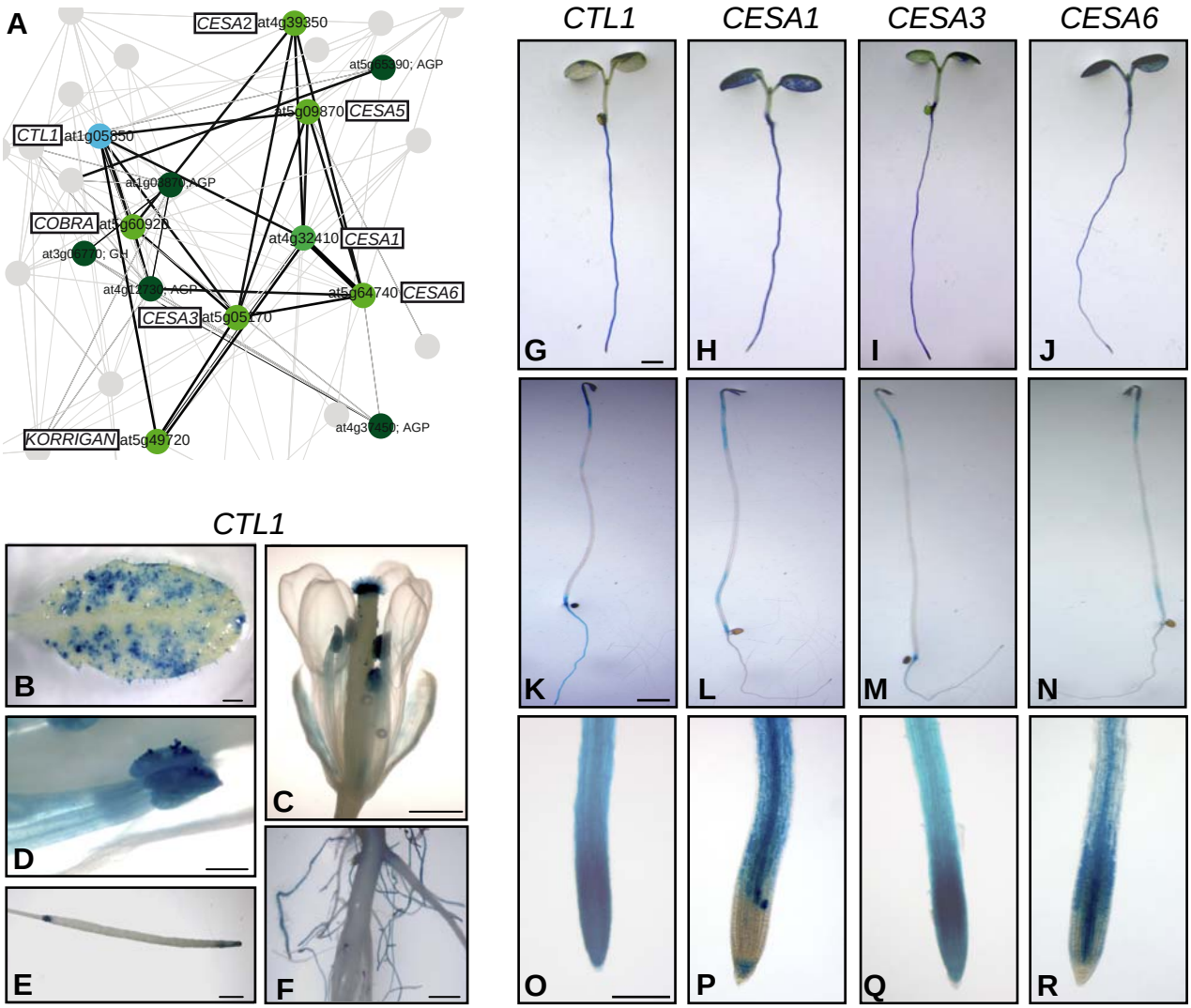

Figure S1. Expression analyses of *CTL1*. A. Co-expression network for *CTL1* generated from AraNet (aranet.mpimp-golm.mpg.de/aranet; (Mutwil et al., 2011)). Primary wall *CESA1*, *2*, *3*, *5* and *6* are in close vicinity to *CTL1* indicating that the genes are co-regulated. *CTL1* is shown in blue and tightly co-expressed with cell wall-related genes in green. **B** to **R**. *CTL1* promoter activity assessed by GUS staining. The *CTL1* promoter is active in rosette leaves (**B**), anthers, pollen and stigma (**C** and **D**), mature roots (**F**), and siliques (**E**). GUS activity was also found in seedling roots (**G**, **K**, and **O**). **G** to **R.***CTL1* and the primary wall *CESAs* are similarly expressed in seedlings. Promoter activities for *CTL1* (**G** and **K**), *CESA1* (**H** and **L**), *CESA3* (**I** and **M**) and *CESA6* (**J** and **N**) as assessed with promoter:GUS constructs in five-days-old light-grown (upper panel) and etiolated (lower panel) seedlings. **O** to **R**. Magnification of GUS activity in the root tips of *CTL1* (**O**), *CESA1* (**P**), *CESA3* (**Q**), and *CESA6* (**R**) in five-days-old light-grown seedlings. Scale bars: (**B, C, E**, and **F**)=1 mm; (**D**)=300 μm; **(G)**=1 mm for (**G** to **J**); (**K**)=1 mm for (**K** to **N**); (**O**)=100 μm for (**O** to **R**)

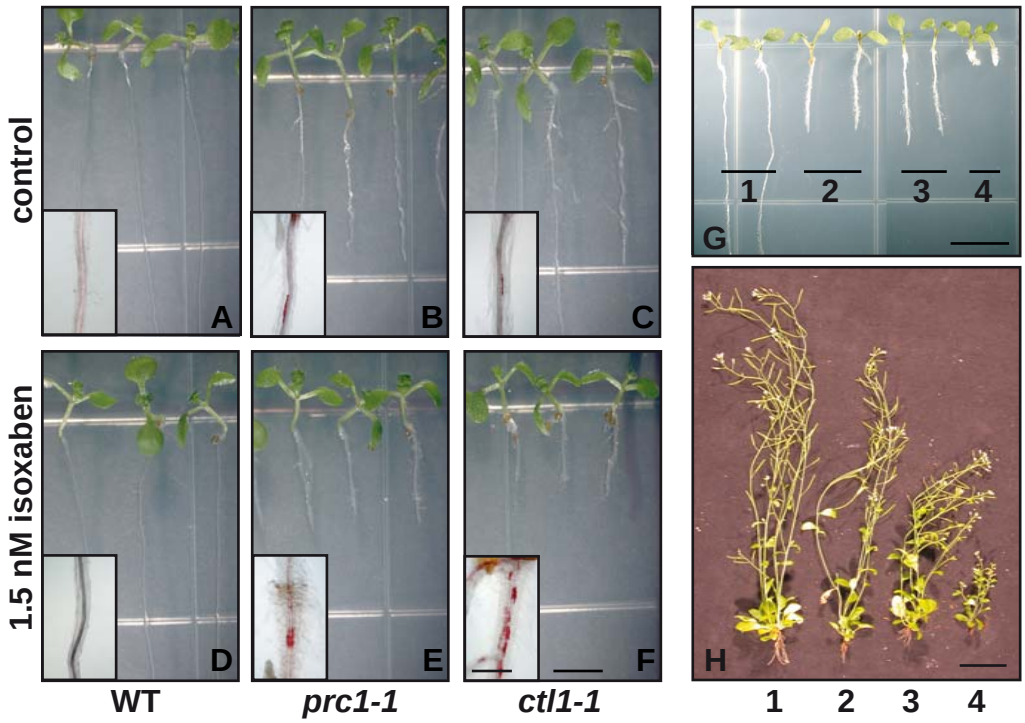

Figure S2. Genetic interactions between *ctl1-1* and *prc1-1*. A to **F.** Isoxaben treatment induces lignification in the *ctl1* mutant. Images are of phloroglucinol stained roots from five-days-old WT (**A** and **D**), *prc1-1* (**B** and **E**), and *ctl1-1* (**C** and **F**) seedlings grown on control media (**A** to **C**), or on media containing 1.5 nM isoxaben (**D** to **F**).**G** and **H**. Additive growth phenotypes for *ctl1-1 prc1-1* double mutants observed in six-day-old light grown seedlings (**G**) and in seven-week-old plants (**H**) 1=WT (Col-0); 2=*prc1-1*, 3=*ctl1-1*, 4=*ctl1-1 prc1-1*. Scale bars: (**F**)= 5 mm and 500 μm for the blown-up phloroglucinol staining (**A** to **F**); (**G**) and (**H**)= 5 mm.

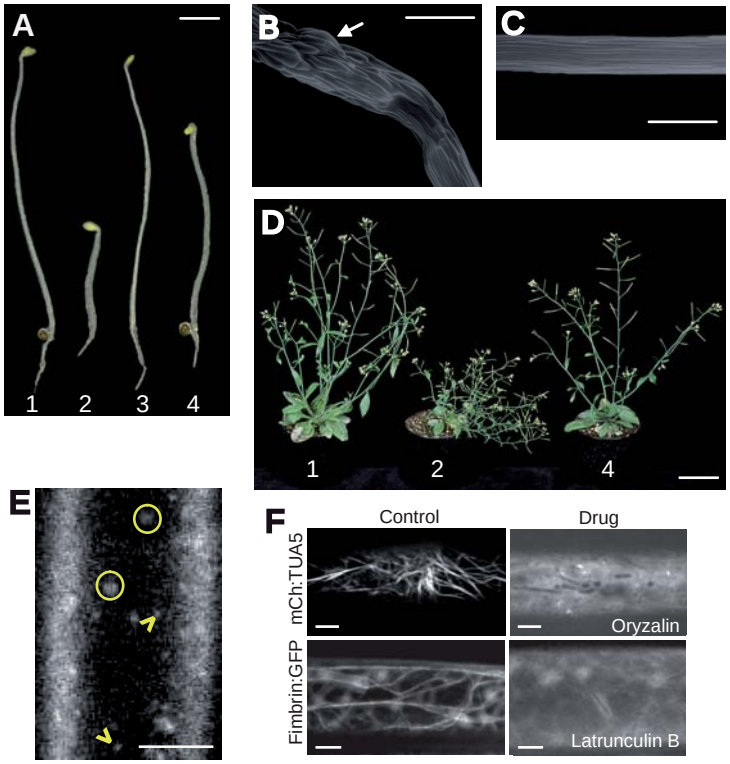

Figure S3. ***ctl1-1* complementation. A** to **E.** Complemented *ctl1* seedlings using a genomic clone of *CTL1*. **A.** Complementation of *ctl1*with *PCTL1:CTL1* and *P35S:CTL1:GFP*. **B** and **C.***CTL1:GFP* complementation of *ctl1-1* eliminates cell swelling in five-days-old *ctl1-1* etiolated hypocotyls. Environmental scanning electron microscopy (ESEM) of *ctl1-1* (**B**) and *ctl1-1;CTL1:GFP* (**C**). Arrow indicates swollen cell in *ctl1-1* (**B**) **D.** *CTL1:GFP* rescues the *ctl1-1* mutant light-grown phenotype observed in seven-weeks-old plants. 1= WT (Col-0); 2= *ctl1-1*, 3= *ctl1-1* complemented with *PCTL1:CTL1*, 4= *pom1-9* complemented with *P35S:CTL1:GFP*. **E.** CLSM image of a cell from a four-day-old etiolated seedling of *ctl1-1* complemented with *PCTL1:CTL1:mChRFP*. The ring shaped Golgi bodies are surrounded by a yellow circle and a CTL1v is marked by a yellow arrowhead. **F**. Average projected z-stacks showing cytoskeleton fibers distribution in control marker lines for microtubules, (mChRFP:TUA5) and actin filaments (Fimbrin:GFP) after incubation in cytoskeleton destabilizing drugs, 20 μM Oryzalin (Ory) 16 h, and 1 μM Latrunculin B (LatB) 3 h, respectively. Scale bars: (**A**)=1 mm; (**B** and **C**)=4 μm; (**D**)=5 cm; (**E** to **G**)=5 μm.

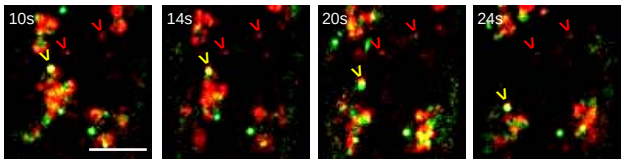

Figure S4. CTL1 co-localizes with oryzalin-insensitive MASCs/SmaCCs in secretory vesicles. Seedling expressing CTL1:GFP and tdT:CESA6 was imaged after 16 h treatment with 20 μM oryzalin. Immobile MASCs/SmaCCs are indicated by red arrowheads. Oryzalin-insenstive motile vesicle containing CTL1:GFP and tdT:CESA6 is indicated by a yellow arrowhead. Scale bar: 5 μm.

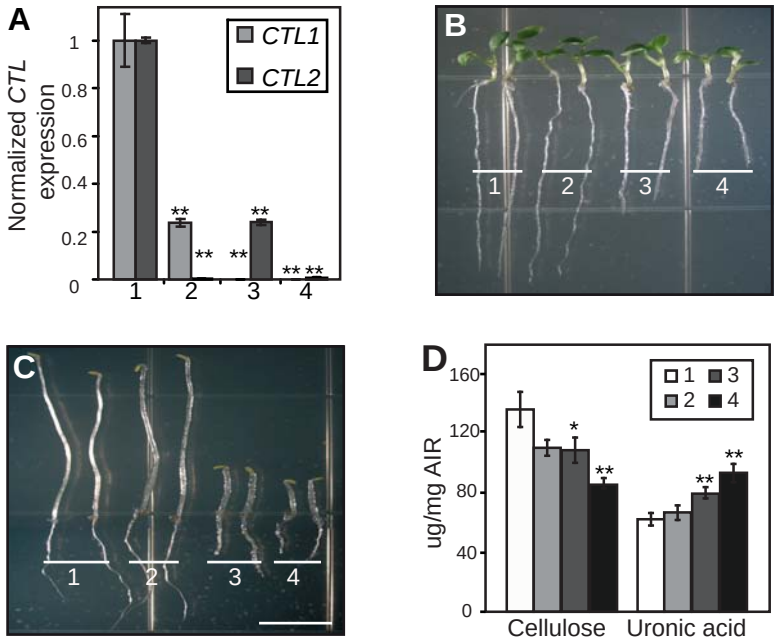

Figure S5. *ctl1 ctl2* double mutant displays subtle additive phenotypes. A. Relative quantification of *CTL1* and *CTL2* levels in 7-week old stems. Values are represented as *n*-fold *CTL* cDNA levels compared to WT. Data represents the average (±SE) of N=3 biological replicates, each with three technical repetitions. **B** and **C**. *ctl1 ctl2* double mutants display subtle additive phenotypes compared to the parental lines. Five-day-old light-grown (**B**) and etiolated seedlings (**C**). **D.** Cellulose and uronic acid content (µg/mg AIR (alcohol insoluble residue)) in seven-week-old stems of WT (Col-0), *ctl1-1*, *ctl2-1* and *ctl1-1ctl2-1* double mutants. (Student's-t test; $P<0.05$ (*) and $P<0.01$ (**)1=WT (Col-0); 2= *ctl2-1*, 3= *ctl1-14*=*ctl1-1 ctl2-1*. Scale bars: (**B**) and (**C**)=5 mm.

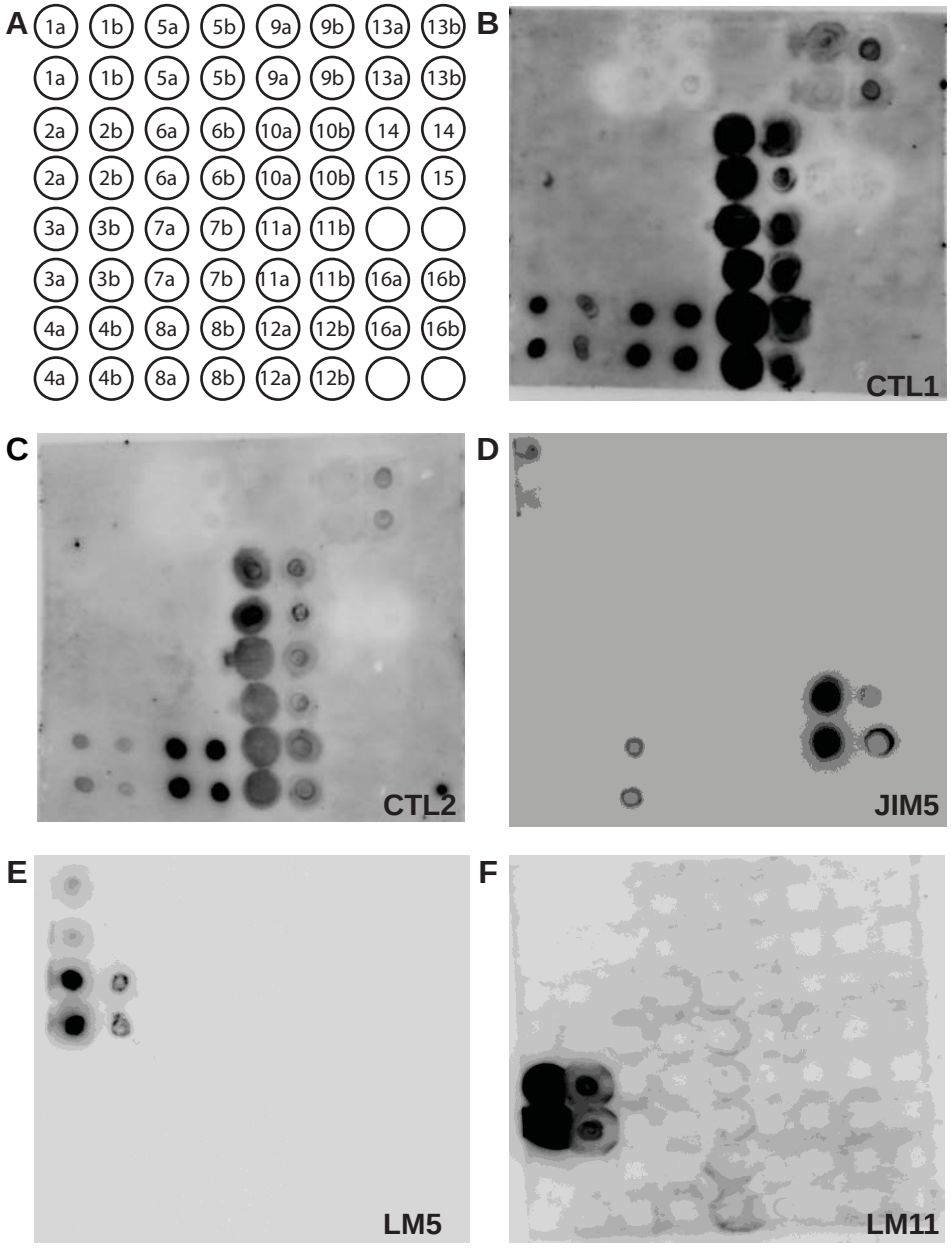

Figure S6. Carbohydrate microarray. A spotting grid for carbohydrate solutions and controls. Numbers indicate individual carbohydrates: pectin esterified form citrus (1), soybean rhamnogalacturonan (2), xylan from birchwood (3), xyloglucan (4), 4 M KOH fraction plant CW (5), lichenan (6), chitosan medium MW (7), chitin from crab shells (8), CM-cellulose (9), cellulose fibrous long (10), sigmacell® cellulose type 20 (11), avicel PH-101 (12), insoluble fraction plant CW (13), PBS (14), Cadoxen (15), pectin from apple (16), letters indicate concentrations of the spotting solution used: 0.5 mg/ml (a) and 0.1 mg/ml (b). **B** to **F**. developed microarrays after incubation with CTL1 incubation (**B**), CTL2 (**C**), JIM5 (**D**; anti-pectin), LM5 (**E**; anti-galactan) and LM11 (**F**; anti-xylan) and respective peroxidase-coupled secondary antibody (see method section)

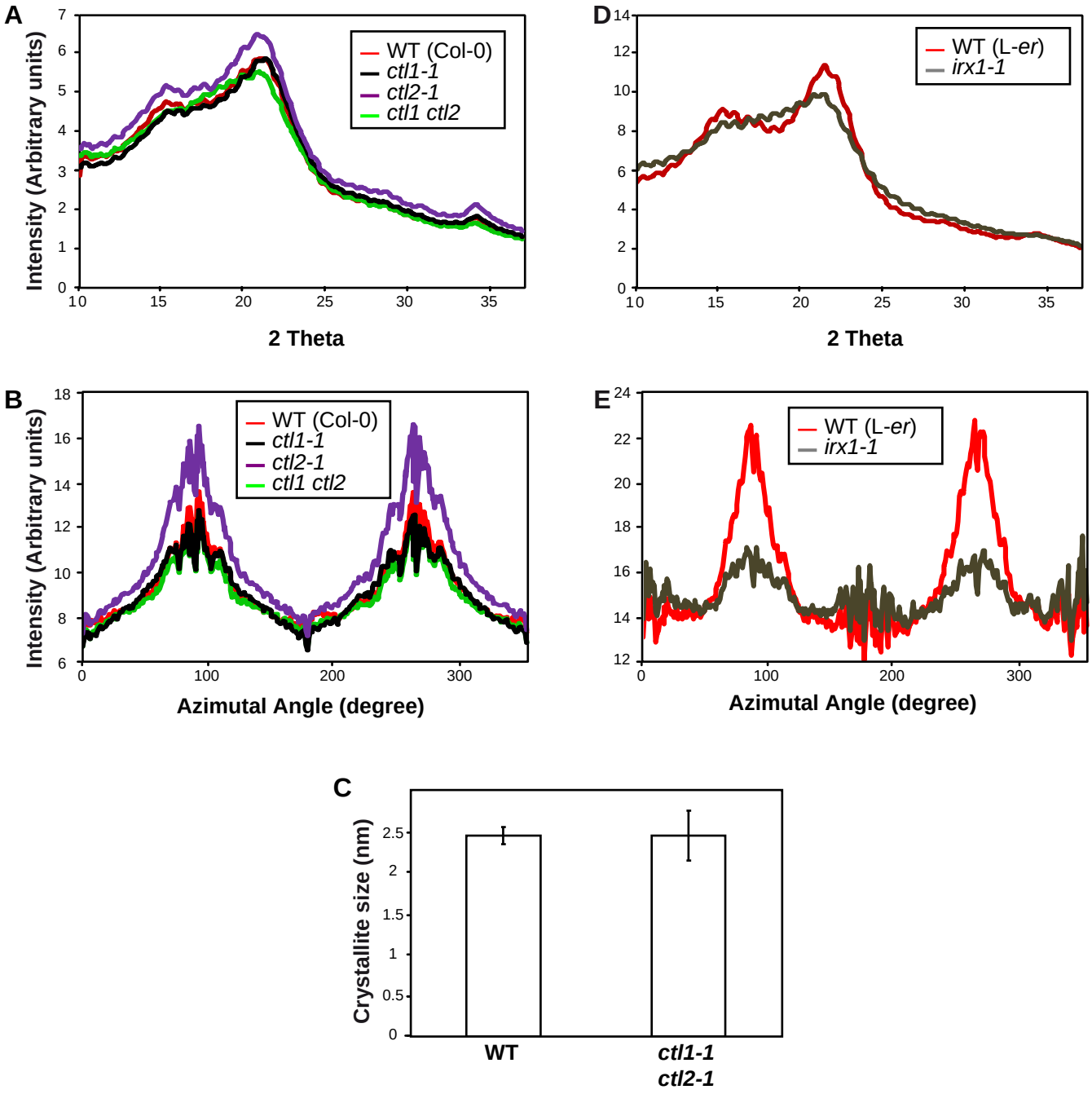

Figure S7. X-ray diffraction analysis of seven-week-old stems of WT plants, *ctl* and *irx1* mutants. A and **D**. Averaged radial profiles from X-ray diffraction. **B** and **E**. Averaged azimuthal integration of the reflection assigned to the 200 plane. **C**. Calculations on crystallite size were made assuming rod shaped crystals. In (**A**), (**B**), (**D**) and (**E**) data represents the average of N= 5 biological replicates with two points measured on each sample. WT (red), *ctl1-1* (yellow), *ctl2-1* (purple), *ctl1 ctl2* double mutant (green), and *irx1-1* (grey). In (**C**) data represents the average (± SE) of N= 5 biological replicates. No statistically significant differences were observed between the samples.

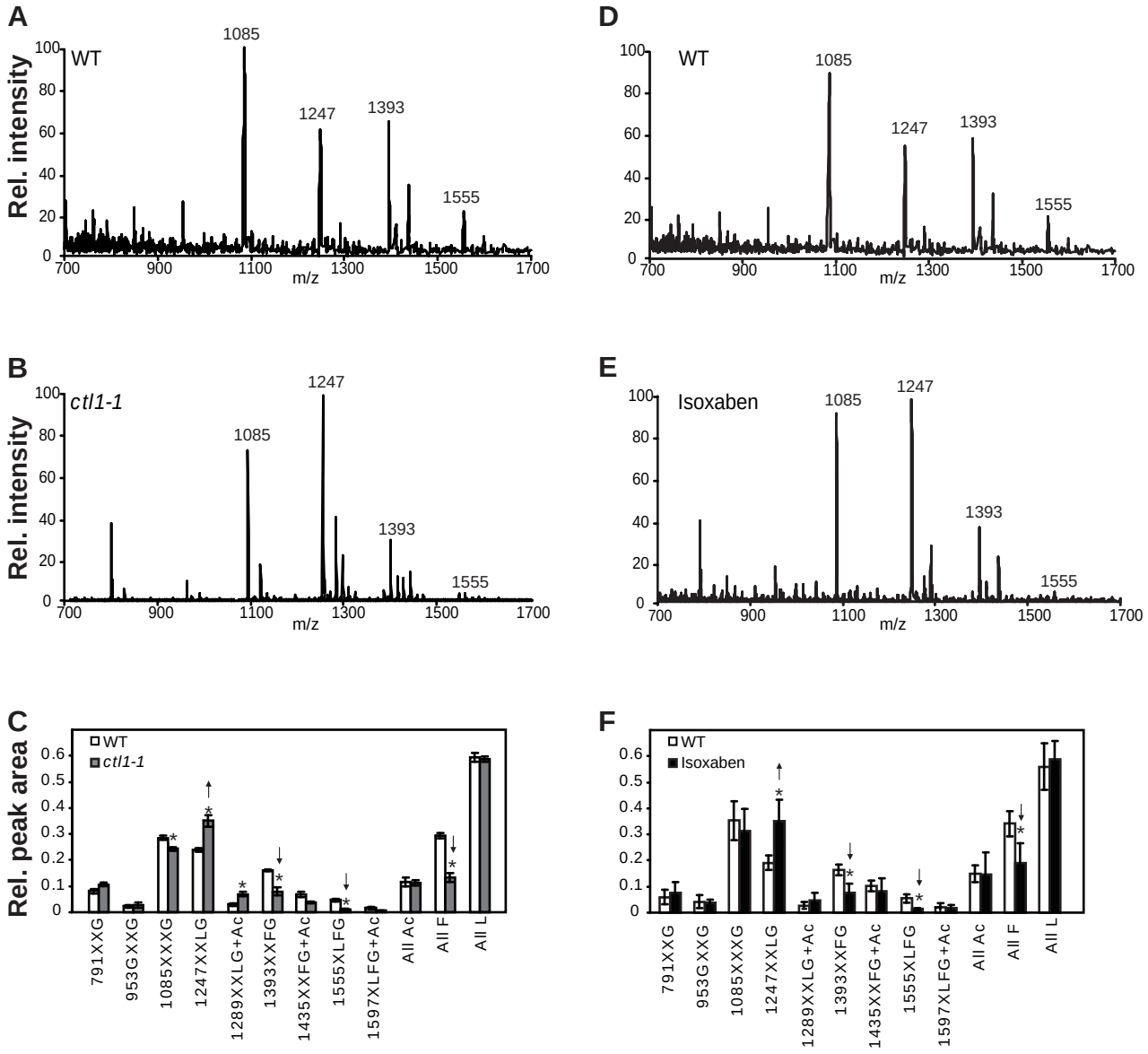

Figure S8. Oligosaccharide Mass Profiling (OLIMP) analyses of *ctl1-1* and isoxaben grown seedlings. A, B, D and **E.** MALDI-TOF mass spectra of crude cell wall fractions from six-days-old dark grown seedlings treated with a xyloglucanendohydrolase for WT (**A** and **D**), *ctl1-1* (**B**), and isoxaben treated WT (**D**).**C**and **F**. Comparative OLIMP analysis of the relative peak intensities for crude cell wall fractions from six-days-old etiolated seedlings of WT (white bars) and *ctl1-1* mutant (grey bars) (**C**) or control WT (white bars) and isoxaben grown WT (black bars) (**F**). Data represents the average (± SD) of N=3 biological replicates. Asterisk indicates significant changes in relative peak intensities (Student's-t test; $P<0.05$). The corresponding mass-to-charge ratios and oligosaccharide fragments are indicated below the bars: G; glucan backbone, X; xylosylatedxyloglucan branch, L; galactosylatedxyloglucan branch, F; Fucosylatedxyloglucan branch, Ac; Acetylated xyloglucan branch.

Table S1. Identification of CTL-like homologs that are co-expressed with primary and secondary *CESA* genes in other plant species using PlanNet (http://aranet.mpimp-golm.mpg.de (Mutwil et al., 2011)) and BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi)

PFAM family	Description		Arabidopsis	Brachypodium	Barley	Medicago	Poplar	Rice	Soybean	Wheat
Cellulose synt	Cellulose synthase catalytic subunits (CESA)	1CW	at4g39350 at4g32410 at5g05170 at5g64740 at5g09870	Bradi1g02510 Bradi2g34240 Bradi1g53210 Bradi1g54250	bab67900.1 aam26299.1 aaf89966.1 aaf89963.1 aaf89963.1 aaf89961.1 aak27814.1 aal38530.1 aaf89964.1	Medtr3g136720.1 Medtr7g099810.1	jgi|poptr1_1|827510| estext_fgenesh4_pg.c_870007 jgi|poptr1_1|835809|e stext_fgenesh4_pm.c_lg_xviii0125	LOC_Os03g62090.1 LOC_Os07g14850.1 LOC_Os07g10770.1 LOC_Os05g08370.1	Glyma02g08920.1 Glyma16g28080.1 Glyma06g07320.2 Glyma05g32100.1 Glyma04g07220.1	UniRef90_A2Y0X2 UniRef90_A2Y0X2 UniRef90_Q69V23 UniRef90_B1P2T4 UniRef90_A2Y0X2 UniRef90_A2Y0X2 UniRef90_Q84ZN6
		2CW	at5g44030 at5g17420 at4g18780	Bradi4g30540 Bradi1g25120 Bradi2g49910 Bradi3g28350	bab67900.1 aam26299.1 aaf89963.1 aaf89963.1 aak27814.1 aaf89964.1	Medtr8g145000.1 Medtr8g145000.2 Medtr4g089980.1 Medtr4g089800.1	jgi|poptr1_1|555650| eugene3.00040363 jgi|poptr1_1|553321| eugene3.00002636	LOC_Os01g54620.1 LOC_Os09g25490.1 LOC_Os10g32980.1	Glyma02g36720.1 Glyma06g06870.1 Glyma09g05630.1 Glyma06g30860.1 Glyma04g06780.1	UniRef90_Q6S348 UniRef90_Q6S350 UniRef90_A2WV32 UniRef90_Q6S350 UniRef90_A2Z1C8 UniRef90_A2Z1C8
Glyco hydro19	Chitinase class I	1CW	at1g05850	Bradi4g34040	AK369795.1	gb|ACJ84914.1|	gb|EEF02193.1| gb|EEE99404.1|	LOC_Os09g32080.2	ACU18968.1	UniRef90_A2Z2P0
		2CW	at3g16920	Bradi4g34040	np_172076.1	medtr2g038810.1	jgi|poptr1_1|723883| estext_genewise1_v1.c_lg_x0543	.	Glyma09g04330.1	.

Table S2. List of substrates tested in ELISA binding studies with CTL1 and CTL2 and resulting relative absorbance units.

	Absorbance (relative units)			
	CTL1		CTL2	
Substrate	**Av**	**SE**	**Av**	**SE**
insoluble fraction plant CW (IF_A.t.)	12.58	2.63	14.56	2.92
4M KOH fraction plant CW (4MKOH_A.t.)	10.30	2.82	13.58	3.95
cellulose, fibrous, long (celluloseFL)	4.71	1.30	8.43	3.97
sigmacell® cellulose type 20 (sigmacell cellulose)	6.70	2.21	10.01	3.80
carboxymethyl cellulose, sodium salt	-0.76	n.a	1.47	n.a
CM-cellulose	-0.54	n.a	1.52	n.a
avicel PH-101(microcrystalline cellulose)	-3.53	n.a	-0.70	n.a
xyloglucan	19.09	1.74	14.71	4.68
isoprimeverose (xyloglucan derived)	-0.76	n.a	0.34	n.a
chitin from crab shells (chitin_crab)	7.13	1.27	10.49	3.48
chitosan medium MW (chitosan mMW)	4.84	1.41	6.54	1.13
chitosan low MW[a]	2.70	1.43	8.28	5.13
lichenan	3.69	0.84	4.32	1.19
laminarin[a]	1.54	n.a	0.36	n.a
beta-glucan from barley	2.84	n.a	1.53	n.a
xylan from birchwood (xylan birchwood)	0.94	n.a	0.34	n.a
xylan (from oat)	1.70	n.a	0.60	n.a
xylan (from larchwood)	1.15	n.a	0.14	n.a
rye arabinoxylan	1.37	n.a	0.64	n.a
pectin esterified from citrus (pectin citrus)	2.77	n.a	0.56	n.a
pectic galactan (lupin)	0.60	n.a	0.05	n.a
rhamnogalacturonan from soybean (RG soybean)	2.16	n.a	0.43	n.a
rhamnogalacturonan I from potato	2.46	n.a	1.66	n.a
galactan (lupin)	1.74	n.a	0.72	n.a
arabinogalactan protein from A. thaliana (AGP_A.t.)	0.56	n.a	0.43	n.a

Relative absorbance units were calculated by the ratio of the (enzyme-blank) absolute measured absorbance at 450 nm and the concentration of protein used (in µg/mL). When a standard error (SE) is given, each value represents the average of a triplicate or a duplicate ([a]) ELISA experiment.

Table S3. Primers sequences used for analyses

Primer name	Primer sequence	Orientation	Restriction
SALK_093049_1	TTTAGACCACCAGCTGCATTC	For	
SALK_093049_2	GATGCCTAGGAGGTTTGGAAG	Rev	
SALK_055713_1	CAGCTTCTTCTCGTCCAACAC	For	
SALK_055713_2	GTTTCGAAACCGCTATTCTCC	Rev	
SALK_insert	CGCTTTCTTCCCTTCCTTTCTC		
CTL1_GUS1	CCATAAGCTTAGATTTGCATCCACTACCAC	For	HindIII
CTL1_GUS2	CCACCCATGGTTGTCACCATAGCTTACAAC	Rev	NcoI
CTL1_GFP1	GTAAGCCATGGTGACAATCAGGAGTGGT	For	NcoI
CTL1_GFP2	ATCTCCATGGAGGAAGAGGAAGGTACAG	Rev	NcoI
CTL1_pCAMBIA1	TTGAGTCGACGATCGGAGATGGATGCC	For	SalI
CTL1_pCAMBIA2	ATAAGGATCCCGAAGAGGAAGAGGAAGGTACAG	Rev	BamHI
mCherry_CTL1_1	GGGATCCACCCTGGCAGTAATCGCCATGGTGAG	For	BamHI
mCherry2_CTL1_2	ACTAGTACGAATGTTACTTGTACAGCTCGTCCATGC	Rev	SpeI
mCherry_FABD_1	CGGGTACCGGTAGAAAAAATGGTGAGCAAGGGCGAGGAGG	For	KpnI
mCherry_FABD_2	GTTGGCGCGCCCTTCTACTTGTACAGCTCGTCCATGCC	Rev	AscI
FABD_1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCACCAAGGGATCCTCTTG AAAGAGCTGAATTGGTTC	For	
FABD_2	GGGGACCACTTTGTACAAGAAAGCTGGGTATCATGACTCGATGGATGCTTCCTC	Rev	
CTL2_PCTL1_1	GTTATATCATGACAATGGTCACATTG	For	BspHI
CTL2_PCTL1_2	TTCACGCGTCAAGAAGAGGAACC	Rev	MluI
CTL1_pichia1	GAAAGCACGTGATATGGTGACAATCAGGAGTGG	For	PmlI
CTL1_pichia2	TACAGTCTAGAGAAGAGGAAGAGGAAGGTACA	Rev	XbaI
CTL2_pichia1	GAAAGCACGTGATATGGTTTCGAAACCGCTA	For	PmlI
CTL2_pichia2	TACAGTCTAGAGAAGAGGAACCAGAACTCGG	Rev	XbaI
CTL1_qRT1	CCCTCAGCTCACGACATCTTTG	For	
CTL1_qRT2	TAGAGGACGTTCATGGTGCTGC	Rev	
CTL2_qRT1	TGCAACAGCGGATTCGATAAC	For	
CTL2_qRT2	TGAGGACCAGCTTCTTCTCGTC	Rev	
GADPH_3end	TTGGTGACAACAGGTCAAGCA	For	
GADPH_3end	AAACTTGTCGCTCAATGCAATC	Rev	
GADPH_600b	AGGTGGAAGAGCTGCTTCCTTC	For	
GADPH_600b	GCAACACTTTCCCAACAGCCT	Rev	