffer at 65 °C
2. Homogenize the oocytes spawned by two females in liquid nitrogen with a mortar and pestle to a homogeneous mass
3. Pour 1ml of preheated buffer into the mortar until the mixture is thawed.
4. Transfer to a test tube and add 1ml of preheated buffer.
5. Distribute the lysate into two test tubes.
6. Incubate for 10 minutes at 65°C. Shake briefly every minute.
7. Add 1ml Phenol/Chloroform/Isoamyl Alcohol mixture (25:24:1) to each tube with lysate.
8. Shake vigorously for 5 minutes
9. Centrifuge at 4°C, max rpm (16100 rcf) for 15 minutes to separate phases.
10. Transfer supernatant into a new tube without touching the interphase
11. Re-add 1 ml of Phenol/Chloroform/Isoamyl Alcohol (25:24:1) to each tube with lysate.
12. Repeat steps 8-10

13. Add an equal volume of 8M LiCl to the supernatant and mix, then centrifuge briefly to dislodge droplets
14. Leave at -20°C in a vertical position overnight
15. Centrifuge at 4°C, max rpm for 45 minutes to precipitate RNA
16. Remove the supernatant, leaving a precipitate containing RNA
17. Add 80% EtOH to the precipitate, break the pellet on a vortex and spin for 10 minutes at 4°C at max rpm
18. Repeat steps 16 and 17
19. Add 96-100% EtOH to the precipitate, break the pellet on a vortex and spin for 10 minutes at +4°C at max rpm.
20. Remove the supernatant and dry for 15 minutes in open-top tubes at room temperature
21. Dissolve in 100 µl of RNAse free H2O 10 minutes at 37°C

File S2. Quantification of RNA Fluorescence: Computed Values. In the provided Excel file, we include computed values for volume (in microns and voxels), integrated density, mean background, and Corrected Total Cell Fluorescence (CTCF) for each examined blastomere in three embryos.

https://docs.google.com/spreadsheets/d/1FhB364N4VoFgEQbLxTQpi-nCsGVrk0_3/edit?usp=sharing&ouid=117228115148361492966&rtpof=true&sd=true

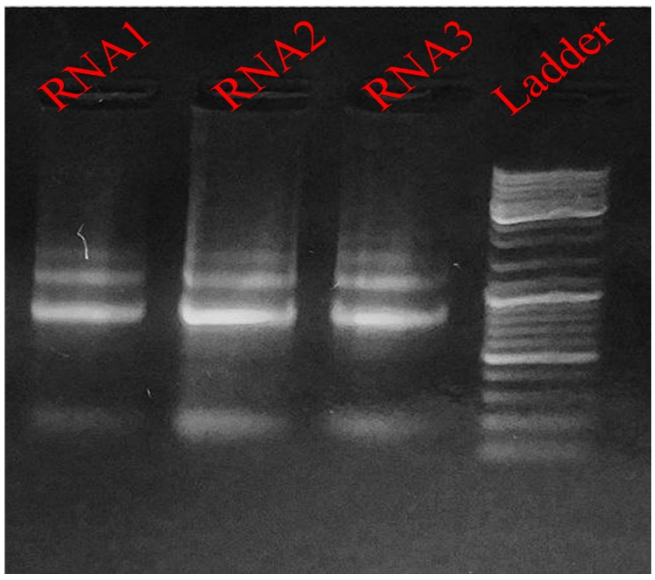

Fig. S1 Agarose gel electrophoresis with extracted oocyte RNA. Three samples were prepared with cetyltrimethylammonium bromide (CTAB), subjected to phenol-chloroform phase separation, and finally precipitated

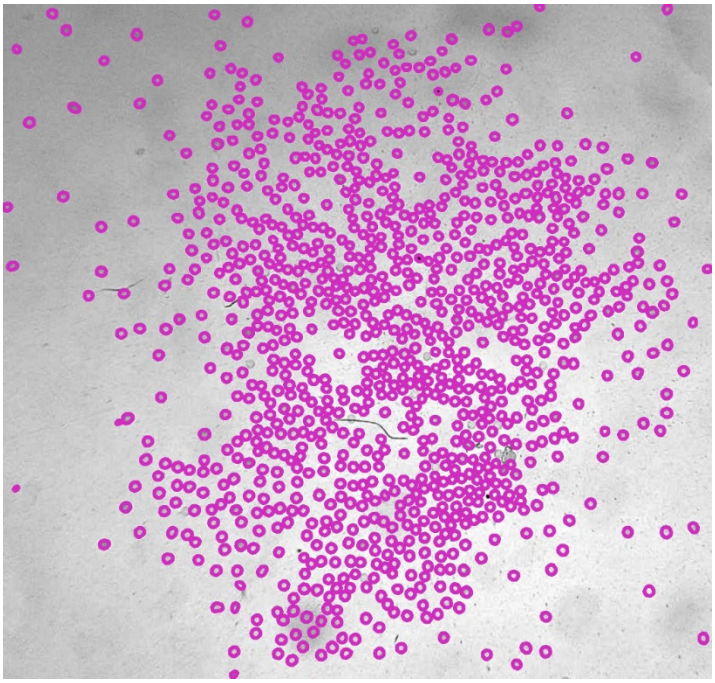

Fig. S2. Counting the number of oocytes in a 100 µl aliquot using machine learning via the DLgram service. In this image, a total of 1044 oocytes were automatically identified. An additional 13 unlabeled oocytes were counted manually using Fiji software

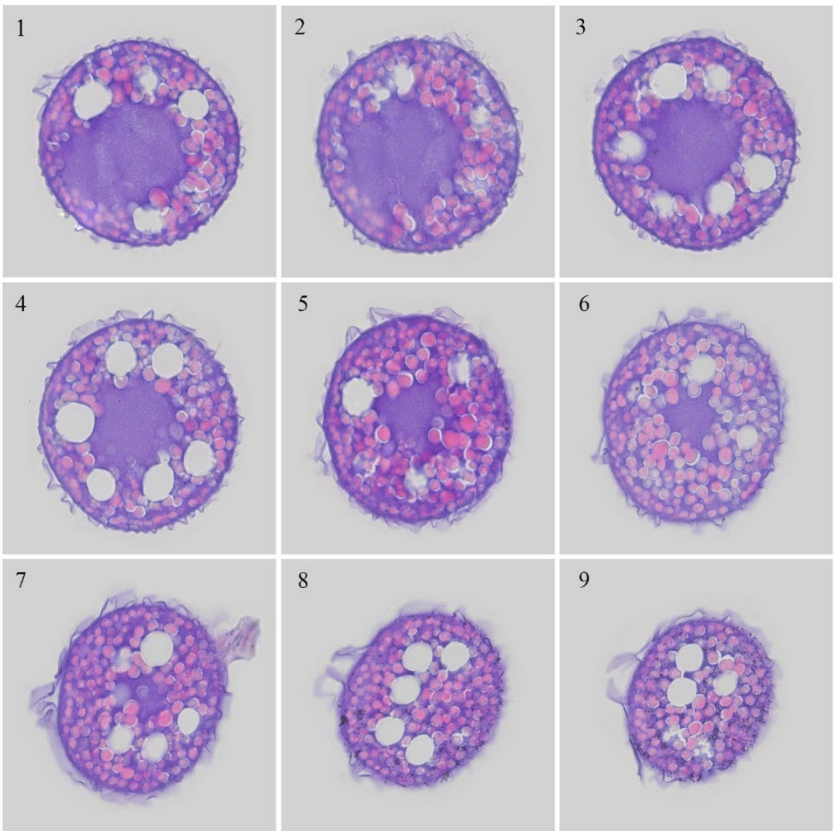

Fig. S3. Complete series of histological sections of *P. dumerilii* zygotes (0.5 hpf) stained with Carazzi's hematoxylin and eosin. 1 — section closest to the animal pole; 9 — section closest to the vegetal pole.

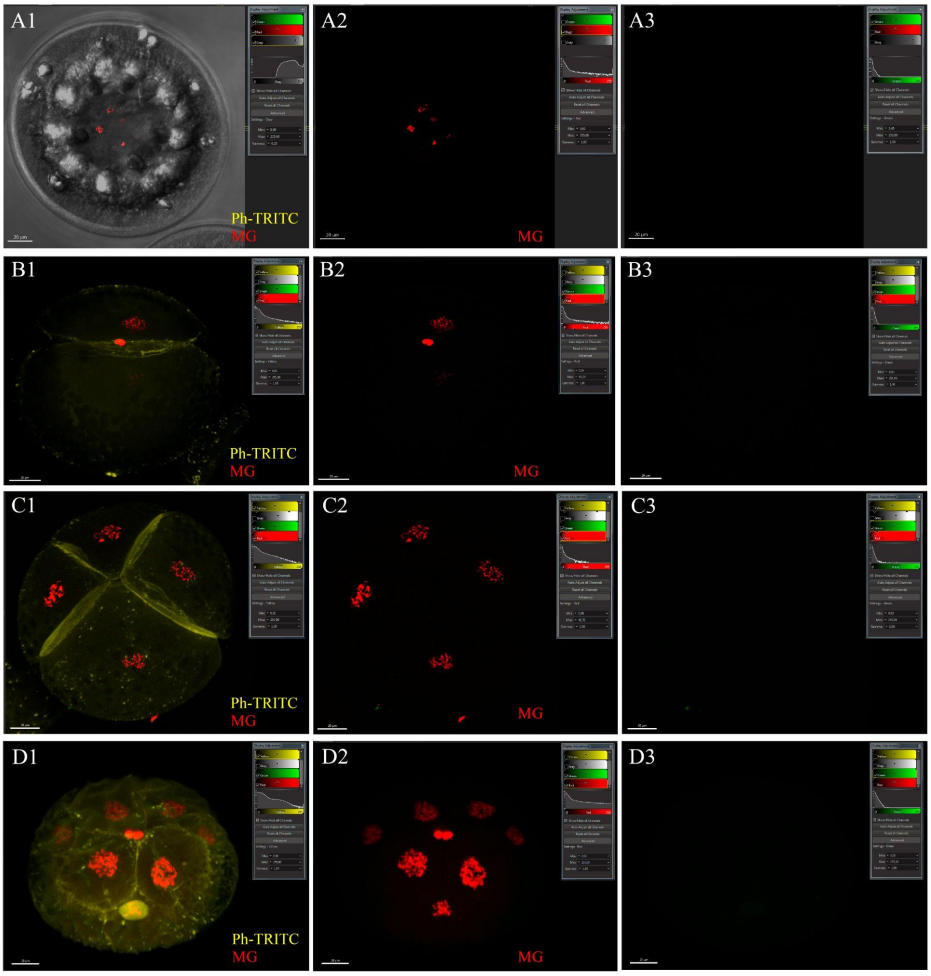

Fig. S4. Control for autofluorescence in the TO-PRO-1 channel (Ex/Em: 515/531 nm). Oocytes and early-stage embryos (A–D) were stained with Phalloidin-TRITC (Ph-TRITC; Ex/Em: 544/570 nm; F-actin; yellow) and Methyl Green (MG; Ex/Em: 633/677 nm upon DNA binding; red). No autofluorescence was detected in the TO-PRO-1 iodide channel (A3–D3).

Fig. S5. The antibodies (primary anti-acetylated tubulin antibody and secondary antibodies (anti-mouse CF633) used in the work stain the nervous system and cilia of the larvae

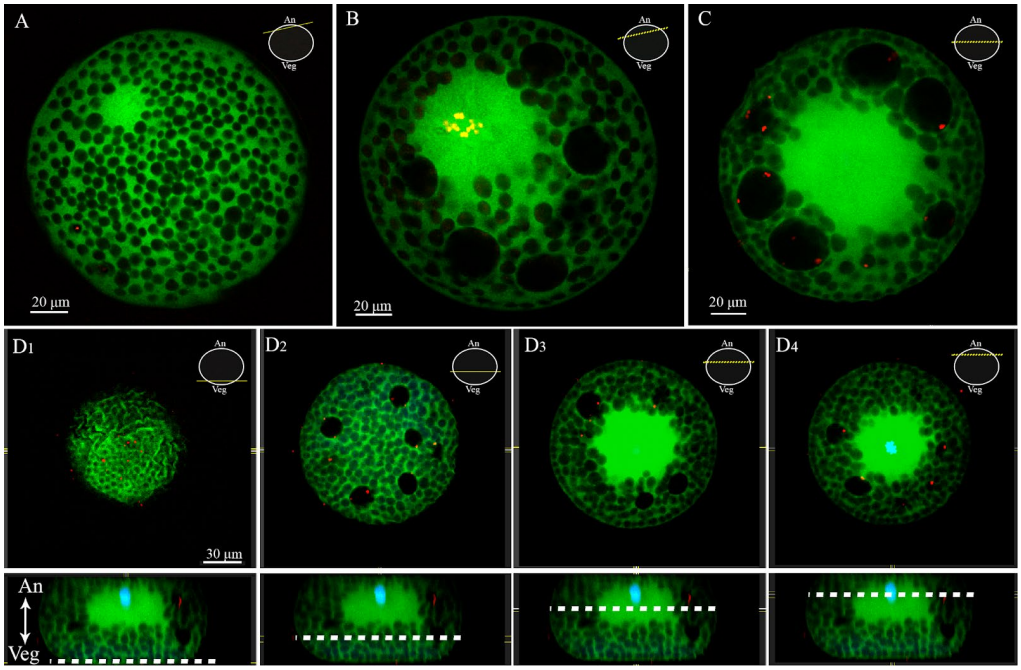

Fig. S6. Figure 3 with the tubulin channel. Zygotes (0.5 hpf). At this stage, antibodies against acetylated tubulin label discrete spots (1–2 µm) on the oocyte surface and round bodies (2–4 µm) within the cavities of lipid droplets. We hypothesize that the surface staining corresponds to sperm, as mammalian sperm are known to contain acetylated tubulin in their centrioles and axoneme (Turner et al., 2022). The stained internal bodies may contain endogenous acetylated tubulin. However, it is also possible that this observation is an artifact introduced during routine fixation and dehydration/rehydration procedures, during which sperm from the cell surface may have been drawn into the interior due to osmotic gradients. A, B — the same cell, scanned near the animal pole surface (A) and closer to the equator (B), RNA (green) is concentrated near the animal pole; C — cell scanned at the equatorial level; D — distribution of maternal RNA and yolk granules along the animal-vegetative axis. Positions of optical sections are indicated by white dashed lines. Staining: TO-PRO-1 (RNA, green), Hoechst 33342 (DNA, yellow, blue), primary anti-acetylated tubulin antibodies (mouse monoclonal) and secondary antibodies (anti-mouse CF633) (red).

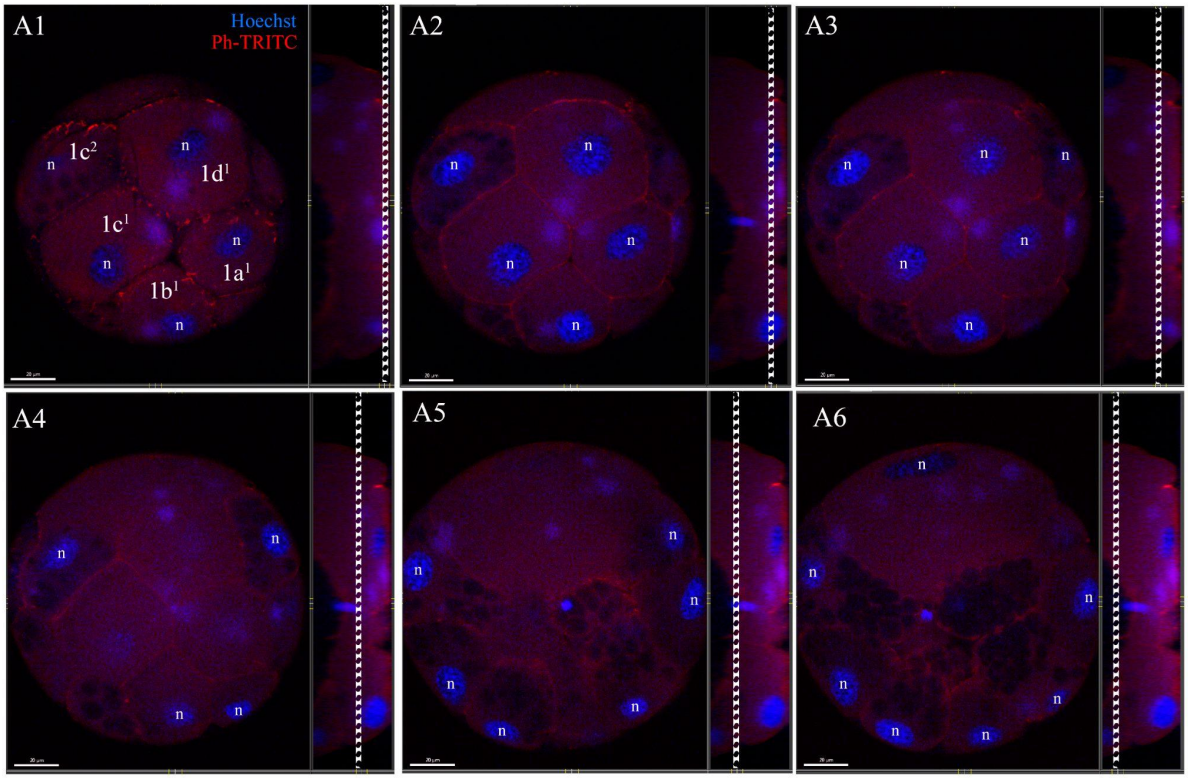

Fig. S7. Embryos at the stage of 16 cells, stained with Hoechst and Phalloidin-TRITC. (A1–A6) Purple focuses in the cytoplasm of blastomeres are supposed accumulations of mitochondria (mitochondrial clusters). Positions of optical sections are indicated by white dashed lines. n - nuclei.