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Supplementary Material

corresponding to:

Enhancement of neural crest formation by mechanical force in *Xenopus* development

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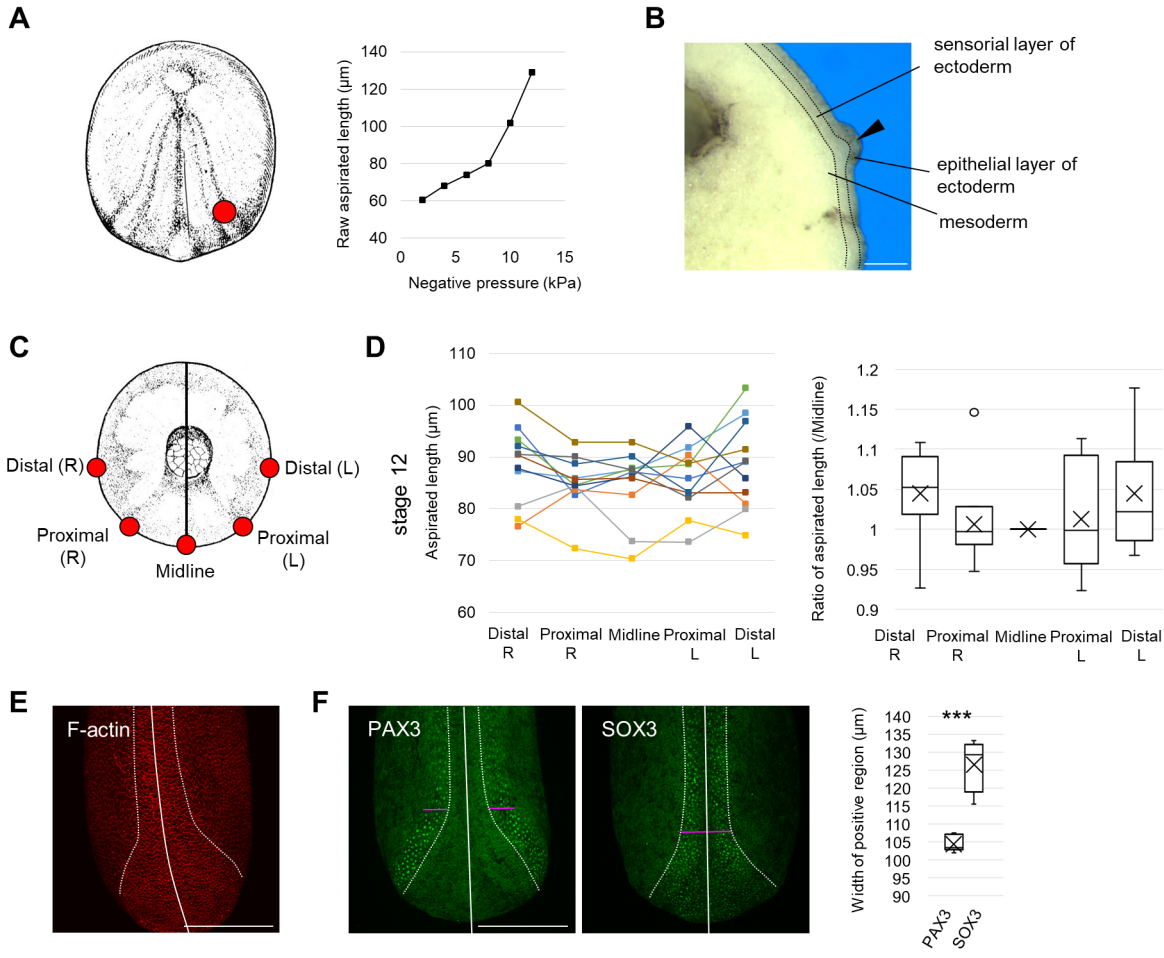

Fig. S1. Determination of appropriate negative pressure for an aspiration assay, and locational information of neuroectodermal patterning (Related to Fig. 1). (A) Determination of appropriate negative pressure for an aspiration assay at the early neurula stage (st. 13). Schematic figure of aspirated region (red circle) is shown on the left. The graph indicates actual length of aspirated tissue with each negative pressure (2, 4, 6, 8, 10, 12 kPa). Embryos applied negative pressure > 14 kPa was wholly aspirated into a glass needle and broken. **(B)** Bright field image of embryos fixed during aspiration. Black dashed lines indicate boundary between sensorial layer and epithelial layer, or between epithelial layer and mesoderm. Scale bar, 100 μm. **(C)** Schematic image of aspirated points at st. 12. Black line indicates the midline of embryo, and red circles indicate aspirated positions: Midline, both sides of Proximal and Distal (see Materials and Methods for details). **(D)** Result of the aspiration assay at st. 12. Length of aspirated tissue is shown in the left graph. Each line indicates actual length of aspirated tissue in each measurement (n = 11). Length ratio normalized by each aspirated length of Midline was shown in the right graph (boxplot: The horizontal line indicates the median. Edges of boxes indicate the first and third quartiles. The cross indicates the mean, and whiskers indicate the minimum and maximum). **(E)** Fluorescent image of Phalloidin in an AFM-measured embryo at the early neurula stage (st. 13). A white line indicates the midline of the embryo. A white dashed line indicates the boundary between NP and NPB. Scale bar, 500 μm. **(F)** Quantification of the width of PAX3- or SOX3-positive region in early neurula (st. 13) with removed vitelline membrane at the same AP coordinate as AFM measurement. Fluorescent images of PAX3 and SOX3 are shown on the left. A white line indicates the midline of the embryo. White dashed lines indicate predicted boundary between NP and NPB. Magenta lines indicate the width of PAX3- or SOX3-positive region. Scale bar, 500 μm. The width of PAX3- or SOX3-positive region is shown in a boxplot (The horizontal line indicates the median. Edges of boxes indicate the first and third quartiles. The cross indicates the mean, and whiskers indicate the minimum and maximum). n = 6 each.

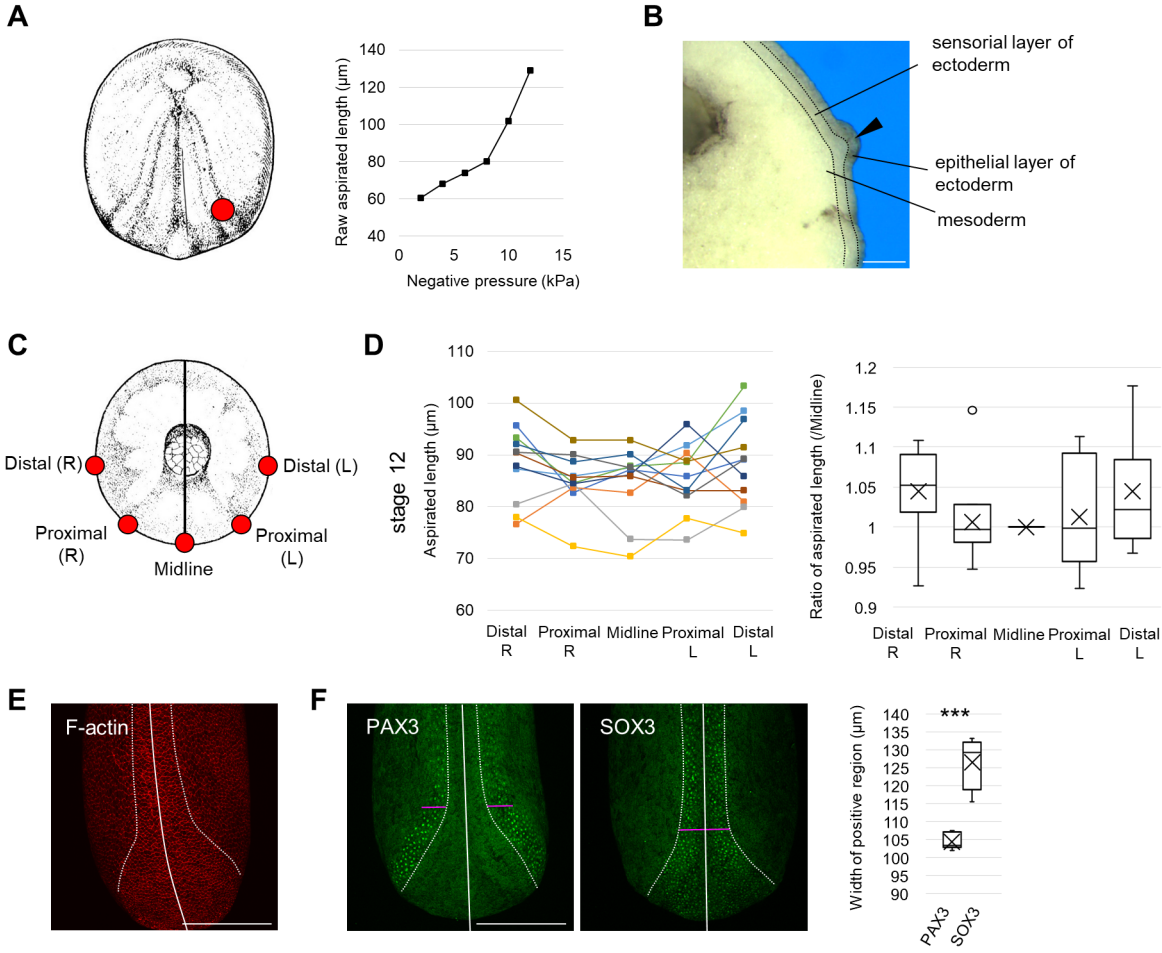

Fig. S2. Individual results of AFM measurements (Related to Fig. 1). Each result of AFM measurements in Fig. 1F-G. Left: Bright field images of measured embryos at the early neurula stage (st. 13). A white line indicates the midline of the embryos. A white dashed line indicates the boundary between NP and NPB. A magenta dashed line indicates the boundary between NPB and epidermis. A white box indicates the region measured with AFM. A, anterior side, P, posterior side, E, epidermis. Scale bar, 500 μm. Right: Calibrated Young's modulus ($\log_{10}E$) on each lateral coordinate (distance from the midline) is shown.

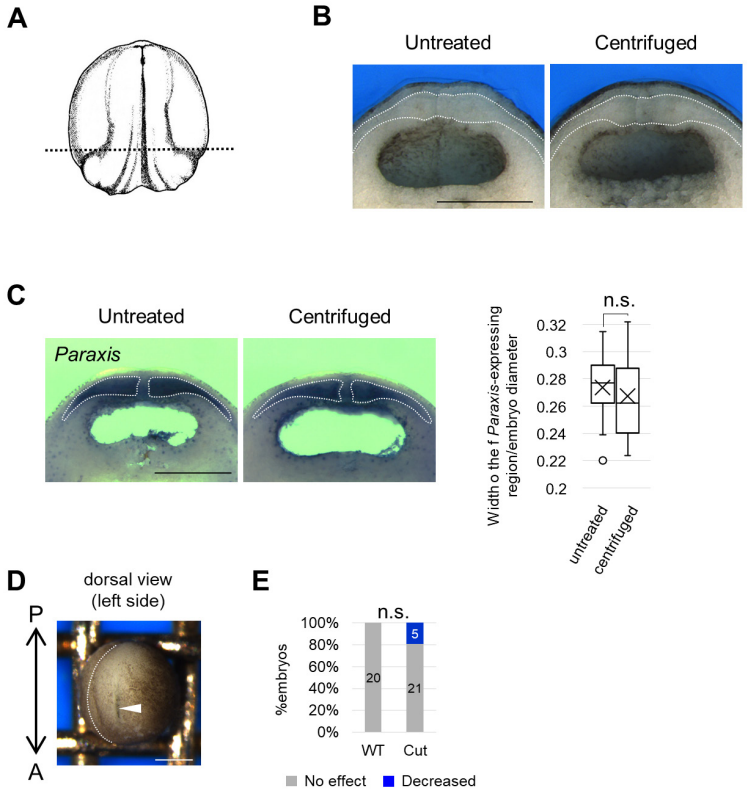

Fig. S3. Effect of centrifugation on mesodermal position and NPB gene expression, and effect of cutting only ectoderm, not including mesoderm, on NC formation (Related to Fig. 2). (A) Schematic figure showing the dissected region in B and C. **(B)** Bright field image of centrifuged embryo at the mid-neurula stage (st. 15) with hemisection. White dashed lines indicate the outline of mesoderm. Scale bar, 500 μm. **(C)** Expression pattern of *Paraxis* in centrifuged embryos at the mid-neurula stage (st. 15) with hemisection. White dashed lines indicate *Paraxis*-expressing region. Scale bar, 500 μm. Ratio of lateral width of the *Paraxis*-expressing region and embryo diameter is shown in a boxplot on right (The horizontal line indicates the median. Edges of boxes indicate the first and third quartiles. The cross indicates the mean, and whiskers indicate the minimum and maximum). n = 10 each. Statistical significance was analyzed with Student *t*-test. **(D)** Bright field image of neurula with cut tissue (cutting only ectoderm). A white arrowhead indicates the cutting site. A white dashed line indicates the midline of the embryo. Scale bar, 500 μm. **(E)** Quantification of expression pattern of *Foxd3* in embryos with cutting only ectoderm at the mid-neurula stage (st. 15). Ratios of each phenotype are summarized in stacked bar graphs. Numbers in the graph indicate numbers of embryos with each phenotype. Statistical significance was analyzed with Fisher's exact test.

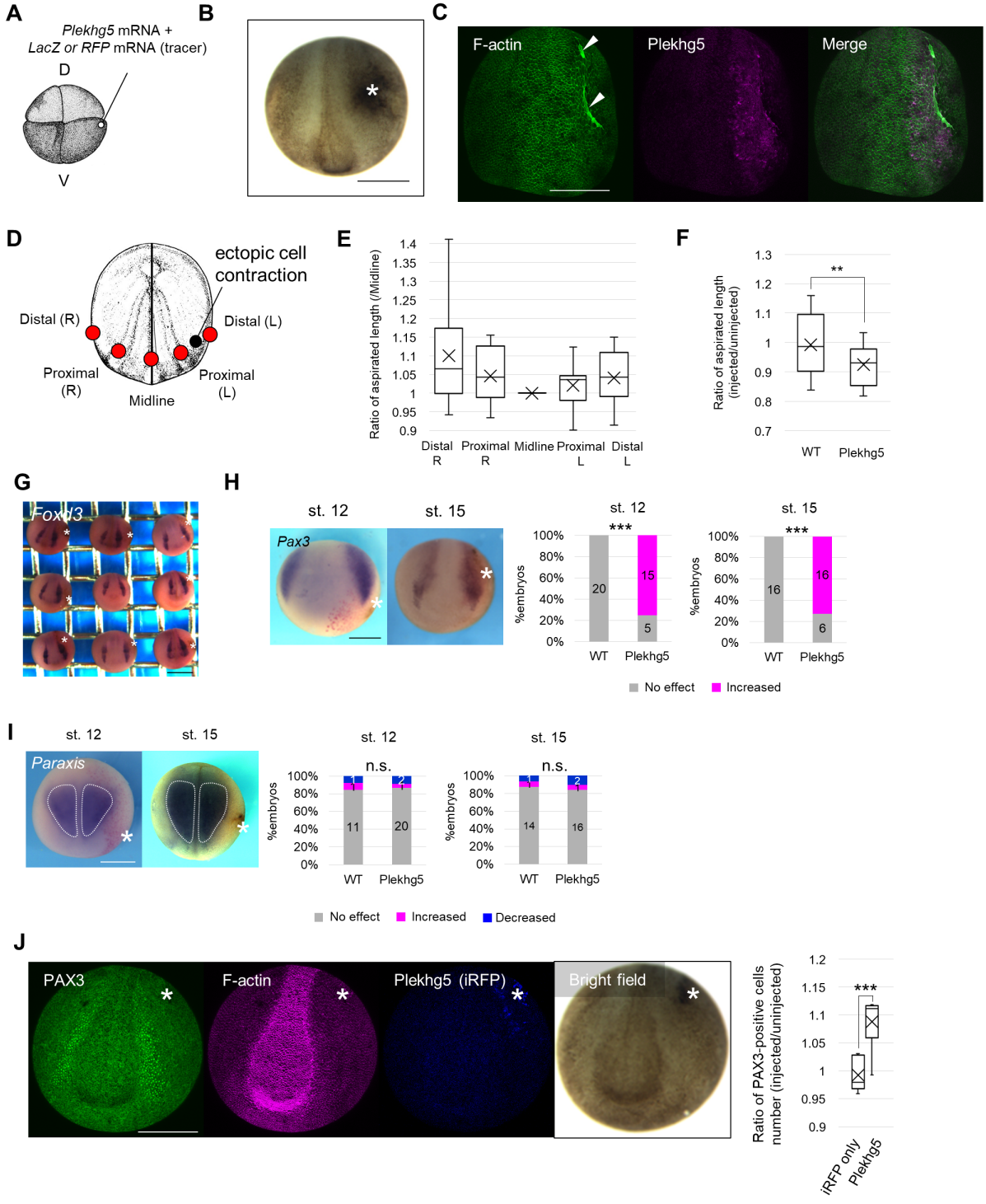

Fig. S4. Effect of Plekhg5-induced ectopic cell contraction on mechanical properties and NPB gene expression (Related to Fig. 3). (A) Schematic figure of ***Plekhg5*** mRNA microinjection. D, dorsal blastomere, V, ventral blastomere. 200 pg ***Plekhg5*** mRNA with ***RFP*** mRNA (tracer) was injected into a ventral blastomere at the 4-8-cell stage. **(B)** Bright field image of a ***Plekhg5***-expressing embryo at the mid-neurula stage (st. 15). Asterisk indicates ectopic accumulation of pigment. Scale bar, 500 μm. **(C)** Fluorescent image of Phalloidin in a ***Plekhg5***-expressing embryo. Green, F-actin (Phalloidin), Magenta, Plekhg5 (RFP). Arrowheads indicate ectopic cell contraction. Scale bar, 500 μm. **(D)** Schematic image of aspirated points at st. 14. Black line indicates the midline of embryo, and red circles indicate aspirated positions: Midline, both sides of Proximal and Distal (see Materials and Methods for details). Black point indicates ectopic contraction caused by Plekhg5. **(E)** Result of the aspiration assay. Length ratio normalized by each aspirated length of Midline was shown in the right graph (boxplot: The horizontal line indicates the median. Edges of boxes indicate the first and third quartiles. The cross indicates the mean, and whiskers indicate the minimum and maximum). n = 8. **(F)** Result of an aspiration assay in ***Plekhg5***-expressing embryos at the early neurula stage (st. 13). Ratio of aspirated length of the injected side normalized by that of the uninjected side is shown in a boxplot (The horizontal line indicates

the median. Edges of boxes indicate the first and third quartiles. The cross indicates the mean, and whiskers indicate the minimum and maximum). n = 10 each. Statistical significance was analyzed with paired *t*-test. ** $p < 0.01$. **(G)** Expression pattern of ***Foxd3*** in ***Plekhg5***-expressing embryos, measured in Fig. 3D. **(H)** Expression pattern of ***Pax3*** in ***Plekhg5***-expressing embryos at the late gastrula stage (st. 12) and the mid-neurula stage (st. 15). Asterisk indicates ectopic cell contraction induced by Plekhg5. Scale bar, 500 μm. Ratios of phenotypes are summarized in stacked bar graphs on right. Numbers in the graph indicates numbers of embryos with each phenotype. Statistical significance was analyzed with Fisher's exact test. *** $p < 0.001$. **(I)** Expression pattern of ***Paraxis*** in ***Plekhg5***-expressing embryos at the late gastrula stage (st. 12) and the mid-neurula stage (st. 15). Asterisk indicates ectopic cell contraction induced by Plekhg5. White dashed lines indicate the expressing region of ***Paraxis***. Scale bar, 500 μm. Ratios of phenotypes are summarized in stacked bar graphs on right. Numbers in the graph indicates numbers of embryos with each phenotype. Statistical significance was analyzed with Fisher's exact test. **(J)** Fluorescent image of PAX3 in a ***Plekhg5***-expressing embryo at the mid-neurula stage (st. 15). Green, PAX3, Magenta, F-actin (Phalloidin), Blue, Plekhg5 (iRFP). Asterisk indicates ectopic cell contraction. Scale bar, 500 μm. Ratio of number of PAX3-positive cells between the ***Plekhg5***-expressing side and the control side is shown in a boxplot (The horizontal line indicates the median. Edges of boxes indicate the first and third quartiles. The cross indicates the mean, and whiskers indicate the minimum and maximum). n - 10 each. Statistical significance was analyzed with Student *t*-test. *** $p < 0.001$.

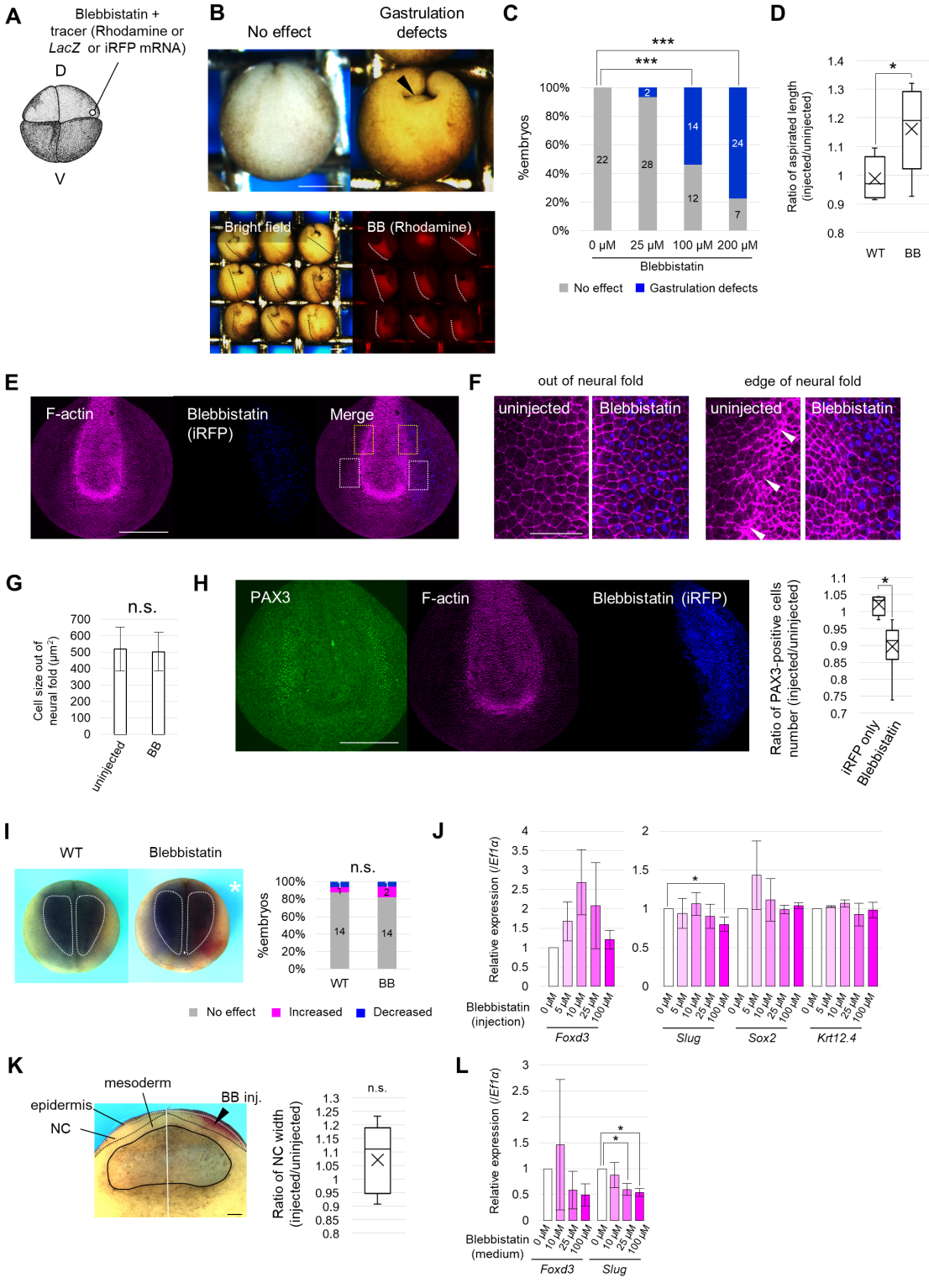

Fig. S5. Effect of Blebbistatin on cell division and mesodermal position (Related to Fig. 4). (A) Schematic figure of Blebbistatin microinjection. D, dorsal blastomere, V, ventral blastomere. A 5 nL of Blebbistatin solution (0 (DMSO), 5, 10, 25, 100 μM) with Rhodamine or ***LacZ*** mRNA (tracer) was injected into a dorsal blastomere at the 4- or 8-cell stage. **(B)** Bright field image of BB-injected embryos. Arrowhead indicates gastrulation defects. Scale bar, 500 μm. **(C)** Ratios of embryos having gastrulation defects caused by Blebbistatin. Numbers in the bar graph indicate numbers of embryos with each phenotype. Statistical significance was analyzed with Fisher's exact test. Each ***p*** value is corrected using Holm method. *** $p < 0.001$. **(D)** Result of an aspiration assay in BB-injected embryos at the early neurula stage (st. 14). Ratio of aspirated length of the injected side by that of the uninjected side was shown in a boxplot. n = 10 each. Statistical significance was analyzed with paired ***t***-test. * $p < 0.05$. **(E)** Fluorescent image of Phalloidin in 100 μM Blebbistatin-injected embryos

at the mid-neurula stage (st. 15). Magenta, F-actin (Phalloidin), Blue, Blebbistatin (iRFP). White (out of neural fold) and yellow (edge of neural fold) dashed boxes indicate enlarged regions in (E). Scale bar, 500 μm. **(F)** Enlarged image of (D). Left, Out of neural fold, Right, Edge of neural fold. Asterisks indicate inhibited cell accumulation. Scale bar, 100 μm. **(G)** Statistical analysis of cell size out of neural fold in Blebbistatin-injected embryos at the mid-neurula stage (st. 15). n = 100 each. Statistical significance was analyzed with Student ***t***-test. **(H)** Fluorescent image of PAX3 in a Blebbistatin-injected embryo at the mid-neurula stage (st. 15). Green, PAX3, Magenta, F-actin (Phalloidin), Blue, Blebbistatin (iRFP). Scale bar, 500 μm. Ratio of number of PAX3-positive cells between the Blebbistatin-injected side and the control side is shown in a boxplot (The horizontal line indicates the median. Edges of boxes indicate the first and third quartiles. The cross indicates the mean, and whiskers indicate the minimum and maximum). n = 10 each. Statistical significance was analyzed with Student ***t***-test. * $p < 0.05$. **(I)** Expression pattern of ***Paraxis*** in 100 μM Blebbistatin-injected embryos at the mid-neurula stage (st. 15). Asterisks indicate the injected side. White dashed lines indicate the expressing region of ***Paraxis***. Scale bar, 500 μm. Ratios of phenotypes are summarized in a stacked bar graph on right. Numbers in the graph indicates numbers of embryos with each phenotype. Statistical significance was analyzed with Fisher's exact test. **(J)** Expression level (RT-qPCR) of ***Foxd3***, ***Slug***, ***Sox2***, and ***Krt12.4*** in Blebbistatin-injected embryos at the mid-neurula stage (st. 15). Expression level of ***Ef1α*** was used as an internal control. Replicates of the experiment: 3 (***Foxd3***, ***Slug***, ***Sox2***, ***Krt12.4*** (5 μM)), 4 (***Foxd3***, ***Slug*** (10 μM)), 3 (***Sox2***, ***Krt12.4*** (10 μM)), 9 (***Foxd3***, ***Slug*** (25 μM)), 3 (***Sox2***, ***Krt12.4*** (25 μM)), 4 (***Foxd3***, ***Slug*** (100 μM)), 3 (***Sox2***, ***Krt12.4*** (100 μM)) (> 5 embryos were used for each replicate). Statistical significance was analyzed with Student ***t***-test, adjusted by Holm method. * $p < 0.05$. **(K)** Bright field image of BB-injected embryos with hemisection. Black arrowhead indicates BB-injected region. White line indicates the midline. Black line indicates archenteron. Black dashed line indicated the boundary between NC and mesoderm. White dashed line indicates the boundary between NC and epidermis. Scale bar, 100 μm. Quantification of NC width is shown in a boxplot on right (The horizontal line indicates the median. Edges of boxes indicate the first and third quartiles. The cross indicates the mean, and whiskers indicate the minimum and maximum). n = 8. Statistical significance was analyzed with paired ***t***-test. **(L)** Expression level (RT-qPCR) of ***Foxd3***, ***Slug*** in Blebbistatin-treated embryos at the mid-neurula stage (st. 15). Expression level of ***Ef1α*** was used as an internal control. Replicates of the experiment: 3 (> 5 embryos were used for each replicate). Statistical significance was analyzed with Student ***t***-test, adjusted by Holm method. * $p < 0.05$.

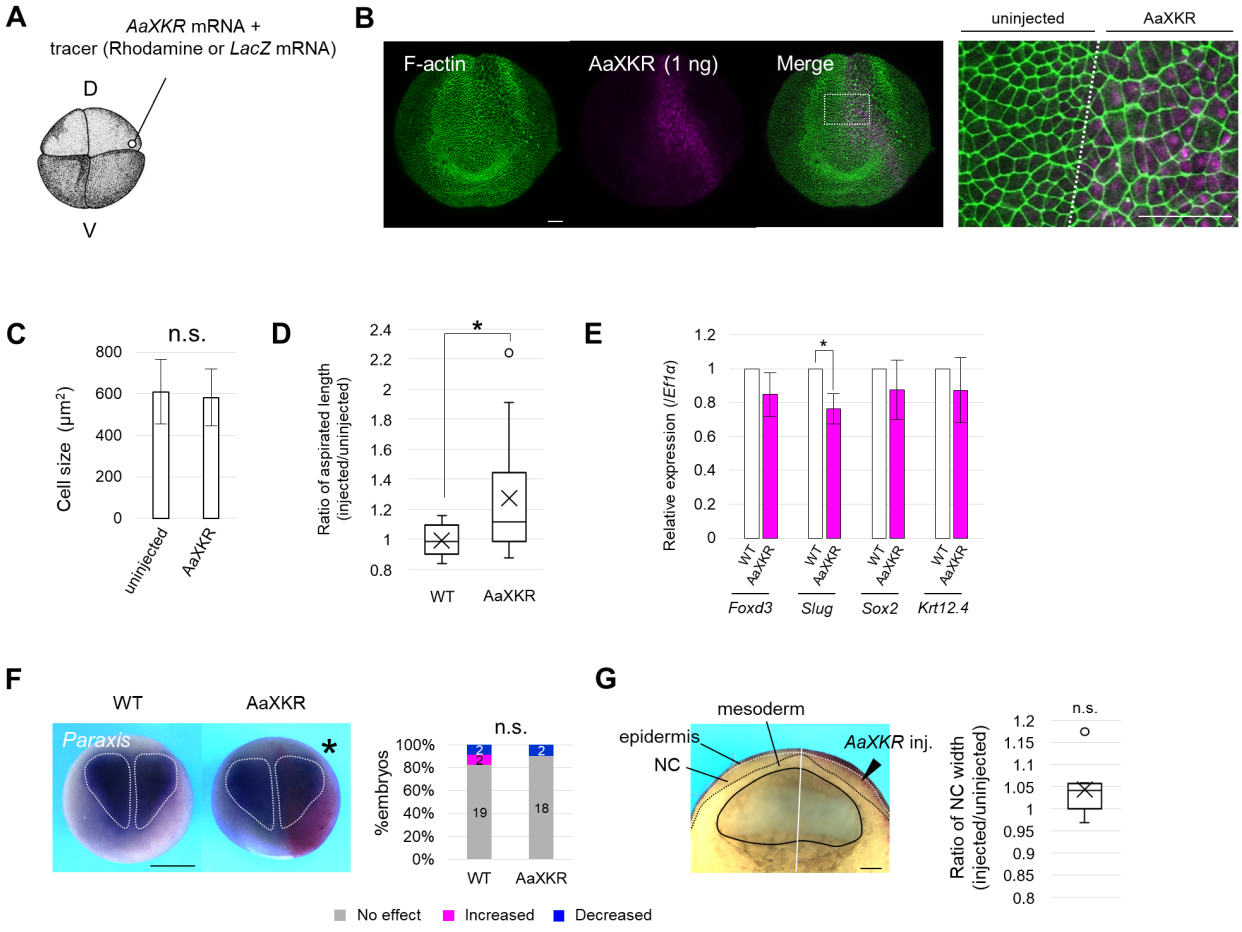

Fig. S6. Effect of AaXKR on mechanical properties and mesodermal position (Related to Fig. 4). (A) Schematic figure of *AaXKR* mRNA microinjection. D, dorsal blastomere, V, ventral blastomere. 400 pg ***AaXKR*** mRNA with Rhodamine (tracer) was injected into a dorsal blastomere at the 4- or 8-cell stage. **(B)** Fluorescent image of Phalloidin in embryo injected with 1 ng mRNA of ***AaXKR*** at the mid-neurula stage (st. 15). Green, F-actin (Phalloidin), Magenta, AaXKR (mRFP). White dashed boxes indicate enlarged regions on right. White dashed line indicates the boundary between ***AaXKR***-expressing cells and uninjected cells. Scale bar, 100 μm. **(C)** Statistical analysis of cell size in ***AaXKR***-expressing embryos at the mid-neurula stage (st. 15). n = 25 each. Statistical significance was analyzed with Student *t*-test. **(D)** Result of an aspiration assay in ***AaXKR***-expressing embryos at the early neurula stage (st. 13). Ratio of aspirated length of the injected side by that of the uninjected side was shown in a boxplot. n = 10 each. Statistical significance was analyzed with paired *t*-test. * $p < 0.05$. **(E)** Expression level of ***Foxd3***, ***Slug***, ***Sox2***, and ***Krt12.4*** in ***AaXKR***-expressing embryos at the mid-neurula stage (st. 15). Expression level of ***Ef1α*** was used as an internal control. Replicates of the experiment: 3 each (> 5 embryos were used for each replicate). Statistical significance was analyzed with Student *t*-test. * $p < 0.05$. **(F)** Expression pattern of ***Paraxis*** in AaXKR-expressing embryos at the mid-neurula stage (st. 15). Asterisk indicates the injected side. White dashed lines indicate the expressing region of ***Paraxis***. Scale bar, 500 μm. Ratios of phenotypes are summarized in stacked bar graphs. Numbers in the graph indicates numbers of embryos with each phenotype. Statistical significance was analyzed with Fisher's exact test. **(G)** Bright field image of ***AaXKR***-expressing embryos with hemisection. Black arrowhead indicates BB-injected region. White line indicates the midline. Black line indicates archenteron. Black dashed line indicated the boundary between NC and mesoderm. White dashed line indicates the boundary between NC and epidermis. Scale bar, 100 μm. Quantification of NC width is shown in a boxplot (The horizontal line indicates the median. Edges of boxes indicate the first and third quartiles. The cross indicates the mean, and whiskers indicate the minimum and maximum). n = 8. Statistical significance was analyzed with paired *t*-test.

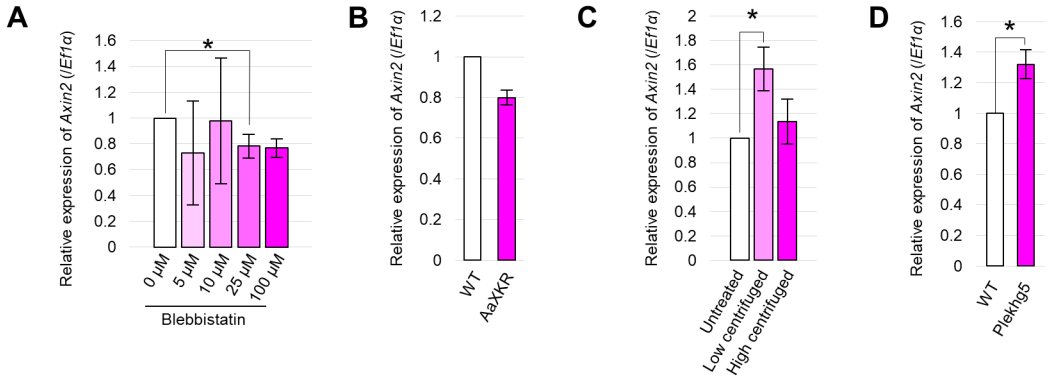

Fig. S7. Quantification of *Axin2* expression, a Wnt target gene (Related to Fig. 7). (A) *Axin2* expression (RT-qPCR) in Blebbistatin-injected embryos at the mid-neurula stage (st. 15). Expression level of ***Ef1α*** was used as an internal control. Replicates of the experiment: 4 (5 µM, 10 µM), 5 (25 µM), 3 (100 µM) (> 5 embryos were used for each replicate). Statistical significance was analyzed with Student ***t***-test. Each ***p*** value is corrected using Holm method. * $p < 0.05$. **(B)** *Axin2* expression in ***AaXKR***-expressing embryos at the mid-neurula stage (st. 15). Expression level of ***Ef1α*** was used as an internal control. Replicates of the experiment: 3 (> 5 embryos were used for each replicate). **(C)** *Axin2* expression in centrifuged embryos (low centrifugation, 200 × g; high, 450 × g) at the mid-neurula stage (st. 15). Expression level of ***Ef1α*** was used as an internal control. Replicates of the experiment: 3 (Low), 6 (High) (> 5 embryos were used for each replicate). Statistical significance was analyzed with Student ***t***-test. Each ***p*** value is corrected using Holm method. * $p < 0.05$. **(D)** *Axin2* expression in ***Plekhg5***-expressing embryos at the mid-neurula stage (st. 15). Expression level of ***Ef1α*** was used as an internal control. Replicates of the experiment: 3 (> 5 embryos were used for each replicate). Statistical significance was analyzed with Student ***t***-test. * $p < 0.05$.