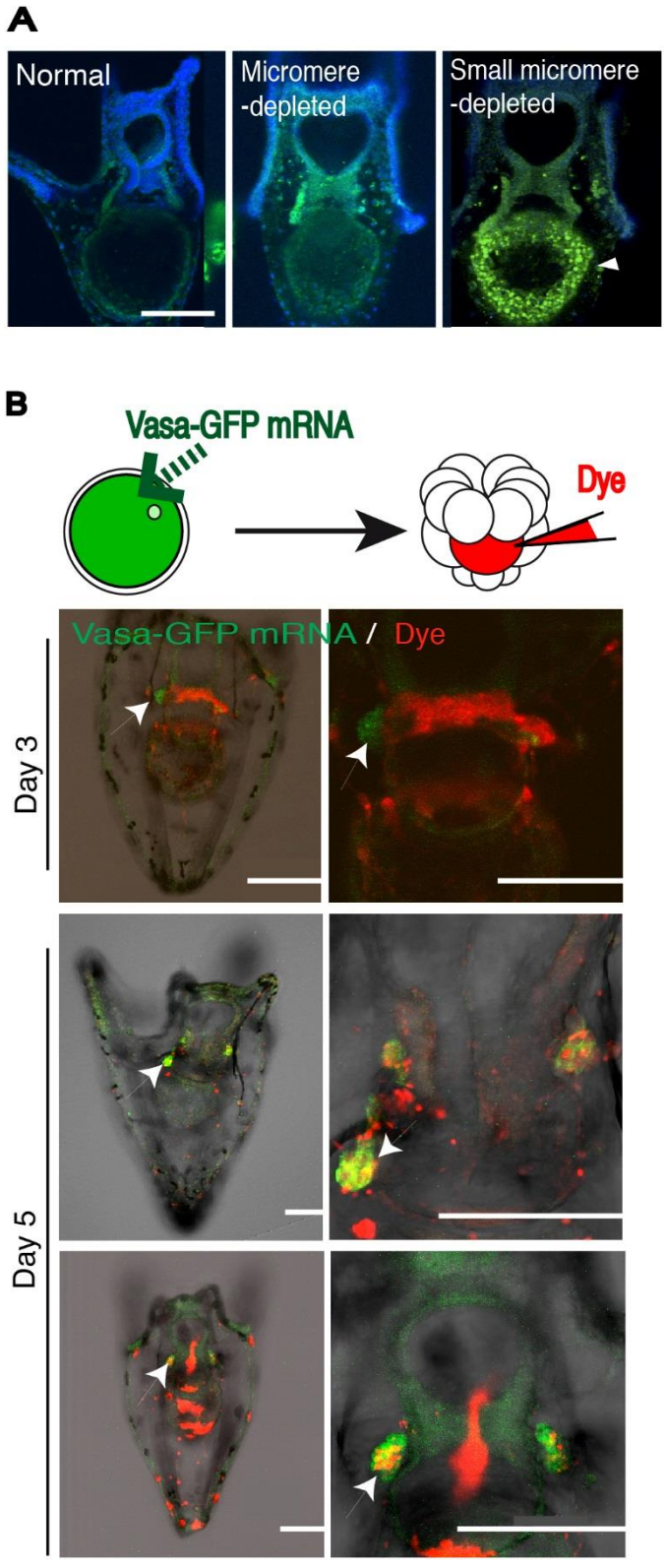


Figure S1, related to Figure 1. A transition from symmetric to asymmetric expression of Vasa in coelomic pouches. **A,** Vasa-positive micromeres /small micromeres were surgically depleted at 16-32 cell stages. Vasa-positive cells were not found during early development (Yajima and Wessel, 2011a), yet reappeared in the coelomic pouches of the Day 5 feeding *L. variegates* larvae, suggesting an involvement of another

lineage in this Vasa expression. Vasa (green), Hoechst (blue). An arrowhead in the stomach indicates background fluorescence derived from algae. Z-stack projection images, focusing only on the surface of coelomic pouches, were taken by confocal microscopy. **B,** To test if macromere descendants are involved, Vasa-GFP mRNA was injected into a fertilized egg and a single macromere was labelled with the fluorescent dye. Vasa-positive cells (green) were restricted into the small micromere lineage until early larvae (Day 3, arrows) yet the labelled macromere descendants (red) appear to become positive with Vasa-GFP signal in late larvae (Day 5, arrows), demonstrating a potential contribution of macromere (non-germ line) lineage to the Vasa-positive population in an expanding coelomic pouch at late larval stages. Scale bars = 50 μm. Images were taken by confocal microscopy and merged over to the bright field.

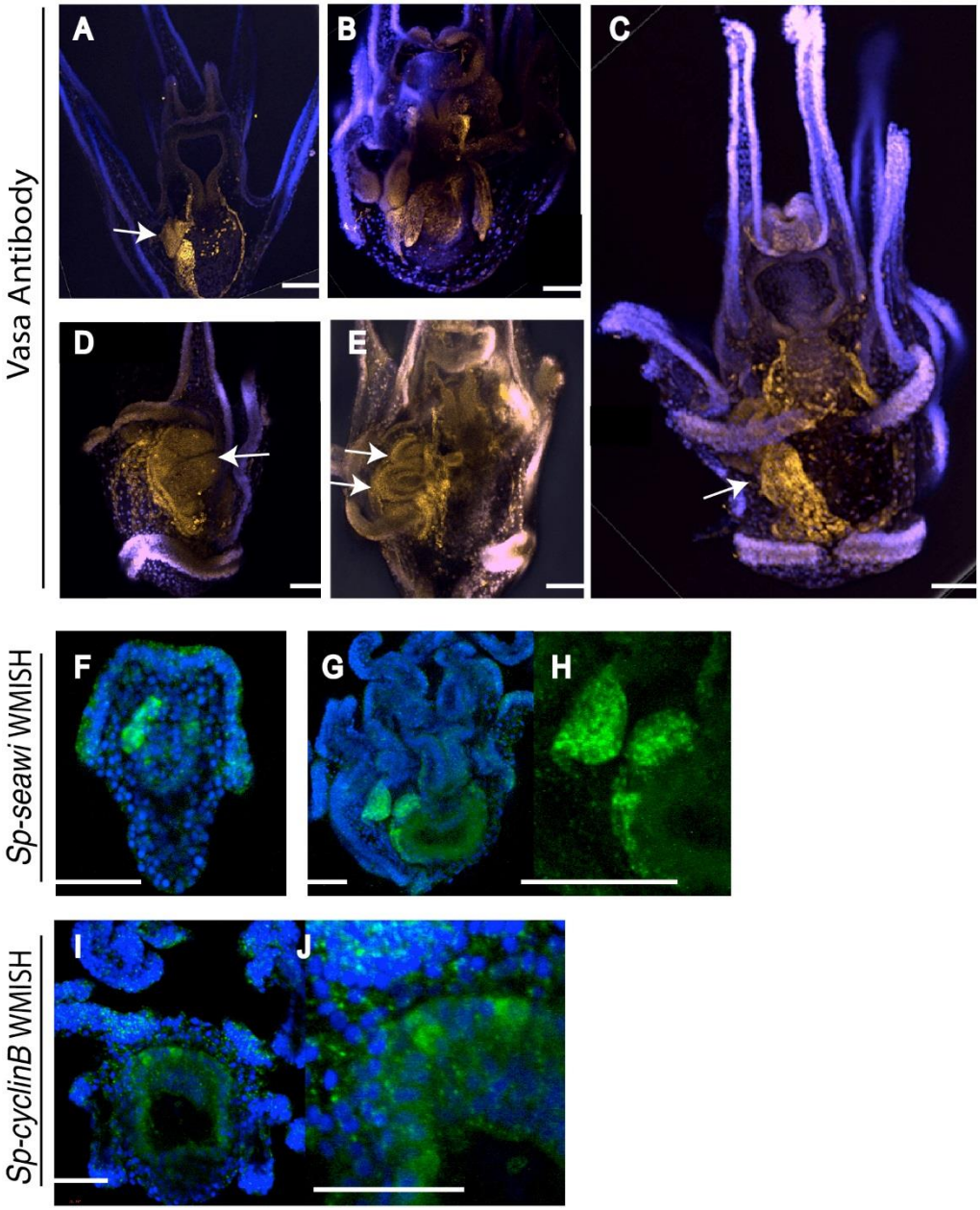


Figure S2, related to Figure 1. Vasa expression during late larval stage. Images were taken by confocal microscopy. **A-E,** High levels of Vasa accumulation continued during development in the entire adult rudiment including somatic adult structures, together with a low signal in the mouth and intestine of the larva. Late larvae from 6-armed pluteus (A), 8-armed pluteus (B and C), and metamorphic larvae (D and E) were immunolabelled with Vasa-antibody (yellow, arrows) and counterstained with Hoechst (blue). From the morphology and the location of labeling, many of the Vasa-positive cells are apparently progenitors of adult structures such as spines, tests, water vascular and neuronal system and tube feet. The levels of Vasa signal were dynamic and its decrease was seen upon differentiation in the adult rudiment by the end of the larval stage and at the onset of metamorphosis. **F-H,** another germ line marker *seawi* mRNA (green) was specifically localized in the coelomic pouch (F) and the adult rudiment (G and H). H is a magnified view of the adult rudiment in G. **I and J,** the positive cell cycle regulator *cyclinB* mRNA (green) was localized in the adult rudiment of the late larvae. J is a magnified view of the adult rudiment in I. Scale bars = 50 μm.

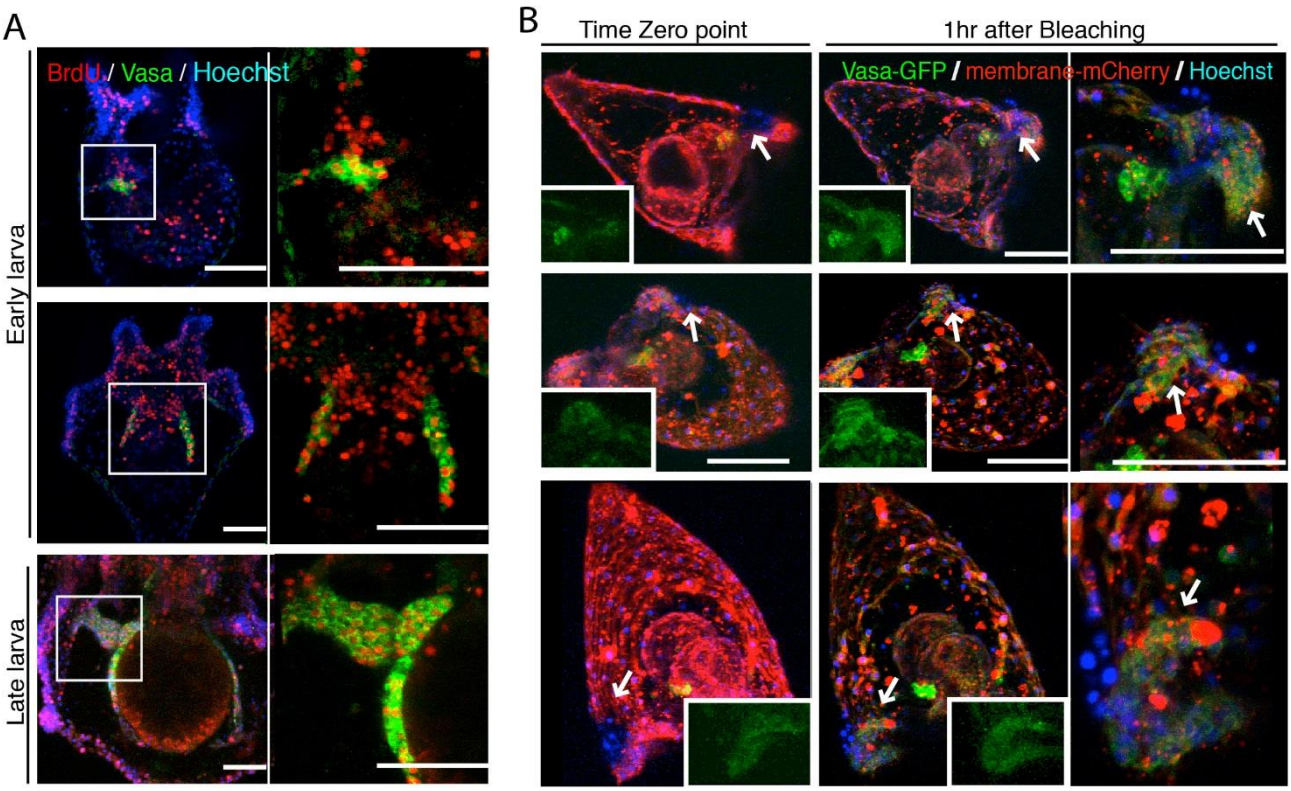


Figure S3, related to Figure 2. Vasa-positive cells in the adult rudiment are highly proliferative and the ectopic Vasa expression was induced in response to the damage. **A,** Upper two panels are 1-week old larvae and the bottom panel is a two-weeks old larva with starting adult rudiment formation. Vasa (green)-positive cells are also co-labeled with BrdU (red) within 1hr of BrdU-treatment, and counterstained with Hoechst (blue). White squares indicate the enlarged region on the right of each panel. Images were taken by confocal microscopy. **B,** Three examples of Day5 larvae injected or labeled with Vasa-GFP (green), membrane-mCherry (Red) and Hoechst (Blue) are shown. A small region of each larval body was damaged by laser-energy (arrows in Left panels) and the ectopic expression of Vasa-GFP around the damaged area was observed after 1hr of the ablation (arrows in Middle and Right panels). Membrane-mCherry that is consistently expressed independent of damage was used to normalize the signal level of Vasa-GFP. Relative signal intensity of GFP to mCherry of the same region was calculated before or after bleaching by *Image J*. Insets are a single channel for GFP. Each right panel is an enlarged view of each middle panel. Middle and Right panels are constructed from a subset of Z-stack images to focus only in the region of interest. Scale bars = 50 μm.

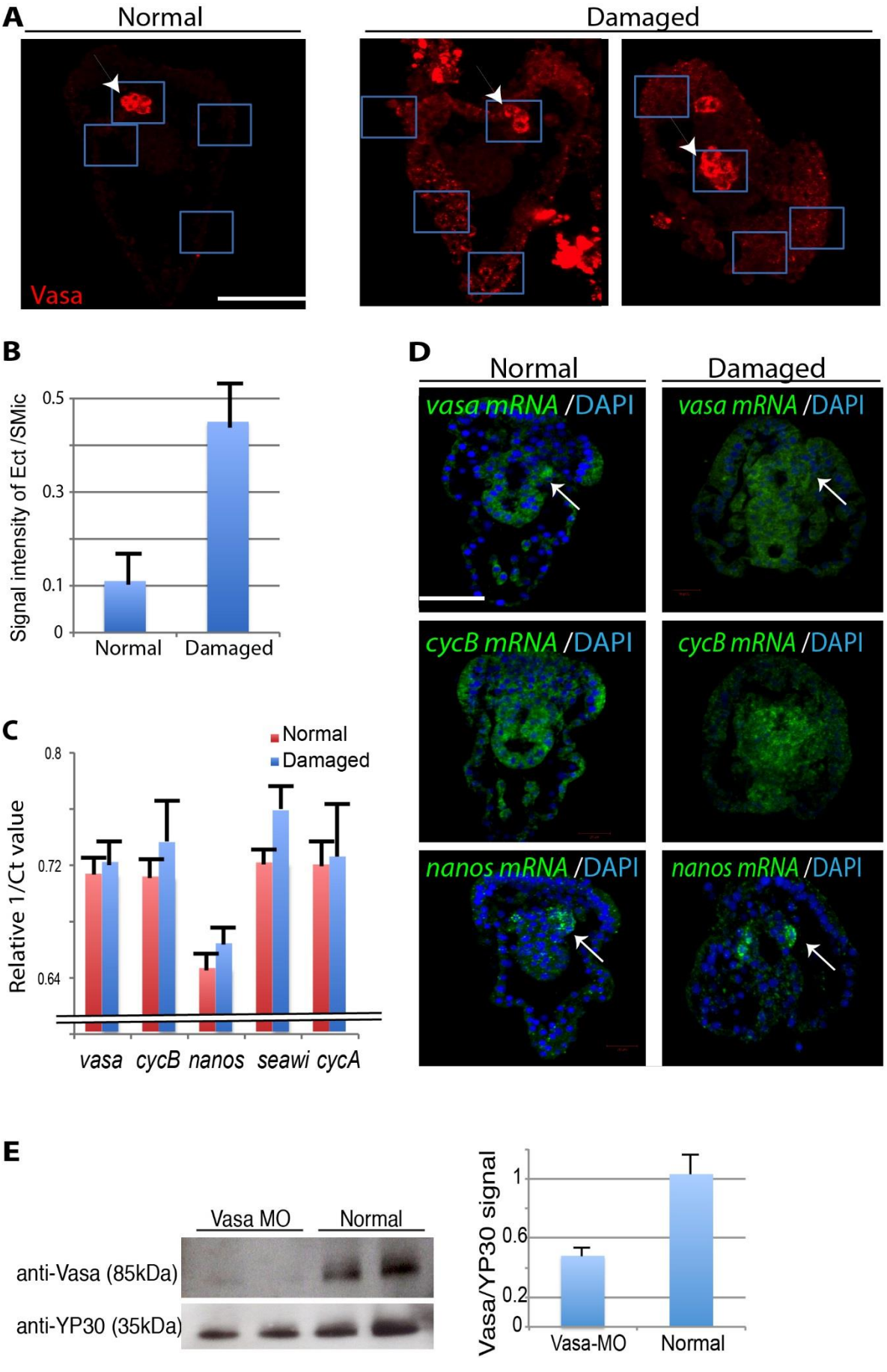
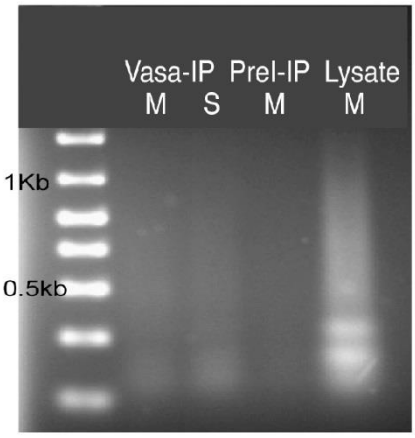


Figure S4, related to Figure 3. The post-transcriptional regulation of Vasa is essential for developmental re-programming. Images were taken by confocal microscopy. **A and B,** A half body of Day3 larvae were

damaged by a glass needle and that induced ectopic Vasa over-expression nearly in the entire larval body (A). The squares are the regions where the ectopic Vasa signal in the ectoderm was quantified by *image J* and compared to the signal in the small micromere descendants (arrows). The small micromeres in this experiment were left intact, and thus Vasa signal in this lineage is unaffected by the damage that serves as a control. Two of the representative images of damaged larvae are shown. In those larvae, over 4 times increase of the Vasa signal was found in the ectodermal tissues within 1hr after damage (B) compared to the non-damaged larvae. Error bars indicate SD of averaged signal level obtained from five individual larvae. **C,** Damaged larvae demonstrated little increase in the transcripts encoding *vasa, cyclinB, nanos2, seawi, cyclinA*. Error bars indicate SD of three independent experiments. **D,** Damaged larvae showed expanded expression of *vasa* and *cyclinB* yet had little effect on *nanos* mRNA localization. Arrows indicate the small micromere descendants. Scale bars = 50 μm. **E,** The immunoblot analysis of Vasa-MO knockdown. Vasa is maternally loaded yet the zygotic Vasa appears to be reduced in Vasa-MO injected embryos (Vasa-MO) compare to the uninjected embryos (Normal). A right graph indicates the signal intensity of each band normalized by YP30 (Yolk protein 30, that is consistently present independent of Vasa function) and quantified by *Image J*. Error bars indicate SD of three independent experiments.

A



B

SAMPLE	Amount of mRNA IP-ed	% of Vasa-interacting mRNA amount in the lysate (mRNA IP-ed / Lysate)
Vasa-IP	1080 ng ± 155. 24	37.6 %
eIF4E-IP	1081 ng ± 243. 66	37.6 %
Nanos2-IP	430 ng ± 163. 71	14.9 %
PreI-IP	470 ng ± 173. 49	16..4 %
Lysate	2870 ng ± 686. 36	-

C

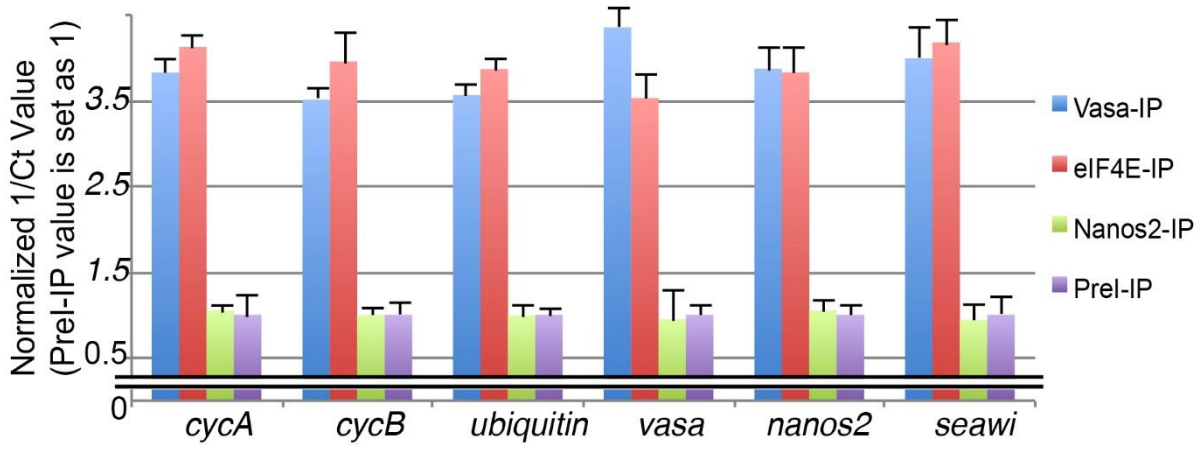


Figure S5, related to Figure 3. Vasa RNA-IP identified a broad range of mRNAs that interacted with Vasa. **A,** The 4-8cell stage embryos were lysed either at M-phase or S-phase. The Vasa interacting mRNAs were Immunoprecipitated (IPed) by Vasa antibody (Vasa-IP) and run on the agarose gel with mRNAs IPed with Pre-immune IgG beads (PreI-IP, negative control), together with lysate. Vasa attracted a large number of RNAs, whereas Pre-immune IgG pulled down reduced amount of RNA IPed in the same experimental condition. **B,** The total amount of RNAs IP-ed with each antibody-beads from 4-8 cell M-phase embryonic lysate is listed. **C,** The same proportion of each IP-ed sample was subjected to RT-qPCR. Vasa- and eIF4E-IPed samples demonstrated enrichment of the transcripts compared to the Nanos and PreImmune (PreI) pullouts. Each 1/Ct value of PreI samples is set as 1. Error bars indicate SD of three independent experiments.

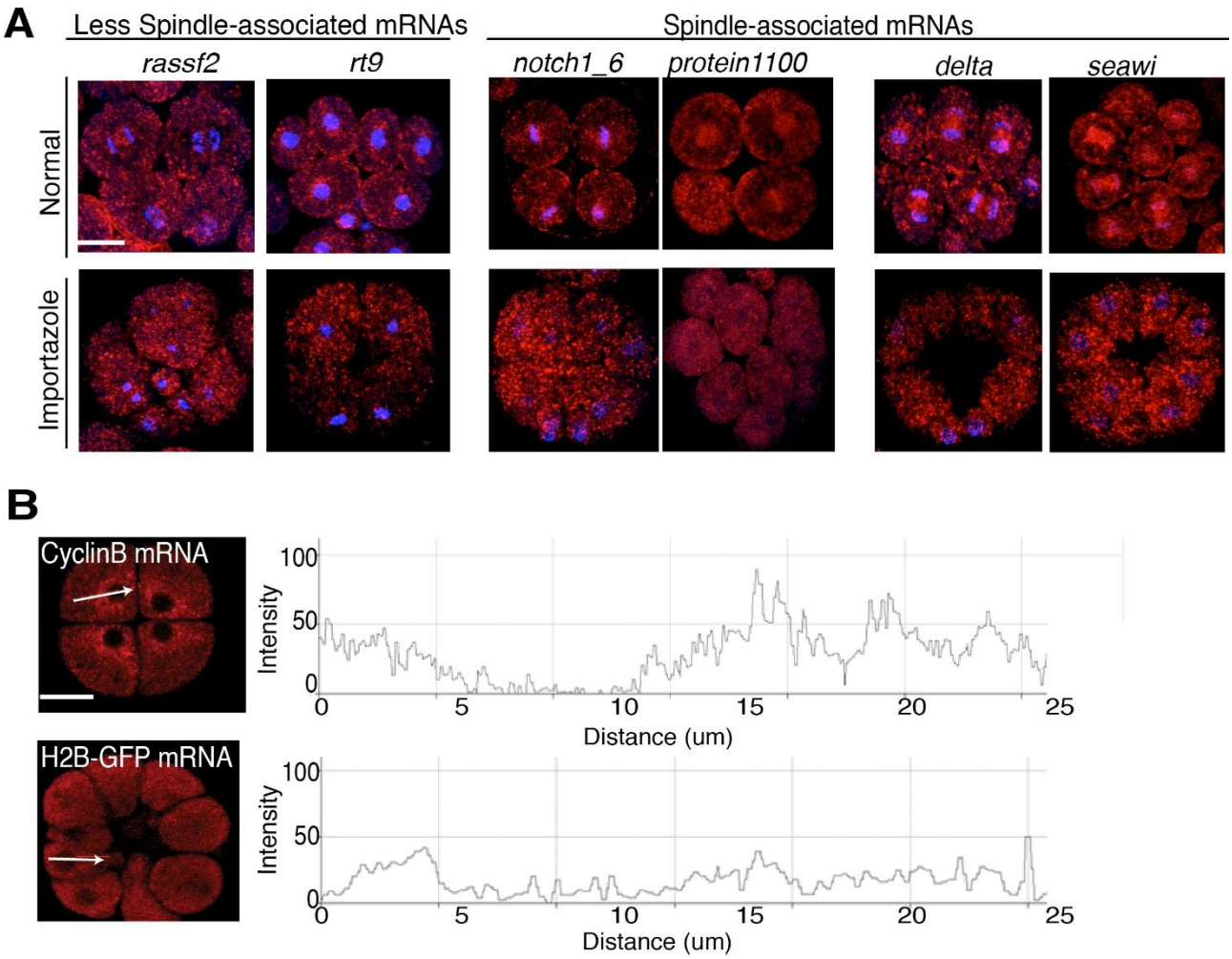
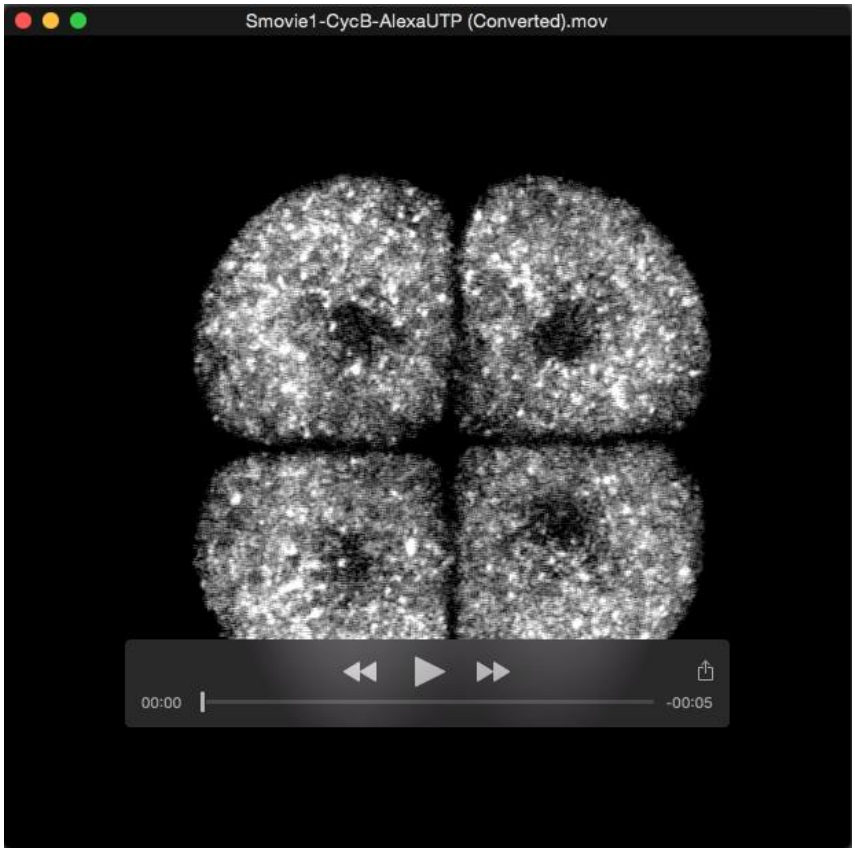
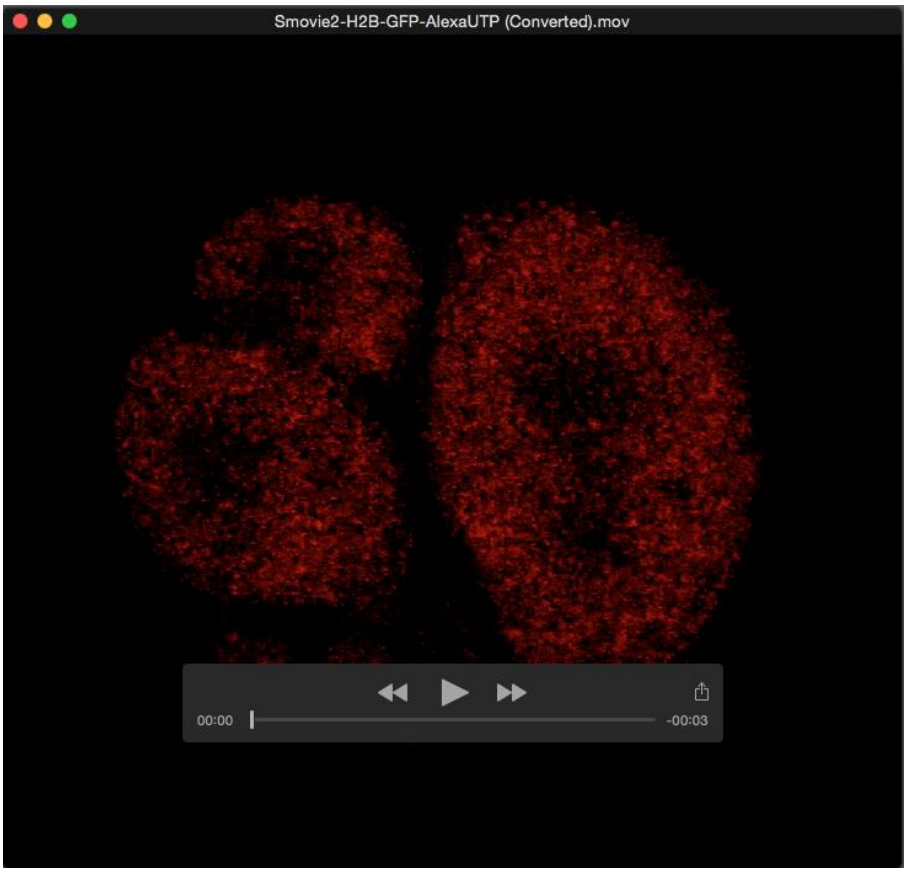


Figure S6, related to Figure 4. Importin is necessary for the mRNA localization in the embryo. Images were taken by confocal microscopy. **A,** Various mRNAs are localized around spindle and perinuclear during cell-cycling yet Importazole diminished their specific localization. Some mRNAs (*rassf2, rt9*) showed less enrichment, whereas the others (*notch1_6, protein1100, delta, seawi*) showed strong enrichment around the spindle and nuclear envelope. **B,** Intensity plot of *cyclinB* or *H2B-GFP* mRNA localization calculated by LSM5 Image Browser (Zeiss). The plot indicates the *cyclinB* mRNA is significantly accumulated around the nuclear envelope yet the *H2B-GFP* mRNA is rather evenly distributed throughout the cytoplasm. The mRNAs were labeled by Alexa546-UTP and injected into fertilized eggs. X-axis indicates the distance from the starting point of an arrow in each panel. Images were obtained at 8-16cell stage. Scale bars = 20 μm.



Movie S1, related to Figure 4. Dynamic distribution of CyclinB-mRNA. The time-lapse images were taken every 10min for 1hr. The movie (4fps) was constructed by *image J* software.



Movie S2, related to Figure 1. Even distribution of H2B-GFP mRNA. The time-lapse images were taken every 10min for 1hr. The movie (7fps) was constructed by *image J* software.

Supplementary Table 1, related to experimental procedure.

SPU #	Primer name	Primer Sequence
	Primers for in situ hybridization	
SPU_020907	*Sp-rassf2*	F: CCTTTACTTTGCTGGTTCAAGACC R: CTTGTCTTGCTGTTATAGAGATGCC
SPU_016539	*Sp-rt9*	F: GGTCCTTGAGAGGATTGTCACAGCTC R: CATGTCAAGATCAGGTAGCACACTG
SPU_007264	*Sp-notchh1_6*	F: GTGAATGGCTTTAGATGTGTCTGT CCCGAGGGTTA R: CATCAAAACCACTAGCACAGCTGCA CAGGTAAC
SPU_023939	*Sp-hypothetical protein-1100*	F: CATGACGAAAGGTCACACACAAAAC R: CGATGACCAGAGCTACCAGGAGACTC
SPU_016689	*Sp-seawi*	F: CACCAAGCATGGATCGTC R: GAAAAGAGAATACATGGTGTCC
SPU_008908	*Sp-vasa*	F: GGACGATCAACTAGCTTCTA R: ACTCCTTCGTCTTTCTTCAT
SPU_015614	*Sp-nanos2*	F: AAGGTGATGAGGGGAGGAAG R: CGCAAATCACCTGTACAAAAA
SPU_015285	*Sp-cyclinB*	F: GGCTTACACCAAGACCCAGA R: GAGGGATCGTATTGCACCAT
	Primers for ORF cloning	
SPU_015285	*Sp-cyclinB ORF (1.2kb)*	F: ATGGCTCATGCCACAAGAAACC R: CTACGATTCTTCCACTAGTGTC
	Primers for qPCR	
SPU_003528	*Sp-cyclinA*	F: GAGATTATCAAGGCCCAAAGG R: GTCTTGTTGCTCGTCTATTC
SPU_015285	*Sp-cyclinB*	F: CTGTAGTGAGTCTACCAGTG R: GCTGAGAAAATGCTTCAATG
SPU_021496	*Sp-ubiquitin*	F: CACAGGCAAGACCATCAC R: GAGAGAGTGCGACCATCC
SPU_008908	*Sp-vasa*	F: TCAACTACGACCTCCCAAGC R: TCTCGCAATGTTAGCATCCTT
SPU_021496	*Sp-seawi*	F: GTGATGGTGTTGGTGACAGC R: TATTGATGCGCTTCTTGACG
SPU_016128	*Sp-delta*	F: ACGGAGCTACATGCCTGAAC R: CTGCCAGTCTGTAGAAGGCTTC