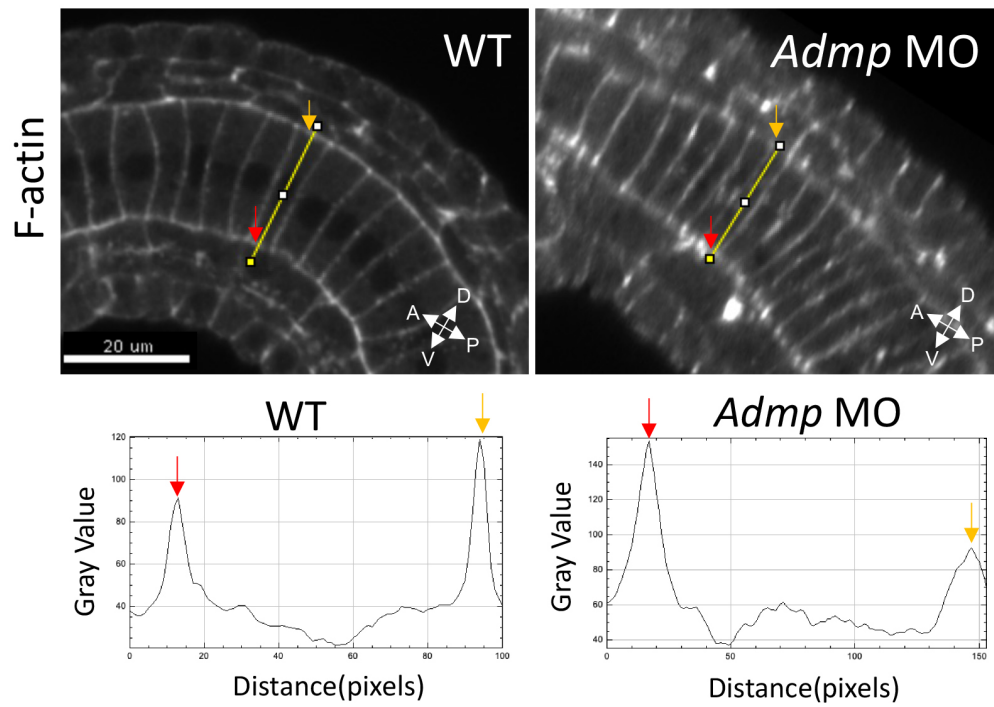


Fig. S1. Morphants of tail bending.

(A) Mid-tailbud stage embryo of WT, DMSO treatment, and dorsomorphin. DMSO and dorsomorphin were treated after the mid neurula stage (st. 15). The dorsomorphin-treated embryo did not bend its tail, similar to the *Admp* MO embryo (Fig. 1A). (B) Midline section view of WT dorsomorphine-treated and *Admp* MO embryo at st. 18, st. 20, and st. 22 by F-actin staining. The dorsomorphin-treated embryo did not bend its tail (N = 10/10, 12/12 and 9/9), similar to the *Admp* MO embryo (N = 5/5, 13/13 and 15/15). (C) The phenotype of *Msx-b* MO embryo at st. 22 in one experiment. The ventral tail bending was normally observed. Note that similar phenotypes were reported (Waki et al., 2015). Abbreviations: A, anterior; D, dorsal; V, ventral; P, posterior

A



B

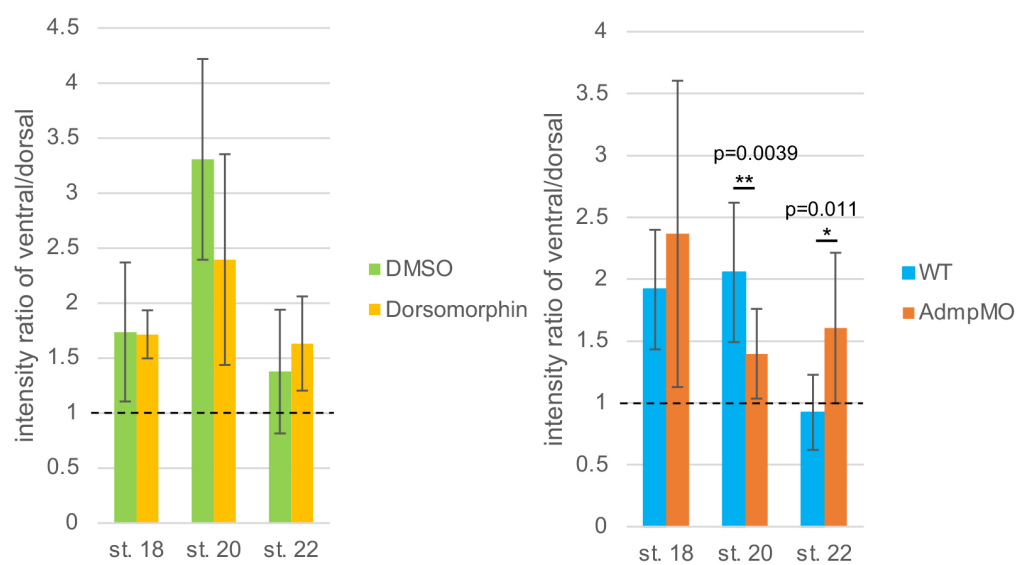


Fig. S2. The asymmetrical actomyosin localization of the notochord.

(A) F-actin localization of notochord in WT and *Admp* MO was measured on the yellow lines across the dorso-ventral (DV) axis. Abbreviations: A, anterior; D, dorsal; V, ventral; P, posterior. (B) The intensity ratio between ventral and dorsal F-actin. The ratio is calculated from the peak intensity values in (A) data. If the intensity of the ventral side (red arrow in A) is stronger than the dorsal side (orange arrow in A), the ratio becomes more than 1. In *Admp* MO embryos, asymmetrical localization remained. F-actin is significantly localized ventrally (WT, N = 8; *Admp* MO, N = 10). * $P < 0.05$, ** $P < 0.01$ (two-tailed Student's t-test). The error bar indicates SD.

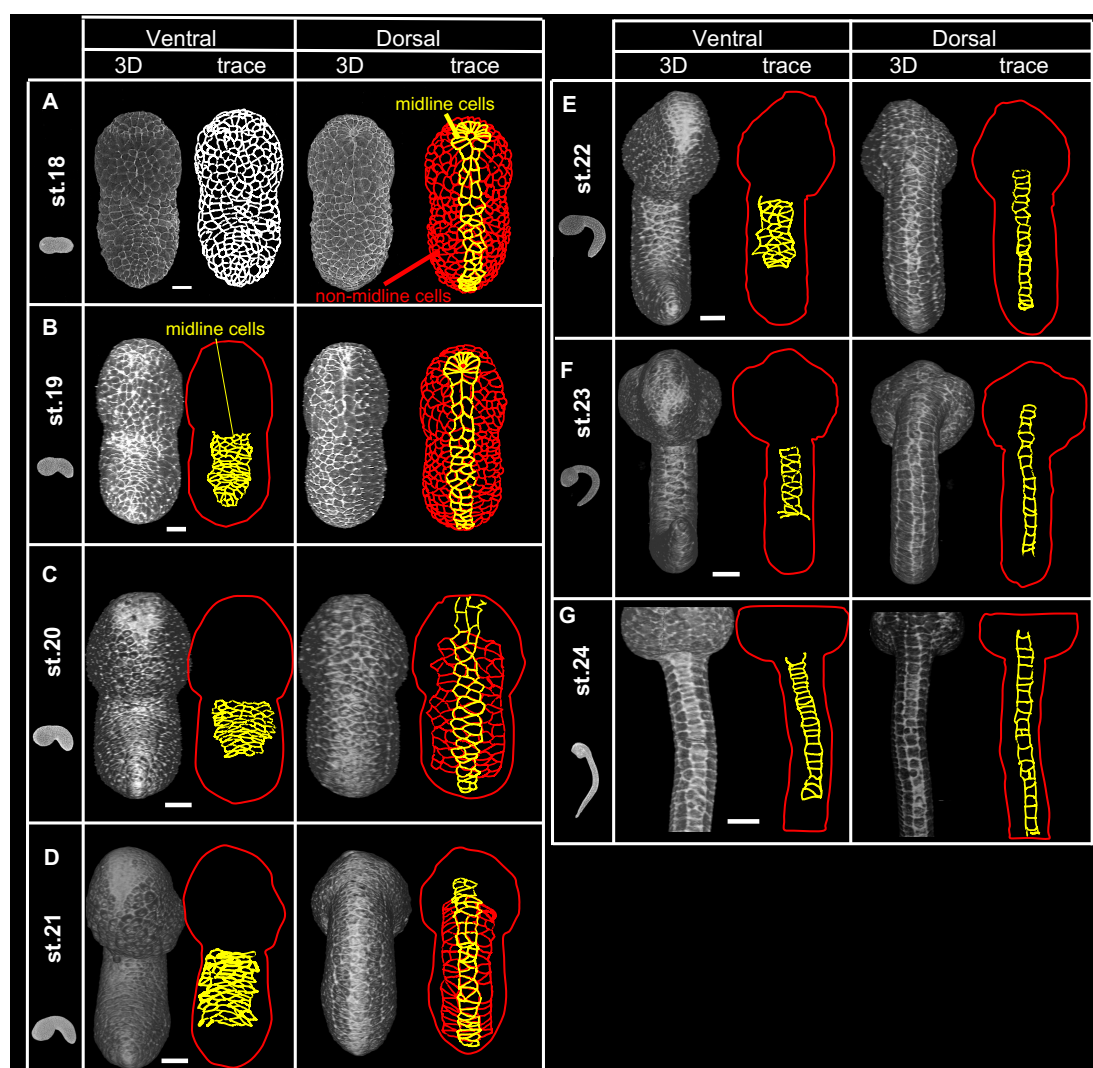


Fig. S3. The alignment of the tail midline epidermal cells during the tailbud period.

From 3D reconstructed confocal stack images by F-actin staining of tailbud embryos (st. 18 in A, st. 19 in B, st. 20 in C, st. 21 in D, st. 22 in E, st. 23 in F and st. 24 in G), cell shapes of both dorsal and ventral midline epidermal cells are traced (yellow lines). Note that cell-cell intercalation completes earlier on the dorsal midline than on the ventral midline.

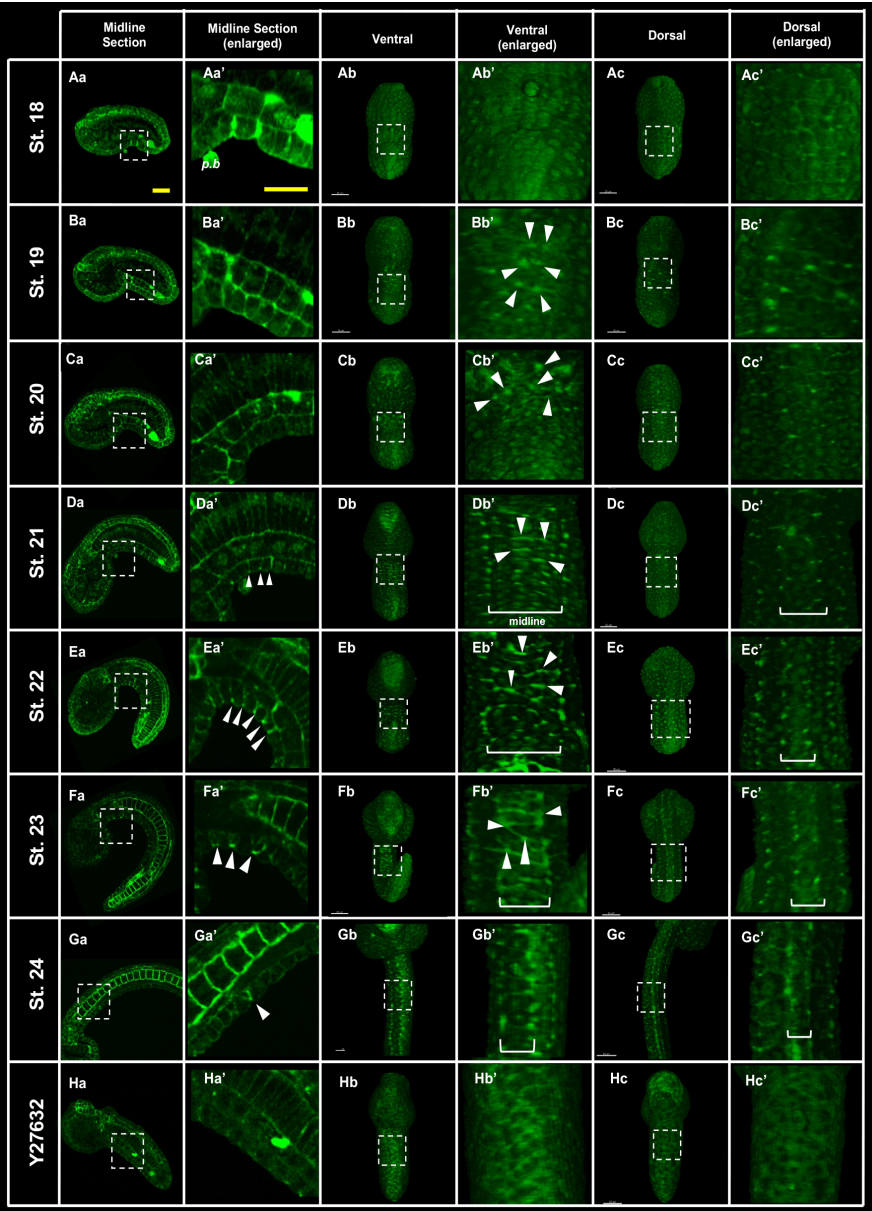
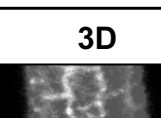
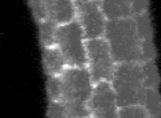
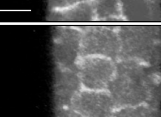
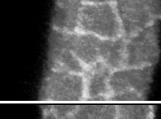
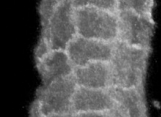
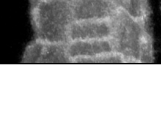

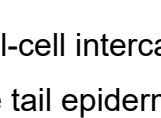
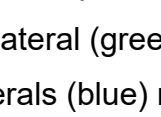
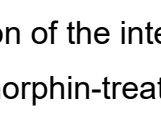
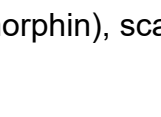



Fig. S4. pMLC antibody staining during tailbud period.

(Aa–Hc') Antibody staining of pMLC from st. 18 to st. 24 in wild type (rows A to G) and st. 22 in Y27632-treated (row H) embryos. Arrowheads show pMLC accumulation in the ventral midline epidermal cells. The part surrounded by the dotted square is enlarged to the panel on the right. Brackets indicate the range of the midline epidermal region. The midline epidermal cells are intercalated, narrowing the region (see Suppl. Fig. 2). Scale bar = 20 μ m.

	DMSO		Dorsomorphin 10 μ M	
	3D	trace	3D	trace
Dorsal				
Ventral				
Latera				

N=2/2

Fig. S5. The alignment of the tail epidermal cells of DMSO-treated and Dorsomorphin-treated embryo at st. 24.

The cell-cell intercalation was completed in the DMSO-treated embryo (left), and the tail epidermal cells consist of eight rows: dorsal (yellow), two dorsal medio-lateral (green), ventral (red), two ventral medio-lateral (orange), and two laterals (blue) rows. On the other hand, there is a ventral-side specific inhibition of the intercalation (red and orange gradation-colored cells) in the dorsomorphin-treated embryo (right). (N = 2 in DMSO and 2 in dorsomorphin), scale bar: 10 μ m.

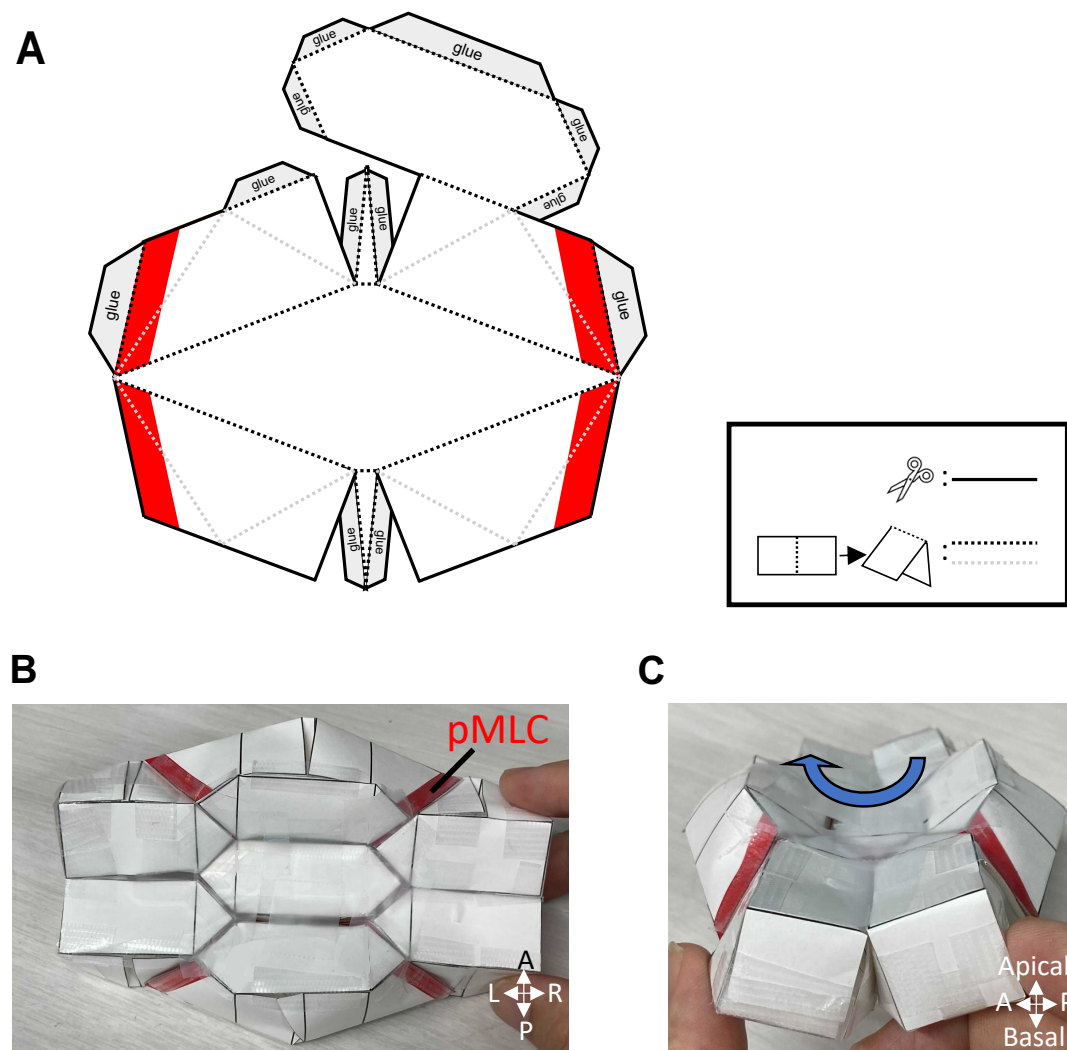


Fig. S6. Boat cells make up the bending of the tissue.

(A) Development view of a 3D model schematically showing the boat cell in the ventral midline epidermis (see Fig. 3A–C). (B) A combination of boat cells, consist of 7 (A), representing the ventral midline epidermis during intercalation. (C) Lateral view of (B). The tissue is bending, as shown in the blue arrow.

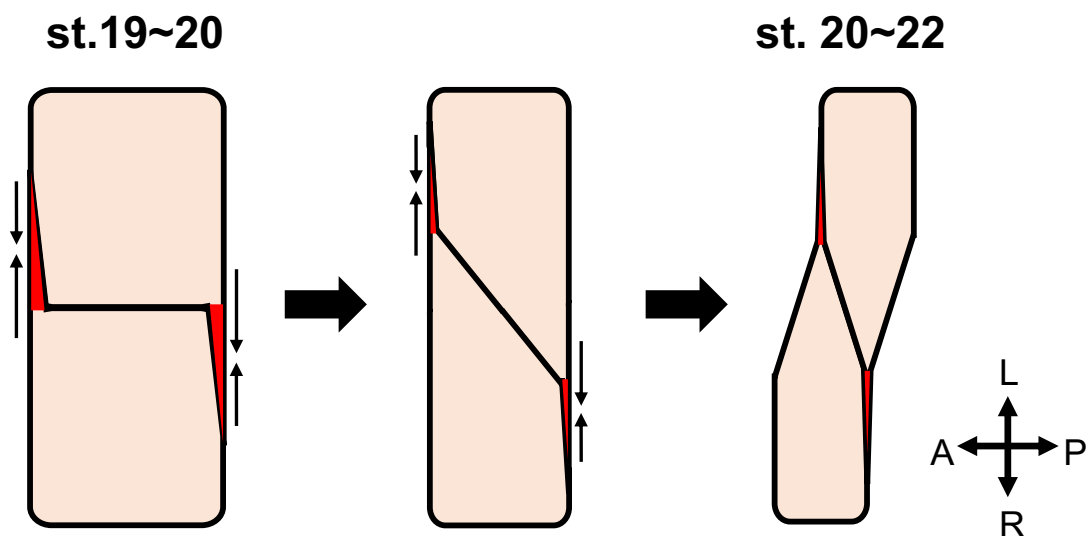
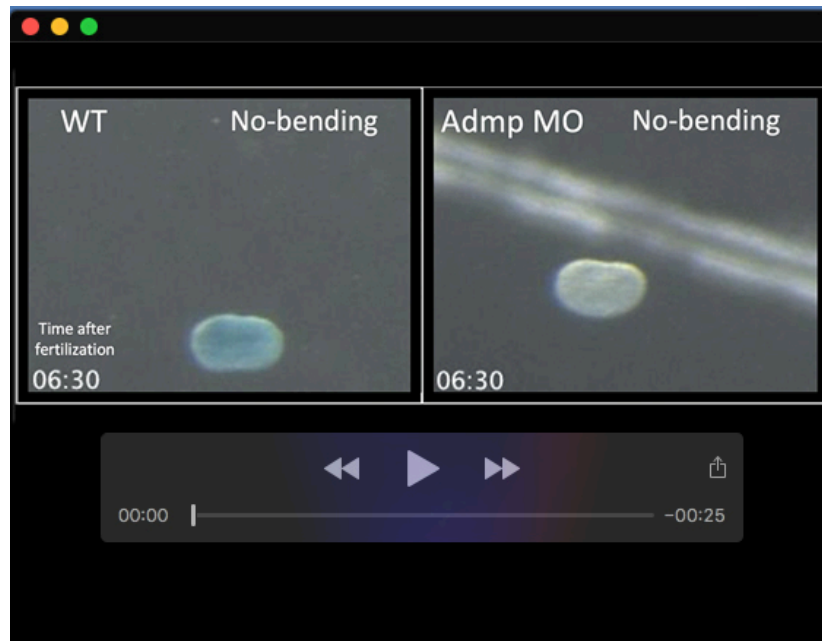


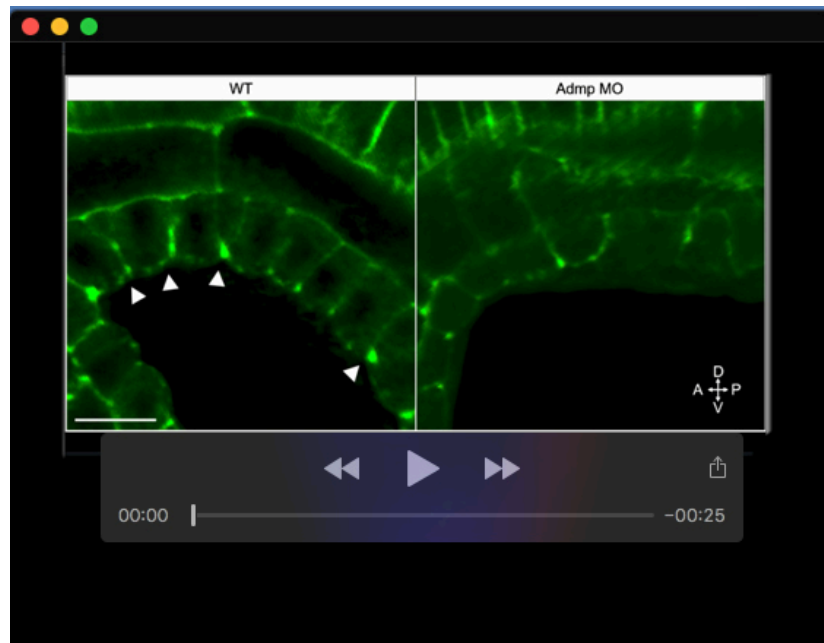
Fig. S7. The cell shape change by ML accumulated pMLC during early intercalation.

In the schematic figure of the apical surface of ventral epidermal cells, the red color indicates the ML localization of actomyosin at the protrusions formed, and the arrows indicate the contractility of actomyosin. This contractility elongates the ventral epidermal cells in the ML direction.



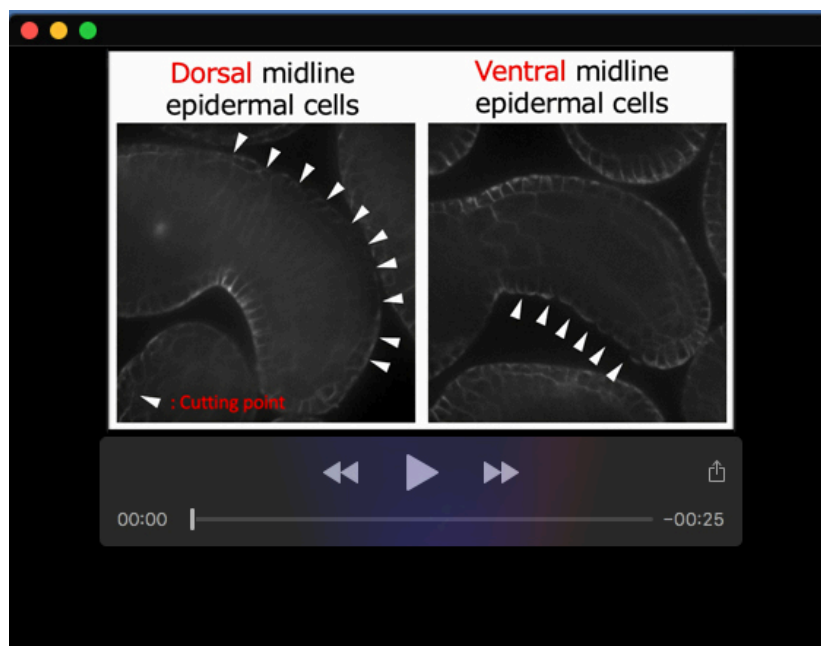
Movie 1. Time-lapse movie of WT (left) and *Admp* MO (right) embryo from late neurula stage 16 to late tailbud stage 25.

Both embryos are incubated in the same dish. The WT embryo was stained with NileBlue B (Matsumura et al., 2020). The movie frame was surrounded by different colors depending on the tail morphology. red: ventroflexion, blue: relaxation of ventroflexion, yellow: dorsiflexion.



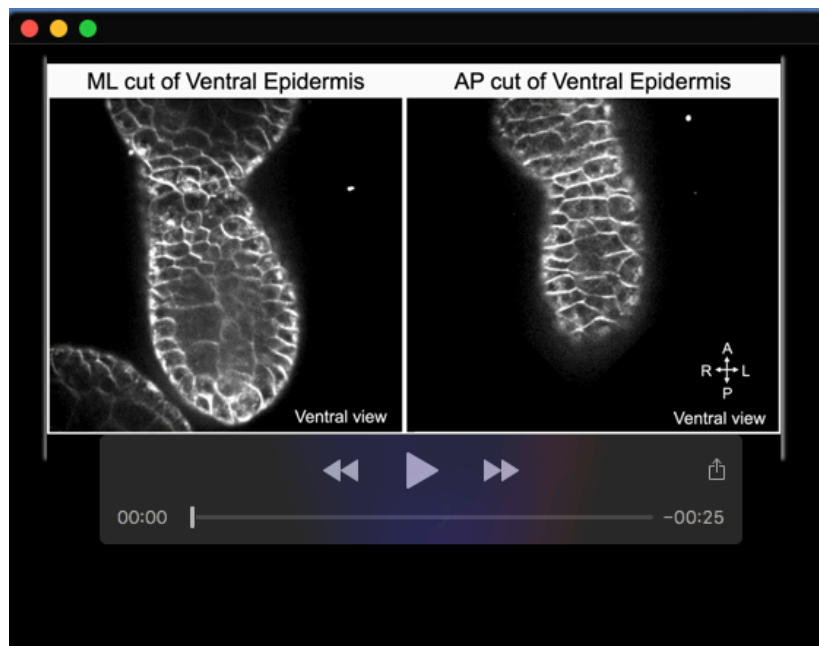
Movie 2. The z-stack section of the ventral epidermis

The z-stack images of the ventral midline of the tailbud embryo by phalloidin staining. All the section of the ventral epidermis of the WT and the *Admp* MO embryo was observed at st.22. The F-actin was stained by phalloidin. The arrowheads indicate the TSBC.



Movie. 3. Laser cutting experiment of the midline tail epidermis.

The dorsal (left) and ventral (right) midline epidermal AP cell borders of the WT mid-tailed embryos were cut with a laser cutter. The arrowheads indicate the cutting point by the laser cutter. The angle of the ventroflexion was relaxed when cut on the ventral side but not on the dorsal side, indicating the AP stress of the ventral midline epidermis (N = 3 each).



Movie 4. The laser cut of the ventral epidermis with ML and AP direction.

The ventral epidermis of the mid-tailbud embryo was cut in AP and ML directions with a laser cutter.