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Supplementary Information for

The synthesis of xyloglucan, an abundant plant cell wall polysaccharide, requires CSLC function.

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Supplementary Information

SI Materials and Methods

Plant material and growth conditions

Homozygous *cslc4-1* (SALK_146718), *cslc4-3* (SAIL_837B10), *cslc5-1* (SAIL_187G09), *cslc6-1* (SALK_088720-11), *cslc8* (WiscDsLox_497-02H) and *cslc12-2* (SAIL_168F02) T-DNA-insertion lines were used in this study after confirmation by PCR based genotyping with specific primers (Table S5). As controls, Arabidopsis wild type (Col-0) and *xxt1 xxt2* mutant were used. Sterilized Arabidopsis seeds were grown on ½ Murashige and Skoog medium (MS with vitamins) with or without 1% sucrose at 23 °C in the dark. Seven-day-old etiolated hypocotyls were collected for transcriptomics (with sucrose), neutral sugar composition (without sucrose), and IP and glycosidic linkage (without sucrose on agar media) analyses. To monitor plant growth, plants were grown at 16 h/ 8 h light/dark (120 µmol/m^2/s) at 22 °C.

Transcriptomics and qRT-PCR analyses

RNA was extracted using plant RNA extraction kit (www.mn-net.com). After RNA isolation, total RNA from 7-day old etiolated hypocotyls of wild type and *cslc456* and *cslc456812* mutant were sent to the Research Technology Support Facility at Michigan State University for generating RNAseq libraries and sequencing the libraries using Illumina platform (paired 125 bp). The RNAseq reads were analyzed with CLC Genomics Workbench version 8.5 (www.qiagenbioinformatics.com/products/clc-genomics-workbench/). Adapter sequence and low-quality regions were removed using the Trim Sequences tool (quality limit: 0.05). Trimmed reads were mapped onto the *Arabidopsis thaliana* reference genome (TAIR10) and alignments over gene regions were quantified using the RNA-Seq Analysis tool (length fraction: 0.8, similarity fraction: 0.8). The resulting read mappings and RPKM normalized expression values were imported into a custom database for further analysis. Total read counts for each gene were analyzed using DESeq2 (1).

For qRT-PCR or RT-PCR of *CSLC* family genes, 1 µg of total RNA was used to investigate expression of *CSLC* genes during Arabidopsis development. qRT-PCR and RT-PCR was performed using *CSLC* gene-specific primers (**Table S5**) as described previously (2). Ubiquitin was used to normalize the expression level of *CSLC* genes. Two to three biological replicates were tested, and a similar pattern of *CSLC* expression was observed among biological replicates.

Root hair measurements and pollination assays.

For root hair measurements, seedlings were grown for 1 week on plates containing ½ MS with 1% sucrose containing 0.7% agar at 16 h/ 8 h light/ dark (120 µmol/m^2/s) at 22 °C. The images of root hairs from seedlings were obtained using Zeiss-Axio imager (www.zeiss.com). Images were analysed using ImageJ software to measure the length of root hairs.

Pollination assays were performed as described previously (3), except that floral buds were processed for microscopy exactly 1 hour after pollination. For each assay, wild-type stigmas were saturated with pollen from wild type or each of the various mutant plants. Pollen tubes were visualized using a Nikon A1 laser scanning confocal microscope by exciting with a 405-nm laser and collecting emission at 525 nm with the green band-pass filter channel. In order to both increase contrast between the pistil tissue and the pollen tubes and emphasize gradations in signal intensities, the "Rainbow Dark" LUT setting was applied to the captured images using NIS-Elements AR software. Pollen tubes that had penetrated the style of each wild-type pistil (n = 6 pistils for each pollen genotype) were counted, and treatments (mutants) that were statistically significantly different from the wild-type control were determined by Dunnett's test ($P < 0.05$) using Prism software.

Glycan array

Glycan array was performed as described previously (4) with slight modification. Two mg of de-starched AIR from Col-0, *cslc456-2, cslc456812,* and *xxt1 xxt2* was sequentially extracted with 200 µl of 1 M and 4 M KOH with 20 mM $NaBH_4$ using a ball mill with two glass balls for 5 min at 30 Hz. The released materials were neutralized with 50% of glacial acetic acid. After generating 5 successive 2-fold dilutions with 0.8 M KOH containing 2.5 mg/ml of oat beta-glucan, 3 µl of each dilution was spotted on a positively charged nylon membrane (www.sigmaaldrich.com) and dried overnight. For immunoblotting the membrane was blocked in PBS with 5% non-fat dairy milk for 15 min. Primary antibodies (LM6, 1:250 dilution; LM15, 1:330 dilution) in PBS with 0.5% non-fat dairy milk and secondary antibody (Goat anti-rat conjugated with HRP, 1:1000 dilution) in PBS with 0.5% non-fat dairy milk were used for the immunoblotting. The blot was developed by chemiluminescence using the SuperSignal West Femto Maximum Sensitivity substrate (www.thermofisher.com). Signals from the blot was obtained using ChemiDoc™ MP and Image Lab software (www.biorad.com).

Xyloglucan Oligosaccharide mass profiling (OLIMP)

OLIMP analysis was performed according to the published protocol (5). Briefly, AIR material was isolated from seven-day-old etiolated Arabidopsis hypocotyls grown in the dark. AIR was suspended in 25 mM ammonium formate buffer, pH 4.5 and digested using 0.2 U of a xyloglucan specific endoglucanase (6) at 37 ˚C for 16 hours. The digested material was analyzed with MALDI-TOF (Bruker rapifleX) using dihydroxy benzoic acid (DHB) as a matrix. Spectra were recorded in positive reflectron mode with an accelerating voltage of 20000V. The spectra from the samples were analyzed using the flexanalysis software (Bruker Daltonics).

Driselase digestion of alcohol-insoluble residue (AIR), and HPAEC and LC/ Qtof MS analyses

The driselase enzyme mix (www.sigmaaldrich.com) was partially purified as previously reported (7). AIR was prepared using 7-day-old etiolated hypocotyls as described (5). Approximately 1.3 mg of AIR obtained from the etiolated hypocotyls were digested with 300 µl of 0.03% of driselase enzyme mix (25 mM NaAc, pH 5.0) at 37 °C for 48 hours, and the reaction was stopped by heating the samples at 95 °C for 5 min. For HPAEC analysis, maltose was added as an internal standard for IP quantification. The digest was analysed using a modified procedure (8). The carbohydrates were separated using a Carbo Pac PA200 column with a linear gradient of 2-10 mM NaOH for 5 min, followed by 10-355 mM NaOH for 20 min. Eluting compounds were detected by pulsed amperometric detection (PAD). For LC/MS analyses, 233 µl of methanol was added to the driselase digest (100 µl) and incubated in 4 °C for 20 min. After centrifugation at 11k x *g* for 10 min, the supernatant containing digested sugars were dried and resuspended in 50% acetonitrile containing 2 µg/ml of ^{13}C-labelled sucrose (monitoring matrix effect) as an internal standard for quantification. IP quantification was done using a Waters Xevo G2-XS Qtof mass spectrometer interfaced with a Waters Acquity UPLC. Samples were injected onto a Waters Acquity BEH-Amide UPLC column (2.1x100 mm) and a 20-min gradient was run as follows: initial conditions were 5% solvent A (10 mM ammonium acetate in water), 95% B (acetonitrile) and held for 1 min, followed by a ramp to 35% A/ 65% B at 14 min, hold at 35% A until 16 min, return to 5% A/ 95% B at 16.01 min and hold until 20 min. Flow rate was 0.3 ml/min and column temperature was 40 ºC. Data were acquired using electrospray ionization in negative-ion mode with a target enhancement function (m/z 325). Peak areas for IP (M-H) and ^{13}C sucrose were processed using Quanlynx (Masslynx software).

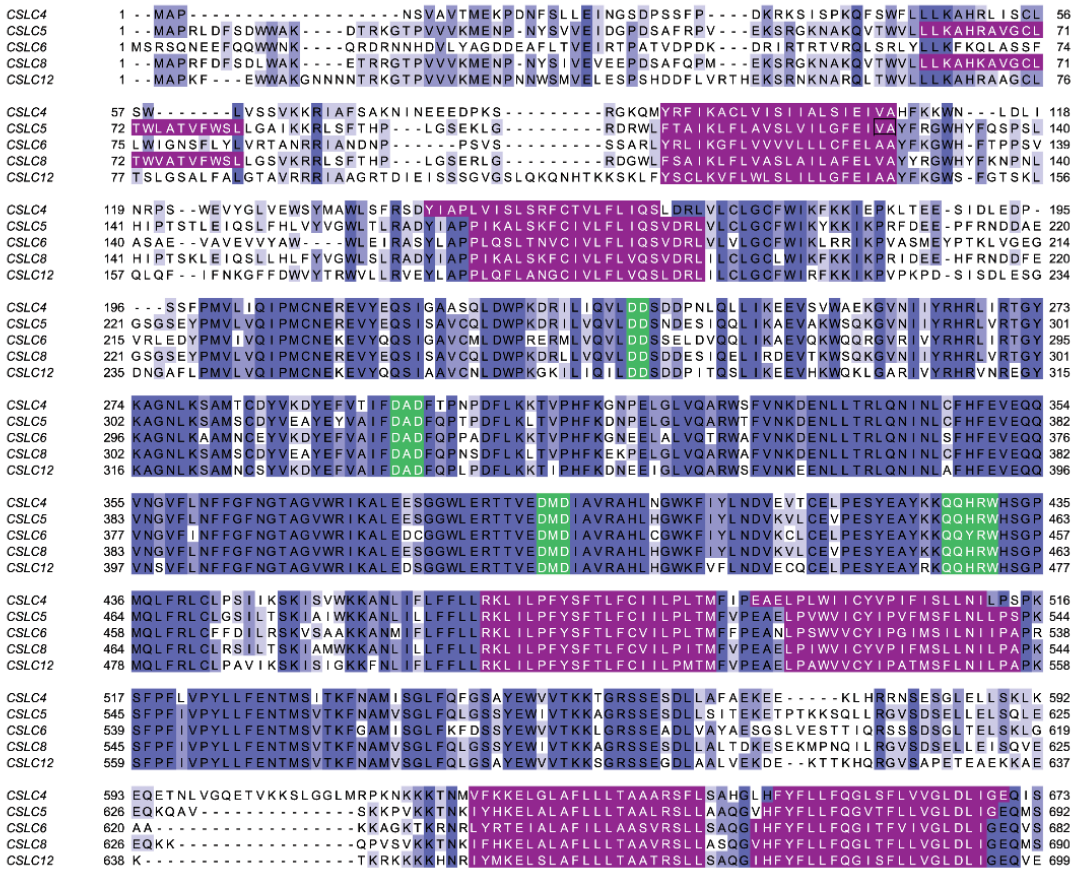
Complementation of the cslc456812 quintuple mutant

To generate Gateway technology-compatible vector containing the Arabidopsis *CSLC4* promoter, amplification of the *CSLC4* promoter (1719 bp) was performed using *CLSC4* promoter-specific primers. The amplified product was introduced in the pEarlygate100 vector using the Infusion system (www.takarabio.com) (9). Full-length coding sequences of *CSLC* family genes with N-terminal T7 tags were synthesized from Thermo-Fisher (www.thermofisher.com). The resulting synthesized genes in the pDONOR221 plasmid were used to generate the final constructs containing *CSLC4pro::T7-CSLC4*, *5*, *6, 8, 12* in the pEarlygate100 plasmid backbone. Arabidopsis transformation by vacuum infiltration was performed to create complementation lines of the *cslc456812* quintuple mutants as previously reported (8). The transformed seedlings were screened by observing root hair growth restoration. Due to difficulty of screening successful complementation lines by *CSLC8* and *CSLC12* with this method, we created *35Spro::T7-CSLC8* or *CSLC12* in pGWB2 via the LR reaction in order to screen transformants using antibiotics (hygromycin). The *CSLC8* or *CSLC12* transformed seedlings were screened on ½ Murashige and

Skoog medium (MS with vitamins) with 0.1% sucrose and 20 µg/ml hygromycin. Primers used are listed in **Table S3**.

Fig. S1. Protein sequence alignment and percent identity among CSLC family members.

A



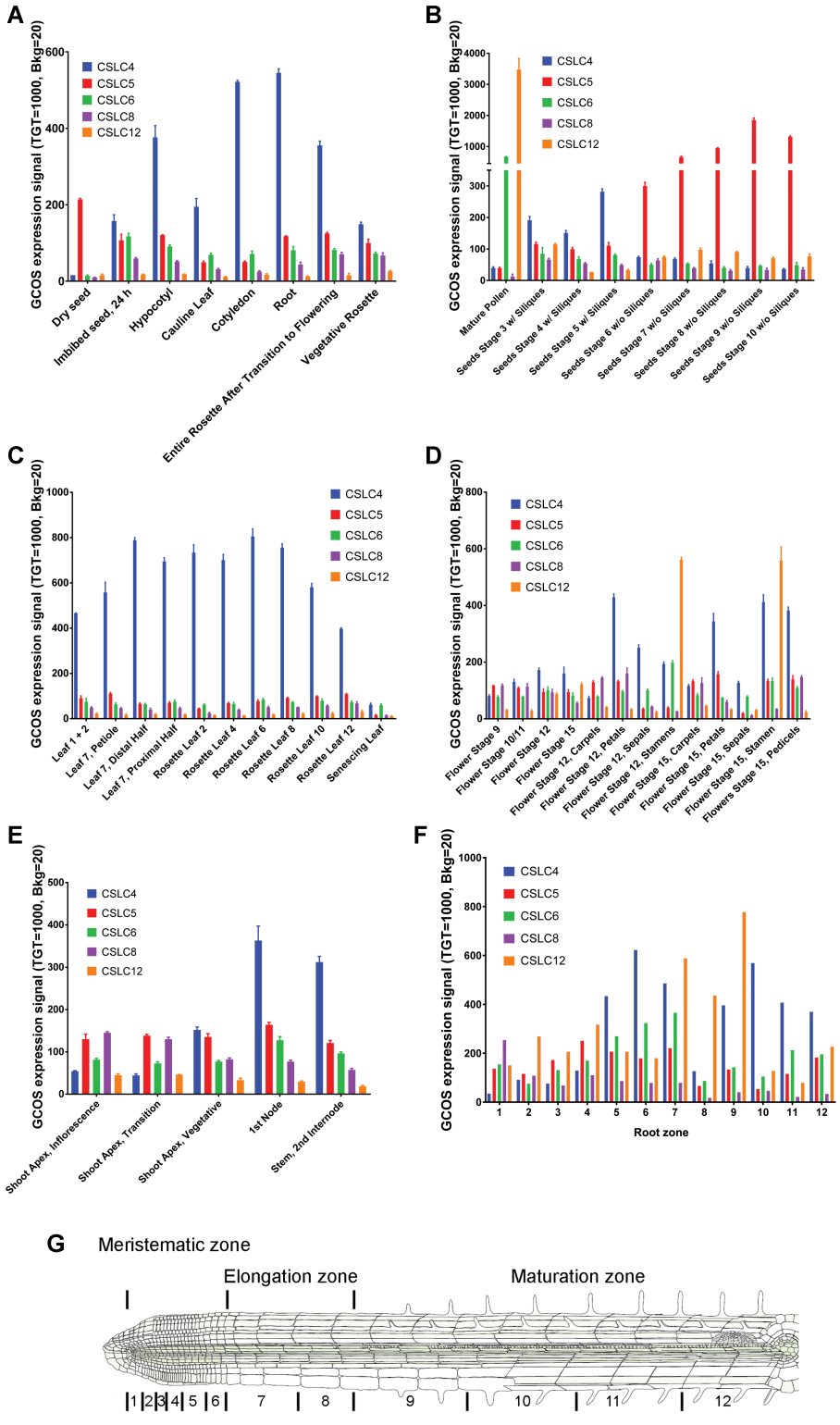
B

Sequence Identity (%)	BLASTp Input				
	CSLC4	CSLC5	CSLC6	CSLC8	CSLC12
CSLC4	**100**	67.25	61	66.38	63.02
CSLC5	66.57	**100**	61.97	87.3	65.78
CSLC6	61	61.97	**100**	61.14	60.69
CSLC8	66.38	88.31	61.14	**100**	64.43
CSLC12	62.59	65.92	60.69	64.2	**100**

(A) Protein model of all five CSLC members. Key domains: D,DxD,D,QxxRW domains are highlighted in green; putative transmembrane domains are indicated in magenta; conserved amino acids are indicated in dark blue (conserved in all 5 CSLCs) and blue (conserved in at least three CSLCs).

(B) Sequence identity (%) among CSLC proteins calculated using the BLASTp algorithm.

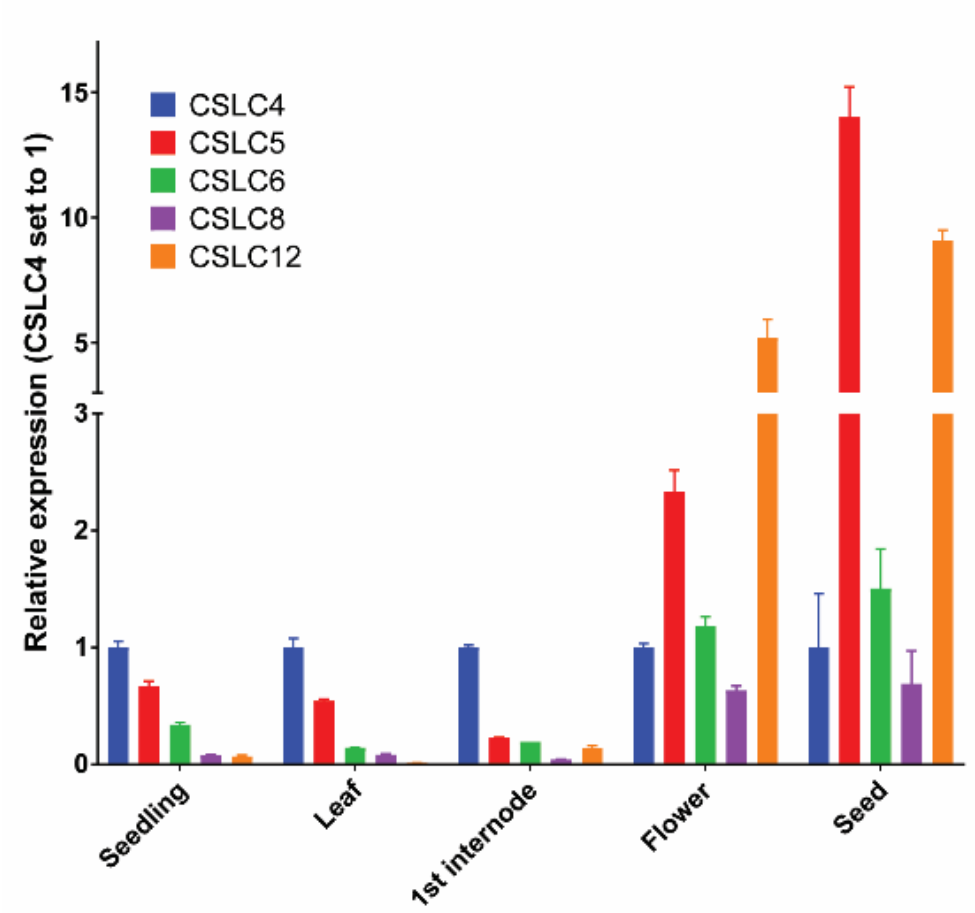
Fig. S2. Expression pattern of *CSLC* genes in Arabidopsis



The expression data of *CSLC*s was obtained from Arabidopsis eFP Browser (http://bar.utoronto.ca/efp2/Arabidopsis/Arabidopsis_eFPBrowser2.html). Mature pollen was combined with seed development to facilitate display of the data.

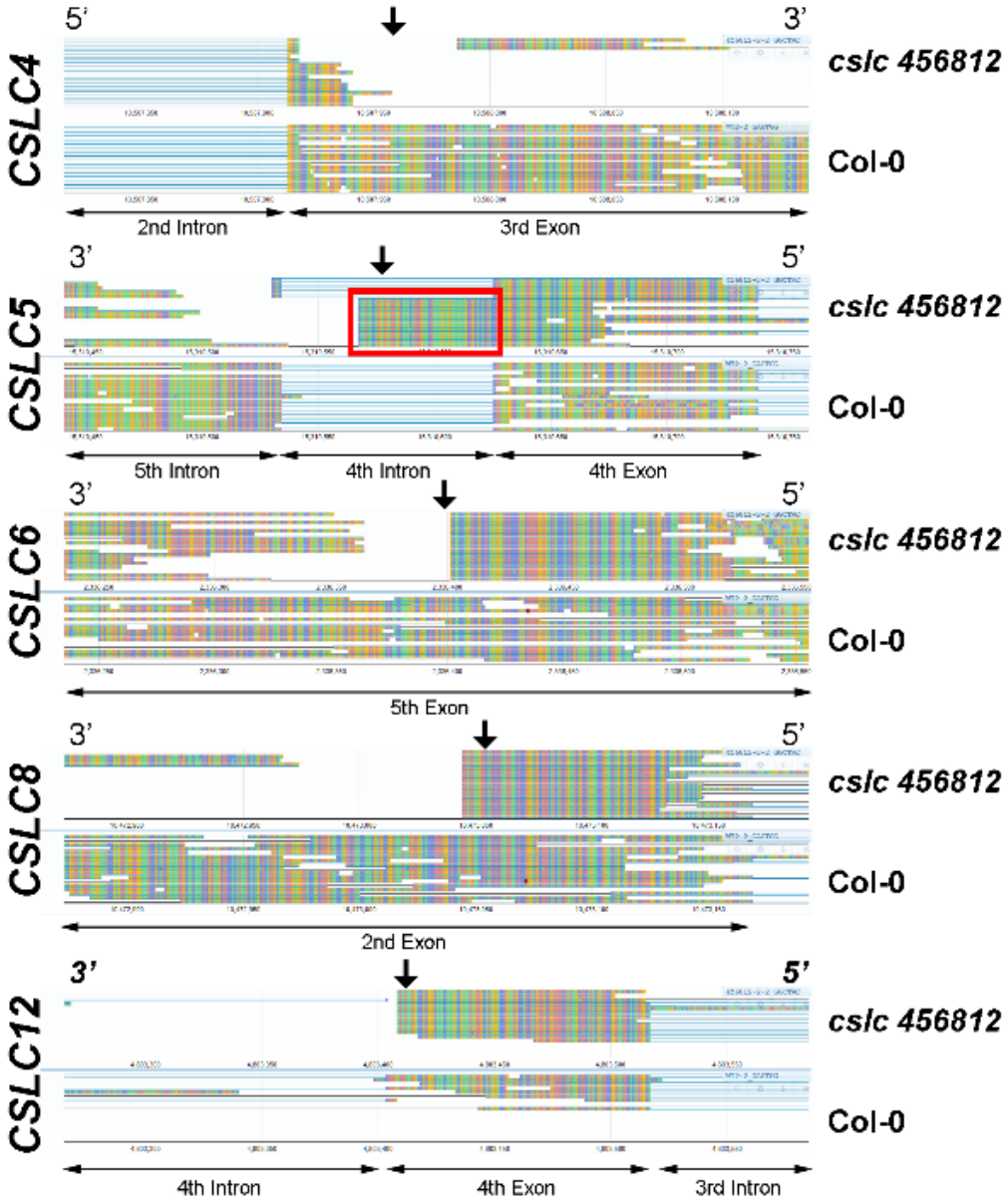
(A) Major plant parts and organs
(B) Mature pollen, and Seed development
(C) Leaf parts and development
(D) Flower parts during development
(E) Shoot development
(F) Root development
(G) Layout of *x*-axis (position of zones) indicated in panel F

Fig. S3. qRT-PCR of *CSLC* gene expression in various plant parts and various developmental stages of Arabidopsis.



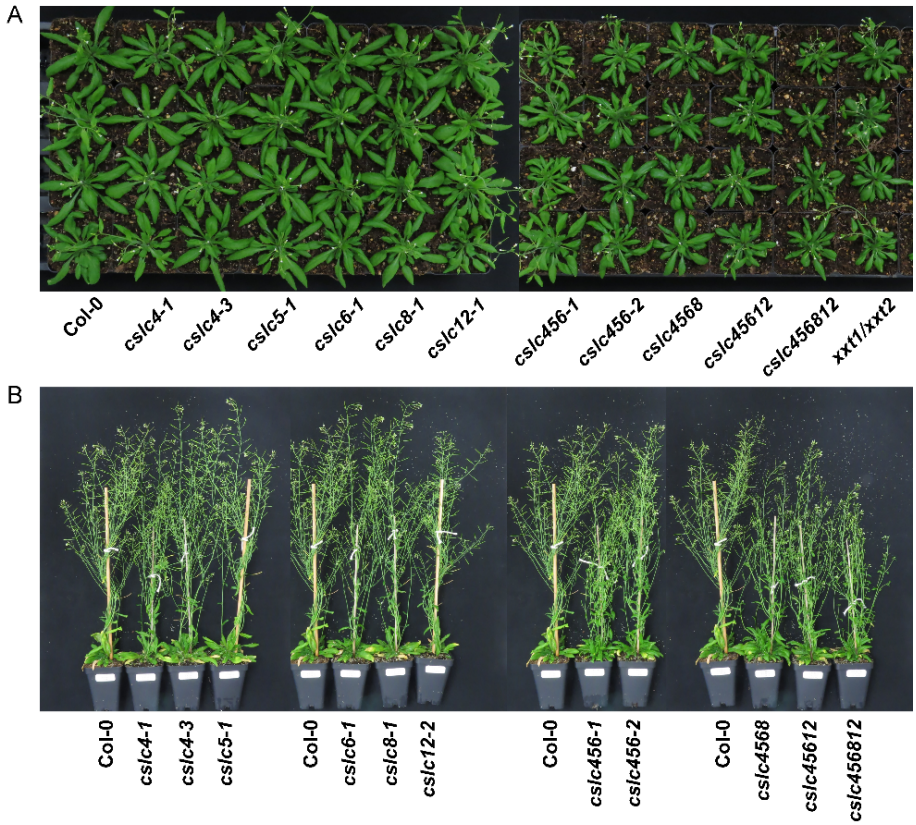
The expression level of each *CSLC* gene was investigated using RNA from wild-type Col-0, and relative expression level of each *CSLC* gene was normalized using the expression level of *CSLC4, n* = 3, ± SD.

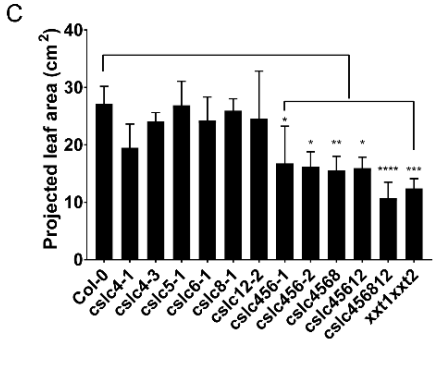
Fig. S4. Confirmation of the lack of full-length transcripts in the *cslc456812* quintuple mutant.

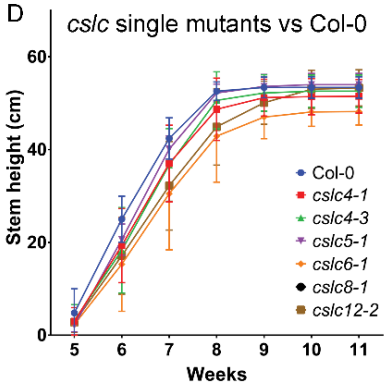


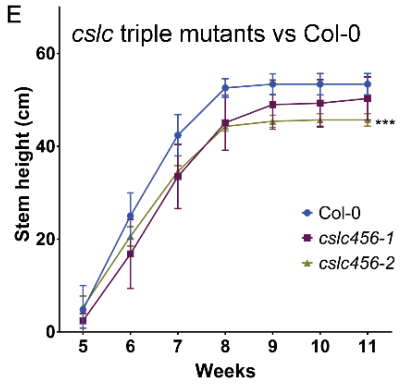
Mapping RNAseq reads onto *CSLC* gene family shows the absence of RNAseq reads covering the T-DNA insertion site of each *CSLC* locus or presence of RNAseq reads containing both T-DNA border and *CSLC* genomic sequences. Arrows indicate T-DNA insertion sites. Each nucleotide is color-coded (A-green, T-red, G-yellow, C-blue). Top 5 reads with a interval covering the 4th intron of *CSLC5* in *cslc456812* do not match the *CSLC5* sequence, and the remaining RNAseq reads at the 4th intron of *CSLC5* in *cslc456812* contain T-DNA left-border sequence (red box).

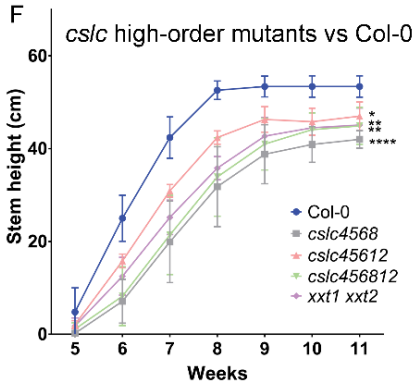
Fig. S5. Growth phenotype of Col-0, *cslc* and *xxt1 xxt2* mutants.









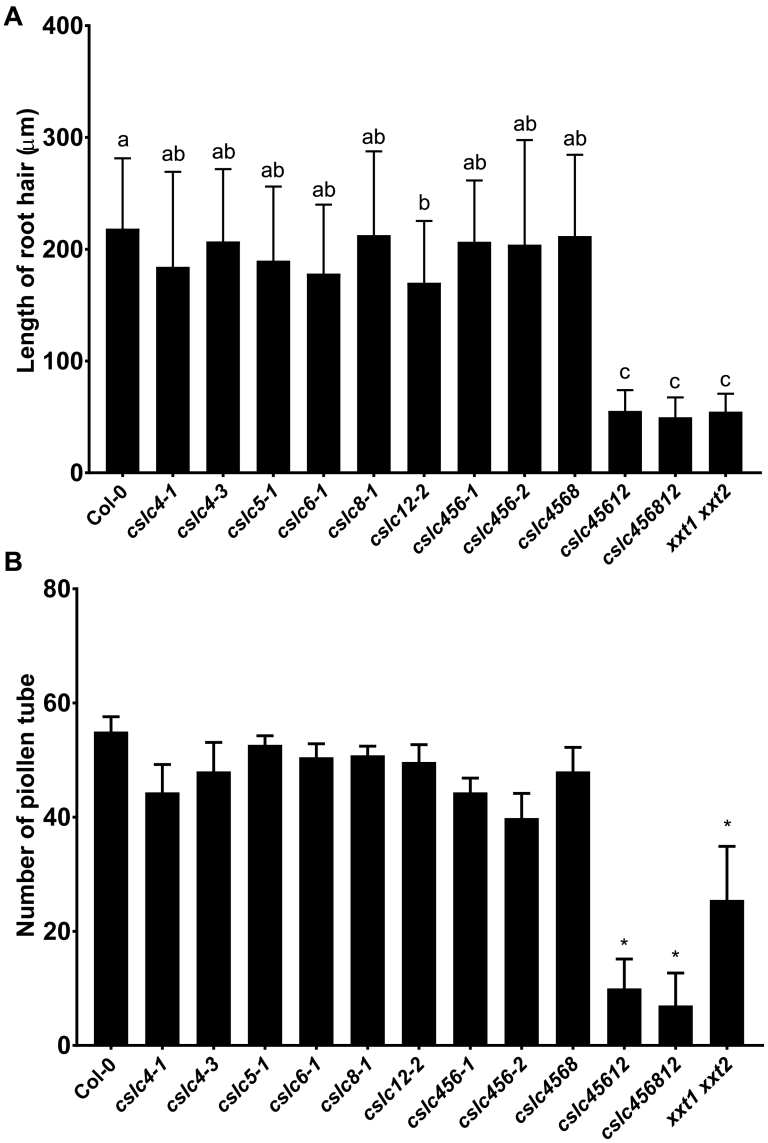


(A) Images of 4.5-week-old rosettes.

(B) Images of 9-week-old plants
(C) Measurements of projected leaf areas at 4.5 weeks (supports Panel A), $n = 4$, ± SD.
(D-F) Measurements of inflorescence stem height during development (supports Panel B), $n = 8$, ± SD.

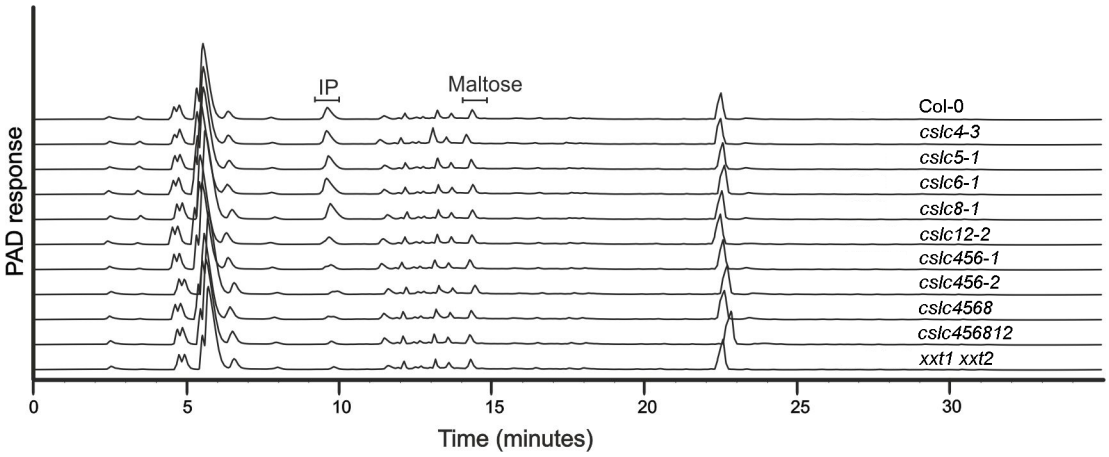
Asterisks indicate statistically significant differences from Col-0 (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ by student's *t*-test).

Fig. S6. Root-hair lengths, and pollen-tube numbers from pollination assays, of various cell wall mutants.



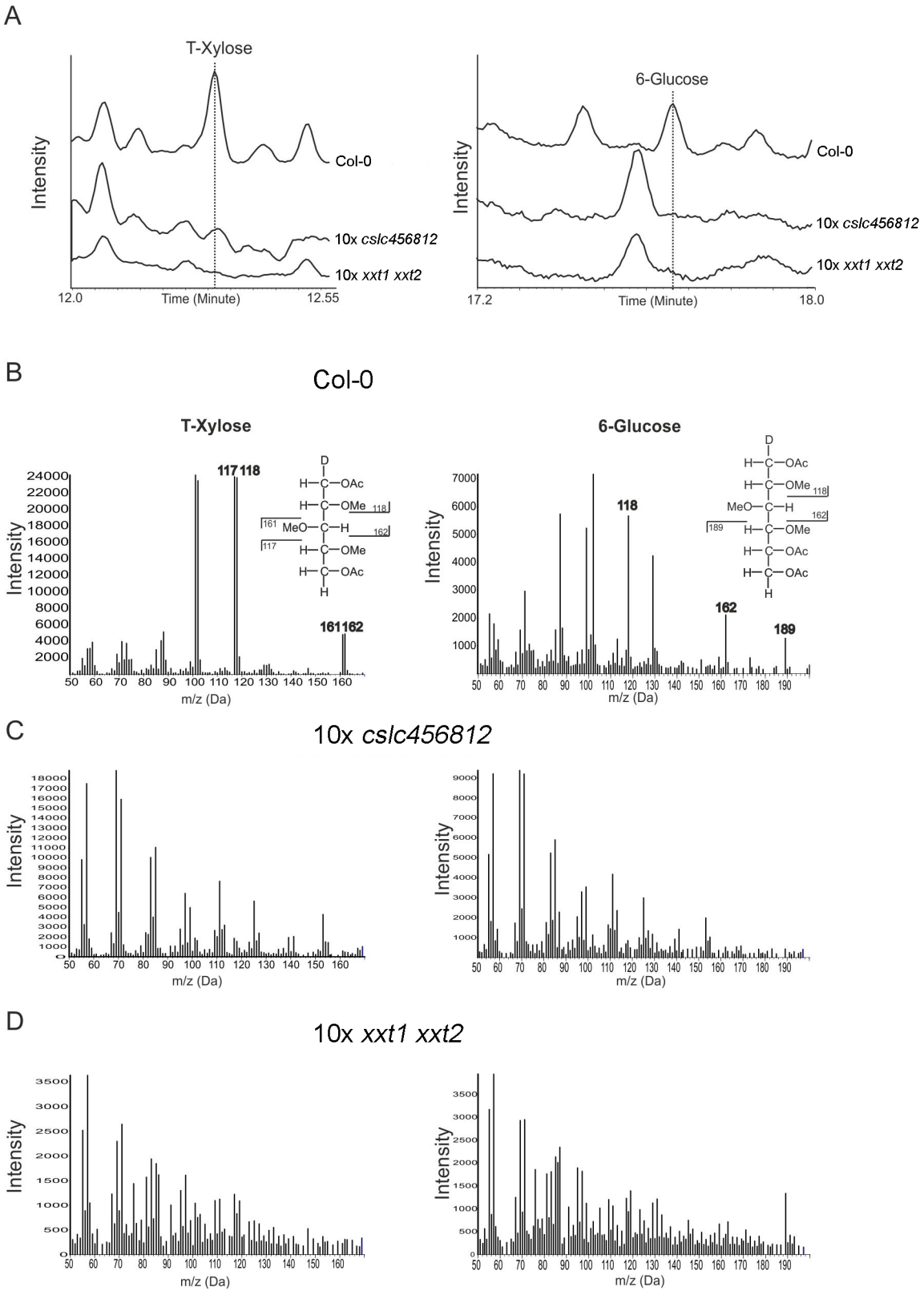
(A) Lengths of root hairs from 1-week-old seedlings (n > 40, ± SD). One-way ANOVA (Tukey's test, P < 0.05) was used to determine statistical differences of root-hair length among genotypes. Each statistical group was labeled with lower-case letters (a, b, and c).
(B) Numbers of pollen tubes that have penetrated the styles of Col-0 pistils, 1 hour after pollination with pollen from each of the indicated genotypes (n = 6, ± SEM). Asterisks denote treatments (mutants) that are statistically significantly different from the Col-0 control (Dunnett's test, *P < 0.05).

Fig. S7. Isoprimeverose profiling of wall preparations from Col-0 and Arabidopsis *cslc* and *xxt1 xxt2* mutants using HPAEC-PAD



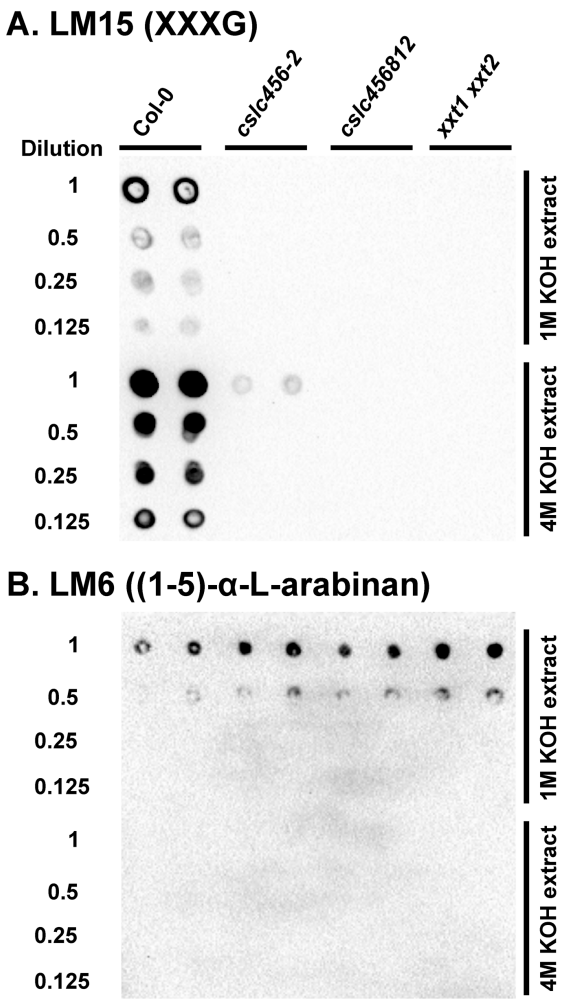
The AIR material obtained from the etiolated hypocotyls of Arabidopsis wild type and different mutants were digested with driselase. The digested material was analysed by HPAEC. The IP present in the digest was quantified using maltose as an internal standard.

Fig. S8. Glycosidic linkage analysis of compounds eluting at the retention time of IP.



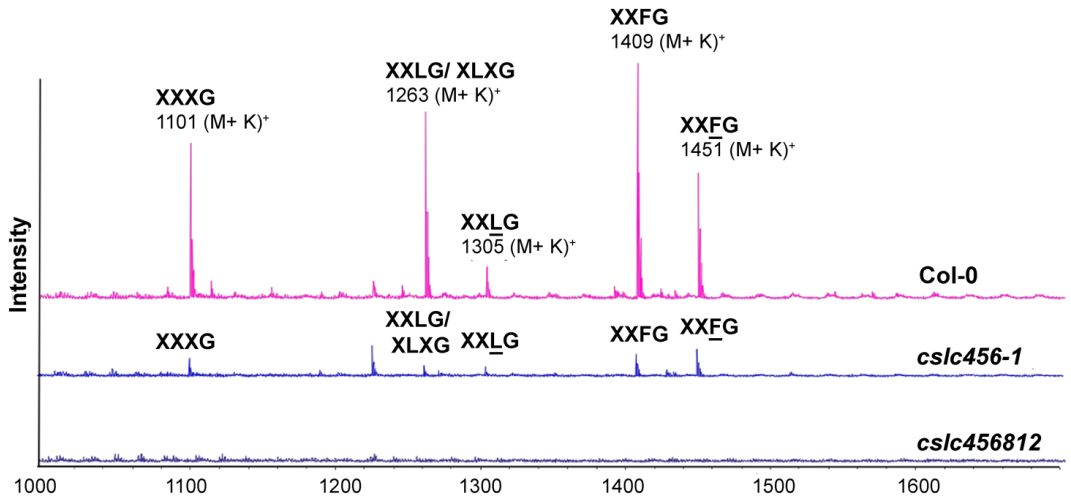
Driselase-digested AIR was subjected to HPAEC (see Fig. 4, Fig. S7) and the compounds eluting at the retention time of IP (approximately 10 min) were collected. To account for the significant reduction in IP abundance in the *xxt1 xxt2* and *cslc456812* mutants (see Fig. 4, Fig. S7), 10 times the amount of driselase digest of the corresponding AIR material was subjected to HPAEC analysis and collected. The collected material was subjected to methylation analysis followed by acid hydrolysis, reduction, and acetylation to yield partially methylated alditol acetates. These were analysed by gas chromatography (panel A) fitted with an electron impact quadrupole detector (GC-MS). An IP standard subjected to this procedure (separation by HPAEC, collection and analysis by GC-MS) results in two peaks that based on their ion fragmentation patterns represent terminal xylose (T-Xylose), and 6-linked glucose (6-Glucose). These two peaks, at 12.3 minutes and 17.6 minutes, respectively, were also visible in the driselase-digested AIR from Col-0. They gave fragmentation patterns characteristic of terminal xylose and 6-linked glucose (panel B). However, similar peaks were not seen in the GC traces of the *cslc* quintuple mutant and the *xxt1 xxt2* mutant (panel A) and the characteristic fragmentation pattern of these two glycosyl-moieties could not be detected in the mass spectra of the material eluting at the appropriate retention times (panels C and D), even though 10 times the material was used for analysis.

Fig. S9. Glycan arrays of XyG and RG-I in XyG mutants.



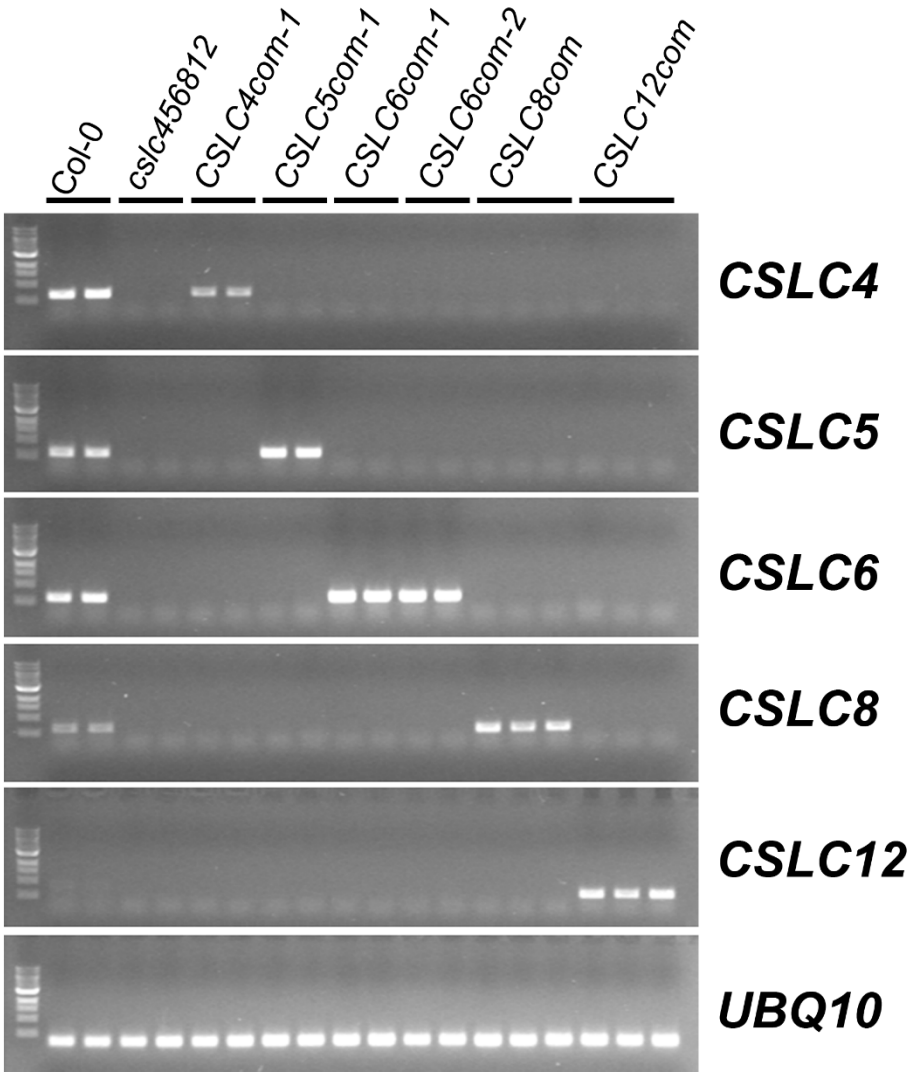
AIR was prepared from 7-day-old etiolated hypocotyls of Col-0, *cslc456-2*, *cslc456812*, and *xxt1 xxt2* and sequentially extracted with 1 M and 4 M KOH with 20mM $NaBH_4$. A series of 2 fold dilutions of the released material was spotted onto a nylon membrane. LM15 and LM6 antibodies were used to detect XyG (XXXG) and RG-I ((1-5)-α-L arabinan), respectively.

Fig. S10. XyG oligosaccharide mass profiling.



AIR materials were prepared from 7-day-old etiolated hypocotyls of Col-0, *cslc456-1*, and *cslc456812,* and digested with a xyloglucan-specific endoglucanase. The released oligosaccharides were analyzed by MALDI-TOF mass spectroscopy. The m/z $(M+K^+)^+$ of known XyG oligosaccharides are labelled with their respective one-letter code nomenclature (10, 11).

Fig. S11. Verification of complementation lines of the *cslc456812* mutant using RT-PCR.



Extracted RNA from each complementation line was used for RT-PCR using *CSLC*-specific primers. *UBQ10* primers were used for the loading control. As positive and negative controls, RNA from Col-0 and the *cslc* quintuple mutant was used to show the specificity of primer sets used in this experiment.

Table S1. Neutral sugar composition of AIR from 7-day-old etiolated seedlings.

Monosaccharides (µg per mg AIR)								
Genotype	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	Crystalline cellulose
Col-0	**19.12 ± 0.36**	**3.82 ± 0.19**	**18.82 ± 0.41**	**36.61 ± 1.74**	**6.94 ± 0.35**	**23.99 ± 1.46**	**14.25 ± 0.39**	**227.07 ± 10.35**
cslc4-1	**20.15 ± 0.32**	**3.90 ± 0.08**	**19.11 ± 0.20**	**32.40 ± 1.22**	**8.12 ± 0.27**	**24.09 ± 0.22**	**14.08 ± 0.75**	**226.00 ± 3.95**
cslc4-3	**20.96 ± 0.14**	**3.87 ± 0.08**	**18.48 ± 0.27**	**30.34 ± 0.95**	**7.87 ± 0.26**	**24.89 ± 0.59**	**16.19 ± 0.40***	**231.28 ± 0.47**
cslc5	**19.77 ± 0.82**	**3.79 ± 0.14**	**18.11 ± 0.15**	**34.87 ± 0.36**	**7.71 ± 0.08**	**25.16 ± 0.36**	**15.77 ± 0.73**	**241.53 ± 2.70**
cslc6	**19.83 ± 0.39**	**3.42 ± 0.12**	**18.87 ± 0.73**	**31.03 ± 0.32***	**8.08 ± 0.15***	**24.55 ± 0.90**	**14.79 ± 1.12**	**213.34 ± 2.38**
cslc8	**20.70 ± 0.85**	**4.15 ± 0.13**	**18.75 ± 0.57**	**33.91 ± 0.71**	**7.85 ± 0.19**	**25.66 ± 1.33**	**15.46 ± 0.40**	**245.39 ± 3.45**
cslc12	**19.76 ± 0.43**	**4.17 ± 0.08**	**18.66 ± 0.32**	**35.77 ± 0.37**	**7.52 ± 0.08**	**24.45 ± 0.20**	**15.43 ± 0.60**	**245.22 ± 2.11**
cslc456-1	**20.01 ± 0.69**	**2.41 ± 0.05**	**18.57 ± 0.08**	**24.25 ± 0.34****	**8.08 ± 0.12***	**20.76 ± 0.20**	**11.43 ± 0.25****	**204.95 ± 5.44**
cslc456-2	**19.67 ± 0.74**	**1.76 ± 0.10****	**19.60 ± 0.27**	**20.84 ± 0.81****	**8.29 ± 0.21***	**21.55 ± 0.45**	**10.19 ± 0.40****	**189.81 ± 9.79**
cslc4568	**21.36 ± 1.13**	**1.67 ± 0.06****	**20.08 ± 0.64**	**20.88 ± 0.65****	**8.38 ± 0.11***	**23.35 ± 1.82**	**10.26 ± 0.16****	**186.99 ± 6.01***
cslc45612	**20.10 ± 0.24**	**1.84 ± 0.07****	**20.56 ± 0.11***	**21.88 ± 0.68****	**8.53 ± 0.23***	**23.03 ± 0.80**	**10.68 ± 0.35****	**194.31 ± 2.31***
cslc456812	**19.82 ± 0.98**	**1.62 ± 0.11****	**20.31 ± 0.17***	**20.09 ± 0.45****	**8.48 ± 0.14***	**21.23 ± 0.89**	**9.76 ± 0.31****	**185.52 ± 1.65***
xxt1 xxt2	**18.50 ± 0.38**	**1.57 ± 0.05****	**20.01 ± 0.34**	**18.10 ± 0.56****	**8.54 ± 0.06***	**20.72 ± 0.50**	**12.21 ± 0.27***	**176.66 ± 2.28****

Means and standard errors of 3 biological replicates with 3 technical replicates are presented (only two biological replicates, each with 3 technical replicates, were used for *cslc4-3*). Significance was based on student's *t*-test (* $P < 0.05$ - yellow shade, ** $P < 0.01$- orange shade).

Table S2. Glycosidic linkage analysis of AIR from 7-day-old etiolated seedlings.

Residue[a]	Col-0	*xxt1 xxt2*	*cslc456812*
T-Fuc*p*	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
2-Rha*p*	3.7 ± 0.5	3.4 ± 0.1	4.0 ± 0.9
T-Ara*f*	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
3-Ara*f*	1.5 ± 0.1	*1.3 ± 0.1	1.4 ± 0.2
5-Ara*f*	3.0 ± 0.8	2.1 ± 0.2	2.2 ± 0.2
T-Ara*p*	1.9 ± 0.4	1.6 ± 0.3	1.8 ± 0.1
T-Xyl*p*	2.5 ± 0.4	**0.9 ± 0.1	**0.9 ± 0.1
4-Xyl*p*	6.2 ± 0.5	5.5 ± 0.8	6.2 ± 2.3
2,4-Xyl*p*	2.5 ± 0.1	2.4 ± 0.3	2.5 ± 0.5
4,6-Man*p*	1.2 ± 0.1	1.3 ± 0.1	1.2 ± 0.2
T-Gal*p*	2.3 ± 0.2	2.3 ± 0.2	2.5 ± 0.2
2-Gal*p*	2.3 ± 0.5	*1.1 ± 0.1	*1.1 ± 0.1
3-Gal*p*[b]	21.0 ± 1.7	*34.2 ± 5.0	27.9 ± 14.1
4-Gal*p*	1.9 ± 0.5	1.6 ± 0.1	1.5 ± 0.1
3,6-Gal*p*	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
4,6-Gal*p*	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
T-Man*p*	1.6 ± 0.1	*1.3 ± 0.1	1.7 ± 0.2
2-Man*p*	1.6 ± 0.4	1.6 ± 0.2	1.4 ± 0.2
2,4-Man*p*	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
T-Glc*p*	0.9 ± 0.1	1.1 ± 0.1	1.2 ± 0.2
4-Glc*p*	34.5 ± 2.4	31.1 ± 4.0	34.8 ± 11.4
6-Glc*p*	1.5 ± 0.2	1.8 ± 0.1	2.1 ± 0.4
4,6-Glc*p*	6.9 ± 0.1	**2.5 ± 0.3	**2.7 ± 0.4

a Glycosyl residues are expressed as percentage of the total glycosyl peak areas. Averages and standard deviations of the biological replicates (n = 3) are presented. Significance was based on student's t-test (* $P < 0.05$, yellow; ** $P < 0.01$, orange).
b Non-plant cell wall component due to contamination of the agar growth medium.

Table S3. Primers used in this study.

Allele	T-DNA insertion line	Sequences	
cslc4-1	Salk_146718	FP	TTCAGCACATGATCCTCGTC
		RP	ATGTACGACCATTCGACAAGC
cslc4-3	Sail_837B10	FP	CAGGGTAATCCAGAGCTAGGG
		RP	GATGATCTCCCGGTTTTCTTC
cslc5-1	Sail_187G09	FP	AAGTTGGCTCAGCTCCAAAA
		RP	TTGACCCGTCTTCAGAACATC
cslc6-1	Salk_088720-11	FP	ACGCTCTTCTGCGTCATTCT
		RP	GTCTTTGGAATCCTCTCCGAC
cslc8	wiscDsLox_497-02H	FP	CACCTAGCCTGAACCAGACC
		RP	TTGAGATCCAGAGCTTGCTT
cslc12-2	Sail_168F02	FP	AGCTCAGCTTCGGGTACAAA
		RP	TACGAATTCGTTGCGATTTTC
	T-DNA RB for Salk line	ATTTTGCCGATTTCGGAAC	
	T-DNA RB for Sail line	TAGCATCTGAATTTCATAACCAATCTCGATACAC	
	T-DNA RB for WiscDsLox line	AACGTCCGCAATGTGTTATTAAGTTGTC	

For cloning complementation line	Sequences	
CSLC4 promoter	FP	TAGGAAGGAAGTTCGAATACTGTTCTAATTTAATCCTGCATC
	RP	AACTTGTGATCTCGAGAAGTAAATAAAGAGGAGAGGAGAAG

For qRT-PCR	Sequences	
CSLC4	FP	GCGAGTTGCCAGAGTCTTATGA
	RP	GGAAGGCATAACCGGAACAG
CSLC5	FP	TCCTGAGTCCTACGAGGCATATAA
	RP	TAGAGGTCAAGATTGAACCAAGACA
CSLC6	FP	CGGAGTCCTATGAGGCATATAAAAA
	RP	GCACAAACGGAACAGTTGCA
CSLC8	FP	CATACAAGAAGCAGCAACATCGA
	RP	GTCAAGATTGATCGCAGACACAA
CSLC12	FP	GAGATCTTGCTGCGTTAGTCGAA
	RP	TCCTCTTAGTCTTTTCCGCCTTT
UBQ10	FP	CGCTTCGTTTTTATTATCTGTGCTT
	RP	TCGCAGAACTGCACTAAACAGAGT

For RT-PCR	Sequences	
CSLC4	FP	GGAGCAGCTTCACAACTTGA
	RP	AGCTGCATAGGACCGGAATG
CSLC5	FP	TTGAGGTGGAGCAGCAAGTG
	RP	ATCCACTCGTAAGAGCTACCC
CSLC6	FP	GAGTGCTGCCAAGAAAGCAAA
	RP	CTGACCTGTTCCCCGATCAAA
CSLC8	FP	GCCGAGAATCGATGAAGAGCA

	RP	**TTCCTCAAGGGCTTTAATTCTCCA**
CSLC12	**FP**	**TTGGTTCAAGCTAGGTGGTCT**
	RP	**GGTACGGGACGATAAACGGA**
UBQ10	**FP**	**TCAATTCTCTCTACCGTGATCAAGATGCA**
	RP	**GGTGTCAGAACTCTCCACCTCAAGAGTA**

Dataset S1. List of differentially expressed genes (DEGs) in the *cslc456-2* and *cslc456812* mutants.

DEGs ($|\log_2 FC|>1$) are summarized in the table after a pair-wise comparison between the *cslc* mutant and wild-type plants. The averages of RPKM and standard deviations ($n = 4$) are presented. Any values showing $|\log_2 FC|>1$ are highlighted.

Dataset S2. Expression levels of genes involved in cell wall synthesis.

Expression levels of genes coding for enzymes involved in cell wall synthesis are summarized. XyG, Xyloglucan; HG, Homogalacturonan; RG-I/II, Rhamnogalacturonan-I/II; XGA, Xylogalacturonan. Averages and standard deviations of biological replicates (n = 4) are presented.

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