Description	Name	Primer sequence	Restriction
(Vectors)			enzyme
Overexpression	ANAC017-OX-F	CTAGAAAGCTT <u>CTGCAG</u> ATGGCG	Pst I
(35S: P-super-1300)		GATTCTTCACCCGATTCGT	
	ANAC017-OX-R	ACTAGTATTTAAAT <u>GTCGAC</u> GTCT	Sal I
		TTCAAGAGAAGACTTCTACCT	
	XTH31-OX-F	CTAGAAAGCTT <u>CTGCAG</u> ATGGCT	Pst I
		TTGTCTCTTATCTTTCTAG	
	XTH31-OX-R	ACTAGTATTTAAAT <u>GTCGAC</u> ACAT	Sal I
		TCTGGTGTTTGGGTATGGTCT	
Complementatio	ANAC017-com-F	AAGCTTGCATGC <u>CTGCAG</u> TTTTC	Pst I
n		TAACACGGTGAATGTAACAGG	
(pCAMBIA1300)	ANAC017-com-R	CATGGTACCCGG <u>GGATCC</u> CTAGT	BamH I
		CTTTCAAGAGAAGACTTCTAC	
Subcellular	ANAC017-CGFP-F	GGGGGACGAGCTC <u>GGTACC</u> ATG	Kpn I
localization		GCGGATTCTTCACCCGATTCGT	
(35S: P-super-C-GFP)	ANAC017-CGFP-R	CCTTGCTCACCAT <u>GTCGAC</u> GTCT	Sal I
		TTCAAGAGAAGACTTCTACCT	
Subcellular	NGFP-ANAC017-F	GAGCTGTACAAG <u>GGTACC</u> ATGGC	Kpn I
localization		GGATTCTTCACCCGATTCGT	
(35S: P-super-N-GFP)	NGFP-ANAC017-R	GTCGACTCTAGA <u>GGATCC</u> CTAGT	BmaH I
		CTTTCAAGAGAAGACTTCTACC	
GUS analysis	ANAC017-promoter-	ATCACTAGTAAAAGGTACCTTTT	Kpn I
	F	CTAACACGGTGAATGTAACAGGC	1
(pOTY)	ANAC017-promoter-	AGATCTACCATGGGGATCCCTAC	BmaH I
	R	GTAACAAATCAAAACCGATCCCT	
		С	
YH1	ANAC017-F	ATGGAGGCCAGTGAATTCATGGC	EcoR I
		GGATTCTTCACCCGATTCGT	
(pGADT7)	ANAC017-R	AGCTCGAGCTCGATGGATCCCTA	BmaH I
· /		GTCTTTCAAGAGAAGACTTCTAC	
		С	
promoter for	XTH31-promoter-F	AAGCTTGAATTCGAGCTCCACTA	Sac I
YH1	- F	AGAAACTGATAGAGTAATTG	
(pAbAi)	XTH31-promoter-R	CATGCCTCGAGGTCGACTTTTGA	Sal I
x /	promotor re	GTGAAGTAAAACCTTTTGA	
	XTH17-promoter-F	AAGCTTGAATTCGAGCTCGCTGC	Sac I
	Promotor 1	AGAAGCTACGTGATGAGAAC	2401
	XTH17-promoter-R	CATGCCTCGAGGTCGACTTTGTT	Sal I
	rinit, promotor K	CTCACAGGAGTATTGATAT	Jul 1
	XTH15-promoter-F		Sac I
	211115-promotor-1	TTATGTTACCGTAAACCCGT	Suc 1
	VTH15_promotor P		Sal I
	zini - promotel-it	CINCELEGAU <u>UICUAC</u> IIUUUI	Suri

Supplemental Table S1. Primers used in the study.

		TTGGTTGATAGAAATGAAA	
Transcription	Plasmid a,b-F	TCAGAGGAGGACCTG <u>CATATG</u> AT	Nde I
activation		GGCGGATTCTTCACCCGATTCGT	
(pGBKT7)	Plasmid c-F	TCAGAGGAGGACCTG <u>CATATG</u> GC	Nde I
		TCCAGGGTTTCGATTTCATCCAA	
	Plasmid d-F	TCAGAGGAGGACCTG <u>CATATG</u> GA	Nde I
		TGAAGATGAACTAGGGAGATGA	
	Plasmid b,c-R	ATGCGGCCGCTGCAG <u>GTCGAC</u> C	Sal I
		ATCGTATACTCATGCATCACCCAA	
	Plasmid a,d-R	ATGCGGCCGCTGCAG <u>GTCGAC</u> GT	Sal I
		CTTTCAAGAGAAGACTTCTACT	
LUC analysis	ANAC017-Infector -	CTCTAGAACTAGT <u>GGATCC</u> ATGG	BmaH I
	F	CGGATTCTTCACCCGATTCGT	
(NONE)	ANAC017-Infector -	TAAGCTTGATATC <u>GAATTC</u> CTAGT	EcoR I
	R	CTTTCAAGAGAAGACTTCTACC	
(190LUC)	XTH31- Reporter -F	GACGGCCAGTGCC <u>AAGCTT</u> CAC	Hind III
		TAAGAAACTGATAGAGTAATTG	
	XTH31- Reporter -R	GGAAGGGTCTTGC <u>AGATCT</u> TTTT	Bgl II
		GAGTGAAGTAAAACCTTTTGA	
Yeast two-hybrid	pGADT7-ANAC017-	ATGGAGGCCAGT <u>GAATTC</u> ATGGC	EcoR I
system	F	GGATTCTTCACCCGATTCGT	
(pGADT7)	pGADT7-ANAC017-	AGCTCGAGCTCGAT <u>GGATCC</u> CTA	BmaH I
(pGBKT7)	R	GTCTTTCAAGAGAAGACTTCTAC	
	pGBKT7-WRKY46-	С	Nde I
	F	TCAGAGGAGGACCTG <u>CATATG</u> AT	
		GATGATGGAAGAGAAACTTGTG	Sal I
	pGBKT7-WRKY46-	А	
	R	ATGCGGCCGCTGCAG <u>GTCGAC</u> CT	Nde I
	pGBKT7-STOP1-F	ACGACCACAACCAATCCTGTCCG	
		TCAGAGGAGGACCTG <u>CATATG</u> AT	Sal I
	pGBKT7-STOP1-R	GGAAACTGAAGACGATTTGTGC	
		А	
		ATGCGGCCGCTGCAG <u>GTCGAC</u> TT	
		AGAGACTAGTATCTGAAACA	
RT-qPCR	ANAC017-qPCR-F	TGAGGTTCAATGGAAAGGCT	
	ANAC017-qPCR-R	CCAGAAGATGGCACACAAAG	
	XTH31-qPCR-F	TGTCACTCTTTGGCTCG	
	XTH31-qPCR-R	ACCTCATCGTGGTCTCC	
	XTH17-qPCR-F	ACTTCTGTTTCTTCTTGCGGCA	
	XTH17-qPCR-R	AGGATTTGTCGAGCGAGAGAGA	
	XTH15-qPCR-F	GGCGACTGTTCTTCTTGTGACA	
	XTH15-qPCR-R	TTGGATTTGAAACCTGACCCGG	
	RAE1-qPCR-F	CCTGATGGTTTGAAGGCGAT	
	RAE1-qPCR-R	CGAATTGGCGATTTGGGTGA	

	HB7-qPCR-F	GCTCAGAAAACGAAGAGAACCG
	HB7-qPCR-R	GCTCCTCAAACCCACCAAAATA
	HB12-qPCR-F	GCAGAGACTAAACGAAGAGATG
	HB12-qPCR-R	TCTTTCCATTATGCGACTCT
	WRKY46-qPCR-F	TGCACGTGTCATTTCTTGAG
	WRKY46-qPCR-R	AACCTGTATGTCCTGCCCCA
	WRKY47-qPCR-F	AAGGGAATCCATGTCCTCGC
	WRKY47-qPCR-R	ACGGGGGAAGAGGATGGTTA
	ALMT1-qPCR-F	ACTTGAGAGAGCTGAGTGACC
	ALMT1-qPCR-R	TCTTCTCGGGTCTTCATTCCC
	MATE-qPCR-F	GCATAGGACTTCCGTTTGTGGCA
	MATE-qPCR-R	CGAACACAAACGCTAAGGCA
	STOP1-qPCR-F	CCAAGTTCCATCTCAAGCTTTTC
		Т
	STOP1-qPCR-R	TGGGACGTAAAACCTGCGAA
	ALS1-qPCR-F	TGAGCAGCAAACGGAATCCT
	ALS1-qPCR-R	CTCGCAAAGCCGTGCATATC
	ALS3-qPCR-F	CAATCGCCGGAATGTTGGTC
	ALS3-qPCR-R	TTGCAACGTCGCTTGTCTTG
	STAR1-qPCR-F	GCCATGCCATCACTTTGGTC
	STAR1-qPCR-R	AGATCCATCGGCGACTC
	TBL27-qPCR-F	GAACAACGTTGAGAGCGGTT
	TBL27-qPCR-R	CTTCGTACGGCTTGGTCATG
	ACTIN2-qPCR-F	GCTGACCGTATGAGCAAAGA
	ACTIN2-qPCR-R	GATCCACATCTGTTGGAACG
	TUBULIN-qPCR-F	AAGTTCTGGGAAGTGGTT
	TUBULIN-qPCR-R	CTCCCAATGAGTGACAAA
anac017-1	SALK_044777-LP	CTCTTTCCTCAGTGGCAACAG
identification	SALK_044777-RP	CAGGGAACTTCTGCAAATCTG
	LBb1.3	ATTTTGCCGATTTCGGAAC
anac017-2	SALK_070231-LP	GACTCATGCTTGTCTTGGAGC
identification	SALK_070231-RP	AACCAAATAACGGATCCGATC
	LBb1.3	ATTTTGCCGATTTCGGAAC
wrky46	SALK_1230_H01-LP	GAGTCTCTTCTCGAAGCTGGG
identification	SALK_1230_H01-	GATCCTTCCCTTTTCGAAGTG
	RP	
	LBb1.3	ATTTTGCCGATTTCGGAAC
stop1	SALK_114108-LP	TCTTAAAGCGGCCATTGGTG
identification	SALK_114108-RP	TTAGAGACTAGTATCTGAAACAG
		ACTCAC
	LBb1.3	ATTTTGCCGATTTCGGAAC



Supplemental Figure S1. Identification of the *anac017* mutants. (A) Schematic structure of the *anac017* mutants. The grey box and green box represent exon and untranslated region (UTR), respectively, while the black bold line indicates the intron. (B) Confirmation of the *anac017* mutant alleles by PCR. DNA molecular weight markers are shown in the left lane in each panel. (C) RT-qPCR analysis of the *ANAC017* expression in WT and *anac017* mutant roots. Seedlings were grown on 1/2 MS medium for four weeks, and the RNA was prepared from root tissues. *TUBULIN* was used as an internal control. Data are means \pm SD (n=4). Different letters above the bars indicate significant differences at P < 0.05 by one-way ANOVA analysis.



Supplemental Figure S2. Phenotype of WT and *anac017* mutants in both vegetative and reproductive stage under normal growth conditions.



Supplemental Figure S3. Phenotypes of WT, *anac017* mutants and *anac017*-com lines (complementary lines). (A) WT, *anac017* mutant (*anac017-1*) and *anac017*-complementation (*anac017-1*-com) lines were grown on 1/2 MS medium with or without 200 μ M Al for 7 days. Seedlings with roots approximately 1 cm long were selected and then transferred to the Al-untreated or Al-treated medium. (B) Root elongation of WT, *anac017-1* and *anac017-1*-com lines in the presence or absence of 200 μ M Al. Root elongation was measured after treatment as indicated by (A). Data are means \pm SD (n=4). Different letters above the bars indicate significant differences at *P* < 0.05 by one-way ANOVA analysis. (C) RT-qPCR analysis of the *ANAC017* expression in WT, *anac017-1* and *anac017-1*-com lines. Seedlings were grown on 1/2 MS medium for four weeks, and the RNA was prepared from root tissues. *TUBULIN* was used as an internal control. Data are means \pm SD (n=4). Different letters above the bars indicate significant differences at *P* < 0.05 by one-way ANOVA analysis.



Supplemental Figure S4. Phenotypes of WT and *ANAC017* overexpression (*ANAC017*-OX) lines. (A) WT and *ANAC017* overexpression (*ANAC017*-OX) lines were grown on 1/2 MS medium with or without 200 μ M Al for 7 days. Seedlings with roots approximately 1 cm long were selected and then transferred to the Al-untreated or Al-treated medium. (B) Root elongation of WT and *ANAC017*-OX lines in the presence or absence of 200 μ M Al. Root elongation was measured after treatment as indicated by (A). Data are means \pm SD (n=5). Different letters above the bars indicate significant differences at *P* < 0.05 by one-way ANOVA analysis. (C) RT-qPCR analysis of the *ANAC017* expression in WT and *ANAC017*-OX lines. Seedlings were grown on 1/2 MS medium for four weeks, and the RNA was prepared from root tissues. *TUBULIN* was used as an internal control. Data are means \pm SD (n=4). Different letters above the bars indicates above the bars indicate differences at *P* < 0.05 by one-way ANOVA analysis.



Supplemental Figure S5. Expression of *ANAN017* and *XTH31* in response to Al, cold and La stresses. (A-B) RT-qPCR analysis of the expression of *ANAC017* (A) and *XHT31* (B) in WT lines. Four-week-old WT seedlings were treated with or without 50 μ M Al and 50 μ M La in the growth chamber with the temperature of 24 °C or 4 °C for 24 h. RNA was prepared from root tissues. *TUBULIN* was used as an internal control. Data are means \pm SD (n=4). Different letters above the bars indicate significant differences at *P* < 0.05 by one-way ANOVA analysis. (C-F) *p*ANAC017-GUS seedlings with roots approximately 1 cm long were treated with or without 100 μ M Al and 100 μ M La in the growth chamber with the temperature of 24 °C or 4 °C for 7 days. Roots were subjected to GUS staining. Scale bars, 100 mm.



Supplemental Figure S6. Total contents of the xyloglucan oligosaccharides by the MALDI-TOF MS analysis. Cell walls were extracted from the roots of Col-0 and *anac017* mutants in the absence of Al and digested with XEG.



Supplemental Figure S7. The subcellular localization of ANAC017 by transient expression into tobacco (*N. benthamiana*) leaves.



Supplemental Figure S8. Relationship between ANAC017 and known Al responsible cascades. (A) The expression *ANAC017* in WT, *stop1* and *wrky46* mutants with or without Al treatment. Seedlings were grown on 1/2 MS medium for four weeks, and the RNA was prepared from root tissues. *TUBULIN* was used as an internal control. Data are means \pm SD (n=4). Asterisk indicate a significant difference at *P*< 0.05 by Student's t test. (B) A pair of plasmid pGBKT7-WRKY46, pGBKT7-STOP1 and pGADT7-ANAC017 were introduced into Y2HGold (AH109) and cultured on the synthetically defined (SD) medium lacking leucine, tryptophan, adenine and histidine (SD-LWHA) at 30 °C for 3 d. A pair of plasmids pGADT7-SV40 and pGBKT7-53 were used as the positive control, while a pair of plasmids pGADT7-SV40 and pGBKT7-Lam were used as the negative control.



Supplemental Figure S9. Occurrence of NACRS in the promoter regions of Al tolerance genes. A stretch of 2 kb upstream of the transcription initiation site was examined. The predicted NACRS-CGTA, CGTG and CACG are represented by triangles, circles and squares, respectively.