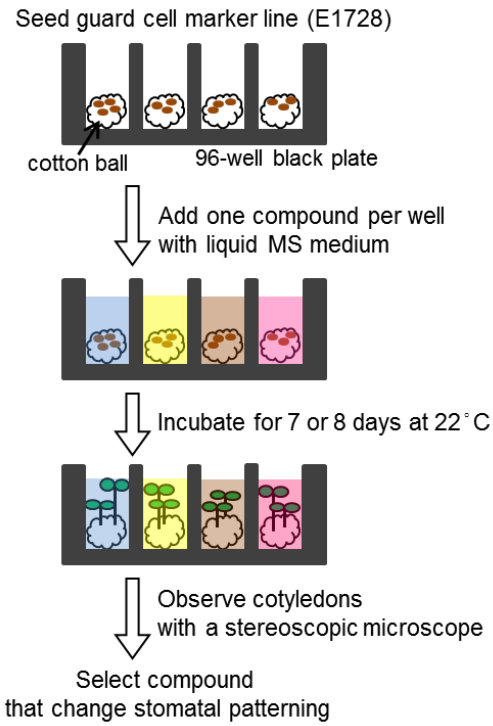


Fig. S1. The high-throughput chemical screening system.
Schematic representation of the high-throughput screening system used to identify compounds that affect stomatal patterning.

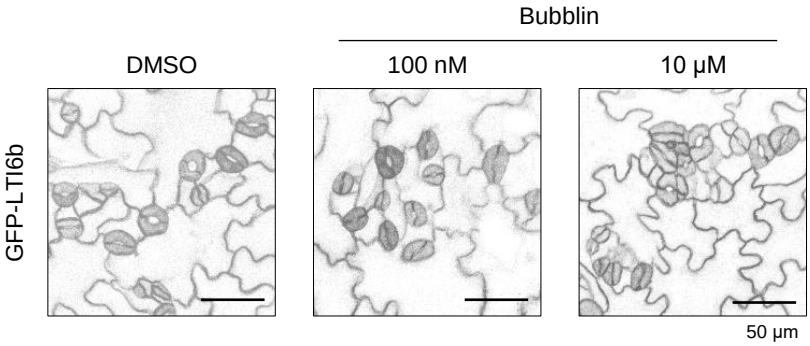


Fig. S2. 100 nM bubblin does not affect stomatal development. Confocal images of abaxial cotyledon epidermis from 10-day-old GFP-LTI6b seedlings treated with DMSO only as a control, 100 nM bubblin, or 10 µM bubblin.

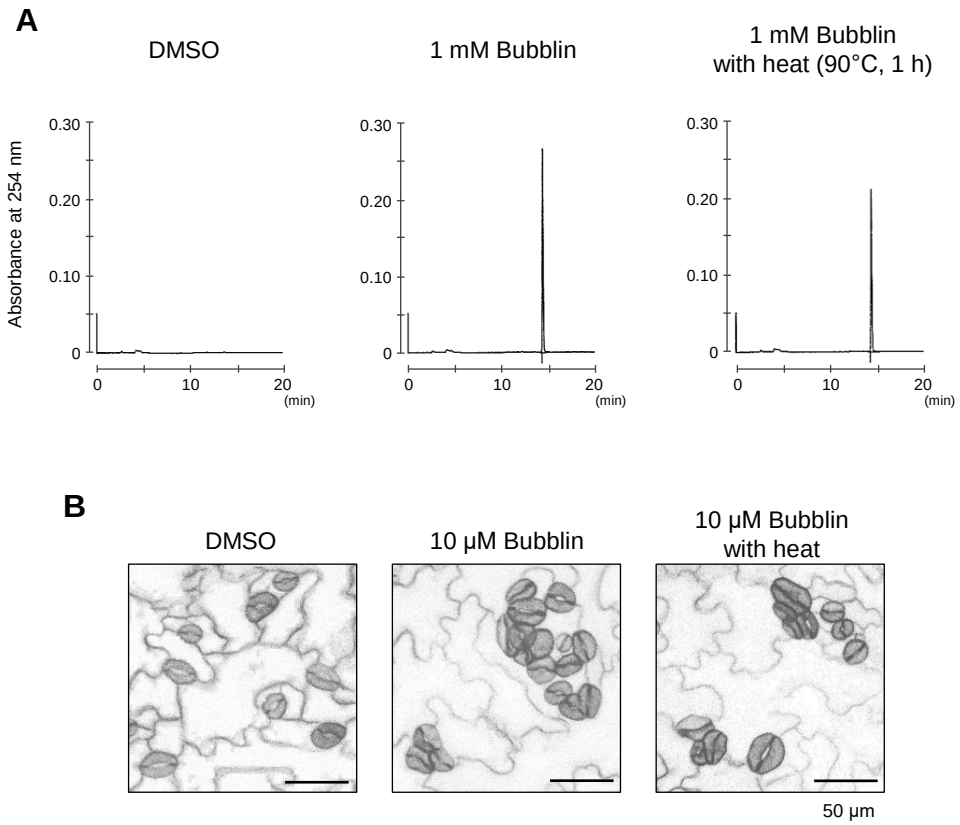


Fig. S3. Bubblin is not degraded and retains stomatal clustering activity after heat treatment. (A) Analytical HPLC profiles of DMSO, 1 mM bubblin, and 1 mM bubblin heated at 90° C for 1 h. (B) Confocal images of abaxial cotyledon epidermis from 10-day-old GFP-LTI6b seedlings treated with DMSO, 10 µM bubblin, or 10 µM bubblin heated at 90° C for 1 h.

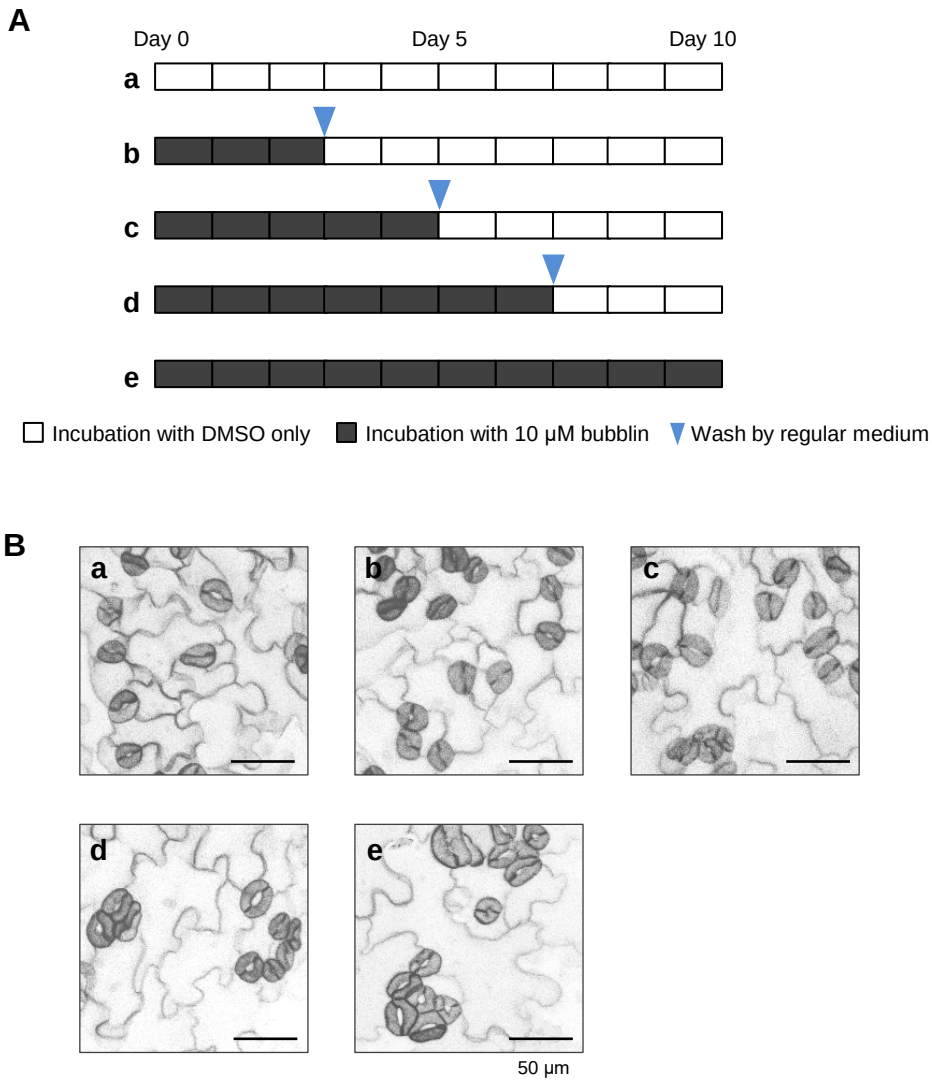


Fig. S4. Time-dependent effect of bubblin. (A) Rescue treatments used for data presented in (B). Bubblin-treated seedlings were transferred to regular medium at the indicated time points. Black boxes indicate the presence of bubblin, white boxes indicate the number of days in regular medium, and blue arrow heads indicate the day when seedlings were washed and transferred. (B) Confocal images of abaxial cotyledon epidermis from 10-day-old GFP-LTI6b seedlings treated with 10 µM bubblin for each time period indicated in (A).

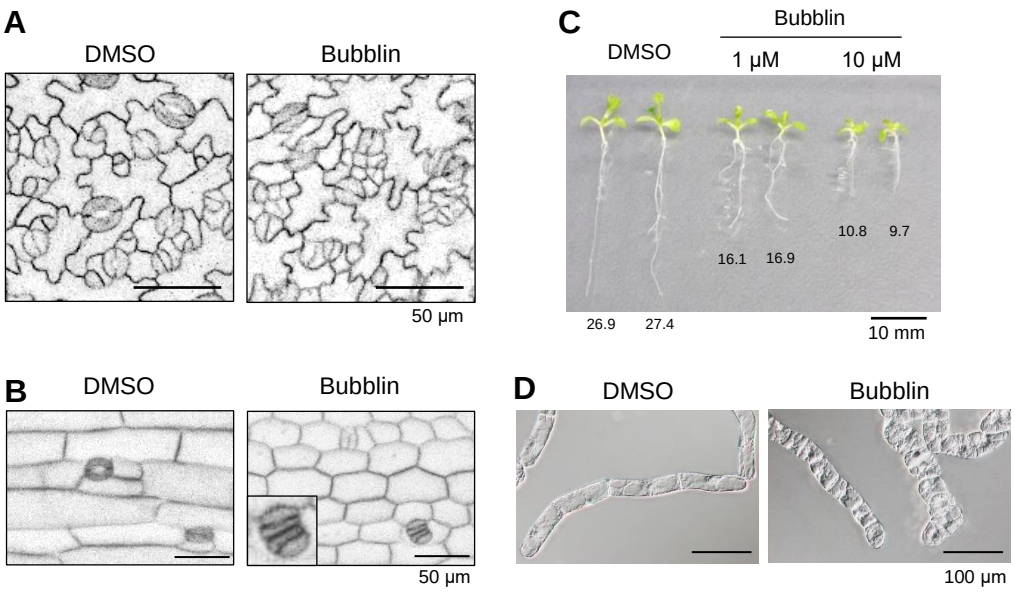


Fig. S5. Bubblin induces stomatal clustering in various tissues, and broadly affects cell size. (A) Confocal images of first leaves from 10-day-old GFP-LTI6b seedlings treated with DMSO or 10 µM bubblin. (B) Confocal images of hypocotyls from 10-day-old GFP-LTI6b seedlings treated with DMSO or 10 µM bubblin. A inset shows a magnified stomatal cluster. (C) Whole 10-day-old plants treated with DMSO, 1 µM bubblin, or 10 µM bubblin. Root lengths (mm) are indicated under each seedling. (D) Tobacco BY-2 cultured cells treated with DMSO or 10 µM bubblin for 3 days.

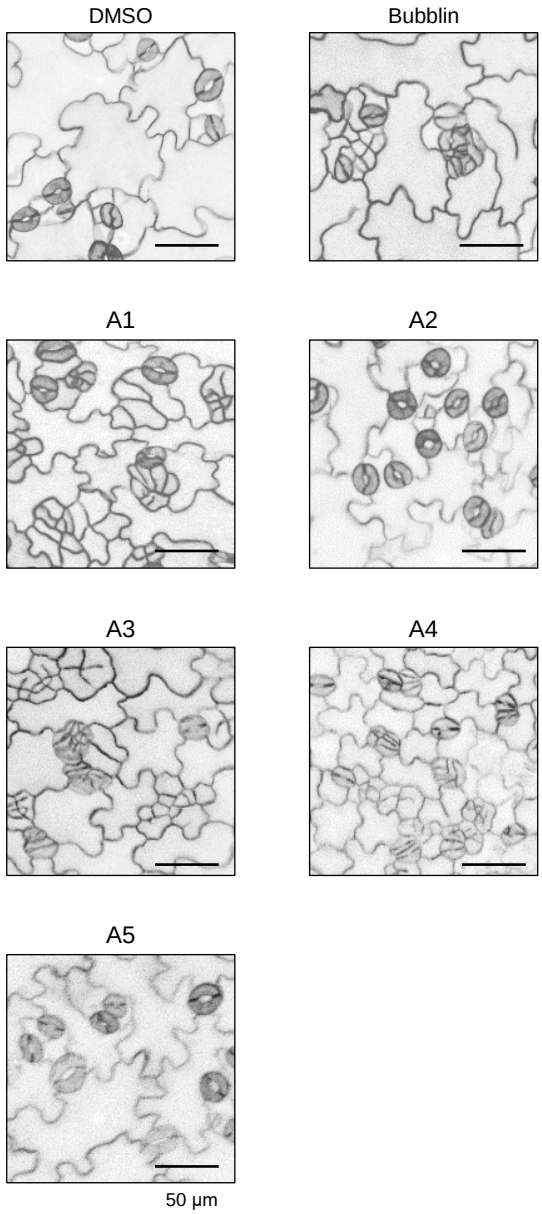


Fig. S6. Effect of 50 µM bubblin and five derivatives on stomatal patterning. Confocal images of abaxial cotyledon epidermis from 10-day-old GFP-LTI6b seedlings treated with the indicated compounds at 50 µM.

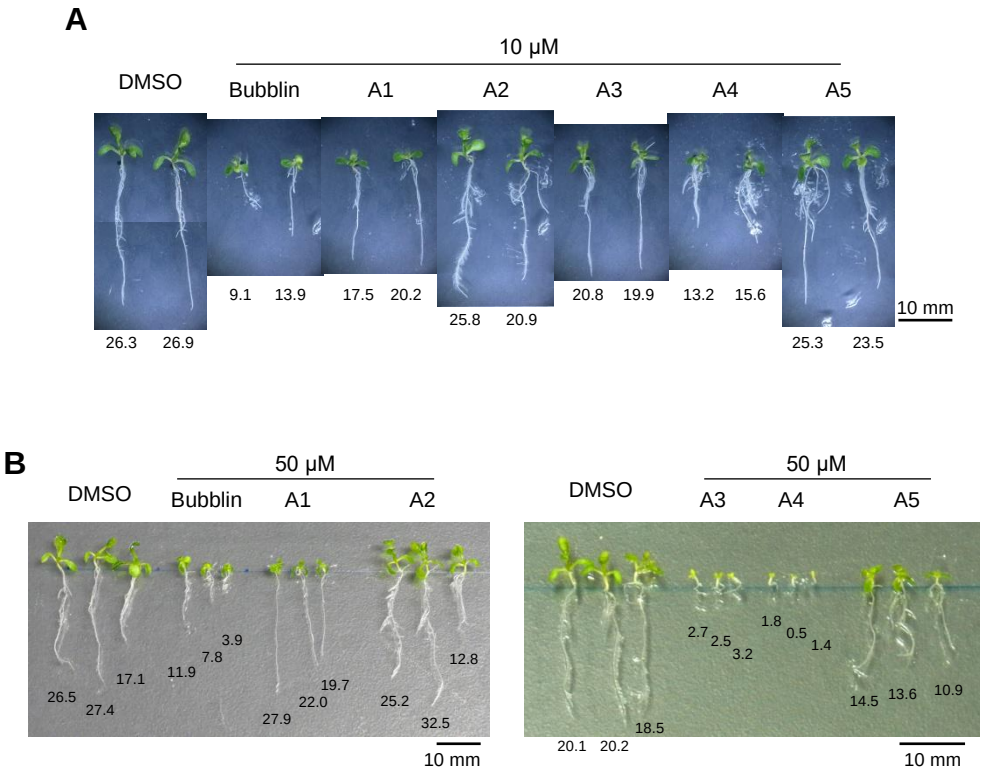


Fig. S7. The effect of five bubblin derivatives on seedling growth. Whole 10-day-old plants treated with the derivatives A1 to A5 at 10 μM (A) or 50 μM (B). Root lengths (mm) are indicated under each seedling.

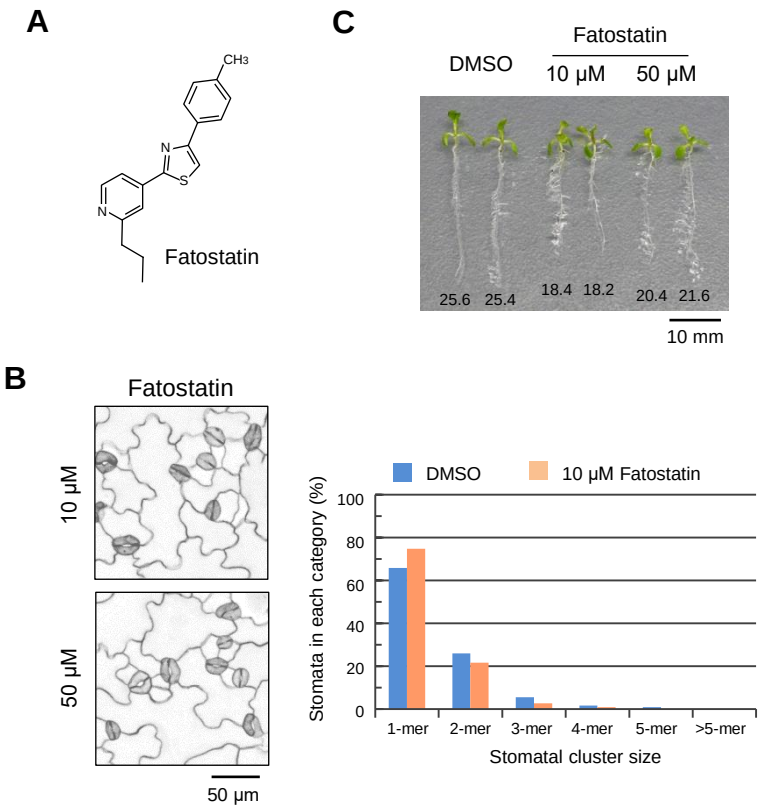


Fig. S8. Fatostatin has no effect on stomatal development or seedling growth. (A) Chemical structure of fatostatin. (B) Confocal images of abaxial cotyledon epidermis from 10-day-old GFP-LTI6b seedlings treated with 10 μM or 50 μM fatostatin. Quantitative analysis indicates that fatostatin has no stomatal clustering activity. The percentage of stomata in each cluster size class is shown. Stomata (n = 485 (DMSO), n = 443 (10 μM fatostatin)) from 10 independent observations were counted for each treatment. (C) Whole 10-day-old plants treated with DMSO, 10 μM fatostatin, or 50 μM fatostatin. Root lengths (mm) are indicated under each seedling.

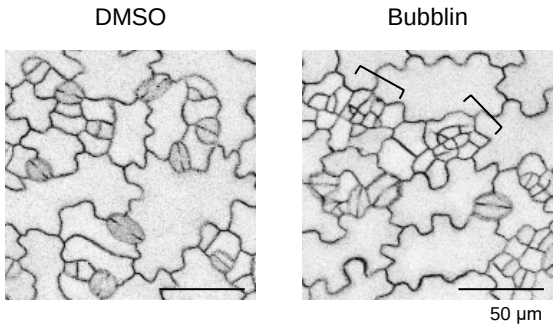


Fig. S9. Bubblin generates clustered small cells. Confocal images of abaxial cotyledon epidermis from 7-day-old GFP-LTI6b seedlings treated with DMSO or 10 µM bubblin. Bubblin-induced small cell clusters are indicated by brackets.

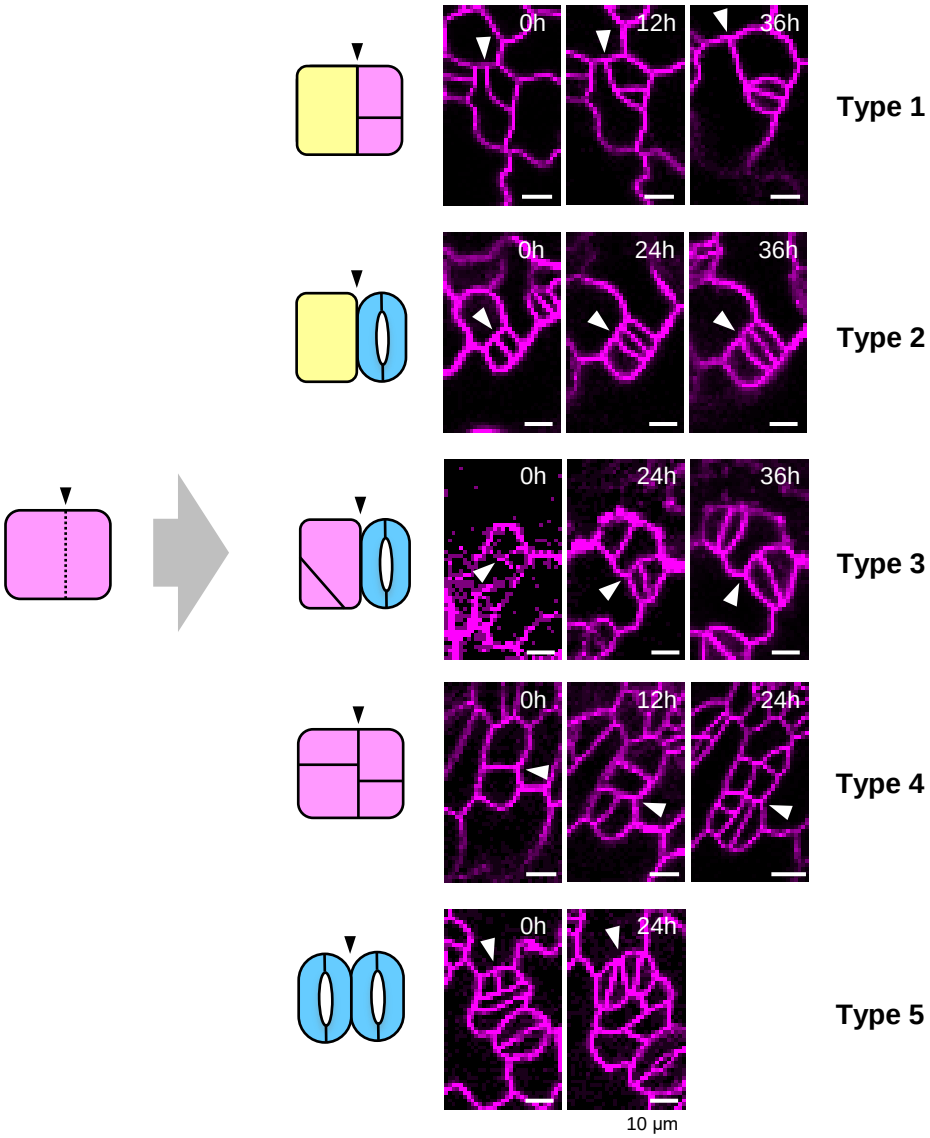


Fig. S10. Five types of divisions exhibited by stomatal lineage cells. Confocal images showing representative divisions categorized into types 1 to 5. Arrowheads indicate the planes of division that were categorized. Divisions of types 1 to 3 produce two daughter cells with different cell fates: type 1 produces a continuously dividing stomatal stem cell (magenta) and an expanding pavement cell (yellow), type 2 results in a pavement cell and a stoma that is composed of a pair of guard cells (blue), and type 3 generates a stoma and a stomatal stem cell. Divisions that produce daughter cells with the same cell fate are categorized into type 4 and type 5: type 4 divisions result in adjacent stomatal stem cells and type 5 divisions result in paired stomata.

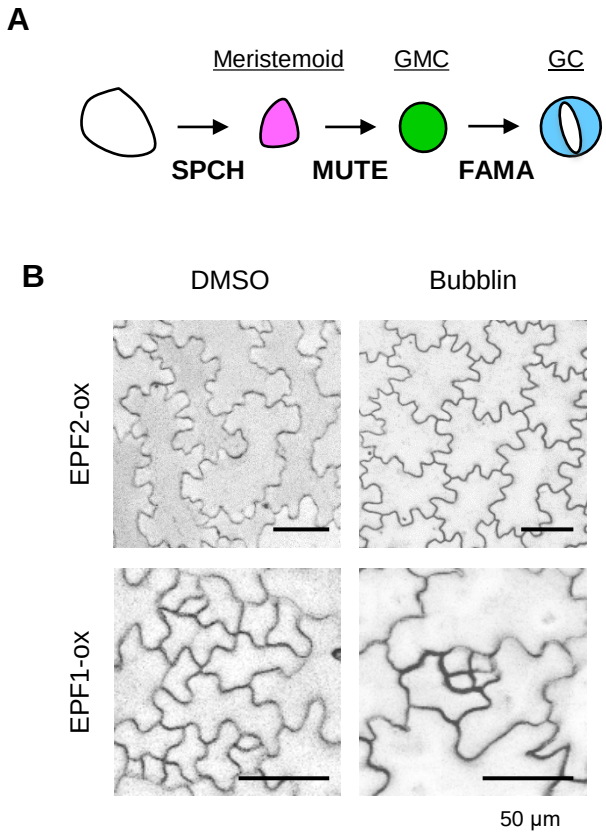


Fig. S11. Effect of bubblin on *EPF*-overexpressors. (A) Three paralogous bHLH transcription factors (SPCH, MUTE, and FAMA) regulating cell-fate transition during stomatal development. (B) Confocal images of abaxial cotyledon epidermis from 10-day-old EPF2-ox seedlings and EPF1-ox seedlings treated with DMSO or 10 µM bubblin.

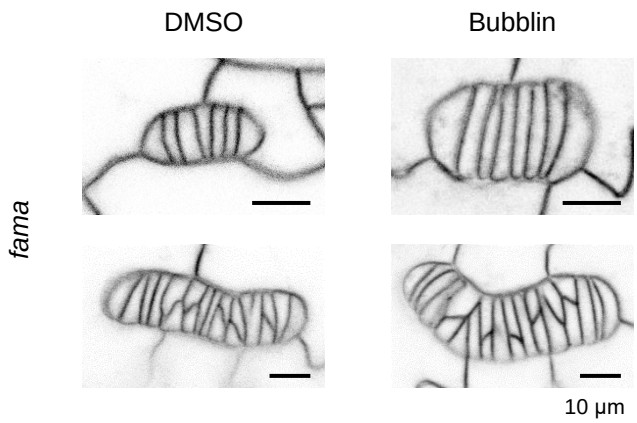


Fig. S12. Bubblin has no effect on the cells within *fama* tumors. High-resolution images of *fama* tumors in 7-day-old cotyledon epidermis treated with DMSO or 10 μM bubblin. There were no significant differences in their dividing patterns between bubblin-treated and mock-treated epidermis (upper). Some excessive divisions in tumor cells were frequently observed in both conditions (lower). Cell shape was visualized by FM4-64 dye staining.

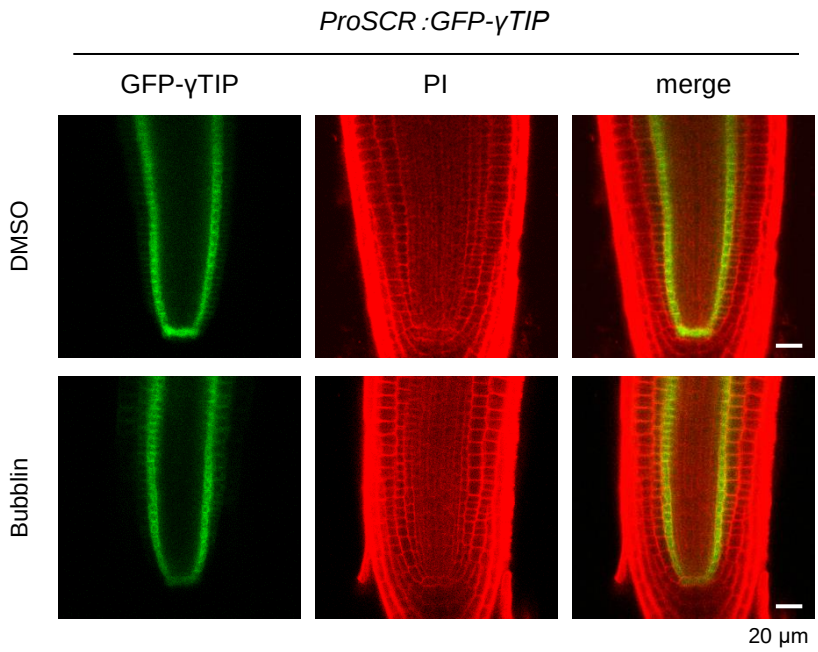


Fig. S13. Bubblin has no effect on asymmetric cell divisions in the root apical meristem. Confocal images of root tip from 8-day-old *ProSCR:GFP-γTIP* seedlings treated with DMSO or 10 μM bubblin. Endodermis is labeled by GFP. Cell shapes were visualized by PI staining.

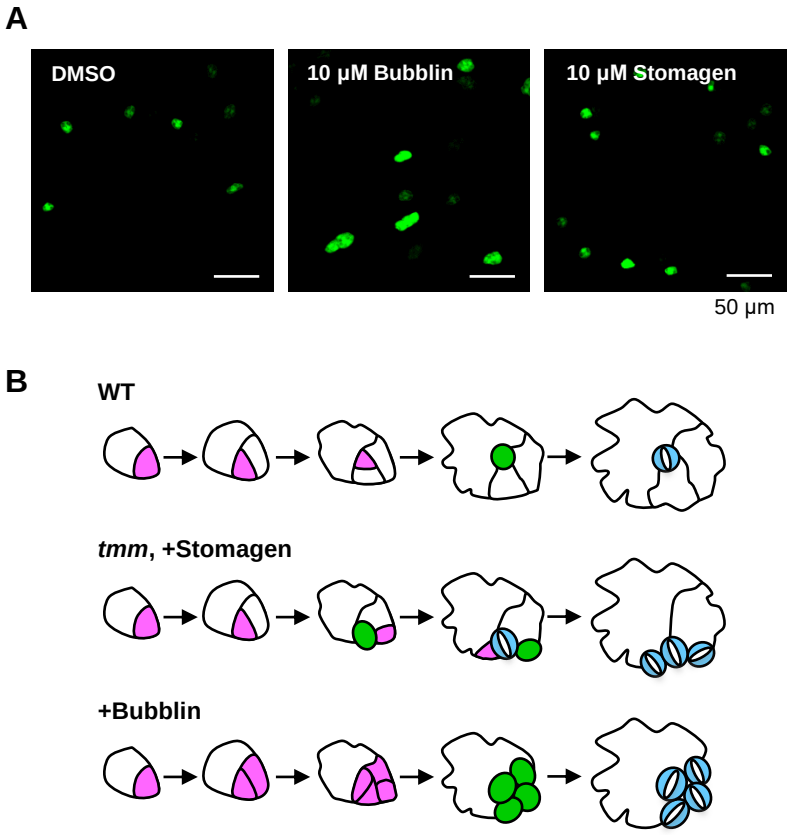


Fig. S14. Effect of bubblin on extrinsic mechanisms of stomatal patterning. (A) Low-magnification confocal images of adaxial cotyledon epidermis from 6-day-old *ProSDD1:GFP* seedlings treated with DMSO, 10 μM bubblin, or 10 μM stomagen. (B) Schematic models of stomatal development in three conditions (wild-type (WT), loss of TMM or stomagen treatment, and bubblin treatment).

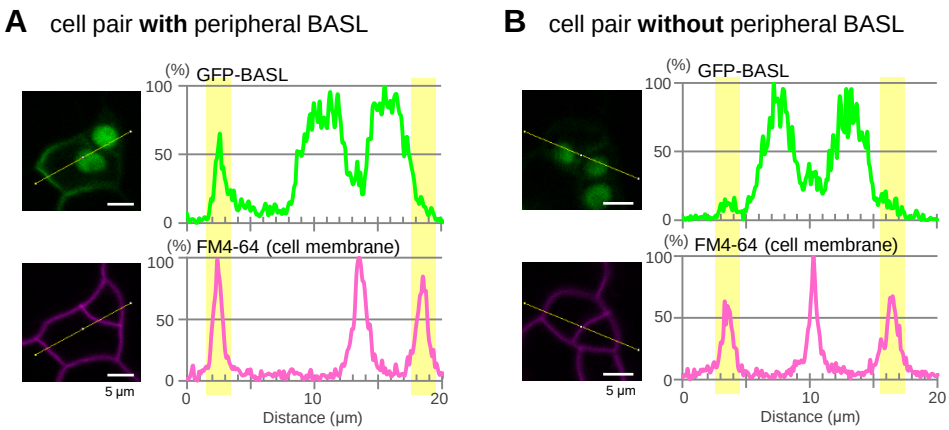


Fig. S15. The divided cell with or without peripheral BASL. (A, B) Left: confocal images of adaxial cotyledon epidermis from 6-day-old and 7-day-old *ProBASL:GFP-BASL* treated with DMSO (A) and with 10 µM bubblin (B), respectively. Cell shape was visualized by FM4-64 dye staining (magenta). Right: relative intensities of GFP-BASL and FM4-64 (cell membrane) were plotted along the lines indicated in the confocal images, with maximum luminance value set to 100%. Yellow bands highlight two distal cell membranes from the division plane between two daughter cells. The cell pair displayed in (A) maintains GFP-BASL signals in the cell periphery, but that in (B) does not maintain GFP-BASL signals in the cell periphery, and loses polarity.

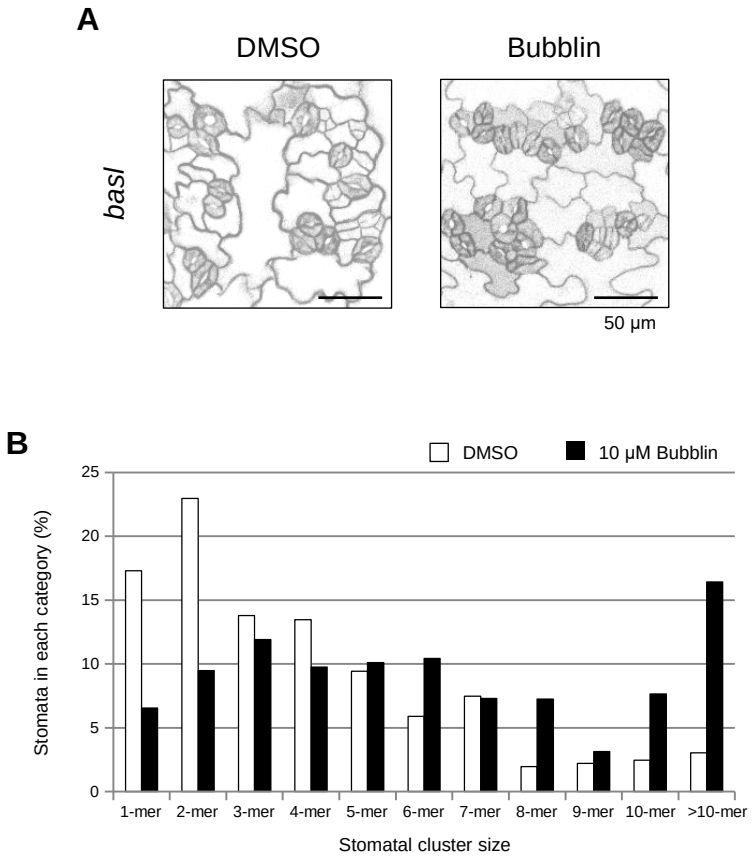
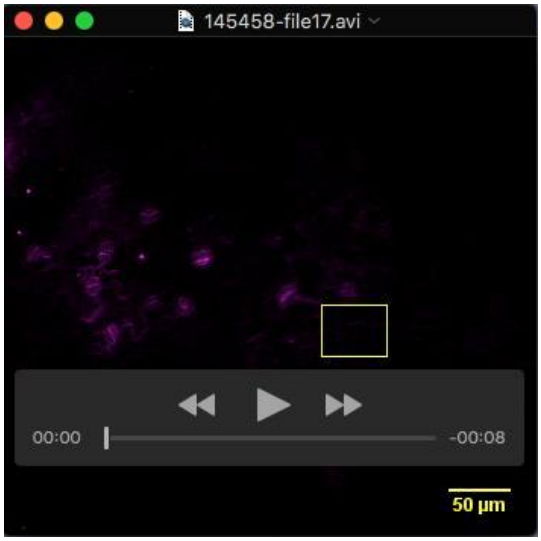
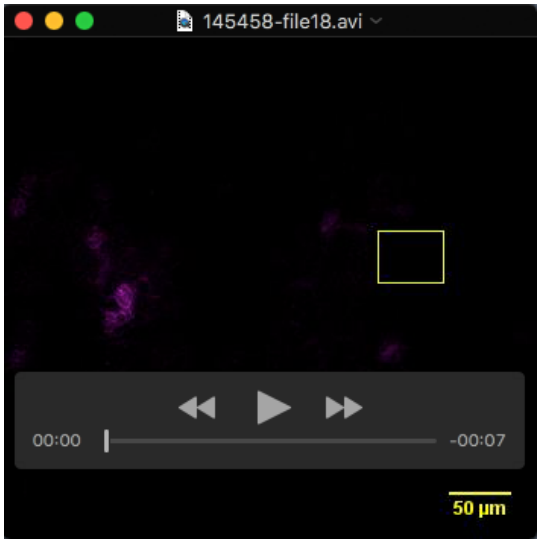


Fig. S16. Bubblin enhances the stomatal clustering phenotype in *basl*. (A) Confocal images of abaxial cotyledon epidermis from 8-day-old *basl* treated with DMSO or 10 µM bubblin. (B) Quantitative analysis of the stomatal clustering displayed in (A). The percentage of stomata in each cluster size class is shown. Stomata (n = 1219 (DMSO), n = 1436 (10 µM bubblin)) from 16 independent observations were counted for each treatment.

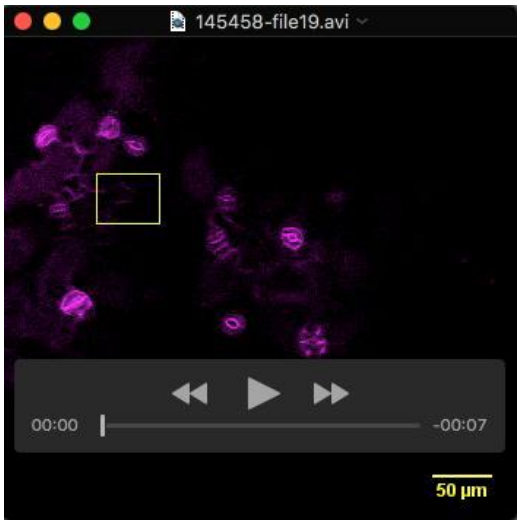
Movies



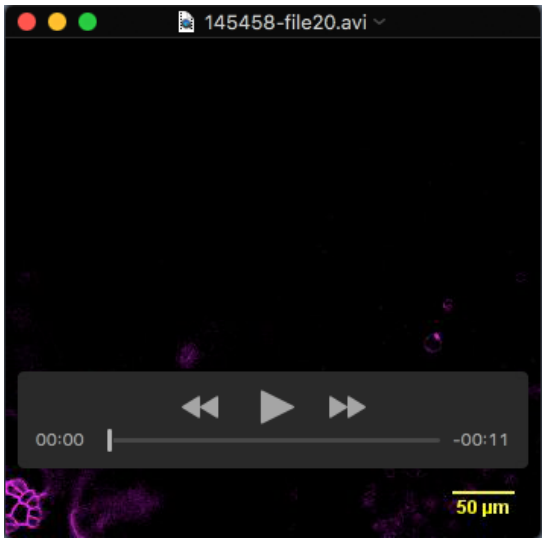
Movie 1. Z-stack series of full-size images of adaxial epidermis from DMSO-treated cotyledon at the experimental starting point (0 h, 4-day-old seedling). A yellow square indicates the area that is shown in Fig. 3A.



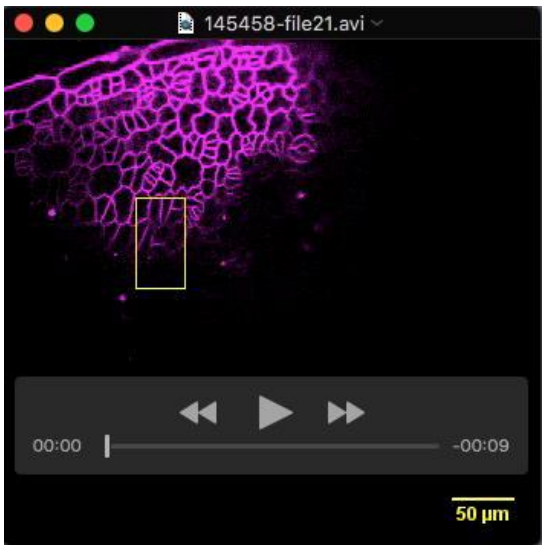
Movie 2. Z-stack series of full-size images of adaxial epidermis from DMSO-treated cotyledon at 12 h after initiation of treatment. A yellow square indicates the area that is shown in Fig. 3A.



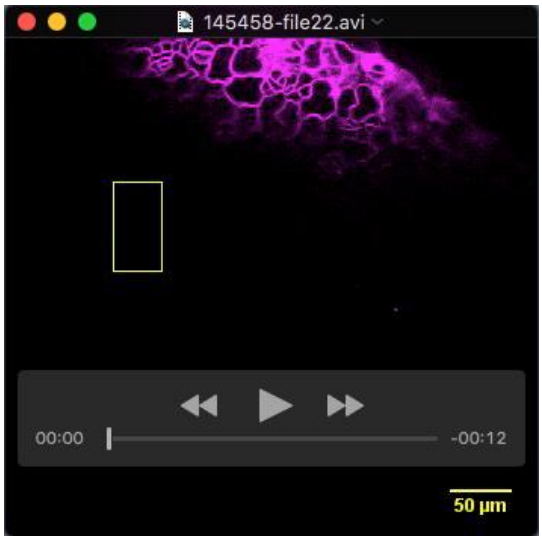
Movie 3. Z-stack series of full-size images of adaxial epidermis from DMSO-treated cotyledon at 24 h after initiation of treatment. A yellow square indicates the area that is shown in Fig. 3A.



Movie 4. Z-stack series of full-size images of adaxial epidermis from DMSO-treated cotyledon at 36 h after initiation of treatment. A yellow square indicates the area that is shown in Fig. 3A.

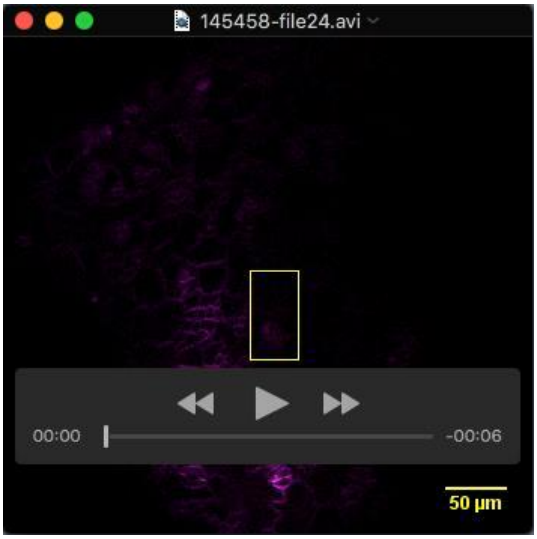


Movie 5. Z-stack series of full-size images of adaxial epidermis from bubblin-treated cotyledon at the experimental starting point (0 h, 4-day-old seedling). A yellow square indicates the area that is shown in Fig. 3B.

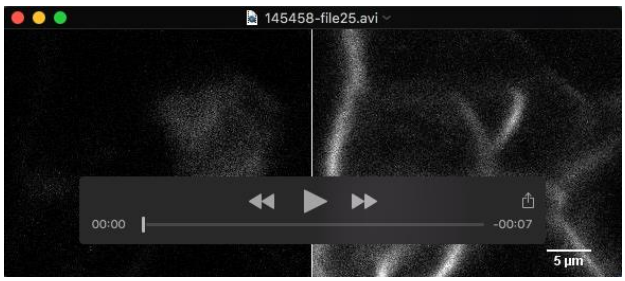


Movie 6. Z-stack series of full-size images of adaxial epidermis from bubblin-treated cotyledon at 12 h after initiation of treatment. A yellow square indicates the area that is shown in Fig. 3B.

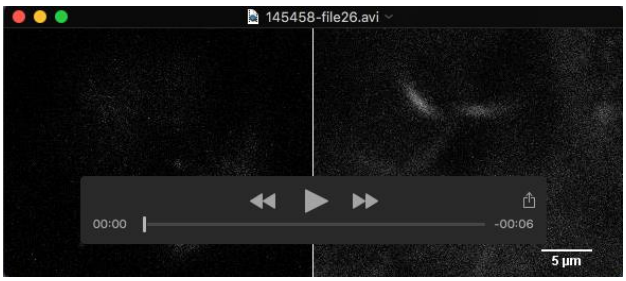
Movie 7. Z-stack series of full-size images of adaxial epidermis from bubblin-treated cotyledon at 24 h after initiation of treatment. A yellow square indicates the area that is shown in Fig. 3B.



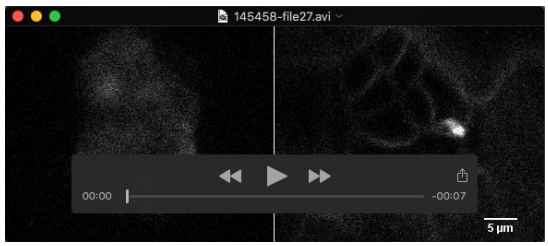
Movie 8. Z-stack series of full-size images of adaxial epidermis from bubblin-treated cotyledon at 36 h after initiation of treatment. A yellow square indicates the area that is shown in Fig. 3B.



Movie 9. Z-stack series of adaxial cotyledon epidermis from *ProBASL:GFP-BASL* treated with DMSO only as a control. Left: GFP-BASL signal; right: FM4-64-stained cell membrane.



Movie 10. Z-stack series of adaxial cotyledon epidermis from *ProBASL:GFP-BASL* treated with 10 µM bubblin. Left: GFP-BASL signal; right: FM4-64-stained cell membrane.



Movie 11. Z-stack series of adaxial cotyledon epidermis from *ProBASL:GFP-BASL* treated with 50 µM bubblin. Left: GFP-BASL signal; right: FM4-64-stained cell membrane.