Supplementary Information for

EXPANSIN A1-mediated radial swelling of pericycle cells positions anticlinal cell divisions during lateral root initiation

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This PDF file includes:

SI Supplemental Figures S1 to S14
SI Materials and Methods
SI Supplemental Table S1
SI Captions for Supplemental Datasets and Movies

Other supplementary materials for this manuscript include the following:

SI Datasets S1-S2
SI Supplemental Movies S1-S3

www.pnas.org/cgi/doi/10.1073/pnas.1820882116

SI SUPPLEMENTAL FIGURES

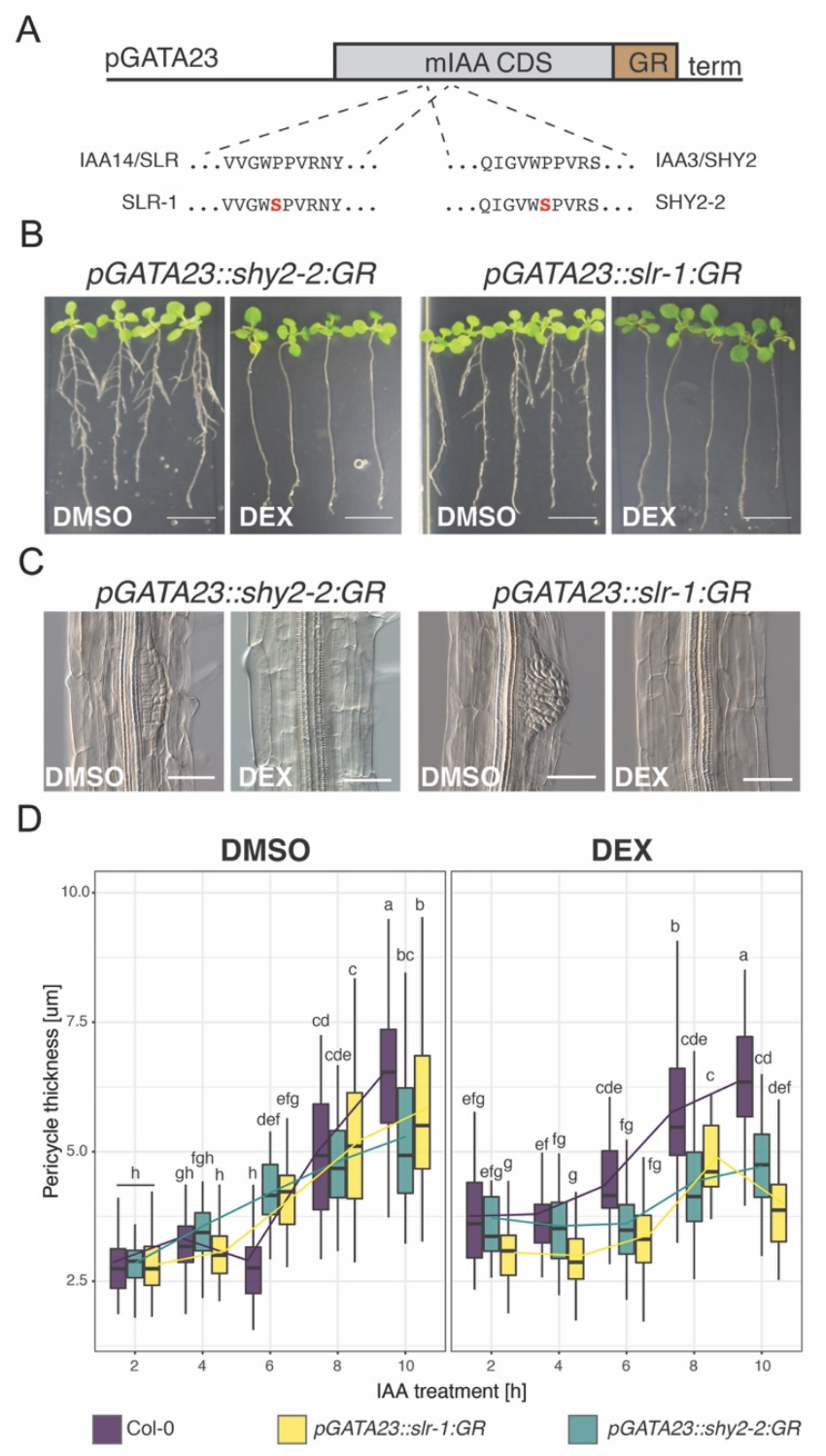

Figure S1. Radial swelling and lateral root formation are impaired by inhibition of auxin signaling in founder cells. (A) Schematic of the IAA14/SLR:GR and IAA3/SHY2:GR fusions used. **(B**) 7-day-old plants expressing non-degradable versions of the AUX/IAAs IAA14 (*slr-1*) or IAA3 (*shy2-2*) fused to the glucocorticoid receptor domain (*slr-1*:GR or *shy2-2*:GR) under

the control of the pericycle and founder cell specific GATA23 promoter do not have emerged lateral roots when grown on dexamethasone (DEX) but do on control medium (DMSO). Scale bar, 1 cm. **(C)** DEX treatment blocks lateral root initiation in lines described in (B). Scale bar, 50 µm. **(D)** Quantification of pericycle thickness in wild type (Col-0), *pGATA23::shy2-2:GR* and *pGATA23::slr-1:GR* plants synchronously induced for lateral root initiation after 2, 4, 6, 8 and 10 h of auxin (IAA) treatment. Boxplots represent distributions of pericycle thickness (µm) measured in 17 to 89 cells. Comparison between samples was performed using ANOVA and Tukey's HSD. Samples with the same letters do not significantly differ (α=0.05).

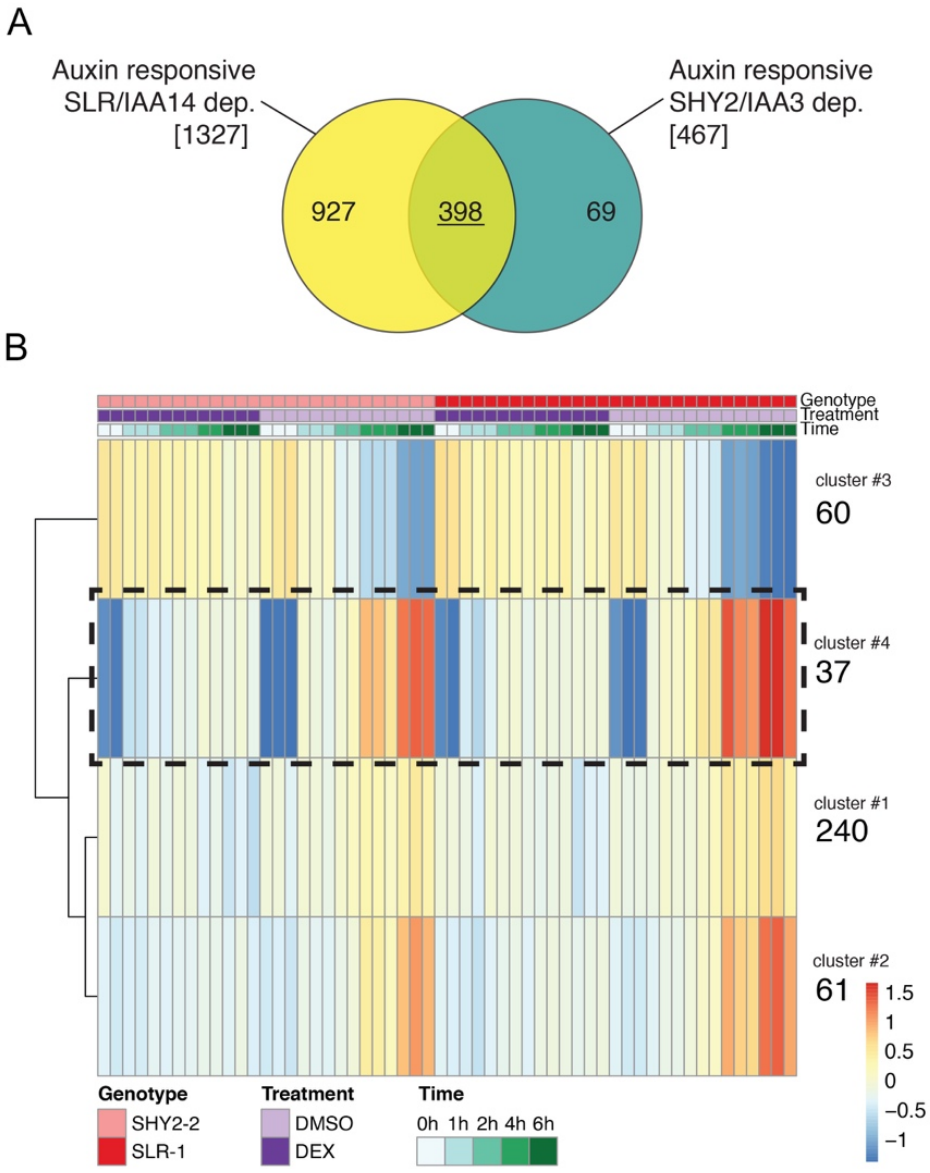

Figure S2. Transcriptome analysis of plants synchronously induced for lateral root initiation after 0, 1, 2, 4 and 6 h of auxin treatment. (A) Venn diagram presenting the overlap between the auxin responsive genes of which the expression is dependent on SLR/IAA14 and SHY/IAA3. Differentially expressed genes were identified as having log2 fold change >1 at FDR≤0.01. This comparison revealed that 398 differentially expressed genes are commonly regulated by SLR/IAA14 and SHY/IAA3. **(B)** Heatmap of normalized expression of the 398 genes commonly regulated. Genes group in four clusters. Clusters #1 and #2 are genes repressed by auxin in a SLR/IAA14 and SHY/IAA3-dependent manner. Clusters #3 and #4 contain genes induced by auxin in a SLR/IAA14 and SHY/IAA3-dependent manner. Cluster #4 (dashed box) contains genes that are highly upregulated by auxin in an IAA14 and IAA3-dependent way.

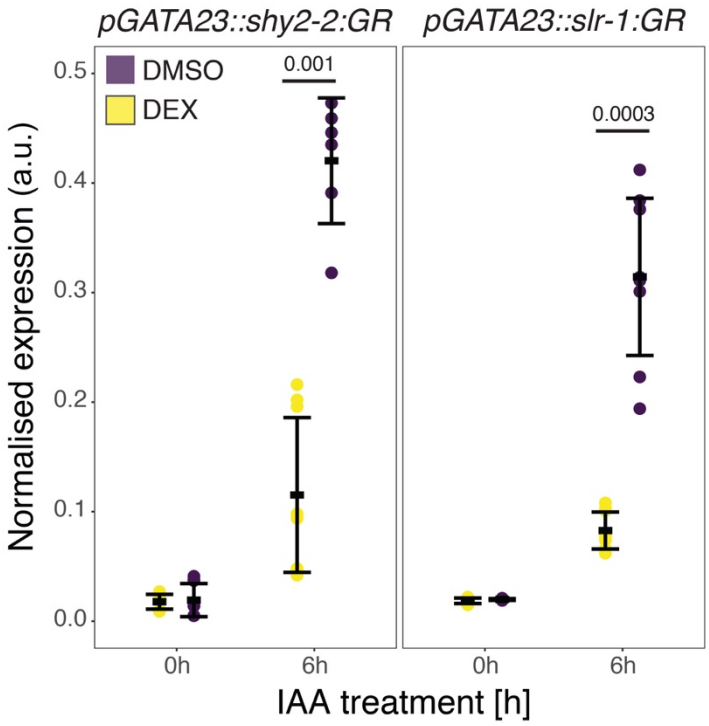

Figure S3. qPCR analysis of *EXPA1* expression in response to auxin treatment and inhibition of SLR/IAA14 or SHY/IAA3 activity. Levels of *EXPA1* mRNA were determined at 0 and 6 h after auxin treatment (10 µM IAA) upon inhibition of SHY2 or SLR activity (DEX) or in control conditions (DMSO). *EXPA1* levels are normalized to those of AT1G13320 (1). Each dot is an individual biological replicate. Bars represent the mean and standard deviation of the distribution. Statistical significance was evaluated by the Kruskal-Wallis test and p values are indicated. a.u., arbitrary units.

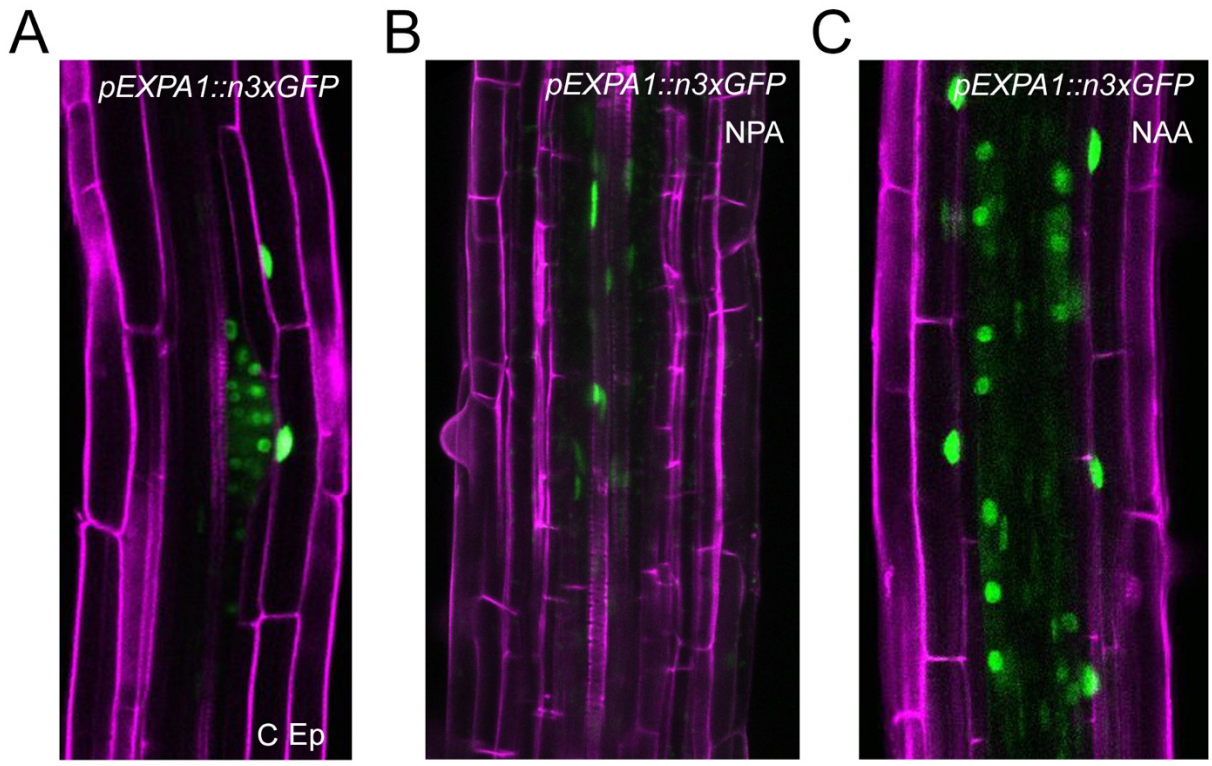

Figure S4. *pEXPA1::n3xGFP* expression during lateral root formation. (A) *pEXPA1::n3xGFP* expression in the cortical cells overlaying the lateral root primordium. C, cortex; Ep, epidermis. **(B-C)** *pEXPA1::n3xGFP* expression in 3 days post germination roots germinated and grown on the auxin transport inhibitor 10 µM NPA (B) and following transfer from 10 µM NPA to 10 µM NAA for 6 h (C). Cell outline visualized by staining with propidium iodide.

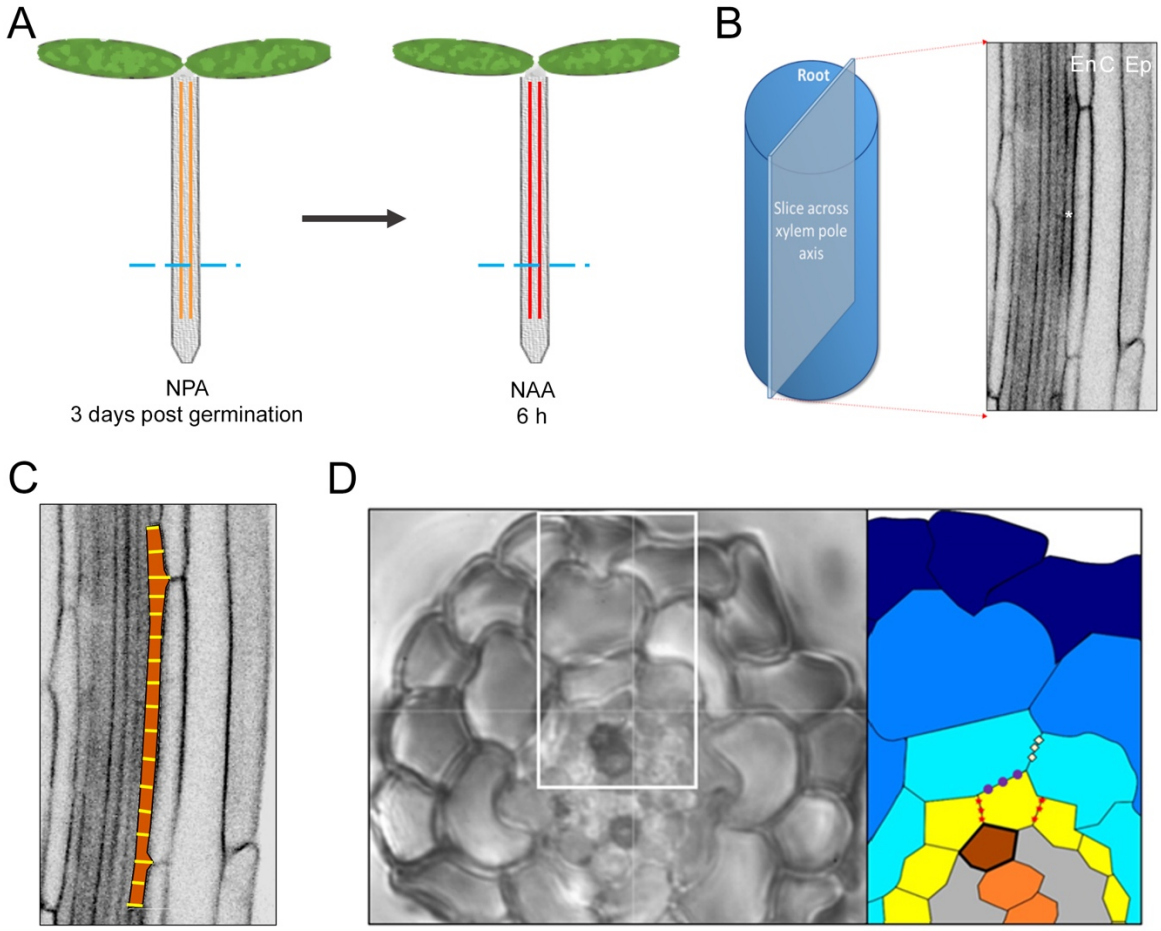

Figure S5. Information on experimental set-ups and measurements. (A) Schematic of the lateral root induction system used for cell width measurements. Seedlings were exposed to the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA), followed by transfer of seedlings to the synthetic auxin 1-naphthaleneacetic acid (NAA) for 6 h. Vertical parallel lines represent the pericycle with red indicative of auxin induction in the pericycle that drives lateral root initiation, the dotted blue lines represent the region where the cell measurements were made (see B). **(B)** Illustration of root confocal slice used for cell width measurements. *, pericycle; En, endodermis; C, cortex; Ep, epidermis. **(C)** Indication of measurement approach to determine pericycle cell (orange) width by averaging multiple positions along the pericycle cell (yellow lines). **(D)** *Arabidopsis* root cross section with white box marking region used for schematic. Schematic of root cross section with the pericycle in yellow and xylem pole pericycle-pericycle junctions marked as red stars, pericycle-endodermis junctions as purple circles and endodermis-endodermis junction as yellow squares.

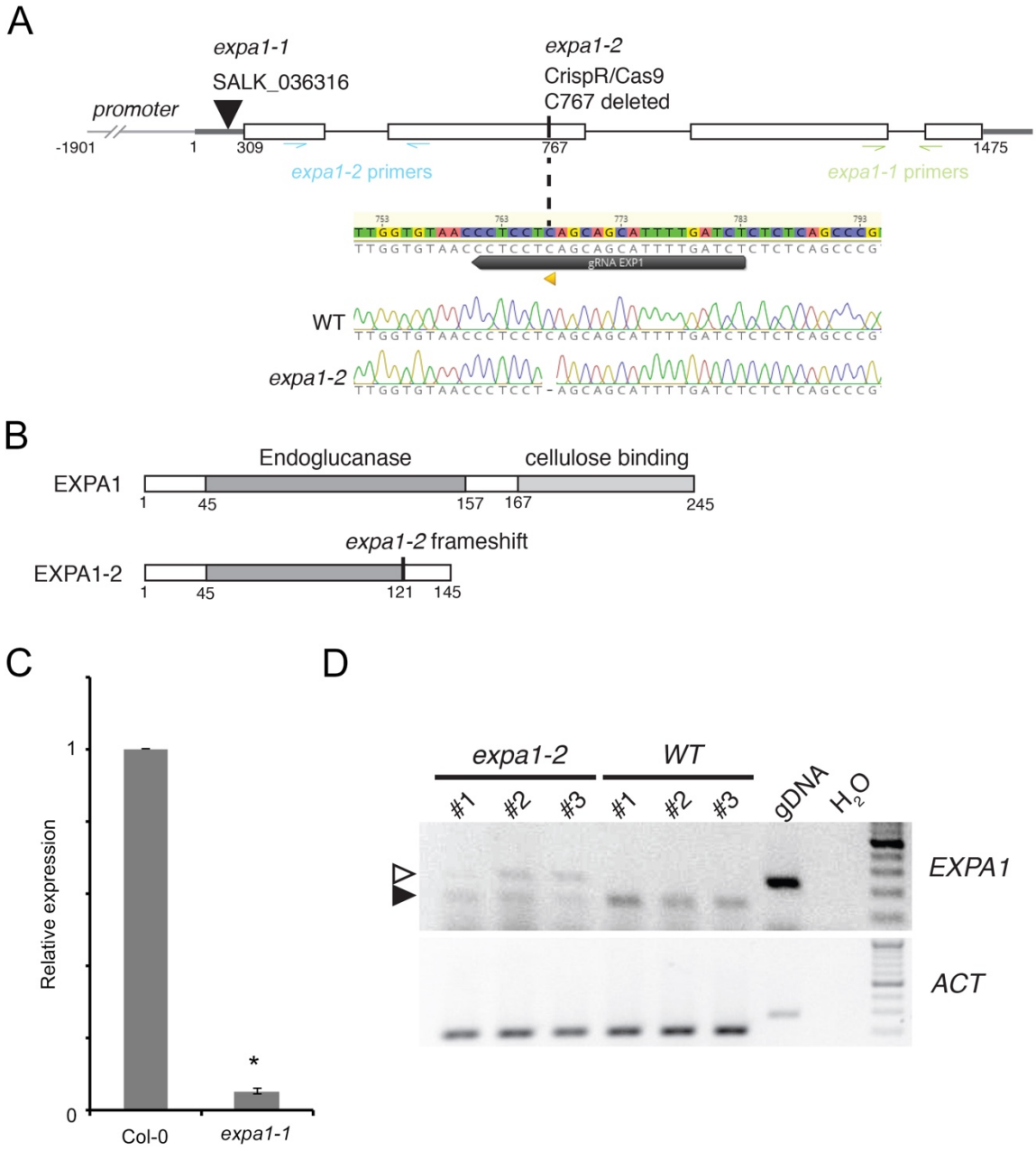

Figure S6. Information on *expa1* alleles. (A) Schematic representation of the *EXPA1* genomic region, indicating the position of the T-DNA insert (60 bp upstream of the transcriptional start site in the 5′ UTR region of *EXPA1*) in *expa1-1* and the CRISPR-Cas9 edit in *expa1-2*. UTRs, dark grey lines. Position of the primers used for (q)RT-PCR are indicated. **(B)** Schematic representation of EXPA1 and the truncated EXPA1-2 allele. The endoglucanase and cellulose binding domains are indicated by grey boxes. **(C)** qRT-PCR analysis of *EXPA1* expression in *expa1-1*. Average of 3-4 biological replicates ± standard error. Statistical significance (Student's t-test) compared with Col-0 is indicated: *, p-value < 0.01. **(D)** RT-PCR analysis of *EXPA1* expression in *expa1-2* and co-segregating wild type (WT). In the *expa1-2* allele, non-

spliced (237 bp, open arrowhead) and spliced (142 bp, closed arrowhead) versions of *EXPA1* mRNA are detected.

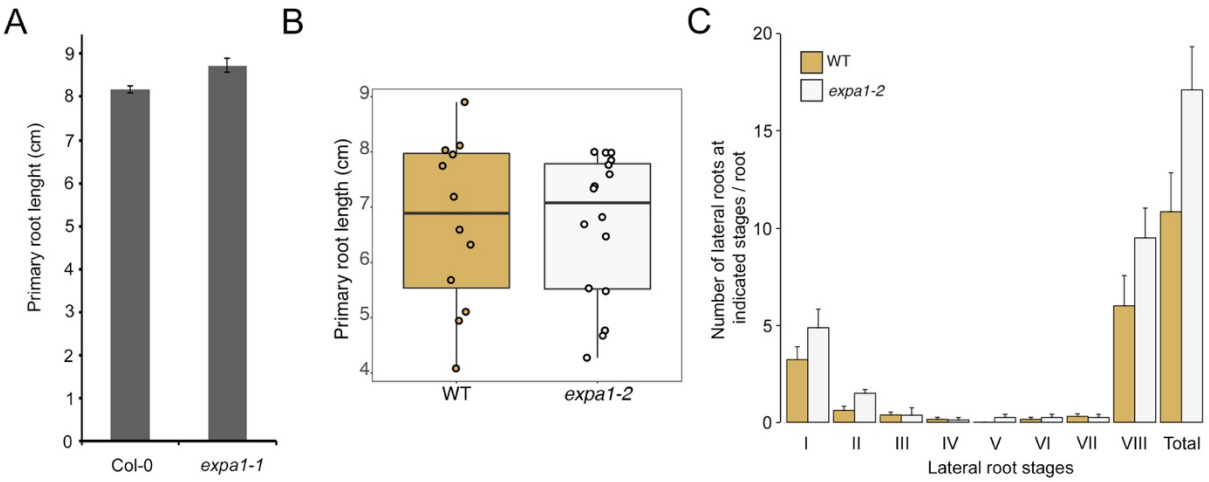

Figure S7. Additional measurements on *expa1-1* and *expa1-2*. (A) Primary root length of Col-0 and *expa1-1* at 10 days after germination. Student`s t-test gave no significant difference. **(B)** Primary root length of Col-0 and *expa1-2* at 9 days after germination. Student`s t-test gave no significant difference. **(C)** Lateral root staging of Col-0 and *expa1-2* roots at 7 days after germination. Bars show the average of 8 to 13 roots ± standard error. No statistical difference (using Student's t-test) was found when compared with co-segregating wild type (WT). VIII, all emerged lateral roots. Total, all lateral root stages combined.

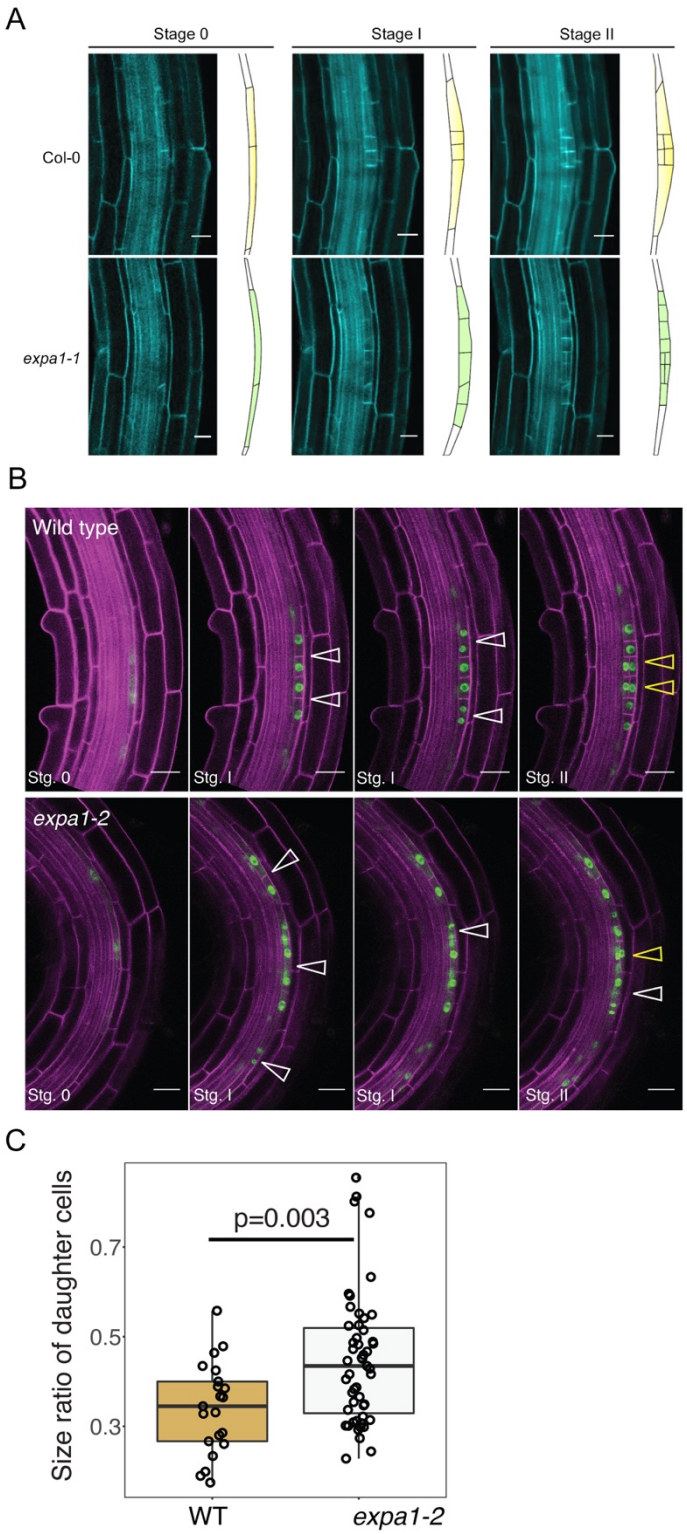

Figure S8. Representative images of cell division patterns in *expa1-1* and *expa1-2*. (A) Visualization of progression through early lateral root development stages in roots of 3 days post germination seedlings that were imaged 12 h after gravitropic root bending in wild type (Col-0) and *expa1-1*. Walls were visualized using the plasma membrane marker *pUBQ10::EYFP:NPSN12* (referred to as WAVE131YFP). **(B)** Confocal time lapse recording

of lateral root initiation in wild type (+/+) or *expa1-2* mutants (*expa1-2/expa1-2*). Nuclei of primed founder cells are labelled in green (*pGATA23::H2B:3xGFP*) and cell contour in magenta (*pUB10::PIP1;4:3xmCHERRY*). Arrowheads indicate the occurrence of anticlinal cell divisions (white) preceding the first periclinal cell division (yellow). Scale bar: 25 µm. See also **Supplemental movie S2**. **(C)** Ratio of length of the two daughter cells of the first anticlinal cell division. A ratio of 1 indicates a symmetric cell division.

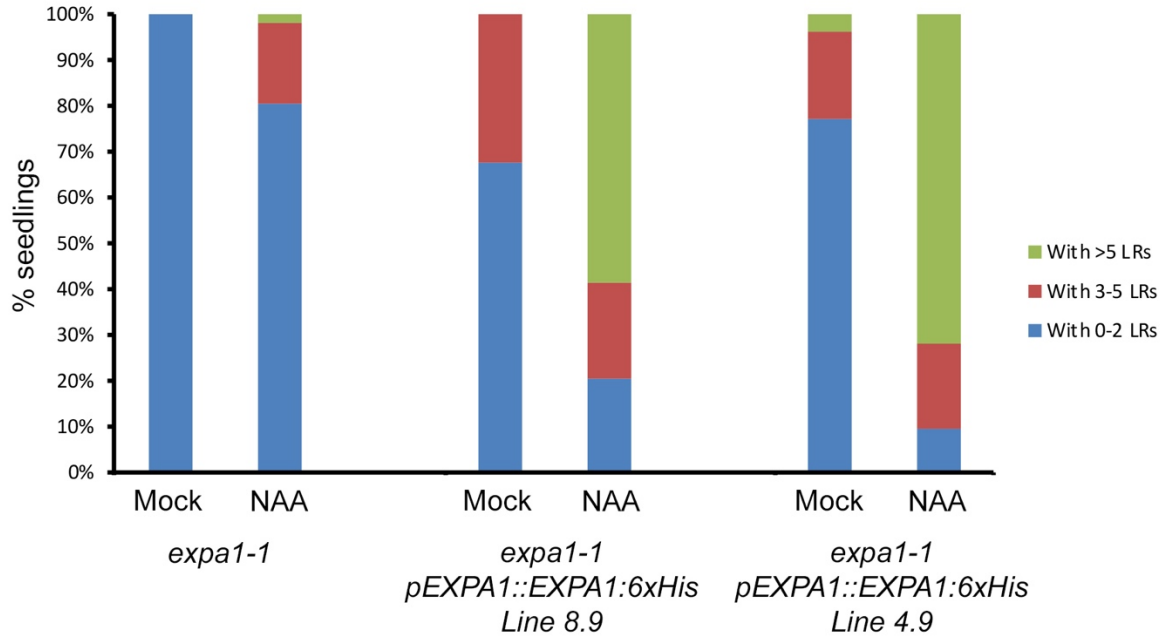

Figure S9. Auxin-induced lateral root formation in *expa1-1*. The fraction of seedling roots with indicated number of emerged lateral roots (LRs) at 5 days after germination upon growing on 100 nM NAA for *expa1-1* and *expa1-1 pEXPA1::EXPA1:6xHIS* control lines. ANOVA with Tukey post hoc test ($p < 0.01$) revealed a significant difference for line:treatment.

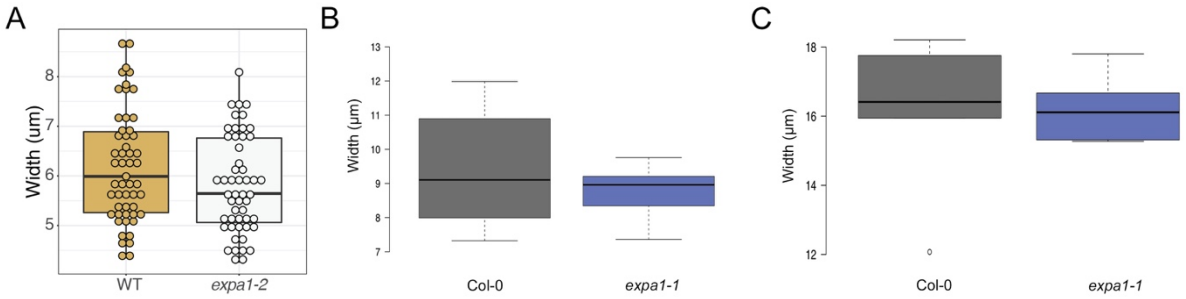

Figure S10. (A) Boxplot of naïve pericycle cell width for *expa1-2* and co-segregating wild type at 5 days after germination. At least 50 cells were measured. There is no statistical difference (Student's t-test) compared with Col-0 at p-value < 0.05. **(B-C)** Boxplots of endodermis (B) and cortex cell width (C) in Col-0 and *expa1-1* roots of seedlings at 3 days after germination on 10 µM NPA. Data from 6-8 roots with at least 8 cells per genotype. There is no statistical difference (Student's t-test) compared with Col-0 at p-value < 0.05.

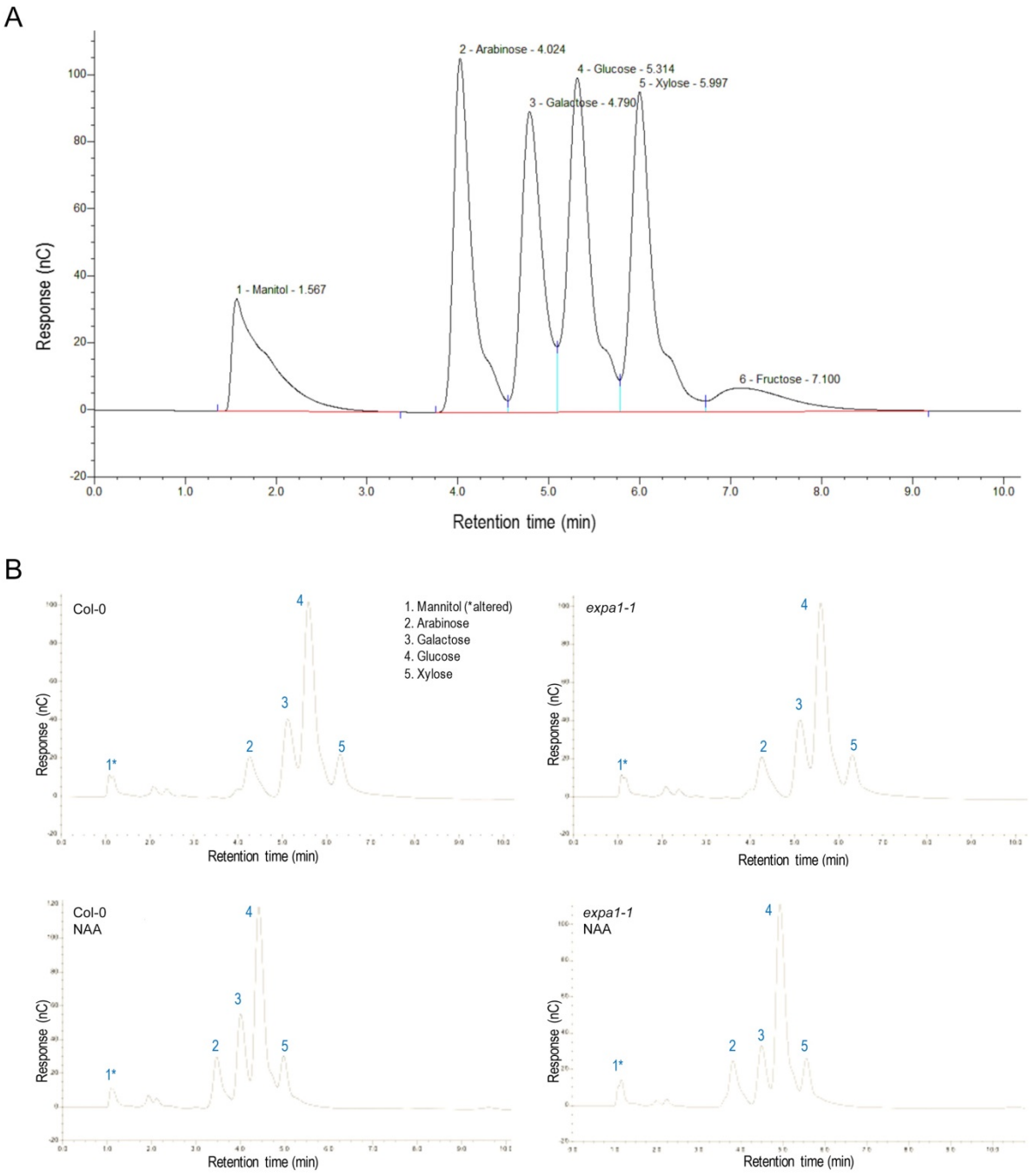

Figure S11. Information related to monosaccharide analyses. (A) Separation of monosaccharide sugar standards analyzed by High Performance Anion Exchange Chromatography - Pulsed Amperometric Detection (HPAEC-PAD) analysis. Peaks represent the sugars present and the area under the peak allows quantification of the amount of sugar based on standards that have been run through the column first to use for quantitative comparison. **(B)** Representative chromatogram of sugar monomers of acid hydrolyzed whole roots of Col-0 and *expa1-1* with and without 6 h 10 µM NAA treatment.

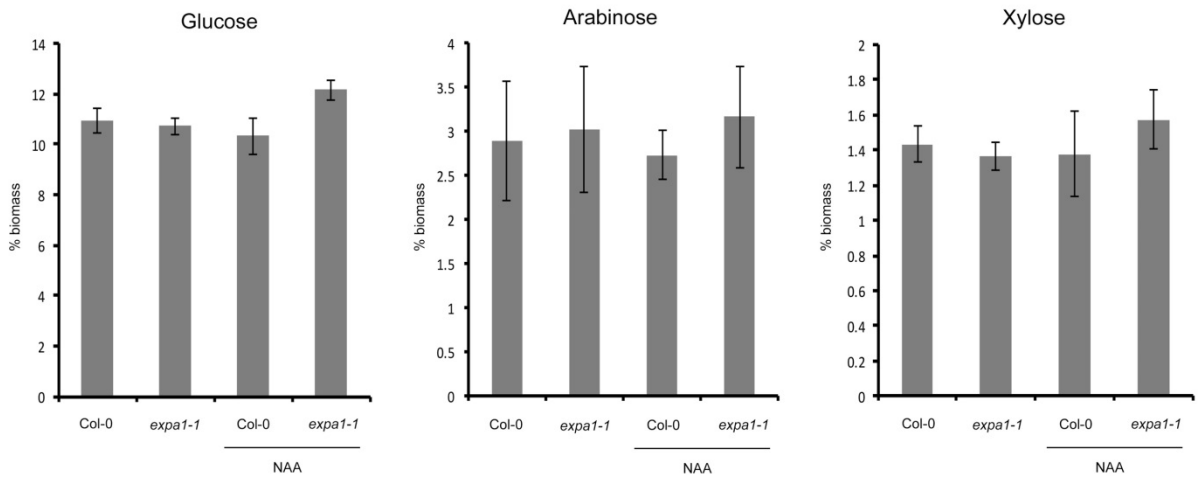

Figure S12. Monosaccharide sugar analysis. Monosaccharide sugar analysis on hydrolyzed Col-0 and *expa1-1* roots at 7 days after germination with and without 6 h of 10 µM NAA treatment using High Performance Anion Exchange Chromatography - Pulsed Amperometric Detection (HPAEC-PAD). Average of two independent biological replicates are shown, each with 2-3 technical replicates, ± standard error. ANOVA with Tukey post hoc test ($p < 0.05$) revealed a significant difference for line:treatment.

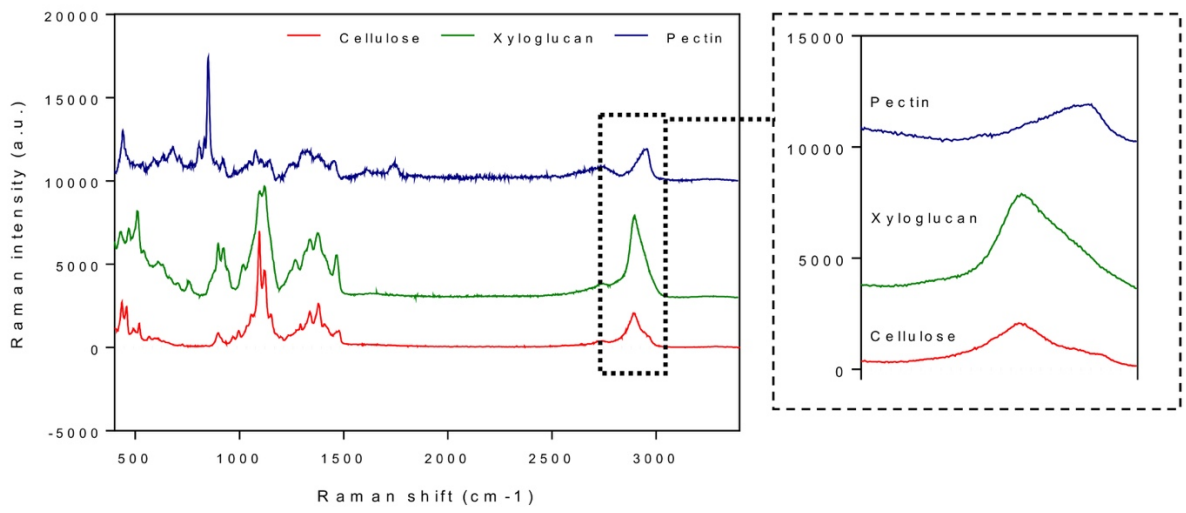

Figure S13. Full range Raman spectra of the pure cell wall compounds (cellulose, xyloglucan and pectin) used for reference. Inset is an expansion of the spectra within the diagnostic C-H stretching region (2750 – 3000 cm^{-1}). Collection of the spectra of the purified commercial xyloglucan and pectin was complicated by autofluorescence and so samples were photobleached for 30 mins prior to spectral acquisition; cellulose did not require photobleaching. Spectra have been offset on the y-axis for visual clarity. a.u., arbitrary unit. It is clear from this analysis that a number of vibrational modes are present in the fingerprint region (400 – 1800 cm^{-1}) of the respective Raman spectra. Although these are potentially suitable for identification of specific components within the spectra collected from the pericycle cell wall junctions, the presence of dominant bands associated with bending and combination of bending and twisting modes of H_2O, D_2O and HOD (a product of isotopic substitution) complicated analysis within this region.

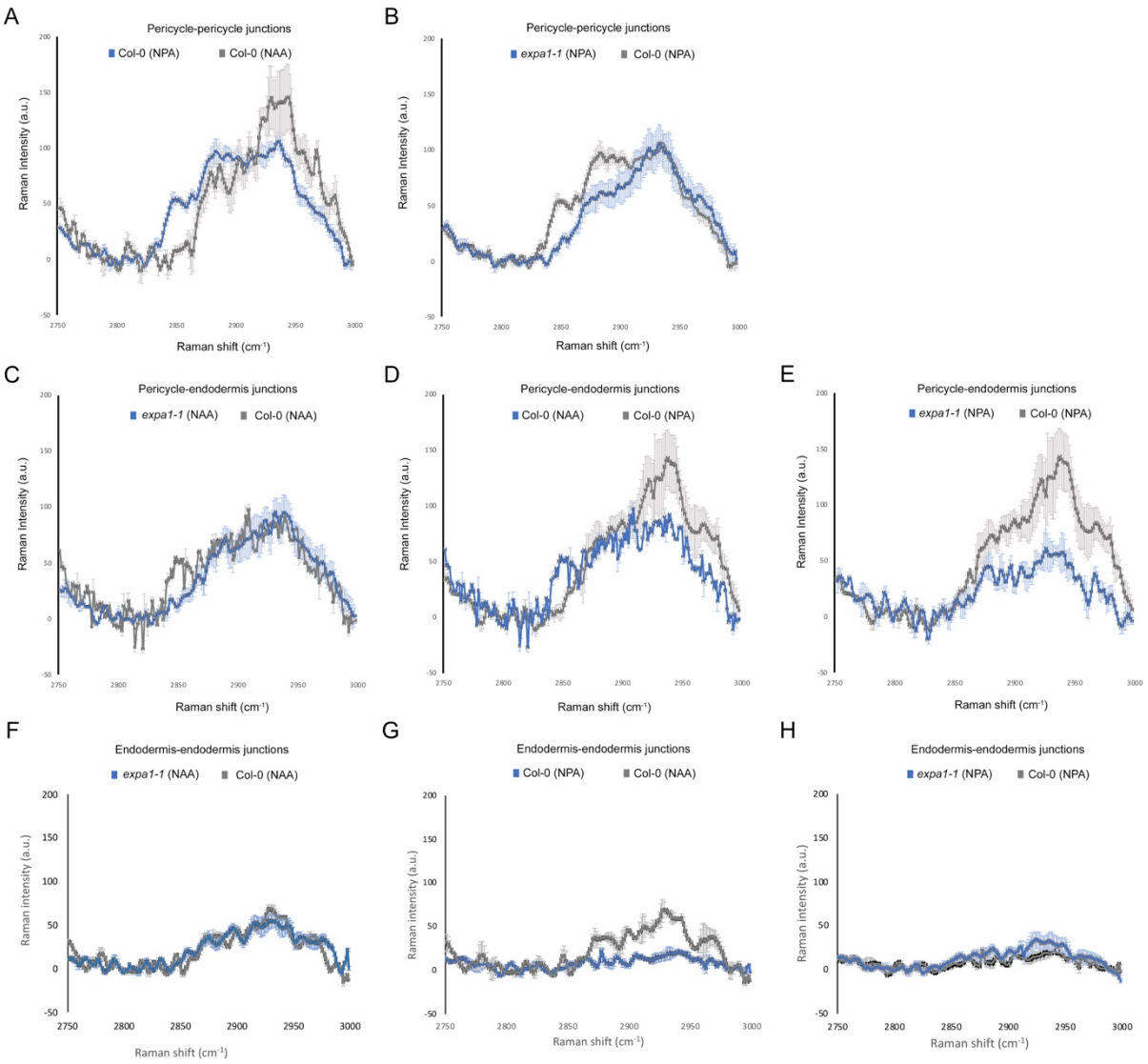

Figure S14. Raman spectra for indicated cell-cell junctions. Chemical spectra in the C-H stretching region 2750 – 3000 cm^{-1} with average of 4-6 measurements (± standard error) of pericycle-pericycle cell junctions (A and B), pericycle-endodermis cell junctions (C-E), and endodermis-endodermis cell junctions (F-H) on sections of Col-0 or *expa1-1* NPA versus NAA-treated roots. a.u., arbitrary unit.

SI MATERIALS AND METHODS

Plant materials and growth conditions

Arabidopsis thaliana accession Columbia (Col-0) was used as wild type throughout the study. The T-DNA line for *EXPANSINA1* (AT1G69530), *expa1-1* (SALK_010506), was obtained from the Nottingham Arabidopsis Stock Centre (NASC) and genotyped using the following gene-specific primers: *CAAAGCAGACCACTATGACCC* and *TGTTCGGTAAGGCGTTGTTAG*. The *pUBQ10::EYFP:NPSN12* (WAVE131YFP) line has been previously described (53). The *expa1-2* allele was generated as previously described (59) in a background expressing *pGATA23::H2B:3xGFP* and *pUB10::PIP1;4:3xmCHERRY*. All lines used for analysis were surface sterilized, stratified for two days at 4°C, and grown on vertical ½ MS plates [(2.154 g/l MS salts (Duchefa Biochemie MS basal salt mixture without vitamins), 0.1 g/l myo-inositol (Sigma), 0.5 g/l MES (Sigma), 10 g/l bacteriological agar (Sigma-Aldrich); pH 5.8 with 1M KOH)] at 22°C under continuous light conditions. For the 1-naphthaleneacetic acid (NAA) and cycloheximide (CHX) assay, 3 DPG (days post germination) roots were transferred onto vertical ½ MS plates supplemented with mock, 10 µM NAA, 10 µM CHX or 10 µM NAA + 10 µM CHX for 6 h and whole roots were analyzed. For the lateral root induction system-based analysis: WAVE131YFP and *expa1-1 x* WAVE131YFP lines were germinated and grown on 10 µM 1-N-naphthylphthalamic acid (NPA) for 72 h and transferred to ½ MS with 10 µM NAA for 6 h before analysis (**SI Appendix, Fig. S5A**).

Microscopy

For localization/expression experiments, a Leica TCS-SP5 confocal microscope (Leica, Milton Keynes, UK) was used. Excitation and emission wavelengths were as follows: GFP – 488 and 485-545 nm; YFP – 514 and 525-600 nm; PI 514 and 570-670 nm. Roots were stained with 10 µg/ml propidium iodide (Sigma) for 2 min, rinsed and mounted in water.

Plasmids and constructs

The *pEXPA1::n3xGFP* line was generated using a previously published vector backbone (2) with the *EXPA1* promoter fragment defined as 1901 base pairs upstream of the start codon. Homozygous transgenic lines were used for all experiments. To generate expression constructs, standard molecular biology procedures and Gateway Cloning Technology (Invitrogen) were

used. For the *pEXPA1::EXPA1:6xHis* complementation construct, the genomic sequence of *AtEXPA1* (fragment containing the 2.5 kb promoter, coding sequence and the UTR's was PCR-amplified using *TTCCAAATATAGCATTGGACCGT* and *AGCACTCGAAGCACCACTT*) was cloned into the entry vector pCR8-GW-TOPO and transferred into the destination vectors pGWB7 (no promoter, 6xHis tag) (3). The *pGATA23::shy2-2:GR* and *pGATA23::slr-1:GR* plasmids were generated by GreenGate based cloning (4). Briefly, the GreenGate A module containing the GATA23 promoter was generated by PCR from genomic DNA. The *shy2-2* and *slr-1* versions of *SHY2/IAA3* and *SLR/IAA14* were created by PCR mutagenesis on *SHY2/IAA3* and *SLR/IAA14* templates cloned from cDNA of auxin treated root RNA and used to generate GreenGate C module entry clones. The 33 amino acid Serine-Glycine linker and Glucocorticoid Receptor tag were cloned as GreenGate D module by PCR from a previously described template (5). The different entry clones were combined to generate transformation vectors (4). The dual reporter *pGATA23::H2B:3xmCHERRY* / *pUB10::3xGFP:PIP1;4* was obtained by GreenGate based cloning (4) by combining two GreenGate intermediary clones *pGATA23::H2B:3xmCHERRY* (assembled from published modules (4)) and *pUB10::3xGFP:PIP1;4* (PIP1;4 D module created by PCR from genomic DNA). All GreenGate transformation vectors carry sulfadizine resistance as described in (4). The sequences of the primers used are listed in **SI Appendix, Table S1**. Plasmids were transformed into *Agrobacterium tumefaciens* strain C58 (pMP90) and *Arabidopsis thaliana* plants were transformed by floral dip as previously described (6). Homozygous, independent transgenic lines with a single insert were analyzed.

Transcriptome profiling of *pGATA23::shy2-2:GR* and *pGATA23::slr-1:GR*

Samples were prepared for harvesting following a modified version of the lateral root induction system protocol (7). Seeds of the *pGATA23::shy2-2:GR* and *pGATA23::slr-1:GR* lines were germinated on Nitex membranes (Sefar) on 0.5X MS medium and transferred 4 days after germination under 16 h light / 8 h dark conditions to 10 µM NPA for 22 h and shifted to plates containing 10 µM NPA and 10 µM DEX or DMSO control for 2 h. Seedlings were then transferred to plates containing 10 µM IAA, and 10 µM DEX and DMSO control. Root tissue was harvested at 0, 1, 2, 4 and 6 h after transfer onto auxin plates. All sampling points were performed in triplicate. For each sample, about 200 segments of the lower two-thirds of the seedling roots were pooled. Total RNA of the 60 samples (2 genotypes x 2 treatments x 5 time points x 3 replicates) was extracted with the Universal RNA purification kit (EURx). Illumina NextSeq libraries were prepared from 2.5 µg of total RNA and sequencing performed on

NextSeq 500 flowcells (12 samples per cell). Reads were mapped onto the *Arabidopsis thaliana* genome (TAIR10) and numbers of reads per transcripts computed using STAR (version 2.5.2b). All the subsequent analysis was done with R (www.r-project.org/) using the DESeq2 package (8). Differentially expressed genes were independently identified for each genotype (*pGATA23::shy2-2:GR* and *pGATA23::slr-1:GR*) using a ~ Treatment + Time + Treatment:Time model (with Treatment: DEX vs DMSO, Time: 0, 1, 2, 4, 6 h post IAA) at log2 fold change >1 and false discovery rate < 0.01. The procedure was inspired by http://master.bioconductor.org/packages/release/workflows/vignettes/rnaseqGene/inst/doc/rnaseqGene.html#time-course-experiments. Differentially expressed genes common to *pGATA23::shy2-2:GR* and *pGATA23::slr-1:GR* were then clustered according to the direction and extent of the temporal change in the DMSO samples compared to the DEX-treated samples. The RNASeq data have been deposited to GEO under the following accession number: GSE122677.

RT-PCR gene expression analysis

Total RNA was extracted from roots using a Qiagen® RNeasy plant mini kit or using phenol (9). Poly(dT) cDNA was prepared from total RNA using the SuperScript II® Reverse Transcriptase (RT) from Invitrogen®. qPCR was performed using PerfeCTa SYBRGreen® FastMix, Low ROX (Quanta) on Roche LightCycler 480 apparatus. Results of the qPCR analysis are from a minimum of two independent biological replicates with four technical replicates in each. Expression of *EXPA1* was determined using the following primers: *GATGTCAAGAAACTGGGGACA* and *GAAAGACCAGCCTGCGTTAG (expa1-1* allele*)* and *CTACGGTGGTGGTGATGCTT* and *CTCGAAGCAAGCACCACAAC (expa1-2* allele*)*. Expression levels were normalized to that of the control gene *ACTIN* or *AT1G13320* (1) using the following primers: *CTGGAGGTTTTGAGGCTGGTAT* and *CCAAGGGTGAAAGCAAGAAGA* (*ACTIN*) or *TAACGTGGCCAAAATGATGC* and *GTTCTCCACAACCGCTTGGT* (*AT1G13320*).

(Lateral) root phenotyping [length, LR numbers, division patterns, cell size measurements]

The root length and number of lateral roots were determined using a dissecting microscope and ImageJ software (imagej.nih.gov/ij/). Lateral root density indicates the number of lateral roots per cm of the primary root. All data are the mean values for each plant considered. Experiments were repeated three times and statistical analysis of data was performed using a Student's *t*-test.

Lateral root bending assays were performed on 3 DPG seedlings according to the method described previously (10). The primordia were considered to be in Stage II when at least one periclinal division was observed. Quantification of frequency of occurrence of normal and disorganized primordia was performed on 7 or 10 DPG seedlings. The primordia were classified as disorganized if the anticlinal divisions of Stage I primordia were misplaced or supernumerary (e.g. Fig. 2C). For cell size measurements following treatment, confocal Z-stacks of roots were collected ~400 microns from the tip with the xylem poles in focus. The stacks were processed into 2D images with the different cell layers in focus using the z-project feature in Fiji (imagej.net/Fiji). Although the curvature of the root surface varies greatly, the surface of the pericycle cells in the longitudinal confocal section is small enough to be locally flattened in 2D without too much cell shape deformation (**SI Appendix, Fig. S5B**). The cell width was measured at multiple points along the cell and averaged out to account for non-uniform cell shapes of the different cell layers (**SI Appendix, Fig. S5C**). To account for membrane thickness, the measurements were taken at midpoint to midpoint of the membrane surface. For the analysis of division patterns in Figure 2E, confocal time series of *expa1-2* and co-segregating wild type were acquired for up to 24h in 5 day-old plants in which lateral root formation was induced by gravistimulation for 4h. Patterns were analyzed in the bends of 16 *expa1-2* and 13 wild type plants (without taking into account that lateral root primordia typically initiate from 3 parallel pericycle cell files).

Statistical analysis

Statistical analyses were performed either with R (www.R-project.org/) or Prism (https://www.graphpad.com/scientific-software/prism/). Plotting was done either in R using the ggplot2 (ggplot2.org) package or with shiny.chemgrid.org/boxplotr/.

Confocal Raman microscopy

Col-0 and *expa1-1* seedlings were subjected to the lateral root induction system described in ***Plant materials and growth conditions*** (**SI Appendix, Fig. S5A**). The roots were embedded in 4% Agarose (BioReagent for molecular biology, low EEO, Sigma-Aldrich) and 100-micron thick root cross sections were prepared with a Vibratome (Leica VT 1000S Vibratome). The sections were sealed in D_2O (Sigma-Aldrich) on a glass slide with nail paint to prevent evaporation of the solvent. Confocal Raman spectroscopy and imaging was performed using a Horiba LabRAM HR microscope equipped with a piezoelectric scan stage (Märzhäuser,

Germany) using either a 532 nm or a 785 nm laser, a 100x air objective (Nikon, NA = 0.9) and 50µm confocal pinhole. To simultaneously scan a range of Raman shifts, a 600 lines mm^{-1} rotatable diffraction grating along a path length of 800 mm was employed. Spectra were detected using a Synapse CCD detector (1024 pixels) thermoelectrically cooled to ~60°C. Prior to spectral acquisition, the instrument was calibrated using the zero-order line and the diagnostic phonon mode at 520.7 cm^{-1} from a Si(100) reference. Single point Raman spectra of approximately 1 and 2 micron spatial resolution (lateral and axial dimensions respectively) were acquired by optically focusing on the point of interest on the sample. The offset between the optical and laser focus was accounted for by maximizing the signal intensity as a function of sample height. For the root cross sections, Raman spectra (λ_{ex} = 532 nm) were acquired with an integration time of 120s (8 accumulations to increase the signal to noise ratio and remove spectral artefacts, e.g. cosmic rays), within the range of 1000-3200 cm^{-1}, from multiple points on the cell-cell junctions and averaged to obtain the final cumulated spectra for comparative analysis (**SI Appendix, Fig. S*5D***). Root sections were taken from at least six roots of each genotype and treatment and the spectral peaks for each line and treatment were compared for consistency in spectral pattern and reproducibility of peaks. Raman spectra (λ_{ex} = 785 nm) of the reference materials were collected to use as the basis for comparative post-analysis (**SI Appendix, Fig. S13**). All spectra were processed using LabSpec 6 software (Horiba Scientific). The spectra were baseline corrected with a local polynomial fit of the data, and subsequently smoothed using the DeNoise filter embedded in LabSpec 6 software. Spectral features within the range 2000 - 2750 cm^{-1} associated with the D-O stretching vibrations in D_2O were removed from the analysis and thus only vibrational modes within the ranges 2750 - 3000 cm^{-1} (C-H stretching region) were compared for differences in the position and intensity of spectral features.

Monosaccharide analysis

Whole roots of 7 DPG Col 0 and *expa1-1* seedlings grown on ½ MS at 22°C under continuous light condition were used for analysis. For auxin treatment, the 7 DPG seedlings were treated with 10 µM NAA in liquid ½ MS medium for 6 h followed by five washes in distilled water. The root fraction was isolated by cutting at the base of the hypocotyl and the samples were air dried at 37˚C for two days. Dried root samples were hydrolyzed by Saeman hydrolysis (60) and monomeric sugar composition determined via High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD). 30 mg of homogenized, dried root tissue was subject to two stage hydrolysis. 12 M sulphuric acid (1 ml)

was added and the sample was incubated for 1 h at 37°C. This was then diluted with 11 ml MilliQ® water and incubated for a further 2 h at 100°C. The sugar monomer content of the supernatant was determined by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD). The hydrolysate was analyzed using Dionex® ICS-3000 comprised of a high-pressure GD 50 gradient pump, an analytical column (Carbopac® PA20, 4 mm × 250 mm). 10 mM NaOH was used as the mobile phase and the column was flushed with 200 mM NaOH between runs. All chromatographic analyses were carried out at 30°C with a flow rate of 1.0 ml/min. Samples were diluted 1:100 in 10 mM NaOH and centrifuged at 4472 g for 10 min before loading to Dionex® vials. Sugar standards ranged from 0.250 to 2 g/L of arabinose, galactose, glucose, and xylose. Representative chromatograms for all lines and treatments are shown in **SI Appendix, Fig. S11**.

SI SUPPLEMENTAL TABLE

Table S1. Primer sequences

Gene	Purpose	Sequence
At5g26930 (*GATA23*)	Promoter cloning	AACAGGTCTCAACCTATAACTTTTCAATAATGGATCTCG
		AACAGGTCTCTTGTTGAGTCATCAAGAAAGGCTTAAG
At1g04240 (*SHY2/IAA3*)	C-clone generation	AACAGGTCTCAGGCTCAACAATGGATGAGTTTGTTAAC
		AACAGGTCTCTCTGATACACCACAGCCTAAACCTTTGG
At1g04240 (*SHY2/IAA3*)	*shy2-2* mutagenesis	GTTGGATGGTCACCAGTTAGATC
		GATCTAACTGGTGACCATCCAAC
At4g14550 (*SLR/IAA14*)	C-clone generation	AACAGGTCTCAGGCTCAACAATGAACCTTAAGGAGACG
		AACAGGTCTCTCTGATGATCTGTTCTTGAACTTCTCC
At4g14550 (*SLR/IAA14*)	*slr-1* mutagenesis	GGGTTGGCCATCGGTGAGGAAC
		GTTCCTCACCGATGGCCAACCC
Linker-GR	D-clone generation	AACAGGTCTCATCAGGTGCAGCGGCCGCTTCAGGGAG
		AACAGGTCTCTGCAGTCATTTTTGATGAAACAGAAGC
AT4G00430 (*PIP1;4*)	D-clone generation	AACAGGTCTCATCAGGTATGGAAGGCAAAGAAGAAGAT
		AACAGGTCTCTGCAGCTAACTCTTGCTCTTGAAAGG

SI CAPTIONS FOR SUPPLEMENTAL DATASETS AND MOVIES

Supplemental Dataset S1. List of 398 differentially expressed genes commonly regulated by SLR/IAA14 and SHY/IAA3.

Supplemental Dataset S2. Raman spectra data and statistical analysis.

Supplemental Movie S1. *pEXPA1::n3xGFP* expression during lateral root initiation (onset of expression in pericycle).

Supplemental Movie S2. *pEXPA1::n3xGFP* expression during lateral root initiation (expression at onset of divisions).

Supplemental Movie S3. Division patterns in *expa1-2* (related to **Figure S8B**).

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