

Fig. S1. The effect of laser ablation on tissue and cell morphology (Related to Figure 2)

(A) Cross-sectional view of the embryo ablated perpendicular to the AP body axis. Arrowhead indicates the ablation site. NE, neuroectoderm.

(B and C) Images of neuroectodermal tissue ablated perpendicular (B) or parallel (C) to the AP body axis. Lower panels indicate aspect ratio of each cell. Cyan line indicates ablation site. Rectangle indicates anterior/posterior (B) or left/right (C) areas of the cut, which were used for quantifying cell aspect ratio.

(D) Change of cell aspect ratio before/after tissue ablation. Embryo number $n=7$ for each condition. The data were obtained from 6 independent experiments. Statistical significance was tested by two-tailed Mann-Whitney U-test.

A, anterior; P, posterior; bp, blastopore. ** = $p < 0.01$. Scale bars, 100 μm .

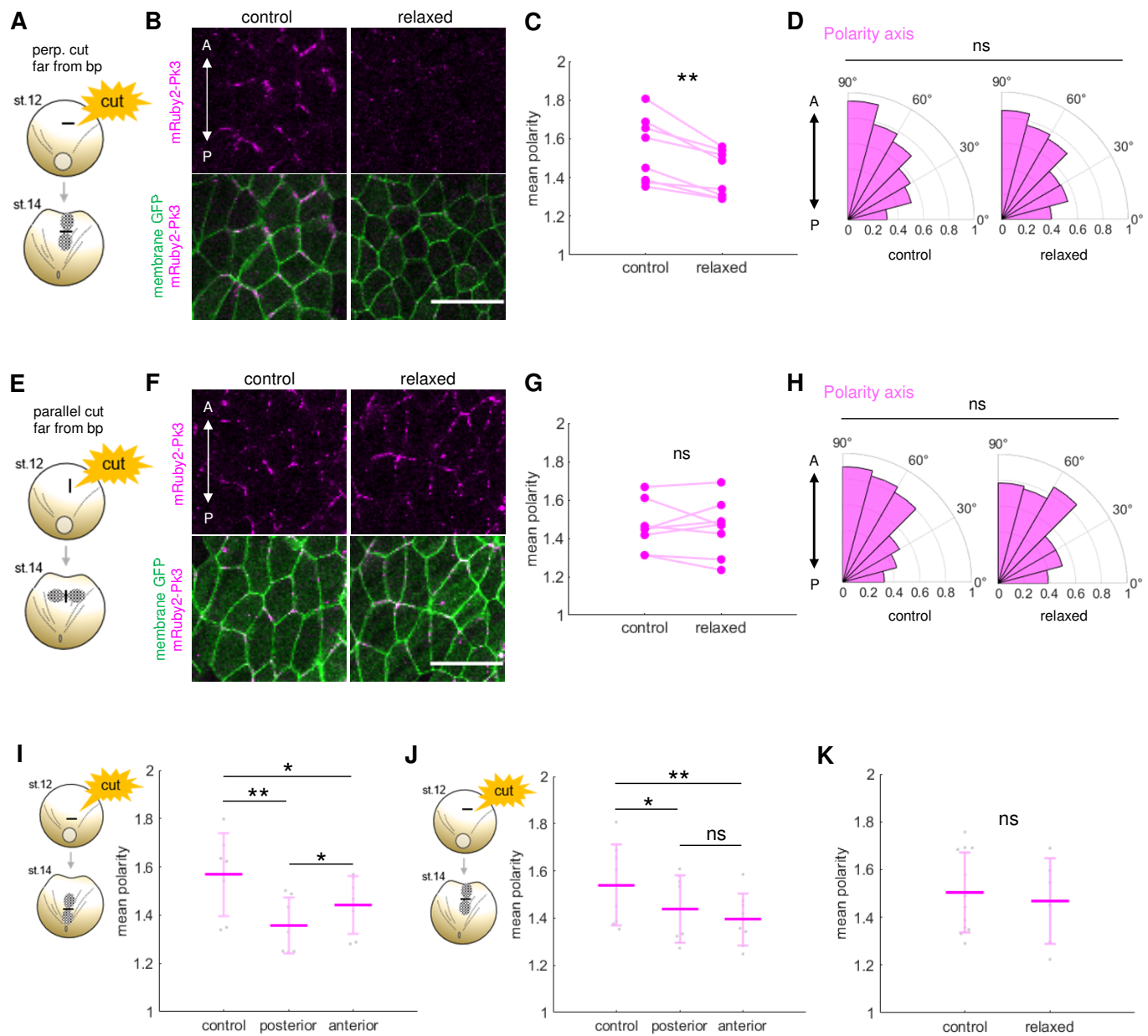


Fig. S2. The effect of laser ablation on polarity formation (Related to Figure 2)

(A and E) Schematic images of laser ablation experiments.

(B and F) Fluorescence images of control and relaxed areas in laser ablation experiments. The 'relaxed' image in (B) shows the posterior side of the cut. Scale bars, 50 μ m.

(C and G) Plots of the mean polarity. Lines connect data from the same embryo.

(D and H) Plots of the polarity axis. The area of each bin represents the relative number of observations. Embryo number $n = 8$ (A-D) and $n = 8$ (E-H).

(I and J) Plots of the mean polarity when the tissue was cut perpendicular to the AP body axis in a region close to (I) or far from (J) the blastopore. Relaxed region was separated into anterior and posterior regions to the cut. Bars show mean \pm s.d.

(K) The mean polarity of the region more than 10 cells away from the cut when the tissue was cut perpendicular to the AP body axis. The data in (I-K) came from the same embryos as in Figures 2D-2G and 2H-2K.

The data were obtained from 4 independent experiments. A, anterior; P, posterior; bp, blastopore. Statistical significance was tested by Wilcoxon signed rank test in (C and G), two-tailed Mann-Whitney U-test in (I-K) and Kuiper test in (D and H). * = $p < 0.05$, ** = $p < 0.01$.

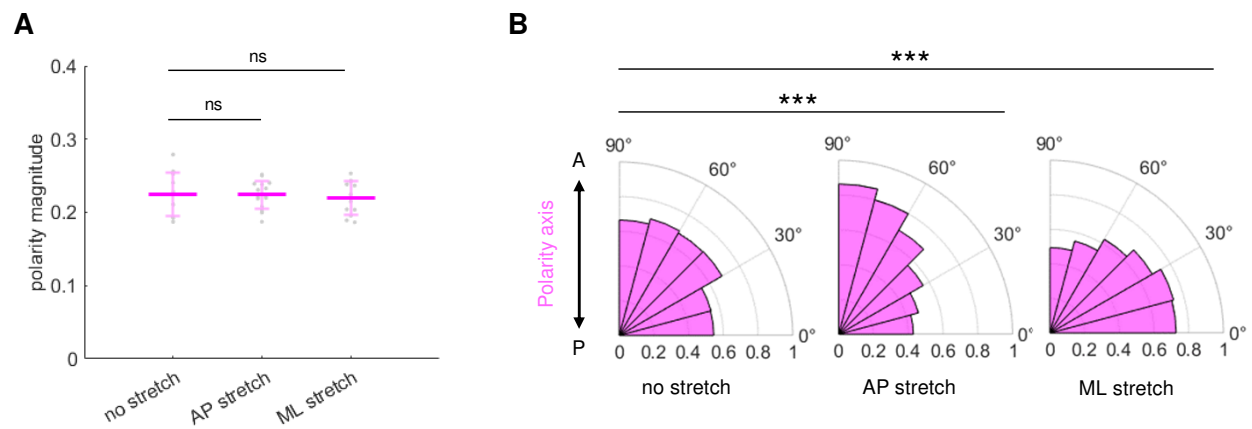


Fig. S3. Polarity analysis by PCA method for the data from explant stretching experiments (Related to Figure 3)

(A) Plots of the polarity magnitude, which corresponds to the mean polarity. Bars show mean \pm s.d.
(B) Plots of the polarity axis. The area of each bin represents the relative number of observations. The same embryos as in Figure 3 were used for the analysis. Statistical significance was tested by two-tailed Mann-Whitney U-test in (A) and by Kuiper test in (B). *** = $p < 0.001$.

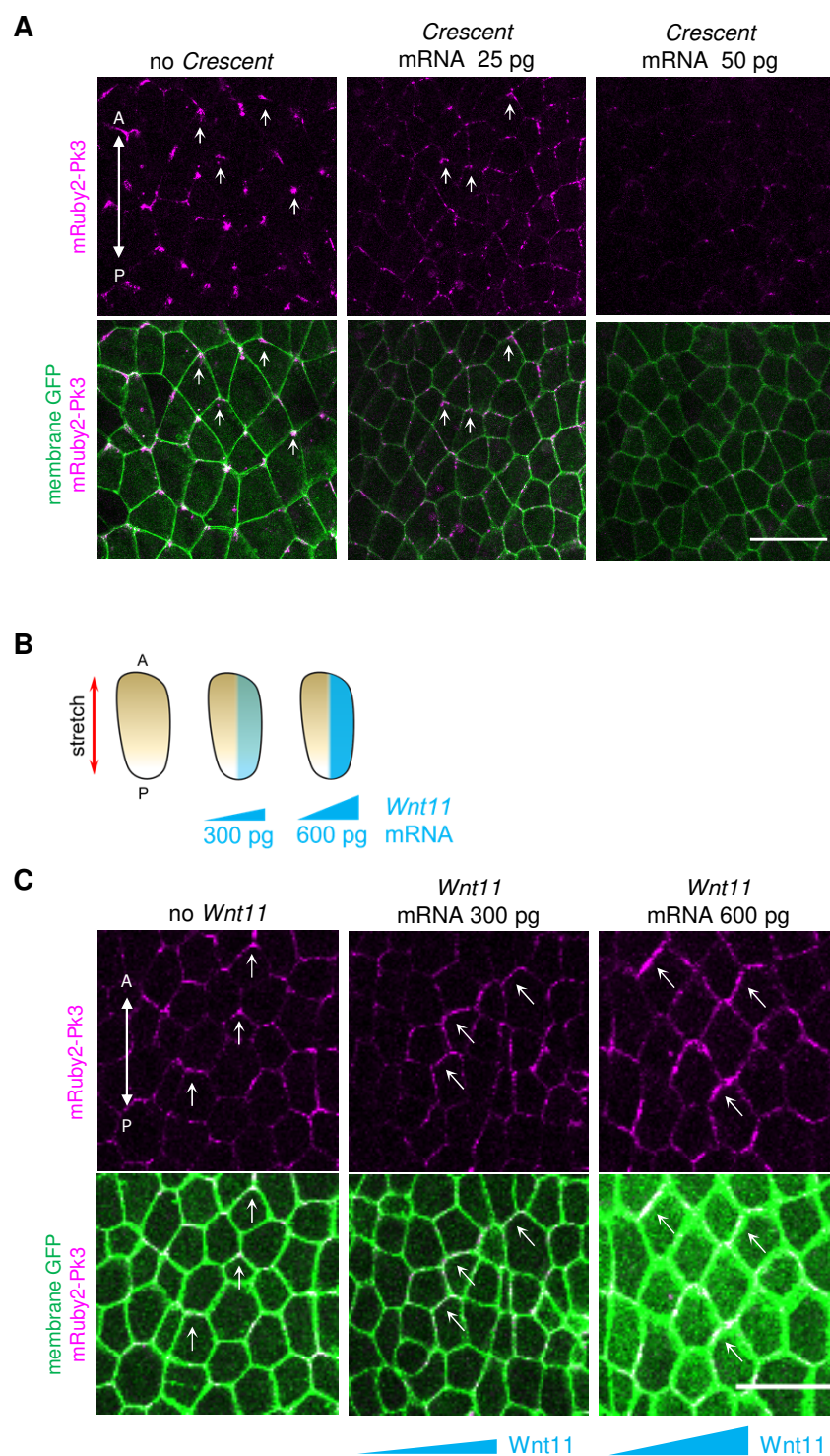


Fig. S4. Crescent or Wnt11 overexpression (Related to Figure 4)

(A) Fluorescence images of embryos overexpressed with Crescent. Arrows indicate the accumulation of mRuby2-Prickle3 at the anterior side of the cell.

(B) Schematic image of stretching experiment. Wnt11 was overexpressed in one side of ventral ectodermal tissue. All the explants were stretched in the AP direction.

(C) Fluorescence images of explants stretched in the method described in (B). Arrows indicate the accumulation of mRuby2-Prickle3. Wnt ligand source is on the right side of the images.

The data were obtained from 2 (A) or 3 (C) independent experiments. A, anterior; P, posterior. Scale bars, 50 μ m.

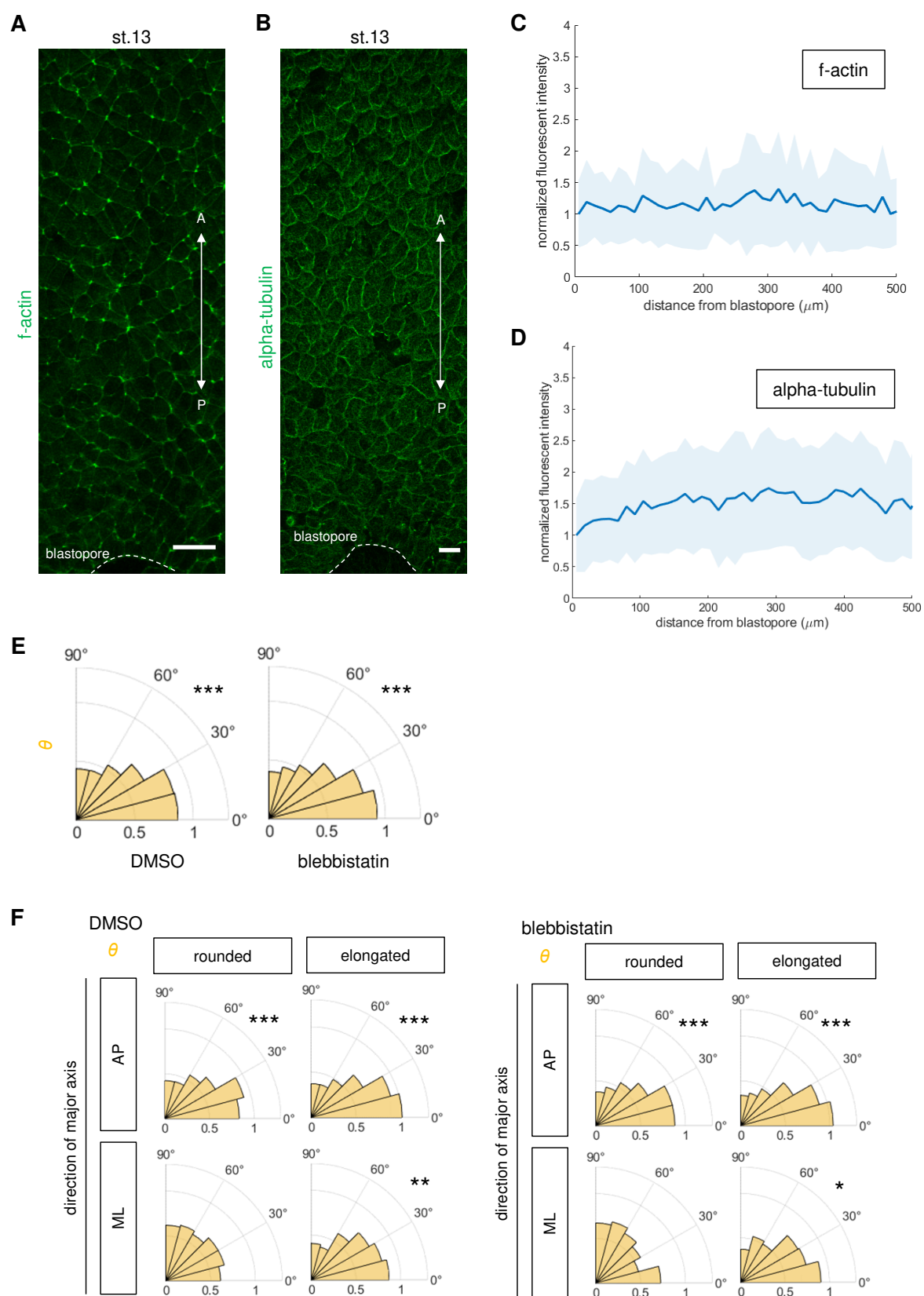


Fig. S5. Cytoskeleton distribution in WT embryos and polarity analysis for blebbistatin-treated embryos (Related to Figure 5)

(A and B) Fluorescence images of stage 13 embryo stained for F-actin (A) or microtubule (B).

(C and D) Plots of fluorescence intensity in (A) or (B) normalized by the average fluorescence intensity of the most posterior region. Line and band show mean \pm s.d. The plots are representative of 6 embryos (C) or 9 embryos (D).

(E) Plots of θ (See Figure 7A) for DMSO- or blebbistatin- treated embryos. Asterisks indicate significant difference from uniform distribution. Embryo number $n = 11$ (DMSO) and $n = 12$ (blebbistatin).

(F) Plots of θ for DMSO- or blebbistatin-treated embryos sorted by cell shape. A cell was sorted as rounded when its aspect ratio was smaller than the median of the distribution ($=1.50$ or 1.52 for DMSO or blebbistatin, respectively). Asterisks indicate significant difference from uniform distribution.

The data were obtained from 3 (A, C), 5 (B, D) or 3 (E-F) independent experiments. Statistical significance was tested by Kuiper test in (E and F). * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

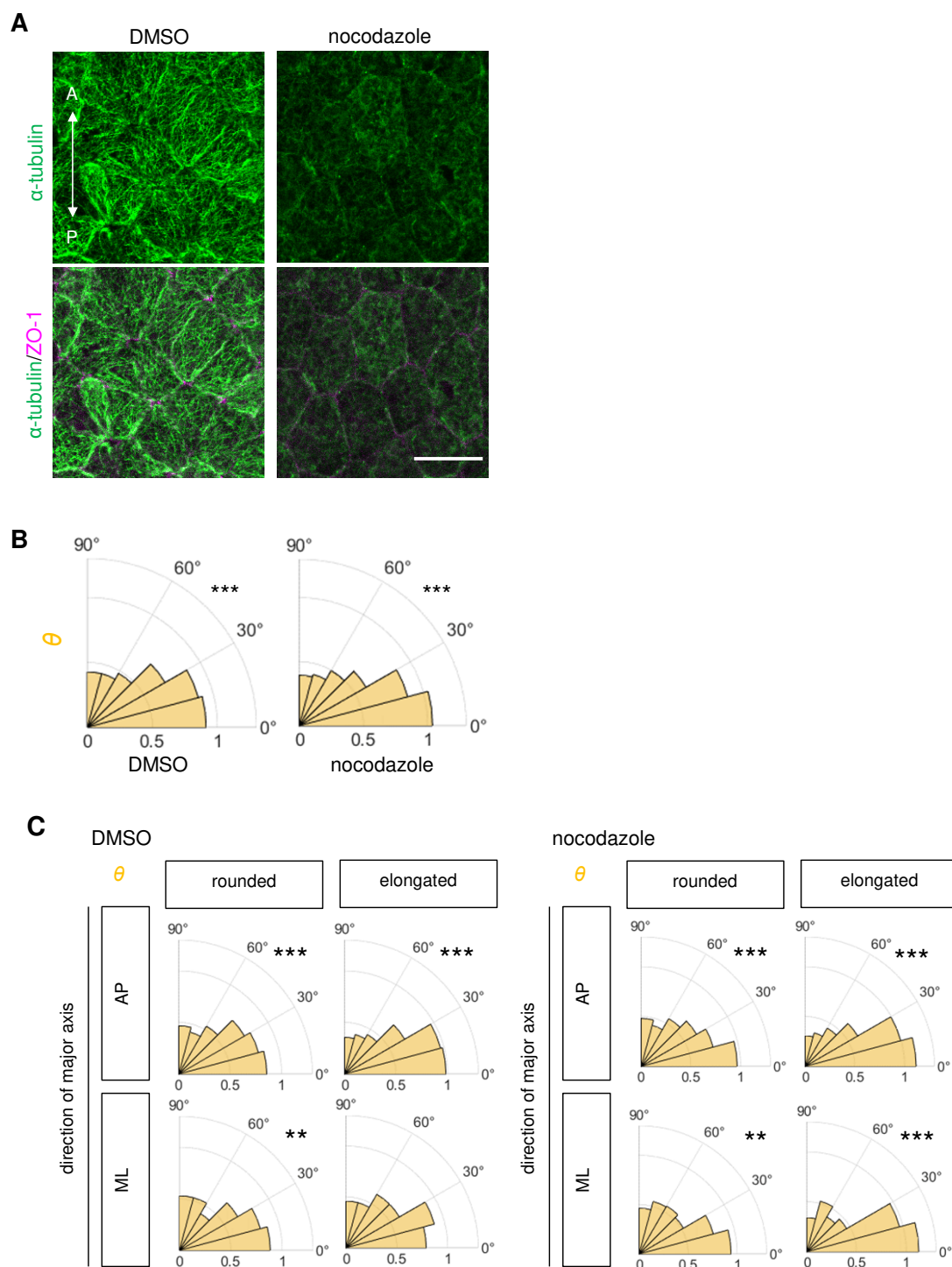


Fig. S6. Nocodazole treatment experiments (Related to Figure 6)

(A) Fluorescence images of immunostained embryos treated with DMSO or nocodazole. Scale bar, 50 μ m.

(B) Plots of θ (See Figure 7A) for DMSO- or nocodazole- treated embryos. Embryo number $n = 17$ (DMSO) and $n = 15$ (nocodazole).

(C) Plots of θ for DMSO- or nocodazole-treated embryos sorted by cell shape. A cell was sorted as rounded when its aspect ratio was smaller than the median of the distribution ($=1.52$ or 1.50 for DMSO or nocodazole, respectively).

The data were obtained from 2 (A) or 3 (B-C) independent experiments. Asterisks indicate significant difference from uniform distribution. Statistical significance was tested by Kuiper test. ** = $p < 0.01$, *** = $p < 0.001$.

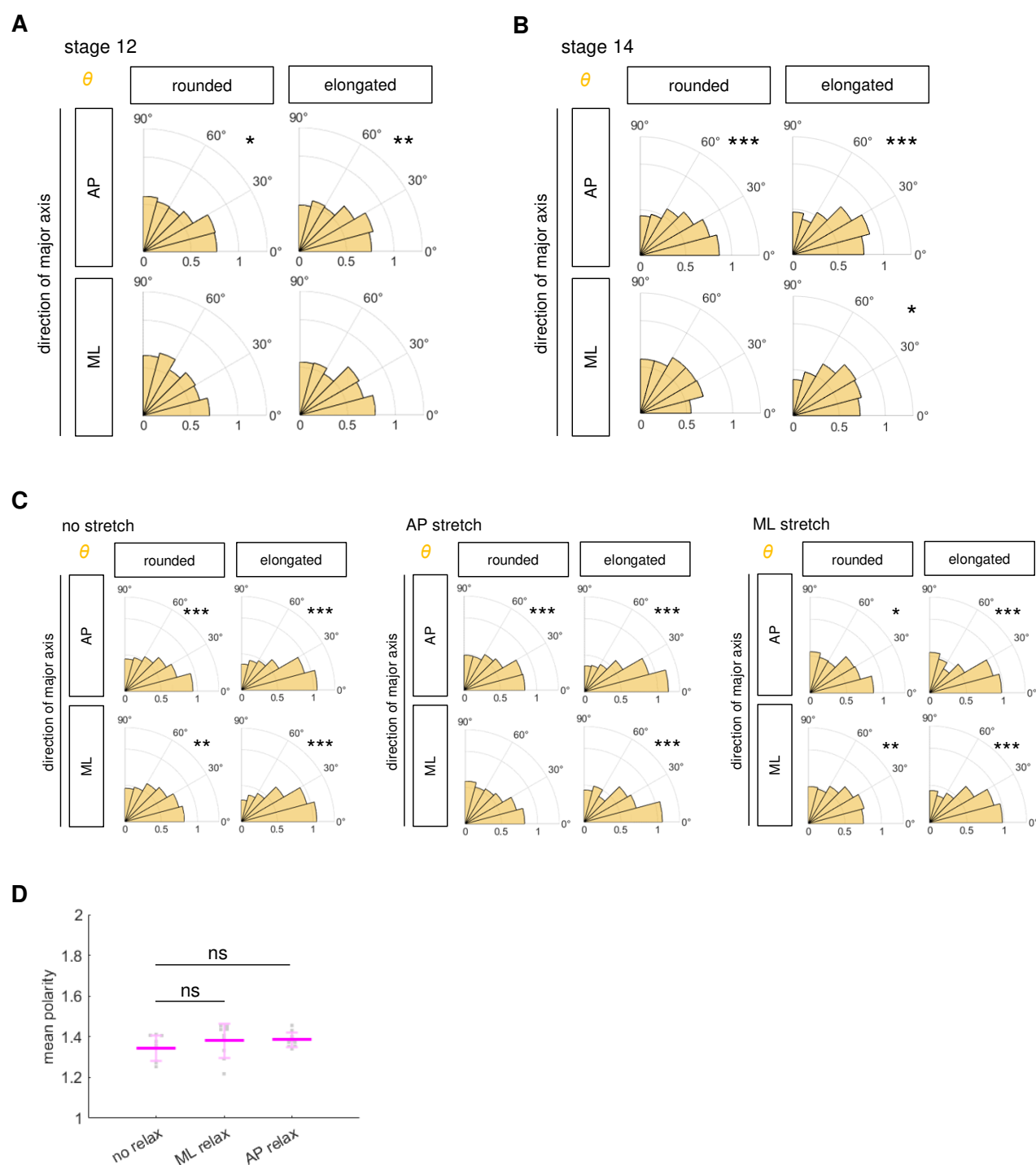


Fig. S7. The coincidence between the major axis and the polarity axis is clearer in elongated cells than in rounded cells (Related to Figure 7)

(A and B) Plots of θ for stage 12 (A) or 14 (B) embryos. Data came from the same embryos as Figure 7B. A cell was sorted as rounded when its aspect ratio was smaller than the median of the distribution ($=1.44$ or 1.55 for stage 12 or 14, respectively). Embryo number $n = 11$ (A) and $n = 11$ (B).

(C) Plots of θ . Data came from the same explants as Figures 7D and 7E. A cell was sorted as rounded when its aspect ratio was smaller than the median of the distribution ($=1.29$, 1.32 or 1.33 for no, AP or ML stretch, respectively). Explant number $n = 11$ (no stretch), $n = 15$ (AP stretch) and $n = 12$ (ML stretch).

(D) Plots of the mean polarity for explant relaxing experiment (Figure 7F-7I). Bars show mean \pm s.d.

The data were obtained from 3 (A-B) 3 (C) or 2 (D) independent experiments. Asterisks indicate significant difference from uniform distribution. Statistical significance was tested by Kuiper test in (A-C) and two-tailed Mann-Whitney U-test in (D). * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.