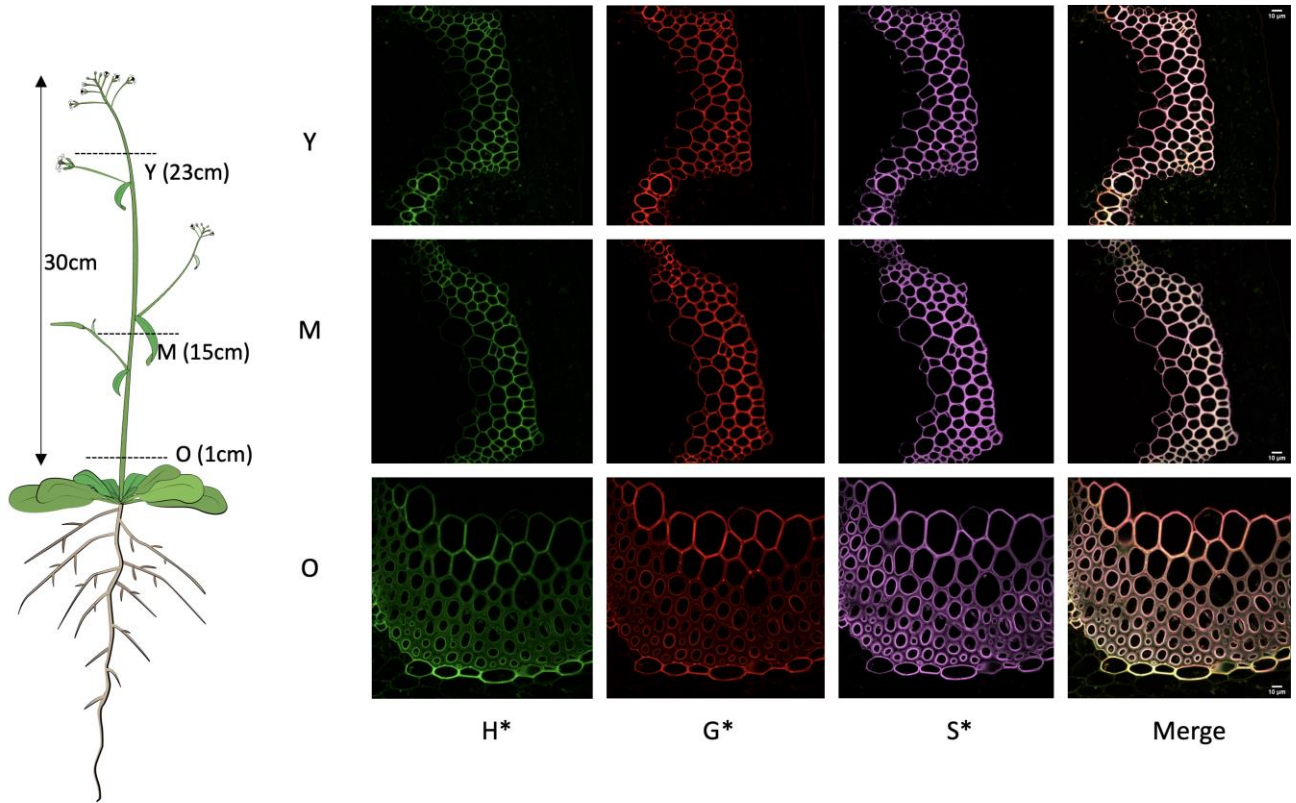


Supplemental data

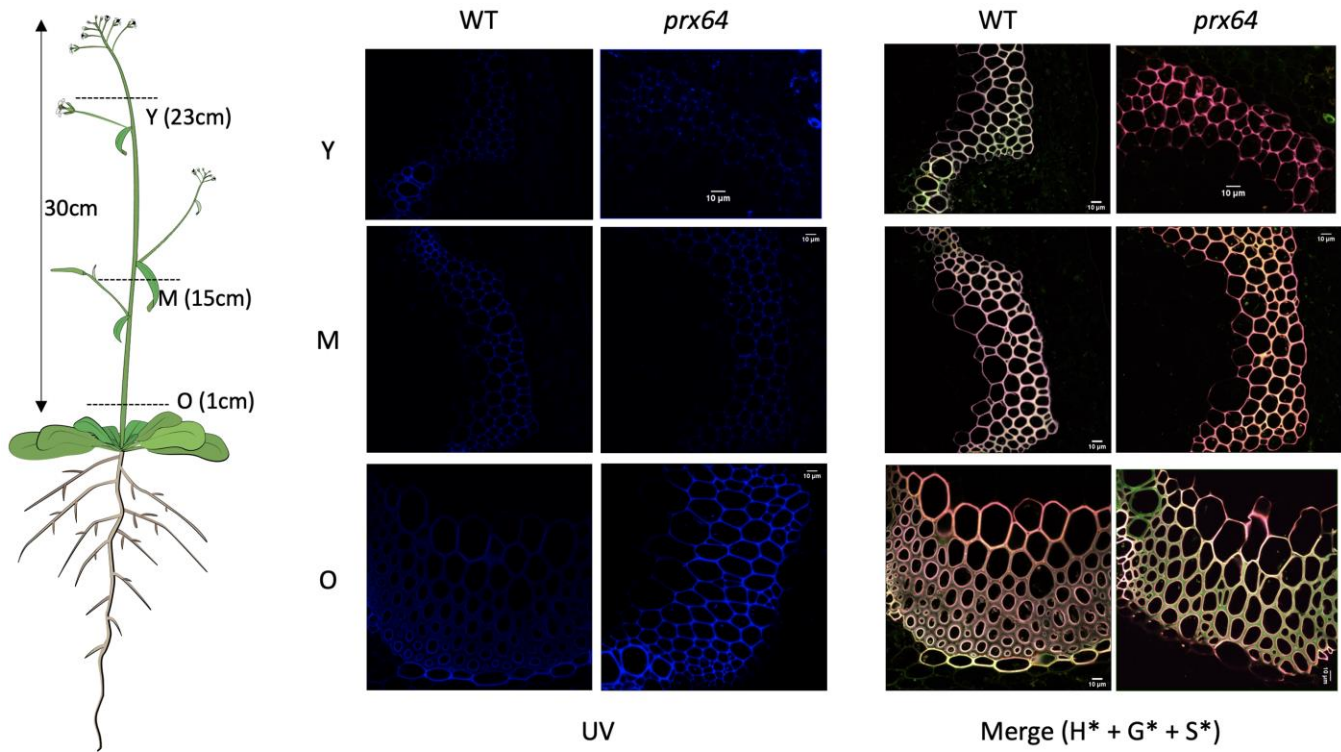
Supplemental Dataset S1: Supplemental dataset provided at <http://doi.org/10.5281/zenodo.4809980>

This dataset contains:

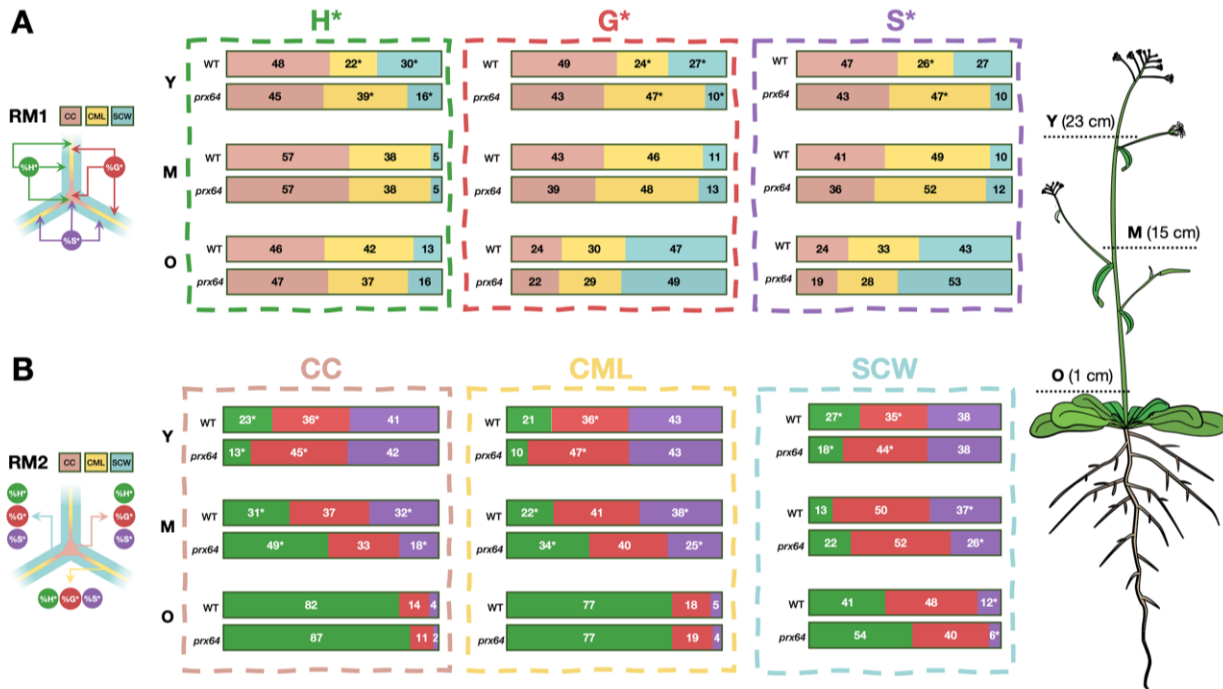
- the algorithm with graphical user interface for imageJ and its installation procedure ("Cell_Wall_Segmentation" and "Cell_Wall_SegmentationTutorial")
- a folder comprising a classifier and a dataset compatible with the machine learning part of the algorithm: "data and classifier for weka"
- representative images adapted for testing: "representative images"
- the macro corresponding to the parametric segmentation procedure (see imageJ documentation for installation instructions): "parametric_segmentation"



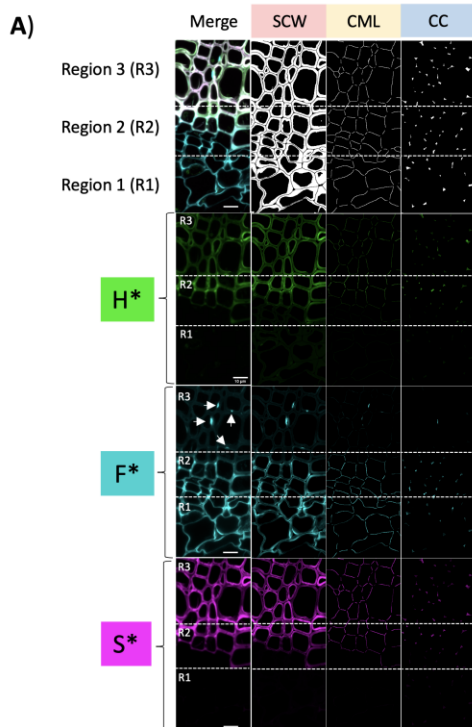
Supplemental Figure S1: Bioorthogonal lignin triple labelling of interfascicular fiber cell walls in cross-sections made at different heights (Y - young, M - medium, O - old) of wild-type (WT) *Arabidopsis* floral stems. H*, G* and S* = green/red/magenta CLSM channel images revealing incorporation of the corresponding H*, G* and S* reporters. Merge = merged channels. Scale bar = 10 μ m.



Supplemental Figure S2: Bioorthogonal lignin triple (H*, G*, S*) labelling in interfascicular fibers of cross-sections made from different heights in Arabidopsis WT and *prx64* mutant floral stems. Lignin autofluorescence (blue) and merged channels. Y = young, M = medium, O = Old. Scale bar = 10 μ m.



Supplemental Figure S3. Comparison of monoglignol reporter incorporation profiles in WT and *prx64* mutant Arabidopsis stem fiber cell walls. **A)** Relative distribution of H*, G* and S* reporters incorporated into different fiber cell wall zones in Y (young), M (medium) and O (old) stem cross sections analyzed by ratiometric method 1 (RM1), CC = cell corner, CML = compound middle lamella, SCW = secondary cell wall; figures represent the percentage of total H*, G* and S* signal incorporated into the different wall zones, Values marked with * indicate significantly different values (Students T-test, p-value = 0.05) between WT and mutant plants for a given stem height (Y, M, O). **B)** Relative contribution of all reporters to total signal in the cell corner (CC) analyzed by ratiometric method 2 (RM2); figures represent the percentage contribution of each reporter's signal to the total signal intensity in different cell wall zones. Values marked with * indicate significantly different values (Students T-test, p-value = 0.05) between WT and mutant plants for a given stem height (Y, M, O).



B)

Reporter	CW zone	% Total Intensity		
		Region 1	Region 2	Region 3
H*	CC	11 %	44 %	45 %
	CML	13 %	38 %	49 %
	SCW	13 %	38 %	49 %
F*	CC	43 %	44 %	13 %
	CML	43 %	40 %	17 %
	SCW	43 %	38 %	20 %
S*	CC	11 %	40 %	49 %
	CML	12 %	35 %	53 %
	SCW	12 %	36 %	52 %

C)

Reporter	CW zone	% Intensity (RM1)		
		Region 1	Region 2	Region 3
H*	CC	34 %	41 %	36 %
	CML	33 %	30 %	33 %
	SCW	32 %	29 %	32 %
F*	CC	40 %	43 %	33 %
	CML	32 %	31 %	34 %
	SCW	27 %	25 %	33 %
S*	CC	16 %	38 %	44 %
	CML	16 %	32 %	46 %
	SCW	67 %	30 %	10 %

Supplemental figure S4. Automatic segmentation of cell wall zones in flax stem tissues showing relative distribution of lignin and non-cellulosic polysaccharide reporters. **A)** The 3 masks (CC, CML, SCW) are applied to three fluorescence channels of CLSM images of flax stem cross-sections incubated with H* and S* monolignol reporters and F* peracetylated alkyne-tagged fucose (Ac₄FucAlk) reporter according to the triple bioorthogonal strategy. **B)** Table indicating the relative percentage of total signal for reporters in a given cell wall zone found in each of the three different tissue regions (1, 2, 3) analyzed. **C)** Ratiometric analysis 1 (RM1) of the relative amounts of total reporter signal found in the different cell wall zones. H*, S* = monolignol reporters; F* = fucose reporter; CC = cell corner, CML = compound middle lamella, SCW = secondary cell wall, scale bar = 10 μm; red values in tables = highest %, green values in tables = lowest %. Region 1 corresponds to the phloem/vascular cambium, Region 2 corresponds to the vascular cambium/young differentiating xylem, Region 3 corresponds to more mature xylem tissue, arrows indicate xylem pits.