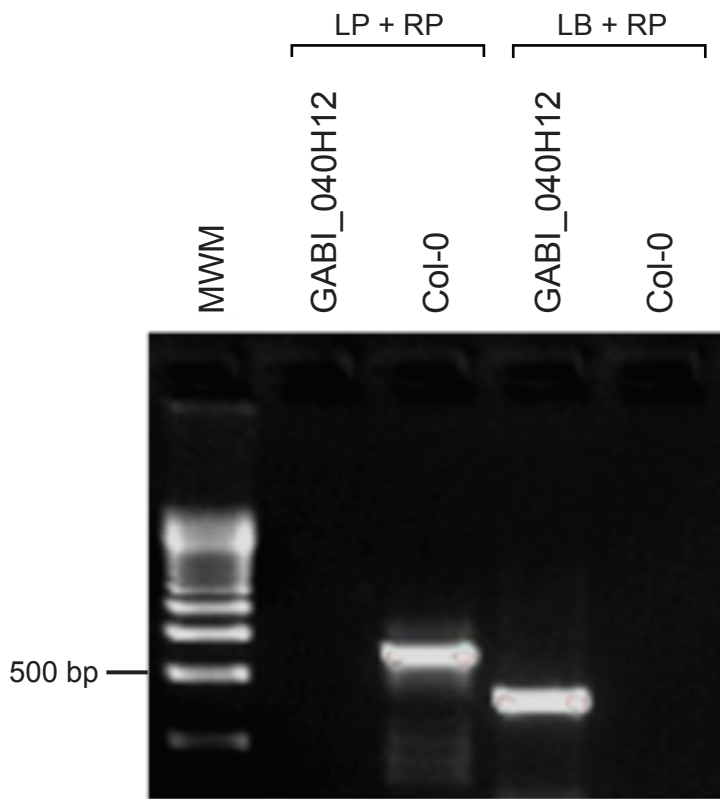
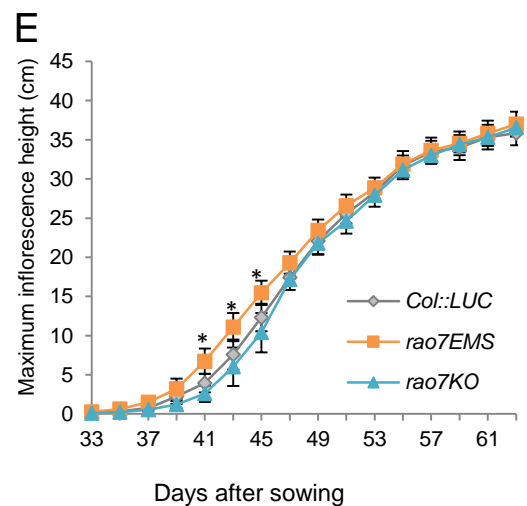
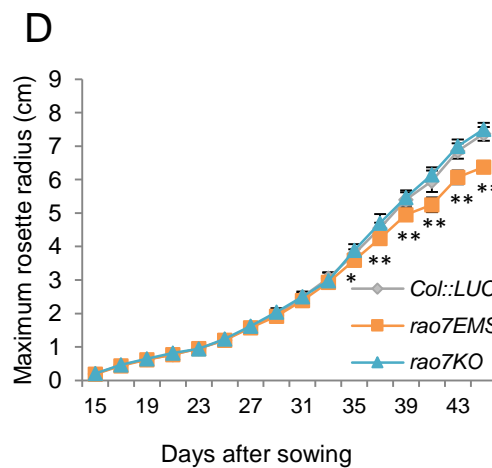
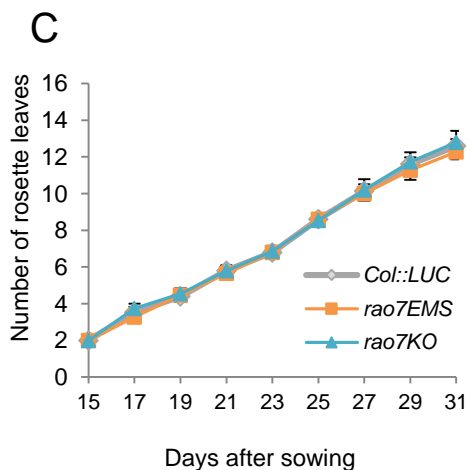
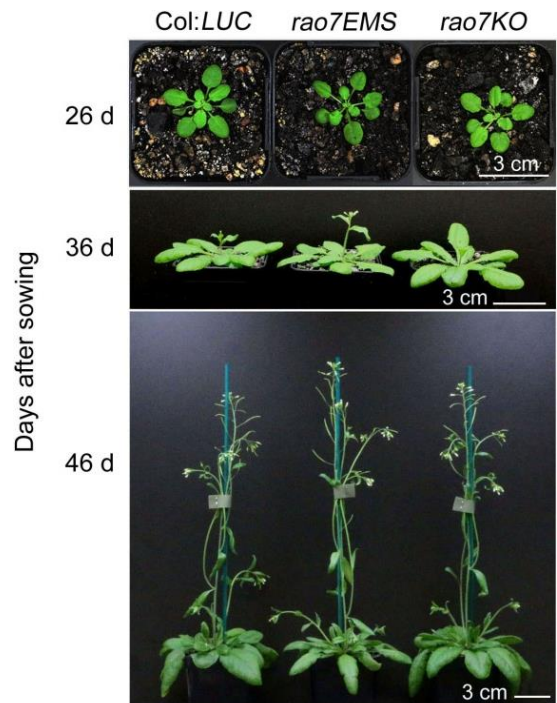
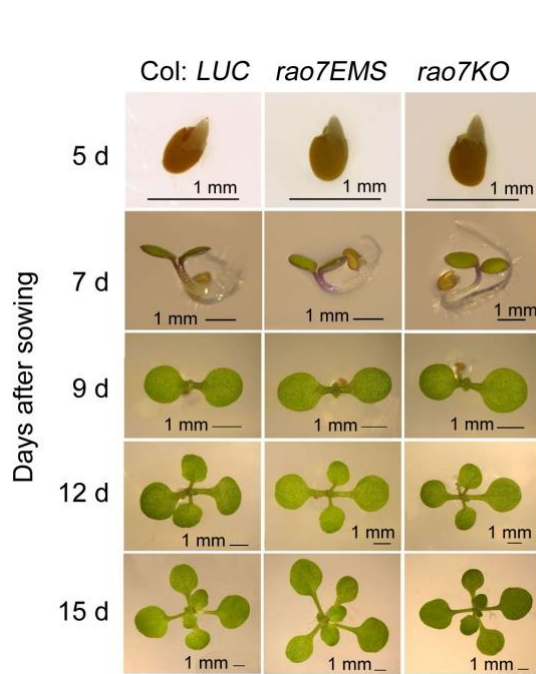
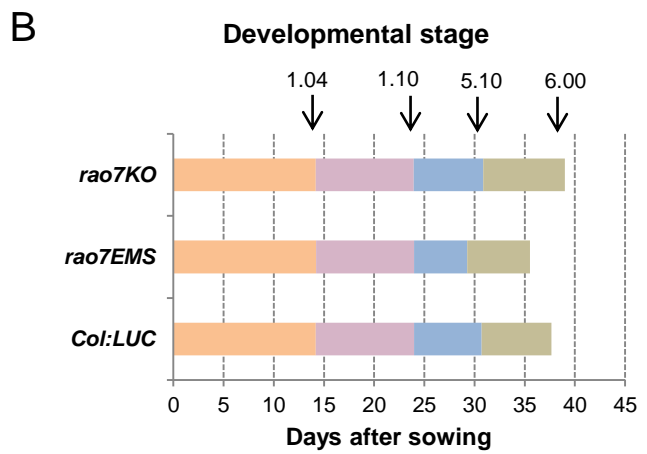
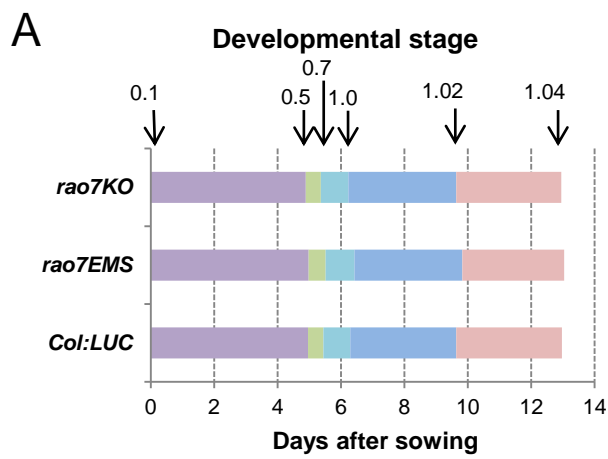


**Supplemental Figure S1. Complementation of *rao7EMS* with wild-type *MYB29* restoring normal antimycin A induction of *PromAOX1a*-driven *LUC*.** Luminescence image of a Petri dish containing *myb29* T-DNA knock-out line (*rao7KO*, see Supplemental Fig. S2) (top left), Col:*LUC* (top right), *rao7EMS* (bottom left), and *rao7EMS* complemented with the expression of the wild-type *MYB29*-coding sequence under the constitutive Cauliflower Mosaic Virus 35S promoter (P35S) (*rao7EMS*+P35S:*MYB29*) (bottom right). Whereas both wild type (Col:*LUC*) and *rao7EMS*+P35S:*MYB29* show induced luminescence upon treatment with antimycin A, *rao7EMS* luminescence is enhanced.



**Supplemental Figure S2. Confirmation of T-DNA knock-out line for the MYB29/RAO7-encoding gene.** PCR with gene (*At5g07690*)-specific primers (LP+RP) and a T-DNA left border (LB) primer. MWM, molecular weight marker.

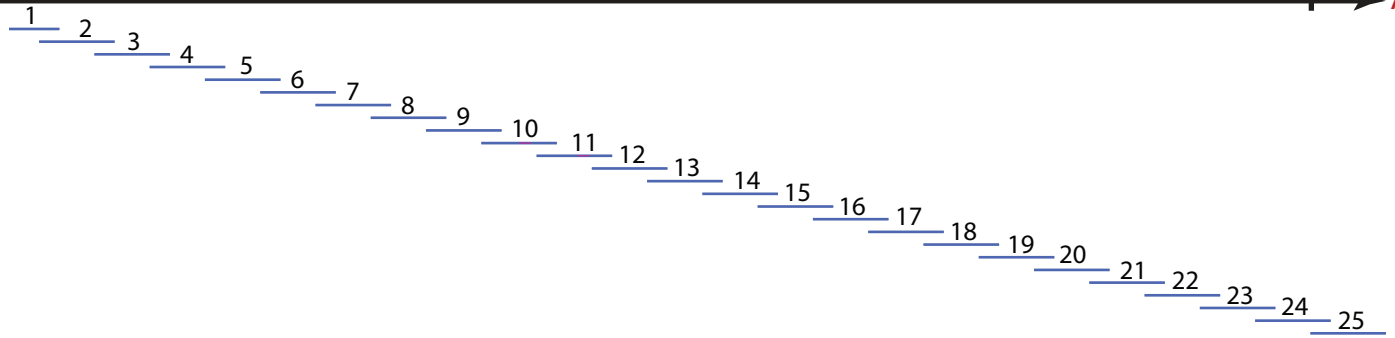


**Supplemental Figure S3. Phenotypic analysis of *rao7* mutants under nonstress conditions.** A, Plate-based growth progression analysis of *Col:LUC*, *rao7EMS*, and *rao7KO*. Arrows define the time (days after sowing) that *Col:LUC* plants have reached the growth stages as defined by Boyes et al. (2001). Stage 0.1, imbibition; stage 0.5, radical emergence; stage 0.7, hypocotyl emergence from seed coat; stage 1.0, cotyledons fully opened; stage 1.02, two rosette leaves >1 mm in length; stage 1.04, four rosette leaves >1 mm in length. Representative images of seedlings at 5, 7, 9, 12 and 15 days after sowing are shown. B, Soil-based growth progression as in (A): stage 1.10, 10 rosette leaves >1 mm; stage 5.10, first flower buds visible; stage 6.00, first flower opens. Representative images of seedlings at 26, 36, and 46 days after sowing are shown. C-E, Representative growth parameters of *Col:LUC* and *rao7* mutants: number of rosette leaves >1 mm, maximum rosette radius (cm), and plant height (cm) over time. Days are relative to the days after sowing after a 3-day stratification at 4°C. Data are given as averages for 15 plants  $\pm$  SE. Asterisk indicates significant differences in mutant compared to the wild type (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ , Student's  $t$  test).

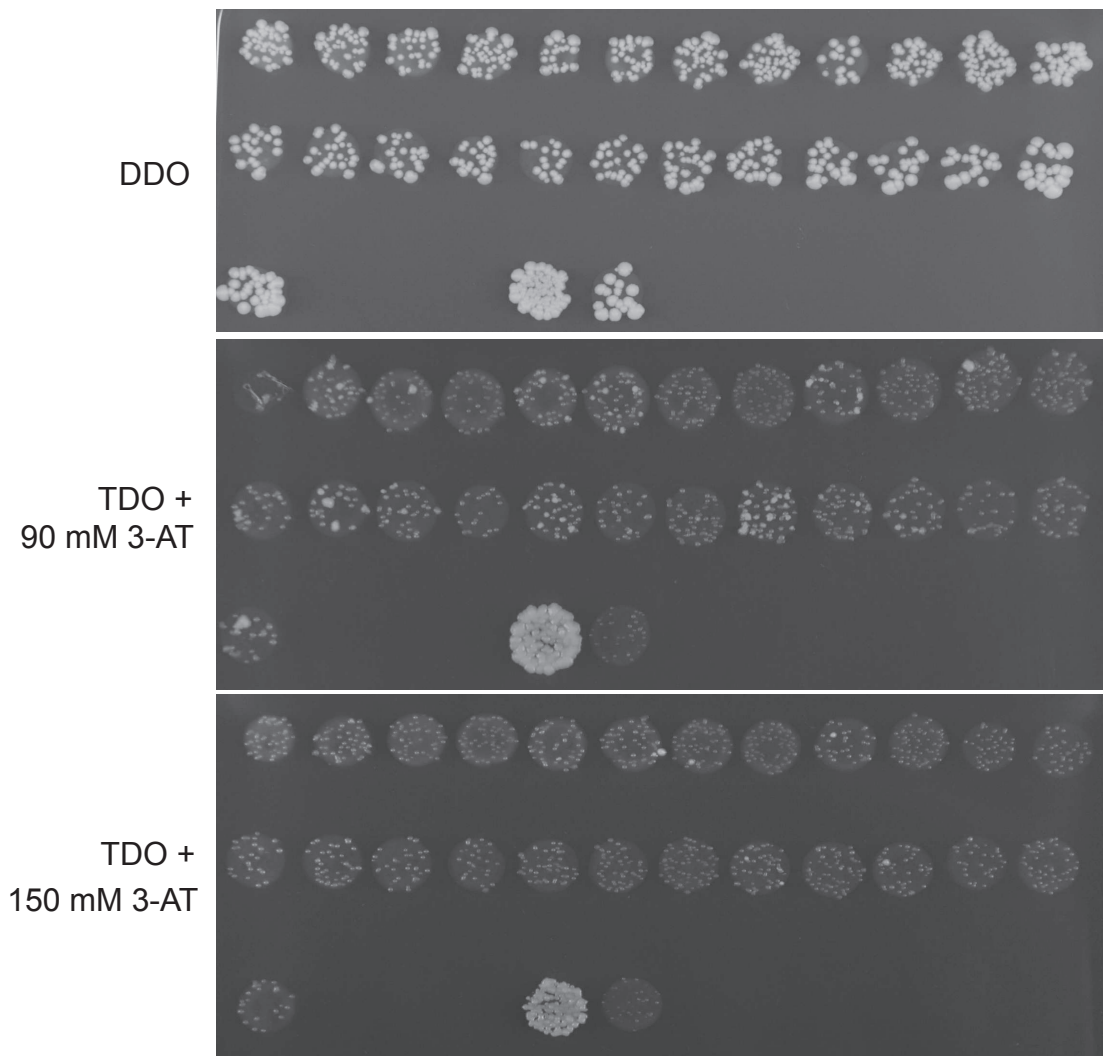
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*AOX1a*

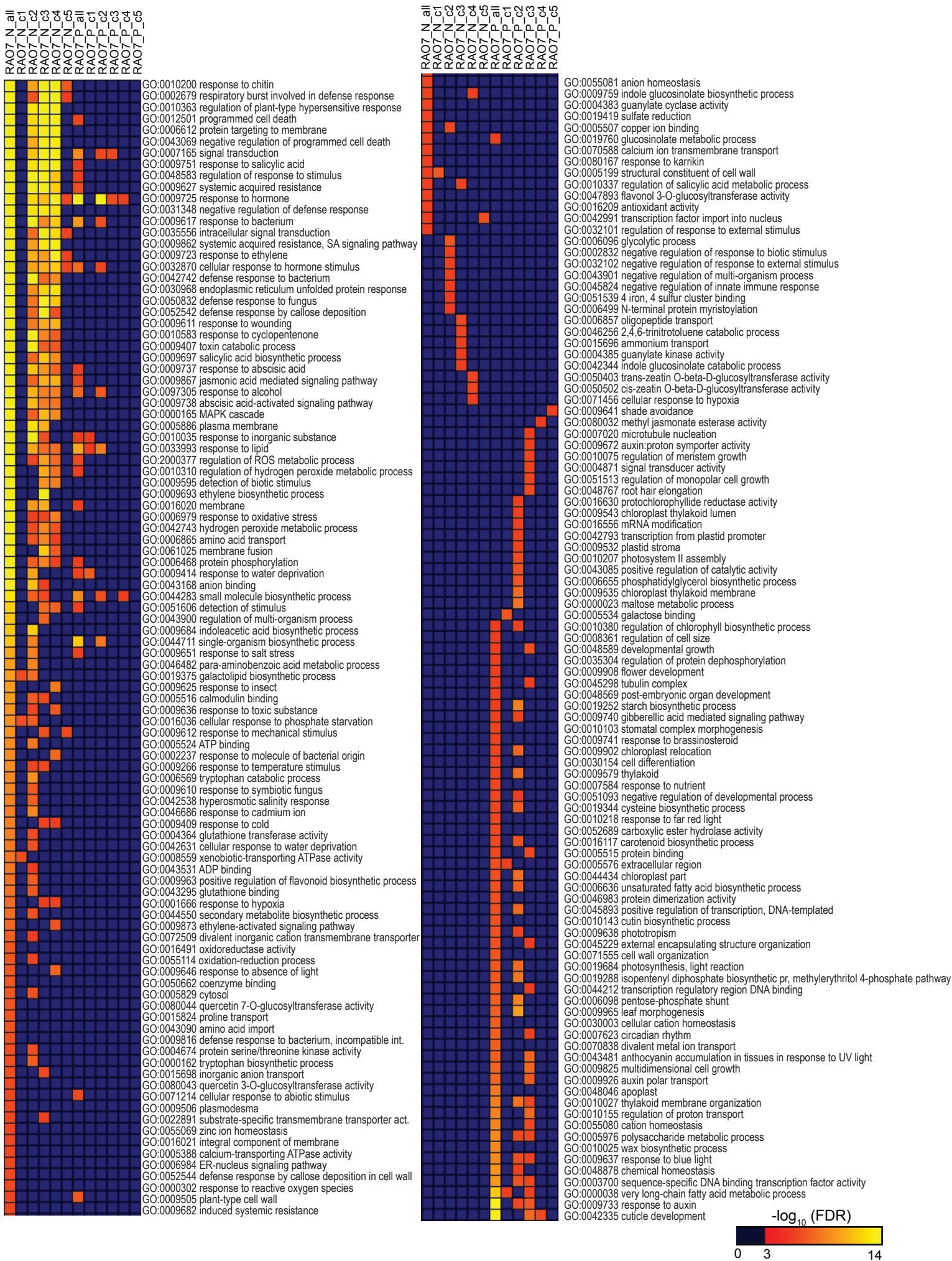
-99 +1  
TSS → ATG



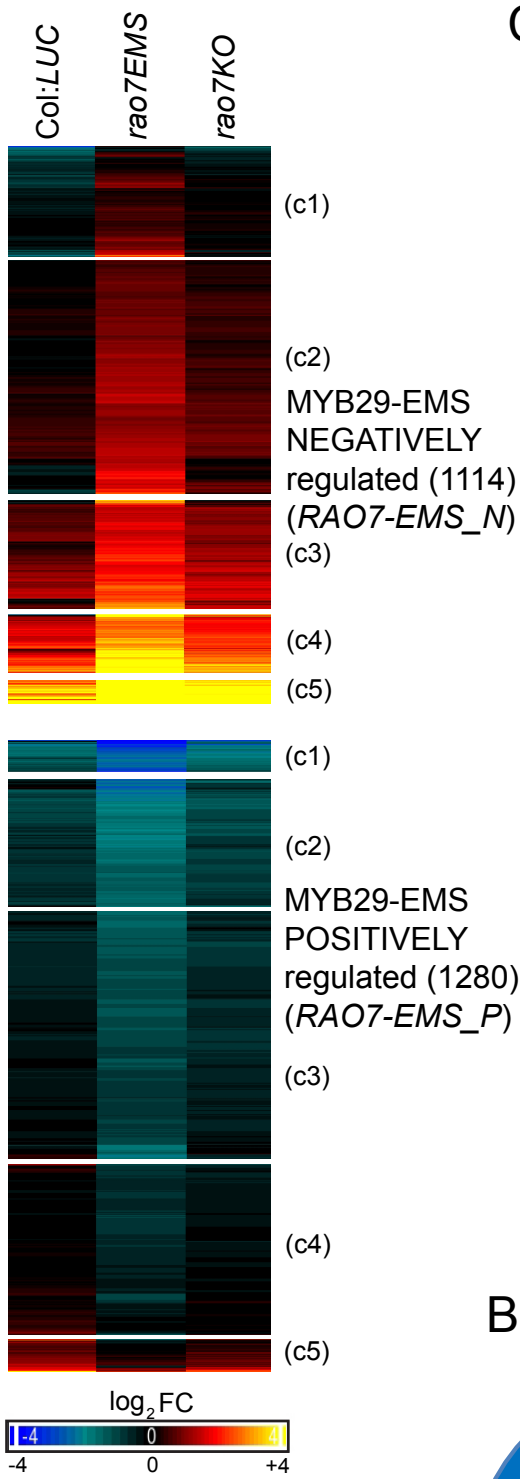
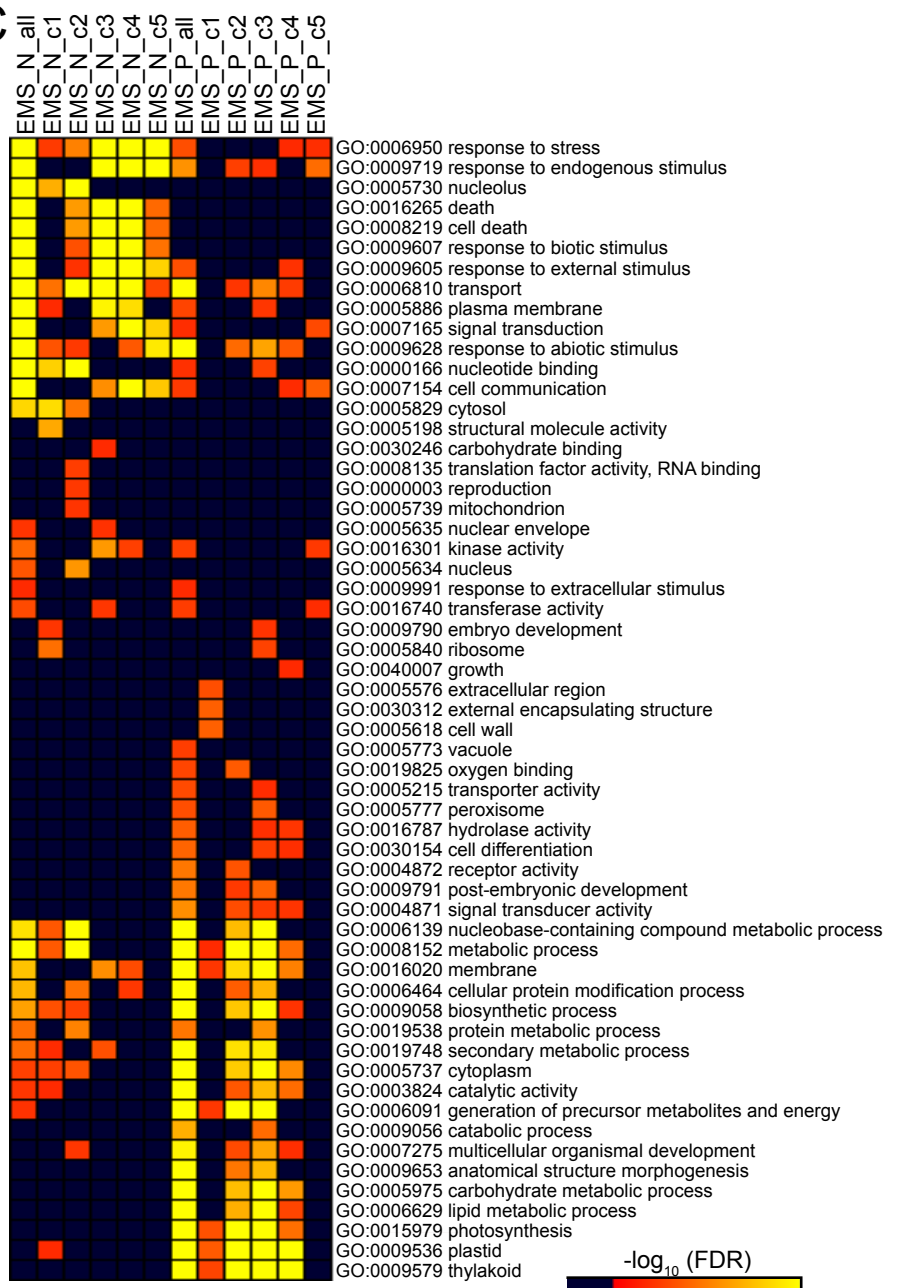
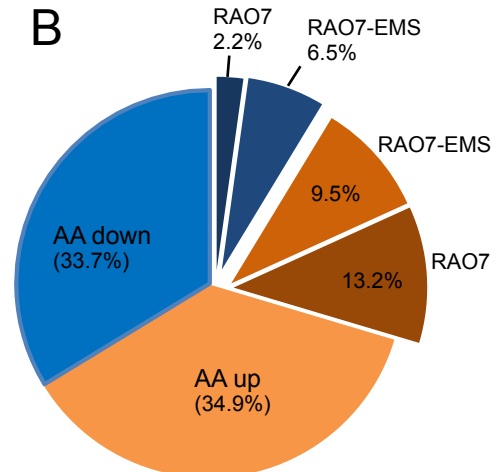
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13	14	15	16	17	18	19	20	21	22	23	24
25				+	-						



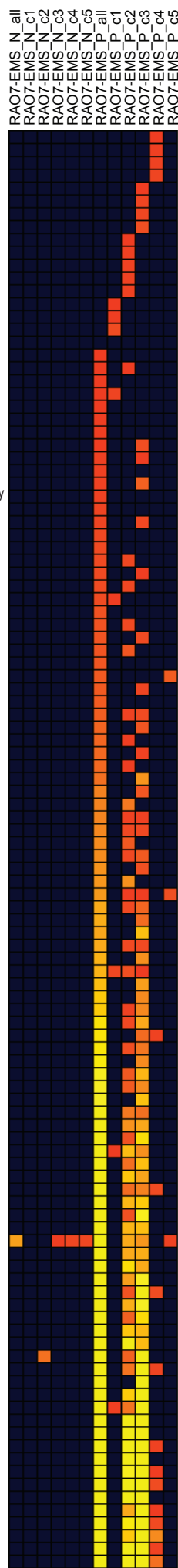
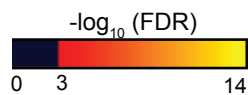
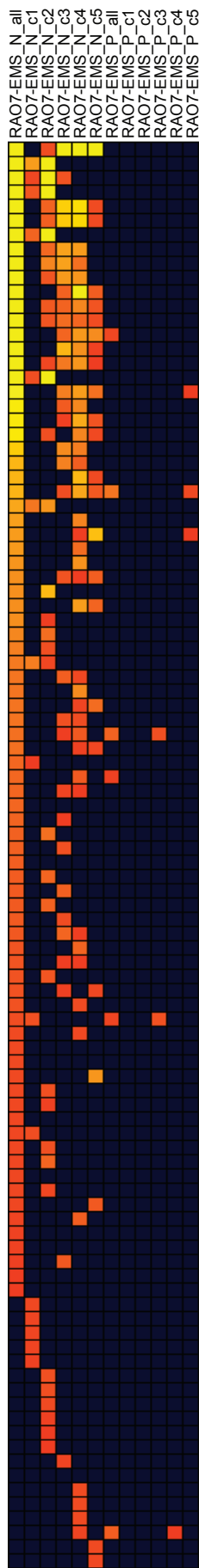
**Supplemental Figure S4. Yeast one-hybrid analysis for MYB29 binding to the *AOX1a* 1.85-kb promoter.** The *AOX1a* promoter was divided into 25 regions of approximately 100 bp with 25 bp overlapping with the previous and next regions. Yeast Y182 cells were cotransformed with the *MYB29* pGADT7-rec2 prey vector and pHIS2 *AOX1a* bait vector. Cells were spotted on double dropout media (DDO; transformation control) and triple dropout media for binding assay (TDO supplemented with 3-aminotriazole [3-AT] to control background growth). Binding of p53 to its p53-binding site or empty pHIS2 vector were used as positive (+) and negative (-) controls, respectively. TSS, transcriptional start site.



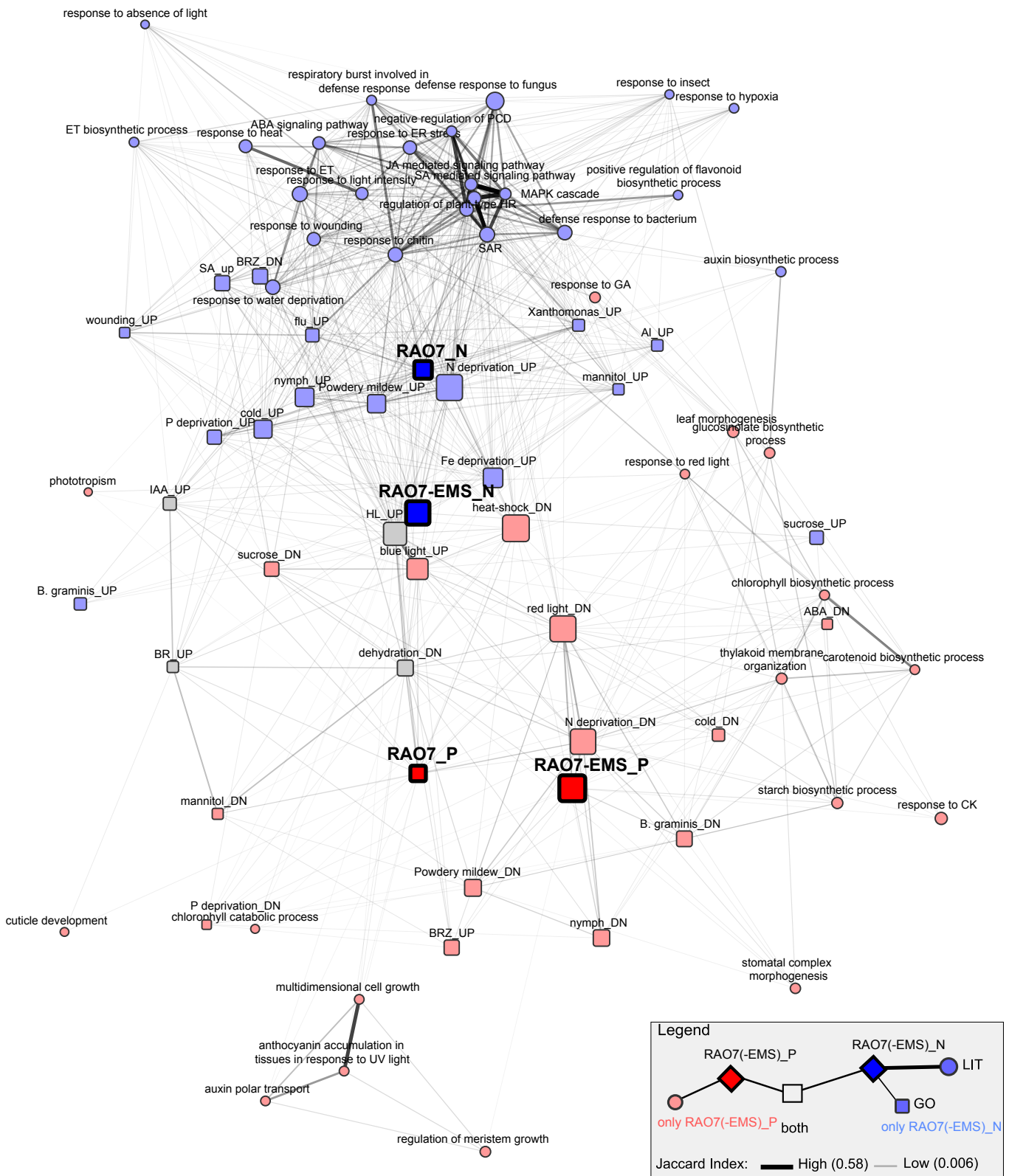
**Supplemental Figure S5. Gene Ontology enrichment analysis of genes regulated by RAO7/MYB29.** RAO7 positively (*RAO7\_P*) and negatively (*RAO7\_N*) regulated genes and their respective coexpression clusters (c1, c2, c3, c4 and c5) were analyzed for enriched Gene Ontology (GO) terms. GO terms enriched with FDR < E-03 ( $-\log_{10}(\text{FDR}) > 3$ ) in at least one of the gene lists are displayed after trimming for redundant GO terms. FDR values smaller and larger than E-03 are displayed in red-yellow and black, respectively.

**A****C****B**

**Supplemental Figure S6. Transcriptional response to antimycin A (AA) regulated through the RAO7/MYB29-EMS function.** A, Heatmap representation of the expression of genes of which the AA response is either negatively or positively regulated specifically through the RAO7/MYB29-EMS function. Genes were classified as negatively and positively regulated by MYB29-EMS only, when their AA fold change was increased (*RAO7-EMS\_N*) and decreased (*RAO7-EMS\_P*) in the *rao7EMS* and not in the *rao7KO* mutants, respectively. Genes were further classified in clusters (c1, c2, c3, c4, and c5) according to their expression characteristics in response to AA in the different genotypes by means of K-means clustering. Colors represent  $\log_2$  fold changes of AA treatment compared to mock treated with blue and red/yellow representing transcripts that are down-regulated and up-regulated by the AA treatment, respectively. B, Proportion of AA up- and down-regulated genes through MYB29 or through the MYB29-EMS function. C, Gene Ontology Slim enrichment analysis of the *RAO7-EMS\_N* and *RAO7-EMS\_P* genes and their respective coexpression subclusters. Color codes represent the negative logarithm (base 10) of the FDR adjusted *P* value. Significantly enriched GO terms (FDR < 0.05) are indicated with a red-yellow color.




































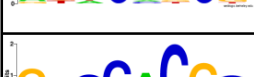
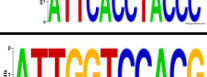


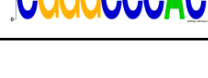


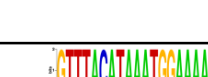









**Supplemental Figure S7. Gene Ontology enrichment analysis of genes regulated by RAO7/MYB29-EMS.** RAO7-EMS positively (*RAO7-EMS\_P*) and negatively (*RAO7-EMS\_N*) regulated genes and their respective co-expression clusters (c1, c2, c3, c4, and c5) were analyzed for enriched Gene Ontology (GO) terms. GO terms enriched with  $\text{FDR} < E-03$  ( $-\log_{10}(\text{FDR}) > 3$ ) in at least one of the gene lists are displayed after trimming for redundant GO terms. FDR values smaller and larger than  $E-03$  are displayed in red-yellow and black, respectively.



**Supplemental Figure S8. Correlation of the RAO7-EMS-regulated gene sets with functional annotations.** The RAO7 and RAO7-EMS positively and negatively regulated genes (*RAO7\_P*, *RAO7\_N*, *RAO7-EMS\_P*, and *RAO7-EMS\_N*) were used for gene set enrichment analysis with GO biological processes (BP) (FDR < 3.00E-05; see Supplemental Fig. S5 and S7) and differentially expressed (DE) genes (UP, up-regulated; DN, down-regulated) after hormone or stress treatments, the latter obtained from the literature through the Plant Gene Set Enrichment Analysis tool (FDR < 3.00E-03) (Yi et al., 2013). Afterwards, all gene lists were compared to each other in a pairwise manner. Only gene list pairs with significant overlaps (FDR < E-03) and containing at least 10% of genes from either of both gene lists were used for network construction and only gene sets directly connected to at least one of the four RAO7-regulated gene lists were retained. Edge thickness and distance in the network correspond to the Jaccard Index of similarity. Node shape refers to the type of gene list (square, GO-BP; circle, DE gene lists from literature) and node color corresponds to nodes connected only with *RAO7\_P* or *RAO7-EMS\_P* (red), with *RAO7\_N* or *RAO7-EMS\_N* (blue) or with both positively and negatively regulated gene sets (grey). ABA, abscisic acid; BRZ, brassinazole; BR, brassinosteroid; CK, cytokinin; ET, ethylene; ER, endoplasmic reticulum; GA, gibberellic acid; HL, high light; HR, hypersensitive response; IAA, indole-3-acetic acid; JA, jasmonic acid; MAPK, mitogen-activated protein kinase; PCD, programmed cell death; SA, salicylic acid; SAR, systemic acquired resistance.



A. RAO7/MYB29-negatively regulated (*RAO7\_N*)

	Amadeus <i>de novo</i> motif	P-value (HG)	STAMP motifs					
			Name	E-value	Sequence	Name	E-value	Sequence
(1)		5.3E-35	WBOXPCWR KY1	3.9E-07		WRKY6_ oneSite	5.8E-07	
(2)		1.0E-30	WRKY6_ oneSite	2.8E-09		WRKY40_ oneSite	1.1E-08	
(3)		4.3E-21	WRKY6_ oneSite	3.2E-08		WBOXPC- WRKY1	3.9E-06	
(4)		3.4E-23	CE3OSOSEM	5.0E-06		ABREMOTIFIII- OSRAB16B	5.7E-06	
(5)		2.1E-20	SP8FBIB- SP8BIB	1.6E-06				
(6)		9.4E-19	RBENTGA3	6.9E-07		O2F1BE2S1	1.2E-06	
						ABREDISTB BNNAPA	4.7E-06	
(7)		7.3E-16	DRECT- COREAT	2.3E-06		AtERF-1_ twoSite AtERF-2_ twoSite AtERF-5_ twoSite	2.9E-06	
(8)		2.3E-12	AtERF-3 AtERF-4	2.8E-08 4.5E-08		AtERF-1_ twoSite AtERF-2_ twoSite AtERF-5_ twoSite	1.6E-07	
(9)		9.6E-16	2SSEEDPROT BANAPA	2.4E-05		WBOXPCWR KY1	2.6E-05	
(10)		1.9E-15	ACIPVPAL2 L1DCPAL1	1.0E-11 9.2E-11		AtMYB- 84_M00970	8.6E-11	
(11)		2.3E-15	UPRE1AT	1.0E-05		GCBP2ZM- GAPC4	2.4E-05	
(12)		1.6E-14	ERELEE4	5.8E-05				
(13)		2.8E-14	MADS- A_M00408* MADS- B_M00404*	2.1E-07 9.6E-07		CArG1 CArG3	1.0E-06 1.0E-06	
(14)		3.1E-14	CBF1*	9.9E-09		LS5ATPR1 LS7	8.3E-08	
(15)		3.1E-14	TATABOX1 TATABOXOSP AL	1.7E-08 2.4E-07		CONSERVED- 11NTZMATP1	1.2E-06	
(16)		7.6E-14	PIATGAPB PI	9.0E-05		RNFG2OS	1.1E-04	
(17)		9.0E-14	AS1LIKECSHP- RA	1.6E-06		27BPDRCON- SENSUSPS25S	1.8E-06	

**Supplemental Figure S9.** De novo promoter motif discovery in RAO7/MYB29-regulated genes. Enriched degenerate 8-mer motifs were identified in the 1-kb intergenic regions upstream of the translation start codon of the *RAO7\_N* (A) and *RAO7\_P* (B) genes with the de novo motif discovery tool Amadeus given the background distribution of the upstream sequences of all the genes in the genome (Linhart et al., 2008). Amadeus large run delivered 20 enriched motifs as output. De novo motif sequences were compared to known plant motifs in the Similarity, Tree-building, and Alignment of DNA Motifs and Profiles (STAMP) database and known motifs with significant matches to the de novo motifs are displayed (Mahony et al., 2007). Sequence logos were made with WebLogo 2.8.2 for 8-mers as input and WebLogo 3.5.0 for position weight matrices (PWM) as input (Crooks et al., 2004). Logos of motifs for which the PWM was not publically available were copied from the STAMP website and indicated with asterisks. HG, hypergeometric.

A. RAO7/MYB29-negatively regulated (*RAO7\_N*) (continued)

	Amadeus de novo motif	P-value (HG)	STAMP motifs					
			Name	E-value	Sequence	Name	E-value	Sequence
(18)		1.3E-13	GLUTAACAO5	5.1E-04		DRE2COREZ MRAB17	5.1E-04	
(19)		2.0E-13	TGA1A-NTPR1A	3.6E-06				
(20)		3.9E-12	ATHB6	1.3E-07		ATHB1	8.5E-07	

B. RAO7/MYB29-positively regulated (*RAO7\_P*)

	Amadeus de novo motif	P-value (HG)	STAMP motifs					
			Name	E-value	Sequence	Name	E-value	Sequence
(1)		4.5E-24	SORLIP2	4.1E-06		GCBP2ZM-GAPC4	1.8E-05	
						SITEIIAOSPCNA	1.8E-05	
(2)		6.8E-22	ABREDISTB-BNNAPA	3.3E-09		ABREAZM-RAB28	6.0E-08	
(3)		7.5E-19	RYREPEAT-BNNAPA	1.3E-04		AUXRETGA1-GMGH3	2.3E-04	
(4)		4.5E-16	TATCCAC-HVAL21	1.9E-11		SREATMSD	4.5E-09	
(5)		5.7E-16	MYB80_M01052*	1.4E-07		TaMYB80	3.7E-07	
(6)		1.4E-14	ACIPVPAL2	3.2E-08		AtMYB15	3.7E-07	
						AtMYB84	7.4E-07	
(7)		4.4E-14	JASE2ATOPR1	1.3E-05		LS5ATPR1 LS7	2.3E-05	
			bZIP911_M003 59*	3.8E-05		QELEMENTZ MZM13	7.8E-05	
(8)		2.0E-14	TATABOX1	4.1E-07		CARG1ATAP3	1.1E-06	
						ATHB6	1.5E-06	
(9)		7.0E-14	SPL1_oneSite	9.2E-07		ACGTOSGLUB1	1.5E-06	
(10)		1.6E-13	TGTCACACM-CUCUMISIN	1.7E-07		SEBFCONSSTP R10A	9.2E-07	
						BIHD1OS	1.9E-06	

**Supplemental Figure S9.** De novo promoter motif discovery in RAO7/MYB29-regulated genes. Enriched degenerate 8-mer motifs were identified in the 1-kb intergenic regions upstream of the translation start codon of the *RAO7\_N* (A) and *RAO7\_P* (N) genes with the de novo motif discovery tool Amadeus given the background distribution of the upstream sequences of all the genes in the genome (Linhart et al., 2008). Amadeus large run delivered 20 enriched motifs as output. De novo motif sequences were compared to known plant motifs in the Similarity, Tree-building, and Alignment of DNA Motifs and Profiles (STAMP) database and known motifs with significant matches to the de novo motifs are displayed (Mahony et al., 2007). Sequence logos were made with WebLogo 2.8.2 for 8-mers as input and WebLogo 3.5.0 for position weight matrices (PWM) as input (Crooks et al., 2004). Logos of motifs for which the PWM was not publically available were copied from the STAMP website and indicated with asterisks. HG, hypergeometric. (Continued)

B. RAO7/MYB29-positively regulated (*RAO7\_P*) (Continued)

	Amadeus <i>de novo</i> motif	P-value (HG)	STAMP motifs					
			Name	E-value	Sequence	Name	E-value	Sequence
(11)		5.3E-13	REBETALGLHC B21	7.5E-07		MYBST1	2.8E-06	
(12)		1.0E-12	MYBST1	1.9E-06		CATATGGMSAUR1.9E-05		
(13)		6.9E-13	PEND*	1.1E-05		ABAREG2	4.9E-05	
(14)		9.6E-13	ATHB5_M00503*	7.1E-06		ATHB6	7.4E-06	
(15)		1.1E-12	TATABOX1	1.3E-08		ATML1_oneSite PDF2_oneSite	4.6E-08	
(16)		2.1E-12	TATABOX3	4.9E-07		SORLIP4	9.1E-06	
(17)		2.4E-12	SP8BFIBSP8BIB	6.4E-07		SORLIP4	3.9E-05	
(18)		2.8E-12	TATABOX1	1.5E-05		ATHB-5_M00503*	1.6E-05	
(19)		3.2E-12	LREBOX2-PSRBCS3	4.0E-05		SE1PVGRP183	5.1E-05	
(20)		8.6E-12	DRE2COREZM-RAB17	2.2E-05		AtWER*	2.9E-05	
						GARE1OSREP1	6.4E-05	

**Supplemental Figure S9.** De novo promoter motif discovery in RAO7/MYB29-regulated genes. Enriched degenerate 8-mer motifs were identified in the 1-kb intergenic regions upstream of the translation start codon of the *RAO7\_N* (A) and *RAO7\_P* (B) genes with the de novo motif discovery tool Amadeus given the background distribution of the upstream sequences of all the genes in the genome (Linhart et al., 2008). Amadeus large run delivered 20 enriched motifs as output. De novo motif sequences were compared to known plant motifs in the Similarity, Tree-building, and Alignment of DNA Motifs and Profiles (STAMP) database and known motifs with significant matches to the de novo motifs are displayed (Mahony et al., 2007). Sequence logos were made with WebLogo 2.8.2 for 8-mers as input and WebLogo 3.5.0 for position weight matrices (PWM) as input (Crooks et al., 2004). Logos of motifs for which the PWM was not publically available were copied from the STAMP website and indicated with asterisks. HG, hypergeometric. (Continued)















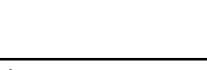




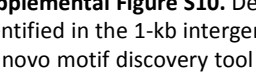
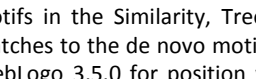
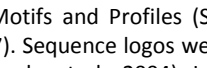
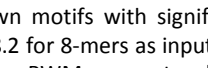
A. RAO7/MYB29-EMS negatively regulated (*RAO7-EMS\_N*)

	Amadeus de novo motif	P-value (HG)	STAMP motifs					
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(1)		5.5E-25	ABREMOTIFIII OSRAB16B	2.8E-06		OCETYPEIINT HISTONE	5.3E-06	
(2)		9.1E-19	ABREMOTIFIII OSRAB16B	2.2E-06		MYBCORE ATCYCB1	4.6E-06	
(3)		3.7E-19	AtSR1	1.0E-06		APOLYA_ M00310	5.7E-06	
(4)		1.1E-18	CEREGLUBO X3PSLEGA	6.8E-06		T-BOX	2.3E-05	
(5)		5.9E-17	TEFBOXATEE F1AA1	9.9E-10				
(6)		2.2E-16	LRENPCABE	1.2E-08		ACGTABREM OTIFA2OSEM	2.7E-07	
(7)		9.3E-16						
(8)		4.6E-14	ABASEED1	1.2E-04				
(9)		5.0E-14	AS1LIKE CSPRA	5.7E-11		RBCSBO X3PS	6.1E-08	
(10)		9.4E-14	ACGTSEED2	1.5E-05		PREATPRODH	7.5E-05	

B. RAO7/MYB29-EMS positively regulated (*RAO7-EMS\_P*)

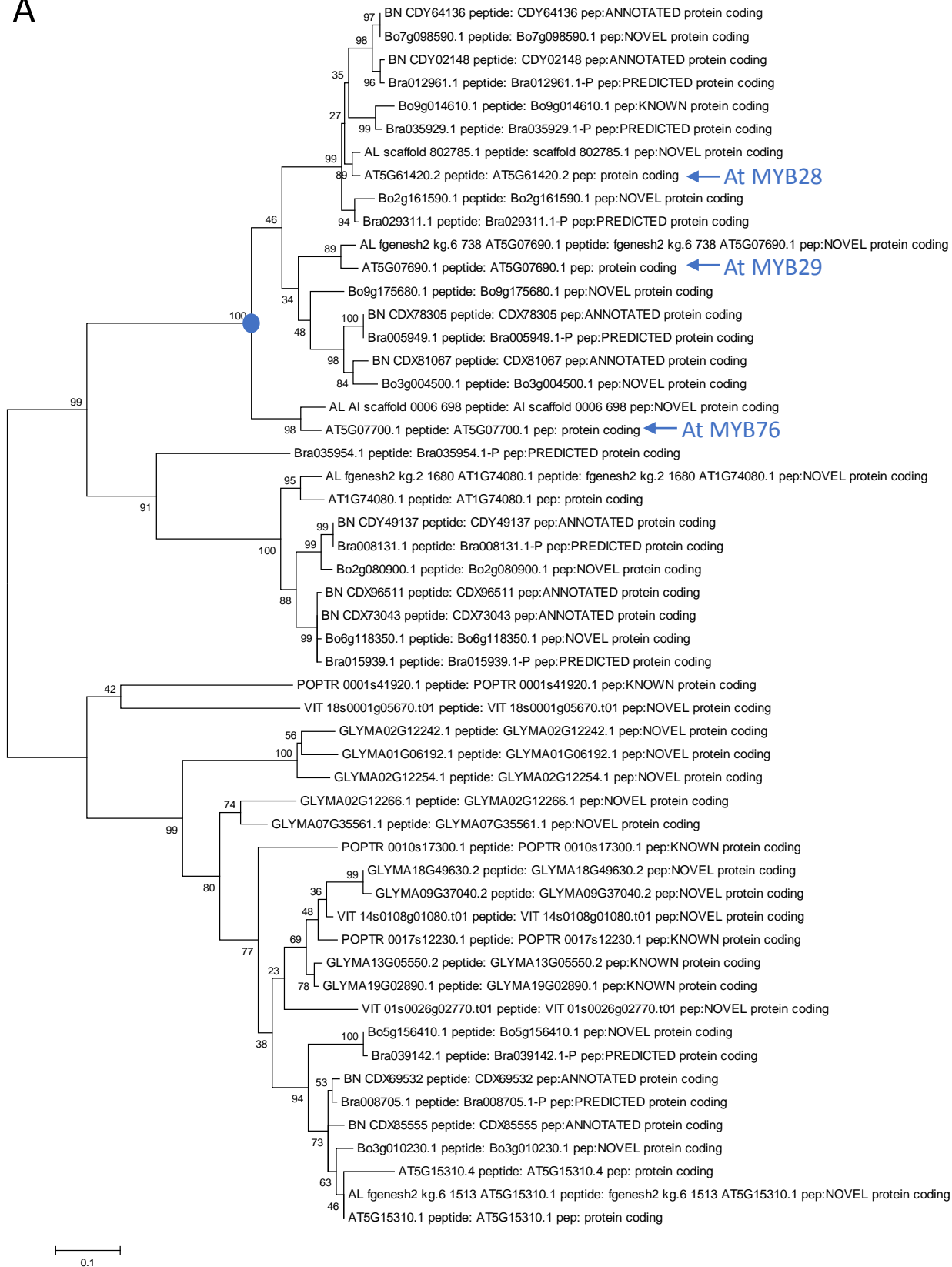
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(2)		1.3E-20	LRENPCABE	1.5E-09		HY5AT	1.9E-07	
(3)		6.0E-17	GLUTEBP1OS	1.4E-07		LREBOX2PSR BCS3	2.3E-07	
(4)		3.8E-16	GGTCCCATG MSAUR	9.5E-13		SITEIIBOS PCNA	7.1E-08	
(5)		2.5E-15	E2FBNTRNR	2.2E-07		ANAERO2CO NSENSUS	3.2E-05	
(6)		4.8E-15	SEBFCONSSTP R10A	7.0E-06		GATA	2.8E-05	
(7)		1.1E-14	NAM_oneSite	1.7E-05		S2FSORPL21	2.2E-04	
(8)		3.1E-14	AMMORESIIU DCRNIA1	5.2E-07		UP2ATMSD	3.1E-05	
						TELO-box	9.6E-05	

**Supplemental Figure S10.** De novo promoter motif discovery in RAO7/MYB29-EMS-regulated genes. Enriched degenerate 8-mer motifs were identified in the 1-kb intergenic regions upstream of the translation start codon of the *RAO7-EMS\_N* (A) and *RAO7-EMS\_P* (B) genes with the de novo motif discovery tool Amadeus given the background distribution of the upstream sequences of all the genes in the genome (Linhart et al., 2008). Amadeus normal run provided 10 enriched motifs as output. The de novo motif sequences were compared to known plant motifs in the Similarity, Tree-building, and Alignment of DNA Motifs and Profiles (STAMP) database and known motifs with significant matches to the de novo motifs are displayed (Mahony et al., 2007). Sequence logos were made with WebLogo 2.8.2 for 8-mers as input and WebLogo 3.5.0 for position weight matrices (PWM) as input (Crooks et al., 2004). Logos of motifs for which the PWM was not publicly available were copied from the STAMP website and indicated with an asterisk (\*). HG, hypergeometric.

	Amadeus de novo motif	P-value	STAMP motifs					
			Name	E-value	Sequence	Name	E-value	Sequence
(6)		4.8E-15	SEBFCONSSTP R10A	7.0E-06		GATA	2.8E-05	
			TGTCACACM CUCUMISIN	6.1E-05				
(7)		1.1E-14	NAM_oneSite	1.7E-05		S2FSORPL21	2.2E-04	
(8)		3.1E-14	AMMORESIIU DCRNIA1	5.2E-07		UP2ATMSD	3.1E-05	
(8)		3.1E-14	AMMORESIIU DCRNIA1	5.2E-07		TELO-box	9.6E-05	
(8)		3.1E-14	AMMORESIIU DCRNIA1	5.2E-07		UP2ATMSD	3.1E-05	
(8)		3.1E-14	AMMORESIIU DCRNIA1	5.2E-07		TELO-box	9.6E-05	
(9)		7.3E-14						
(10)		6.4E-13	CDA1ATCAB2	4.2E-06		PROXBBNNAPA	4.7E-05	

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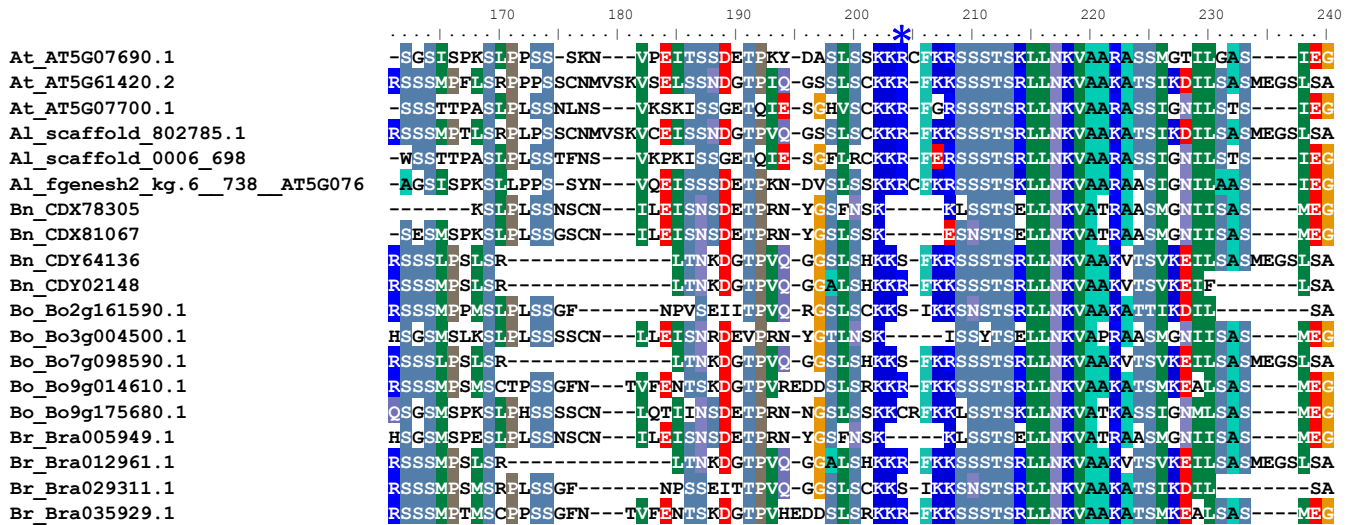
A



**Supplemental Figure S11. Sequence conservation of potential MYB29 homologs.** A, Phylogenetic tree of MYB29 sequence-similar proteins from dicots obtained through the Ensembl Plants release 33 platform with BLAST in Ensembl (E-value < 1.00E-65). *Glycine max* (GLYMA), *Populus trichocarpa* (POPTR), and *Vitis vinifera* (VIT) protein sequences similar (E-value < 1.00E-62) to MYB29 were included as an outgroup of the tree. MYB29 was identified in a Brassicaceae-specific clade, including *Arabidopsis thaliana* (At) MYB28 and MYB76 and *Arabidopsis lyrata* (AL), *Brassica nap*a (BN), *Brassica oleracea* (Bo), and *Brassica rapa* (Bra) homologs. These results indicate that the *Arabidopsis thaliana* MYB29, MYB28, and MYB76 genes and their respective orthologous genes were obtained from a common ancestral gene. The phylogenetic tree was constructed in Mega 5 with the maximum likelihood method and bootstrap values were estimated with 1,000 replicates (Tamura et al. 2011). B, Analysis of the conservation of MYB29 arginine (R) 178 (highlighted with asterisk) in the homologous proteins. Conservation was found in all included species in the clade: *A. lyrata* (3/3), *B. nap*a (1/4), *B. oleracea* (1/5), *B. rapa* (2/4). Sequences were aligned by means of the ClustalW algorithm in BioEdit (version 7.2.5) with default alignment parameters (Hall, 1999). Residues common or similar in at least half of the sequences are marked with colored boxes.

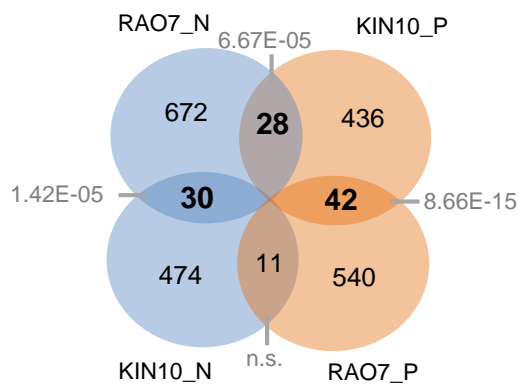
B

At MYB29  
Arg (R)178

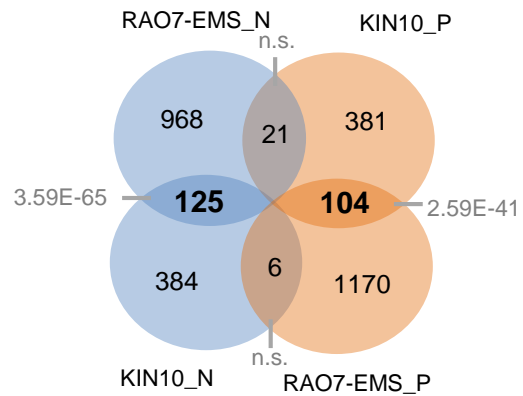


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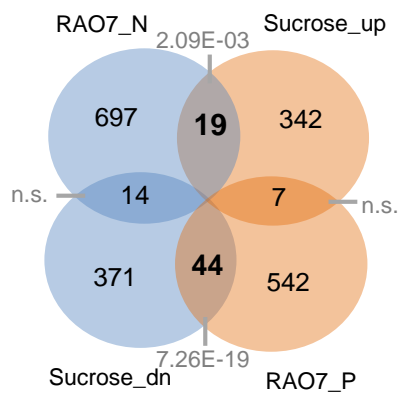
### A RAO7 V KIN10



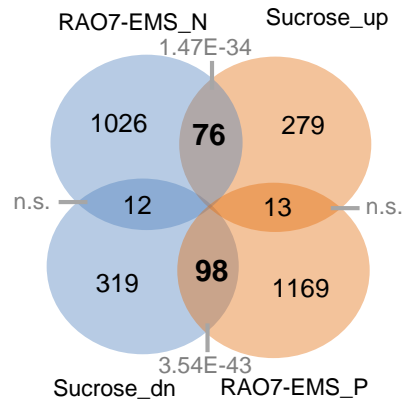
### B RAO7-EMS V KIN10



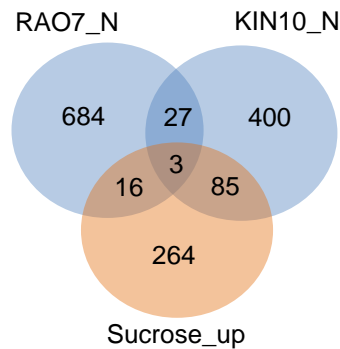
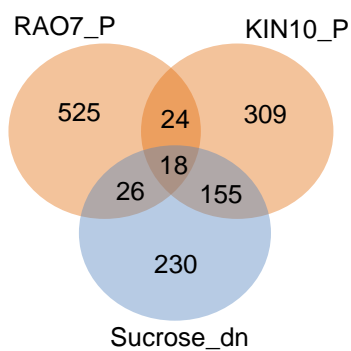
### C RAO7 V sucrose



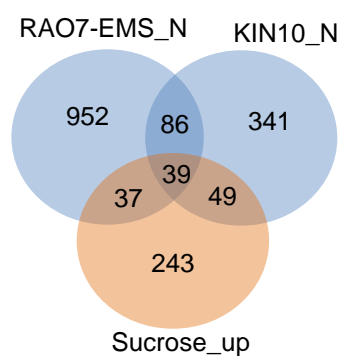
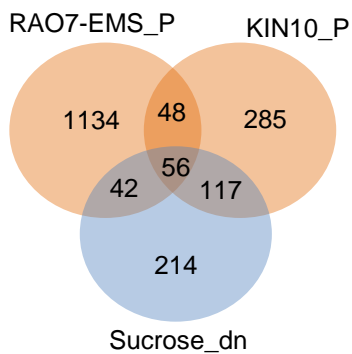
### D RAO7-EMS V sucrose



### E RAO7 V KIN10 V sucrose

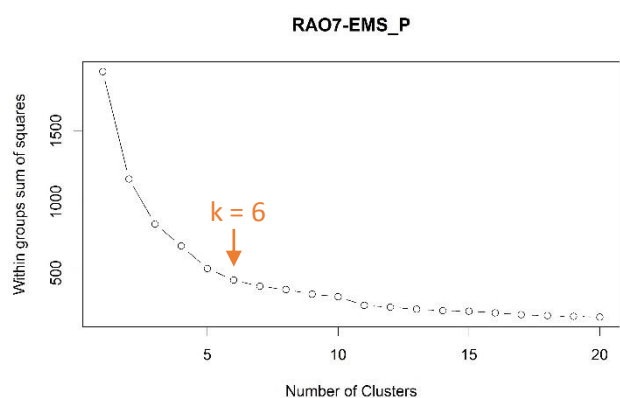
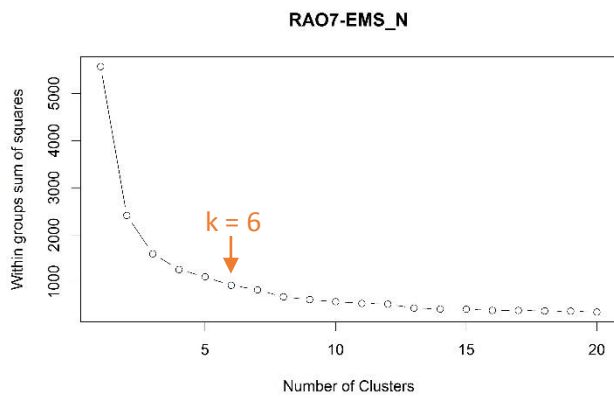
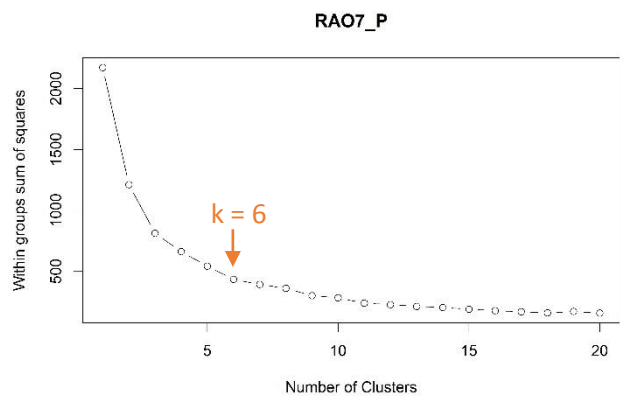
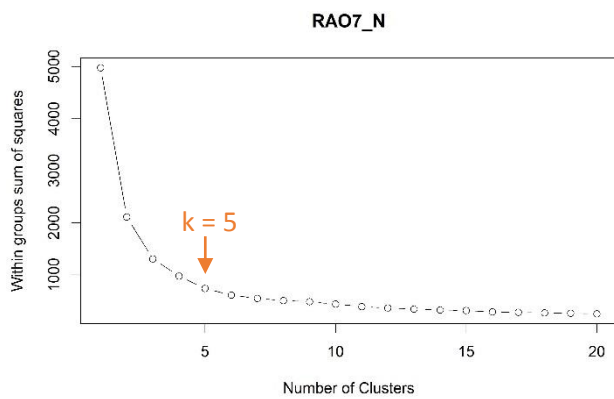


### F RAO7-EMS V KIN10 V sucrose



**Supplemental Figure S12. Comparison of genes regulated by RAO7, KIN10, and sucrose.** Venn diagrams depicting overlap between genes regulated by RAO7 and SNF1 KINASE HOMOLOG 10 (KIN10) (A), RAO7-EMS and KIN10 (B), RAO7 and sucrose (C), RAO7-EMS and sucrose (D), RAO7, KIN10, and sucrose (E), and RAO7-EMS, KIN10, and sucrose (F). KIN10-regulated genes were obtained from Baena-Gonzalez et al. (2007) and differential genes upon sucrose treatment from Gonzali et al. (2006). The probability ( $P$  value) of having an overlap size equal or greater than observed was calculated with a cumulative hypergeometric test in R (v.3.2.3). N.s., not significant.





**Supplemental Figure S13. Plot of the total within-group sum of squares against the number of clusters in K-means solutions.** For each gene list (*RAO7\_N*, *RAO7\_P*, *RAO7-EMS\_N*, and *RAO7-EMS\_P*), genes with similar expression patterns ( $\log_2$  AA-fold changes in *Col:LUC*, *rao7EMS*, and *rao7KO*) were grouped by K-means clustering (with  $k = 2$  to 20 number of clusters) with the *kmeans* function of R (Hartigan and Wong, 1979; R Core Team, 2014) and total within-group sum of squares were calculated. An appropriate number of clusters ( $k$ ) was chosen for which the decrease of the within-group sum of squares dropped off.