

Current Biology

Supplemental Information

Active Control of Cell Size Generates Spatial Detail during Plant Organogenesis

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Figure S1

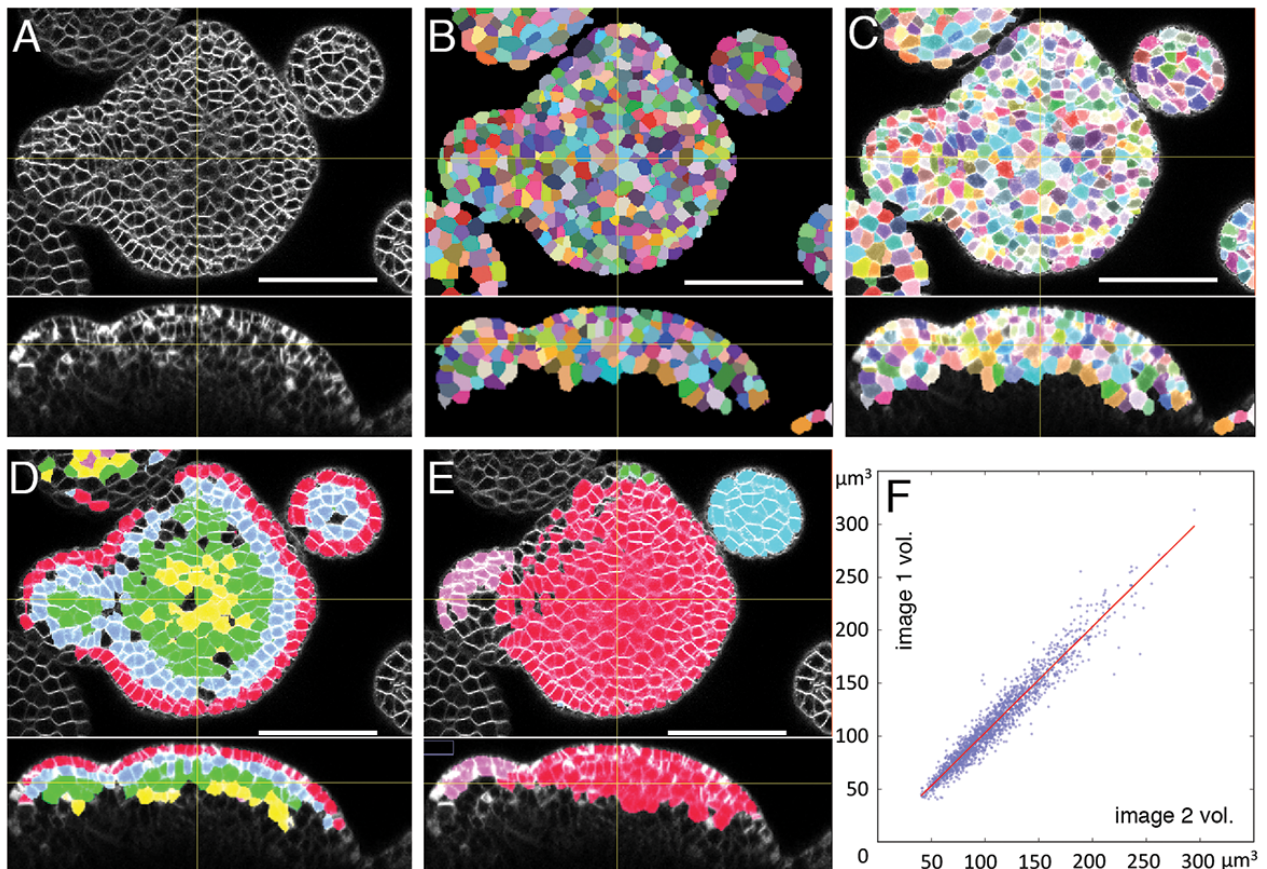


Figure S1 (related to Figure 1): Accuracy of automated segmentation and post-segmentation steps used to select cells for measurements

A: orthogonal views of a representative confocal image of an inflorescence meristem stained with FM4-64. B: corresponding image after watershed segmentation. C: overlap between images A and B. D: overlap between A and image in which the segmented cells were attributed to cell layers (L1-L4 in red, blue, green and yellow, respectively). E: overlap between A and image in which segmented cells were assigned to regions of interest (inflorescence meristem in red, floral bud primordia 2, 4 and 5 in green, magenta and cyan, respectively). F: Reproducibility of measured cell volumes in confocal stacks of the same inflorescence apex imaged independently at two different angles, segmented and matched (three apices, n = 1902)

Figure S2

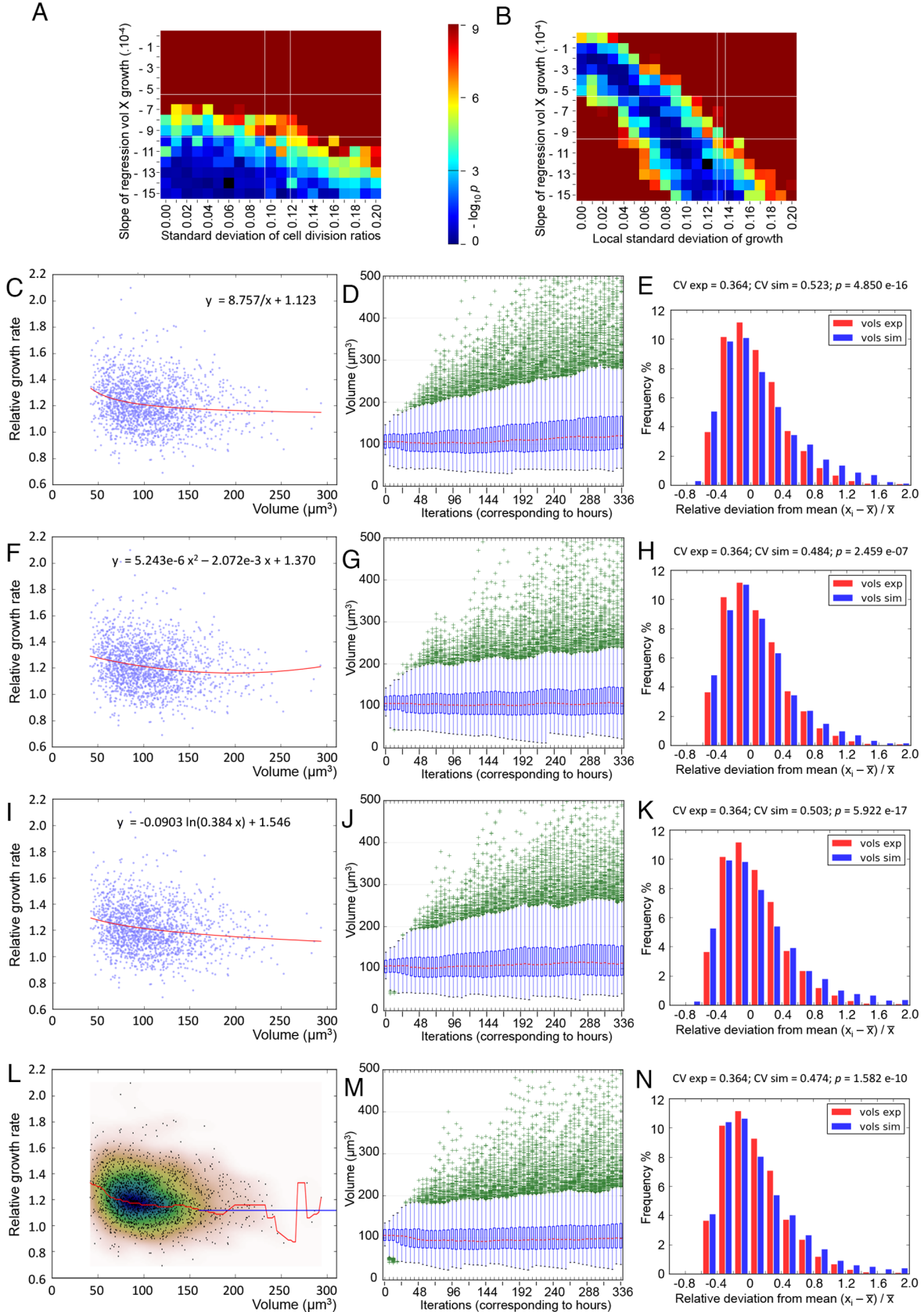


Fig. S2 (related to Figure 2): Sensitivity of the simulations from Figure 2 to parameter values and to the type of function used to adjust cell growth to cell volume.

A, B: sensitivity of simulations (sim_a, Fig.2A) to the slope of the linear function over a range of values for the standard deviation of division ratios (A) and local standard deviation of growth rates (LSDG) (B); each element in the graph indicates the $-\log p$ -value (see colorbar) for the equality of coefficients of variation between the experimental data and the simulations, calculated by applying Levene's test on the relative deviations from the mean [32]; the white lines mark the 95% confidence interval for measured parameters (Table S1). C-N: results of simulations in which cell growth rates were adjusted to cell volumes using fitted inverse (C-E), quadratic (F-H), logarithmic (I-K) and probability density (PDF) functions (L-N). C, F, I: functions were fitted to the data shown in figure 1H, using the least squares method; function coefficients giving the best fit to the data are shown in the top right of each panel. L: growth rates at different volumes were based on the line of maximum probability across the PDF (red); above $160 \mu\text{m}^3$, where the data were too sparse and the PDF became erratic, growth rates were kept constant (blue line). D, G, J, M: boxplots of simulated volumes at different iteration numbers, corresponding to C, F, I, L; for simulation parameters other than function coefficients, the default values used in Table S1 were used. E, H, K, N: frequency histograms of the deviation from the mean cell volume for simulations (sim) compared with the experimental data (exp), corresponding to C, F, I, L; for each set of values, data were pooled from five simulations (2000 cells); p -values are for the hypothesis that simulated and observed cell volumes had the same coefficients of variance (CV) (Levene's test on relative deviations from mean) [32].

Figure S3

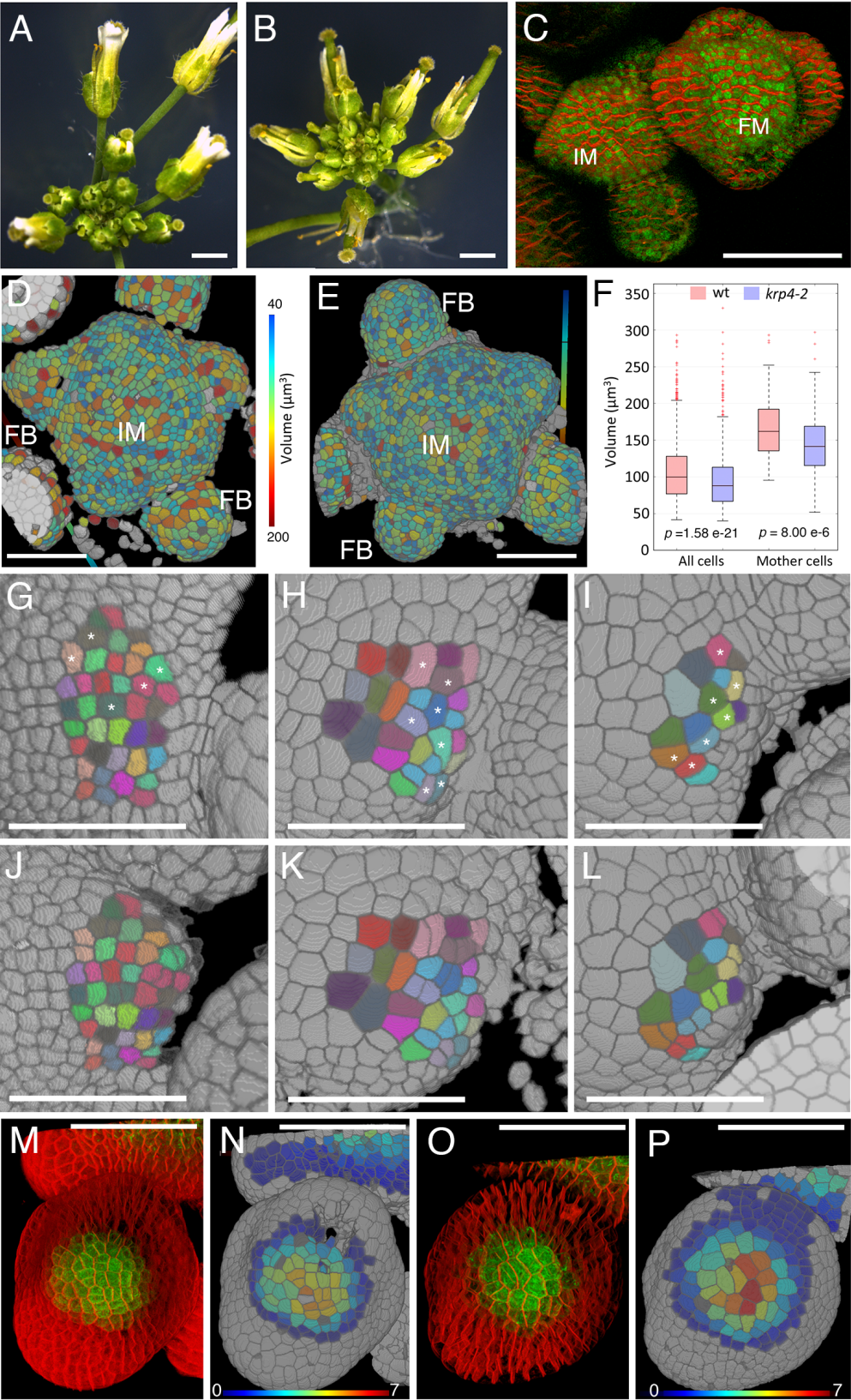


Figure S3 (related to Figure 3): *KRP4* controls meristem cell size.

A, B: inflorescence apices of *jag-2 krp4-2* (A) and *jag-2 krp4-2* transformed with *pKRP4:KRP4-GFP* (B); the KRP4-GFP fusion reverted the partial suppression of *jag-2* by *krp4-2* (11), resulting in a plant similar to the *jag-2* single mutant; C: 3D rendering of confocal images of a *krp4-2 pKRP4:KRP4-GFP* inflorescence apex, showing KRP4-GFP expression in the inflorescence meristem (IM) and floral meristem (FM). D, E: top view of segmented images of wild-type (D) and *krp4-2* (E) inflorescence apices, with cells colored by volume (colorbar between D and E); IM as in C, FB indicate floral buds. F: box plots of cell volumes for the wild type (red, 5 apices) and *krp4-2* (blue, 4 apices) for all cells (wt n = 1746; *krp4-2* n = 1416) or mother cells (wt n = 99; *krp4-2* n = 73); *p*-values are for equality of medians (Mann-Whitney test). G-L: additional replicates for the experiment showing recovery of cell sizes after developmentally transient expression of KRP4; segmented images show cells at the edge of the GFP-expressing region in the inflorescence meristem of *CLV3>>(GFP)* (G,J) and *CLV3>>(GFP, KRP4)* (H,K and I,L) at 0h (G,H,I) and 24h later (J,K,L); matching cells are in the same color and asterisks mark cells in G-I that divided after 24h. M-P: GFP expression and cell sizes in the *CLV3>>(GFP)* control (M,N) and in *CLV3>>(GFP, KRP4)* floral buds (O,P), shown in confocal images (M,O) and in segmented images (N,P) with cells labeled by GFP expression (relative to the median signal of GFP-positive cells in each bud, see color bars at the bottom of each panel); note the increased cell sizes in the central region the floral meristem in *CLV3>>(GFP, KRP4)*, but not in the peripheral region of the meristem and in organ primordia. Size bars: 1 mm (A, B), 50 μ m (C-P).

Figure S4



Figure S4 (related to Figure 4): Overall appearance of wild-type (wt) and *CLV3>>KRP4* inflorescences.

A, B: side view of wt (A) and *CLV3>>KRP4* (B) inflorescences at the stage when apices are dissected for imaging; size bars: 10 mm.

C, D: top view of wt (A) and *CLV3>>KRP4* (B); size bars = 1 mm

Table S1 (related to Figure 2): Parameter values for meristem cell growth

Values and confidence intervals were calculated from the data in Table_S2.csv, using the Python script statistical_analysis.py (see Materials and Methods and Supplemental_software). Column 2 indicates the parameter names used in simulations (script cell_growth_simulation.py, see Materials and Methods and Supplemental_software); default values used in the simulations are underlined.

Parameter name	Parameter in simulations	Value	95% confidence interval	n apices	n cells
Mean cell volume	v0	<u>105.6</u>	103.9 to 107.5	5	1746
Mean relative growth (24h)	gr24	<u>1.217</u>	1.209 to 1.225	5	1746
Standard deviation of cell division ratio	sigma	0.1067	<u>0.09419</u> to 0.1184	5	98
Local standard deviation of relative growth (LSDG)	-	0.1499	0.1465 to 0.1532	5	1843
Local standard deviation of volume ratios (LSDR)	-	0.06885	0.06758 to 0.07014	3	1902
Corrected LSDG	sdgr	0.1331	<u>0.1294</u> to 0.1369	5	1843
Slope of regression cell vol X growth rate	a	- 7.593 e-4	- 5.620 e-4 to - <u>9.574 e-4</u>	5	1746
Intercept of regression cell vol X growth rate	b	<u>1.297</u>	1.275 to 1.320	5	1746
Central zone mean cell volume	-	123.6	117.3 to 130.2	5	185
Central zone mean relative growth (24h)	-	1.262	1.240 to 1.284	5	185
Central zone LSDG	-	0.132	0.126 to 0.138	5	199

Central zone slope of regression vol X growth	-	- 7.183 e-4	-2.951 e-4 to - 11.54 e-4	5	185
Central zone intercept of regression vol X growth	-	1.351	1.275 to 1.320	5	185

Table S2 legend (related to Experimental Procedures):

Raw values and summary statistics for genotypes and treatments analyzed in Figures 1-3 and S1.

Lines 1 to 4 are used by the script `statistical_analysis.py` to read the data, perform statistical tests and produce boxplots (see Materials and Methods and Supplemental_software).

Lines 6 to 9 specify the data source and where it was used. The line “Source image folders” gives the names of image folders containing the replicates for each treatment (the folders can be found in <https://open-omero.nbi.ac.uk>, username “shared”, password “Op3n-4cc0unt”). “Source table type” specifies the cell data table used; `_a_cell_data_checked.csv` corresponds to the tables with matched cell data after manual quality control; `_cell_data.csv` corresponds to the tables with cell data from each single image. “Analyzed data in Figure/Table” indicates the figure or table where the data were used.

Lines 11 to 20 indicate how the data was filtered. “Variable” corresponds to the heading of the column in the cell data tables that contained the raw values; “Filter n” lines specify the headings of columns in the cell data tables that were used to select cells with values between at least “Filter n min” and less than “Filter n max”; for “ROI number”, 1 corresponds to the inflorescence meristem.

Lines 22 to 25 provide summary statistics for each treatment (n, median, mean and standard deviation). Lines 27 to 31 give summary statistics for each inflorescence apex. Raw values for each treatment are listed below line 33.

Table S3 legend (related to Experimental Procedures):

Floral organ numbers in wild-type, *krp4-2* and *AP1>>KRP4* plants. For each plant, flowers number 4 to 18 (in order of emergence) were dissected and each type of floral organ (Se: sepals; Pe: petals; St: stamens; Ca: carpels) were counted.

Supplemental_software (related to Experimental Procedures)

List of scripts included in Supplemental Software

Python scripts:

alignment_images.py

boxplot.py

bud_analysis_lib.py

cell_data_table.py

cell_growth_simulation.py

cell_layers.py

correct_matched_cells.py

delete_or_include_cells.py

heatmap.py

match_cells.py

neighbours_growth.py

optimise_segmentation.py

rib_zone.py

score_ER.py

select_ROIs.py

statistical_analysis.py

stats_tables.py

subtract_images.py

tiff.py

track_cell_divisions.py

watershed_segmentation.py

Fiji macros:

confocal_to_TIF.ijm

landmarks_3D.ijm

landmarks_alignment.ijm

match_cells.ijm

overlap_seg_signal.ijm

reset_LUT.ijm

Installing and using image analysis, simulation and statistical scripts

The scripts were written in Python 2.7.3 using an Apple computer running MacOS X 10.9.4 - small changes may be needed to install and run them on a different platform.

To install and run the image analysis scripts, expand the file Supplemental_software.zip on the Desktop, open the file 3D_meristem_analysis/Image_processing_instructions.pdf and follow the instructions. Fiji [S2] was used to visualize and interact with processed images, using macros included in Supplemental_software.

To run the statistics and simulation scripts, use a script editor to open the corresponding files (3D_meristem_analysis/python_scripts/statistical_analysis.py and

3D_meristem_analysis/python_scripts/cell_growth_simulation.py) and follow the instructions annotated in the section “Main programme”. Functions need to be copied and pasted from the scripts directly to a Python shell.

Supplemental Experimental Procedures

Overview of image analysis

The Supplemental_software file contains the annotated source code and detailed instructions on how to install and use the Python scripts and Fiji macros used for 3D image analysis. Briefly:

1. 3D segmentation used the morphological watershed algorithm [S1] implemented in SimpleITK, after Gaussian blurring with given sigma values for the x, y and z dimensions. Cell volumes were measured by counting voxels in each segmented cell.

2. To define regions of interest (inflorescence meristem and floral buds), four points per bud were manually selected around the base of 3 to 5 bud primordia. For each primordium, a sphere was drawn around the center of mass of the four landmark points and reaching up to the furthest point. The region of interest was defined as the section of this sphere above the best-fitting plane for the four points. The inflorescence meristem was defined in a similar way, using as landmarks the point closest to the meristem for each bud. The main axis of the inflorescence meristem was defined as the vector normal to the best-fitting plane for the meristem landmarks and crossing their center of mass.

3. To define cell layers, cells touching the image borders or facing the bottom of the image were masked, then binary erosion was used to identify cells in the outermost layer; cells considered abnormally thick for their layer (more than 1.3 times the median thickness) were masked and not attributed to any layer; cells already attributed to a layer were deleted and the process was repeated until all cells had been assigned to a layer.

4. To measure GFP, the signal was first corrected for diminishing intensity with increasing depth in the confocal stack, based on the FM4-64 signal within the boundaries of

segmented cells. To detect regions with GFP signal, a filtering element of 5 x 5 x 5 voxels was centered on each voxel; voxels were marked as positive if the median value within the filtering element exceeded a selected threshold value, which was kept constant in comparisons across genotypes. For each cell scored positive, the total GFP signal within the positive region was collected and normalized to the median intensity in all positive cells. In the case of ER-localised GFP (lines expressing *Op:ER-GFP*), the normalised signal was also divided by cell volume to allow comparison of signal density in genotypes with different cell volumes. For the weak nuclear signal detected in *pCUC1::CUC1-GFP* buds, the automatic selection of positive cells was checked and corrected manually.

5. To align cells across time course images, four landmarks were placed manually on matching cell vertices in 3D renderings of each confocal stack. These points were used for rigid registration, then for each cell in image 1, a sphere of given radius (5 or 8 μm) was drawn around the cell in the corresponding position in image 2. Within this sphere, the shape of each cell and its neighbors was compared with the shape of the initial cell and its neighbors in image 1 and the best match was selected. Cell divisions were detected when shape matches were better for pairs of fused cells in image 2 than for each cell separately. Images of the matched cells were used to manually identify and delete incorrect matches (2-10% of cells in each image pair).

6. To calculate the local standard deviation of growth (LSDG), binary dilation was used to find the immediate neighbors of each cell that had been matched between images 1 and 2. For each cell and neighbor that had been successfully matched across the two images, relative growth rates were calculated as the ratio between cell volumes in image 2 and image 1. The standard deviation of the relative growth rates (defined as the LSDG) was calculated if n was at least 3.

Overview of simulations

All simulations used functions defined in the Python script
/3D_meristem_analysis/python_scripts/cell_growth_simulation.py (Supplemental_software).

The functions were used directly in a Python shell, following the instructions annotated on the script.

The simulations `sim_a` described in Fig. 2A assumed that the cell cycle length T is constant for all cells and coincides with the number of hours taken to double the tissue volume, based on the measured cellular growth rates over 24 h. A typical simulation ran for 336 iterations, corresponding to approximately 4 cell cycles. Starting populations of unsynchronized cells with narrow volume distributions were created by allowing identical cells to grow uniformly for a random number of iterations between 0 and one cell cycle length, then normalizing cell volumes to the experimentally measured average. During subsequent cycles, cell growth rates were adjusted to cell volume at each iteration (based on the linear regression between experimentally measured cell volumes and growth rates) and were multiplied by a random variability factor k (based on the experimentally measured standard deviation of growth rates of neighboring cells), which was re-set at the start of each cell cycle. At the end of each cycle, normal noise in cell division was added based on the standard deviation of cell division ratios; if unequal division resulted in a daughter cell smaller than the minimum volume accepted in the experimental data (40 μm), both sister cells were removed from the simulation. After each time all cells were allowed to grow and divide for a full cycle, a random subset of cells was removed to maintain the population size around 400 (similar to the number of cells measured in one inflorescence apex).

Simulations `sim_b` described in Fig. 2A were as described above for `sim_a`, except that the number of cell growth iterations (corresponding to cell cycle length) of individual cells was adjusted to compensate for cell growth heterogeneity by dividing it by the growth variability factor k .

For both `sim_a` and `sim_b`, default parameter values (Table S1) were chosen to minimize cell variability (lower bounds of the 95% confidence intervals for the SD of cell division ratio and for the mean LSDG) and maximize the correction of cell volumes (most negative value in the 95% confidence interval for the slope of the regression between cell volumes and growth rates). Artifactual variability in growth rates introduced by imaging and image processing was estimated by independently imaging the same apices from different angles at a single time

point and processing the images as if they corresponded to different time points (Table S2, treatments 4-7). The resulting local standard deviation of volume ratios (LSDR, calculated as described above for the LSDG) was used to calculate the corrected LSDG (Table S1) as the square root of the difference between the squares of the measured mean LSDG and the mean LSDR.

During each simulation, cell volumes were collected every six iterations to produce the boxplots shown in Fig. 2C,D and in Fig. S2 D,G,J. To compare simulated and experimentally measured cell volume distributions (Fig. 2B), data were pooled from 5 simulations and from 3-5 inflorescence apices, and the *p*-values for equality of coefficients of variation were calculated as described above (Statistics). When simulations were run over a range of parameter values (Fig. S2 A, B), parameters that were not varied remained at the default values described above.

To test different functions for the relation between cell volume and growth rate, inverse, quadratic and logarithmic functions were fitted to the cell volume and growth rate data shown in Figure 1H, using the least squares method (source code in Supplemental Software, script /3D_meristem_analysis/python_scripts/statistical_analysis.py). The functions and best-fitting coefficients were used instead of the linear function in simulation sim_a as described above to produce the data shown in Figure S2 C-K. The probability density function for growth rate as a function of cell volume was calculated using Gaussian kernel density (source code in statistical_analysis.py).

Supplemental References

- S1. Soille, P. (2013). Morphological image analysis: principles and applications, (Springer Science & Business Media).
- S2. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., and Schmid, B. (2012). Fiji: an open-source platform for biological-image analysis. Nature Methods 9, 676-682.