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Supplementary Materials for

FERONIA and wall-associated kinases coordinate defense induced by lignin modification in plant cell walls

Chang Liu *et al.*

Corresponding author: Richard A. Dixon, richard.dixon@unt.edu; Chang Liu, chang.liu@nefu.edu.cn

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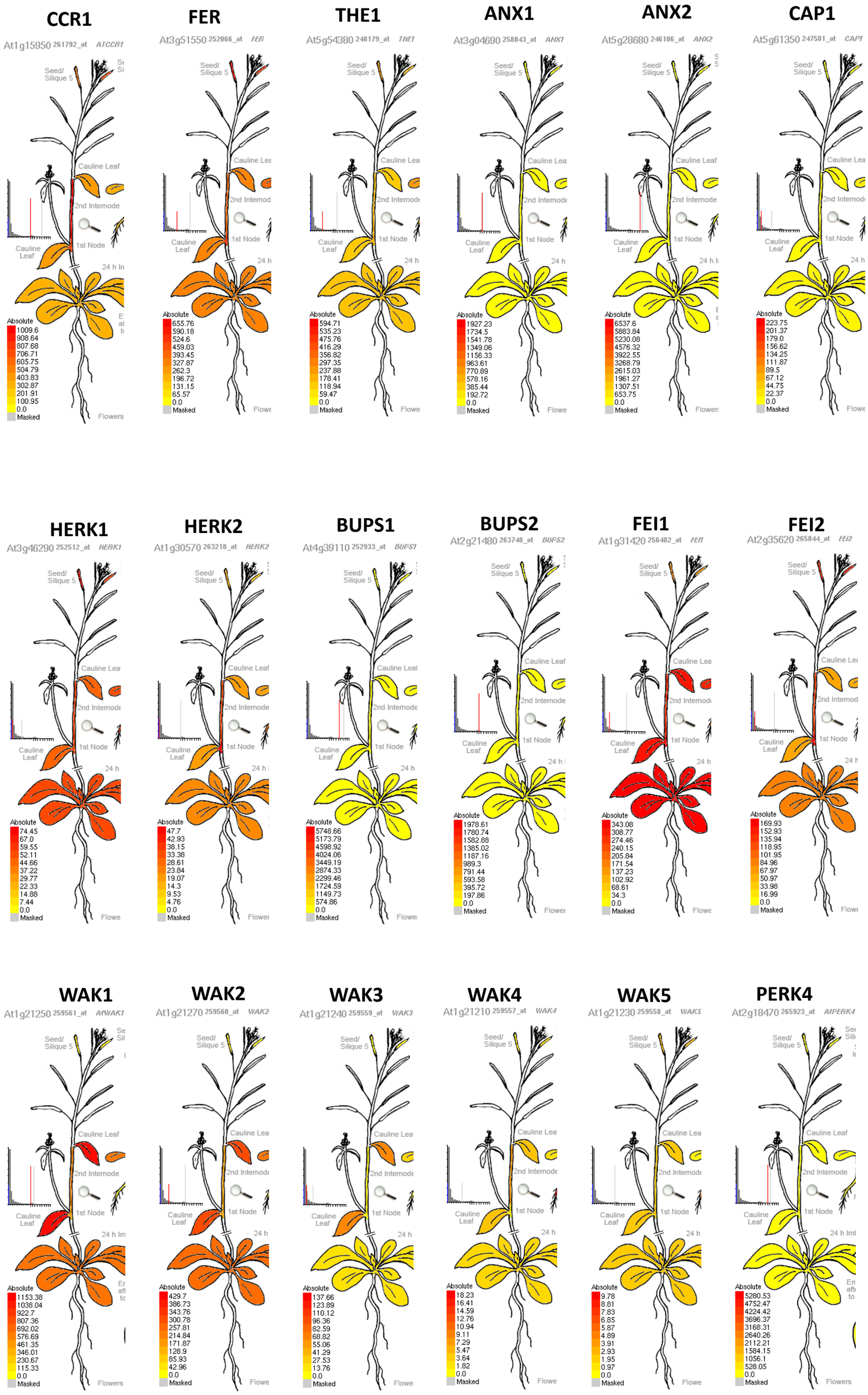
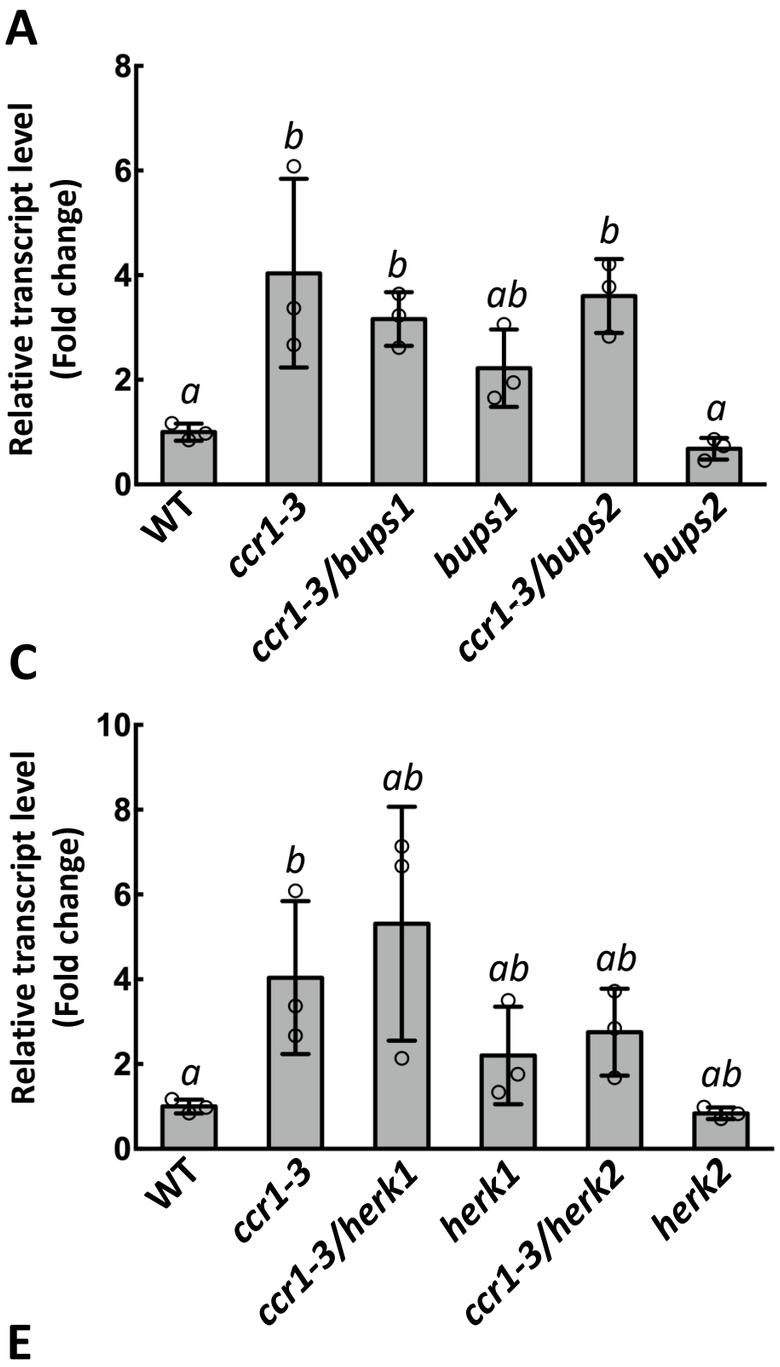
The PDF file includes:

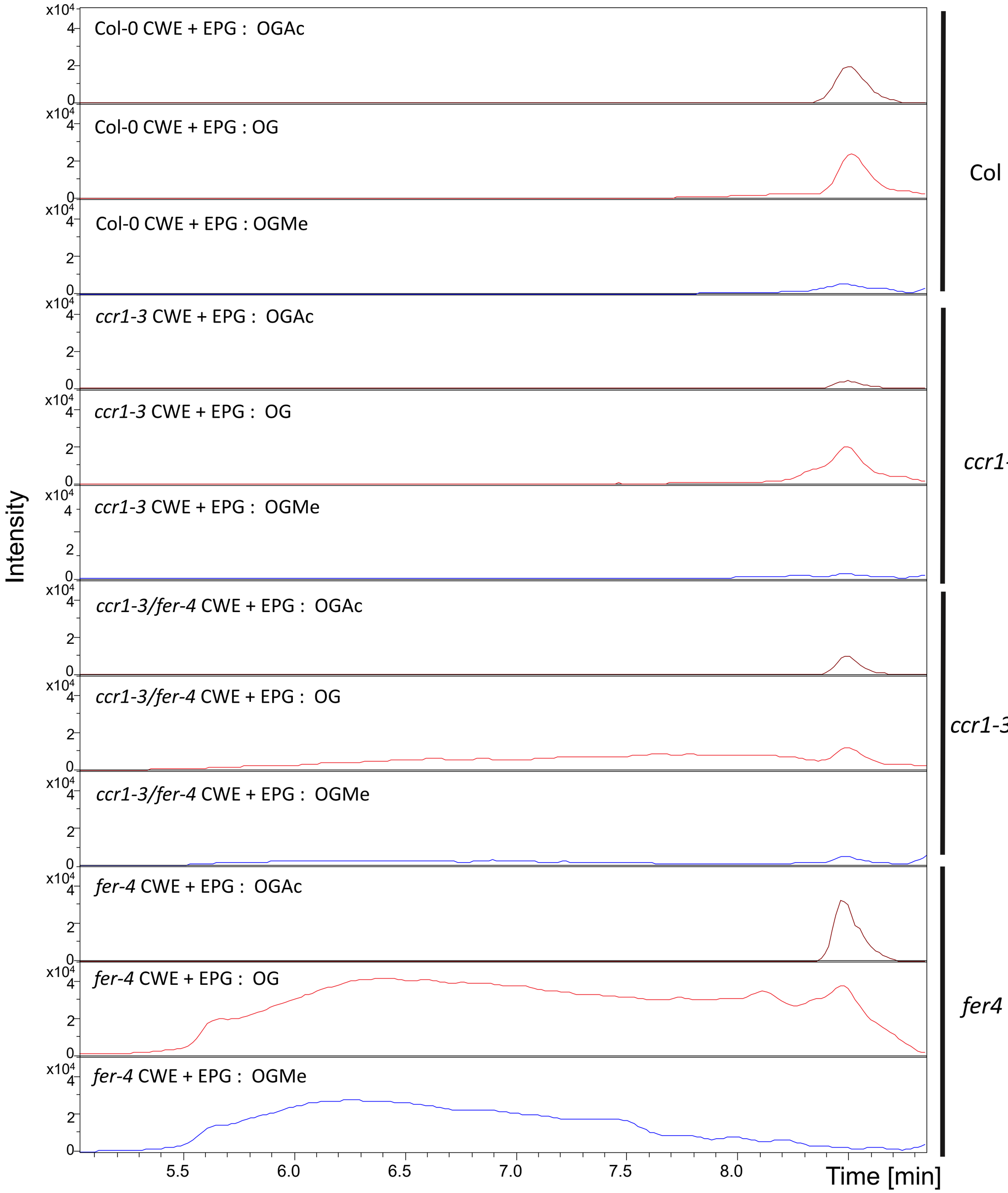
Other Supplementary Material for this manuscript includes the following:



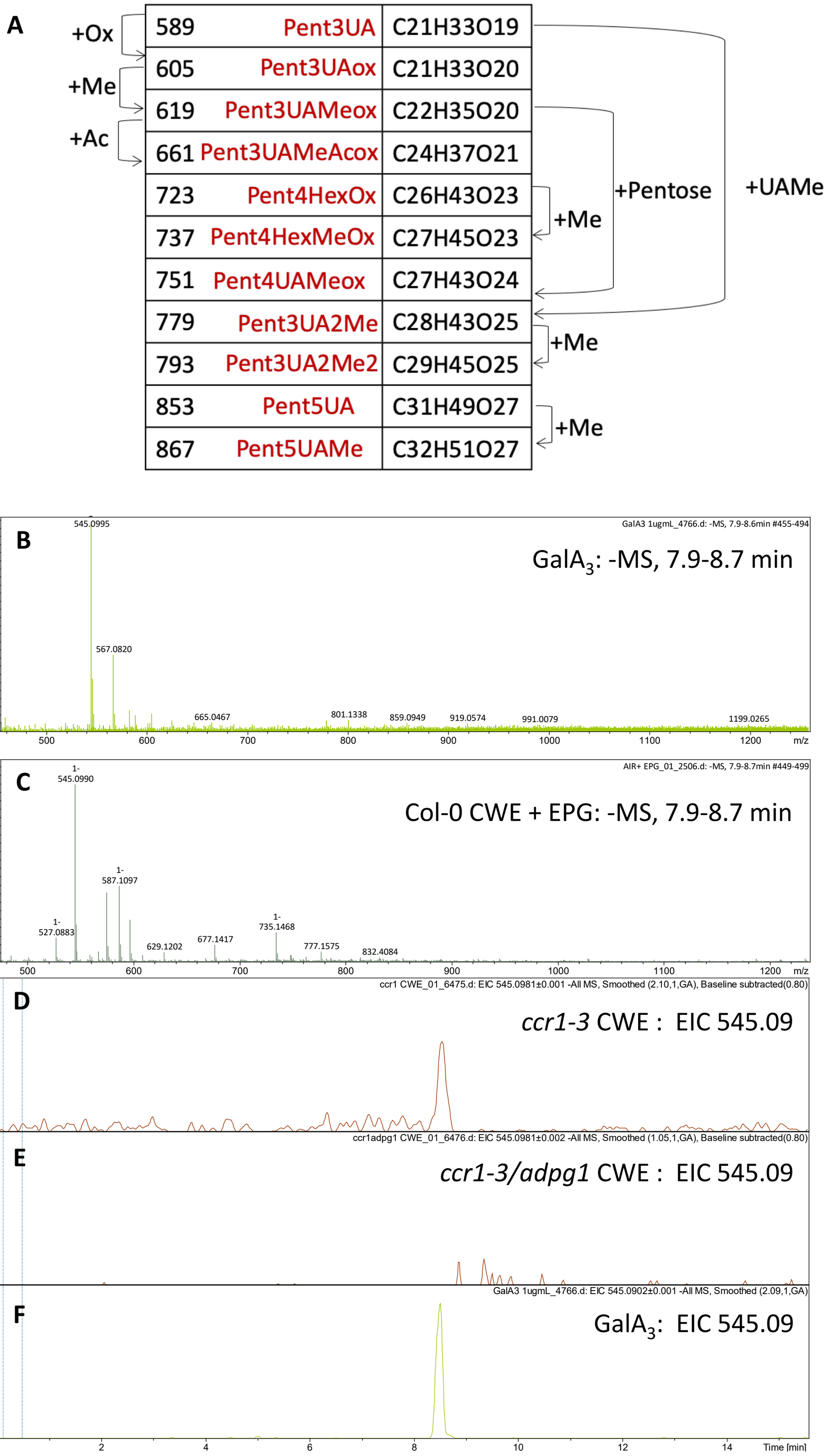
Supplementary Fig. 1



Supplementary Fig. 2.



Supplementary Fig. 3.



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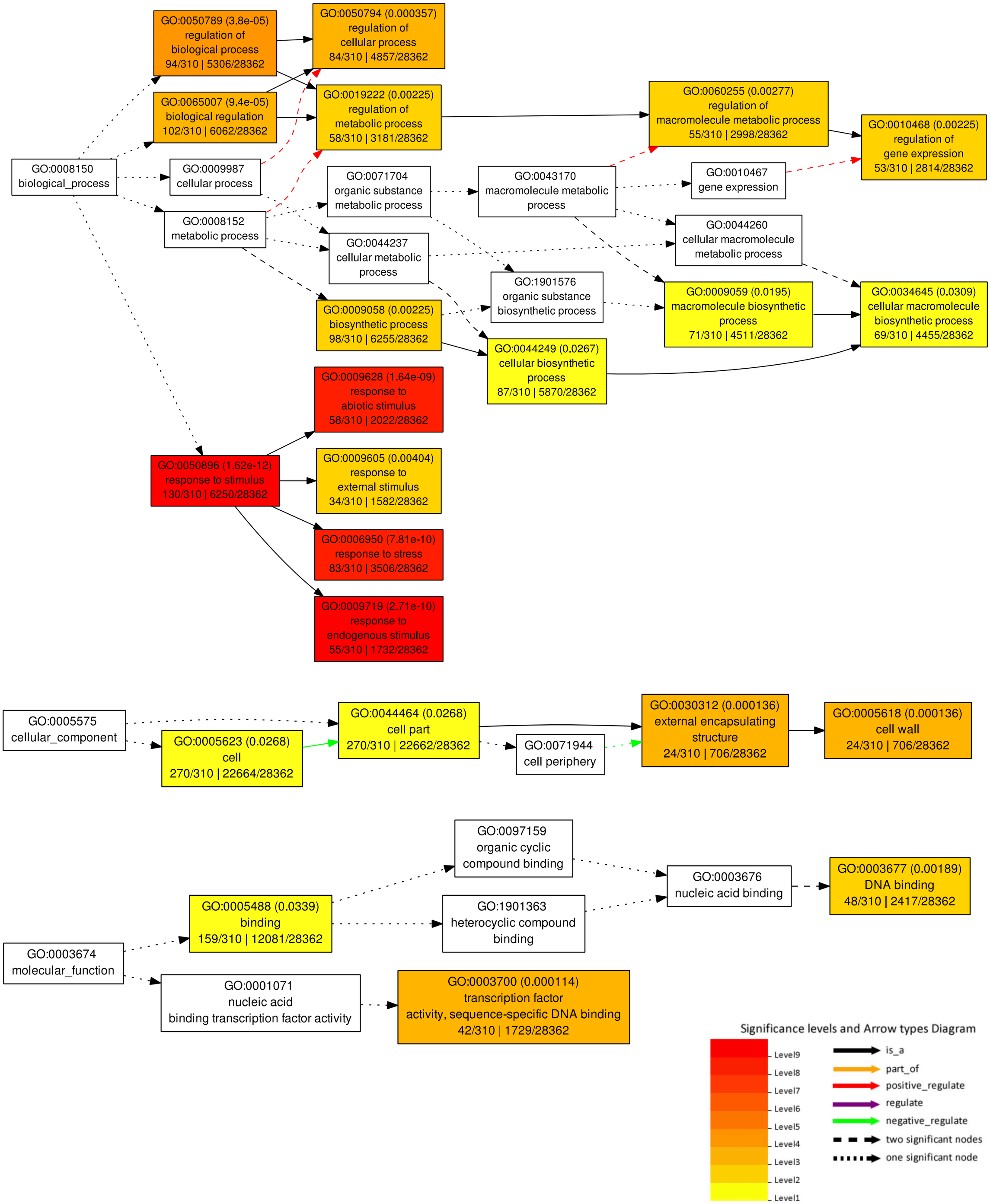
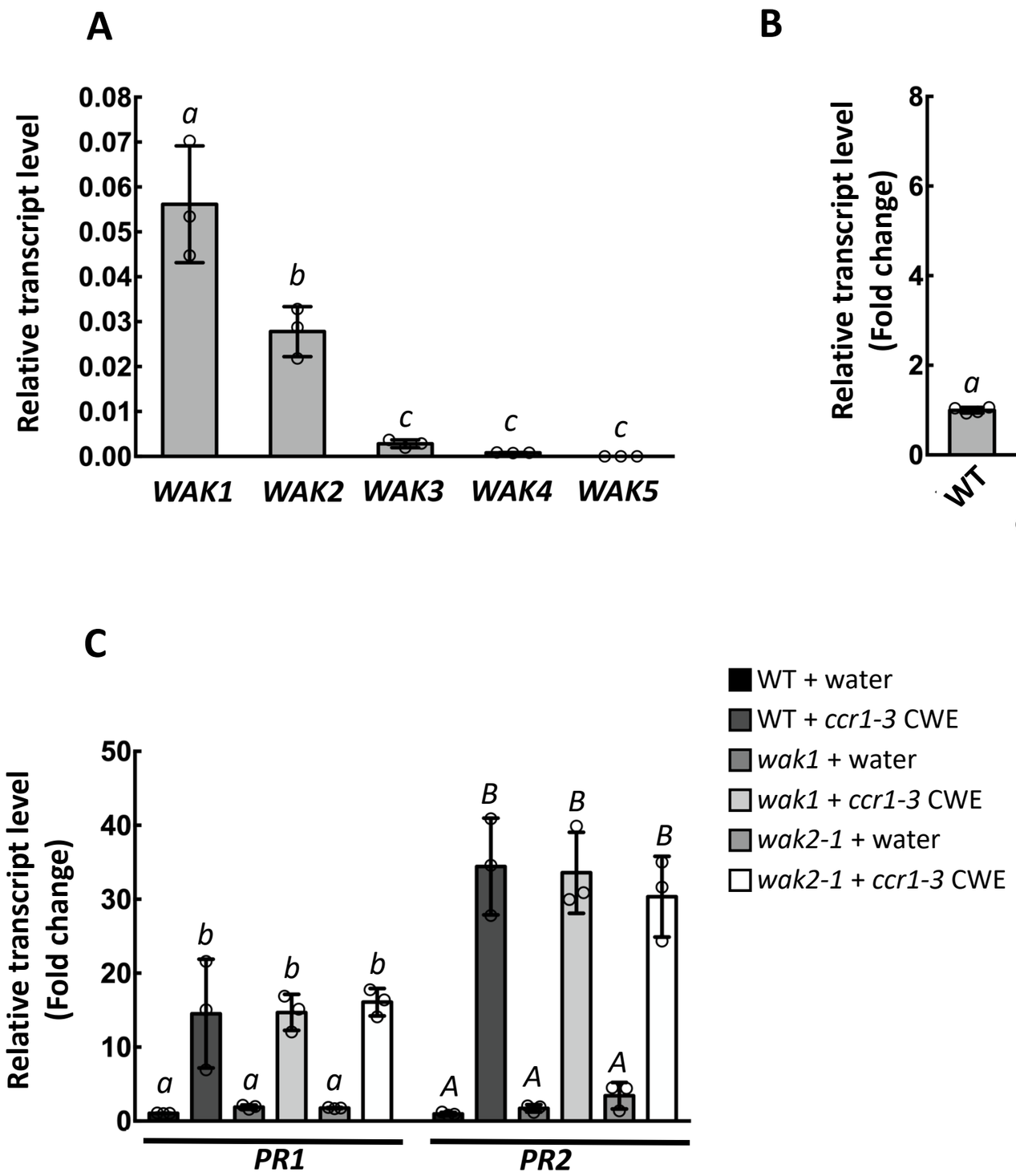


Supplementary Fig. 5.

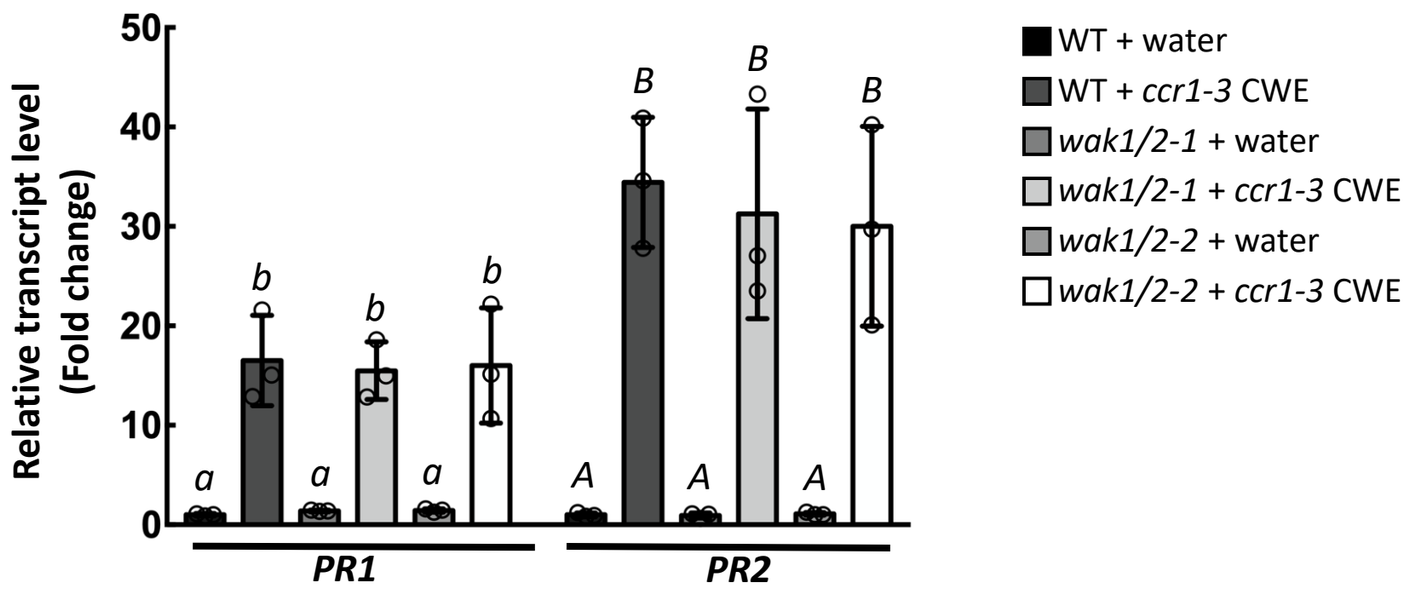


Supplementary Fig. 6.

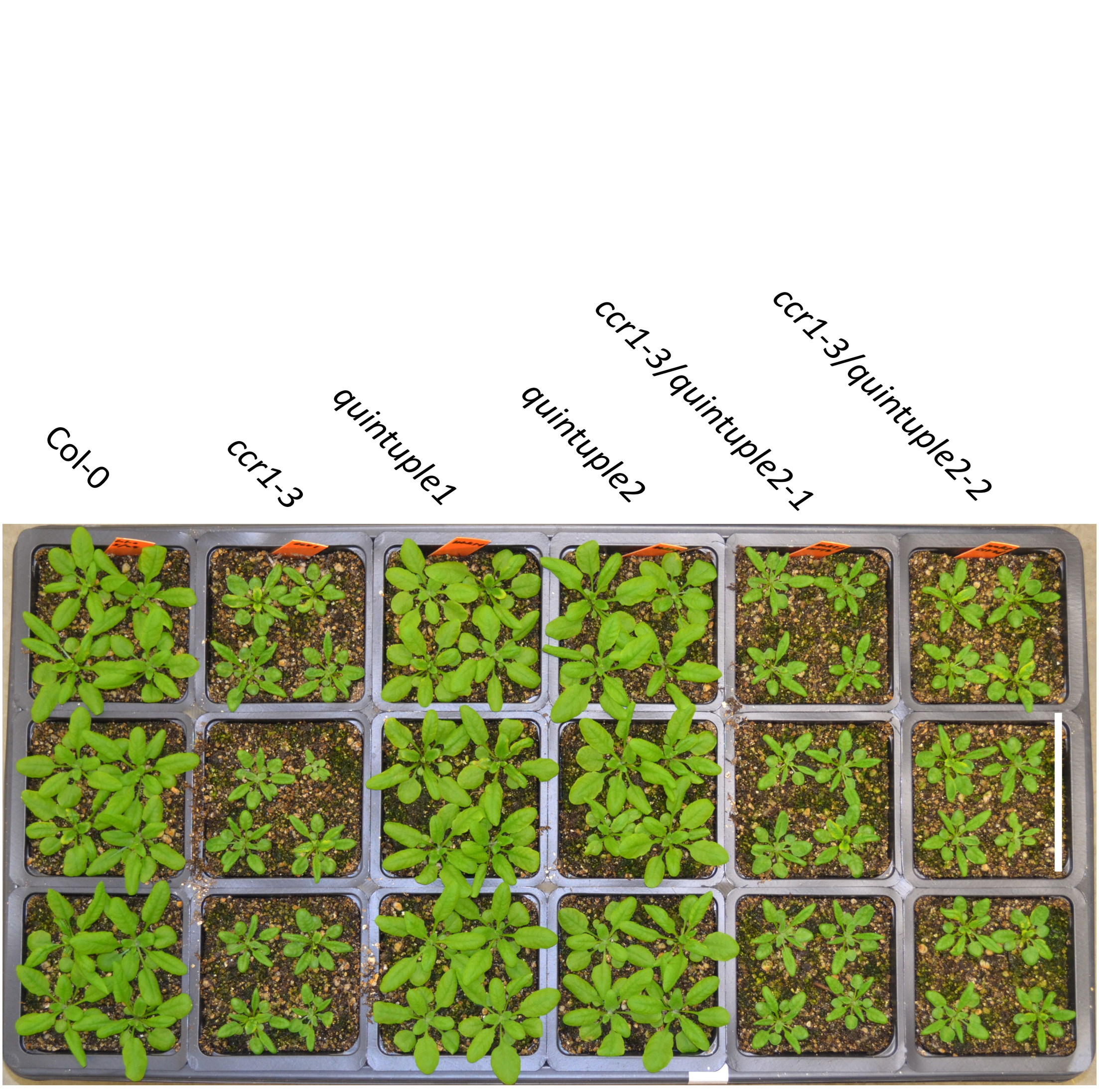
A

Transgenic line	Target sequence (N19NGG)	Indel
Wild type WAK1	GGTCAGGGTGGCCAAGGAACAGTGTACAA**AGG**GATATTGCCGGAC	-
wak1/2-1	GGTCAGGGTGGCCAAGGAACA----ACAA**AGG**GATATTGCCGGAC	-4
wak1/2-2	GGTCAGGGTGGCCAAGGAACAGTGTACAA**A**AGGGATATTGCCGGAC	+1
Wild type WAK2	GGTCAGGGAGGCCAAGGAACAGTGTACAA**AGG**GATATTGCCGGAC	-
wak1/2-1	GGTCAGGGAGGCCAAGGAACAGTGT--AA**AGG**GATATTGCCGGAC	-2
wak1/2-2	GGTCAGGGAGGCCAAGGAACA----ACAA**AGG**GATATTGCCGGAC	-4

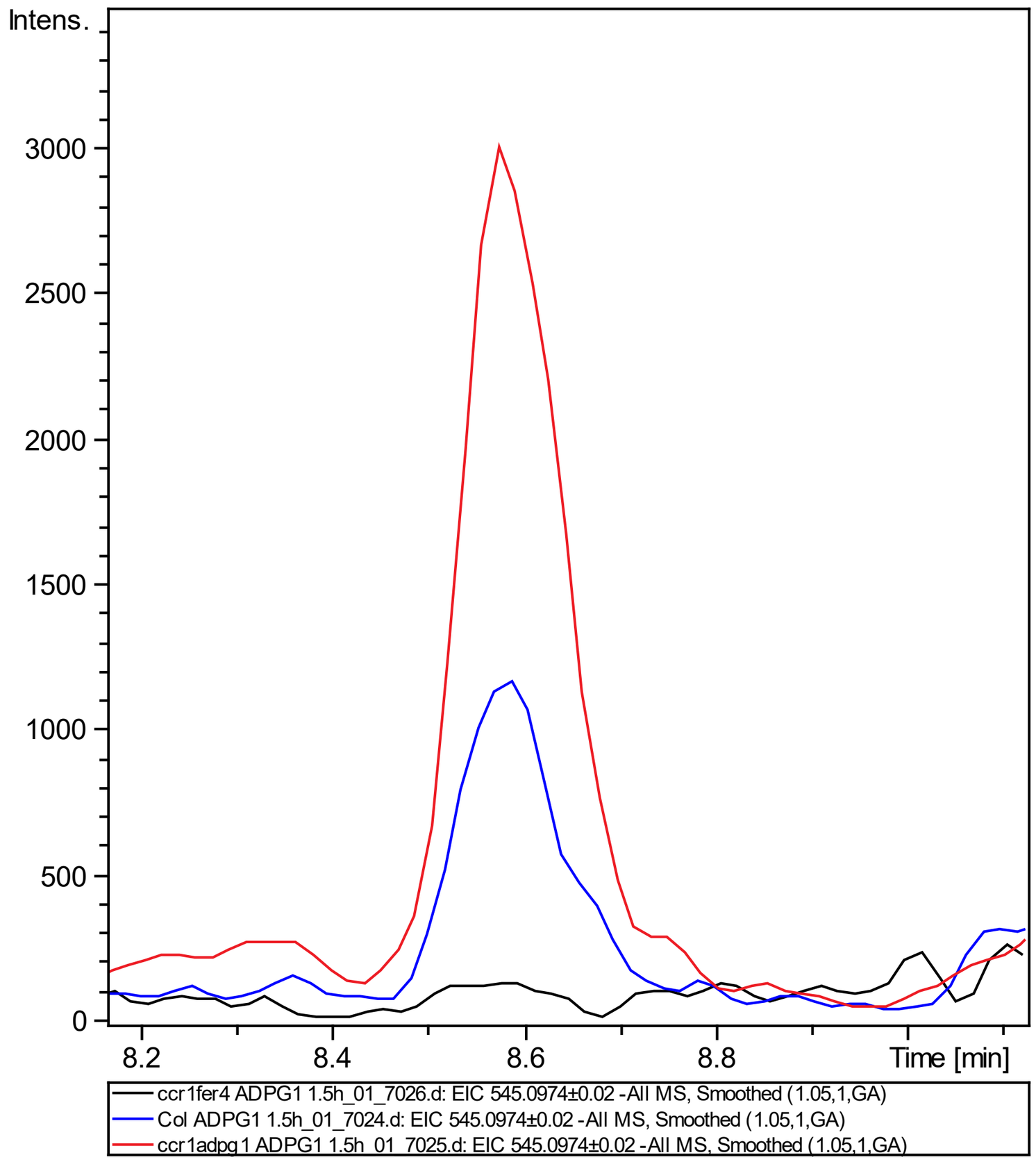
B



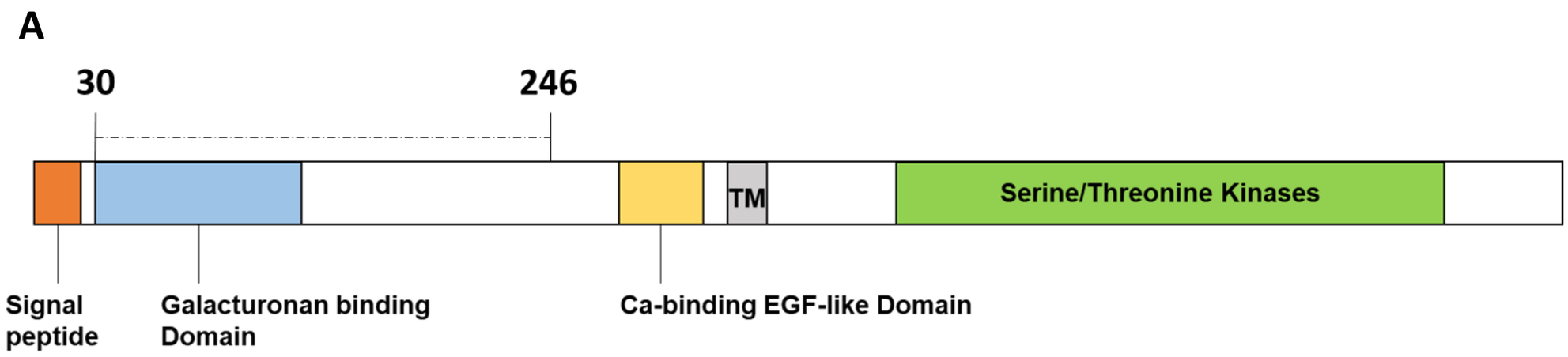
Supplementary Fig. 7.



Supplementary Fig. 8.



Supplementary Fig. 9.



Supplementary Fig. 10.

Supplementary Table1. Mutants used in this study

Gene	Name	Stock number	Mutant allele	Reference
AT1G15950	*CINNAMOYL COA REDUCTASE 1 (CCR1)*	SALK_123689	*ccr1-3*	30
AT5G54380	*THESEUS1 (THE1)*	CS829966	*the1-4*	23
AT3G51550	*FERONIA (FER)*	CS69044	*fer-4*	28
AT3G04690	*ANXUR1 (ANX1)*	SALK_016179	*anx1-1*	26
AT5G28680	*ANXUR2 (ANX2)*	SALK_133057	*anx2-2*	26
AT3G46290	*HERCULES RECEPTOR KINASE 1 (HERK1)*	SALK_008043	*herk1-1*	23
AT1G30570	*HERCULES RECEPTOR KINASE 2 (HERK2)*	SALK_105055	*herk2*	22
AT4G39110	*BUDDHAS PAPER SEAL 1 (BUPS1)*	SALK_061000	*bups1*	Name used in the current paper
AT2G21480	*BUDDHAS PAPER SEAL 2 (BUPS2)*	CS837126	*bups2*	Name used in the current paper
AT5G61350	*ERULUS (ERU)*	SALK_083442C	*cap1/eru*	29
AT1G31420	*FEI1*	SALK_080073	*fei1*	24
AT2G35620	*FEI2*	SALK_044226	*fei2-2*	24
AT1G21250	*WALL-ASSOCIATED KINASE 1 (WAK1)*	SALK_107175	*wak1*	Name used in the current paper
AT1G21270	*WALL-ASSOCIATED KINASE 2 (WAK2)*	CS813270	*wak2-1*	21
AT1G21240	*WALL-ASSOCIATED KINASE 3 (WAK3)*	SALK_080632C	*wak3*	Name used in the current paper
AT1G21210	*WALL-ASSOCIATED KINASE 4 (WAK4)*	CS842650	*wak4*	Name used in the current paper
AT2G18470	*PROLINE-RICH EXTENSIN-LIKE RECEPTOR KINASE 4 (PERK4)*	SALK_026953	*perk4-2*	25

Supplementary Table 2. Oligonucleotide primers used in this study.

Oligos used for genotyping		
Primer name	Sequence (5`-3`)	Mutant allele
SALK_123689 LP	GTGTCGTAGAGGCTTTGCTTG	*ccr1-3*
SALK_123689 RP	TTGTGGAAATATTTCCGGTTG	
CS829966 LP	CCGGGTCTAGATAACCAAAGC	*the1-4*
CS829966 RP	TGTTTTAACCGTTAGCGTTGG	
CS69044 T-DNA F	ACGGTCTCAACGCTACCAAC	*fer-4*
CS69044 T-DNA R	TTTCCCGCCTTCGGTTTA	
CS69044 WT F	GATTACTCTCCAACAGAGAAAATCCT	
CS69044 WT R	CGTATTGCTTTTCGATTTCCTA	
SALK_016179 LP	TCCTTTGACGACGGTTGTTAC	*aux1-1*
SALK_016179 RP	CGCAGGCAGATACTACTCCTG	
SALK_133057 LP	TAAATCCACAGAACCACCACC	*aux2-2*
SALK_133057 RP	TATCGGGTAAGAGCAACAACG	
SALK_008043 LP	ATGTGACTTGGGAGTTCGATG	*herk1-1*
SALK_008043 RP	TGCAGATTTCACGTCTCTGTG	
SALK_105055 LP	ACTGGTCACAATGCTACTGCC	*herk2*
SALK_105055 RP	CTTACCAAACCCTCCAACTCC	
SALK_061000 LP	GATCAGCCTGGAAGAAGATCC	*bups1*
SALK_061000 RP	ATCACCAACGAAACAAACGAG	
CS837126 LP	GCCCTAAGGTTCAAGCCTATG	*bups2*
CS837126 RP	ACCGATGATCTCTGATGCATC	
SALK_083442C LP	TTTATCAACGCCGTTGAAATC	*cap1/eru*
SALK_083442C RP	ATTTTGTGTCGCGGTCTGTAG	
SALK_080073 LP	CTTTTTCAATTCAGGTGCGTC	*fei1*
SALK_080073 RP	TTCAAGTAACTGGACAACCCG	
SALK_044226 LP	TTGCCATCTATGGGAACTTTG	*fei2-2*
SALK_044226 RP	ATTACAACCATTGTTGCAGGC	
SALK_107175 LP	GCTTCTTGGTCATTCTGCTTG	*wak1*
SALK_107175 RP	TTGTGCTGACAAGATGTGACC	
SAIL_286_E03 LP	CATGTGCTGTTACCACCACAC	*wak2-1*
SAIL_286_E03 RP	ATTCCTTGCAAGTTGCAACTG	
SALK_080632C LP	ATCTCACCTGTGTCGTGGAAG	*wak3*
SALK_080632C RP	CTCAAAGAATTGTCGTCGGAG	
CS842650 LP	ACTATCTCTTTGAGCGGCTCC	*wak4*
CS842650 RP	TTAACCAATTGTTCCCTGCAG	
SALK_026953 LP	AAATGTTCAAAAACTCTCTCCCC	*perk4-2*
SALK_026953 RP	CTCCCTGTCCCAAAAGGTTAG	
LBb1.3 for SALK lines	ATTTTGCCGATTTCGGAAC	
LB3 for SAIL lines	TAGCATCTGAATTTCATAACCAATCTCGATACAC	
Oligos used for quantitive PCR		
Primer name	Sequence (5`-3`)	Gene name
AT1G15950 F	ACGTTATCTCCTAGCCGAGAGTGC	*CCR1*
AT1G15950 R	GGGTTCTTCTCGTCCTTGCACTTG	
AT3G57510 F	AAGGACAAATGCGAAGACCAAGAG	*ADPG1*
AT3G57510 R	ACGTTATCGCCACATCCGTAGC	
AT5G54380 F	TCGGCTTTGATGGAGCCTGATG	*THE1*
AT5G54380 R	CGCCATTGGAATCCCTGGAATG	
AT3G51550 F	ACCTCAAGGGCAAGATCACACC	*FER*
AT3G51550 R	CACACTTCATCGCGGTTTCAGC	
AT3G04690 F	GCGTTAAACCCGAGTTTGCCTAAG	*AUX1*
AT3G04690 R	TGCAATTCATTGCCCAGTCTCC	
AT5G28680 F	AGCAATGGTGGTGGTTCTGTGG	*AUX2*
AT5G28680 R	TTTCTCCGGCGCTGATGTTCAC	
AT3G46290 F	TGGCAGAAGAAAGGGCAACTGG	*HERK1*
AT3G46290 R	GGTCTGATATTTCCGCGAAGCG	
AT1G30570 F	GCTTGGCTTCGCAAACAGAATGG	*HERK2*
AT1G30570 R	TTCTTCTACCGCTTGGCTGCTC	
AT4G39110 F	AGAGGCATTCACTCAGGGCAAAG	*BUPS1*
AT4G39110 R	TGTCACCACATCAGGCTTAGCG	
AT2G21480 F	AGCCGAATGGGCTATGCTATGG	*BUPS2*
AT2G21480 R	TCACAGCACCAACGAGATGAGG	
AT5G61350 F	ACCGCGGTAAAGGGTAGTTTCG	*CAP1/ERU*
AT5G61350 R	TCAGTGAGTTGTTGTCTGCGGAAG	
AT1G31420 F	TGCACAGCATTGGAGGAAATTCAC	*FEI1*
AT1G31420 R	TTTCAGCTGGGATTGGTCCAGTG	
AT2G35620 F	TGGCTCCAGAGTATATGCAAAGCG	*FEI2*
AT2G35620 R	TGAATGAGGCATCCGTGGGAAG	
AT1G21250 F	ACCTTTCAGCTGGTTGCCAAGAC	*WAK1*
AT1G21250 R	TGGTGGTATCTAAGCGGTAACCAG	
AT1G21270 F	AGAAGCCGATGGTGGTTCCAAG	*WAK2*
AT1G21270 R	TGCCCATCTGGTTACCGCAAAG	
AT1G21240 F	ACCGTTCAGAGGGTTGCAAAGAC	*WAK3*
AT1G21240 R	ACTTACAATCGAAGCCTCCATCCC	
AT1G21210 F	CTTTGCCTCAGCCACGAAAGAG	*WAK4*
AT1G21210 R	TCTCGTTCATCACTTGGCCATC	
AT2G18470 F	ATCGCCTTAGGTGCTGCGAAAG	*PERK4*
AT2G18470 R	TGAATGATCCGAGGATGGCAGTC	
AT3G18780 F	TCTTCCGCTCTTTCTTTCCAAGC	*ACT2*
AT3G18780 R	ACCATTGTCACACACGATTGGTTG	
Oligos used for sgDNA		
Primer name	sequence	
CRISPR-Wak1/2-F	gattgGCCAAGGAACAGTGTACAA	
CRISPR-Wak1/2-R	aaacTTGTACACTGTTCCTTGGCc	
CRISPR-Wak1/2/3/4/5-F	gattgAATTCTTCGAGCAAAATGG	
CRISPR-Wak1/2/3/4/5-R	aaacCCATTTTGCTCGAAGAATTc	
Oligos used for constructs		
Primer name	sequence	
PGEX-6p-1-WAK1OG-F	CTGTTCCAGGGGCCCCTGGGATCC ATGGATCTGTACGACGATGACGATAAG TGCCAAAATAAATGTGGCAACATC	
PGEX-6p-1-WAK1OG-R	CCGCTCGAGTCGACCCGGGAATTC TATGCTTGTGCTTCCAACTTGCTC	

Liu et al Supplementary Material

Supplementary Fig. 1 Transcript expression levels of candidate receptor kinase genes in Arabidopsis stems. Relative transcript levels of cell wall receptor kinase genes in different tissues were obtained from the Arabidopsis eFP Browser. The expression pattern of *CCR1* is included for comparison. Details of the genes are presented in Table S1.

Supplementary Fig. 2. PR1 transcript levels in inflorescence stems of wild type (WT, Col-0), ccr1, and homozygous double mutants obtained by crossing *ccr1-3* with a range of cell wall receptor kinase mutants as listed in Table S1. PR1 transcript levels were determined by qRT-PCR and are expressed relative to the level in WT. Crosses of *ccr1-3* were made with (**A**) *bups1* and *bups2*, (**B**) *anx1*and *anx2*, (**C**) *herk1*and *herk2*, (**D**) *fei1* and *fei2*, (**E**) *the1*, (**F**) *cap1* and (**G**) *perk4*. Bars represent the means ± SD, n=3. Hollow Dots represent individual data points. Letters indicate statically significant differences among samples according to one-way ANOVA followed by Tukey`s HSD test, $\alpha = 0.05$.

Supplementary Fig. 3. Pectin enzymatic fingerprinting of CWEs. Extracted ion chromatograms obtained by high-performance size-exclusion chromatography mass spectrometry analysis in negative ionization mode of oligogalacturonides (OGs) with degrees of polymerization (DP) 3 to 8 obtained following digestion of cell wall water extracts by commercial endopolygalacturonase (EPG) from *Aspergillus aculeatus*. In blue, methylesterified OGs; in red, non-methylesterified OGs, in dark red: acetylated OGs.

Supplementary Fig. 4. Peak identification for LC-MS oligosaccharide profiling. (**A**), Fragmentation and relationships among pentose/uronic acid containing oligosaccharides from **Fig.3**. Numbers indicate molecular masses, arrows indicate relationships between ions. (**B,C**), Mass spectra of $GalA_3$ standard (**B**) and $GalA_3$ identified in EPG-treated crude Col-0 cell wall water extract (**C**). (**D**) Extracted ion chromatograms (EICs)of $GalA_3$ for *ccr1-3* cell wall water extract (CWE), *ccr1-3/adpg1* CWE and $GalA_3$ standard.

Supplementary Fig. 5. Gene ontology enrichment analyses of genes up-regulated in *ccr1-3* compared with Col-0 but down-regulated *in ccr1-3/fer-4* double mutant compared with *ccr1-3* . The paths are in three categories: the biological process, the cellular component, and the molecular function. The analysis and visualization were done by AgriGO v2.0 with default parameters (49, 50).

Supplementary Fig. 6. Loss of function of single *WAK* genes does not prevent elicitor reception. (**A**) WAK transcript levels in Arabidopsis inflorescence stems as determined by qRT-PCR. (**B**) PR1 transcript levels in stems of *ccr1-3* and homozygous *ccr1-3*/*wak* double mutants (with *wak1*, *wak2* and *wak3*). (**C**) Induction of PR1 and PR2 transcripts following injection of water (control) or *ccr1-3* CWE into leaves of wild type, *wak1* and *wak2* mutants. Bars represent the means ± SD, n=3 (A-C), hollow dots represent individual data points. Letters indicate statically significant differences among samples according to one-way ANOVA followed by Tukey`s HSD test, $\alpha = 0.05$.

Supplementary Fig. 7. The *wak1/2* double mutant still responds to *ccr1*-CWE. (**A**) Generation of the *wak1*/*2* double mutant by CRISPR/Cas9 gene editing, showing mutations generated in the target sites of *WAK1* and *WAK2* in two independent alleles of each. (**B**) Induction of PR1 and PR2 transcripts following injection of water (control) or *ccr1-3*-CWE into leaves of wild type, *wak1* and *wak2* mutants. Bars represent the means ± SD, n=3 (B). Hollow Dots represent individual data points. Letters indicate statically significant differences among samples according to one-way ANOVA followed by Tukey`s HSD test, $\alpha = 0.05$.

Supplementary Fig. 8. **Four-week-old Arabidopsis plants of wild type (Col-0), *ccr1-3*, *wak* quintuple mutant, and *ccr1-3/waks* sextuple mutant.**
Bar = 10 cm.

Supplementary Fig. 9. Selected ion monitoring for $GalA_3$ (at m/z = 545) of LC-MS traces of oligosaccharides from CWEs from Col-0 (blue), *ccr1-3/fer-4* (black) and *ccr1-3/adpg1* (red). CWEs were pre-incubated with recombinant ADPG1.

Supplementary Fig. 10. Expression of the WAK1 OG-binding domain recombinant protein for elicitor pull-down assay. (**A**) Diagram of the open reading frame of the WAK1 receptor kinase, showing the oligogalacturonan binding domain (blue). (**B**) SDS PAGE analysis of the purification of recombinant OG-binding domain GST-fusion from *E. coli* lysates (left) and confirmation of expression by protein gel-blot analysis (right). (**C**) Oligogalacturonides with degree of polymerization (DP) 2 to DP20, released from bound material (from *ccr1-3* (red) or wild-type (blue) cell wall extracts in pull down assays with immobilized OG-binding domain fusion protein, with the buffer alone spectrum as the baseline. Bound oligosaccharides were digested with endo-polygalacturonase M (EPG) prior to separation and analysis of fragments by LC-MS.

Supplementary Table1. Mutants used in this study

Supplementary Table 2. Oligonucleotide primers used in this study.

Supplementary Dataset 1. List of genes that are highly expressed in *ccr1-3* compared with Col-0 but decreased *in ccr1-3/fer-4* compared with *ccr1-3*. P-value<0.05 and fold change >2 in Col-0 and ccr1-3.