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Supplementary Materials for

The ABA Receptor PYL8 Promotes Lateral Root Growth by Enhancing MYB77-Dependent Transcription of Auxin-Responsive Genes

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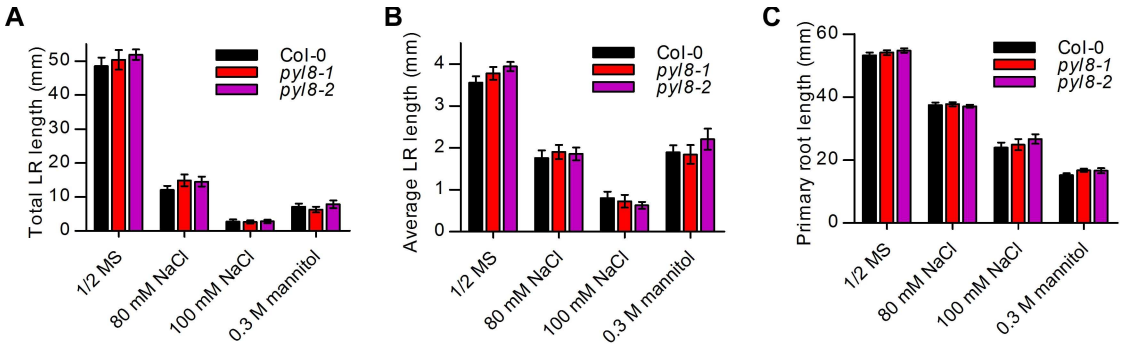

Fig. S1. *pyl8* mutants show no difference in root growth compared to the wild type when exposed to NaCl or mannitol stress. Lateral root length **(A-B)** and primary root length **(C)** of Col-0 and *pyl8* mutant seedlings at five dpt to media with or without NaCl or mannitol. N ≥ 8 seedlings. Data are mean ± S.E.M., **p*<0.05, Student's *t*-test.

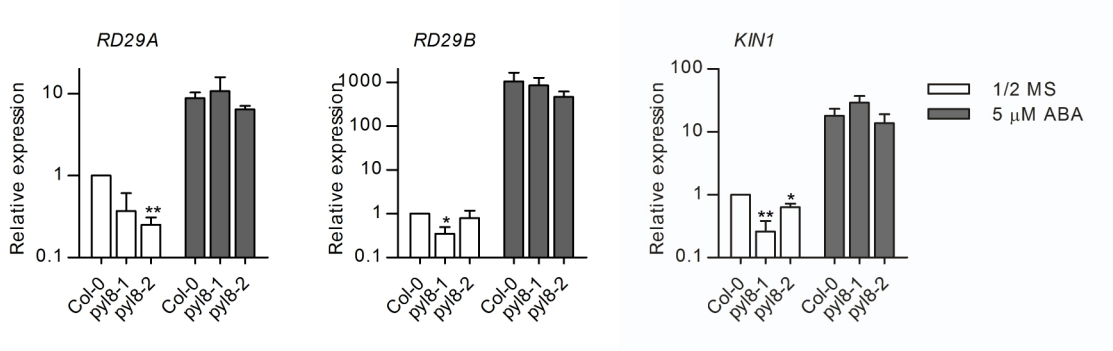

Fig. S2. Expression of stress-responsive genes in roots of *pyl8* mutant seedlings.

Quantitative RT-PCR was conducted on 3-day-old seedlings at 12 hour post transfer to media (1/2 MS, 1% sucrose) with or without 5 μM ABA. The expression levels of *RD29A*, *RD29B* and *KIN1* in Col-0 without ABA treatment were set as 1. N = 3 experiments. Data are mean ± S.E.M., *p<0.05, ** p<0.01, Student's t-test.

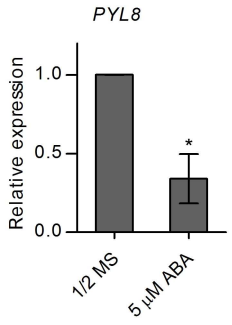

Fig. S3. Expression of *PYL8* in wild-type seedlings exposed to ABA. Quantitative RT-PCR was conducted on 3-day-old seedlings at 12 hour post transfer to media (1/2 MS, 1% sucrose) with or without 5 µM ABA. The expression level of *PYL8* in Col-0 without ABA treatment was set as 1. N = 3 experiments. Data are mean ± S.E.M., **p*<0.05, Student's *t*-test.

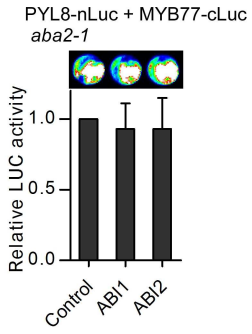

Fig. S4. The interaction between PYL8 and MYB77 is not reduced by overexpression of ABI1 or ABI2. PYL8-MYB77 interaction in LUC complementation assays in *aba2-1* mutant protoplasts with 5 µM ABA. N = 6 experiments. Data are mean ± S.E.M., **p*<0.05, Student's *t*-test.

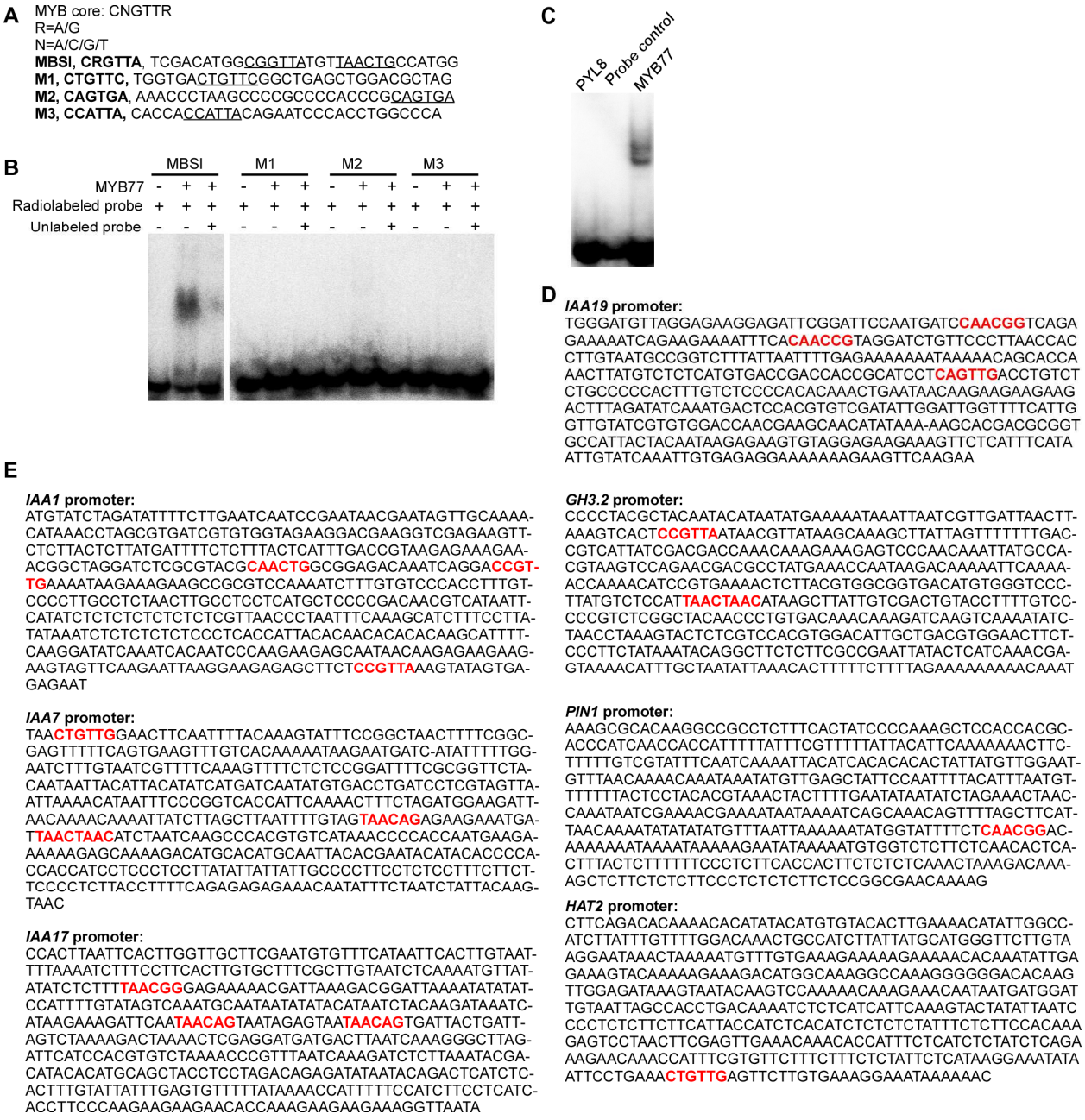

Fig. S5. The promoter region of auxin-regulated genes contains MBSI elements recognized by MYB77 and MYB44. (A) Oligonucleotides used in EMSA assay. Sequences of interest are underlined. **(B)** Binding between MYB77 and DNA oligonucleotides containing MBSI motif and mutated MBSI motif (M1-3) in EMSA assay. **(C)** Binding between PYL8 and MBSI motif-containing DNA oligonucleotides. **(D)** The 439-bp region of the translational start of *IAA19* is shown. MBSI elements are marked with red. **(E)** The 500-bp regions of the translational start of the indicated auxin-regulated genes are shown. MBSI and MBSII (GTTAGTTA) elements are marked with red.

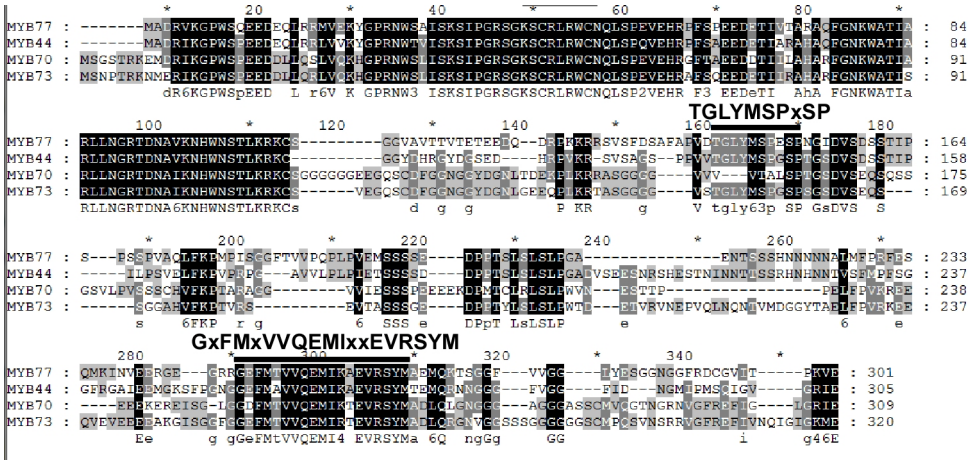

Fig. S6. Sequence alignment of MYB77 and its paralogs. MYB77, MYB44, MYB70 and MYB73 from *Arabidopsis* were aligned using ClustalX 2.0.5 with the default settings and were viewed using the GeneDoc software (http://www.nrbsc.org/gfx/genedoc/). R2R3 MYB subgroup 22 contains two conserved motifs: TGLYMSPxSP and GxFMxVVQEMIxxEVRSYM.

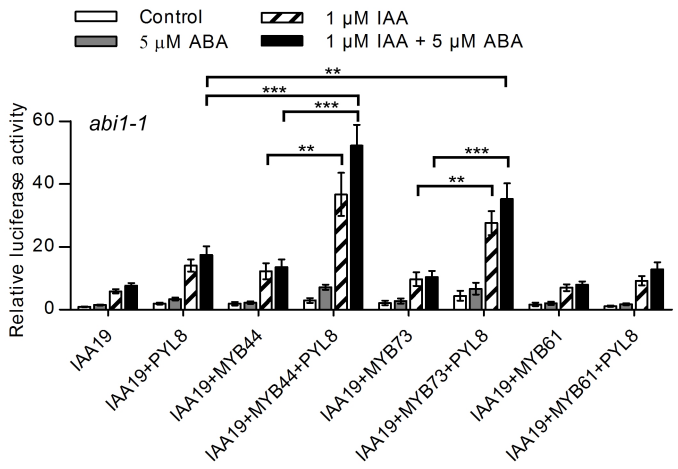

Fig. S7. PYL8 enhances the abilities of MYB44 and MYB73 to activate *IAA19pro-LUC* expression in *abi1-1* mutant protoplasts. *IAA19pro-LUC* expression in *abi1-1* mutant protoplasts co-transformed with the indicated combinations of *PYL8*, *MYB44*, *MYB73* and *IAA19pro-LUC* with varying concentration of ABA and IAA. N = 7 experiments. Data are mean ± S.E.M., ***p*<0.01, ****p*<0.001, Student's *t*-test.

Table S1. Primers used for plasmid constructions.

Plasmids	Forward primers	Reverse primers
IAA19pro-LUC	*IAA19pro*F: AGAGGATCCTGGGATGTTAGGAGAAGGAGATTCG	*IAA19pro*R: CTTCCATGGTTCTTGAACTTCTTTTTTTCCTCTCAC
pHBT95-MYB44	CGGCTCCCTCTCCCCTTGCTCCGTGGATCC ATGGCTGATAGGATCAAAGGTCCAT	GTAGTCTGGAACGTCGTATGGGTAAGGCCT CTACTCGATTCTCCCAACTCCAATTTGAC
pHBT95-MYB73-HA	CGGCTCCCTCTCCCCTTGCTCCGTGGATCC ATGTCAAACCCGACCCGTAAG	GTAGTCTGGAACGTCGTATGGGTAAGGCCT CTCCATCTTCCCAATTCCG
pHBT95-MYB77	CGGCTCCCTCTCCCCTTGCTCCGTGGATCC ATGGCGGATCGTGTTAAAGG	GTAGTCTGGAACGTCGTATGGGTAAGGCCT CTACTCAACCTTAGGTGTTATTACTCC
pHBT95-MYB61-HA	CGGCTCCCTCTCCCCTTGCTCCGTGGATCC ATGGGGAGACATTCTTGCTGTTAC	GTAGTCTGGAACGTCGTATGGGTAAGGCCT AAGGGACTGACCAAAAGAGACG
AD-MYB44	GCTCATATGGCCATGGAGGCCAGTGAATTC ATGGCTGATAGGATCAAAGGTCCAT	ATTCATCTGCAGCTCGAGCTCGATGGATCC CTACTCGATTCTCCCAACTCCAATTTGAC
AD-MYB73	GCTCATATGGCCATGGAGGCCAGTGAATTC ATGTCAAACCCGACCCGTAAG	ATTCATCTGCAGCTCGAGCTCGATGGATCC CTCCATCTTCCCAATTCCG
AD-MYB77	GCTCATATGGCCATGGAGGCCAGTGAATTC ATGGCGGATCGTGTTAAAGG	ATTCATCTGCAGCTCGAGCTCGATGGATCC CTACTCAACCTTAGGTGTTATTACTCC
AD-MYB61	GCTCATATGGCCATGGAGGCCAGTGAATTC ATGGGGAGACATTCTTGCTGTTAC	ATTCATCTGCAGCTCGAGCTCGATGGATCC CTAAAGGGACTGACCAAAAGAGACG
6P1-MYB77	GAAGTTCTGTTCCAGGGGCCCCTGGGATCC ATGGCGGATCGTGTTAAAGG	ATGCGGCCGCTCGAGTCGACCCGGGAATTC CTACTCAACCTTAGGTGTTATTACTCC
pET28a-PYL8	ATCGGATCC ATGGAAGCTAACGGGATTG	ATCGAATTC CTATAGTCTCGGGGTGAAG
pENTR-MYB77	CACCATGGCGGATCGTGTTAAAGG	CTCAACCTTAGGTGTTATTACTCC
cLUC-ABI1	CAGATCTCGTACGCGTCCCGGGGCGGATCC ATGGAGGAAGTATCTCCGGC	TGTTTGAACGATCTGCAGTCTAGAGTCGACTTA TCAGTTCAAGGGTTTGCTCTTG