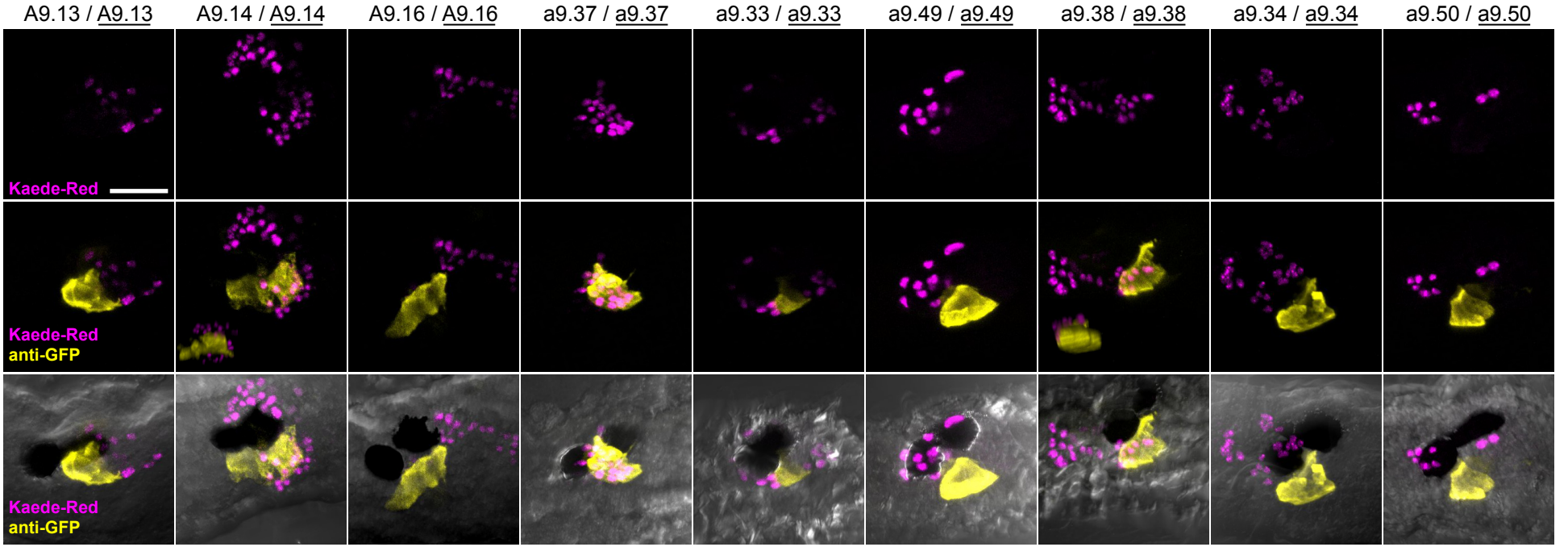


Fig. S1. Little position variation of cells labeled with the photo-converted Kaede among individuals. Other individuals with the larvae shown in Fig.1,2. Anterior is to the left. Lateral views. Scale bars: 10 μm.

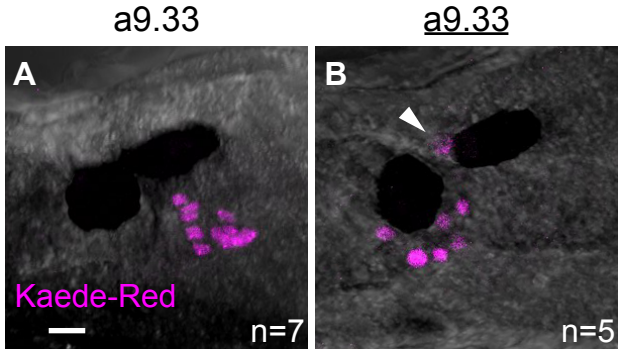


Fig. S2. Movement of a9.33/a9.33 cells through the embryogenesis. (A,B) Asymmetric distribution of descendants of left and right a9.33 cells. Anterior is to the left. Dorsal views. Nuclei of left (A) or right (B) a9.33-lineage cells were labeled by Kaede-red fluorescence (*magenta*). *White arrowhead*: A right a9.33-derived cell that did not move to the left side of the brain vesicle. Scale bars: 10 µm.

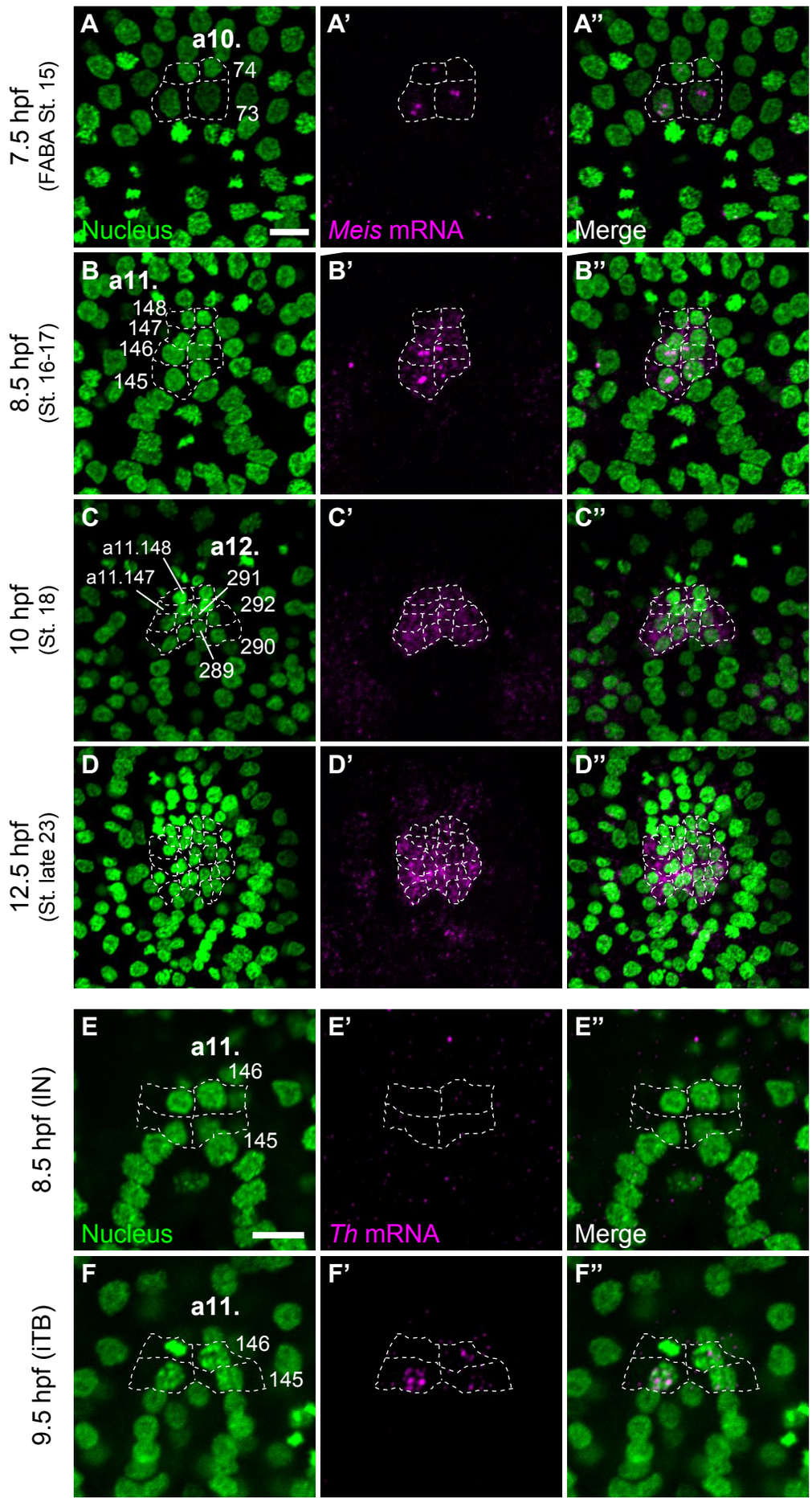


Fig. S3. Spatio-temporal expression patterns of *Meis* and *Th*. (A–F") Anterior is to the top. Dorsal views. *Meis* mRNA (A–D") and *Th* mRNA (E–F") (*magenta*) were detected by WISH at the indicated stages (hpf and FABA stages). Nuclei were counter-stained with DAPI (*green*). *White dotted lines:* a9.37-lineage cells. iTB, initial tailbud; lN, late neurula. Scale bars: 10 µm.

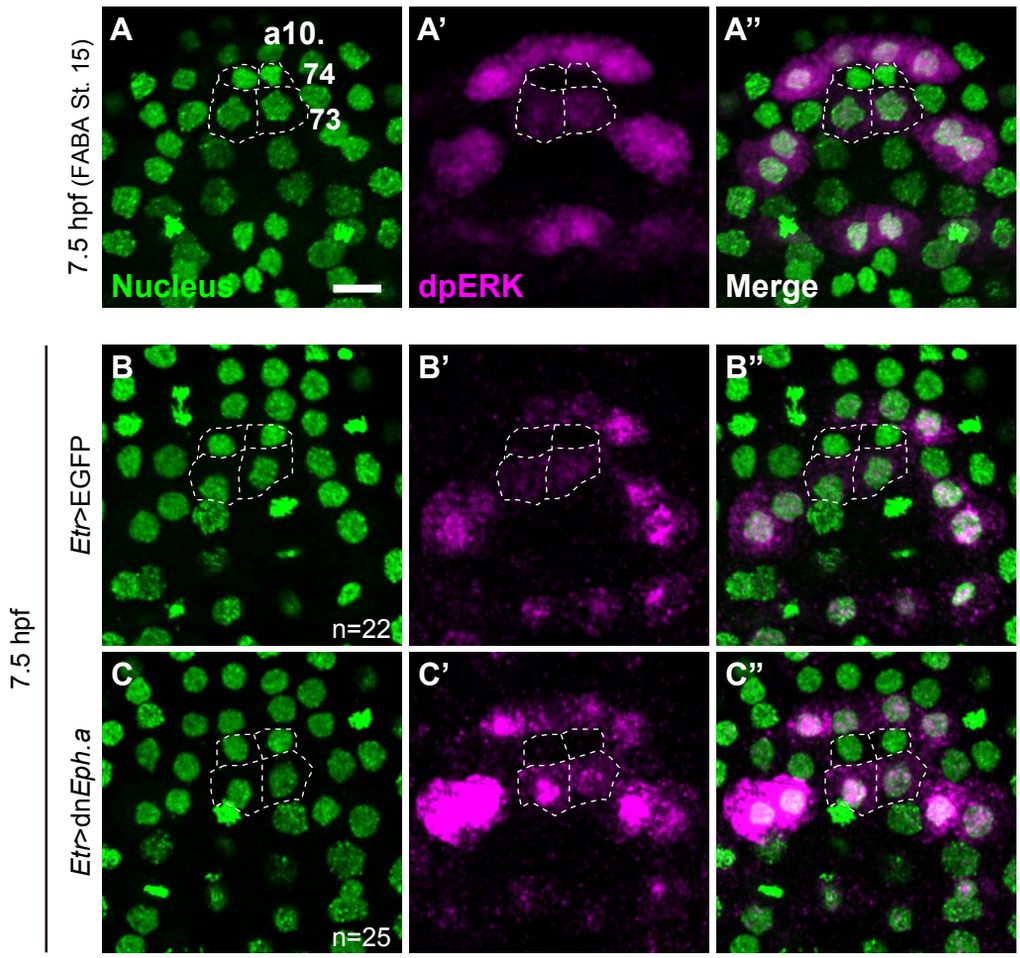


Fig. S4. Cells in which the MAPK pathway is activated in the neural plate and overexpression of the dominant negative form of *Eph.a* (dn*Eph.a*) receptor. (A–C") The immunofluorescent staining of dpERK (*magenta*) at 7.5 hpf. Nuclei were counter-stained with DAPI (*green*). White dotted lines: The daughter cells of a9.37 cells. (B-C") dpERK was not detected in a10.74 cells of the control embryos (B-B") and the embryos overexpressing dnEph.a (C-C"). Anterior is to the top. Dorsal views. Scale bars: 10 μm.

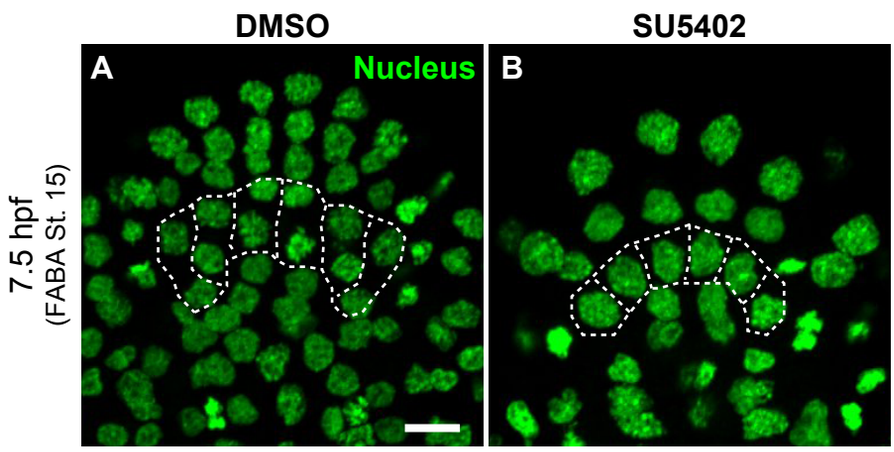


Fig. S5. Cells in row III of the neural plate do not divide by the treatment with SU5402. (A,B) Neurula embryos treated with DMSO (A) or an FGF receptor inhibitor, SU5402 (B). Anterior is to the top. Dorsal views. Nuclei were stained with DAPI (green). White dotted lines indicate cells in the row III of the neural plate including the a9.37-lineage cells. Scale bars: 10 μm.

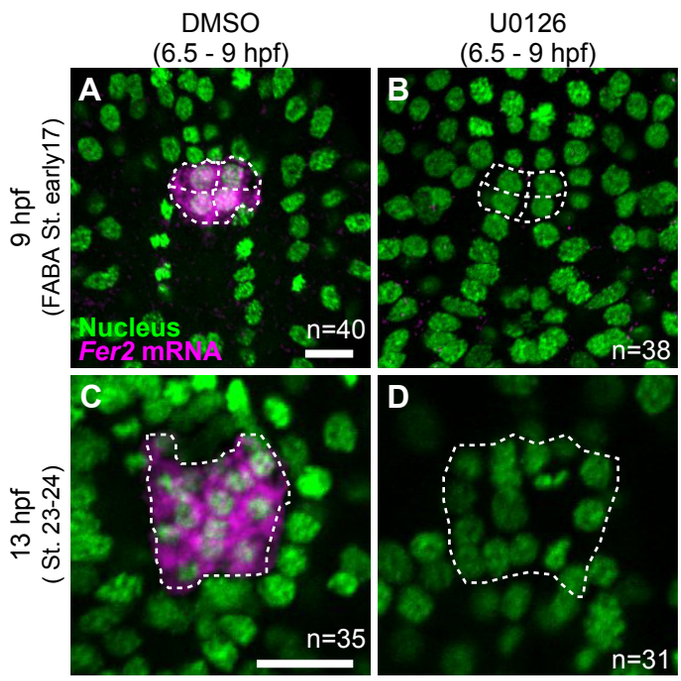


Fig. S6. MAPK pathway is required for the maintenance of *Fer2* expression after the neurula stage. (A–D) *Fer2* mRNA (*magenta*) was detected in embryos treated with DMSO (A,C) or U0126 (B,D) by the fluorescent-WISH. *Green*: Nuclei counter-stained with DAPI. Anterior is to the top. Dorsal views. *White dotted lines*: descendants of a10.73 cells. Scale bars: 10 µm.

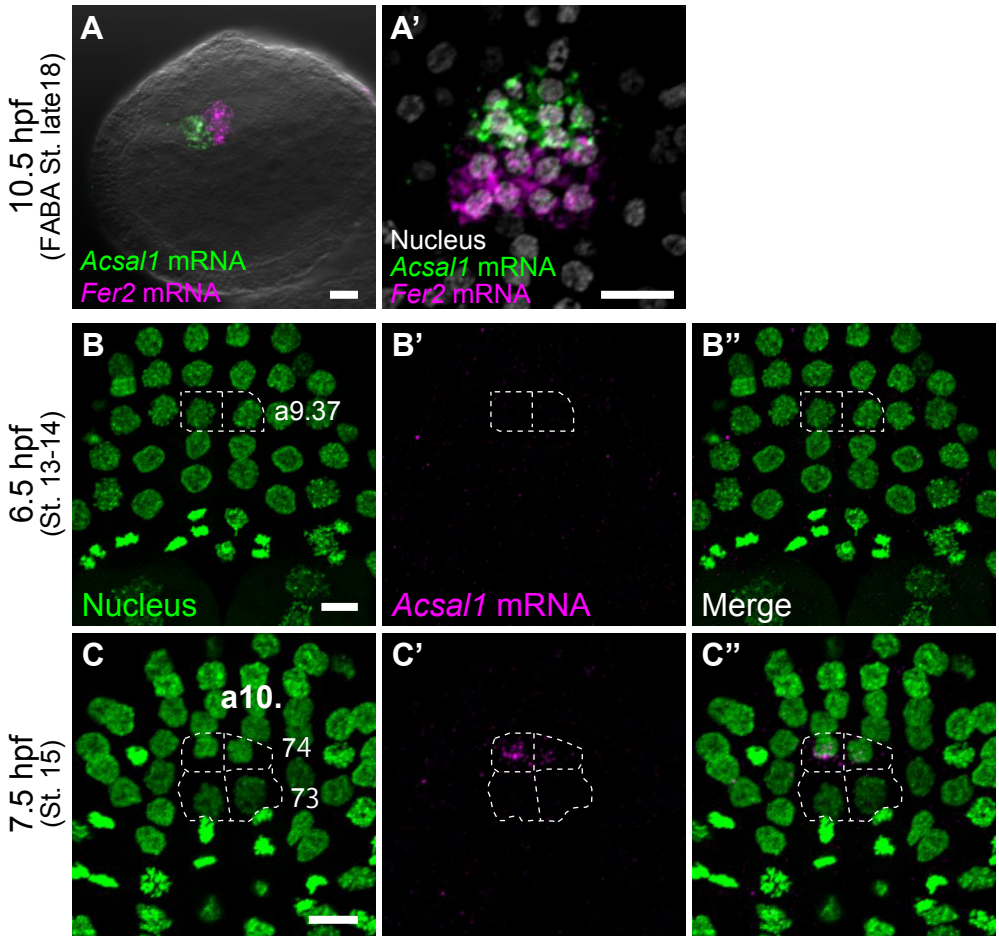


Fig. S7. The Acsal1 gene is specifically expressed in the a10.74-lineage cells. (A,A') Comparison of expression patterns between *Acsal1* and *Fer2* genes at the early tailbud stage (10.5 hpf) by WISH. (A) Anterior is to the left. Lateral views. (A') Nuclei were counter-stained with DAPI (*gray*). Anterior is to the top. Dorsal views. (B–C") *Acsal1* mRNA (*magenta*) was detected at 6.5 hpf (B–B") and 7.5 hpf (C–C") by fluorescent-WISH. Nuclei were counter-stained with DAPI (*green*). Anterior is to the top. Dorsal views. *White dotted lines*: a9.37 cells (B–B"), a10.73 and a10.74 cells (C–C"). Scale bars: 10 µm.

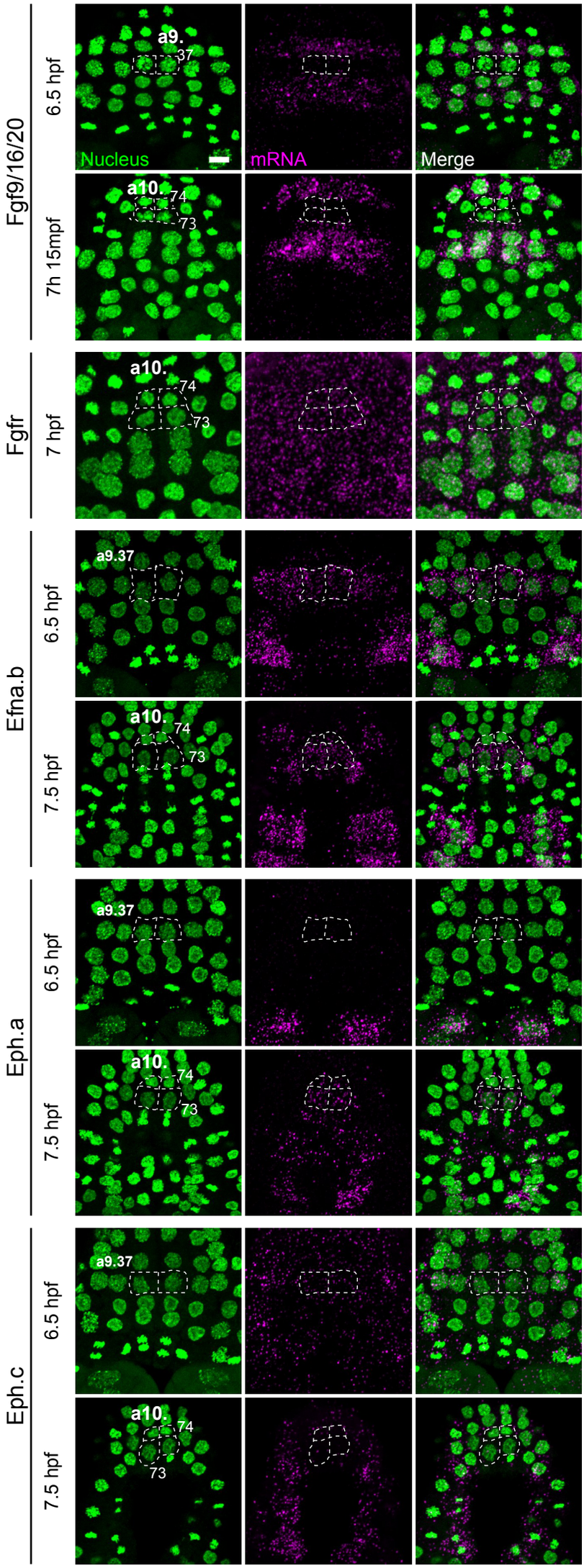


Fig. S8. The expression patterns of *Fgf9/16/20, Fgfr, Efna.b, Eph.a* and *Eph.c* before and after the a9.37 cell division. The expression of each gene was detected by WISH (*magenta*). Nuclei were counter-stained with DAPI (*green*). Anterior is to the top. Dorsal views. *White dotted lines:* a9.37-lineage cells. Scale bar: 10 μm.

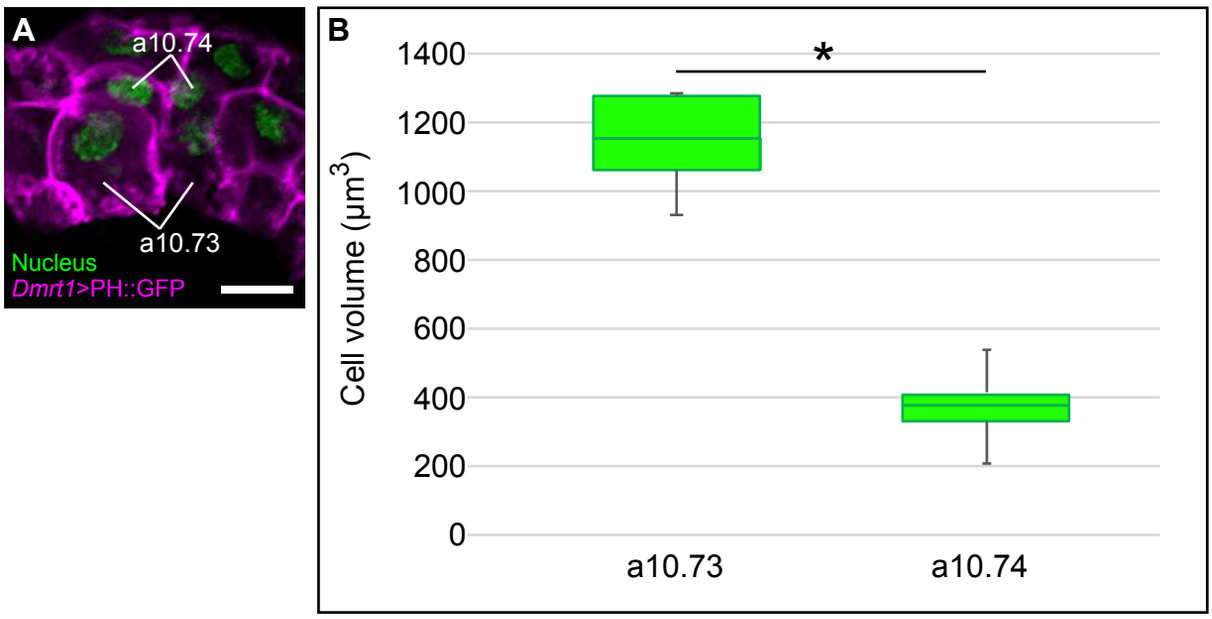


Fig. S9. The cell volume of a10.73 cells is higher than that of a10.74 cells. (A) The detection of membrane localized GFP (PH::GFP) expression (*magenta*) by the immunofluorescent staining against GFP. Nuclei were counter-stained with DAPI (*green*). Anterior is to the top. Dorsal views. (B) Boxplots indicate the cell volumes of a10.73 and a10.74 cells (n=5). The average volumes are 1120.52 µm³ (a10.73) and 361.376 µm³ (a10.74), respectively. Scale bar: 10 µm. Statistical analysis was done by standard Student *t*-test. (*$P < 0.001$).

Table S1. The average number of cells expressing *Fer2* reporter at the larval stage

	Cells expressing *Fer2* reporter		
labeled cells†	only Kaede-Green positive	Kaede-Red positive	n=
Left a9.37	7.6 ± 0.40	8 ± 0.00	5
Right a9.37	7.25 ± 0.48	8 ± 0.00	4

†The cells labeled with Kaede red fluorescence at the late gastrula stage.

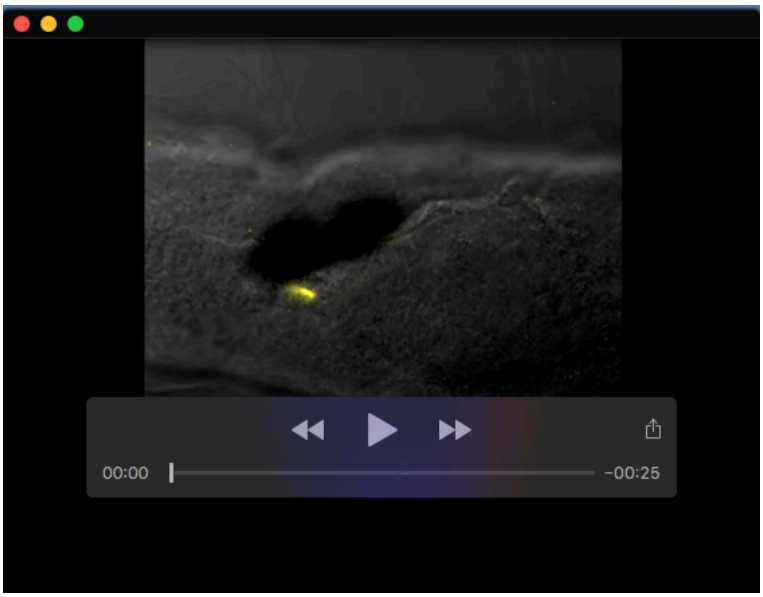
The numbers of cells are presented as mean ± standard error (s.e.m.).

Table S2. The average number of cells in the larval brain vesicle derived from each pair of neural plate cells

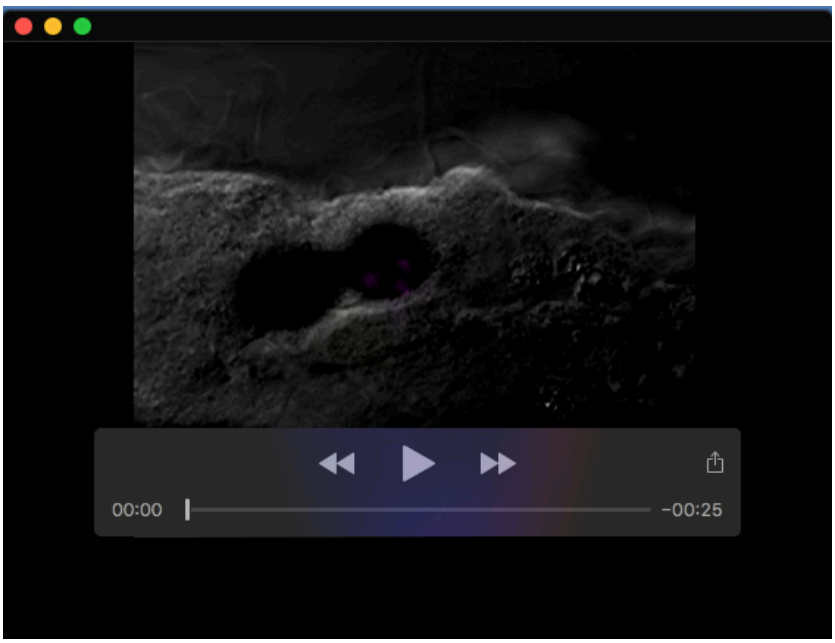
Labeled cells†	The average labeled cell number	s.e.m.	n=
a9.37/a9.37	23.5	0.50	4
a9.33/a9.33	17.7	0.95	6
a9.49/a9.49	9.5	0.29	4
a9.38/a9.38	23.6	0.24	5
a9.34/a9.34	24.3	0.25	4
a9.50/a9.50	12.2	1.36	5
A9.13/A913	24.8	0.77	6
A9.14/A914	48	1.22	5
A9.16/A916	48.3	1.11	4

†The cells labeled with red fluorescence of photo-converted Kaede at the late gastrula stage.

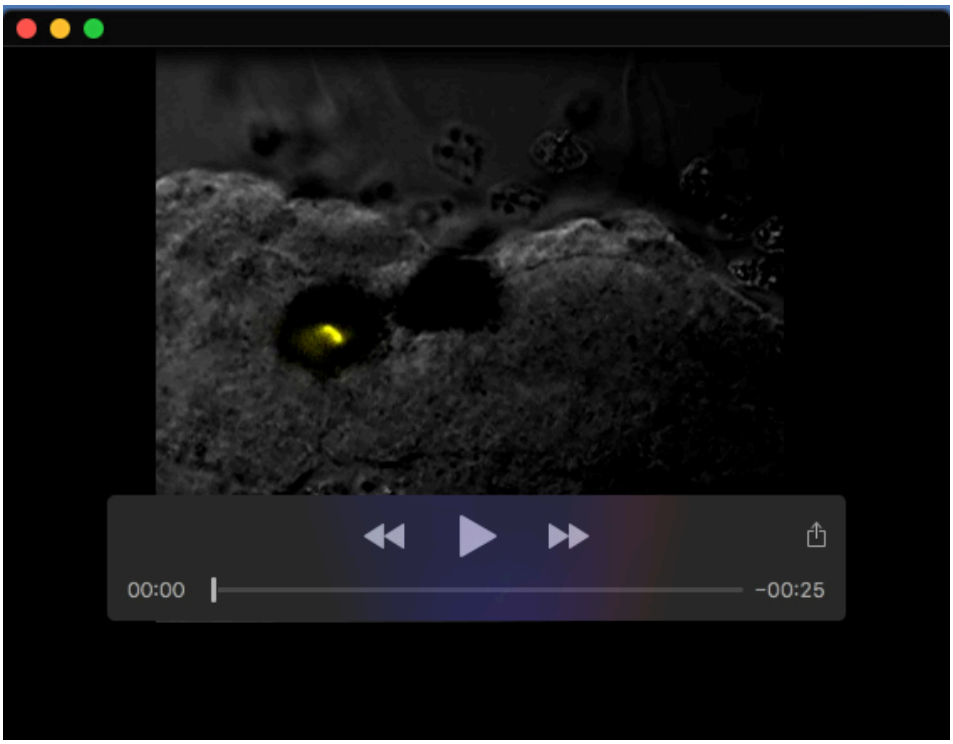
The cell numbers and standard error of the mean (s.e.m.) are shown.



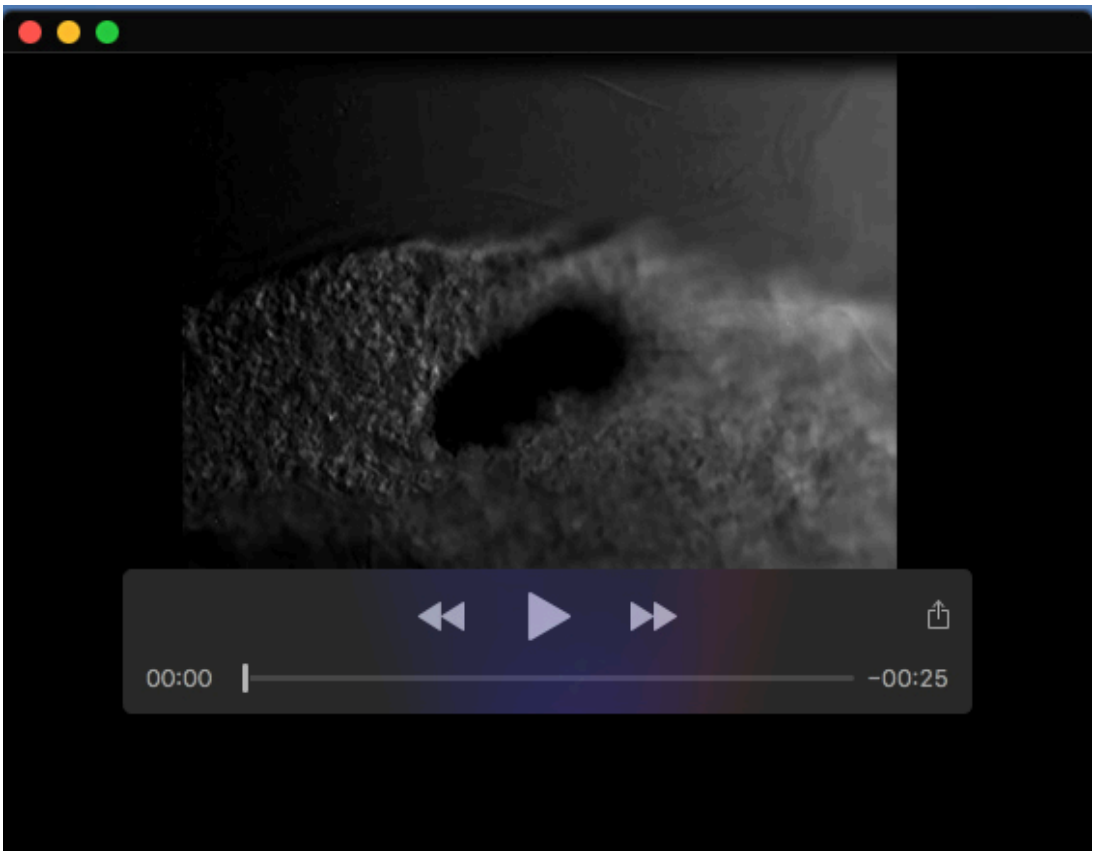
Movie 1. Serial optical sections of confocal images of A9.13/A9.13 lineage-cells in the *Ciona* larva shown in Fig. 1E–E''. Localization of the fluorescence of Kaede-red and *Fer2*>EGFPv were visualized in magenta and yellow, respectively. Anterior is to the left. Lateral views. This movie progresses from dorsal to ventral regions of the larva.



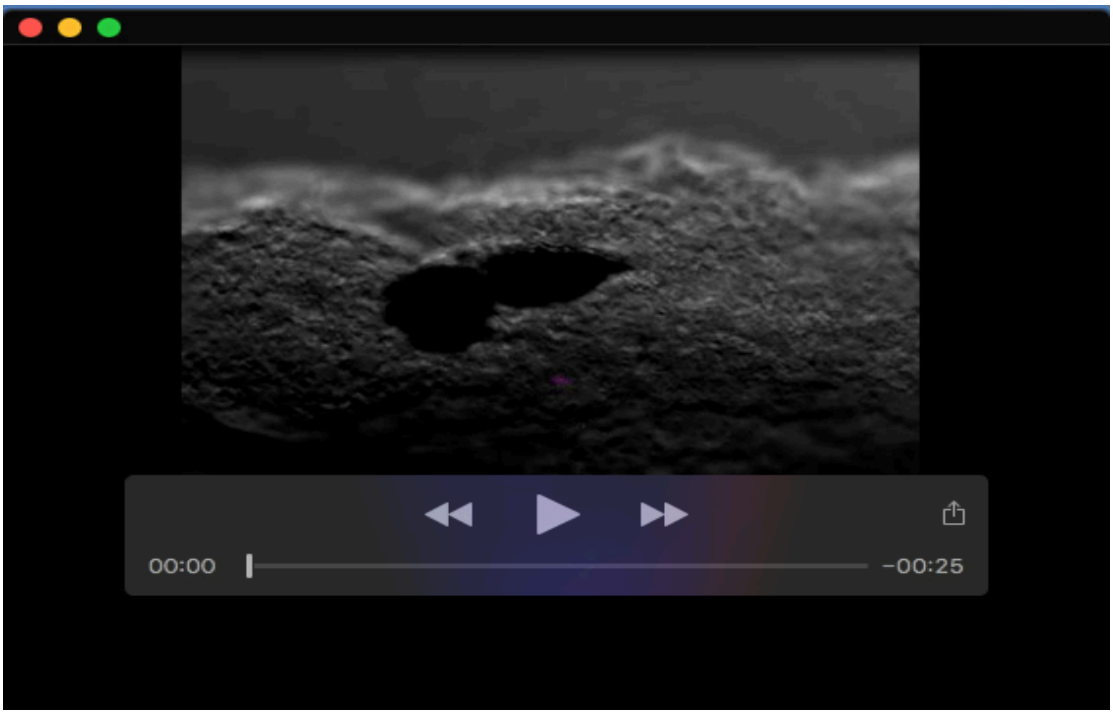
Movie 2. Serial optical sections of confocal images of A9.14/A9.14 lineage-cells in the *Ciona* larva shown in Fig. 1G–G''. Localization of the fluorescence of Kaede-red and *Fer2*>EGFPv were visualized in magenta and yellow, respectively. Anterior is to the left. Lateral views.



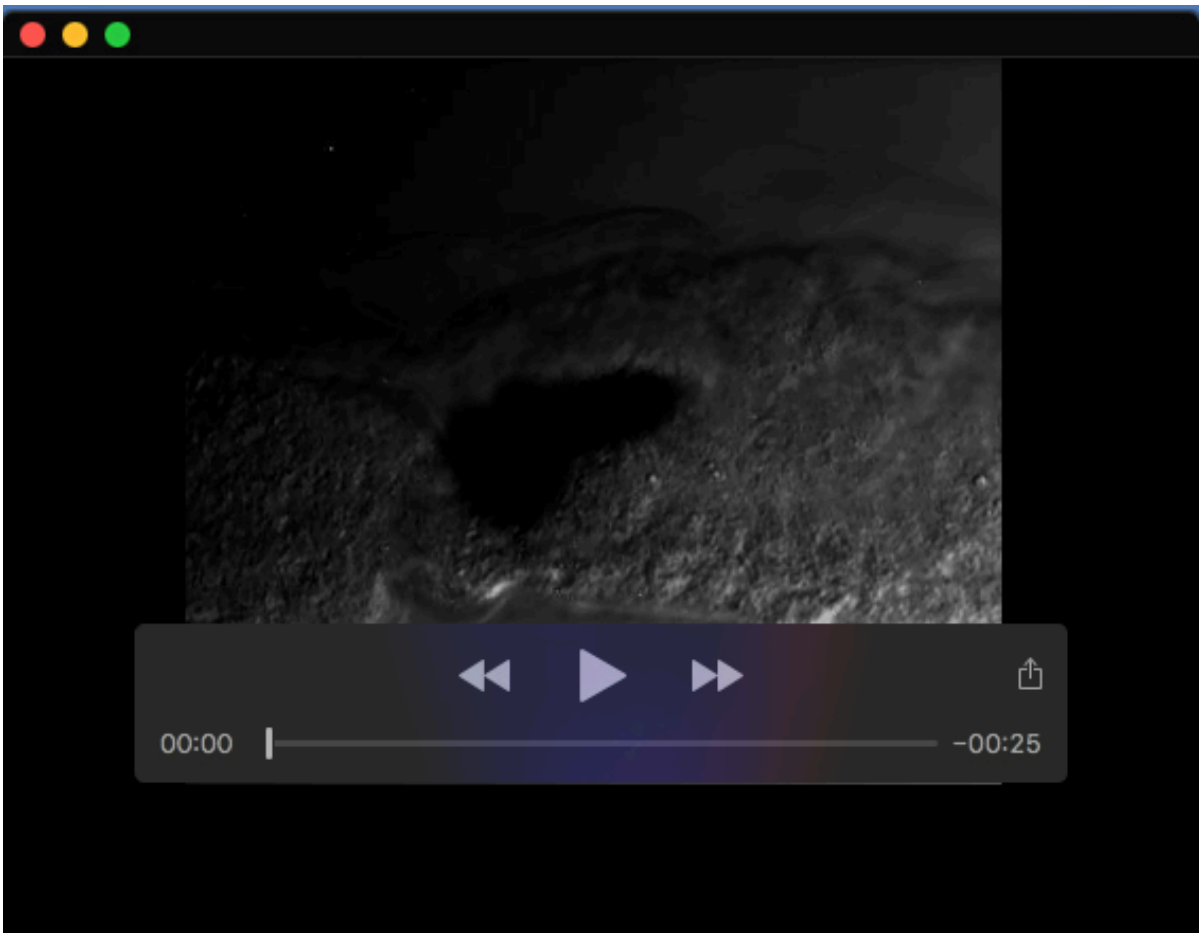
Movie 3. Serial optical sections of confocal images of A9.16/A9.16 lineage-cells in the *Ciona* larva shown in Fig. 1I–I''. Localization of the fluorescence of Kaede-red and *Fer2*>EGFPv were visualized in magenta and yellow, respectively. Anterior is to the left. Lateral views.



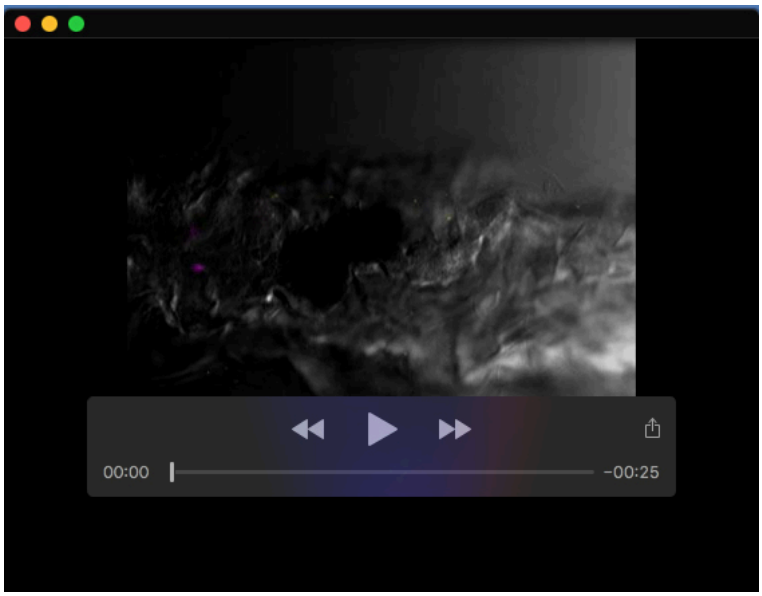
Movie 4. Serial optical sections of confocal images of a9.37/a9.37 lineage-cells in the *Ciona* larva shown in Fig. 2G–G''. Localization of the fluorescence of Kaede-red and *Fer2*>EGFPv were visualized in magenta and yellow, respectively. Anterior is to the left. Lateral views.



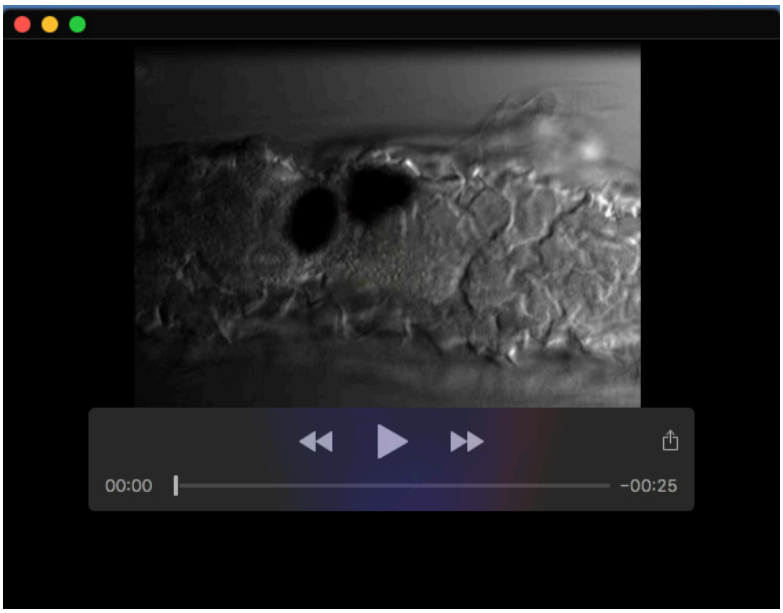
Movie 5. Serial optical sections of confocal images of a9.33/a9.33 lineage-cells in the *Ciona* larva shown in Fig. 2H–H''. Localization of the fluorescence of Kaede-red and *Fer2*>EGFPv were visualized in magenta and yellow, respectively. Anterior is to the left. Lateral views.



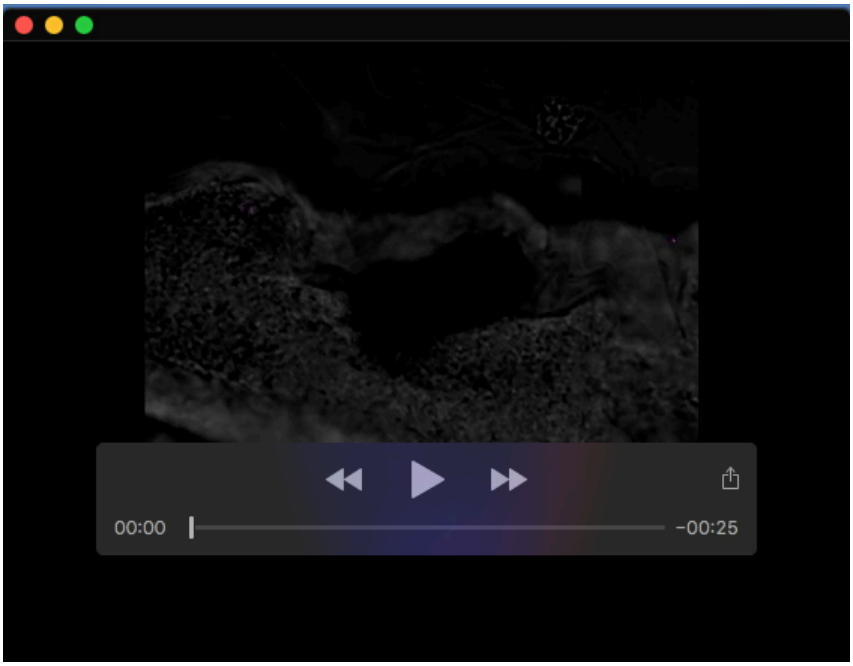
Movie 6. Serial optical sections of confocal images of a9.49/a9.49 lineage-cells in the *Ciona* larva shown in Fig. 2I–I''. Localization of the fluorescence of Kaede-red and *Fer2*>EGFPv were visualized in magenta and yellow, respectively. Anterior is to the left. Lateral views.



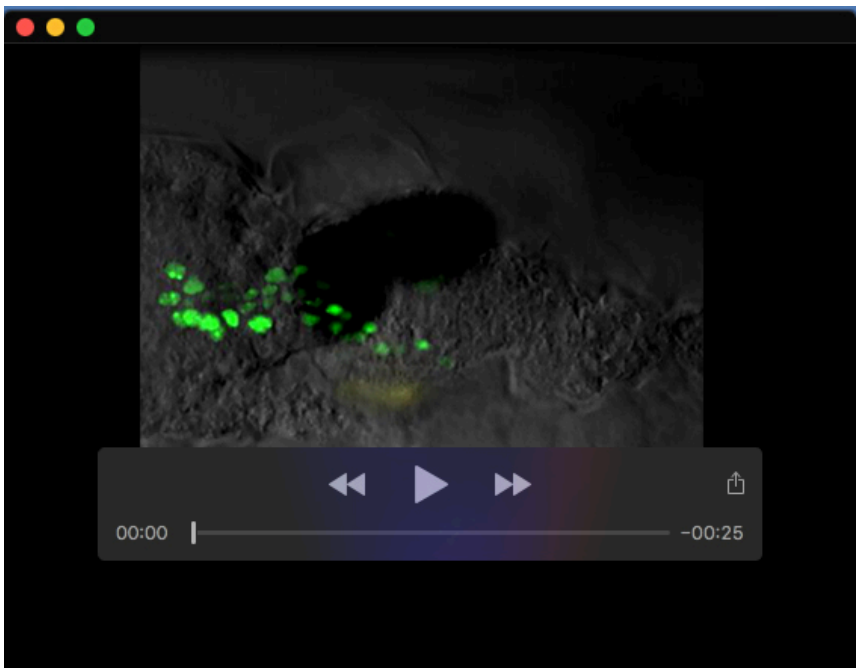
Movie 7. Serial optical sections of confocal images of a9.38/a9.38 lineage-cells in the *Ciona* larva shown in Fig. 2J–J''. Localization of the fluorescence of Kaede-red and *Fer2*>EGFPv were visualized in magenta and yellow, respectively. Anterior is to the left. Lateral views.



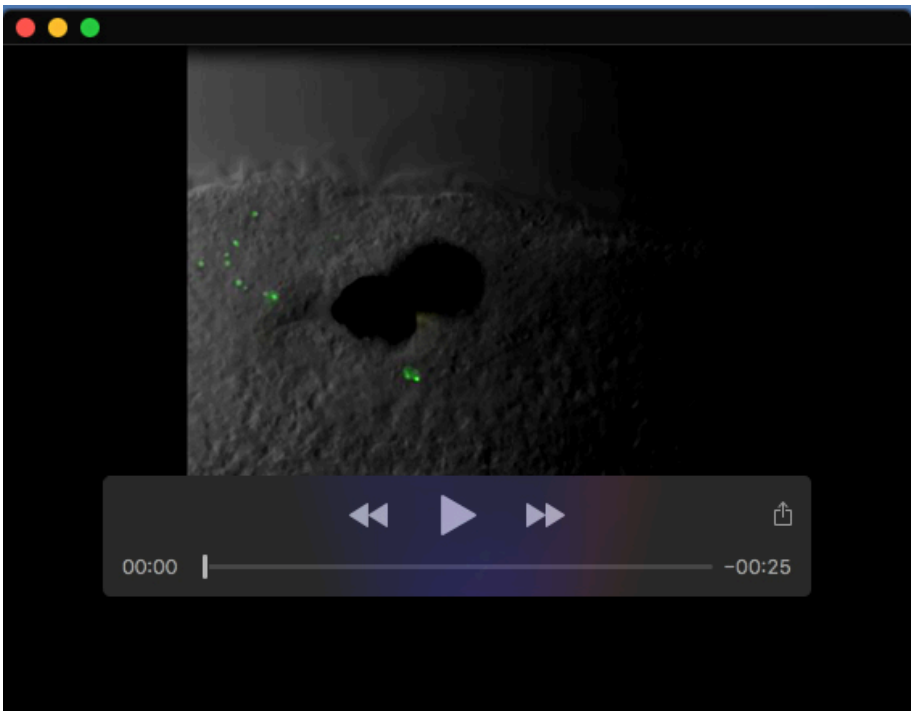
Movie 8. Serial optical sections of confocal images of a9.34/a9.34 lineage-cells in the *Ciona* larva shown in Fig. 2K–K''. Localization of the fluorescence of Kaede-red and *Fer2*>EGFPv were visualized in magenta and yellow, respectively. Anterior is to the left. Lateral views.



Movie 9. Serial optical sections of confocal images of a9.50/a9.50 lineage-cells in the *Ciona* larva shown in Fig. 2L–L''. Localization of the fluorescence of Kaede-red and *Fer2*>EGFPv were visualized in magenta and yellow, respectively. Anterior is to the left. Lateral views.



Movie 10. Serial optical sections of confocal images of left a9.37 lineage-cells in the *Ciona* larva shown in Fig. 3B–B''. Localization of the fluorescence of Kaede-green, Kaede-red and *Fer2*>EGFPv were visualized in green, magenta and yellow, respectively. Anterior is to the left. Lateral views.



Movie 11. Serial optical sections of confocal images of right a9.37 lineage-cells in the *Ciona* larva shown in Fig. 3D–D''. Localization of the fluorescence of Kaede-green, Kaede-red and *Fer2*>EGFPv were visualized in green, magenta and yellow, respectively. Anterior is to the left. Lateral views.