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Supplemental Information

SPIRAL2 Determines Plant

Microtubule Organization

by Modulating Microtubule Severing

Raymond Wightman, Guillaume Chomicki, Manoj Kumar, Paul Carr,
and Simon R. Turner

Supplemental Inventory

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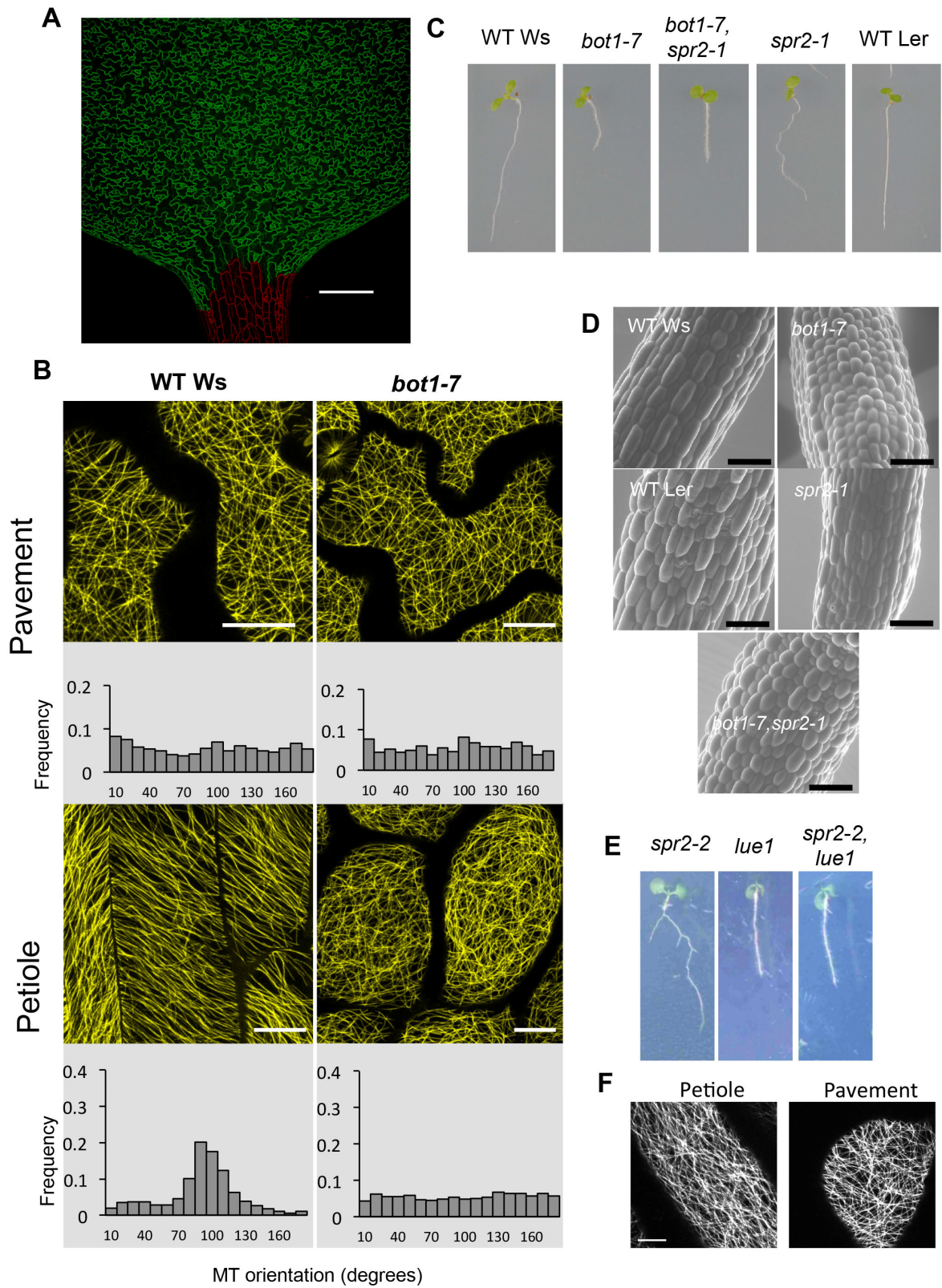


Figure S1.

Figure S1. Cell Shape and Microtubule Organization in the Cotyledon Epidermis, Related to Figure 1

- (A) Cell outlines in the epidermis of an Arabidopsis cotyledon visualized using a plasma membrane reporter attached to YFP with high levels of expression maintained at the epidermis using *pML1::LhG4*. Outlines of petioles cells are colored red while those of pavement cells are green. Bar = 2 μ m.
- (B) Microtubule alignment in wild-type Ws and *bot1-7* mutant pavement and petiole cells. Graphs show normalized distribution of microtubule alignment. For pavement, the angles of 1145 MTs have been measured in a total of 25 cells; for petiole, 1073 MTs in 35 cells have been measured. Bars = 20 μ m.
- (C) Phenotype *spr2-1* and *bot1-7* mutants seedling grown side by side on agar plates.
- (D) Scanning electron micrographs showing cell from Arabidopsis seedling hypocotyls. Bar = 100 μ m.
- (E) Representative seedlings grown on agar plates of *lue1*, *spr2-2* mutant combinations.
- (F) Microtubule organization in *lue1,spr2-2* cotyledon epidermal cells. Note the lack of orientation in petiole cells and the decrease in lobe formation in pavement cells.

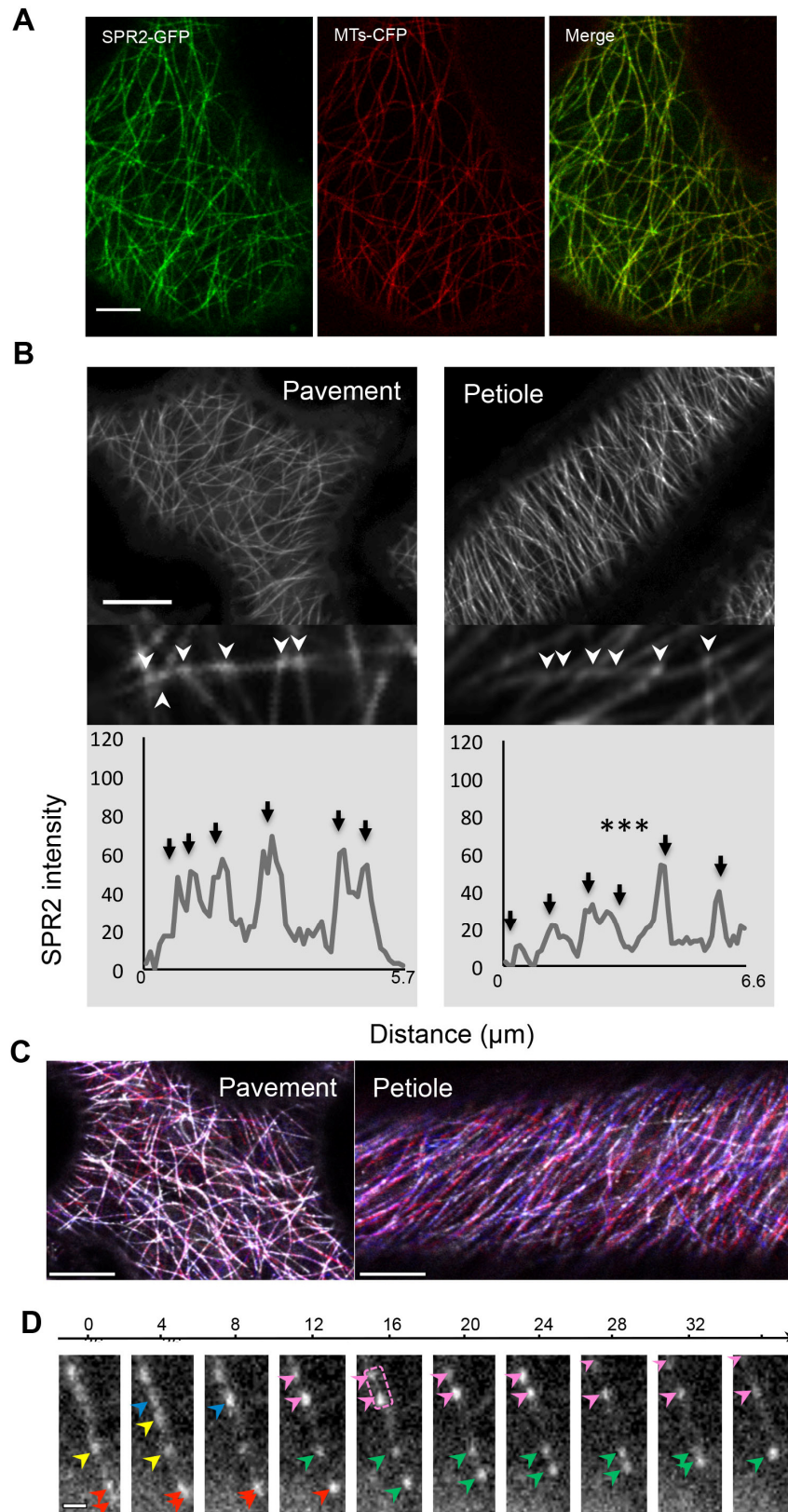


Figure S2.

Figure S2. SPR2 Dynamics, Related to Figure 3

(A) SPR2 localizes along microtubule lattice. Confocal images of pavement cells taken from a line with a SPR2-GFP fusion construct transformed in a line containing a CFP microtubule reporter. Bar = 5 μm .

(B) Average intensity of 200 time lapse images at one frame per second of a pavement cell and petiole cell from a SPR2-GFP line. Both datasets were from the same seedling taken using identical settings. Bars = 20 μm . Middle panels show a close up of an individual microtubule from each cell type and the corresponding SPR2 intensity along the microtubule is shown below (The minimum pixel value along the microtubule was set to zero). Arrowheads in pavement cells highlight sites of microtubule crossover and correspond to the peaks of SPR2 intensity (arrows).

(C) SPR2 dynamics in pavement and petiole cells. Three images have been taken 10 sec apart, colored in white (0 sec), blue (10 sec) and red (20 sec) and merged. Different colors along a single MT depict SPR2 movement. Note that in pavements cells most stable MTs are entirely white, meaning that there little movement of SPR2-GFP particles. In contrast in petiole cells MTs are multi-colored, indicating that SPR2-GFP particles have moved between successive images. Bars = 10 μm .

(D) The basic mechanisms of SPR2 movement in petiole cells. SPR2-GFP particles can fuse together (red, blue and green). SPR2-GFP can also split in two distinct particles (yellow). Another type of interaction between SPR2-GFP particles is depicted in purple whereby two particles next to each other do not merge but exchange particle material (pink frame at 16 sec) that results in the movement of SPR2-GFP particles in relay. Bar = 1 μm .

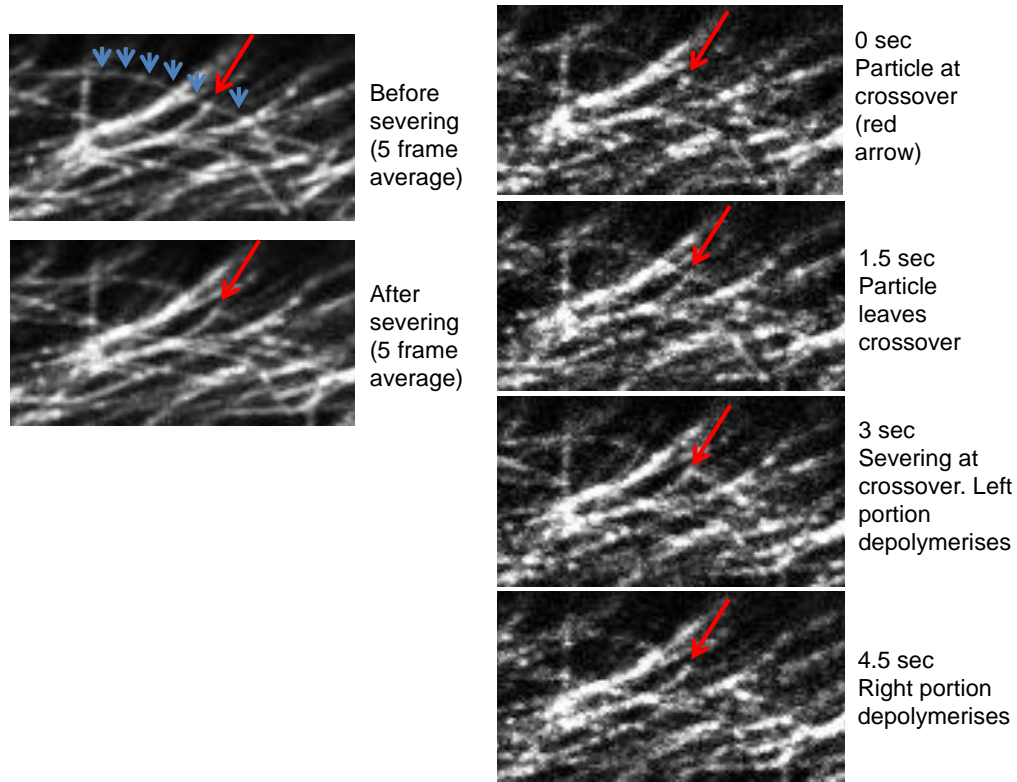


Figure S3. SPR2 Dynamics during Microtubule Severing in Petiole Cell, Related to Figure 2

SPR2-GFP localization and microtubule depolymerization at a site of microtubule severing in a wild-type petiole cell.

Table S1. Parameters Used to Quantify Microtubule Severing, Related to Figure 2A

	WT Ler		<i>spiral2-1</i>		WT Ws		<i>botero1-7</i>	
	PAV	PET	PAV	PET	PAV	PET	PAV	PET
Total severing events	1	26	48	85	1	22	0	0
Severing at crossover events	1	24	42	78	1	20	0	0
Area analyzed (cm²)	24.05	16.69	19.41	28.06	14.01	14.47	12.05	8.90
Total video time (min)	19	23	43	45	16	13	33	35
Number of cells observed	26	16	21	26	4	8	6	6

Supplemental Experimental Procedures

Transgenic and Mutant Arabidopsis Lines

SPR2:GFP line, as *SPR2* GFP fusion transformed into *spr2-2*, was a courtesy of Professor Takahashi Hashimoto. *spr2-1* was crossed with *bot1-7* in order to obtain a double mutant. F2 plants that exhibited a *botero* phenotype were genotyped to check for the presence the *spr2-1* mutant allele. For *spr2-1* genotyping, a region that encompasses the single adenine deletion of *spr2-1* was amplified using primers *SPR21-fwd* (5'-GCAATCCGTGGAAGTAGTAGTTCCTCGT-3') and *SPR21-rev* (5'-CATTATACTTTCCAGTTGGATAGTTAGC-3'). Sequencing of the amplified region was carried out using primer *SPR21-fwd* and the data compared with that of WT genomic DNA. The sequence traces showed the presence of a peak corresponding to adenine for WT samples, overlapping adenine and guanine peaks for heterozygous *spr2-1* and a single guanine peak for homozygous *spr2-1*. For microtubule visualization, a YFP-MBD reporter [10] was transformed into wild-type Landsberg (WT Ler), WT Wassilewskija (WT Ws), *spr2-1*, *bot1-7*, *spr2-1 bot1-7* and *spr2-2 lue1* using the floral dip method. To visualise *SPR2* on microtubules, a CFP-MBD reporter was transformed into the *SPR2-GFP* line.

Plant Growth Conditions for Microscopy

Seeds were sterilized in a solution containing 30% (v/v) sodium hypochlorite and 1% Triton X-100 during 15 minutes and further rinsed five times before suspension in 0.1% (w/v) sterile agar (Sigma-Aldrich) solution. Seeds were placed on 1.5% (w/v) agar plates containing 2.2 g/L MS supplemented with Gamborg B5 vitamins (Melford Laboratories). The seeds were kept in the dark for 2 days at 4°C and subsequently placed in a growth chamber (Gallenkamp) under constant light ($\sim 100 \mu\text{mol.m}^{-2}.\text{sec}^{-1}$) at 22°C. Microscopy was carried out on cotyledons of 6 day old seedlings.

Imaging of Microtubule Dynamics and SPR2:GFP

For the imaging of both microtubules and *SPR2:GFP*, cotyledons were cut in half and mounted in water. Confocal microscopy of the YFP-MBD reporter was either carried out on a Leica TCS SP5 upright equipped with HyD detectors or a Zeiss LSM780 upright confocal microscope. YFP was excited at 514 nm with the pinhole diameter set at 1 airy unit and images acquired through a 63x NA 1.4 oil immersion objective lens. Z-stacks were taken at an optimal interval using manufacturer's settings. For the analysis of microtubule dynamics and severing, all frames were taken at 1 second intervals. *SPR2:GFP* imaging was performed on a Leica TCS SP5 confocal (488nm excitation and 4x line average), Zeiss LSM 780 (488nm, a 2 line average). Each frame was typically a 3 frame average. Epifluorescence microscopy of YFP-MBD and *SPR2-GFP* was carried out on a Leica DMR microscope equipped with a SPOT Xplorer 4MP camera (Image Solutions, UK) and a Leica HCX PL APO X100 oil NA 1.4-0.7 objective.

Scanning Electron Microscopy

Hypocotyl images were acquired on a FEI Quanta 200 environmental scanning electron microscope equipped with a peltier stage held at 5°C. The beam HV was set to 20.0 kV, spot size was set to 4 and chamber pressure was maintained at about 5.5 Torr. Hypocotyls were placed on the stage and water droplets were placed around the samples to maintain hydration during the pump down sequence.

Image Analysis

Image analysis was performed in ImageJ (<http://rsb.info.nih.gov/ij>).

Angle Measurements

Angle measurements of microtubules were performed using the measure tool of ImageJ, which takes 0 degree as reference angle and were normalized similarly to the method used by Yao et al. [27]. The initial angles measured were between -180 and +180 and consequently negative angle values were normalized by adding 180 degrees. For WT pavement cells and *bot1-7* pavement and petiole cells, no further normalization was applied since the MT arrays are unaligned. However, for WT petiole cells and *spr2-1* pavement and petiole cells, it was necessary to ensure that for all cells measured, each line were measured using the same array orientation relative to the reference angle. Furthermore, for visualization purposes, the alignment orientation of the array was set at 90 degrees. Henceforth, final angles (A_{final}) were obtained from the measured angles ($A_{measured}$) using equation (S1); all angles used were positive.

$$(S1) A_{final} = A_{measured} - (A_{array} - 90)$$

The number of cells and microtubules (MTs) recorded for the analysis of microtubule angle distribution is as follows: *Pavement cells*. WT Ler, Cells: 38; MTs: 1013. *spr2-1*, Cells: 32; MTs: 1023. *bot1-7 X spr2-1*, Cells: 22; MTs: 1098. *Petiole cells*. WT Ler, Cells: 34; MTs: 1228. *spr2-1*, Cells: 27; MTs: 1012. *bot1-7 X spr2-1*, Cells: 20; MTs: 1020.

Severing Quantification

Severing events were recorded using the point picker tool of ImageJ. *boterol-7* (*bot1-7*), which does not possess a functional katanin, was used as a negative control [7]. In order to determine accurately the level of severing in different cell types, the total number of severing events in all cells recorded for a particular cell type was normalized to acquisition time (in minutes) and visible area (cm^2) (Table S1).

SPR2:GFP Mobility

For the analysis of SPR2-GFP particles dynamicity, kymograph analysis was performed using the MultipleKymograph tool of ImageJ. The quantitative analysis of SPR2-GFP particle dynamicity was also performed in ImageJ. The (x, y) position of single SPR2-GFP particles along MTs was manually tracked, using the measure tool of ImageJ, until the particle underwent a fusion/merging with another SPR2-GFP particle, was disrupted by microtubule dynamics or became unrecognizable for technical reasons. The distance travelled during the tracking of each particle was then divided by the corresponding time of the tracking in order to obtain the dynamicity. For petiole cells the mean SPR2-GFP tracking time was 25.7 seconds and 75.9 seconds for pavement cells. A total of 253 particles in 18 cells were recorded for pavement cells and 258 particles in 16 cells for petiole cells. Values are expressed with the associated standard error.

Movie Image Manipulations

Movie S4 was processed with the running z-projector plugin (written by Nico Stuurman) of ImageJ to reduce noise. Movie S5 (right panel) has been processed with a Gaussian filter using a 1 pixel radius and has undergone further processing using contrast enhancement and the fire look

up table (ImageJ). Both panels have had the resolution increased 4-fold without interpolation to aid annotation. For movies, S6 and S7, resolution has been increased with bilinear interpolation to aid visualization and annotation.

SPR2 Intensity

SPRAL2 intensity along microtubules or specifically at crossover sites was measured using the plot profile tool of image J. Crossover intensity was measured after subtraction of MT (basal) signal.

Crossover Type Distribution

For each cell types, 5 representative cells were selected. Within each of these cells, a rectangular zone was chosen and all crossovers were counted per category with the help of the point picker plugin of ImageJ. The total number of crossover recorded for each cell type is as follow: WT Ws [pavement cells: $n = 833$ crossovers; petiole cells: $n = 578$]. *bot1-7* [pavement cells: $n = 714$ crossovers; petiole cells : $n = 760$]. WT Ler [pavement cells: $n = 695$ crossovers; petiole cells : $n = 531$]. *spr2-1* [pavement cells: $n = 601$ crossovers; petiole cells : $n = 600$]. *bot1-7 X spr2-1* [pavement cells: $n = 520$ crossovers; petiole cells : $n = 696$].

Correlation of Severing Frequency and Crossover Density

In order to determine the relationship between severing frequency, (i.e. katanin activity) and crossover density (i.e. substrate concentration), the two parameters were jointly quantified in severing active cells of WT Ler petiole. Each cell was subdivided in distinct subpopulations, i.e. arbitrary zones with seemingly distinct levels of MT order, as shown in Fig. S4A. Crossover density was measured in the distinct delimited subpopulations on average intensity frames. Severing frequency was quantified as for the rest of the study.

Statistical Analysis

Analyses were all performed using R (the R foundation for statistical computing). The significance of tests performed in this study was reported as ‘*’ for a P-value <0.05; ‘**’ for a P-value <0.01; and ‘***’ for a P-value <0.001. Significance of the difference in angle distribution (Fig. 1) of the different cell types and mutants was evaluated with Fmax tests (Hartley’s test); null hypothesis (H_0): true ratio of variances equal to 1. Differences in severing frequencies (Fig. 2A) were confirmed by two-sample t-tests; null hypothesis (H_0): difference of means is null. Because the SPR2-GFP particle distribution was too far from a normal distribution and homoscedascity was not verified, a non-parametric test was used. The Kolmogorov-Smirnov test confirmed statistical significance of the distribution; null hypothesis (H_0): the two samples have the same distribution. The distribution of SPR2 intensity in pavement and petiole cells was also probed using a Kolmogorov-Smirnov test.