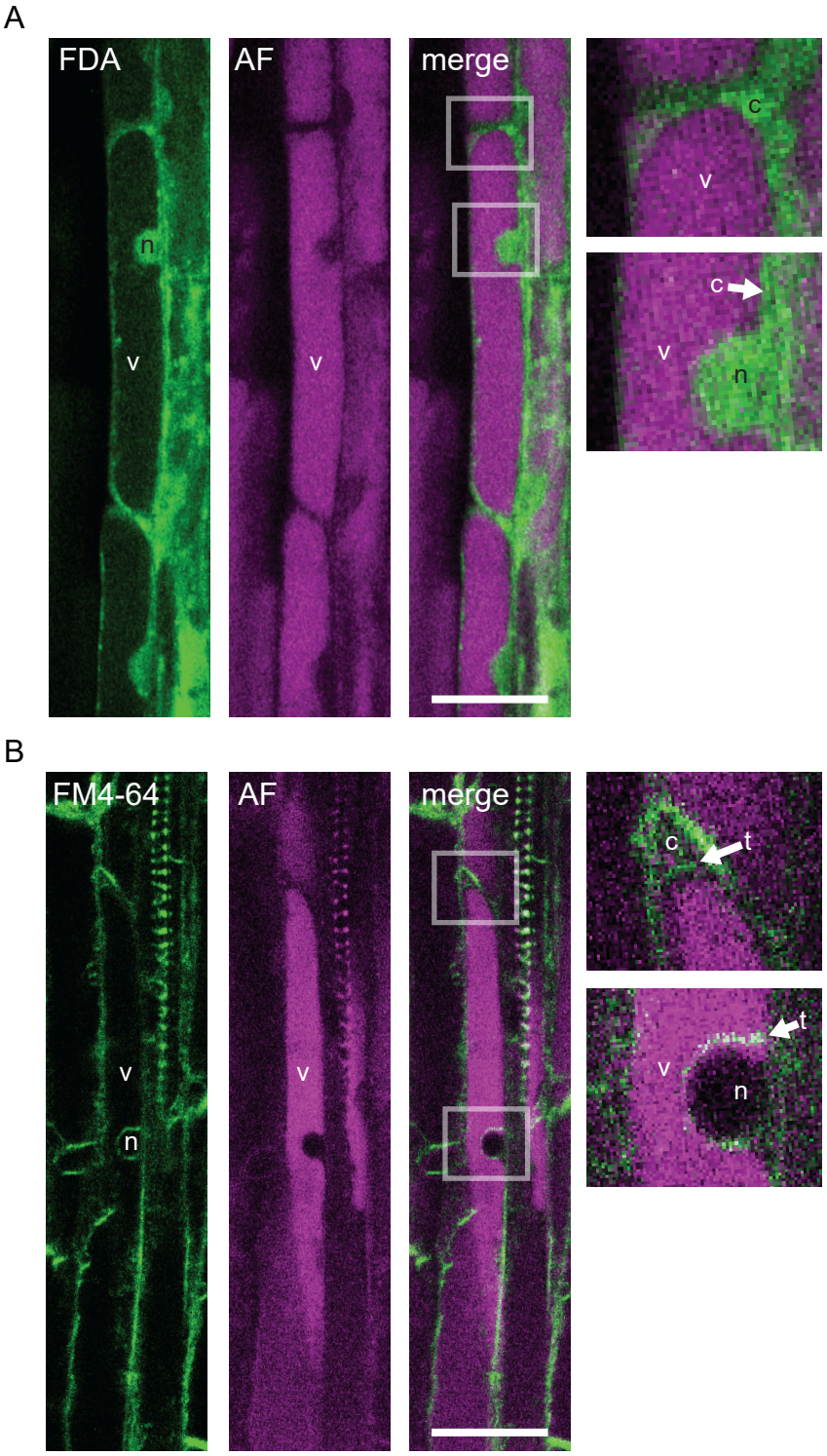
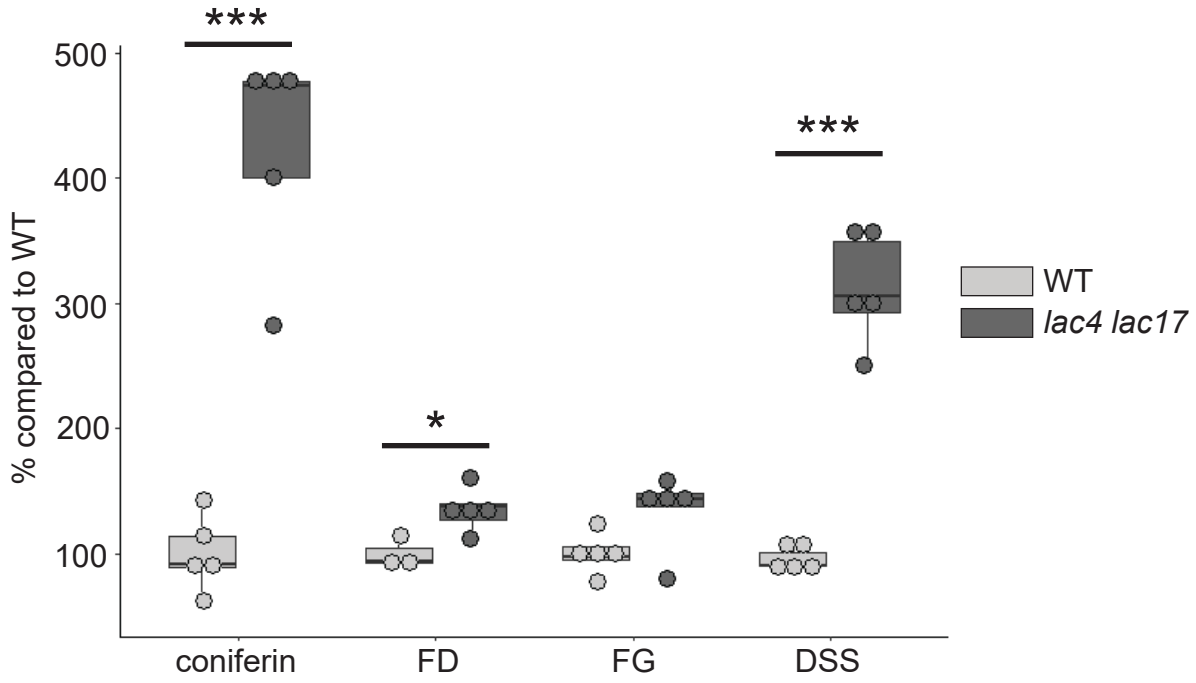
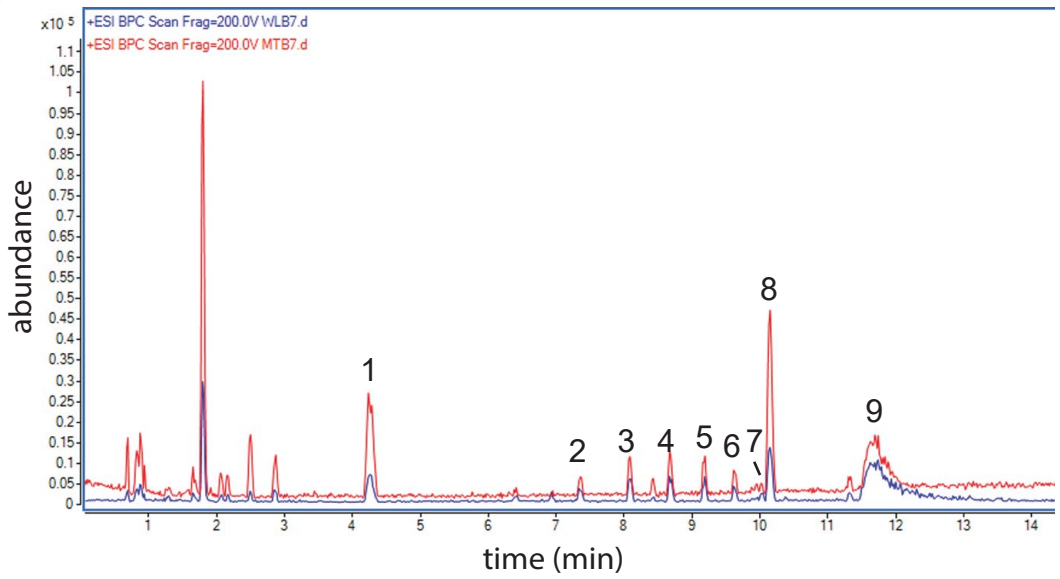


Supplemental Figure S1. Vacuolar localization of soluble autofluorescent signals. Signals localized within both the peripheral cytoplasm and tonoplast demonstrate vacuolar localization. Supports Figure 1, 2 and 3.
(A) Fluorescein diacetate (FDA) stained cytoplasm in *lac4 lac17* mutant Arabidopsis roots (green). Autofluorescent signal (magenta). n indicates nucleus. v indicates vacuole. c indicates cytoplasm. Scale bar indicates 25µm. Transparent boxes indicate regions magnified in inset images.
(B) FM^{TM}4-64 stained membranes, including the tonoplast, in *lac4 lac17* mutant Arabidopsis (green). Autofluorescent signal (magenta). n indicates nucleus. v indicates vacuole. t indicates tonoplast membrane. Scale bar indicates 25µm. Transparent boxes indicate regions magnified in inset images.



Supplemental Figure S2. Quantified changes in soluble phenolics in *lac4 lac17* roots compared to WT Col-0 determined by HPLC/QTOF. Supports Figure 2. FD = feruloyl derivatives (peaks 5 and 6 in Supplemental Figure S3), FG = flavonol glycosides (peaks 2, 3, 4 and 7 in Supplemental Figure S3), DSS = N′, N″ - disinapoylspermidine (peak 8 in Supplemental Figure 3). Two sample T – tests: * indicates $p < 0.05$, *** indicates $p < 0.001$. n = 5 biological replicates of 2-3 plates with ~200 seedlings each. Identification of compounds described in Supplemental Figure S3.

A



B

peak #	Identification	MS main ions (m/z)	Molecular structure	Type of identification
1	Coniferin	365.1188	365.1213 $[M+Na]^+$	Pure standard, Molecular structure of the main ions
3	FG - quercetin rutinoside	611.1633, 303.0473	611.1612 $[M+H]^+$, 303.0504 $[M\text{-}rut+2H]^+$	
9	Internal standard - morin	303.0503	303.0504 $[M+H]^+$	
-	Syringin	N.D.	395.1317 [M+Na]+	
peak #	**Most probable identification**	**MS main ions (m/z)**	**Molecular structure**	**Type of identification**
2	FG - quercetin hexoside	465.0962, 303.0497	465.1033 $[M+H]^+$, 303.0504 $[M\text{-}hexoside+2H]^+$	Molecular structure the main ions, Order of elution
4	FG - kaemferol rutinoside	595.1645, 449.1087	595.1663 $[M+H]^+$, 449.1084 $[M\text{-}rham+2H]^+$	
7	FG - isorhamnetin rutinoside-rhamnoside	609.3876	609.3876 $[M\text{-}O\text{-}rham+2H]^+$	

C

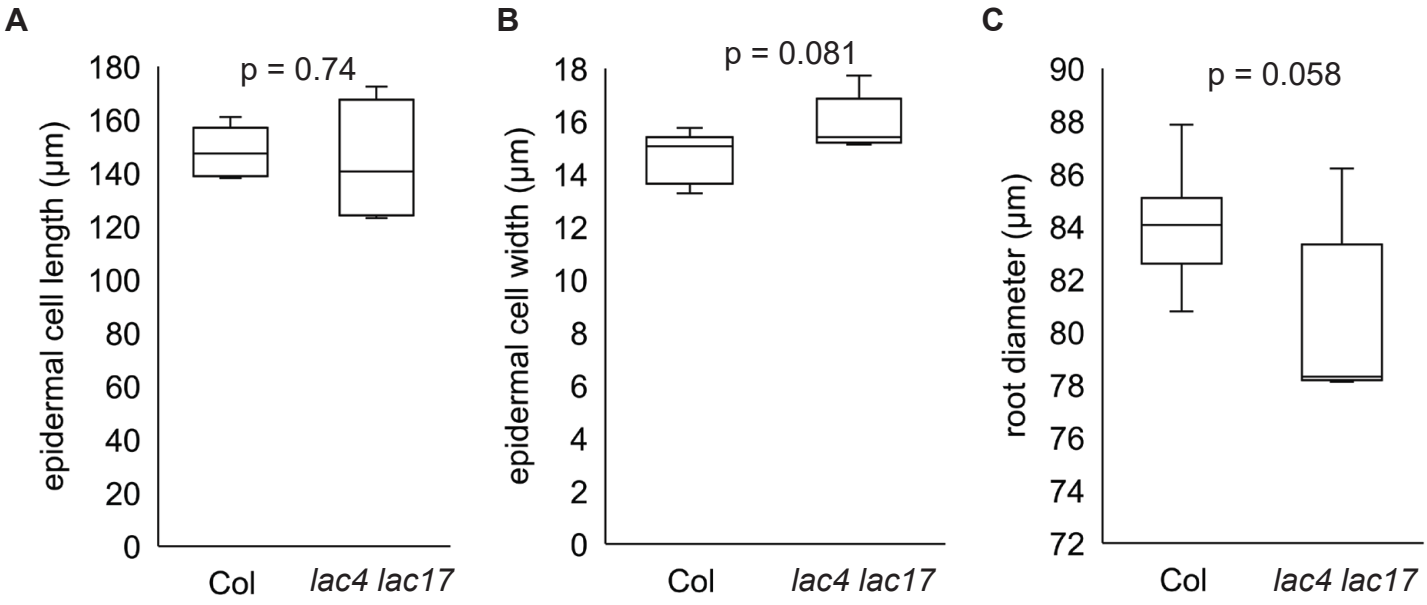
peak #	Most probable identification	Precursor ion (m/z)	Molecular structure precursor ion	MS/MS main product ions (m/z)	Type of identification
5	FD - feruloyl derivative 1	520.3315	Unknown	177.1103, 133.0848	Presence of m/z 177.11 *, Order of elution
6	FD - feruloyl derivative 2	564.3578	Unknown	177.1135, 133.0807	
8	DSS - N′, N″ - disinapoylspermidine	558.2806	558.2806 $[M+H]^+$	207.0631, 175.0369, 264.1216, 352.2200	Molecular structure of the main ions, both for precursor and product ions **

Supplemental Figure S3. Characterization of targeted and untargeted soluble phenolics by High Performance Liquid Chromatography/ Quadrupole-Time of Flight-Mass Spectrometry (HPLC/QTOF-MS). Supports Figure 2.

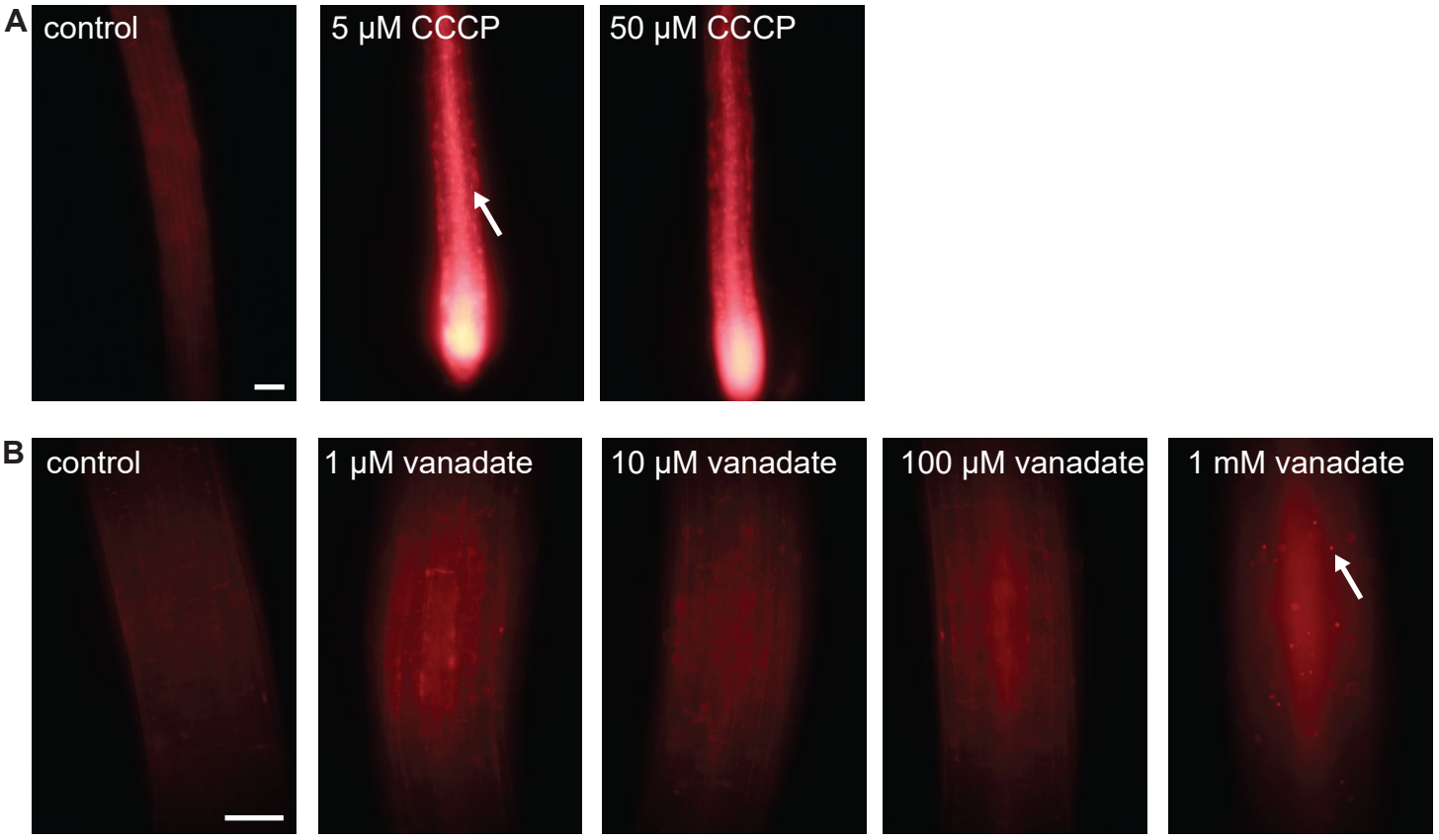
(A) Base peak chromatogram of soluble phenolic extracts. Representative sample of Col-0 WT in blue and *lac4 lac17* in red. Numbered peaks explained in tables B and C. FG (flavonol glucosides) and FD (feruloyl derivatives) indicate compounds grouped into the corresponding categories in Supplemental Figure S2.

(B) Identification/Tentative identification of the main soluble phenolics found among the Col-0 WT and *lac4 lac17* samples injected in MS mode. Peaks 1,3, and 9 and syringin were identified by pure standards and fragmentation patterns in the spectra. Peaks 2, 4, and 7 were identified based on the fragmentation patterns in the spectra and the order of elution. “N.D”. means “not detected” or “under the limits of detection of the method”.

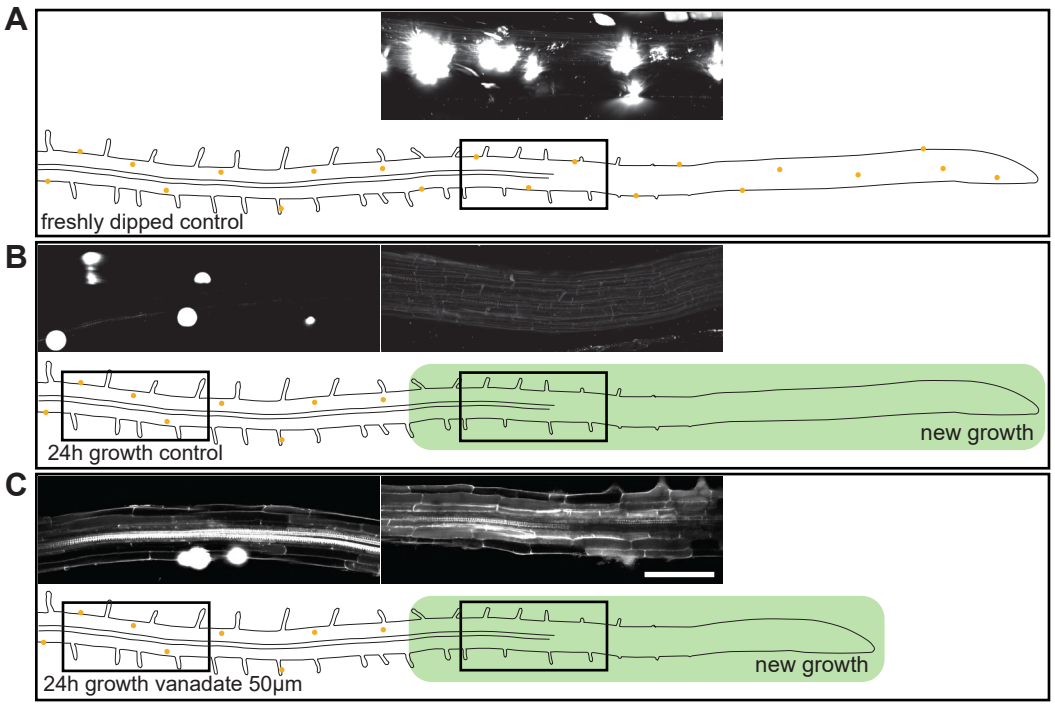
(C) Tentative identification of peaks 5, 6, and 8 found among the Col-0 WT and lac4lac17 samples, injected in “product ion mode” (HPLC-MS/MS) for a better structural characterization. * The m/z 177.11 has been commonly reported as feruloyl derivative of phenolic acid (see https://www.mdpi.com/2304-8158/9/10/1471). ** Precursor and product ions of N′, N″ - disinapoylspermidine fully match the MS/MS spectra found in Luo et al. (2009), accessible at www.plantcell.org/cgi/doi/10.1105/tpc.108.063511.



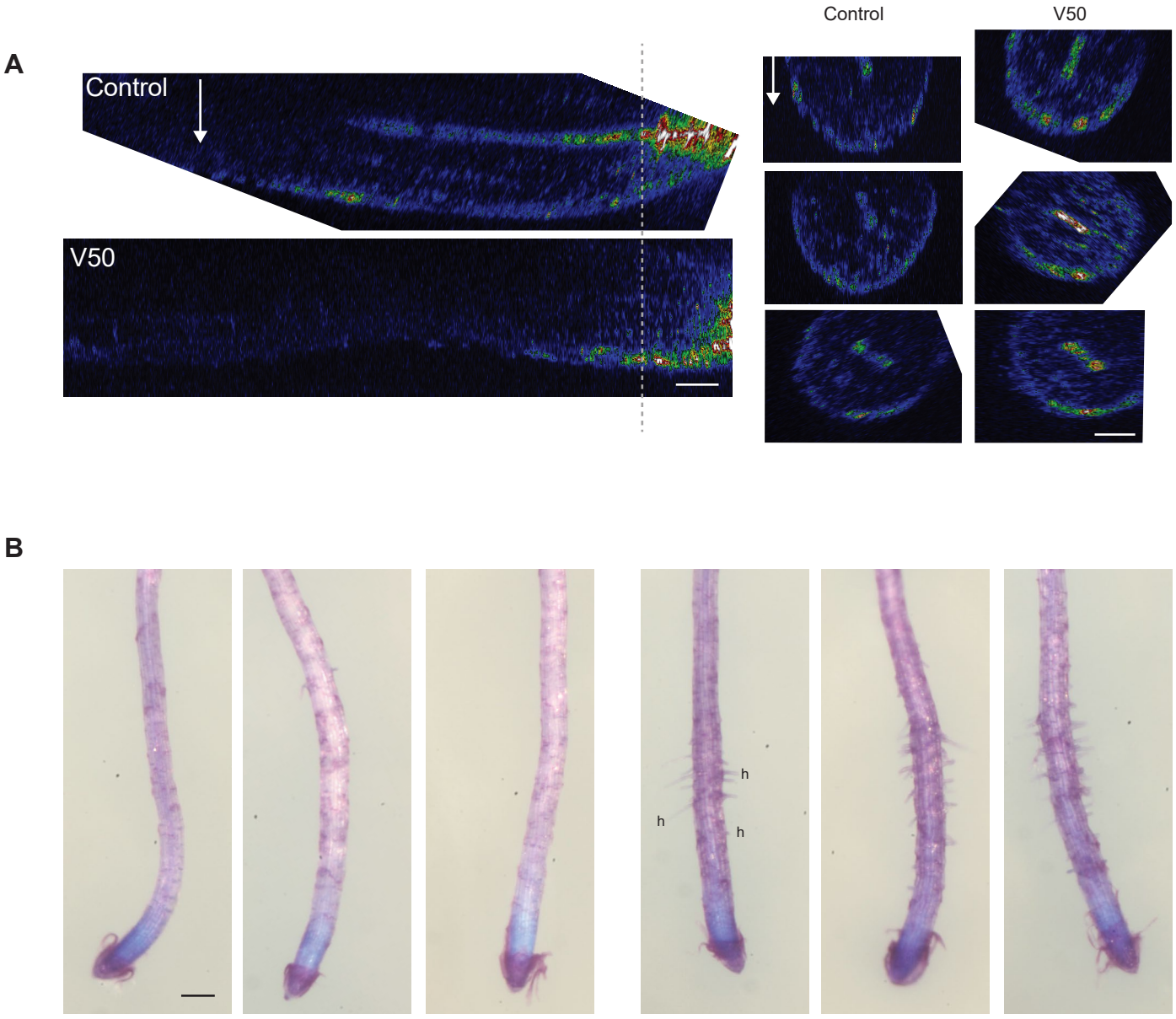
Supplemental Figure S4. No size difference was detected between WT Col-0 and *lac4 lac17* roots. Supports Figure 2. Box and whiskers represent median values and quartiles.
(A) Comparison of epidermal cell length. Three representative cells measured and averaged per root, n = 5 roots. Two sample t test, p = 0.74.
(B) Comparison of epidermal cell width. Three representative cells measured and averaged per root, n = 5 roots. Two sample t test, p = 0.08.
(C) Comparison of root diameter. Roots measured at three points and averaged per root, n = 5 roots. Two sample t test, p = 0.06.



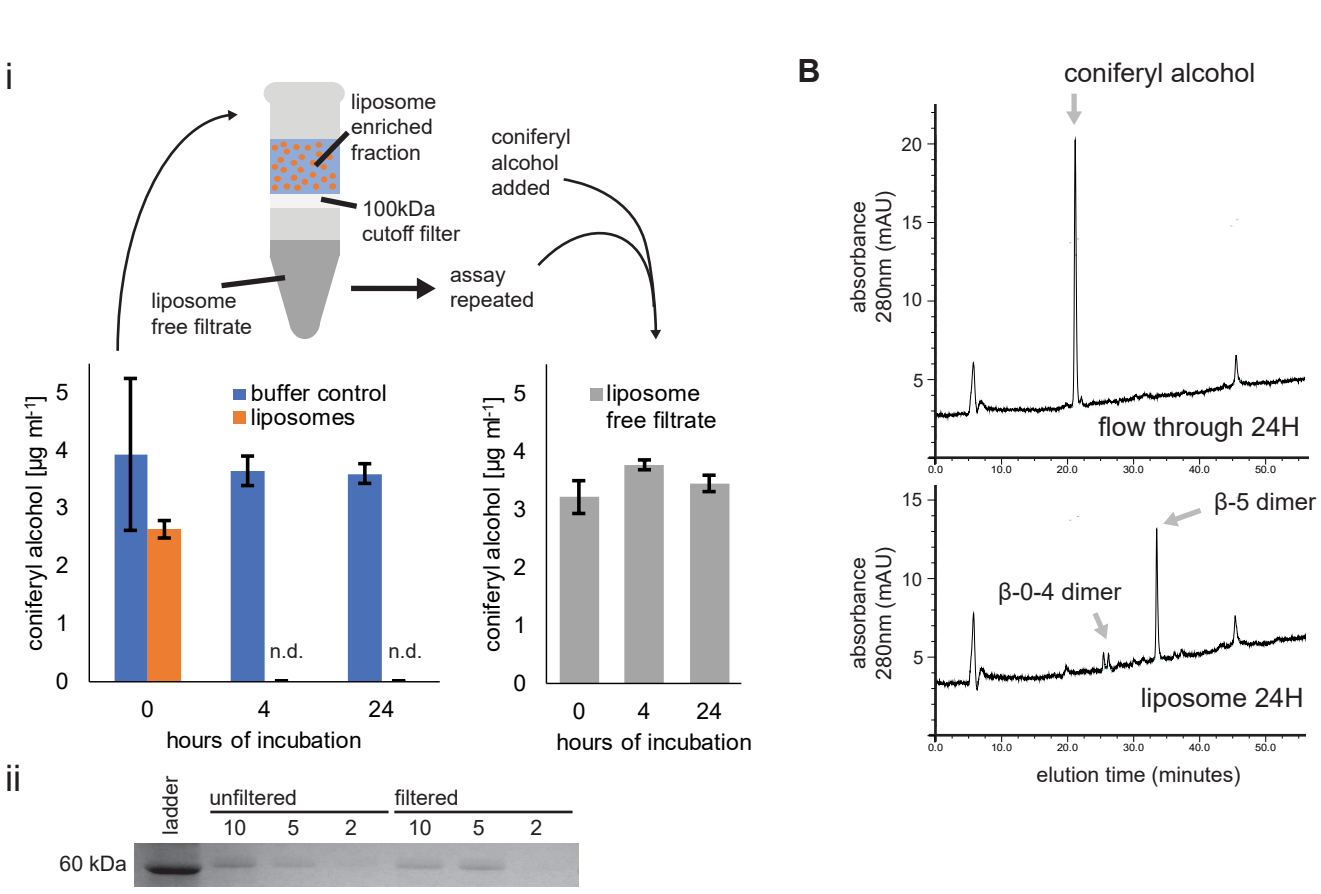
Supplemental Figure S5. Effect of inhibitor treatments on the viability of Arabidopsis roots. Supports Figure 3. Roots were grown for 5 days on control medium, transferred to the indicated concentration of inhibitor containing medium or inhibitor free control medium for 24h, and then stained with propidium iodide. Scale bar indicates 50µm. Arrows indicate nuclei stained with propidium iodide indicating cell death.
(A) Carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP)-treated roots.
(B) Vanadate-treated roots.



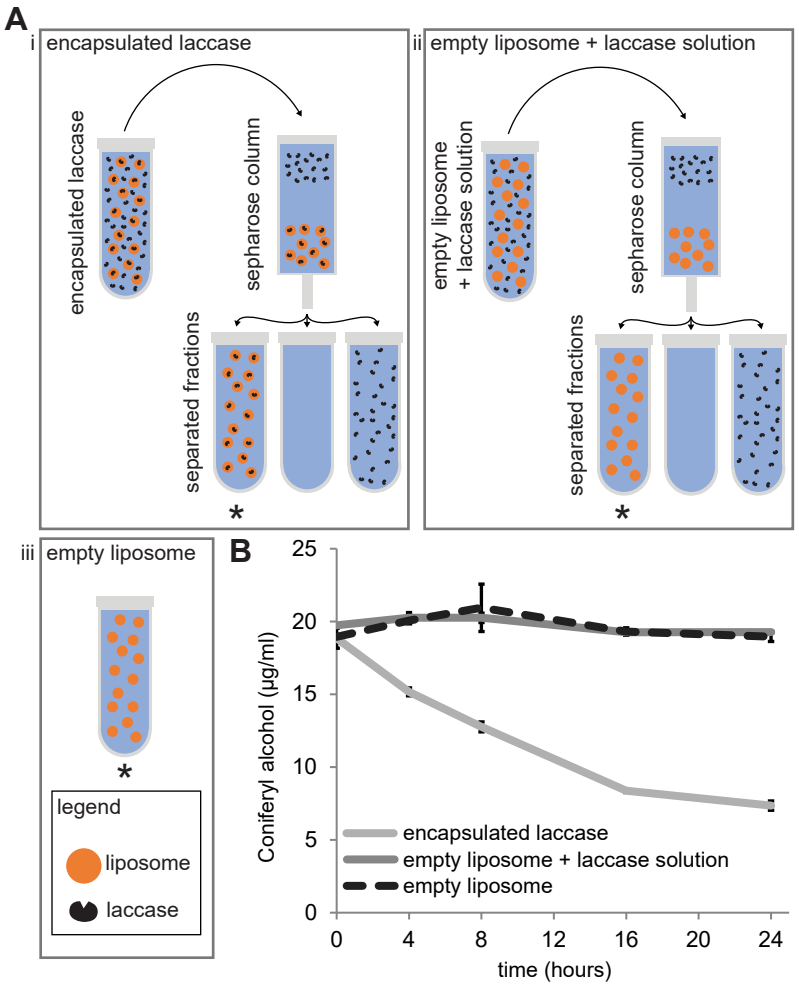
Supplemental Figure S6. Vanadate slows root growth but cell division and differentiation continue. Images are all maximum intensity projections of Z-stack, so that any beads present appear as bright signals. Supports Figure 3.
(A) Roots at time zero were dipped in fluorescent beads (MagSphere Orange Fluorescent Aminated PS Microspheres 6.8 µm AMOF007UM) for ~1 minute, rinsed briefly in water 2 times and imaged immediately. The box on the root schematic indicates the area near the most recently lignified xylem vessel.
(B) Control roots grown on GM medium for 24h following bead treatment. Two regions of interest were imaged (indicated by boxes). The region of the root where the first lignification occurred (right) lacks beads and while the more mature root (left) was labelled at time zero.
(C) Col-0 roots were transferred to 50µM vanadate containing GM media for 24h following bead treatment and imaged as in (B). Beads (left) that were added at time zero appear as either circles or out of focus bright patches. No beads appear in regions of new growth (right). Scale bar 100µm.



Supplemental Figure S7. Positive controls for effective ABC transporter inhibition by vanadate in gravitropic auxin responses and root hair development. Supports Figure 3.
(A) ABC transporter-mediated auxin distribution (*ProDR5:GFP*) during a gravitropic response of roots was altered. The *ProDR5:GFP* construct is transcribed in proportion to the quantity of auxin in a cell. When tilted, a gravitropic response is elicited and auxin accumulates asymmetrically with an accumulation on the downward side of the root. The mutant *mdr4-1 (abcb4)* exhibited an increase in both the intensity and width of fluorescent signal induced by a change in direction of gravity (Lewis *et al.*, 2007). White arrows indicate direction of gravitropic stimulus. Exposure to 50μM vanadate for 6h altered the gravity-induced auxin asymmetry in a manner similar to the *mdr4-1* (*abcb4)* mutant as described by Lewis et al. 2007 in Figure 5b and c. A wider region of brighter auxin signal was observed in the 50μM vanadate. Grey dashed line indicates approximate location of computed transverse sections near the root tip which was excluded due to the overwhelmingly bright auxin maxima there. Scale bar indicates 25μm.
(B) Vanadate exposure elicits a characteristic abundance of root hairs. Control 8-day-old dark grown Col-0 Arabidopsis roots stained with toluidine blue. Scale bar = 100μm. Vanadate treated Arabidopsis roots stained with toluidine blue. Roots were grown for 6 days on GM medium and transferred to 50μM vanadate media for 48h. h indicates root hairs.



Supplemental Figure S8. Laccase activity remains within liposomes throughout the duration of monolignol uptake assays. Supports Figures 4 and 5.
(A) Schematic of the assay testing for residual laccase activity in the liquid medium. Coniferyl alcohol or MES buffer controls were incubated with laccase-containing liposomes + buffer for 24h, and similar to Figure 4, CA was rapidly depleted in the liposome fraction within 8 h (orange bars on right). To make the control more stringent, 2.5X higher concentration of laccase-containing liposomes and a 24-hour incubation period were used. The liposome-containing mixture was filtered with an Amicon ultra 100kDa filter devices (Millipore Ltd.) to remove the liposomes from the buffer, leaving only the surrounding buffer. This filtered buffer was re-incubated with additional coniferyl alcohol to test for free laccase activity. In contrast to the original liposome + buffer fraction (orange bar on left chart), which depleted the coniferyl alcohol within 4 h, there was no change in coniferyl alcohol levels when incubated with liposome-free filtrate (grey bars on right chart). Samples (n=3) were run on HPLC to quantify coniferyl alcohol levels. **ii**, To ensure laccase could pass through the filter device, solutions of pure laccase were sampled before (unfiltered) and after (filtered) being run through the Amicon ultra 100kDa filter, then run on a SDS-PAGE gel. Filtered laccase solutions of different concentrations (10mg/mL, 5mg/mL and 1mg/mL) all showed protein of the predicted molecular weight at levels comparable to unfiltered control (left, ladder of 60 kd reference). This demonstrates that if any laccase enzyme had leaked into the solution, it would have been able to pass through the filter and be detected in the assay. Therefore, we conclude that liposomes with encapsulated laccase did not release free laccase into the surrounding solution.
(B) Upper: HPL chromatograph of phenolics (coniferyl alcohol) following a 24h incubation of CA in liposome-free filtrate (flow through) from the experiment in (A). Lower: HPL chromatograph of phenolics (dimers) following 24h incubation of CA with laccase-containing liposomes.



Supplemental Figure S9. Laccase activity is not carried over on the exterior of liposomes following purification. Supports Figures 4 and 5.
(A) Schematic of laccase-containing and empty liposomes undergoing size exclusion purification. **i**, Liposomes were prepared as previously described, with laccase encapsulated inside, followed by Sepharose column purification. * Indicates encapsulated laccase fraction assayed in (B). **ii**, Control liposomes were prepared with no laccase inside, then laccase was added to the solution surrounding the liposomes to test if laccase sticks to the exterior of liposomes. Following the column purification, control liposomes (empty liposomes + laccase solution, starred) were assayed in B. **iii**, In addition, a preparation containing no laccase at any stage was prepared. * Indicates liposome containing fractions assayed in (B).
(B) Preparations were assayed for their ability to consume coniferyl alcohol over time as in Figure 4. Both the empty liposomes and liposomes that had been surrounded in laccase solution exhibited no ability to consume coniferyl alcohol suggesting that the column purification step was highly effective at removing laccase activity from the solution and outer surface of the liposomes. n = 3 replicated independent liposome reactions.

Supplemental Table S1. Summary of statistical tests performed in the figures and supplementary figures.

Figure	Test	Degrees of freedom	Test statistic	p value
2C	two sample T test	8	t = 7.9693	< 0.0001
2Ei	two sample T test	20.771	t = 2.4225	0.025
2Fi	two sample T test	15.969	t = -4.3402	0.00051
2Eii	ANOVA	3	f = 4.205	0.0159
2Eii	Levene's test	3	f = 0.5641	0.6439
2Fii	ANOVA	3	f = 15.96	$6.5 * 10^{-6}$
2Fii	Levene's test	3	f = 2.3931	0.093
3B	two sample T test	26.914	t = -0.029	0.98
5Ci	Kruskal-Wallis	3	$\chi 2 = 89.742$	$2.2 * 10^{-16}$
5Cii	Kruskal-Wallis	3	$\chi 2 = 59.28$	$8.4 * 10^{-13}$
S2	DSS two sample T test	8	f = 108.62	$6.3 * 10^{-6}$
S2	FD two sample T test	8	f = 8.84	0.025
S2	coniferin two sample T test	8	f = 63.509	$4.5 * 10^{-5}$
S2	FG two sample T test	8	f = 4.5347	0.067
S4A	two sample T test	9	t = 0.3383	0.74
S4B	two sample T test	9	t = -1.9670	0.081
S4C	two sample T test	9	t = 2.1667	0.058