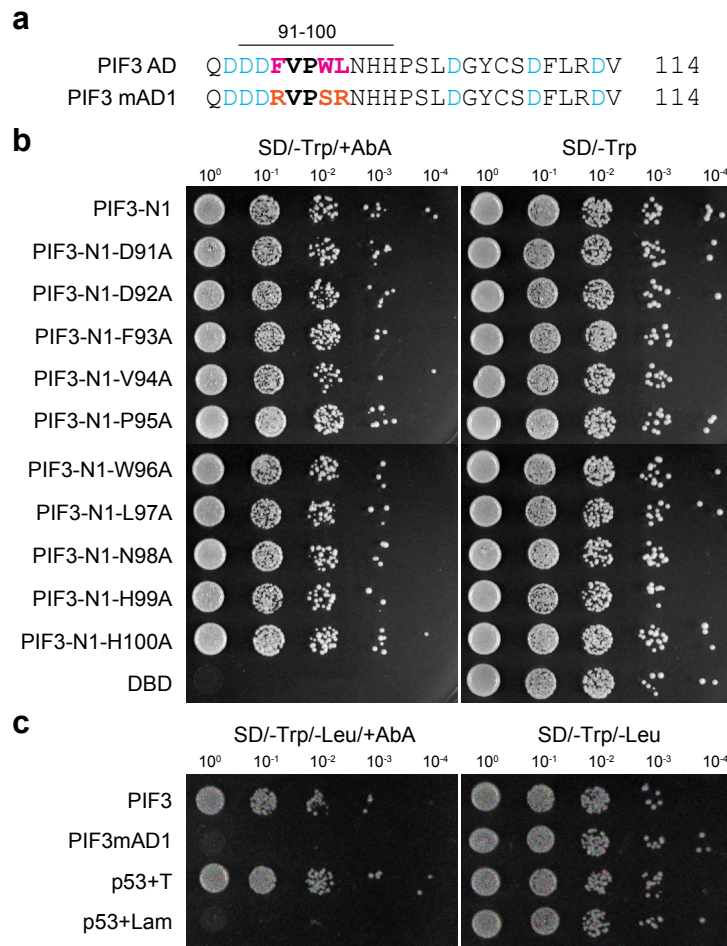
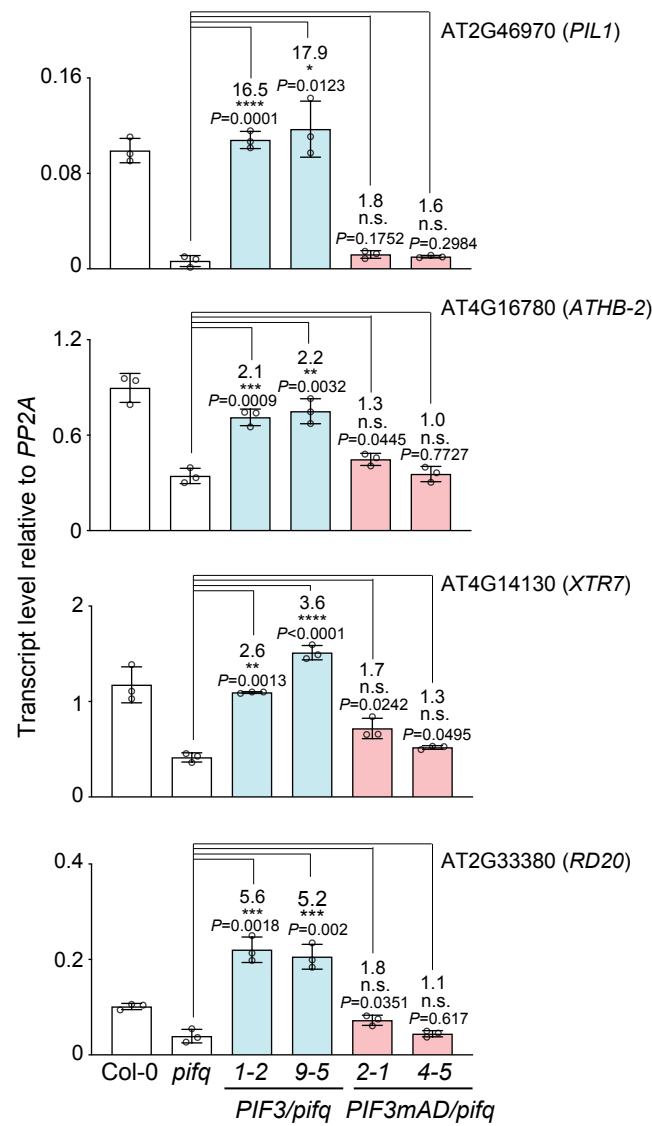


Supplementary Fig. 1. The aa₁₋₉₀ region does not exhibit transactivation activity in yeast. A series of N-terminal fragments of PIF3 between amino acids 1 and 101 were fused with Gal4-DBD as shown in the schematics and examined for their self-activation activity in yeast. Serial dilutions of the yeast strains containing the respective constructs were grown on either SD/-Trp/+AbA or SD/-Trp (control) media.



Supplementary Fig. 2. Abolishing the transactivation activity of PIF3 requires multiple mutations in the AD. **a** Sequence alignment of the PIF3 AD and the mAD1 mutant, in which the three hydrophobic residues in the Φ xx Φ activator motif were replaced with either an arginine or a serine (labeled in orange). The black bar indicates the region of amino acids 91 to 100, containing the conserved activator motif. **b** Yeast transactivation assays of DBD-PIF3-N1 (PIF3-N1) and DBD-PIF3-N1 mutants with individual amino acids between 91 and 100 replaced with an alanine. The yeast strains containing the respective constructs were grown on either SD/-Trp/+AbA or SD/-Trp (control) media. **c** Yeast transactivation assays of DBD-PIF3 (PIF3) and DBD-PIF3mAD1 (PIF3mAD1). Serial dilutions of the yeast strains containing the respective constructs were grown on either SD/-Trp/-Leu/+AbA or SD/-Trp/-Leu (control) media. Yeast strains containing p53 and either the SV40 large T-antigen (p53+T) or lamin (p53+Lam) were used as positive and negative controls, respectively.



Supplementary Fig. 3. *PIF3mAD/pifq* lines exhibit reduced activity in PIF target gene expression in R light. qRT-PCR results showing the steady-state transcript levels of select PIF3 target genes in 4-d-old R-light-grown seedlings of Col-0, *pifq*, and the *PIF3/pifq* and *PIF3mAD/pifq* lines. The transcript levels were calculated relative to those of *PP2A*. Error bars represent the s.d. of three biological replicates. Numbers indicate fold changes relative to *pifq*; the statistical significance was analyzed using two-tailed Student's t-test (** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$); n.s. indicates the difference is either less than 2-fold or not statistically significant.

Supplementary Table 1. PCR primers used for making the constructs of PIF3 and PIF3 paralogs.

	Insert	Vector	Forward primer	Reverse primer
pBridge-PIF3	<i>PIF3</i>	pBridge	acagttgactgtatcgccggctatgcctctgtttgagctttcagg	ggaattagctggctgcaggtcacgacgatccacaaaactg
pBridge-PIF3-N1	<i>PIF3-N1</i>	pBridge	acagttgactgtatcgccggctatgcctctgtttgagctttcagg	ggaattagctggctgcaggtcaacctgcttcttccatc
pBridge-PIF3-N2	<i>PIF3-N2</i>	pBridge	acagttgactgtatcgccggctatgcctctgtttgagctttcagg	ggaattagctggctgcaggttacgggctttgaggactctttc
pBridge-PIF3-N3	<i>PIF3-N3</i>	pBridge	acagttgactgtatcgccggctatgcctctgtttgagctttcagg	ggaattagctggctgcaggttacggattggtttggtgtagg
pBridge-PIF3-N4	<i>PIF3-N4</i>	pBridge	acagttgactgtatcgccggctatgcctctgtttgagctttcagg	ggaattagctggctgcaggttagggatgatgattcaacctg
pBridge-PIF3-N5	<i>PIF3-N5</i>	pBridge	acagttgactgtatcgccggctatgcctctgtttgagctttcagg	ggaattagctggctgcaggtcagggatgatgattcaacct
pBridge-PIF3-N6	<i>PIF3-N6</i>	pBridge	acagttgactgtatcgccggctatgcctctgtttgagctttcagg	ggaattagctggctgcaggtcagcttgactcaaacccgtc
pBridge-PIF3-N7	<i>PIF3-N7</i>	pBridge	acagttgactgtatcgccggctatgcctctgtttgagctttcagg	ggaattagctggctgcaggtcagatctctgaccatagtc
pBridge-PIF3-N8	<i>PIF3-N8</i>	pBridge	acagttgactgtatcgccggctatgcctctgtttgagctttcagg	ggaattagctggctgcaggtcaaggaatgttctctgatcta
pBridge-PIF3-C1	<i>PIF3-C1</i>	pBridge	acagttgactgtatcgccggctatccctatgtcagtccatc	ggaattagctggctgcaggtcacgacgatccacaaaactg
pBridge-PIF3-C2	<i>PIF3-C2</i>	pBridge	acagttgactgtatcgccggctgatgactttgttccatggt	ggaattagctggctgcaggtcacgacgatccacaaaactg
pBridge-PIF3-C3	<i>PIF3-C3</i>	pBridge	acagttgactgtatcgccggctccctccctgatggatatt	ggaattagctggctgcaggtcacgacgatccacaaaactg
pBridge-PIF3-M1	<i>PIF3-M1</i>	pBridge	acagttgactgtatcgccggctatccctatgtcagtccatc	ggaattagctggctgcaggttagggatgatgattcaacctg
pBridge-PIF3-M2	<i>PIF3-M2</i>	pBridge	acagttgactgtatcgccggctgatgactttgttccatggt	ggaattagctggctgcaggttagggatgatgattcaacctg
pET42b-GST-PIF3	<i>PIF3</i>	pET42b	ggcgaattctatgcctctgtttgagctttcag	ccgctcgagcgacgatccacaaaactgatc
pJHA212G-3HA-YFP-PIF3	<i>PIF3pro</i>	pJHA212 G-RBCSt	catgattacgaattcgagctataaaccagaagatgcaac	ggtgtgcttttacagaa
	<i>3HA-YFP</i>		gtaaaacgcaacacatgtaccatacagatgttc	gaaaagctcaaacagaggcataggggtgggagttggtg
	<i>PIF3</i>		atgcctctgtttgagcttt	gcaggtcgactctagagtcacgacgatccacaaaac
pJHA212G-3HA-YFP-PIF3mAD	<i>PIF3pro</i>	pJHA212 G-RBCSt	catgattacgaattcgagctataaaccagaagatgcaac	ggtgtgcttttacagaa
	<i>3HA-YFP</i>		gtaaaacgcaacacatgtaccatacagatgttc	gaaaagctcaaacagaggcataggggtgggagttggtg
	<i>PIF3</i>		atgcctctgtttgagcttt	gcaggtcgactctagagtcacgacgatccacaaaac
pBridge-PIF3/PHYB	<i>PIF3</i>	pBridge	acagttgactgtatcgccggctatgcctctgtttgagctttcagg	ggaattagctggctgcaggtcacgacgatccacaaaactg

pBridge-PIF3/PHYB	<i>PHYB-NLS</i>	pBridge	catccatacaaatgggccatatggtttccggagtcggggg	agatcttcgggctaatagcttacaccttctcttcttaggatatggc atcatcagcat
pBridge-PIF3/PHYB-C	<i>PIF3</i>	pBridge	acagttgactgtatcgccggctatgcctctgtttgagctttcagg	ggaattagctggctgcaggtcacgacgatccacaaaactg
	<i>PHYB-C-NLS</i>		catccatacaaatgggccatatgaactctaaagttgtcgat	agatcttcgggctaatagcttacaccttctcttcttaggatatggc atcatcagcat
pBridge-PIF3/NGB	<i>PIF3</i>	pBridge	acagttgactgtatcgccggctatgcctctgtttgagctttcagg	ggaattagctggctgcaggtcacgacgatccacaaaactg
	<i>NGB</i>		catccatacaaatgggccatatggtttccggagtcggggg	aagatcttcgggctaatagcctatcctccaaccttctc
pBridge-PIF3/PHYA	<i>PIF3</i>	pBridge	acagttgactgtatcgccggctatgcctctgtttgagctttcagg	ggaattagctggctgcaggtcacgacgatccacaaaactg
	<i>PHYA-NLS</i>		catccatacaaatgggccatatgtcaggctctaggccga	agatcttcgggctaatagcctaaaccttctcttcttaggctgtttg ctgcagcgag
pBridge-PIF1/PHYB	<i>PIF1</i>	pBridge	acagttgactgtatcgccggctatgcatcattttgtccctg	ggaattagctggctgcaggttaacctgttggtggttt
	<i>PHYB-NLS</i>		catccatacaaatgggccatatggtttccggagtcggggg	agatcttcgggctaatagcttacaccttctcttcttaggatatggc atcatcagcat
pBridge-PIF1/NGB	<i>PIF1</i>	pBridge	acagttgactgtatcgccggctatgcatcattttgtccctg	ggaattagctggctgcaggttaacctgttggtggttt
	<i>NGB</i>		catccatacaaatgggccatatggtttccggagtcggggg	aagatcttcgggctaatagcctatcctccaaccttctc
pBridge-PIF1/PHYA	<i>PIF1</i>	pBridge	acagttgactgtatcgccggctatgcatcattttgtccctg	ggaattagctggctgcaggttaacctgttggtggttt
	<i>PIF1/PHYA-NLS</i>		catccatacaaatgggccatatgtcaggctctaggccga	agatcttcgggctaatagcctaaaccttctcttcttaggctgtttg ctgcagcgag
pBridge-PIF1	<i>PIF1</i>	pBridge	acagttgactgtatcgccggctatgcatcattttgtccctg	ggaattagctggctgcaggttaacctgttggtggttt
pBridge-PIF4	<i>PIF4</i>	pBridge	acagttgactgtatcgccggctatggaacaccaaggttg	ggaattagctggctgcaggctagtggccaacagaga
pBridge-PIF5	<i>PIF5</i>	pBridge	gactgtatcgccggctatggaacaagtgttgctga	ttagcttgctgcaggtcagcctattttaccat
pBridge-PIF7	<i>PIF7</i>	pBridge	gactgtatcgccggctatgcaattatggagtaa	ttagcttgctgcaggctaatactctttctcatgat
pBridge-PIF8	<i>PIF8</i>	pBridge	gactgtatcgccggctatgtccaatgtgtccaaa	ttagcttgctgcaggctatttggattcgaaggaggag
pBridge-PIL1	<i>PIL1</i>	pBridge	ttgactgtatcgccggctatggaagcaaaccttagc	ttagcttgctgcaggtagttggcgagcgataat
pBridge-PIL2	<i>PIL2</i>	pBridge	gactgtatcgccggctatgatgttcttaccacccga	ttagcttgctgcaggctatctgttagtttctctg

Supplementary Table 2. Primers used for generating the mutant constructs of PIFs and PHYB.

Plasmid name	Gene name	Vector	Forward primer	Reverse primer
pBridge-PIF3-N1m1	<i>PIF3-N1m1</i>	pBridge	gagctggtgtgggaaaatggtc	ccatttcccacaccagctcagcggctgcggcagcaggtggagaagggtcctgtc
pBridge-PIF3-N1m2	<i>PIF3-N1m2</i>	pBridge	aatggtcagatatcaactcaaag	tgagttgatatctgaccattagcggctgcggcagccacaactcatctacaggtgg
pBridge-PIF3-N1m3	<i>PIF3-N1m3</i>	pBridge	actcaaagtcagtcagtagatc	ctactgactgactttgtagtagcggctgcggcagctcccacaccagctccacaac
pBridge-PIF3-N1m4	<i>PIF3-N1m4</i>	pBridge	agtagatcgaggaacattcctc	ggaatgttctcgatctactagcggctgcggcagctgatatctgaccatttcccac
pBridge-PIF3-N1m5	<i>PIF3-N1m5</i>	pBridge	attcctccaccacaagcaaac	ttgcttgggtggaggaatagcggctgcggcagctgactgactttgagttgatatc
pBridge-PIF3-N1m6	<i>PIF3-N1m6</i>	pBridge	gcaaactctctagagctagag	ctagctctagaagagtttcagcggctgcggcagcgttctctgatctactgactg
pBridge-PIF3-N1m7	<i>PIF3-N1m7</i>	pBridge	gctagagagattggaatggc	ccatttcaatctctctagcagcggctgcggcagcttgggtggaggaatgttctc
pBridge-PIF3-N1m8	<i>PIF3-N1m8</i>	pBridge	aatggctcaaagacgactatg	atagtcgtcttgagccattagcggctgcggcagctctagaagagtttctgtgtgg
pBridge-PIF3-N1m9	<i>PIF3-N1m9</i>	pBridge	actatggtggacgagatccc	gggatctcgccaccatagtagcggctgcggcagctccaatctctctagctctagaag
pBridge-PIF3-N1m10	<i>PIF3-N1m10</i>	pBridge	cttcagtggagactgatatgcc	gatggcactgacatagggatagcggctgcggcagccgttcttgaccatttccaatc
pBridge-PIF3-N1m11	<i>PIF3-N1m11</i>	pBridge	ccatcactaatgacgggttg	caaaccctgattagtgatggagcggctgcggcagcctctgcccaccatagctctct
pBridge-PIF3-N1m12	<i>PIF3-N1m12</i>	pBridge	ggtttgagcaagacgatgac	tcctgctctgactcaaacaccagcggctgcggcagccactgacatagggatctctg
pBridge-PIF3-N1m13	<i>PIF3-N1m13</i>	pBridge	gatgactttgtccatggtg	aaccatggaacaaagtcacagcggctgcggcagccgtcattagtgatggcactgac
pBridge-PIF3-N1m14	<i>PIF3-N1m14</i>	pBridge	tggtgaaatcatcatccctcc	gagggatgatgattcaaccaagcggctgcggcagcgtcttgactcaaacctgctcattag
pBridge-PIF3-N1m15	<i>PIF3-N1m15</i>	pBridge	ccctcccttgatggatattgc	caatccatcaagggaggagcggctgcggcagctggaacaaagtcacgtctgtg
pBridge-PIF3-N1m16	<i>PIF3-N1m16</i>	pBridge	tattgctctgattcttgcg	gcaagaaatcagagcaaatatgcagcagcggcggcatgatgattcaaccatgga
pBridge-PIF3-N1m17	<i>PIF3-N1m17</i>	pBridge	ttgctgtagtgctgctc	gacgacacatcacgcaaggcagcagcggcagctccatcaagggaggatg
pBridge-PIF3-N1m18	<i>PIF3-N1m18</i>	pBridge	tcgtctcctgtactgtca	gacagtaacaggagacgacgacgagcggcggaaatcagagcaatatccatc
pBridge-PIF3-N1-D91A	<i>PIF3-N1-D91A</i>	pBridge	agtcaagacgctgactttgtccatggtga	aacaaagtcagcgtctgactcaaacctg

pBridge-PIF3-N1-D92A	<i>PIF3-N1-D92A</i>	pBridge	caagacgatgcctttgtccatggtgaat c	tgaacaaaggcatcgtcttgactcaaacc
pBridge-PIF3-N1-F93A	<i>PIF3-N1-F93A</i>	pBridge	agacgatgacgctgtccatggtgaatc at	ccatggaacagcgcatcgtcttgactcaa
pBridge-PIF3-N1-V94A	<i>PIF3-N1-V94A</i>	pBridge	gatgactttgctccatggtgaatcatcat	caaccatggagcaaagtcacgtcttgactc
pBridge-PIF3-N1-P95A	<i>PIF3-N1-P95A</i>	pBridge	tgactttgtgcatggtgaatcatcatc	attcaaccatgcaacaaagtcacgtcttg
pBridge-PIF3-N1-W96A	<i>PIF3-N1-W96A</i>	pBridge	caagacgatgactttgtccagcattgaa tcatcatccctccctg	caagggagggatgatgattcaatgctggaacaaagtcacgtcttg
pBridge-PIF3-N1-L97A	<i>PIF3-N1-L97A</i>	pBridge	gacgatgactttgtccatgggcaaatca tcatccctccctgatg	catcaagggagggatgatgattgccatggaacaaagtcacgtc
pBridge-PIF3-N1-N98A	<i>PIF3-N1-N98A</i>	pBridge	gatgactttgtccatggtggcacatcat ccctccctgatgg	ccatcaagggagggatgatgccaaccatggaacaaagtcac
pBridge-PIF3-N1-H99A	<i>PIF3-N1-H99A</i>	pBridge	gactttgtccatggtgaatgcacatccct cccttgatggatattg	caatatccatcaagggagggatgtgattcaaccatggaacaaagtc
pBridge-PIF3-N1-H100A	<i>PIF3-N1-H100A</i>	pBridge	ctttgtccatggtgaatcatgcaccctcc cttgatggatattg	caatatccatcaagggagggatgattcaaccatggaacaaag
pBridge-PIF3mAD	<i>PIF3 mAD</i>	pBridge	gctgctgcagcggcgaatcatcatccct ccc	cgccgctgcagcagcgtcatcgtcttgactcaa
pBridge-PIF3/NGB-R110Q	<i>NGB-R110Q</i>	pBridge	aatccagcaaggtggttacattcagcc	tgtaaccacctgctggattcgagagagat
pBridge-PIF3/NGB-G111D	<i>NGB-G111D</i>	pBridge	tccagcgagatggttacattcagcctttc	gaggagccttaagagtagaaccaaccaag
pBridge-PIF3/NGB-R352K	<i>NGB-R352K</i>	pBridge	tctactctaaggctcctcatggttgca	gaggagccttaagagtagaaccaaccaag
pBridge-PIF3mAD1	<i>PIF3mAD1</i>	pBridge	cggttccatctcgtaatcatcatccctccc ttgatgga	atgattacgagatggaacacggcatcgtcttgaca
pBridge-PIF1mAD	<i>PIF1mAD</i>	pBridge	gatgaagctgcggcggctgcgcattatc ctctccgt	gataatgcgagccgccgagcttcatcttctgaata
pBridge-PIF4mAD	<i>PIF4mAD</i>	pBridge	atcaagaagctgcggcggctgcgcaat accctccagatgaag	gtattgcgagccgccgagcttcttgatcttcaagaaag
pBridge-PIF5mAD	<i>PIF5mAD</i>	pBridge	caagaagctgcggcggctgcgcaatac cctccgatgacgtc	agggattgcgagccgccgagcttcttgatcatctag

pBridge-PIF7mAD	<i>PIF7mAD</i>	pBridge	cacctcggctgcggcggctgcgactca aagtctcaacggt	gacttgagtcgcagccgccgcagccgaggtggtggt
pBridge-PIF8mAD	<i>PIF8mAD</i>	pBridge	aggctcggcggctgcgcatcatcgctc ctcca	atgcgcagccgccgcagcctcatccgtgg

Supplementary Table 3. Primers for qRT-PCR analyses.

Accession	Gene name	Forward primer	Reverse primer
AT1G69960	<i>PP2A</i>	TATCGGATGACGATTCTTCGTGCAG	GCTTGGTCGACTATCGAATGAGAG
AT2G46970	<i>PIL1</i>	AAATTGCTCTCAGCCATTCGTGG	TTCTAAGTTTGAGGCGGACGCAG
AT4G16780	<i>ATHB2</i>	TCACAGTACTCTCAATCCGAAGC	CCGTAAGAACTCGCAGTCTAC
AT4G14130	<i>XTR7</i>	CACCGTCACTGCTTACTACTTG	CATTGGTGTGAAGAACATAAG
AT4G32280	<i>IAA29</i>	CACCATCATTGCCCGTATCA	CCACAGTAGCCGTTGTTGGA
AT2G33380	<i>RD20</i>	AAGGACGAAGATGGTTTCCTATC	CGAGAATTGGCCCTCTCTTT
AT4G14690	<i>ELIP2</i>	GGCAGAGGCAAAGTCAAAAGG	CGCAACGAGACCGAGCAT

Supplementary Table 4. Primer sets for ChIP-qPCR analysis of the *PIL1* locus.

Primer set	Forward primer	Reverse primer
1	ATGAATCACGCGGCATTC	ACGTGAGCGGAAAGAACC
2	GGATGAACAATGCACCACCAC	ACACGAAGGCACCACGAATG
3	CTCTATGACAGGAACATCACACC	ACAATGACTTGCCTTGTTTACAG
4	TCGAAGCAAAACCAATCCAAAC	GAATTGTGACCATTCTTTGTTTCAG
5	AGTTGCATTATTGTTGGAGCATC	AAGGTCAGGAAAACACAATGTAG
6	CTGTGTCATAATCACGATTCAAGG	TTGGGGTTAATGAAGAGCAGC