SUPPLEMENTAL DATA:

EXPERIMENTAL PROCEDURES

Plant growth. Wild type was *Arabidopsis thaliana* ecotype Columbia (Col-0). *PIL1* T-DNA lines, *pil1-4* (Salk_043937C) (Roig-Villanova et al., 2006), *pil1-6* (Salk_025598C) and *hfr1-4* (Sessa et al., 2005) were obtained from the ABRC (Alonso et al., 2003). Transgenic lines were generated by transforming plants with *Agrobacterium* harboring constructs of 35S::*PIL1-YFP* and p*PIL1*::*LUC* in the Col-0 background, or 35S::*PIL1-CFP* in the p*PIL1*::*LUC* background. Seeds were germinated on solid agar LS plates without sucrose. After stratification, plates were incubated in growth chambers under continuous white light (30-50 $\mu E \cdot m^{-2} \cdot s^{-1}$, R:FR ratio of 1.2) for 3 days at 22 ℃, then the plates were either left in white light or transferred to simulated shade (LED light, R: 13 $\mu E \cdot m^{-2} \cdot s^{-1}$ and Blue: 1.23 $\mu E \cdot m^{-2} \cdot s^{-1}$; FR light: 20.2 $\mu E \cdot m^{-2} \cdot s^{-1}$, R:FR ratio of 0.7, 22 ℃) for 5 days before hypocotyl measurements were made.

Hypocotyl measurements. Quantitative measurements of hypocotyls were performed on scanned images of seedlings using Scion Image software. For all measurements, at least 16 seedlings were used per treatment or genotype.

LUC activity measurement. 4 mm diameter leaf discs from 3-week-old plants were placed into a 96-well white plate and incubated overnight in 100 µl solution containing 80 µl ½ LS and 20 µl 2.5 mM D-Luciferin with 0.01% Tween 80 under white light. LUC activity in white light was first measured using Gilma microplate luminometer, and then measured after the plate was transferred to shade conditions for 2 hours. Luciferase activity in tobacco transactivation experiment was measured using the Dual-Luciferase reporter assay system (E1980, Promega, San Luis Obispo, CA) according the manufacturer's instructions.

Constructs. To develop the *PIL1* overexpression line, *PIL1* cDNA was PCR-amplified from wild-type total cDNA and cloned into the pCHF3-YFP vector using *Sal* I and *BamH* I sites. *PIL1* 1.5 Kb promoter regions were amplified from wild-type genomic DNA and cloned to pJHA212K vector using *BamH* I and *Nco* I. The *LUC* gene was cloned to the same vector using *Nco* I and *Xba* I to create the p*PIL1::LUC* construct. To over express *PIL1* in p*PIL1::LUC* background, we cloned *PIL1* cDNA into pENTR^{tm}/D-TOPO by BP reaction and into pB7CWG2-CFP by LR reaction. To generate mutant bHLH domain E238D, we used a primer based mutation strategy. For Y2H, *PIL1, PIF4, PIF5* and *PIF7* cDNAs were PCR-amplified from wild-type total cDNA and cloned into the pDBleu and pEXP-AD502 vector using *Sal* I and *Not* I sites. For DNA binding assay, *PIL1* and *HFR1* cDNAs were inserted into pIX-3xHA Gateway vector. All primers are listed in Table S1.

We used a modified Multisite Gateway Pro protocol to construct the Dual LUC assay vectors (Life Technologies, Carlsbad, CA). Transcription factors flanked by attB1 and attB4 recombination sites were cloned into pDONR P1-P4. The 1.5Kb *PIL1* promoter region flanked by attB3 and attB2 was cloned into pDONR P3-P2. The Nos terminator with attB4r and attB3r was cloned into pDONR P4r-P3r. The final vector with effector, reporter and internal control was generated using a multisite Gateway Pro LR recombination reaction including the three entry clones and an attR1, attR2-containing destination vector (pMDC-LRA). The primers used are listed in Table S1.

Immunoblotting. Seedling samples for immunoblots are described in the figure legend and resolved on 4-12% SDS-PAGE gel, followed by a wet transfer to nitrocellulose. The GFP tag was detected by probing the membrane with rat anti-GFP-horseradish peroxidase antibody at a dilution of 1:5000. FLAG was detected with anti-FLAG M2 monoclonal antibody from Sigma (St. Louis, MO). HA was detected with anti-HA tag antibody (BBI) from Sangon

(Shanghai, China). Loading was checked by directly reprobing membranes using a mouse anti-actin antibody at a dilution of 1:5000. Signal was detected using the Thermo scientific ECL kit. MG132 is from Sigma (St. Louis, MO) and working concentration is 50 μM. In Figure 2, the PIL1ox transgenic line was grown in white light on ½ LS plates for 3 days. For the last hour in white light, half of the seedlings were treated with DMSO and half were treated with MG132, and then treated with light as described in figures.

DNA binding assay. DNA binding assays were performed as described (Vert and Chory, 2006) with the following modifications. 3xHA-PIL1 and 3xHA-HFR1 were synthesized i*n vitro* using TNT-T7 wheat germ expression system (Promega, WI) using linearized pIX-3xHA-PIL1 (*Spe* I for linearization) and pIX-3xHA-HFR1 (*Not* I for linearization). Complimentary biotinylated oligos were annealed to generate DNA fragments which was then incubated with Streptavidin Dynabeads M-280 (Invitrogen, CA) for 15 minutes and washed extensively 5 times. The probes used were CCA1: 5'-ttctagtgtatcagaCACGTGtcgacaaactggtgg-3'; PIL1:-1A: 5' acgcggcattCACGTGaagtg CACGTGaacttggcca-3'. For competition assay, proteins were incubated with unlabeled probe (20x labeled probe) before added to DNA-bound Dynabeads.

Protein Pull-Down Assay. For pull-down assay, recombinant GST-PIL1 and GST were expressed in *Escherichia coli* and purified using glutathione beads. 5 days white light grown pFlash-PIF4 and pFlash-PIF7 seedlings were collected in protein extraction buffer (100mM Tris-HCL, 300mM NaCl, 2mM EDTA, 10% glycerol, 1% Triton X-100, pH7.5). Reactions were incubated in at 4 for 2h. The beads were then washed three times with buffer (1XPBS, 0.1% Triton X-100) and eluted samples were analyzed by western blotting using anti-myc (Sigma 9E10) antibody.

Bimolecular Fluorescence Complementation Assay. Full-length coding sequences of PIL1 was cloned into SPYCE and full length coding sequences of PIF4 and 7 were cloned into SPYNE gateway-compatible vectors. For expression in the *Nicotiana benthamiana*, the bacterial suspension was injected into leaves from lower epidermis. Tobacco plants were kept in the greenhouse for at least 36 hours at 22°C to allow the expression of the transfected DNA. All fluorescence observations were made with a Leica microscope. Images in Figure show overlay of fluorescence and bright field.

SUPPLEMENTARY FIGURE AND TABLE

Figure S1. Immunoblot showing PIL1 levels from 4-day-old dark grown *35S::PIL1-YFP#17* seedlings treated with different time of Red light (10uE) or Far red light (10uE). Anti-GFP antibody was used to detect PIL1-YFP. Actin is shown as the loading control.

Figure S2. PIL1 can interact with PIF7 in yeast. pDBLeu-PIF7 as bait was transformed with pEXP-AD502, pEXP-AD502-PIL1and pEXP-AD502-PIF7. pDBLeu as abit was transformed with all pEXP-AD502 constructs. Beta-Gal activity (AU: Arbitrary Unit) was shown from four clones with each combination of plasmids. Error bars represent SD.

Figure S3. Ponceau S stainning of the member used in GST-Pull down assay. Arrows indicates GST-PIL1 and GST.

Figure S4. Endogenous PIL1 and luciferase gene expression levels of Col-0 and Col-0 harboring a *pPIL1::LUC* transgene. Gene expression levels from 5-day-old white light grown seedlings exposed for 1 hr to white light or shade light were measured using quantitative real-time PCR. Values are the average of three replicates and normalized by the expression level of the reference gene AT2G39960. Error bars represent SD.LUC activity fold change after

shade treatment from leaf discs of Col-0 and *pil1-4* mutant plants harboring a *pPIL1::LUC* transgene.

Figure S5. Expression patterns of *PIL1*, *IAA29*, *IAA5*, *YUC8* in Col-0 and *35S::PIL1-YFP#17*. 5-day-old white light grown seedlings were treated by 1 hr shade. Expression levels were quantified using quantitative real-time PCR in triplicates and normalized by the expression level of the reference gene AT2G39960. Error bars represent SD.

Table S1. Primers used in this study.

Figure S1

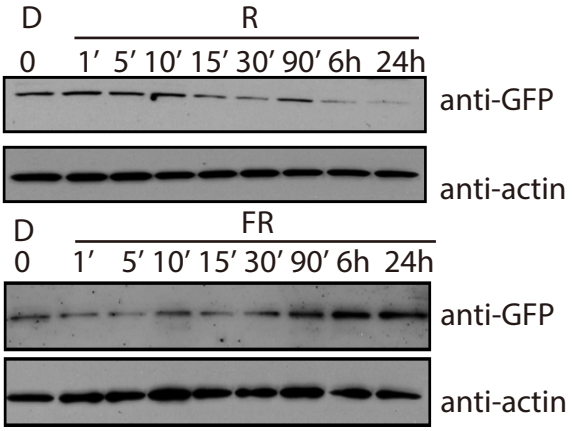


Figure S2

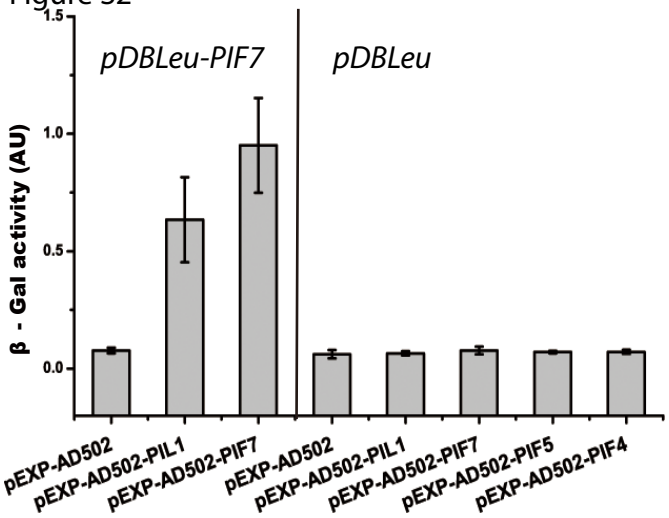
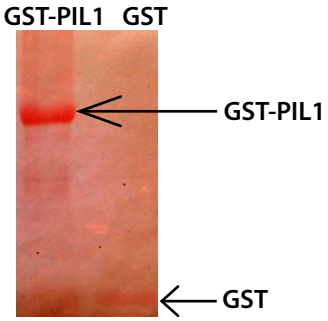


Figure S3



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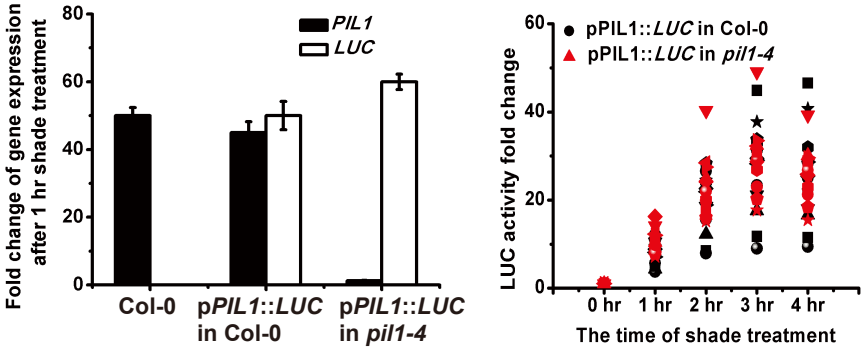

Figure S5

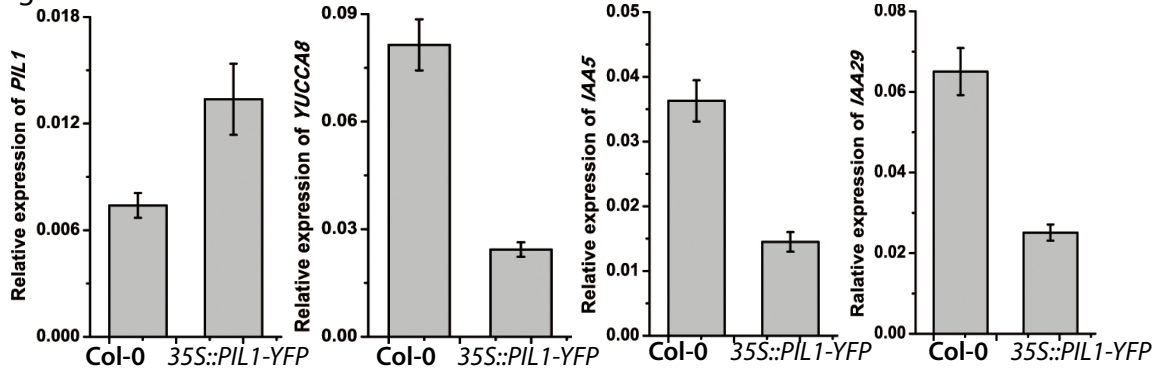

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Table S1. Primers used in this study.

Primer sequence	Note
GGCGGATCC ATGGAAGCAAAACCCTTAGCA	Clone PIL1 to pCHF3-YFP
GGCGTCGAC GTTTGGCGAGCGATAATAAC	Clone PIL1 to pCHF3-YFP
GCGGGATCC GTCTGATCCGTCGTCTGATGAGC	Clone 4 Kb PIL1 promoter
GCGGGTACC GGGTTTTGCTTCCATTGAAG	Clone 4 Kb PIL1 promoter
GCGGGTACCATGGTCACCGACGCCAAAAAC	Clone LUC gene
GCGTCTAGATTACACGGCGATCTTTCCGCC	Clone LUC gene
CACC ATGGAAGCAAAACCCTTAGC	PIL1 Gateway primer
GTTTGGCGAGCGATAATAAC	PIL1 Gateway primer
CACC ATGTCGAATAATCAAGCTTT	HFR1 Gateway primer
TCATAGTCTTCTCATCGCAT	HFR1 Gateway primer
CACC ATGTCGAATTATGGAGTTAA	PIF7 Gateway primer
ATCTCTTTTCTCATGATTCG	PIF7 Gateway primer
CACC ATGGAACACCAAGGTTGGAG	PIF4 Gateway primer
GTGGTCCAAACGAGAACCGT	PIF4 Gateway primer
CACC ATGGAACAAGTGTTTGCTGA	PIF5 Gateway primer
GCCTATTTTACCCATAT	PIF5 Gateway primer
GGGG ACA ACT TTG TAT AAT AAA GTT G GAATTTAGAATGTGTCGAGAG	1.5kb PIL1 promoter
GGGG ACC ACT TTG TAC AAG AAA GCT GGG TA ACCGGGTTTTGCTTCCATTGAAG	1.5kb PIL1 promoter
GGGG ACA AGT TTG TAC AAA AAA GCA GGC T ATGGAAGCAAAACCCTTAGCA	PIL1 for Dual LUC assay
GGGG ACA ACT TTG TAT AGA AAA GTT GGG TGA TTAGTTTGGCGAGCGATAAT	PIL1 for Dual LUC assay
GGGG ACA AGT TTG TAC AAA AAA GCA GGC T ATGGAACAAGTGTTTGCTGA	PIF5 for Dual LUC assay
GGGG ACA ACT TTG TAT AGA AAA GTT GGG TGA TCAGCCTATTTTACCCATAT	PIF5 for Dual LUC assay
TGGACTAATTCCAAACACTCCTATCTT	PIL1 qRT-PCR primer
CACACGAAGGCACCACGA	PIL1 qRT-PCR primer
GGATGGATGGCTACATTCTGGA	LUC qRT-PCR primer
TTCAGGCGGTCAACGATGA	LUC qRT-PCR primer
TGAAACAAAACAACCCACGA	YUCCA8 qRT-PCR primer
TTGATTCGCTTTGGGTCTTC	YUCCA8 qRT-PCR primer
AAGAGTCAAGTTGTGGGTTGGC	IAA5 qRT-PCR primer
AATGCAGCTCCATCTACACTCACT	IAA5 qRT-PCR primer
ACCGTGTGCATATACAAGATGTTTG	IAA29 qRT-PCR primer
TCCGATTTGAACGCCTATCCT	IAA29 qRT-PCR primer
CCATCGACAGTGCTGATCCA	AT2G39960 reference primer

CCATTGGGTGACACTTTTGGT	AT2G39960 reference primer
AAA GTCGAC C ATGGAAGCAAAACCCTTAGC	Clone PIL1 for Y2H
AAA GCGGCCGC TTAGTTTGGCGAGCGATAAT	Clone PIL1 for Y2H
AAA GTCGAC C ATGTCGAATTATGGAGTTAA	Clone PIF7 for Y2H
AAA GCGGCCGC CTAATCTCTTTTCTCATGAT	Clone PIF7 for Y2H
AAA GTCGAC C ATGGAACAAGTGTTTGCTGA	Clone PIF4 for Y2H
AAA GCGGCCGC TCAGCCTATTTTACCCATAT	Clone PIF4 for Y2H
AAA GTCGAC C ATGGAACACCAAGGTTGGAG	Clone PIF5 for Y2H
AAA GCGGCCGC CTAGTGGTCCAAACGAGAAC	Clone PIF5 for Y2H