Extended experimental procedures

Plant materials and growth conditions

The wild type (WT) Arabidopsis seedlings used in this study is Columbia-0 ecotype. All ethylene mutants [1, 2], PIF3OX [3], iE/*qm* [4] , *pif1 pif3 pif4 pif5* and *pif3-3* [5] were lab stocked. *pif1* (SALK_131872C) [6], *pif3* (SALK_081927C) [2], *pif3-1* (SALK_030753) [7], *pif4* (SALK_140393C), *pif5* (SALK_087012C) [8], *erf1* (CS481507), *erf6* (SALK_030723), *erf11* (SALK_116053) mutants were acquired from ABRC and identified by PCR genotyping. Double, triple and quadruple mutants were generated by crossing and homozygous lines were genotyping confirmed.

Surface-sterilized seeds were sown on MS medium with half dosage of MS salts and sucrose (2.2g/L MS salt, 0.5% sucrose, PH 5.7, and 8g/L agar) and imbibed for 3d at 4°C in dark.For ethylene treatment, plants were grown on MS medium supplemented with 10 μM ACC. Germination was induced by 6h of irradiation with white light at 22°C before transferring to different growth conditions. Light-grown seedlings were grown under fluorescence white light (about 130μmol $m^{-2}s^{-1}$) with a 12h light/12h dark photoperiod unless indicated. Dark-grown seedlings were exposed to 5-min far-red light (about 10μmol/m^2/s) as previous described before transferring to dark condition [5]. ACC and β-estradiol treatments were conducted as described in previous studies [1, 4]. Hypocotyl lengths were surveyed from the intersection of cotyledon to root by ImageJ software and more than 20 seedlings were measured for each set of experiments.

Generation of transgenic *Arabidopsis* plants

To generate the *PIF3*-promoter: GUS transgenic line used in Figure 2C, about 1.2 kb of the promoter sequence upstream of ATG was cloned into the pBI101.1 vector harboring the GUS reporter gene. The construct was introduced into Col-0 Arabidopsis by Agrobacterium-mediated (GV3101) transformation. Transformants were selected by Kanamycin resistance, and strongly expressing T3 homozygous lines were selected for GUS staining analysis. Introduction of *ctr1* and *ein3 eil1* mutations into the *PIF3*-promoter: GUS transgenic line was conducted by genetic crosses, and the homozygous lines were confirmed by genotyping.

To further examine the regulation of ethylene on PIF3 promoter in Figure 2H, about 3kb of the sequence upstream of the ATG of PIF3 was PCR amplified from Col-0 genomic DNA. The PCR product was cloned into pENTR/SD/D-TOPO vectors (Invitrogen). Then the pENTR-PIF3p was used as the template to produce mutated pENTR-PIF3p (mutations of the three EIN3 binding elements) according to the manufacturer's instruction for the QuickChange Site-Directed Mutagenesis Kit (Stratagene). Finally, the WT and mutated pENTR-*PIF3*p were subcloned into gateway compatible pGWB3 by using LR reaction kit (Invitrogen) to get the binary vector of *PIF3*p-GUS for plants transformation. Full-length cDNA of PIF4, PIF5 and ERF1 without stop codons were amplified by PCR from Col-0 cDNA. The PCR fragments were cloned into pENTR/SD/D-TOPO vectors (Invitrogen) and then subcloned into gateway compatible pGWB17 to generate 35S: 4xMYC fused binary vectors for plants transformation. All the gateway cloned transformants were selected by Kanamycin and Hygromycin resistance [9].

Microscopy

To detect hypocotyl cell elongation, 7-day-old light-grown seedlings were stained by 10μg/mL propidium iodide (PI, Sigma) for 5 min and then observed using a Leica TCS SP2 inverted confocal laser microscope (Leica). The wavelength for excitation was 543nm and band-pass filter 580 to 595nm was used for emission.

RNA extractions and qRT-PCR

Total RNA was extracted using Trizol reagent according to manufacture procedures (Invitrogen). After RNase-Free DNase (Qiagen) treatment, spectrophotometric and gel electrophoretic analysis were performed to detect RNA quality. Two micrograms of RNA was added in a 20μL reaction system using the M-MLV reverse transcriptase (Promega). Products were diluted 4-fold for later analysis. Real-time PCR was performed using SYBR Green Mix (Takara) in an MX3305P machine (Stratagene). The gene expression results were normalized by *UBIQUTIN5* (*UBQ5*).

Protein extraction and immunoblot assays

For each sample, approximately 100 seeds were grown on indicated medium and growth condition. Etiolated seedlings were collected under a dim-green safe light and quickly thrown into liquid nitrogen. Seedlings were ground to fine powder in liquid nitrogen and total protein extracts were subjected to immunoblot with indicated antibody. The assays were performed as previously described and a nonspecific band was used as loading control [1].

Yeast-one-hybrid assays

EIN3 and HY5 full-length coding sequences were amplified by PCR and cloned into the EcoRI-XhoI sites of the pB42AD vector (Clontech). Fragments of PIF3 and EBF2 promoters were amplified and cloned into the KpnI-XhoI sites of the pLexA LacZ reporter vector (Clontech). Plasmids of AD-fused protein and LacZ reporter were co-transformed into the EGY48 yeast strain. Yeast transformation and growth were conducted by the Yeast Protocols Handbook (Clontech). Transformants were transferred to grown on proper drop-out medium plates containing X-gal for blue color development.

Chromatin immunoprecipitations and DNA gel-shift assays

Five-day-old white light-grown seedlings were treated with 10μM β-estradiol for inducement or DMSO as control in the same growth condition for 4h. Chromatin immunoprecipitation assays were performed as described [10], using anti-FLAG monoclonal antibody (Sigma) for immunoprecipitation. Sonicated input DNA (1%) was used for quantitative control, and the precipitated DNA without antibody as negative control in PCR amplification.

DNA gel-shift assay was performed using 3' end biotin-labeled probes and Lightshift Chemiluminescent EMSA kit (Pierce). Single chain probes were annealed with equal anti-chain in buffer (10mM Tris-HCl, 1mM EDTA, 50mM NaCl, PH=8.0). Labeled probes were diluted to 10nM and un-labeled cold probes to 2μM for reactions. EMSA assay were carried out using recombinant EIN3 protein (1-314aa) purified from bacteria and the biotin-labeled probes were synthesized by IDT (US).

Supplementary Figures and Table

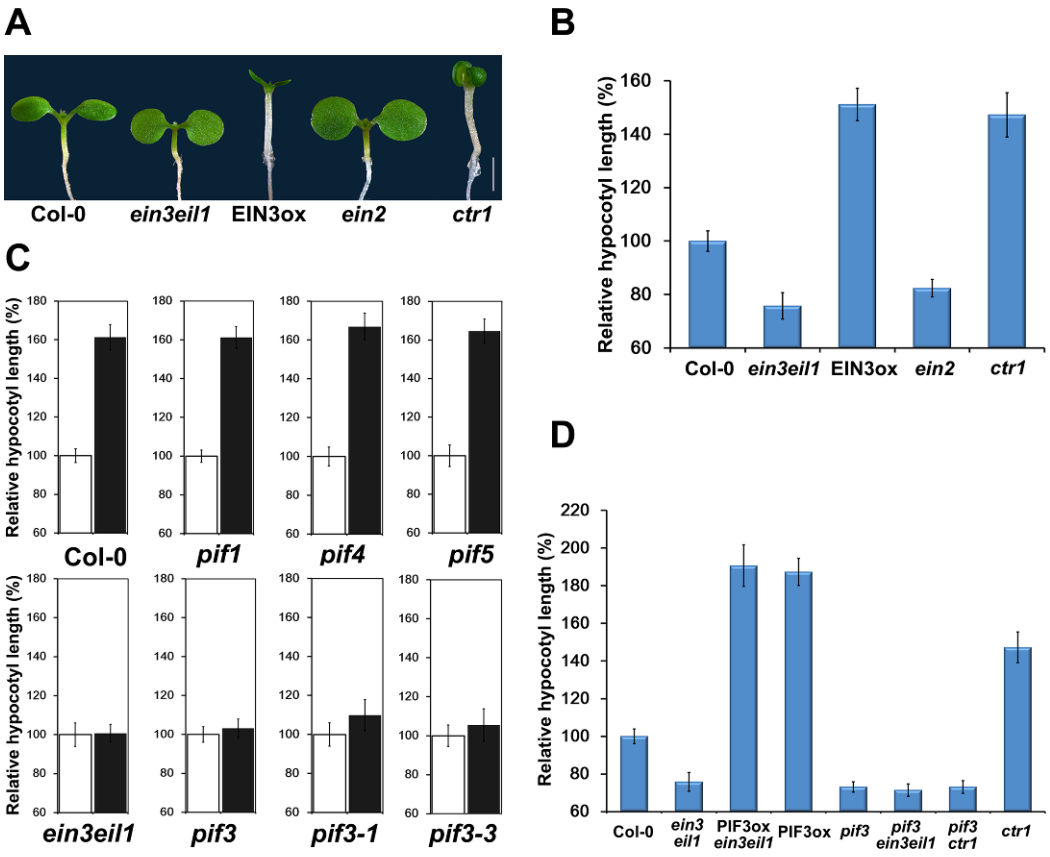

Figure S1. EIN3/EIL1 and PIF3 are required for ethylene-induced hypocotyl elongation in light, related to Figure 1.

(A-B) Hypocotyl phenotype (A) and lengths (B) of 5-day-old light-grown seedlings on MS medium. Mean ±s.d., n>20.

(C) Hypocotyl lengths of 5-day-old light-grown seedlings on MS medium supplemented without (white) or with (black) 10μM ACC. Mean ±s.d., n>20.

(D) Hypocotyl lengths of 5-day-old light-grown seedlings on MS medium. Mean ±s.d., n>20.

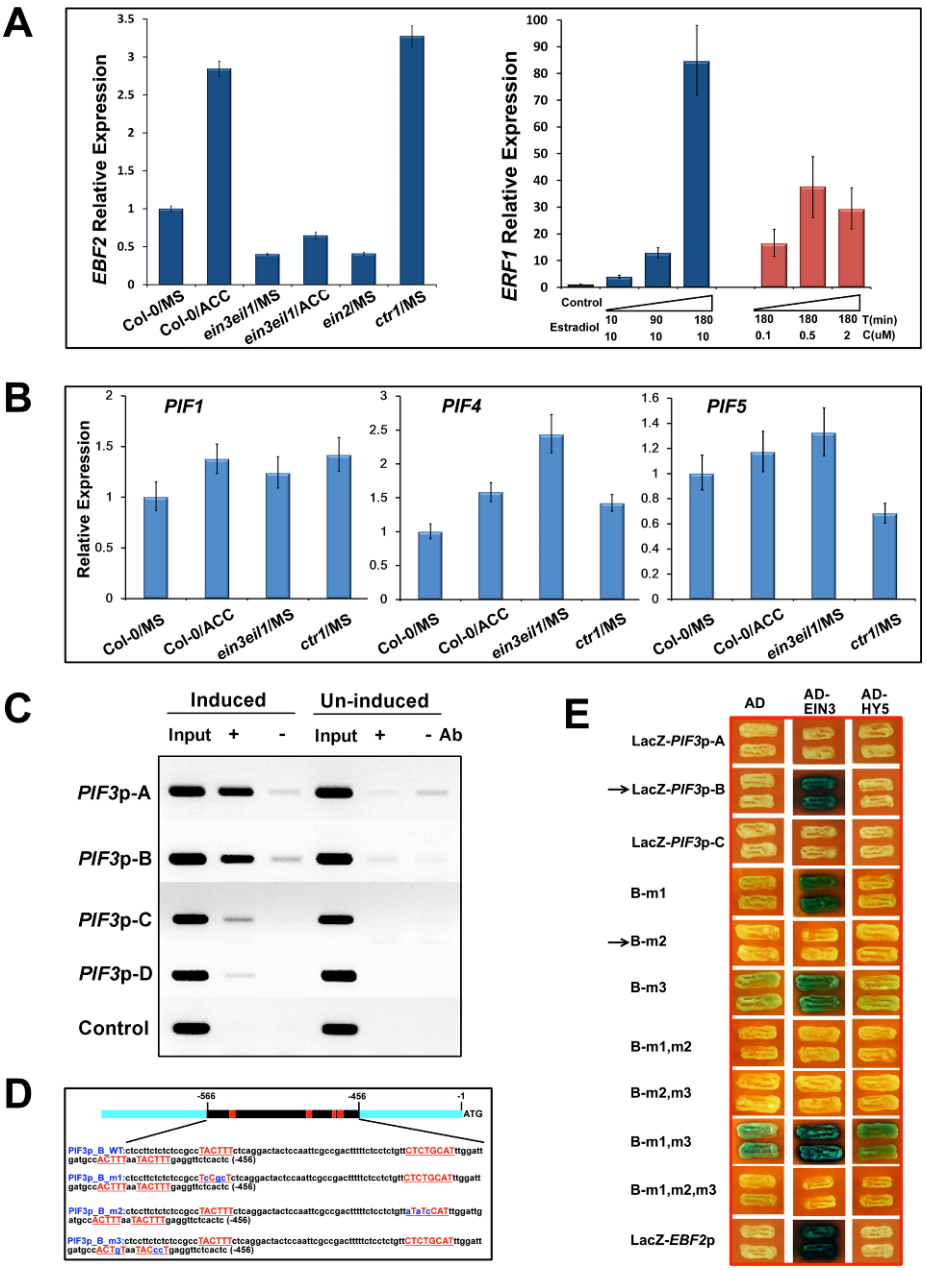

Figure S2. EIN3 directly binds to specific elements of the PIF3 promoter to regulate PIF3 transcription, but not other PIFs, related to Figure 2.

(A) Left panel: quantitative RT-PCR analysis of EBF2 expression in 5-day-old continuous light-grown seedlings on MS with or without 10μM ACC as indicated. Right panel: ERF1 expression in 5-day-old continuous light-grown seedlings with gradually

induced EIN3 protein synthesis. Estradiol-inducible EIN3-FLAG protein in *ein3 eil1 ebf1 ebf2* mutant background (iE/*qm*) seedlings were treated with estradiol gradient of concentrations (C) or treatment time (T).

(B) qRT-PCR analysis of *PIF1*, *PIF4*, and *PIF5* genes expression in 5-day-old continuous light-grown seedlings on MS with or without 10μM ACC as indicated. Mean ±s.d., n=3.

(C) ChIP using anti-FLAG antibody to the induced EIN3-FLAG protein, followed by PCR detection of the four PIF3 promoter regions in 5-day-old continuous light-grown iE/qm seedlings.

(D) The sequences of the PIF3 promoter regions used for Yeast-one-hybrid in (E). Underlined red bases in capitals indicate the predicted EIN3 binding motifs, and the base changes in the mutant sequences are marked in blue and lower case.

(E) Yeast-one-hybrid assays using the same regions as the ChIP-PCR. EBF2 was used as a positive control while AD and AD-HY5 as negative controls.

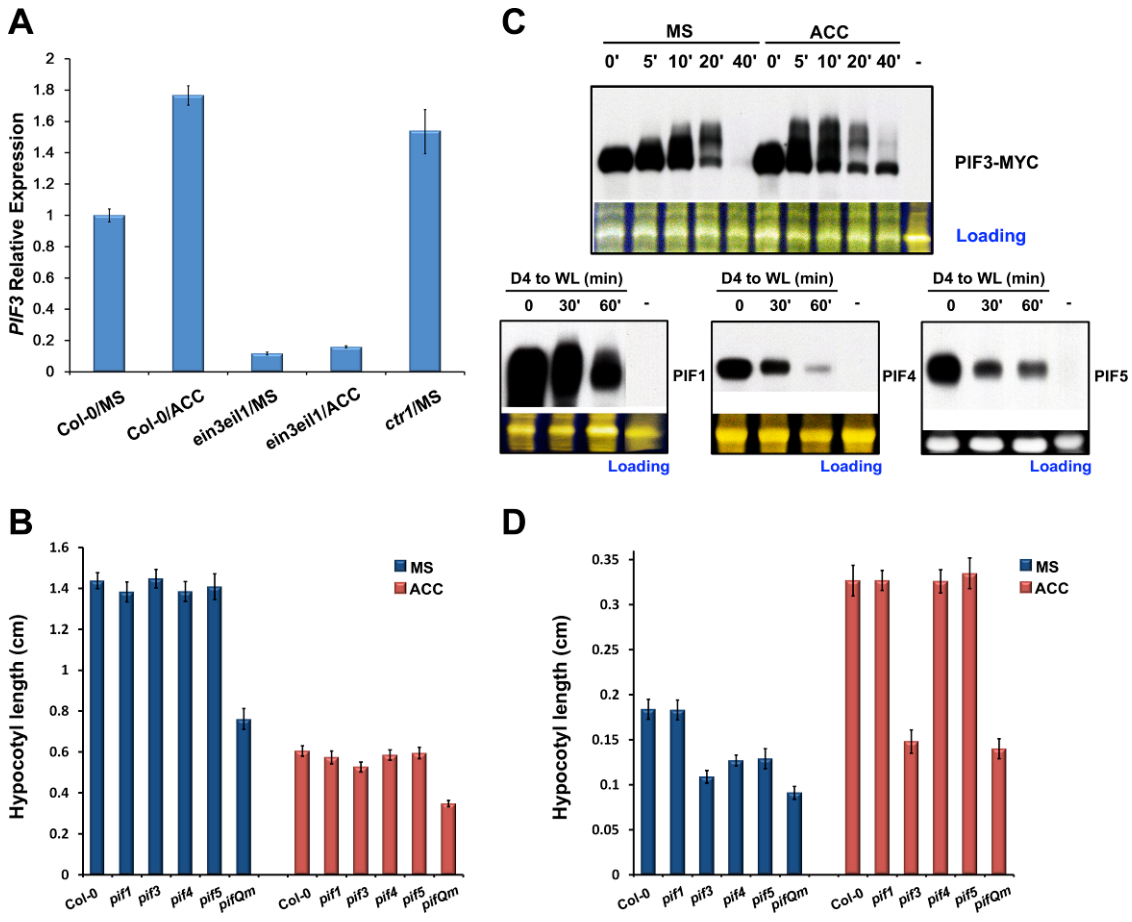

Figure S3. Ethylene-activated PIF3 has an undetectable effect on hypocotyl elongation in darkness, under which other PIFs are abundant and a functional redundancy is present, related to Figure 3.

(A) qRT-PCR analysis of PIF3 gene expression in 4-day-old dark-grown etiolated seedlings on MS±10μM ACC. Mean ±s.d., n=3.

(B and D) Hypocotyl lengths of 5-day-old seedlings grown in darkness (B), or in continues white light (40μmol $m^{-2}s^{-1}$) (D) on MS±10μM ACC. Mean ±s.d., n>20.

(C) Immunoblot assays of PIF proteins (by anti-MYC antibody) in etiolated seedlings constitutively over-expressing individual PIF-MYC proteins grown on MS for 4 days or 3

days on MS followed by 1 day of 10μM ACC treatment (ACC) then transferred into white light.

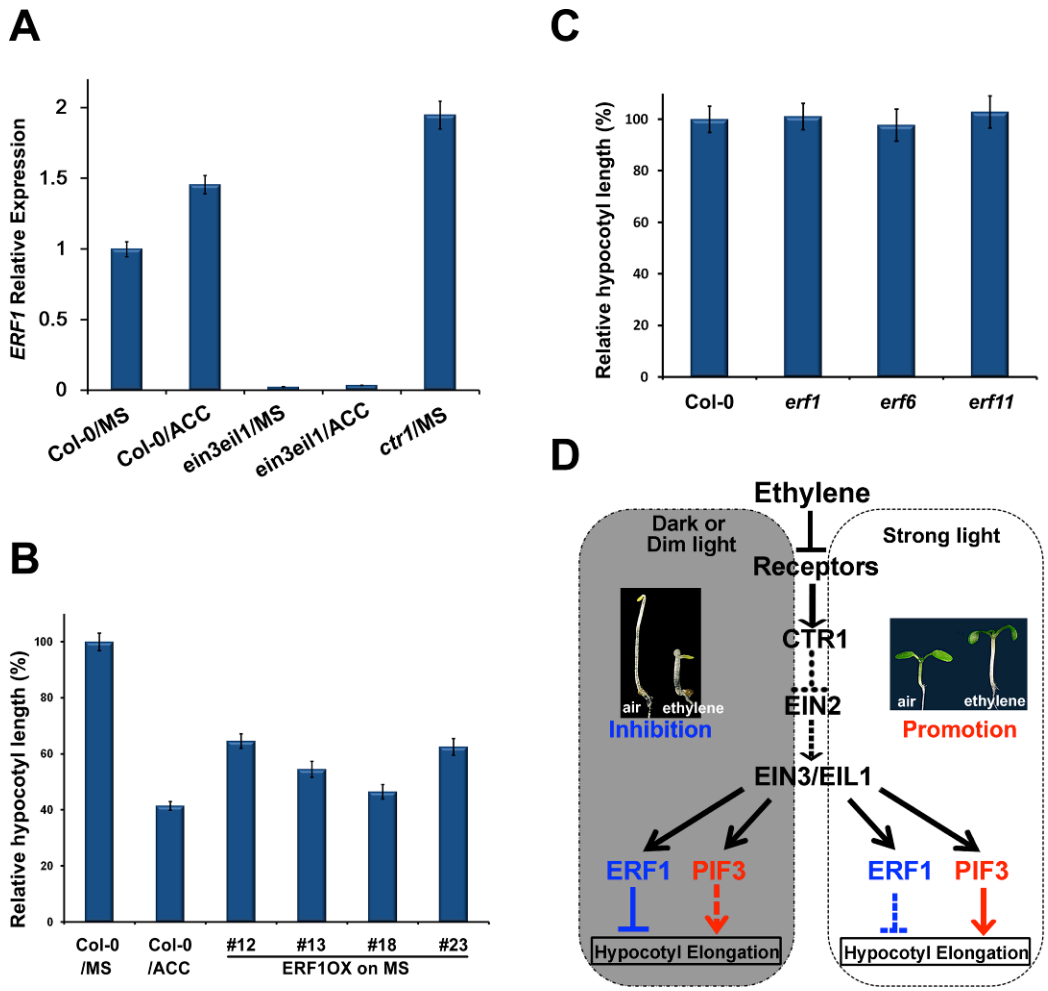

Figure S4. Ethylene concomitantly activates ERF1 and PIF3 pathways in regulating hypocotyl elongation, and light oppositely regulates these two pathways, related to Figure 4.

(A) qRT-PCR analysis of ERF1 gene expression in 4-day-old dark-grown etiolated seedlings on MS±10μM ACC. Mean ±s.d., n=3.

(B) Hypocotyl lengths of 4-day-old light-grown seedlings on MS±10μM ACC. Four individual transgenic lines of ERF1OX were measured. Mean ±s.d., n>20.

(C) Hypocotyl lengths of 5-day-old light-grown seedlings on MS. Mean ±s.d., n>20.

(D) A model illustrating how light reverses the ethylene effect on hypocotyl elongation. Ethylene concomitantly activates two contrary pathways in regulating hypocotyl elongation: a PIF3-mediated promoting pathway and an ERF1-mediated inhibiting pathway. The dark-accumulated PIF proteins are functionally saturated so that the ethylene-activated PIF3 activity is not manifested in dark. On the other hand, light robustly stabilizes ERF1 to saturate ERFs function, which masks the ethylene-activated ERF1 activity in light. The PIF3 and ERF1 pathways seem to operate in parallel and cannot affect each other. As a result, ethylene utilizes the PIF3 pathway to promote hypocotyl elongation in light, whereas takes the ERF1 pathway to suppress hypocotyl elongation in darkness.

Table S1. List of primers used in the study.

Gene expression qPCR primers:

PIF1-F	GATCCTCAGCAGCAACCTTCTTC
PIF1-R	ACCTGCGTAGATTCTCTCACAACC
PIF3-F	CTGAAAGGAGACGGCGTGATAG
PIF3-R	CAGATAGTAACCAGACGCCATTGAC
PIF4-F	CCGAAAGGAGGAGGAGAGATAGG
PIF4-R	CATTCCACTCCCCATCCACATC
PIF5-F	CCAAGTCCAAACCCACAAACAAG
PIF5-R	GCCTCATGCTTAACTGTCTCTTCG
HY5-F	ATCAAGCAGCGAGAGGTCATCAAG
HY5-R	CTCTCTCTTGCTTGCTGAGCTGAAAC
ERF1-F	ACCGCTCCGTGAAGTTAGATAATG

ERF1-R	ATCCTAATCTTTCACCAAGTCCCAC
EBF2-F	CTTTGGTTTAGGTTCGTGATAGTGC
EBF2-R	CGAAGAGTTGTAATGGCGGATTAAG
ACTIN-F	GACGTGACCTTACTGATTACCTC
ACTIN-R	TATCCACATCACACTTCATGATT
UBQ5-F	AAGGTTCAGCGTTTGAGGAAGG
UBQ5-R	TCTTTCTGGTAAACGTAGGTGAGTC

ChIP-PCR primers:

PIF3A-F	TGACTAATCATGTTGCCATTAAACTAAGTG
PIF3A-R	CAGAAACAATCTTTTACGAAGTCTTGATCCTA
PIF3B-F	ATCCTCAGTTACAGACGATTTGGTCC
PIF3B-R	GGTACTGAAGCAATCGAAGTCAGCG
PIF3C-F	CTACTAATAGCCTAAAACCACTACTAATACAACAAC
PIF3C-R	GCGTATGTATTGTTTTATCAAATAGTGTTCATTC
PIF3D-F	GTGTATCACAGGCCCATGTCACTTTAAG
PIF3D-R	CTTCAATTGATATGGTAACTGCTTTCATAAG
Control-F	CCGAAAGGAGGAGGAGAGATAGG
Control-R	CATTCCACTCCCCATCCACATC

ChIP-qPCR primers:

PIF3A-F	CCTCAGTTACAGACGATTTGGTCC
PIF3A-R	GTACTGAAGCAATCGAAGTCAGCG
PIF3B-F	CGTTCTTCAGTGTGATTAAACAATTGGTT
PIF3B-R	GACGAATTAGGCCAAGAAAAACTTGC
PIF3C-F	TCTTATGAAAGCAGTTACCATATCAATTGAAGG
PIF3C-R	TACTATCTATAGAACCATCTCACTGAGATCTTTG
PIF3D-F	GTGTATCACAGGCCCATGTCACTTTAAG
PIF3D-R	CTTCAATTGATATGGTAACTGCTTTCATAAG
Control-F	GGAGTTGTTGATGGTTCCTGACATGG
Control-R	TCTTCGAGCTGGTCATTGTTGTTCTG

Genotype primers:

pif1-LP	CTCTTTTGGATCTTTCTGGGG
pif1-RP	GACTTGCGCACGATAGCTAAC
pif3-LP	AGTCTGTTGCTTCTGCTACGC
pif3-RP	AAGAACCGGCAAAGATACCAC

pif4-LP	AATTCATCATCGGGGATTAGG
pif4-RP	TCGTCGTTTAATAAACACGGC
pif5-LP	CGATTTGTTACCCATGGTTTG
pif5-RP	CCTTGCTCGATTTTTGTTACG
LB1.3	ATTTTGCCGATTTCGGAAC
ein3-1-F-Hae□	GAGCAAGCTAGGAGGAAGAAATGTCTAG
ein3-1-R	TTTAGGCAAACCAAGTTGGATGCCAC
ctr1-1-F	ATTCCTCTTGTGAGACAAGC
ctr1-1-R-EcoR1	CCTTCAATCGCGAGAGACCGAA
eil1-F-440	GATCGTAATGGTCCAGCT
eil1-R-Bam302	ATAAAGGATCCGAGCCACAACCTCTTC
EIN3OX-LP	TCGTGGCTCCAAATTGCATTAC
EIN3OX-RP	GAGGTCACGGATGTGCATTAAA
EIN3OX-RB	CTCTGTATGAACTGTTCGCCAG

EMSA primers:

EMSA-PIF3p-B1-F	CTGTTCTCTGCATTTGGATTGATGCCACTTTAATACTTTGAGGTTC
EMSA-PIF3p-B1-R	GAACCTCAAAGTATTAAAGTGGCATCAATCCAAATGCAGAGAACAG
EMSA-PIF3p-B2-F	TCTCCATCTCCTTCTCTCTCCGCCTACTTTCTCAGGACTACTC
EMSA-PIF3p-B2-R	GAGTAGTCCTGAGAAAGTAGGCGGAGAGAGAAGGAGATGGAGA
EMSA-PIF3p-B-C1-F	CTGTTCTCTGCATTTGGATTGATGCCACTTTAATACTTTGAGGTTC
EMSA-PIF3p-B-C1-R	GAACCTCAAAGTATTAAAGTGGCATCAATCCAAATGCAGAGAACAG
EMSA-PIF3p-B-C2-F	CTGTTCTCTGCATTTGG
EMSA-PIF3p-B-C2-R	CCAAATGCAGAGAACAG
EMSA-PIF3p-B-C3-F	ATTGATGCCACTTTAATACTTTGAGGTTC
EMSA-PIF3p-B-C3-R	GAACCTCAAAGTATTAAAGTGGCATCAAT
EMSA-PIF3p-B-C4-F	CTGTTATATGCATTTGG
EMSA-PIF3p-B-C4-R	CCAAATGCATATAACAG
EMSA-PIF3p-B-C5-F	ATTGATGCCACTTTAACGAGCAGAGGTTC
EMSA-PIF3p-B-C5-R	GAACCTCTGCTCGTTAAAGTGGCATCAAT
EMSA-PIF3p-B-C6-F	ATTGATGCCGAGACAACGAGCAGAGGTTC
EMSA-PIF3p-B-C6-R	GAACCTCTGCTCGTTGTCTCGGCATCAAT
EMSA-PIF3p-B-C7-F	TCTCCATCTCCTTCTCTCTCCGCCTACTTTCTCAGGACTACTC
EMSA-PIF3p-B-C7-R	GAGTAGTCCTGAGAAAGTAGGCGGAGAGAGAAGGAGATGGAGA
EMSA-PIF3p-B-C8-F	TCTCCATCTCCTTCTCTCT
EMSA-PIF3p-B-C8-R	AGAGAGAAGGAGATGGAGA
EMSA-PIF3p-B-C9-F	CCGCCTACTTTCTCAGGACTACTC
EMSA-PIF3p-B-C9-R	GAGTAGTCCTGAGAAAGTAGGCGG
EMSA-PIF3p-B-C10-F	CCGCCCGATTTCTCAGGACTACTC

EMSA-PIF3p-B-C10-R	GAGTAGTCCTGAGAAATCGGGCGG
EMSA-PIF3p-B-C11-F	CCGCCTACGCACTCAGGACTACTC
EMSA-PIF3p-B-C11-R	GAGTAGTCCTGAGTGCGTAGGCGG

Clone constructing primers:

pBI101.1-PIF3p-GUS-F	GCTCTAGAGCAAAGATCTCAGTGAGATGGTTC
pBI101.1-PIF3p-GUS-R	AGGGGGCCCCCTTGCGTTTTACAGAAACAA
AD-HY5-F	ATGCGAATTCATGCAGGAACAAGCGACTAG
AD-HY5-R	ATGCCTCGAGTCAAAGGCTTGCATCAGCATT
AD-EIN3-F	GAACTCAATTGATGATGTTTAATGAGATGGG
AD-EIN3-R	GAACTCTCGAGTTAGAACCATATGGATACATC
LacZ-PIF3A-F-Kpn1	GGGGTACCTGACTAATCATGTTGCCATTAAACTAAGTG
LacZ-PIF3A-R-Xho1	CGCTCGAGCAGAAACAATCTTTTACGAAGTCTTGATCCTA
LacZ-PIF3B-F-Kpn1	GGGGTACCATCCTCAGTTACAGACGATTTGGTCC
LacZ-PIF3B-R-Xho1	CGCTCGAGGGTACTGAAGCAATCGAAGTCAGCG
LacZ-PIF3C-F-Kpn1	GGGGTACCCTACTAATAGCCTAAAACCACTACTAATACAACAAC
LacZ-PIF3C-R-Xho1	CGCTCGAGGCGTATGTATTGTTTTATCAAATAGTGTTCATTC
LacZ-EBF2F-Kpn1	GGGGTACCCGCAACACTTGCTCTTAATCCTC
LacZ-EBF2R-Xho1	CGCTCGAGCGAAGAAGATTTGAAGAAGAAAGACG
LacZ-PIF3Bm1-F	CATCTCCTTCTCTCTCCGCCTCCGCTCTCAGGACTACTCCAATT
LacZ-PIF3Bm1-R	AATTGGAGTAGTCCTGAGAGCGGAGGCGGAGAGAGAAGGAGATG
LacZ-PIF3Bm2-F	CGCCGACTTTTTCTCCTCTGTTATATCCATTTGGATTGATGCCACTTTA
LacZ-PIF3Bm2-R	TAAAGTGGCATCAATCCAAATGGATATAACAGAGGAGAAAAAGTCGGCG
LacZ-PIF3Bm3-F	CTGTTCTCTGCATTTGGATTGATGCCACTGTAATACCCTGAGGTTCTCACTC
LacZ-PIF3Bm3-R	AGAGTGAGAACCTCAGGGTATTACAGTGGCATCAATCCAAATGCAGAGAA
For the m2m3 and m1m2m3 mutating, using the m3-2 primers.	
LacZ-PIF3Bm3-2-F	CTCTGTTATATCCATTTGGATTGATGCCACTGTAATACCCTGAGGTTCTCAC
LacZ-PIF3Bm3-2-R	GAGAGTGAGAACCTCAGGGTATTACAGTGGCATCAATCCAAATGGATATAA
TOPO-PIF3P-F	CACCAATTGTGGCATATGCGAA
TOPO-PIF3P-R	CATGGTGTTGCGTTTTACAGAAACAATC
For the mutantion of TOPO-PIF3p, using the same method and primers as Y1H cloning.	
TOPO-PIF4-F	TCAGTCGACATGGAACACCAAGGTTGGAGTTTTG
TOPO-PIF4-R	TCAGCGGCCGCGAGTGGTCCAAACGAGAACCGTCG
TOPO-PIF5-F	TCAGTCGACATGGAACAAGTGTTTGCTGATTG
TOPO-PIF5-R	TCAGCGGCCGCGAGCCTATTTTACCCATATGAAGACTG

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