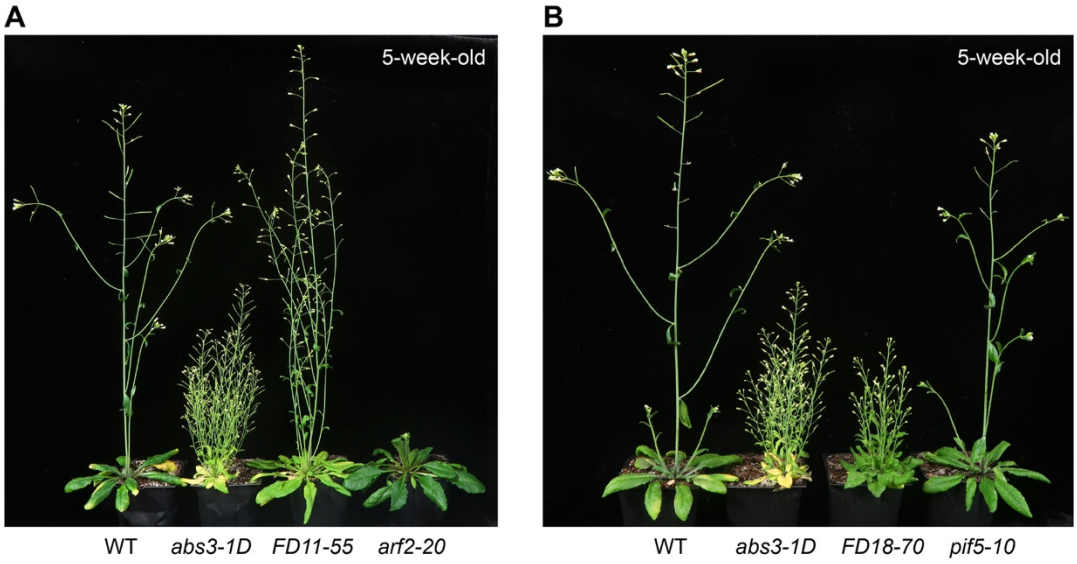
Appendix

ARF2-PIF5 interaction controls transcriptional reprogramming in the ABS3-mediated plant senescence pathway

Xue et al.

Table of Contents

Appendix Figure S1

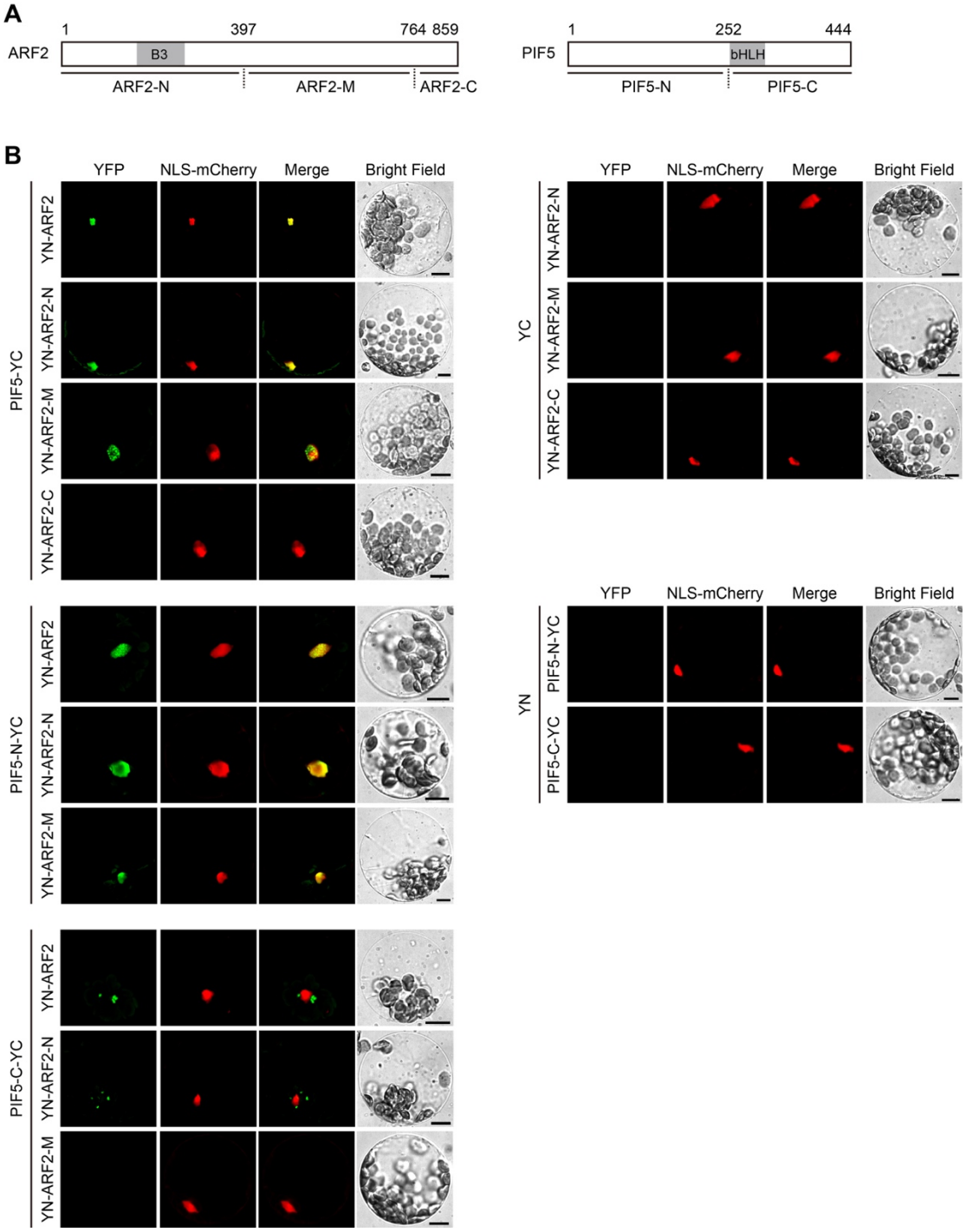


Appendix Figure S1. Supporting data for Figure 1.

A, Phenotypes of soil grown 5-week-old WT, *abs3-1D*, *FD11-55*, and *arf2-20.*

B, Phenotypes of soil grown 5-week-old WT, *abs3-1D*, *FD18-70*, and *pif5-10*.

Appendix Figure S2



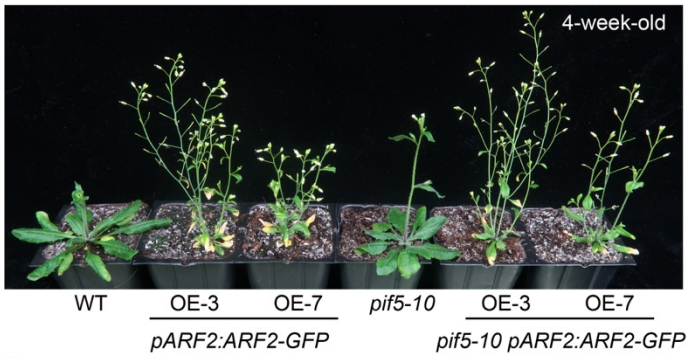
Appendix Figure S2. Supporting data for Figure 3.

A, Schematic diagrams showing truncated forms of ARF2 and PIF5 used in BiFC assays.

B, Protoplasts coexpressing indicated *YN* and *YC* vectors. Bars, 10 μm.

Appendix Figure S3

A



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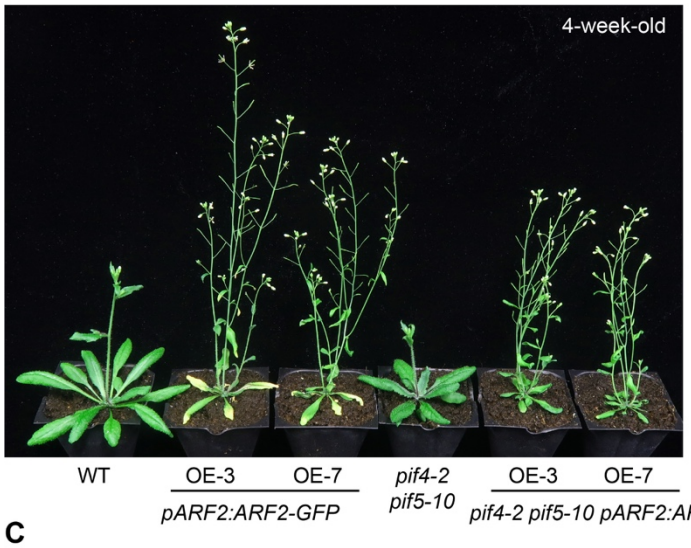

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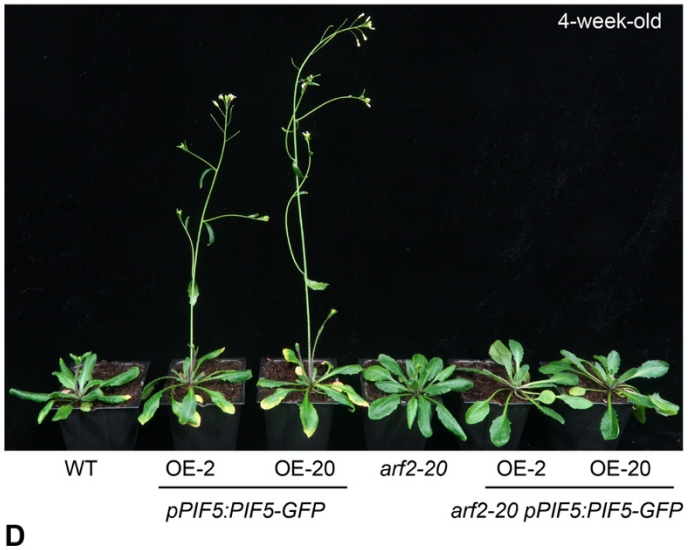

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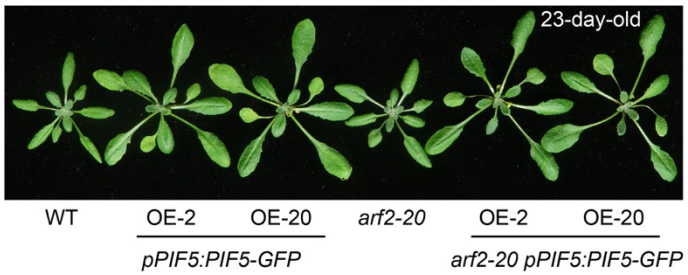


Figure S3. Supporting data for Figure 4.

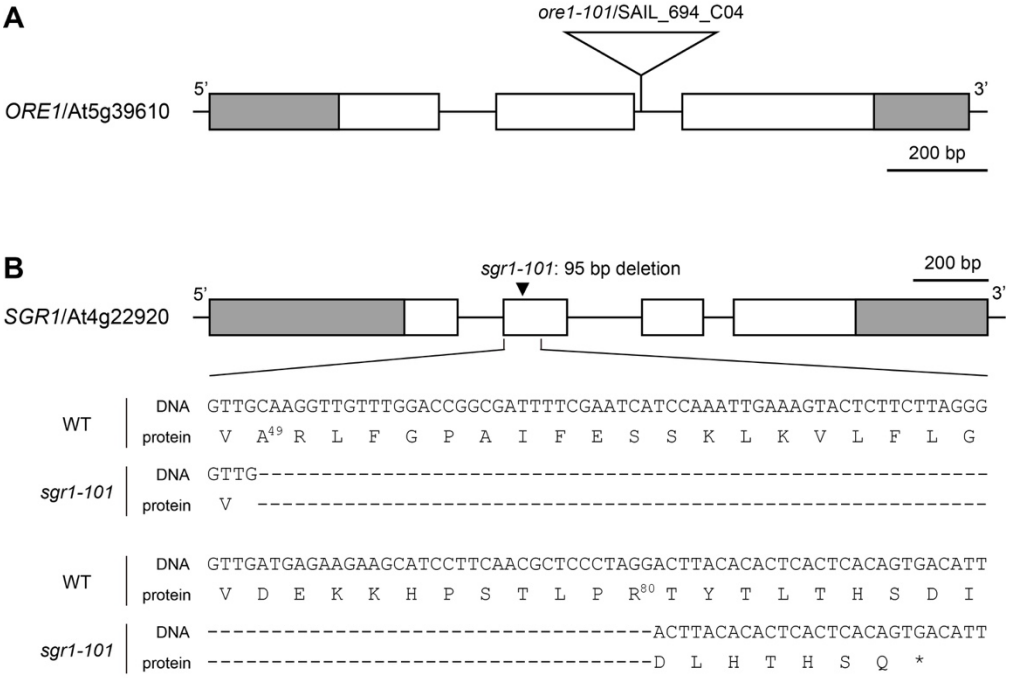
A, Phenotypes of soil grown 4-week-old WT, *pARF2:ARF2-GFP* OEs in the WT background, *pif5-10*, and *pARF2:ARF2-GFP* OEs in the *pif5-10* background.

B, Phenotypes of soil grown 4-week-old WT, *pARF2:ARF2-GFP* OEs in the WT background, *pif4-2 pif5-10*, and *pARF2:ARF2-GFP* OEs in the *pif4-2 pif5-10* background.

C, Phenotypes of soil grown 4-week-old WT, *pPIF5:PIF5-GFP* OEs in the WT background, *arf2-20*, and *pPIF5:PIF5-GFP* OEs in the *arf2-20* background.

D, Phenotypes of soil grown 23-day-old plants of the same genotypes as shown in C.

Appendix Figure S4



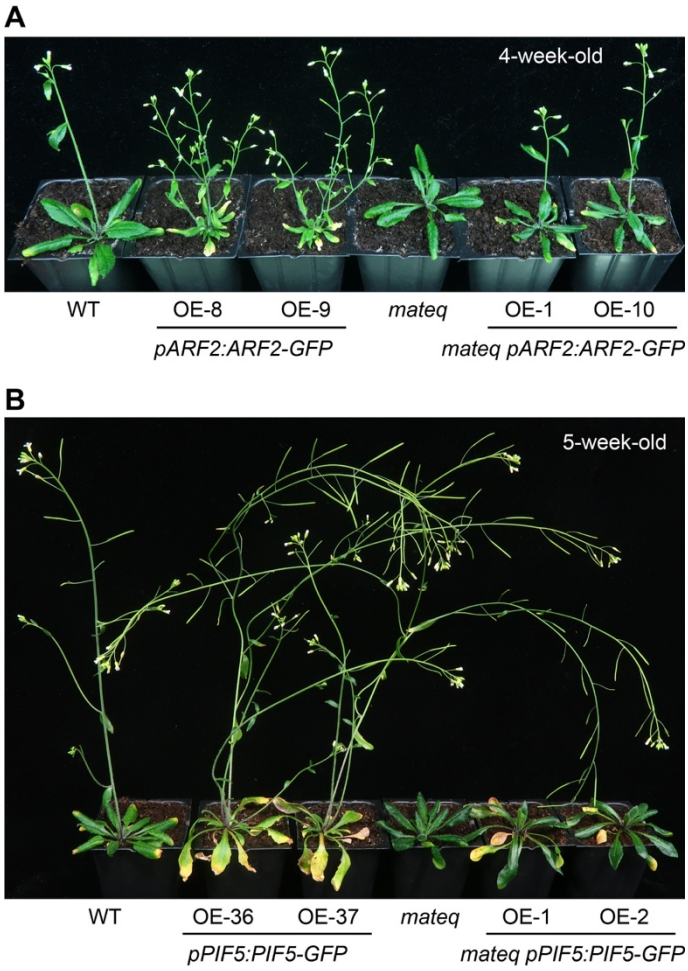
Appendix Figure S4. Supporting data for Figure EV5.

A, Schematic diagram showing the T-DNA insertion site in *ore1-101*.

B, Schematic diagram showing the deletion mutation in *sgr1-101*.

In gene models, boxes and lines represent exons and introns, respectively. 5'- and 3'-untranslated regions are shaded.

Appendix Figure S5

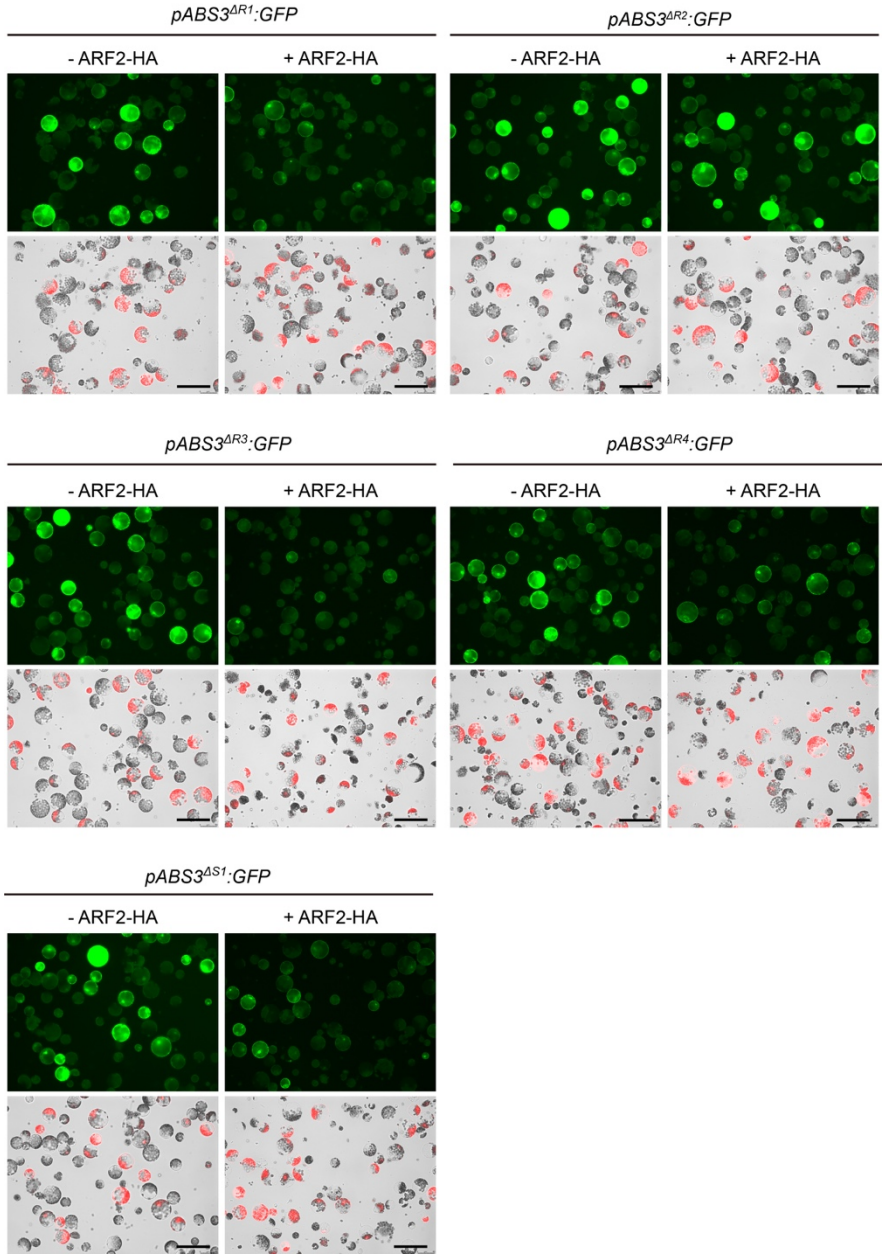


Appendix Figure S5. Supporting data for Figure 6.

A, Phenotypes of soil grown 4-week-old WT, *pARF2:ARF2-GFP* OEs in the WT background, *mateq*, and *pARF2:ARF2-GFP* OEs in the *mateq* background.

B, Phenotypes of soil grown 5-week-old WT, *pPIF5:PIF5-GFP* OEs in the WT background, *mateq*, and *pPIF5:PIF5-GFP* OEs in the *mateq* background.

Appendix Figure S6



Appendix Figure S6. Supporting data for Figure 7.

WT and mutant forms of *pABS3:GFP* were expressed in WT mesophyll protoplasts with or without *p35S:ARF2-HA*. *p35S:mCherry* was co-transfected and served as a transfection control. Protoplasts were examined with fluorescence microscopy. Experiments were independently repeated 3 times with similar results. Bars, 100 μm.

Appendix Table S1. Primers used in this study.

	Primer Name	Primer Sequence (5′ to 3′)
Vector construction		
pUC18-p35S:YN-ARF2	ARF2 BamHI F	CAT GGATCC ATGGCGAGTTCGGAGGTTTC
pGEX 4T-1 GST-ARF2	ARF2 BamHI R	CAT GGATCC gtTTAAGAGTTCCCAGCGCT
pGBKT7-ARF2	ARF2 BamHI F2	CATGGATCCTCATGGCGAGTTCGGAGGTTTC
	ARF2 BamHI R	CAT GGATCC gtTTAAGAGTTCCCAGCGCT
pUC18-p35S:YN-ARF2-N	ARF2 BamHI F	CAT GGATCC ATGGCGAGTTCGGAGGTTTC
	ARF2-N-BamHI-R	CATGGATCCTTAAGCAAGAGCTGGCTCTAC
pUC18-p35S:YN-ARF2-M	ARF2_M-BamHI-F	CATGGATCCATGCCTCCTGCTTTGAGTCCTGT
	ARF2-M-BamHI-R	CATGGATCCTTACAGCTCAGCGACTAACTC
pUC18-p35S:YN-ARF2-C	ARF2_C-BamHI-F	CATGGATCCATGGACAGGCTGTTTGAGTTCAA
	ARF2 BamHI R	CAT GGATCC gtTTAAGAGTTCCCAGCGCT
pCambia1300-pARF2:ARF2-GFP	pARF2 XbaI F	CAT TCTAGA tgaatgaaagagtcgcagcg
	pARF2 BamHI R	CAT GGATCC accttccgaagctcagatct
	ARF2 BamHI F	CAT GGATCC ATGGCGAGTTCGGAGGTTTC
	ARF2 BamHI R1	CAT GGATCC AGAGTTCCCAGCGCTGGACA
HBT95-p35S:ARF2-HA	GB-P35SPPDK-Rv	ggatccacggagcaagggga
	35S-ARF2-F	tccccttgctccgtggatccATGGCGAGTTCGGAGGTTTC
	HBT-ARF2-R	GTCGTATGGGTAAGGCCTaccAGAGTTCCCAGCGCTGGAC
	HBT-HA-GvF	GGTAGGCCTTACCCATACGAC
pUC18-p35S:PIF5-GFP *pUC18-p35S:PIF5-YC*	PIF5 BamHI F	CATGGATCCATGGAACAAGTGTTTGCTGA
	PIF5 BamHI R1	CATGGATCCGCCTATTTTACCCATATGAAG
pGAD7-PIF5	PIF5 EcoRI F	CATGAATTCATGGAACAAGTGTTTGCTGA
	PIF5 BamHI R3	CATGGATCCTCAGCCTATTTTACCCATATG
pMAL-c4X-MBP-PIF5-His	MBP-PIF5-F	GGATTTCAGAATTCGGATCCATGGAACAAGTGTTTGCTGA
	HIS-PIF5-R	cagtggtggtggtggtggtgctcgagGCCTATTTTACCCATATGAAG
	MBP-R	GGATCCGAATTCTGAAATCCTTC
	HIS-F	caccaccaccaccaccactgagatccggctgctaacaaag
pUC18-p35S:PIF5-N-YC	PIF5 BamHI F	CATGGATCCATGGAACAAGTGTTTGCTGA
	PIF5_N XhoI R	CATCTCGAGAGTAGATCCTGACCGTTGGC
	PIF5_C BamHI F	ACTGGATCCATGCGAAGAAGCCGTGCAGCTGA

pUC18-p35S:PIF5-C-YC	PIF5 XhoI R	CATctcgagGCCTATTTTACCCATATGAAG
pCambia1300-pPIF5:PIF5-GFP	PIF5 BamHI pF	CATGGATCCGATCGGTAGGGGTTATGCAC
	PIF5 BamHI R1	CATGGATCCGCCTATTTTACCCATATGAAG
pUC18-p35S:PIF4-GFP	PIF4 XbaI F	CATTCTAGAATGGAACACCAAGGTTGGAG
	PIF4 XbaI R1	CATTCTAGAGTGGTCCAAACGAGAACCGT
pUC18-p35S:PIF4-YC	PIF4 KpnI F	CATGGTACCATGGAACACCAAGGTTGGAG
	PIF4 KpnI R	CATGGTACCCTAGTGGTCCAAACGAGAAC
pGAD7-PIF4	PIF4 EcoRI F	CATGaattCATGGAACACCAAGGTTGGAG
	PIF4 EcoRI R	CATGaattCCTAGTGGTCCAAACGAGAAC
pMAL-c4X-MBP-PIF4-His	PIF4 EcoRI F	CATGaattCATGGAACACCAAGGTTGGAG
	PIF4 XhoI R1	CATctcgagGTGGTCCAAACGAGAACCGT
pCambia1300-pORE1:ORE1-GFP	ORE1 F1	cacgctatgtcttgaaatgg
	ORE1 R1	GAAATTCCAAACGCAATCCA
	1300-ORE1 F1	GGATTGCGTTTGGAATTTCggatccatggtgagcaagggcgag
	1300-ORE1 R1	catttcaagacatagcgtgaagcttggcactggccgtcgttttac
pHEE2A-dual-sgSGR1	SGR1-DT1-F	TGgggatcggtgcagGTTGCAGTTTTAGAGCTAGAAATAGC
	SGR1-DT1-BsF	ATATATGGTCTCGATTGggatcggtgcagGTTGCAGTT
	SGR1-DT1-R	AACTAGGACTTACACACTCACTCAATCTCTTAGTCGACTCTAC
	SGR1-DT1-BsR	ATTATTGGTCTCGAAACTAGGACTTACACACTCACTC
pCambia1300-pSGR1:SGR1-GFP	P1300-SGR1-F1	TAAAACGACGGCCAGTGCCATTCTTACTGCTCTCGTCTTC
	GFP-SGR1-R1	ctcgcccttgctcaccatggatccGAGTTTCTCCGGATTTGG
pUC18-pABS3:GFP	pABS3 F	cataagctttaaacaatggtagcgacgctaag
	pABS3 R	catggatcctgaagagctctctaatgagtatg
pUC18-pABS3$^{\Delta R1}$:GFP	pUC18-pABS3-F	ACGGCCAGTGCCAAGCTTtaaacaatggtagcgacgctaag
	Δ12R	acttaactttggtgctgaatttcttatacaaatag
	Δ12F	gaaattcagcaccaaagttaagtaaatgcaaacac
	GFP-pABS3-R	CCTTGCTCACCATGGATCCtgaagagctctctaatgagtatg
pUC18-pABS3$^{\Delta R2}$:GFP	pUC18-pABS3-F	ACGGCCAGTGCCAAGCTTtaaacaatggtagcgacgctaag

	Δ34R	ctaggtataccagttcaatgtaacgtctaaat
	Δ34F	cattgaactggtatacctagattatttttgctt atcc
	GFP-pABS3-R	CCTTGCTCACCATGGATCCtgaagagctctct aatgagtatg
pUC18-pABS3$^{\Delta R3}$:GFP	pUC18-pABS3-F	ACGGCCAGTGCCAAGCTTtaaacaatggtagc gacgctaag
	Δ56R	ctagctaggtcaagggaacattgtagagcatc caatgg
	Δ56F	ttcccttgacctagctagtagg
	GFP-pABS3-R	CCTTGCTCACCATGGATCCtgaagagctctct aatgagtatg
pUC18-pABS3$^{\Delta R4}$:GFP	pUC18-pABS3-F	ACGGCCAGTGCCAAGCTTtaaacaatggtagc gacgctaag
	Δ78R	tggataaataccaactaaaatcaaatacttac ccaaatg
	Δ78F	tgattttagttggtatttatccatataaagtg acttctaaaag
	GFP-pABS3-R	CCTTGCTCACCATGGATCCtgaagagctctct aatgagtatg
pUC18-pABS3$^{\Delta S1}$:GFP	pUC18-pABS3-F	ACGGCCAGTGCCAAGCTTtaaacaatggtagc gacgctaag
	Δ3R	cttttatctaattcaatgtaacgtctaaataa tttaaag
	Δ3F	cgttacattgaattagataaaagatgtactgt aatattgg
	GFP-pABS3-R	CCTTGCTCACCATGGATCCtgaagagctctct aatgagtatg
pUC18-pABS3$^{\Delta S2}$:GFP	pUC18-pABS3-F	ACGGCCAGTGCCAAGCTTtaaacaatggtagc gacgctaag
	Δ4R	ttatgaattaagatggccaatattacagtaca tc
	Δ4F	atattggccatcttaattcataattttatttt agtatacc
	GFP-pABS3-R	CCTTGCTCACCATGGATCCtgaagagctctct aatgagtatg
Genotyping		
arf2-20	ARF2 dCAPs F2	CGACGAACCAGGCGGCAGTA
	ARF2 dCAPs R2	ctcttgttagctgacaatcc
pif4-2 (SAIL_1288_E07)	SAIL_LB3	TAGCATCTGAATTTCATAACCAATCTCGATAC AC
	43010F1	CACACTGACGAATCTGTATC

	43010R2	gtggctcaccaacCTAGTGG
pif5-10	PIF5 dCAPs F1	CGAGTCATTGCGGCAGGATC
	PIF5 dCAPs R	CCTGACGAAGTGTCAAGTCT
ore1-101 (SAIL_694_C04)	SAIL_LB3	TAGCATCTGAATTTCATAACCAATCTCGATACAC
	ORE1SeqF2	ctaaagaagaacttgatacg
	ORE1R1	GAAATTCCAAACGCAATCCA
sgr1-101	SGR1-seq-F	AGAACCAATCGATTGTTCCC
	SGR1-seq-R	acagagccatgtagatatgt
RT-qPCR		
GAPDH (AT1G13440)	GAPDHqF	TTGGTGACAACAGGTCAAGCA
	GAPDHqR	AAACTTGTCGCTCAATGCAATC
ABS3 (AT4G29140)	29140qF2	CTCGAACCGGACTCTTCCTC
	29140qR2	GGTAGAGGACAAGAGCCGTC
ARF2 (AT5G62000)	ARF2qF	CCTCATCCGAAGGATGCTCAAACG
	ARF2qR	GGAGCCATCAACTCTCCATTGAACTC
PIF5 (AT3G59060)	PIF5qF	CAACTCCAAGTGATGTGGATG
	PIF5qR	CAATTGCATCTGACTTTGCAT
ORE1 (AT4G03280)	ORE1qF2	TCTTCCCCAAACAGCTAAGAACGA
	ORE1qR2	GGCTGGTTCCATTCGGTTAATGTG
SGR1 (AT4G22920)	SGR1qF1	GCGGTGGCCATTTCCTTTTA
	SGR1qR1	AGTTCCCATCTCCATGCACA
PIL1 (AT2G46970)	PIL1qF	TATGCGGACCCTTCAACTTC
	PIL1qR	GGCAACATCGTAGGTGGTCT
YUCCA8 (AT4G28720)	YUC8qF2	GGTCAACGGACCGGTCATCG
	YUC8qR2	GTTCATGGAGACAAGCCGCC
ChIP-qPCR		
PP2A (AT1G13320)	PP2A F2	GAGGCAGAAGTTCGGATAGCAG
	PP2A R2	gtgtgtacCTTTACACAGGGAAG
ORE1 (AT4G03280)	ORE1-ChIP-2F	tagagtgcacatgttgtagcaatg
	ORE1-ChIP-3R	gaacaactttacttaccttcgcc
PIL1 (AT2G46970)	PIL1-ChIP-F	tgaatcacgcggcattcacg
	PIL1-ChIP-R	ccacgtgagcggaaagaacc
YUCCA8 (AT4G28720)	YUC8-CHIP-F	cacagcttaccttcctcatcctc
	YUC8-CHIP-R	ggttagagaagggaagtgatgg
SGR1 (AT4G22920)	SGR1-CHIP-F	cgactgacaactacagtacgtgagttag
	SGR1-CHIP-R	ctgaacacgagagagtgccac
ABS3 (AT4G29140)	ABS3-1F	caaaaagaattttacaaggacattttcgtg
	ABS3-1R	cattgcacgtgaactctcgaatc
	ABS3-2F	ggattcgagagttcacgtgcaatg

	ABS3-2R	ggtcttgaatactttgtaaaatcagtaaagg
	ABS3-3F	cctttactgattttacaaagtattcaagacc
	ABS3-3R	gacaaaacattgtagagcatccaatgg
	ABS3-4F	cctcatatctttcaaccactttttcataag
	ABS3-4R	ctggtggaccatacacaaagatg
	ABS3-5F	catctttgtgtatggtccaccag
	ABS3-5R	cttttagaagtcactttatatggataaatac
	ABS3-U1F	cctgccaactcctttattaagatgg
	ABS3-U1R	gacggtgatgatgatgatggtgttg

Appendix Table S2. Vectors used in this study.

Vector Name	Source
For transient expression in protoplasts	
pUC18-p35S:GFP	Wang *et al*, 2015a
pBS-p35S:mCherry	Wang *et al*, 2015a
pUC18-p35S:NLS-mCherry	Jia *et al*, 2019
pUC18-p35S:PIF5-GFP	This study
pUC18-p35S:PIF4-GFP	This study
HBT95-p35S:ARF2-HA	This study
pUC18-p35S:YN	Jia *et al*, 2019
pUC18-p35S:YC	Jia *et al*, 2019
pUC18-p35S:YN-ARF2	This study
pUC18-p35S:PIF5-YC	This study
pUC18-p35S:PIF4-YC	This study
pUC18-p35S:YN-ARF2-N	This study
pUC18-p35S:YN-ARF2-M	This study
pUC18-p35S:YN-ARF2-C	This study
pUC18-p35S:PIF5-N-YC	This study
pUC18-p35S:PIF5-C-YC	This study
pUC18-pABS3:GFP	This study
pUC18-pABS3$^{\Delta R1}$:GFP	This study
pUC18-pABS3$^{\Delta R2}$:GFP	This study
pUC18-pABS3$^{\Delta R3}$:GFP	This study
pUC18-pABS3$^{\Delta R4}$:GFP	This study
pUC18-pABS3$^{\Delta S1}$:GFP	This study
pUC18-pABS3$^{\Delta S2}$:GFP	This study
For Yeast two hybrid	
pGADT7	630442, Takara Bio
pGBKT7	630443, Takara Bio
pGADT7-PIF5	This study
pGADT7-PIF4	This study
pGBKT7-ARF2	This study
For expression in *E. coli*	
pGEX-4T-1	27-4580-01, GE Healthcare
pGEX-4T-1 GST-ARF2	This study
pET28a-MBP-HIS	Li *et al*, 2021
pMAL-c4X-MBP-PIF5-HIS	This study
pMAL-c4X-MBP-PIF4-HIS	This study

For plant transformation	
pCambia1300-pARF2:ARF2-GFP	This study
pCambia1300-pPIF5:PIF5-GFP	This study
pCambia1300-pORE1:ORE1-GFP	This study
pCambia1300-pSGR1:SGR1-GFP	This study
pHEE2A-dual-sgSGR1	This study