

Supplemental Experimental Procedures.

Calculation of number of wild type meristems for linkage analysis.

The upper part of the meristem dome can be assumed to be approximately 15 μm from the top of a spherical cap of radius 50 μm . Assuming the volume of this region, 32000 μm^3 , weighs 32 ng, it would require 1.26×10^5 dissected SAMs to generate the 4 mg fresh weight to generate approximately 1 mg of cell wall preparation (alcohol insoluble residue; AIR) required for reliable polysaccharide linkage analysis.

Generating sufficient meristematic material.

The above figures illustrate that harvesting from carefully dissected WT meristems is not feasible. We made use of the enlarged, stem-cell enriched SAMs of the *clv3* mutant (Figure S1). Columbia genetic background *clv3* mutant apices have very thin, fasciated SAMs lined with numerous small overhanging flowers making dissection difficult and we were unable to harvest enough cell wall material. However, for the Landsberg erecta background the *clv3-2* mutant yields greatly enlarged SAMs of minimum 1 mm radius with developing flowers arranged around the periphery (Figure S1). After minimal dissection, a thin slice of apical tissue (SAM sample) is harvested in the absence of young floral tissue. The enlarged *clv3-2* SAM is enriched in central zone (stem cells) and rib meristem tissue [S1]. In our hands, sixty plants yield at least 1 mg of cell walls (AIR). Young *clv3-2* flowers (stage 6-7) were collected independently to represent a developing and rapidly growing tissue sample (floral sample). Samples were then prepared for either polysaccharide linkage analysis (AIR) or transcriptomics.

Validation of transcriptome data and data clustering based on gene ontology definitions.

Graphical representations of examples of mapped reads that peak either in the SAM, flower or pooled sample are given in Figure S2A. To provide validation i.e. to check the RNA samples are of sufficient quality to mine for expressed GTs, known transcripts with expected enrichment in a sample were examined. Transcription factors controlling floral patterning such as *AGAMOUS* [S2] and *APETALA1* [S3] are enriched in the *clv3-2* floral samples whereas *PIF4/SRL2* [S4] and *PUMILIO10* [S5], genes that have been shown to play a role in the SAM, exhibit expected *clv3-2* SAM enrichment. The root-hair specific *EXP7* [S6] is detected in trace amounts only in whole plant tissues and *TED6* [S7], *CESA7* [S8], *COBL4* [S9], *MYB46* [S10], *LAC11* [S11], *SND2* [S12], expressed during xylem development, are also enriched in the whole plant sample (Figure S2A)

Normalizing read counts

The total number of mappable reads varied slightly among the six samples and details are given as follows:

	<i>clv3-2</i> SAM	<i>clv3-2</i> floral tissue	<i>clv3-2</i> pooled tissue
No. of biological replicates	2	2	2
Total no of raw reads	28742265/41004746	35522653/33809171	30268068/30110535
Genome coverage	28.76/40.06	37.24/36.20	20.45/20.95
Mean insert sizes (bp)	237.89/240.18	243.14/240.14	234.32/236.40

In order to visualize mapped reads of different libraries side-by-side we normalized the read counts in each sample with the average genome-wide coverage per bp, and visualised using the Integrative Genome Viewer (IGV) [S13].

Only genes with TPM values > 1 were included in the subsequent analyses. Expression estimates of replicate experiments were averaged and the fold change between the tissue-specific samples and the whole plant sample was calculated for every gene. Subsequently, a Z-score for each fold change was

generated analogous to Busch et al., 2010 [S14]. For Figure S2C, transcripts that displayed a fold change > 1.5 and a Z-score greater than 2 were considered as increased, and transcripts with a fold change > 1.5 and a Z-score smaller than -2 were considered as decreased.

Gene Ontology Analysis

Gene ontology (GO) enrichment analysis was performed using BINGO [S15] implemented in Cytoscape 3.0[S16]. A hypergeometric test was conducted and the Benjamini & Hochberg False Discovery Rate (FDR) was calculated. A list of GO categories was retrieved containing enriched terms with a p value lower than 0.05 after FDR correction.

Construction of a pairwise correlation coefficient network.

All GT transcripts present at levels >10 TPM were used as a query list for the CORNET co-expression tool [S17] using method "Pearson" and the output showing pairwise correlations for values above 0.5 (Figure S2D). A subset of data series, totalling 958 experiments, were used to generate the network as follows: Development, Flower, Leaf, Compendium 1 and Whole plant.

Two-colour fluorescent in situ hybridization.

Processed sections were hybridized with a mixture of two gene-specific probes labelled with digoxigenin and fluorescein, respectively. For detection of digoxigenin labelled probe, the sections were incubated with anti-digoxigenin antibody conjugated to horseradish peroxidase (Anti-DIG-POD, Roche). Hybridization signal was detected with Tyramide Signal Amplification (TSA) System (PerkinElmer). After the first TSA reaction, 3% H₂O₂ (Sigma) was applied to quench peroxidase activity. The sections were processed with anti-fluorescein antibody binding (Anti-FITC-POD, Roche) and subsequently TSA reaction. Images were taken with a Zeiss LSM700 confocal equipped with a 20x 0.8NA dry objective. For colocalization analysis with *CSLD5* the Manders' coefficient M1, representing the fraction of the GT signal that overlaps with the cyclin B signal, were determined using the JACoP plugin of ImageJ [S18]. To test the degree of localisation with the expressed *GALS2* signal, the Manders coefficient M2 (fraction of cell cycle marker that overlaps with *GALS2*) was used. The lower cut-off for determining values was a pixel intensity of 35.

Making a DNA construct for visualisation of At1g32930 gene expression and GFP-GATL6.

A 3,145 bp region upstream of the start of the At1g32930 open reading frame was amplified using primers PGT31a_f (5'-GCCGCGGCCGCGTTTAAACTcttgacacaaatcacaacgagtgag) that incorporates both NotI-PmeI restriction sites at the 5' end and PGT31a_r (5'-GCCGCGCGCCttttcttaactcttttcgaa) that incorporates an AscI restriction site. After PCR, the promoter fragment was cloned in to a NotI-AscI digested vector containing a polylinker with NotI and AscI restriction sites, followed 3' by a Gateway recombination cassette and then 3' by the OCS terminator from pBJ36 with a PmeI restriction site at the 3' end. A PmeI fragment containing the promoter::Gateway-OCS sequence was transferred to the PmeI-digested binary vector pMoA34 to generate the destination vector pM34_GT31aGW. The tdTomato gene fused in frame to a nuclear localization sequence N7 and cloned within a Gateway entry vector was used in a recombination reaction with the pM34_GT31aGW destination vector. The construct was validated by DNA sequencing and transformed into Arabidopsis plants containing an acylated YFP that labels the plasma membrane.

For *GFP-GATL6*, a 3Kb fragment upstream of GATL6 start codon was amplified using primers GATL6_p_F (5'CTGCAGTCTCTTCGTCCGCCCTTGG3') and GATL6_p_R (5'AAGCTTCGAAACCCTAGAAATAAC3'). The GFP coding sequence was amplified using primer EGFP_F (5'AAGCTTATGGTGAGCAAGGGCGAG3') and EGFP_R (5'ATCGATCTTGTACAGCTCGTCCAT 3'). A 3Kb fragment containing GATL6 genomic region as well as 1.6Kb 3 UTR was amplified using primers GATL6_DNA_F (5'ATCGATATGCTTTGGATAACGAGATT 3') and GATL6_DNA_R

(‘GTCGACGAAGCTCGACCTCTTTCTTC’). The three fragments were digested with *Pst*I, *Hind*III (GATL6_p) *Hind*III, *Cla*I (EGFP) *Cla*I, *Sal*I (GTL6_DNA) and then ligated in a standard cloning vector. The whole DNA construct, isolated as a *Pst*I/*Sal*I fragment, was ligated into the binary vector pCambia1300, giving rise to the construct pGATL6::EGFP-GATL6 and transformed into *Arabidopsis*. After selection and propagation, plants were subject to confocal microscopy.

References

- S1. Fletcher, J. C., Brand, U., Running, M. P., Simon, R., and Meyerowitz, E. M. (1999). Signaling of cell fate decisions by CLAVATA3 in *Arabidopsis* shoot meristems. *Science* 283, 1911–4.
- S2. Bowman, J. L., Drews, G. N., and Meyerowitz, E. M. (1991). Expression of the *Arabidopsis* floral homeotic gene AGAMOUS is restricted to specific cell types late in flower development. *Plant Cell* 3, 749–58.
- S3. Mandel, M. A., Gustafson-Brown, C., Savidge, B., and Yanofsky, M. F. (1992). Molecular characterization of the *Arabidopsis* floral homeotic gene APETALA1. *Nature* 360, 273–7.
- S4. Huq, E., and Quail, P. H. (2002). PIF4, a phytochrome-interacting bHLH factor, functions as a negative regulator of phytochrome B signaling in *Arabidopsis*. *EMBO J.* 21, 2441–50.
- S5. Yadav, R. K., Girke, T., Pasala, S., Xie, M., and Reddy, G. V. (2009). Gene expression map of the *Arabidopsis* shoot apical meristem stem cell niche. *Proc. Natl. Acad. Sci. U. S. A.* 106, 4941–6.
- S6. Lin, C., Choi, H.-S., and Cho, H.-T. (2011). Root hair-specific EXPANSIN A7 is required for root hair elongation in *Arabidopsis*. *Mol. Cells* 31, 393–397.
- S7. Endo, S., Pesquet, E., Yamaguchi, M., Tashiro, G., Sato, M., Toyooka, K., Nishikubo, N., Udagawa-Motose, M., Kubo, M., Fukuda, H., et al. (2009). Identifying New Components Participating in the Secondary Cell Wall Formation of Vessel Elements in *Zinnia* and *Arabidopsis*. *PLANT CELL ONLINE* 21, 1155–1165.
- S8. Taylor, N. G., Scheible, W. R., Cutler, S., Somerville, C. R., and Turner, S. R. (1999). The irregular xylem3 locus of *Arabidopsis* encodes a cellulose synthase required for secondary cell wall synthesis. *Plant Cell* 11, 769–80.
- S9. Brown, D. M., Zeef, L. A. H., Ellis, J., Goodacre, R., and Turner, S. R. (2005). Identification of novel genes in *Arabidopsis* involved in secondary cell wall formation using expression profiling and reverse genetics. *Plant Cell* 17, 2281–95.
- S10. Zhong, R., Richardson, E. A., and Ye, Z.-H. (2007). The MYB46 transcription factor is a direct target of SND1 and regulates secondary wall biosynthesis in *Arabidopsis*. *Plant Cell* 19, 2776–92.
- S11. Zhao, Q., Nakashima, J., Chen, F., Yin, Y., Fu, C., Yun, J., Shao, H., Wang, X., Wang, Z.-Y., and Dixon, R. A. (2013). Laccase is necessary and nonredundant with peroxidase for lignin polymerization during vascular development in *Arabidopsis*. *Plant Cell* 25, 3976–87.
- S12. Hussey, S. G., Mizrachi, E., Spokevicius, A. V, Bossinger, G., Berger, D. K., and Myburg, A. A. (2011). SND2, a NAC transcription factor gene, regulates genes involved in secondary cell wall development in *Arabidopsis* fibres and increases fibre cell area in *Eucalyptus*. *BMC Plant Biol.* 11, 173.
- S13. Thorvaldsdóttir, H., Robinson, J. T., and Mesirov, J. P. (2013). Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Brief. Bioinform.* 14, 178–92.
- S14. Busch, W., Miotk, A., Ariel, F. D., Zhao, Z., Forner, J., Daum, G., Suzaki, T., Schuster, C., Schultheiss, S. J., Leibfried, A., et al. (2010). Transcriptional control of a plant stem cell niche. *Dev. Cell* 18, 849–61.
- S15. Maere, S., Heymans, K., and Kuiper, M. (2005). BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. *Bioinformatics* 21, 3448–9.
- S16. Shannon, P. T., Grimes, M., Kutlu, B., Bot, J. J., and Galas, D. J. (2013). RCytoscape: tools for exploratory network analysis. *BMC Bioinformatics* 14, 217.

- S17. De Bodt, S., and Inzé, D. (2013). A guide to CORNET for the construction of coexpression and protein-protein interaction networks. *Methods Mol. Biol.* 1011, 327–43.
- S18. Bolte, S., and Cordelières, F. P. (2006). A guided tour into subcellular colocalization analysis in light microscopy. *J. Microsc.* 224, 213–32.

Supplemental Tables

Table S1. Calculation of polysaccharide composition based on linkage analysis, related to Figure 2.

Table S2. Transcriptome data for GT family genes arranged by polysaccharide, related to Figure 3.

Table S3. *In situ* hybridizations showing mRNA of SAM-expressed GTs grouped according to their expression patterns. Related to Figure 3

Table S4. List of probes and primers.