Supplementary Information

Actomyosin contractility drives apical polarization and membrane transport during tubulogenesis

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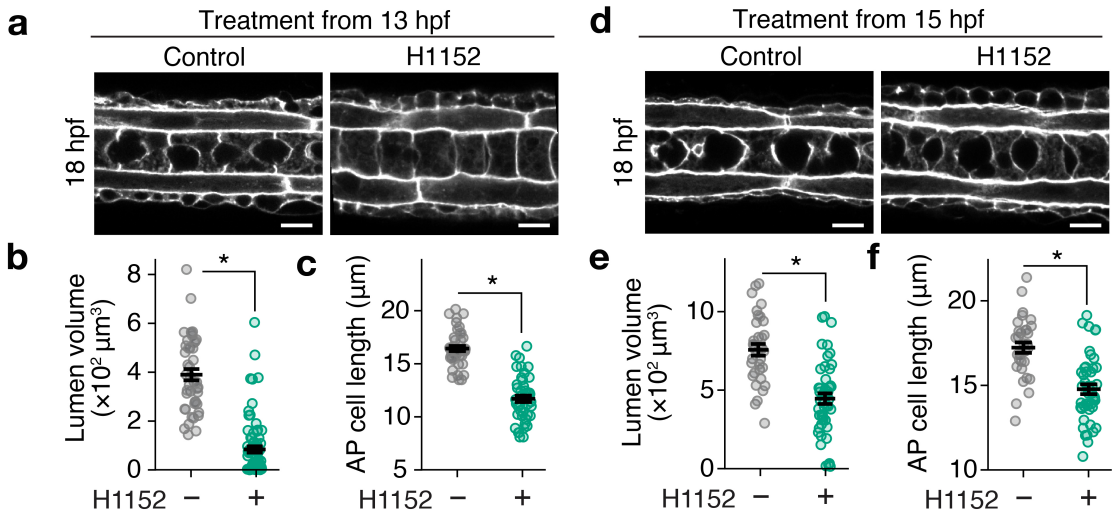
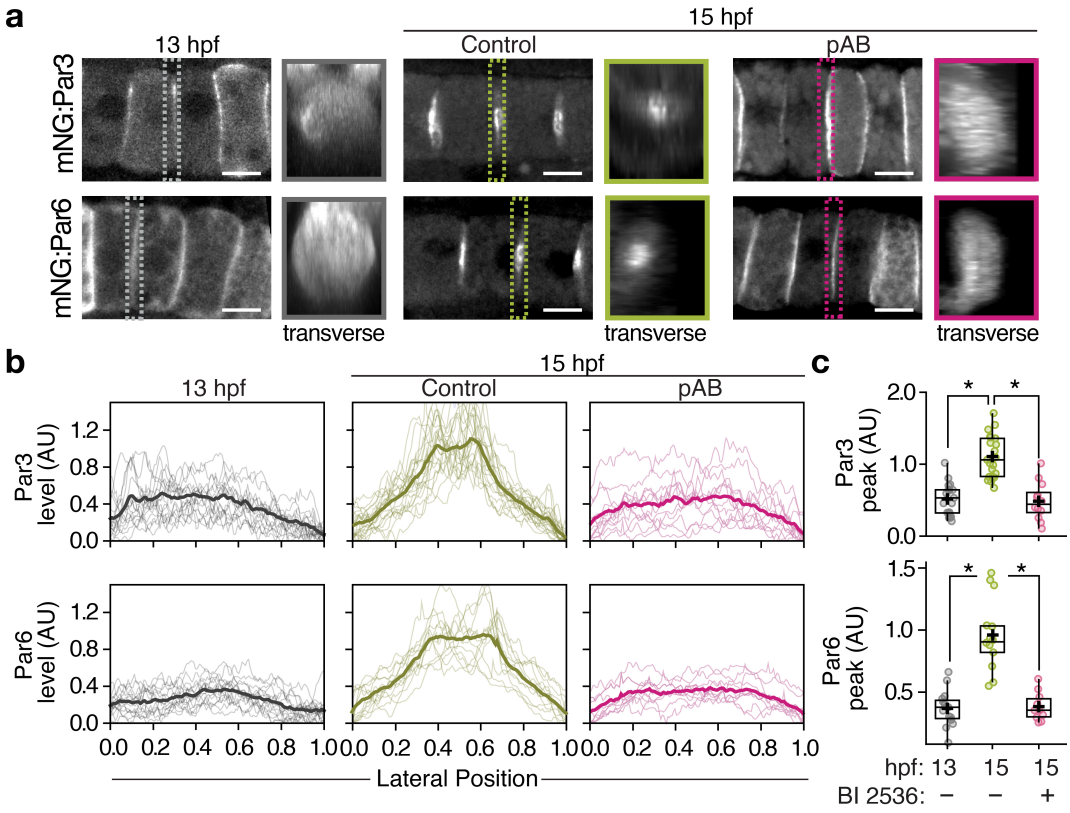
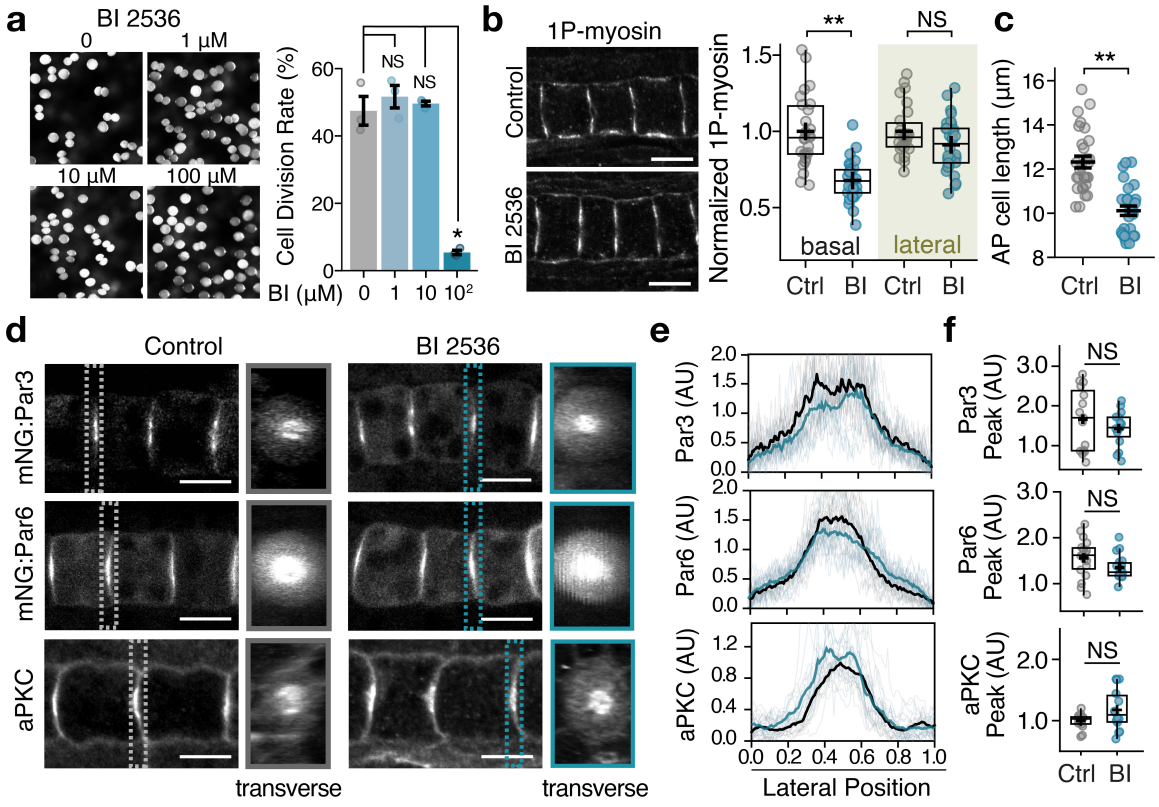


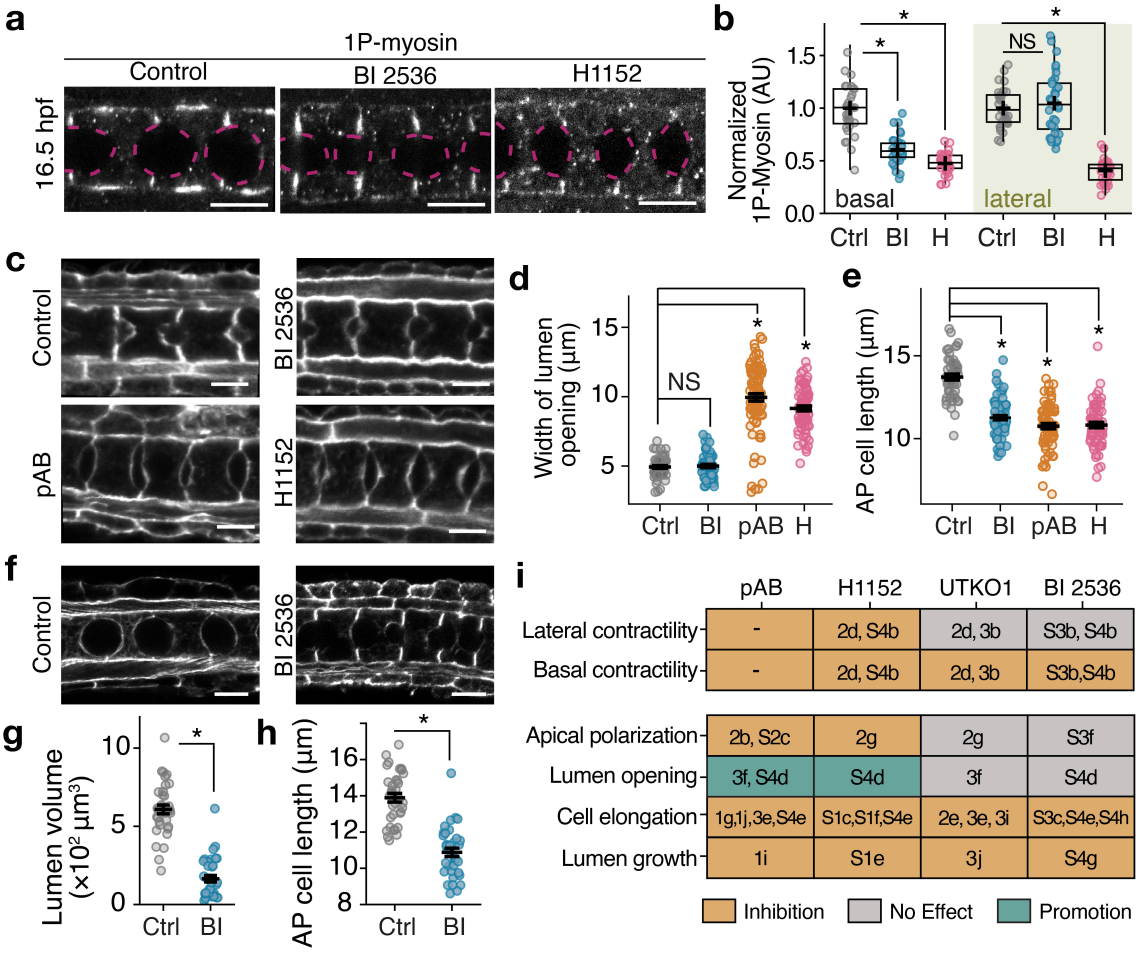
Supplementary Fig. 1. Rho Kinase is required for both lumen initiation and growth (related to Fig. 1). a–f Quantitative analysis of notochord morphology in embryos treated either with vehicle control (DMSO) or with 10 µM H1152 starting from 13 hpf (**a**–**c**) or 15 hpf (**d**–**f**), fixed at 18 hpf and stained with Alexa 488 Phalloidin to label F-actin. (**a, d**) Representative micrographs; (**b**, **e**) measurements of lumen volume; (**c**, **f**) measurements of notochord AP cell length. Scale bars: 5 µm. Horizontal bars in (**b**), (**c**), (**e**), (**f**) show mean ± SEM (Welch's t-test). NS, not significant; *P < 0.001.



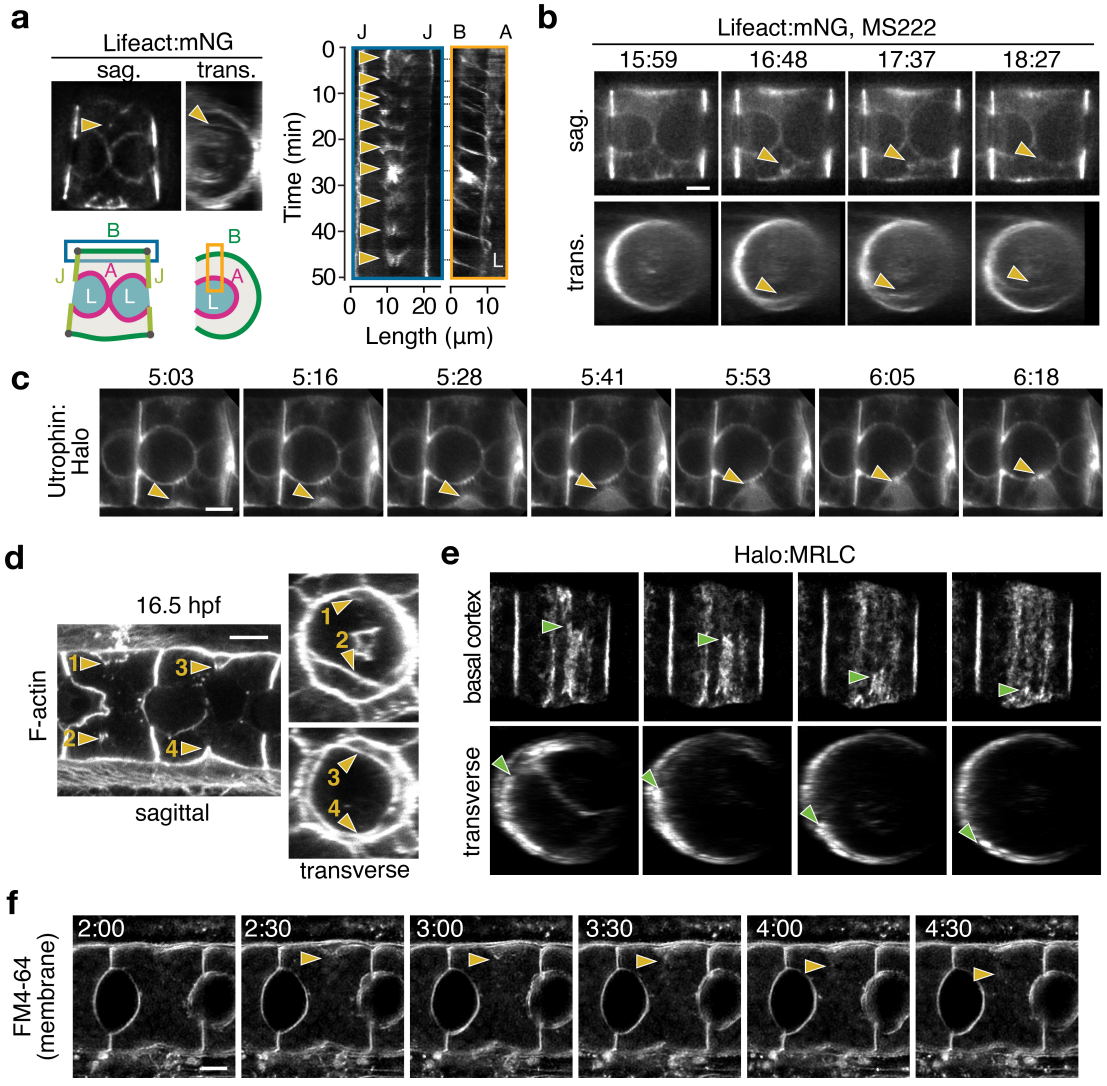
Supplementary Fig. 2. Lateral actomyosin is required for polarization of Par3 and Par6 (related to Fig. 2). a Representative micrographs showing sagittal and transverse views of notochord cells expressing mNG:Par3 (top) or mNG:Par6 (bottom), before (13 hpf) and after (15 hpf) treatment with vehicle control (DMSO) or pAB. Dashed rectangles indicate axial positions used for transverse views. **b** Quantification of the signal intensities along lateral cell contacts for the indicated treatments. Faint thin lines indicate measurements on individual contacts; thick lines show the average. **c** Boxplots show peak signal intensities on individual contacts for the indicated treatments. Horizontal lines indicate median, + indicate mean, boxes indicate second and third quartiles, and whiskers indicate 95% confidence interval. Scale bars: 5 µm. Mann-Whitney U test with Bonferroni's correction; NS, not significant; *P < 0.001.



Supplementary Fig. 3. BI 2536 is a basal selective inhibitor of actomyosin that does not inhibit apical polarization (related to Fig. 2). a Representative images (left) and quantification of successful division rates (right) in embryos treated with the indicated concentrations of BI 2536 up to ~ 1 hpf, when embryos normally complete first cleavage. **b**, **c** Representative micrographs (**b**, left) and quantification of basal and lateral 1P-myosin staining (**b**, right) and AP cell length (**c**) in embryos treated with vehicle control (DMSO) and BI 2536 from 13–15 hpf. **d** Representative micrographs of notochord cells expressing mNG:Par3, mNG:Par6, or aPKC at 15 hpf after the treatment with vehicle control (DMSO) or BI 2536 from 13–15 hpf. Dashed rectangles indicate axial positions used for transverse views. **e** Quantification of the signal intensities along lateral cell contacts for the indicated treatments. Faint thin lines indicate measurements on individual contacts; thick lines show the average. **f** Boxplots show peak signal intensities on individual contacts for the indicated treatments. Horizontal lines indicate median, + indicate mean, boxes indicate second and third quartiles, and whiskers indicate 95% confidence interval. In dot plots, data are mean ± SEM. Welch's t-test with Bonferroni's correction for (**a**); Mann-Whitney U test for (**b**), (**c**), and (**f**); NS, not significant; *P < 0.05, **P < 0.001.

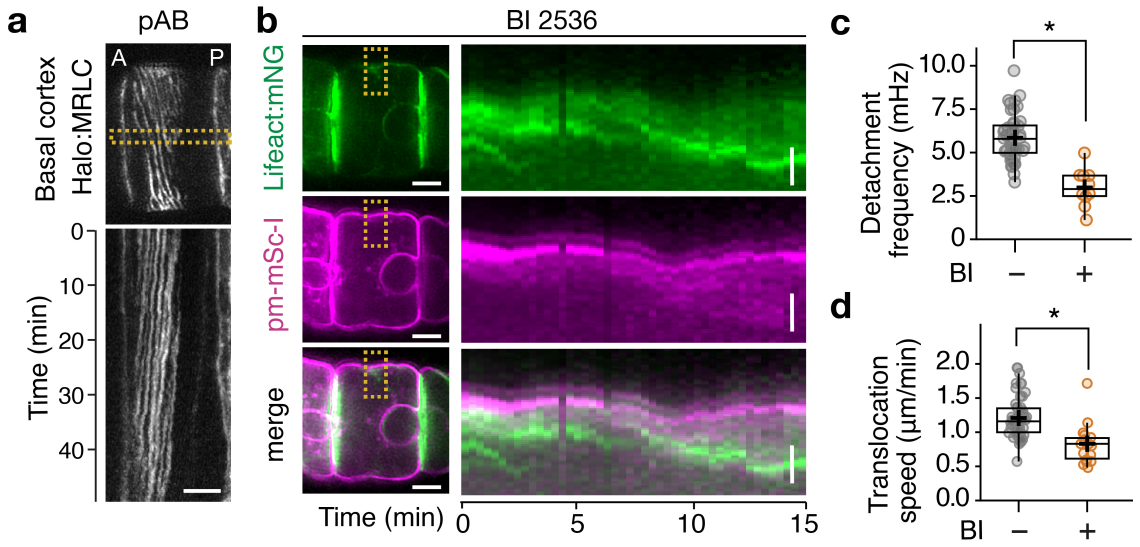


Supplementary Fig. 4. BI 2536 is a basal selective inhibitor of actomyosin that inhibits lumen formation and cell elongation without affecting lumen opening along lateral axis (related to Fig. 3). a, b Representative micrographs (**a**) and quantification of basal and lateral 1P-myosin staining (**b**) in 16.5 hpf embryos treated with vehicle control (DMSO), BI 2536, or H1152 from 15 hpf. Pink dashed lines indicate the positions of lumens. **c**–**e** Representative micrographs (**c**) and quantification of lateral lumen opening (**d**) and AP cell length (**e**) in 16 hpf phalloidin-stained embryos, treated with vehicle control (DMSO) and UTKO1 from 15–16 hpf. **f**–**h** Representative micrographs (**f**) and quantification of lumen volume (**g**) and AP cell length (**h**) in 18 hpf phalloidin-stained embryos, treated with vehicle control (DMSO) and UTKO1 from 15–18 hpf. **i** Summary of the notochord cell phenotypes caused by the treatment with pAB, H1152, UTKO1, and BI 2536. Figure numbers and letters indicate the corresponding data. Scale bars: 10 μm. In boxplots, horizontal lines indicate median, + indicate mean, boxes indicate second and third quartiles, and whiskers indicate 95% confidence interval. In dot plots, data are mean ± SEM. Mann-Whitney U test with Bonferroni's correction for (**b**); Welch's t-test with Bonferroni's correction for (**d**), (**e**), (**g**), and (**h**); NS, not significant; *P < 0.001.

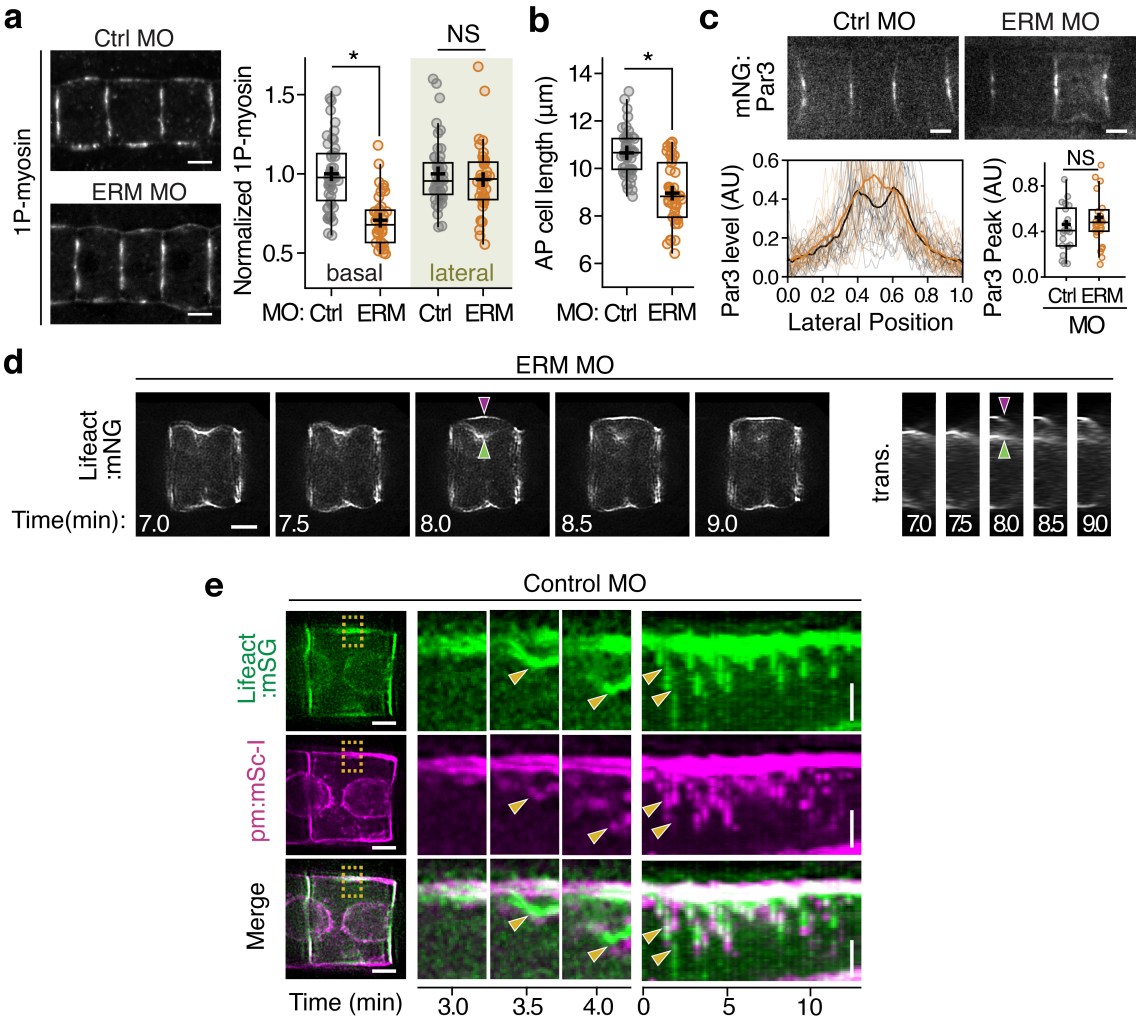


Supplementary Fig. 5. Actomyosin moves inward to cytoplasm and moves toward the apical domain (related to Fig. 4). a Representative sagittal and transverse sections (top left), and corresponding schematic views (bottom left), and kymographs (right) of a notochord cell expressing Lifeact:mNG. J = Lateral junction, L = lumen, A = apical and B = basal. Blue and orange rectangles indicate the regions used to construct the kymographs. Yellow arrowheads indicate detached sections of basal actin rings undergoing internalization. **b** Live images of cells expressing Lifeact:mNG immobilized by paralyzer MS-222, shown in sagittal and transverse sections. Yellow arrowheads indicate detached sections of actin rings undergoing internalization. **c** Live images of cells expressing Utrophin: Halo, and labelled with JF549. Yellow arrowheads indicate detached sections of basal actin rings undergoing internalization. **d** Micrographs of F-actin in fixed embryos stained with Alexa 488 Phalloidin, shown in sagittal and transverse sections. Yellow arrowheads indicate detached sections of basal actin rings undergoing internalization. **e** Basal myosin filaments showing local cortical tearing, followed by rapid lateral retraction of myosin, which remains associated with the basal surface (green arrowheads). **f** A 16

hpf embryo stained with FM4-64 to label membranes. Yellow arrowheads indicate internalizing membrane. Scale bars in all images: 5 μm.

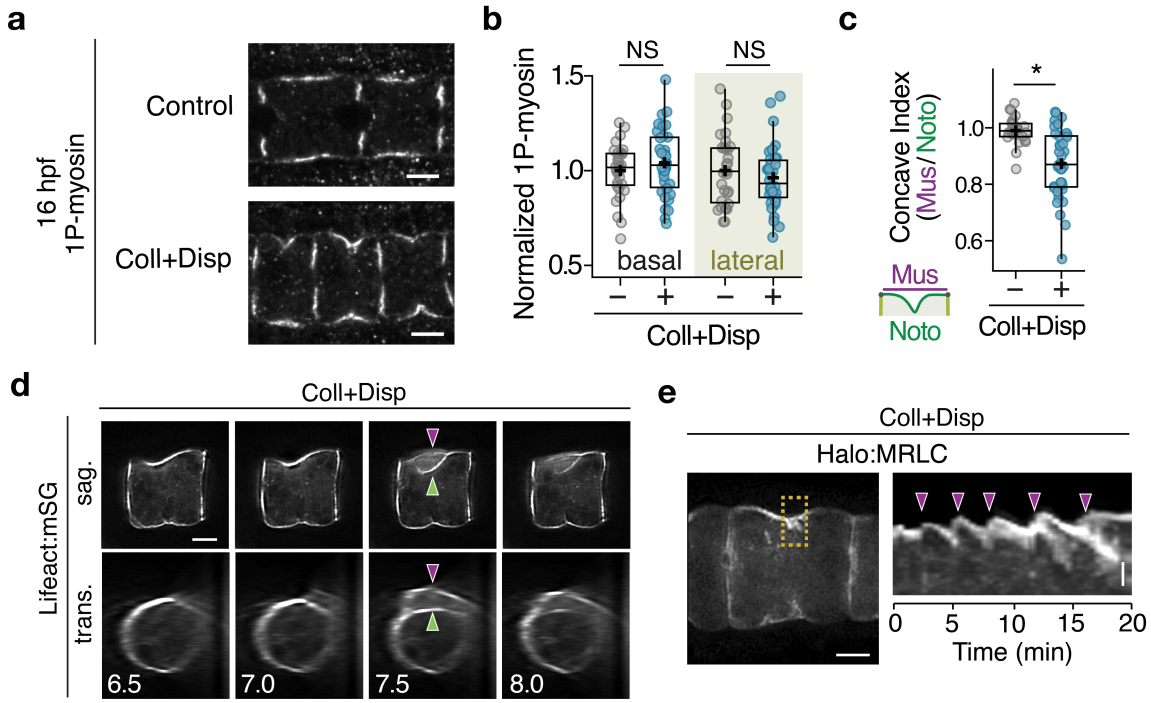


Supplementary Fig. 6. Basal actomyosin inhibition suppresses local detachment and inward movement of basal cortex and adjacent membranes (related to Fig. 4). a Basal cortical view (top) and corresponding kymograph (bottom) of a 16 hpf notochord cell, expressing Halo:MRLC, and treated with pAB from 15 hpf. A = anterior, P = posterior. Dashed yellow box indicates the region used for the kymograph. **b** Representative micrographs (left) and corresponding kymograph (right) of notochord cells expressing markers for F-actin (Lifeact:mNG, top, green), plasma membrane (pm:mSc-I, middle, magenta), and their merge (bottom). Dashed yellow boxes indicate the regions used for the kymographs. **c**, **d** Quantification of speed (**c**) and frequency (**d**) of actomyosin movement from the basal cortex to cytoplasm. In boxplots, horizontal lines indicate median, + indicate mean, boxes indicate second and third quartiles, and whiskers indicate 95% confidence interval. Mann-Whitney U test: NS, not significant; *P < 0.001.



Supplementary Fig. 7. ERM promotes both basal activation of myosin II and cortex-membrane linkage to direct internalization and directed movement of basal membranes toward the apical lumen surface (related to Fig. 5). a, b Representative micrographs (a, left) and quantification of basal and lateral 1P-myosin staining (b, middle) and AP cell length (**b**) in 15 hpf embryos after microinjection of eggs with control or ERM MO. **c** Representative micrographs (top), and measurements of lateral (bottom left) and peak (bottom right) signal intensities for notochord cells expressing mNG:Par3 at 15 hpf after microinjection of eggs with control or ERM MO. **d** Dynamics of Lifeact:mNG in 16 hpf embryos viewed in sagittal (left) or transverse (right) sections after injecting eggs with ERM MO. Green arrowheads indicate detached and internalized sections of the basal contractile ring; magenta arrowheads indicate outward displacement of the basal membrane just after local detachment of the actin ring from the basal surface. **e** Representative images (left), magnified snapshots (middle) and kymographs (right) from a 16 hpf embryo, expressing markers for F-actin (Lifeact:mSG (mStayGold)) and plasma membrane (pm:mSc-I) from an egg microinjected with a control morpholino. Yellow rectangles at left indicate regions used for magnified snapshots and kymographs. Yellow arrowheads indicate the movement of actin and membrane from the basal to apical domain. In

boxplots, horizontal lines indicate median, + indicate mean, boxes indicate second and third quartiles, and whiskers indicate 95% confidence interval. Mann-Whitney U test: NS, not significant; *P < 0.001.



Supplementary Fig. 8. Anchorage to basal ECM promotes the normal cycle of cortical detachment and internalization of basal membranes (Related to Fig. 5) a, b Representative micrographs (**a**) and quantification of basal and lateral 1P-myosin staining (**b**) at 16 hpf in embryos treated with (+) or without (-) Collagenase and Dispase (Coll+Disp). **c** Quantification of concavity index in embryos treated with (+) or without (-) Coll+Disp. Bottom schematic: The concavity index is defined to be the ratio of basal surface lengths, measured in sagittal cross sections, for muscle and notochord membranes. **d** Live images of cells expressing Lifeact:mSG (mStayGold), at the indicated timepoints (min), treated with Coll+Disp, shown in sagittal and transverse section. Green arrowheads indicate detached and internalized sections of the basal contractile ring; magenta arrowheads indicate outward displacement of the basal surface just after local detachment of the actin ring from the basal surface. **e** Representative images (left), and kymographs (right) from a 16 hpf embryo, expressing Halo:MRLC, and treated with Coll+Disp from 15 hpf. Dashed yellow box indicates the region used for the kymographs, and magenta arrowheads indicate repeated cycles of detachment and bleb-like protrusion. In boxplots, horizontal lines indicate median, + indicate mean, boxes indicate second and third quartiles, and whiskers indicate 95% confidence interval. Mann-Whitney U test: NS, not significant; *P < 0.001.

Supplementary Movie 1 (Related to Fig. 4a). Dynamics of Lifeact:mNG (actin) and Halo:MRLC (myosin II) in a control embryo at 16–17 hpf shown in sagittal and transverse views. Time compression = 300x.

Supplementary Movie 2 (Related to Supplementary Fig. 5a). Dynamics of Lifeact:mNG (actin) in a control embryo at 16–17 hpf shown in sagittal and transverse views. Time compression = 300x.

Supplementary Movie 3 (Related to Supplementary Fig. 5b). Dynamics of Lifeact:mNG (actin) in an embryo at 16–17 hpf paralyzed with MS-222, shown in sagittal and transverse views. Time compression = 300x.

Supplementary Movie 4 (Related to Supplementary Fig. 5c). Dynamics of Utrophin:Halo (actin) in an embryo at 16–17 hpf stained with JF549, shown in sagittal view Time compression = 300x.

Supplementary Movie 5 (Related to Fig. 4b–d). Dynamics of Halo:MRLC (myosin II) in a control embryo at 16–17 hpf, shown in the basal cortex (left) and transverse view (middle), along with the kymograph for basal cortex (right). Time compression = 300x.

Supplementary Movie 6 (Related to Fig. 4e). Dynamics of Lifeact:mNG (actin) and pm:mSc-I (membrane) in a control embryo at 16–17 hpf shown in sagittal view. Time compression = 300x.

Supplementary Movie 7 (Related to Supplementary Fig. 5e). Dynamics of membrane internalization, visualized by FM4-64, in a control embryo at 16–17 hpf shown in sagittal view. Time compression = 300x.

Supplementary Movie 8 (Related to Supplementary Fig. 6a). Dynamics of Halo:MRLC (myosin II) on the basal cortex in an embryo treated with pAB 16–17 hpf shown in basal cortex view. Time compression = 300x.

Supplementary Movie 9 (Related to Fig. 4f). Dynamics of Lifeact:mNG (actin) and pm:mSc-I (membrane) in an embryo treated with pAB at 16–17 hpf shown in sagittal view. Time compression = 300x.

Supplementary Movie 10 (Related to Fig. 4g). Dynamics of Lifeact:mNG (actin) and pm:mSc-I (membrane) in an embryo treated with UTKO1 at 16–17 hpf shown in sagittal view. Time compression = 300x.

Supplementary Movie 11 (Related to Supplementary Fig. 6b). Dynamics of Lifeact:mNG (actin) and pm:mSc-I (membrane) in an embryo treated with BI 2536 at 16–17 hpf shown in sagittal view. Time compression = 300x.

Supplementary Movie 12 (Related to Fig. 5b). Dynamics of Lifeact:mNG (actin) and pm:mSc-I (membrane) in an embryo injected with ERM MO 16–17 hpf shown in sagittal view. Time compression = 300x.

Supplementary Movie 13 (Related to Supplementary Fig. 7e). Dynamics of Lifeact:mNG (actin) and pm:mSc-I (membrane) in an embryo injected with control MO 16–17 hpf shown in sagittal view. Time compression = 300x.

Supplementary Movie 14 (Related to Supplementary Fig. 7d). Dynamics of Lifeact:mNG (actin) in an embryo injected with ERM MO at 16–17 hpf shown in sagittal and transverse view. Time compression = 300x.

Supplementary Movie 15 (Related to Fig. 5h). Dynamics of Lifeact:mNG (actin) and pm:mSc-I (membrane) in an embryo treated with Coll+Disp at 16–17 hpf shown in sagittal and transverse view. Time compression = 300x.

Supplementary Movie 16 (Related to Supplementary Fig. 8d). Dynamics of Lifeact:mNG (actin) in an embryo treated with Coll+Disp at 16–17 hpf shown in sagittal and transverse view. Time compression = 300x.

Supplementary Movie 17 (Related to Supplementary Fig. 8e). Dynamics of Halo:MRLC (myosin II) in an embryo treated with Coll+Disp at 16–17 hpf shown in sagittal and transverse view. Time compression = 300x