Mechanical regulation of organ asymmetry in leaves

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Supplementary Figures

Supplementary Fig. 1. Dynamic changes of the wall stiffness of epidermal cell walls.

(a and b) Quantification of epidermis cells apparent Young’s modulus obtained by AFM using the ramp mode in P_2 (a) and P_3 (b). (a) A total of 14 positions in 4 leaf samples for P_2 adaxial, 17 positions in 5 leaf samples for P_2 abaxial, and 81 positions in 4 leaf samples for P_2 middle domain were recorded to calculate elastic modulus. (b) A total of 19 positions in 3 leaf samples for P_3 adaxial, 19 positions in 3 leaf samples for P_3 abaxial, and 26 positions in 3 leaf samples for P_3 middle domain were recorded to calculate elastic modulus. At least 5 force curves were recorded per position. Values are mean ± standard error. *Student's t-test P < 0.0001 and the value changes above two-fold. (c-e) Histograms of individual samples for AFM data in Fig.1d, h and i. (f) Elastic moduli of positive controls used for AFM calibration. Probe’s spring constant was calibrated by the relative method on the Bruker’s polystyrene test sample (PDMS-SOFT-2 with 3.5 MPa) for QNM measurements.
Supplementary Fig. 2. Cell wall thickness and the distribution of indentation depth.

(a) Epidermal cell wall thicknesses of P$_2$. (b) Histogram of indentation depth of AFM measurements of P$_2$ Ab and Ad epidermal cells. Junctions between periclinal and anticlinal cell walls (margin) and inner regions of cells (inner) were separately quantified. (c-f) Force curves of representative samples in (b) showing indentation curve. Red, extending trace of a force curve, blue, retracting trace of a force curve.
Supplementary Fig. 3. Methyl-esterification of cell wall pectin in leaf primordium detected by 2F4 antibody.

(a-d) Transverse sections stained with PI (red) showing 2F4 labeling (green) of pectin de-methyl-esterification in (a-c) tomato and (f) Arabidopsis shoot apexes. Sections are ordered from most apical to most basal (a to c); approximate distance (in
micrometers) from the summit of the SAM to the section is given in the top right-hand corner of each image. (d, e, g and h) Longitudinal sections stained with PI (red) showing 2F4 labeling (green) of pectin de-methyl-esterification in (d and e) tomato and (g and h) Arabidopsis P1 (arrowheads). Similar results were obtained from 13 samples from tomato and 15 samples from Arabidopsis. (e and h) View of the same shoot apex section as in (d and g) with maximum intensity projections of Nomarski interference contrast images overlaid with single optical sections of 2F4 labeling. (i) An enlarged tomato P3 leaf primordium showing 2F4 (green) labeling in cell layers 1, 2 and 3. (j and k) Transverse sections showing 2F4 labeling (green) of pectin methyl-esterification status in Arabidopsis mutant shoot apexes. (j) pMP::MPΔ with abaxialized leaves; and (k) phv-1d with adaxialized leaves. (l-n) Positive control for cell wall pectin methyl-esterification in leaf primordium stained with PI. After NaOH treatment to hydrolyze pectin methyl-esterification, uniform immunolabeling was detected in tomato (l) Col-0 (m) and phv-1d (n). Fraction of samples showing the displayed features are in parentheses. Scale bars, 100 μm.
Supplementary Fig.4. Methyl-esterification of cell wall pectin in leaf primordia.

(a-c and e-g) Series of transverse sections stained with DAPI (red) showing (a-c) LM19 and (e-g) JIM5 labeling (green) of pectin de-methyl-esterification in tomato shoot apexes. Sections are ordered from most apical to most basal (a-c, and e-g); approximate distance (in micrometers) from the summit of the SAM to the section is given in the top right-hand corner of each image. (d) LM19 and (h) JIM5 are NaOH-treated positive controls. (i) A longitudinal section stained with DAPI (red) showing JIM5 labeling (green) of pectin-methyl-esterification in a tomato P₃. (j) LM20 labeling (green) in tomato. (k) Transverse section stained with DAPI (red) showing LM19 labeling (green) of pectin de-methyl-esterification in Col-0 shoot apexes. (l) LM19 NaOH-treated positive controls for Arabidopsis shoot apexes. (m-n) No primary antibody and only the Alexa Fluor 546-labeled secondary antibody was used as a negative control in Arabidopsis (m) and tomato (n). Fractions of samples showing the
displayed features are in parentheses. (o) Quantification of epidermal cells’ apparent Young’s modulus obtained by AFM using the QNM mode in untreated and treated P1. Elastic modulus values are from Fig. 1d, Supplementary Fig. 6g and 7e, where more controls are included. Raw AFM measurements are provided in Supplementary Table 2. Values are mean ± standard error. *Student’s *t*-test *P* < 0.0001 and the value change above two-fold. Scale bars, 100 μm.
Supplementary Fig. 5. Manipulation of pectin methyl-esterification status changes cell wall elastic modulus.

(a) Control microapplication treatment of tomato leaf primordium. A Sephacryl HR S300 bead (light blue) at the adaxial domain of an emerging primordium (P₁). (b-d) Transverse sections stained with PI (red) showing 2F4 labeling (green) of pectin de-methyl-esterification in (b) untreated, (c) EGCG treated, and (d) PME treated tomato P₁. Arrows highlight the lack of abaxial 2F4 signal (c) and the ectopic adaxial 2F4 signals (d). Arrowheads highlight the boundary. Plants were fixed 48 hr after
treatment. Fraction of samples showing the displayed features are in parentheses. (e and f) 3D rendering of epidermal cell topography and stiffness obtained by AFM using the PeakForce QNM mode, with (e) from EGCG treated abaxial cells, and (f) from PME treated adaxial cells. Cell topography is overlaid with stiffness. (g) Quantification of epidermis cells apparent Young’s modulus obtained by AFM using the QNM mode. 24 cells in 6 leaf samples were recorded per region under each treatment, with raw AFM measurements provided in supplemental information (Supplementary Table 2). (h) Histograms of individual samples for AFM data in (g) Values are mean ± standard error. *Student's t-test P < 0.0001 and the value changes above two-fold. Scale bars, 50 μm in a-d; and 5 μm in e and f.
Supplementary Fig. 6. Manipulation of auxin distribution or auxin signaling changes pectin methyl-esterification status and cell wall elastic modulus.

(a and b) Transverse sections stained with PI (red) showing 2F4 labeling (green) of pectin de-methyl-esterification in (a) auxinole treated, and (b) IAA treated tomato P1. Arrowheads highlight the lack of abaxial 2F4 signal (a) and the ectopic adaxial 2F4 signals (b). Plants were fixed 48 hr after treatment. Fraction of samples showing the displayed features are in parentheses. (c and d) 3D rendering of epidermal cell topography and stiffness obtained by AFM using the QNM mode, with (d) from
auxinole treated abaxial cells, and (e) from IAA treated adaxial cells. Cell topography is overlaid with stiffness. (e) Quantification of epidermis cells apparent Young’s modulus obtained by AFM using the QNM mode. 24 cells in 6 leaf samples were recorded per region under each treatment, with raw AFM measurements provided in supplemental information (Supplementary Table 2). (f) Histogram of individual samples for AFM data in (f). Values are mean ± standard error. *Student's t-test P < 0.0001 and the value changes above two-fold. Scale bars, 50 μm in a and b; and 5 μm in c and d.
Supplementary Fig. 7. Schematic of model setting, model robustness, and stress and strain analysis of the epidermal layer upon different relaxation cycles.

(a) Geometry of the model; the outmost layer (green), the polygonal elements (grey), and the interspace (blue) represent the epidermis, the inner cells, and the cushion zone, respectively. (b) Generalized Maxwell model. In our model setting, the generalized
Maxwell model (GMM) contains two Maxwell elements, i.e., $M = 2$. By performing appropriate parameter controls, i.e., $\tau_j = \eta_j/E_x = 1000$ and $\gamma_j = E_j/E_x = 0.25$, the GMM can simulate a slow and significant creep behavior. (c and d) Parametric analysis of elastic modulus and resistance coefficients. For the sake of simplicity, the difference in division area of distinct inner cells was neglected and all were set equal to 3; other non-mechanical parameters remained the same as in Fig. 3a. (e) A series of snapshots showing the leaf morphogenesis corresponding to the parameter settings in (d). Morphogenesis similarity is attributed to the implementation of “three domain of mechanical property dynamics”, while morphogenesis variation is due to the different actual growth rate of inner cells, which can be approximately reflected by $N_{\text{stiff}}/N_{\text{soft}}$.

(d) The ratio of number of stiff cells to number of soft cells ($N_{\text{stiff}}/N_{\text{soft}}$) as a function of both the ratio of long-term elastic modulus ($E_{\text{stiff}}/E_{\text{soft}}$, varying from 1/0.9 to 1/0.1) and that of the resistance coefficient ($\lambda_{\text{stiff}}/\lambda_{\text{soft}}$, varying from 2 to 6). The largest $N_{\text{stiff}}/N_{\text{soft}}$ appears at the maximum $\lambda_{\text{stiff}}/\lambda_{\text{soft}}$ and minimum $E_{\text{stiff}}/E_{\text{soft}}$. As $\lambda_{\text{stiff}}/\lambda_{\text{soft}}$ increases, stiff cells offer more contributions to the system’s potential energy, and thus, their growth rate also increases, hereafter, higher division frequency, whereas, as $E_{\text{stiff}}/E_{\text{soft}}$ decreases, soft inner cells experience less resistance from its neighboring epidermal cells, which in turn promotes their growth and division. (e-h) Stress and strain analysis of the epidermal layer upon different relaxation cycles. The abscissa is the dimensionless time, and the ordinate $\theta$, taking values between $-180^\circ$ and $180^\circ$, is the position on the leaf profile (as shown in a). (e) Spatiotemporal responses of strain in early stage of leaf primordium with two domains; epidermis experiences isotropic squeezing force from the inner cells during growth. (f) Spatiotemporal changes of stress corresponding to (e); note the small stress anisotropy is generated due to anisotropic geometry. (g) Spatial response of strain in a later stage of leaf primordium with three domains; the epidermis experiences the anisotropic squeezing force related to the rigidity of the adjacent inner cell. (h) Spatiotemporal changes of stress corresponding to (g); anisotropic distribution of stress is mainly caused by the applied squeezing force.
Supplementary Fig. 8. Morphology and LeREV transcript pattern changes after treated in tomato.

(a-c) Transverse sections showing different morphology changes in tomato leaf primordia after abaxial microapplication of EGCG-containing lanolin as in Fig. 4f. Note different levels of vascular defects. (d-f) Patterns of LeREV transcript accumulation in transverse sections through mock treated (d), PME treated (e), and EGCG treated (f) P1. Samples were fixed 48 hr after treatment. LeREV enriched in the adaxial domain in leaves of mock-treated samples, but is uniformly distributed after treated with PME or with EGCG. Note that mis-expression may be underestimated due to difficulties in
unambiguously scoring images. Scanning electron microscopy of tomato leaf epidermis (g-j). Leaf primordia 7 d after (g) adaxial PME treatment, (h) abaxial EGCG treatment, and (i) simultaneous adaxial PME and abaxial EGCG treatment. (j) Magnification of regions in the white line box in (i). Fraction of samples showing the displayed features are in parentheses. Scale bars, 50 μm in a-f and g-i; and 100 μm in j.
Supplementary Fig. 9. Manipulations of pectin methyl-esterification status lead to leaf morphology defects in *Arabidopsis*.
(a and b) Patterns of AS2 transcript accumulation in transverse sections through vegetative shoot apex of 25 d-old Col-0 (a), and rev-6 (b) plants. AS2 accumulation in both Col-0 and rev-6 is adaxialized. (c-e) Patterns of FIL transcript accumulation in transverse sections through vegetative shoot apex of 25 d-old Col-0 (c), rev-6 (d), and pAS2::PME5 rev-6 (e) plants. FIL accumulation in Col-0 and rev-6 is excluded from the adaxial domain, but is invaded or even enriched in the adaxial domain (highlighted by a red circle in e) in pAS2::PME5 rev-6. Note that mis-expression may be underestimated due to difficulties in unambiguously scoring images. (f and g) Patterns of ATML1 transcript accumulation in transverse sections through vegetative shoot apex of 25 d-old Col-0 (f), and as2-2 (g), plants. ATML1 accumulation in both Col-0 and as2-2 is epidermis-specific. (h and i) Arabidopsis pAS2::PME5 and pFIL::PMEI3 plants showing leaf morphology defects in Col background. Arrowheads highlight trumpet-like leaves in pAS2::PME5 Col (h) and pFIL::PMEI3 Col (i). (j and k) Arabidopsis pATML1::PME5 and pATML1::PMEI3 plants showing leaf morphology defects. Arrowheads highlight trumpet-like leaves in pATML1::PME5 (j) and pATML1::PMEI3 (k). Fraction of samples showing the displayed features are in parentheses. Scale bars, 50 μm in a-g; and 1 mm in h-k.
Supplementary Fig. 10. Epidermis morphology of *Arabidopsis* leaves.

Scanning electron microscopy of *Arabidopsis* Col-0 wild-type (a and b), rev-6 (c and d), pAS2::PME5 rev-6 (e), and pFIL::PMEI3 rev-6 (f) plants showing leaf epidermis. The low trichome density and pavement cell morphology in both transgenic lines resemble abaxial epidermis. Scale bars, 100 μm.
Supplementary Fig. 11. Methyl-esterification of cell wall pectin in transgenic Arabidopsis leaf primordium.

Transverse sections stained with DAPI (red) showing LM19 labeling (green) of pectin de-methyl-esterification in Arabidopsis Col-0 (a), as2-2 (b) pAtML1::PME5 as2-2 (c), and pAtML1::PMEI3 as2-2 (d) leaf P₃ stage primordia. Fraction of samples showing the displayed features are in parentheses. Note reduced LM19 staining of cell walls between L1 and L2 layers in pAtML1::PMEI3 as2-2. Scale bars, 50 μm.
### Supplementary Tables

#### Supplementary Table 1. Leaf adaxial-abaxial polarity phenotypes associated with alterations of mechanical properties.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Plants with polarity defects in leaves</th>
<th>Total plants counted</th>
<th>Percentage of polarity defects, %</th>
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<tr>
<td>Col-0</td>
<td>0</td>
<td>100</td>
<td>0%</td>
</tr>
<tr>
<td>as2-2</td>
<td>0</td>
<td>100</td>
<td>0%</td>
</tr>
<tr>
<td>rev-6</td>
<td>0</td>
<td>100</td>
<td>0%</td>
</tr>
<tr>
<td>pAS2::PME5 rev-6</td>
<td>27</td>
<td>75</td>
<td>36%</td>
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<tr>
<td>pFIL::PMEI3 rev-6</td>
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<td>24</td>
<td>33%</td>
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<tr>
<td>pAS2::PME5 Col-0</td>
<td>1</td>
<td>18</td>
<td>5.6%</td>
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<tr>
<td>pFIL::PMEI3 Col-0</td>
<td>1</td>
<td>60</td>
<td>1.7%</td>
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#### Supplementary Table 2. Raw AFM measurements for Fig. 1 and Supplementary Fig. 4-6. (In Excel format)
## Supplementary Table 3. List of primers used for RT-qPCR.

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<th>Gene name</th>
<th>Description</th>
<th>Gene ID</th>
<th>Forward primer</th>
<th>Reverse primer</th>
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<td><strong>LeActin</strong></td>
<td>Actin</td>
<td>Solyc03g078400</td>
<td>CCTGTTCTCCTG</td>
<td>TGCTCCTAGCGG</td>
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<td></td>
<td></td>
<td></td>
<td>ACTGAGGC</td>
<td>TTTCAAGT</td>
</tr>
<tr>
<td><strong>LeFIL</strong></td>
<td>YABBY-like transcription factor</td>
<td>Solyc01g091010</td>
<td>ACTGTTCTCGCG</td>
<td>TTCTCCGGAGGT</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>GTGAGTGTT</td>
<td>CTGTTTGC</td>
</tr>
<tr>
<td><strong>LeREV</strong></td>
<td>Class III homeodomain-leucine zipper</td>
<td>Solyc11g069470</td>
<td>TTCAGAACAGAA</td>
<td>TGGTAGTAGTCC</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>GGTGTGAGAGA</td>
<td>AGCAGGGTTG</td>
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<tr>
<td><strong>LeWOX1</strong></td>
<td>WUSCHEL-related homeobox</td>
<td>Solyc03g118770</td>
<td>GAATCAGGGGCA</td>
<td>CCATCTTTTCTGC</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>AATAGGACAGT</td>
<td>TGCTGCTACTC</td>
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### Supplementary Table 4. Scaling parameters for simulation of a normal leaf.

<table>
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<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
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</thead>
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<td>$\lambda_{\text{stiff}} / \lambda_{\text{soft}}$</td>
<td>Ratio of area resistance of stiff inner cells to soft inner cells</td>
<td>2</td>
</tr>
<tr>
<td>$A^{(1)} : A^{(2)} : A^{(3)}$</td>
<td>Ratio of target area of inner cells at region I, II, and III</td>
<td>5:4:4</td>
</tr>
<tr>
<td>$a^{(1)} : a^{(2)} : a^{(3)}$</td>
<td>Ratio of division area of inner cells at region I, II, and III</td>
<td>4:3:3</td>
</tr>
<tr>
<td>$E_{\text{stiff}} / E_{\text{soft}}$</td>
<td>Ratio of long-term elastic modulus between stiff and soft epidermis</td>
<td>1/0.7</td>
</tr>
<tr>
<td>$\phi^{(1)} : \phi^{(2)} : \phi^{(3)}$</td>
<td>Ratio of squeezing force coefficient at region I, II, and III</td>
<td>1:3:0.5</td>
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<tr>
<td>$\xi$</td>
<td>Metropolis algorithm step size</td>
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</tr>
<tr>
<td>$\kappa$</td>
<td>Fluctuation energy</td>
<td>0.2</td>
</tr>
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</table>
**Video Legends**

**Video 1. Normal leaf growth, related to Fig. 2i-l.**

Numerical simulation shows the development of a normal leaf with normal wall rigidity.

**Video 2. Hastened adaxial cell wall loosening leads to reduced asymmetry, related to Fig. 3c.**

Numerical simulation shows the development of a leaf with hastened adaxial cell wall loosening, in which the adaxial and abaxial domains have identical wall stiffness patterns.

**Video 3. Two-domain partition leads to reduced asymmetry, related to Fig. 3d.**

Numerical simulation shows the development of a leaf with the abaxial domain acquiring the middle domain cell wall property, i.e., constitutively stiffening in the abaxial and middle domains.

**Video 4. Reduced epidermal restriction leads to reduced asymmetry, related to Fig. 6a.**

Numerical simulation shows the development of a leaf with uniformly reduced epidermal restriction.

**Video 5. Enhanced epidermal restriction leads to reduced asymmetry, related to Fig. 6b.**

Numerical simulation shows the development of a leaf with uniformly enhanced epidermal restriction.
Supplementary Methods

Model assumptions

Considering that the outmost epidermis has much higher stiffness than inner tissues\(^1\), partially because epidermal cell walls are thicker than inner cell walls\(^2\) and the epidermis is expected to provide strong physical constraints. In the models, the epidermis was simplified as viscoelastic biological materials with continuum properties of mechanics, rather than explicitly considering each individual epidermal cell. The expansion of epidermis was considered non-autonomous, and was driven by the squeezing forces generated from the growth of inner cells. Unlike the epidermis, we considered the expansion of each inner cell as an autonomous process, which obeys the widely-accepted concept of turgor-driven plant cell growth. In the model, the following assumptions were taken for inner cells: (i) Cells do not slide against neighboring cells, and remain contact to each other. (ii) No additional meristem cells are incorporated into leaf primordia, and new cells only come from cell division of existing cells. (iii) Each cell has an idealized growth rate, at which the cell would have when isolated from the tissue; this idealized growth rate is negatively correlated with and is exclusively affected by the wall stiffness\(^1\). (iv) In a multicellular context, a cell has an actual growth rate, which is further determined by the mechanical constrain imposed by its neighbors. (v) Cells divide once their size reaches a specific threshold. Following our experimental observations (Fig. 1), we implemented different wall stiffness, which was negatively reflected by the idealized growth rate, to the adaxial, middle and abaxial domains, with the abaxial domain first, then the adaxial domain, and finally the middle domain to acquire decreased wall rigidity.

Mechanical modeling

In our model, inner cell walls were represented in two dimensions using polygonal
elements. Adjacent cells share a common cell wall, which prohibits cell movement relative to each other (Supplementary Fig. 7a). We implemented different idealized growth rate, which is exclusively affected by the wall stiffness, to different leave domains (i.e., adaxial, middle and abaxial). We also assumed that cells divide along the short axis once their sizes reach a division threshold. The growth coordination of neighboring cells was achieved through solving the generalized potential energy or Hamiltonian energy (\( H \)) upon an energy minimization principle.

\[
H = \sum_{i} \lambda^a (a_i - A_i)^2 + \sum_{i} \lambda^p (l_i - L_i)^2,
\]

where \( a_i \) and \( A_i \) are the current and target area of \( i^{th} \) individual inner cell, and \( \lambda^a \) is a parameter that describes the resistance to deviation from the target area. Similarly, \( L_i \) is the target perimeter of an individual inner cell, and \( \lambda^p \) describes the resistance to change in the perimeter \( l_i \) away from \( L_i \), and is positively correlated to \( \lambda^a \).

We considered the non-autonomous expansion of epidermis as a viscoelastic creep process, and then modeled using the generalized Maxwell model (a parallel connection of several Maxwell arms and a spring \( E_\infty \), see Supplementary Fig. 7b). The time-dependent elastic modulus for linear viscoelastic materials has been written in a form of Prony series:

\[
E(t) = E_\infty + \sum_{j=1}^{M} E_j e^{(-t/\tau_j)},
\]

where \( M \) denotes the number of Maxwell elements, \( E_\infty \) is the long-term elastic modulus corresponding to the long-term elastic response, \( E_j \) is the elastic coefficient of the \( j^{th} \) Maxwell element, and \( \tau_j \) is the relaxation time related to the damping coefficients (\( \eta_j \)) of dashpots, as \( \tau_j = \eta_j / E_\infty \).
Considering that an arbitrary strain input is obtained through superposition of small strain increments $\varepsilon$, the constitutive equation has been written in the following form:

$$\sigma(t) = E,\varepsilon(t) + \sum_{j=1}^{M} \int_{e}^{t} E_j \left( \frac{\partial E}{\partial s} \right) ds, \quad (3)$$

$$= \sigma_0(t) + \sum_{j=1}^{M} h_j(t).$$

where $h_j$ is the stress on $j^{th}$ Maxwell element, and $s$ is any arbitrary past time between 0 and $t$.

For a non-homogeneous epidermis layer in quasi-static condition, assuming a boundary value problem with $u_i$ displacement on boundary $\Omega$, the equilibrium relationship has been written as:

$$\sigma_{ij} + f_i = 0, \quad j = 1, 2 \quad u_i = 0, \quad \text{on} \ \Omega, \quad (4)$$

where $f_i$ is the squeezing force generated from the growth of the inner cells.

The coordinated growth between the epidermis and the inner cells was established though the introduction of the following assumptions: (i) Mechanical properties of the epidermis are differentially regulated among abaxial, middle and adaxial regions. Local softening of inner cell wall is in accord with that of the neighboring epidermis. (ii) Rather than explicitly considering the mechanical interactions between the inner cells and the epidermis, a cushion zone between them is introduced to value the squeezing forces generated from the growth of inner cells. (iii) The squeezing forces suffered by the epidermis is anisotropic, and time-dependent; the magnitude of the squeezing force dependents on both the size of the cushion zone and the stiffness of neighboring inner cells, while the direction of the squeezing forces is along the outward unit normal to the current configuration.

**Numerical calculations**

The complete numerical algorithm involves two coupled modules, namely the top (or
growth) module for the inner cells and the bottom (or relaxation) module for the epidermis. In the top module, we used the Metropolis dynamics to minimize the potential energy\(^3\) and to straightforwardly describe cell behaviors. In the bottom module, the mechanical equilibrium equation was solved by a viscoelastic finite element method (FEM) approach described previously\(^4\). During modeling simulation, switching of the two modules was dependent on the area of the cushion zone: the Monte-Carlo simulations were repeated until the cushion zone was decreased to a threshold, and then, a relaxation cycle was initiated to inflate the epidermis and to enlarge the cushion zone.

For mimicking the softening behavior, as observed experimentally, in our model setting, we let a certain number of inner cells and their adjust epidermis cells to change their properties, when the area of leaf has surpassed a threshold value. After that, these inner cells were allowed to divide and to grow according to their newly assigned properties.

Additionally, to cross the gap between the static AFM measurement and the dynamic material information required for numerical simulation, we adopted the following strategies. First, all model variables and the elastic modulus data from AFM measurement were set in the dimensionless form, i.e., the length unit was scaled by the length of inner cell while the time unit by the cell division cycle. Second, the maximum elastic module of the generalized Maxwell solid model,\( E_5 = E_x + E_1 + E_2 \) was determined from AFM measurements to reflect the instantaneous elastic response. Third, the damping coefficient of the dashpot (\( \eta \)) in each Maxwell arm was further estimated according to the relaxation time (\( \eta = E_x \tau \)), which could be estimated from the timescale of growth, since we took the epidermis growth as a creep process. Forth, the extrusion pressure generated from inner cells toward the epidermis, which cannot be measured directly, was assumed to achieve reasonable strain increment upon one relaxation cycle.

In the following sections, the details of the numerical method are presented.
Top module for inner cells

During each time interval, each inner cell node was moved in a random order
\( x_{\text{new}} = x_{\text{old}} + \xi r \), where \( r = \{ \rho, \varphi \} \) is a random vector chosen uniformly from the circle
(i.e., \( \rho \in [0,1] \), and \( \varphi \in [0,2\pi] \)), and \( \xi \) is the step size. In the case of \( x_{\text{new}} \in \Omega \), where \( \Omega \) represents the surrounding region of the epidermis, the system would accept any movement if \( x_{\text{new}} \) leads to an energy drop, and the system accepts movement with a Boltzmann probability function if \( x_{\text{new}} \) leads to an energy rise. In the case of \( x_{\text{new}} \not\in \Omega \), the system would reject any movement. The formula noted above can be simply expressed as:

\[
P = \begin{cases} 
1 & \text{if } (\Delta H < 0) \& (x_{\text{new}} \in \Omega) \\
\exp(-\Delta H/\kappa) & \text{if } (\Delta H > 0) \& (x_{\text{new}} \in \Omega), \\
0 & \text{if } (x_{\text{new}} \not\in \Omega)
\end{cases}
\]

where \( P \) represents the probability for accepting a stepwise movement, \( \kappa \) allows us to tune the magnitude of stochastic fluctuations in the model, \( \Delta H \) is the energy difference between the energy of the current configuration \( (H_{\text{new}}) \) and the previous configuration \( (H_{\text{old}}) \), i.e., \( \Delta H = H_{\text{new}} - H_{\text{old}} \).

Bottom module for epidermis

During each relaxation cycle, time duration was also divided into equal steps of duration \( \Delta t \) \( (t_n = n\Delta t) \), and the continuous model of the epidermal layer was divided into finite elements (the linear beam element is adopted in this work). According to the standard FEM formalism, the internal force within each element \( (e) \) has been written as:

\[
\left( F^{\text{int}}_{e,n} \right)_k = \int B^T \sigma(t_n) d(\nu_e)_k,
\]
where \((\ast)_k\) denotes the value of \((\ast)\) at \(k^{th}\) relaxation cycle, \(B\) is the strain-displacement matrix, which is defined by the shape functions, and \(v_e\) is the element’s volume.

Combining Eqs. 5 and 6 and rewriting with matrix notation lead to:

\[
(f^\text{ext}_{i,n,k}) = (K_e)_k(u_{e,n})_k + h^\text{hist}_{e,n} - (K^\text{hist}_e)_k(u_{e,n-1})_k,
\]

where \((K_e)_k = B^T D^\text{elastic}_e B(v_e)_k (1 + \sum_{j=1}^{M} \gamma_j A_j)\) is the element stiffness matrix with the following definition of \(A_j\),

\[
A_j = \frac{1 - e^{(-\frac{\tau_j}{\tau})}}{-t}.
\]

and \(\gamma_j = E_j/E_e\) is the \(j^{th}\) normalized elastic modulus, \(D^\text{elastic}_e\) is the stress-strain matrix relating to material properties. \((K^\text{hist}_e)_k = B^T D^\text{elastic}_e B(v_e)_k \sum_{j=1}^{M} \gamma_j A_j\) is the history element stiffness matrix, and \(h^\text{hist}_{e,n} = v_e B^T \sum_{j=1}^{M} e^{(-\frac{\Delta t}{\tau_j})} h_{j,n-1}\) is the history force vector, with

\[
h_{j,n} = e^{(-\frac{\Delta t}{\tau_j})} h_{j,n-1} + \gamma_j A_j D^\text{elastic}_e B((u_n)_k - (u_{n-1})_k).
\]

Next, we denoted \((f^\text{ext}_{i,n,k})\) as the nodal external force vector at time step \(n\) of relaxation cycle \(k\). The exact form of \((f^\text{ext}_{i,n,k})\) can be achieved by adopting spatial integration:

\[
(f^\text{ext}_{i,n})_k = \frac{1}{2} \sum_{\text{elem}(i)} (T_{e,n})_k (l_{e,n})_k (\zeta_{e,n})_k,
\]

where \(w(i)\) represents a set of elements containing node \(i\), \(\zeta_{e,n}\) is the outward unit normal vector, \(l_{e,n}\) is the length of the beam element that is revalued at the initial of each relaxation cycle, and \(T_{e,n}\) stands for the squeezing force density at the beam element.
We assumed that, at each time step of the $k^{th}$ relaxation cycle, the external force is completely counter-balanced by the inner force, i.e., $(F_{\text{ext}})_k = (F_{\text{int}})_k$, the nodal displacements at each time step $n$ can be obtained by solving,

$$
(U_n)_k = (K_G)^{-1}_k((F_{\text{ext}})^{n}_k + (K_{\text{hist}})^{n}_k(U_{n-1})_k - (H_{\text{hist}}^n)_k),
$$

(10)

where $K_G$ is the global stiffness matrix, $K_{\text{hist}}$ the global history stiffness matrix, and $H_{\text{hist}}^n$ is the history vector, which are assembled from $K_e$, $K_{\text{hist}}^e$, and $H_{\text{hist}}^{e,n}$ respectively.

**Coordination of two modules**

We assumed that $T_{e,n}$ is related with the area of cushion zone, and can be written in the following form:

$$
(T_{e,n})_k = \begin{cases} 
(\phi_e)_k, & \text{if } (S_n)_k \leq (I)_k \\
(\phi_e)_k((2(I)_k - (I)_k), & \text{if } (S_n)_k > (I)_k
\end{cases}
$$

(11)

where $\phi_e$ is the squeezing force coefficient. $S_n$, is the surrounding area of the epidermis at the current time step $n$. $I$ is the target surrounding area of the epidermis and is assigned at the beginning of the $k^{th}$ FEM calculation cycle, i.e., $(I)_k = (Q)_k + (Y)_k \Delta l,$

where $Q$ is the overall area of the inner cells, $Y$ is the perimeter of the inner tissue, and $\Delta l$ is the width of the cushion zone. To achieve stable simulations, $\Delta l$ should be valued with a relatively large constant (i.e., $\Delta l = 0.5$). Note that, by introducing Eq. 11, the squeezing force becomes a function of the size of the cushion zone.

The algorithm repeats this procedure until the real surrounding area of the epidermis reaches its “target”, which also means that the squeezing force has been reduced significantly. Then, the algorithm solves the $k+1^{th}$ cycle of inner cell growth/division again.

**Supplementary References**

