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Supplemental Information

The ELF3-PIF7 Interaction Mediates the Circadian Gating of the Shade Response in *Arabidopsis*

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Transparent Methods

Plant material and growth conditions. All *Arabidopsis thaliana* plants used in this study were of the Columbia-0 ecotype. The following mutants were used in this study and have been described previously: *pif7-1, PIF7-Flash* (*PIF7ox*), *toc1-2*, *prr5-11*, *cca1-1 lhy*, *elf3-1*, *elf3-7* and *elf3-8*. The *ELF3ox* overexpression line was previously published (Liu et al., 2001). Plants overexpressing *ELF3* were obtained by in planta transformation of Col-0 with a pBI121 vector (Clontech, CA) containing the *35S* promoter and *ELF3* cDNA. *ELF3ox* overexpression was confirmed by immunoblots of total protein with anti-ELF3 antibodies.

For the phenotypic analysis, seeds were germinated on plates that contained 1/2-strength Murashige and Skoog (MS) medium (Duchefa Biochemie, Netherlands) with 1% agar (Sangon, China) but without sucrose. After seed stratification was performed, the plates were incubated in growth chambers under continuous LED white light conditions. The plates were then either left in white light (R: ~25 $\mu mol \times m^{-2} \times sec^{-1}$, B: ~27 $\mu mol \times m^{-2} \times sec^{-1}$, FR: ~5 $\mu mol \times m^{-2} \times sec^{-1}$) or transferred to simulated shade (R: ~25 $\mu mol \times m^{-2} \times sec^{-1}$, B: ~27 $\mu mol \times m^{-2} \times sec^{-1}$, FR: ~50 $\mu mol \times m^{-2} \times sec^{-1}$) before hypocotyl measurements were made. For EOD shade treatment, the plates were either left under short days conditions (8 hours light/16 hours dark) or transferred to shade (R: ~25 $\mu mol \times m^{-2} \times sec^{-1}$, B: ~27 $\mu mol \times m^{-2} \times sec^{-1}$, FR: ~50 $\mu mol \times m^{-2} \times sec^{-1}$) for 2 hours before darkness. At least three independent biological replicates were evaluated for the phenotypic analysis.

Measurement of hypocotyl growth kinetics via DynaPlant. Kinetics of hypocotyl growth was measured by a commercial high-throughput imaging platform, DynaPlant® (Microlens Technology, Beijing, http://www.dynaplant.cn/en). Seedlings for kinetics measurement were sown on 1/2 MS medium containing 2% phytagel (Solarbio, P8170) and grown under SD conditions and then transferred to continuous white light with plates

vertically positioned, after which the shade treatment was started or the seedlings were kept under continuous white light for 2 hours. The images of hypocotyl growth were captured by the DynaPlant® platform once every 10 min for each seedling with the physical resolution of 1.2 μm per pixel. The length of new hypocotyl growth in the time-series images were quantified by DynaPlant Analysis software which was provided by the manufacturer. The values shown indicate the means with SEMs.

Quantitative RT-PCR analysis. Approximately 100 mg of seedlings grown on 1/2-strength MS media supplemented with 1% agar under different light conditions were collected in Eppendorf tubes, frozen in liquid nitrogen, and ground to a fine powder. Total RNA was extracted using a Trizol kit (Promega, USA). One microgram of total RNA was used for reverse transcription using a First Strand cDNA Synthesis Kit (TIANGEN, China) according to the manufacturer's instructions. The cDNAs were then subjected to real-time qPCR using a CFX Connect Real-Time System (Bio-Rad, USA) and SYBR Green qPCR Mix (Mei5 Biochem, China). Three biological replicates per sample were used for qRT-PCR analysis. The data are presented as the means with the SEMs of three biological replicates normalized to the expression of the reference gene *AT2G39960* (Li et al., 2012). The comparative ΔΔCt method was employed to evaluate the relative quantities of each amplified product in the samples. The specificity of the qRT-PCR reactions was determined by melt curve analysis of the amplified products using the standard method of the system. The primers used are listed in Supplemental Table S1.

Firefly luciferase complementation imaging assays. An LCI assay was performed as described previously (Yang et al., 2018). Briefly, the fragments encoding PIF7 were amplified by PCR and ligated into a pCAMBIA2300-nLUC vector to produce nLUC-PIF7. The coding regions of *ELF3* were amplified by

PCR and ligated into pCAMBIA2300-cLUC to produce ELF3-cLUC. The resulting constructs were transformed into *Agrobacterium* strain GV3101. *Agrobacterium* cells harboring different constructs were then infiltrated into *N. benthamiana* leaves. Three days after infiltration, luciferin (Promega, USA) (2.5 mM, 0.1% Triton X-100) was spread before LUC activity was monitored by a Tanon 5500 chemical luminescence imaging system (Tanon, China).

***In vitro* pull-down assays.** To detect the interaction between PIF7 and ELF3, seedlings from ELF3 transgenic plants or from PIF7-Flash transgenic plants were ground in liquid nitrogen and resuspended in extraction buffer (100 mM Tris-HCl [pH 7.5], 300 mM NaCl, 2 mM EDTA [pH 8.0], 1% Triton X-100, 10% glycerol, and protease inhibitor cocktail). The protein extracts were centrifuged at 20,000 x g for 10 min, and the resulting supernatant was incubated with preclarified PIF7-GST/ELF3-GST beads for 1 hour. GST was used as a negative control. The beads were washed in extraction buffer five times, resuspended in SDS-PAGE loading buffer and analyzed by both SDS-PAGE and immunoblotting with anti-ELF3 antibodies or anti-Myc (Sigma-Aldrich, USA). The polyclonal antibodies against ELF3 were produced by GL Biochem (Shanghai, China). The peptide CSIQEERKRYDSSKP was used to immunize rabbits to raise polyclonal antibodies. A protein affinity column was then used to purify the ELF3 antibodies. Western blotting was performed to determine the specificity of the ELF3 antibodies, as shown in Figure 3B.

Co-IP assays. Total protein extracts were prepared from *PIF7oxELF3ox* and *ELF3ox* seedlings. A Co-IP assay was performed as described previously (Peng et al., 2018). IP was performed using an anti-Flag affinity gel (Sigma, USA). Input and IP-resulting fractions were analyzed by Western blots using anti-Myc and anti-ELF3 antibodies. The production of anti-ELF3 antibodies has been published previously (Ding et al., 2018).

ChIP-PCR. ChIP was performed as previously described (Peng et al., 2018). Col-0, *PIF7oxELF3ox* and *ELF3ox* seedlings were grown under white light for 4 days and then treated with 1 hour of shade. The seedlings were then harvested and cross-linked for 15 min under vacuum in cross-linking buffer (extraction buffer 1 with 1% formaldehyde). Cross-linking was stopped with 125 mM glycine (pH 8.0) under vacuum for 5 min, and then seedlings were washed three times in double-distilled water and rapidly frozen. Bioruptor was used at high power with 30 s on/30 s off cycles fifteen times until the average chromatin size was approximately 300 bp. Anti-Flag M2 affinity gel (Sigma, USA) was used for IP. Quantitative real-time PCR was performed with a kit from Takara to determine the enrichment of DNA immunoprecipitated in the ChIP experiments using the gene-specific primers listed in Supplemental Table S1. To facilitate comparisons, fold-change values were obtained from the enrichment data, expressed as the percent input, by using *AT2G39960* as an internal reference gene.

Reference

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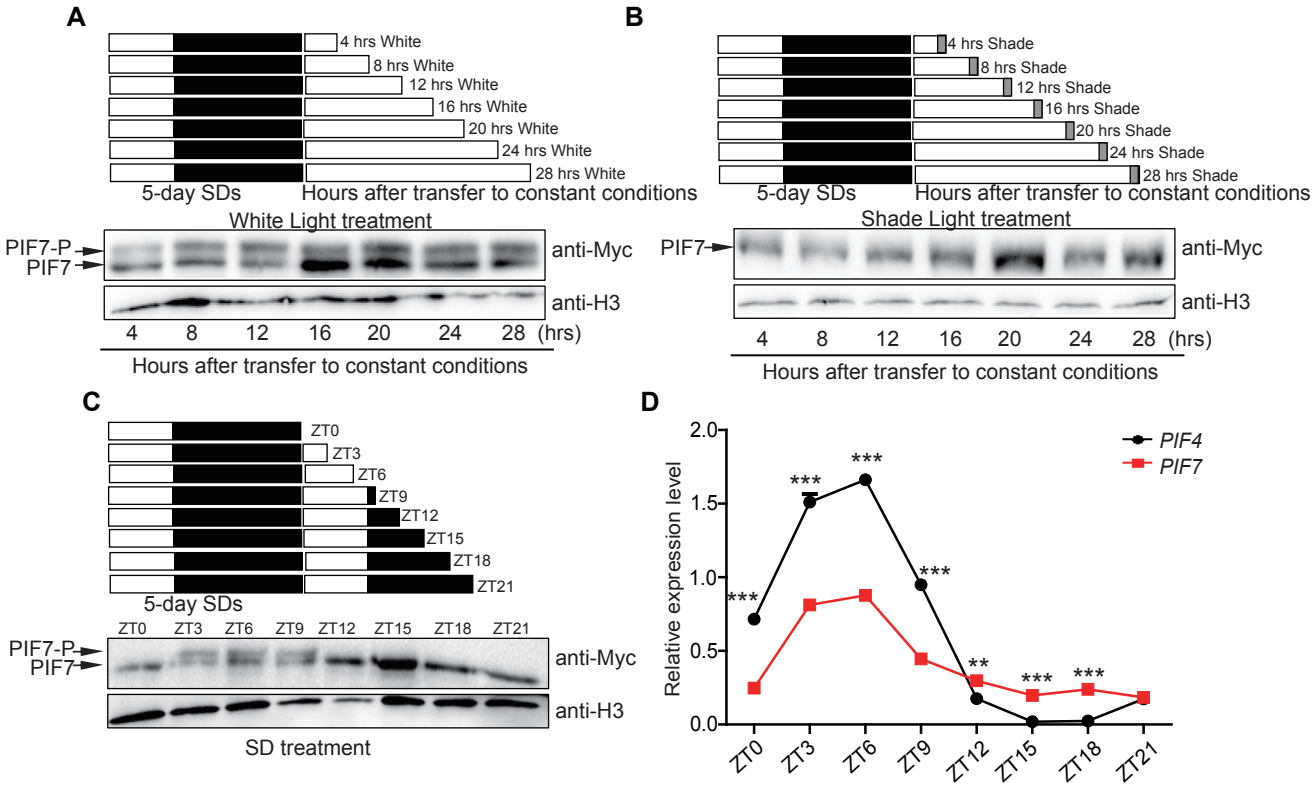

Figure S1. Transcriptional and translational level of *PIF7* at the different time of day with white light or shade treatments, Related to Figure 1.

(A-B) Phosphorylation of PIF7 is regulated by light, not circadian clock. Top panel represents light treatment for the detections of phosphorylation of PIF7. *PIF7ox* seedlings were grown under SD conditions for 5 days and then transferred to continuous white light, after which the continuous white light (A) or the shade treatment (B) was started for 1 hour. The white, black and gray colors represent white light, darkness and shade, respectively. The bottom panels represent the phosphorylation level of PIF7. (C) Phosphorylation and protein level of PIF7 under SDs. Five-day-old SD-grown *PIF7ox* seedlings were kept under SD conditions. The protein level of PIF7 was detected every 3 hours from ZT0 to ZT21 using anti-Myc antibody. (D) The transcriptional level of *PIF4* and *PIF7* under short day. Transcriptional levels of *PIF4* and *PIF7* were detected every 3 hours from ZT0 to ZT21 by qRT-PCR. Data was presented as mean with SEMs of three biological replicates. The asterisk indicates statistically significant differences between mean values according to Student's t-test (** $P < 0.01$, *** $P < 0.001$).

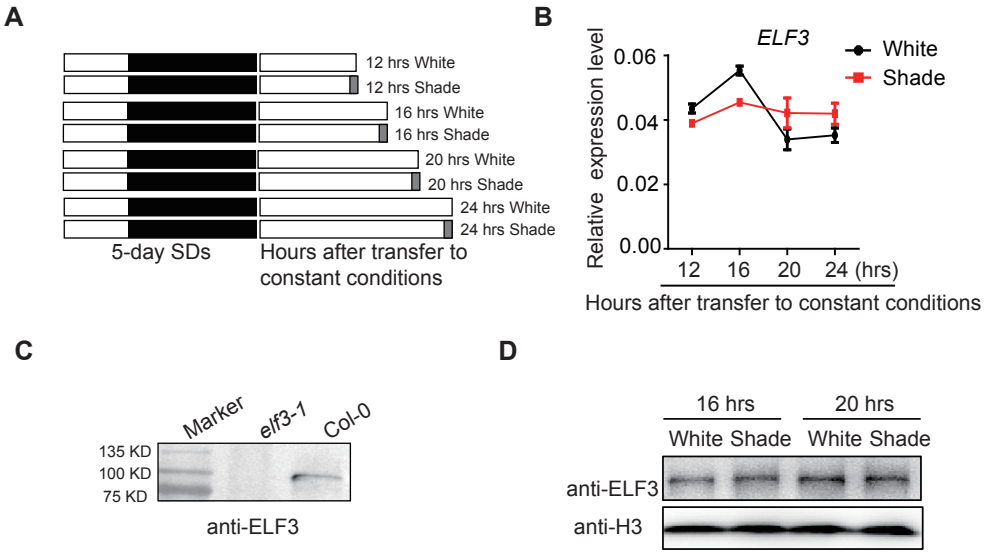

Figure S2. Transcriptional and translational level of *ELF3* after shade treatment, Related to Figure 2.

(A) Light treatment for the detection of shade-induced gene expression and hypocotyl growth. Wild-type seedlings were grown under SDs for 5 days and then transferred to continuous white light, after which the shade treatment was started or the seedlings were kept under continuous white light for 1 hour. The white, black and gray colors represent white light, darkness and shade, respectively. (B) Transcriptional level of *ELF3* after shade treatment. The error bars indicate the SEMs of three independent studies. (C) Detection the specificity of anti-ELF3 antibody. Seedlings were grown for 5 days under white light, the same number of seedlings were taken, total protein was extracted, and the content of the target protein ELF3 was detected by anti-ELF3 antibody. (D) Translational level of ELF3 at 16 hours and 20 hours after transfer to constant conditions. Equal loading of samples is shown by anti-H3 antibodies.

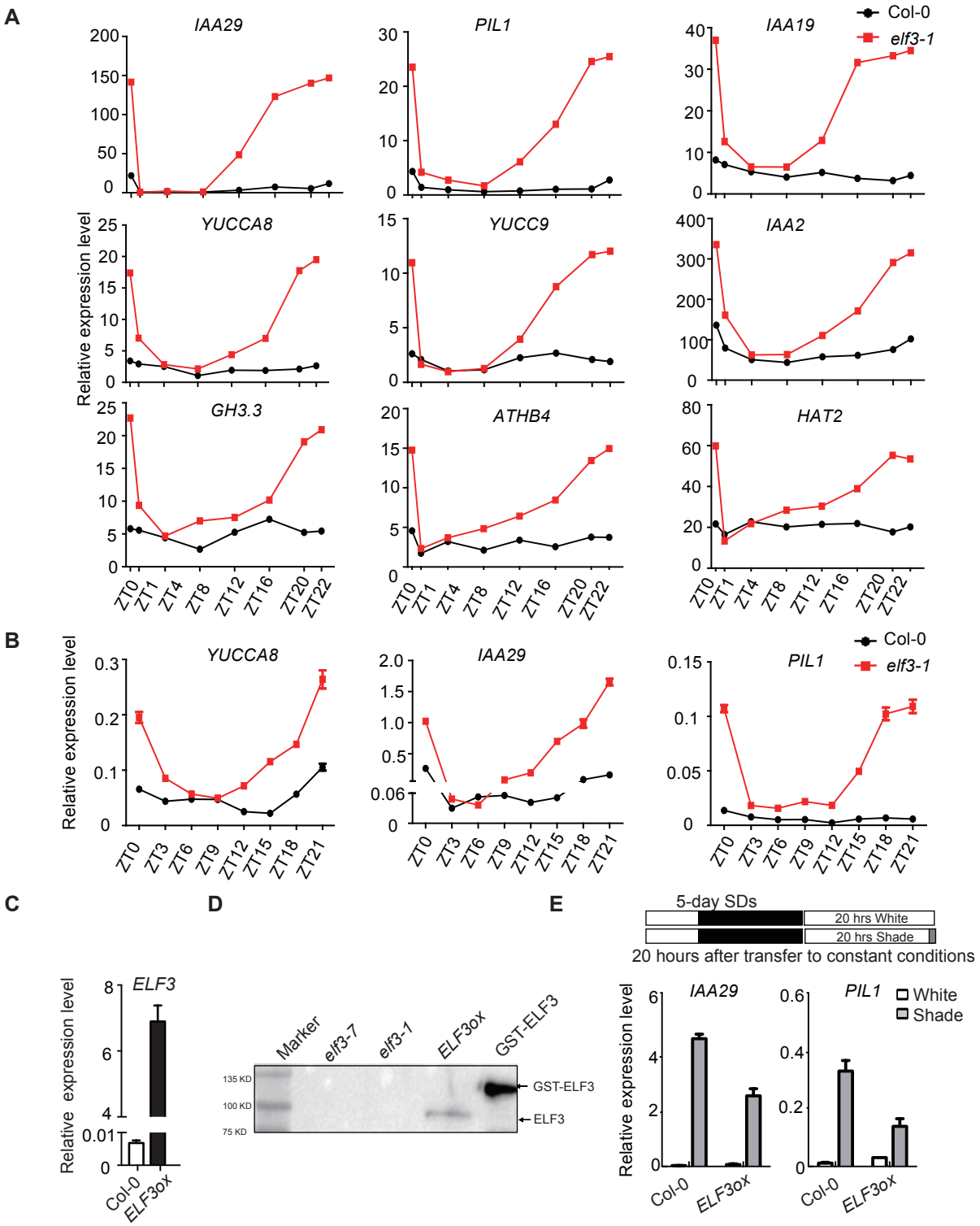

Figure S3. Effect of ELF3 on the expression level of PIF7's targets, Related to Figure 2.
(A) Transcriptional level of *IAA29*, *PIL1*, *IAA19*, *YUCCA8*, *YUCCA9*, *IAA2*, *GH3.3*, *ATHB4,* and *HAT2* from published RNA-sequencing data of *elf3-1 (Ezer et al., 2017)*. (B) Transcriptional levels of *YUCCA8, IAA29 a*nd *PIL1* were confirmed by qRT-PCR. Five-day-old SD-grown wild-type seedlings were collected every 3 hours from ZT0 to ZT21. The error bars indicate the SEMs of three independent studies. (C) Relative transcriptional level of *ELF3* in Col-0 and *ELF3ox* seedings. Seedlings were grown for 5 days in white light. (D) Relative protein level of ELF3 in *ELF3ox* seedings. Seedlings were grown for 5 days under white light, the same number of seedlings were taken, total protein was extracted, and the content of the target protein ELF3 was detected by anti-ELF3 antibody. ELF3-GST was purified from *E. coli.* (E) Relative expression of *IAA29* and *PIL1* in in Col-0 and *ELF3ox* seedings. Seedlings were grown under SD conditions for 5 days and transferred to continuous white light, and started shade treatment or kept in continuous white light for 1 hour. The white, black and gray colors represent white light, darkness and shade, respectively. The error bars indicate the SEMs of three independent studies.

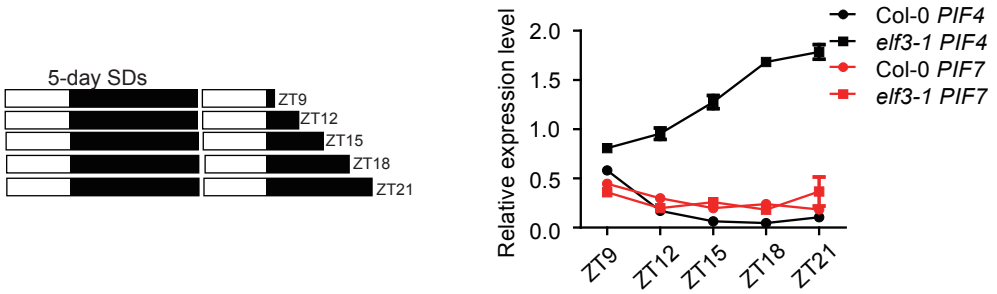

Figure S4. Effect of ELF3 on the expression level of *PIF4* and *PIF7* at the different time of short day, Related to Figure 3.

Transcriptional levels of *PIF4 a*nd *PIF7* were confirmed by qRT-PCR. Five-day-old SD-grown wild-type seedlings were collected every 3 hours from ZT9 to ZT21. The error bars indicate the SEMs of three independent studies.

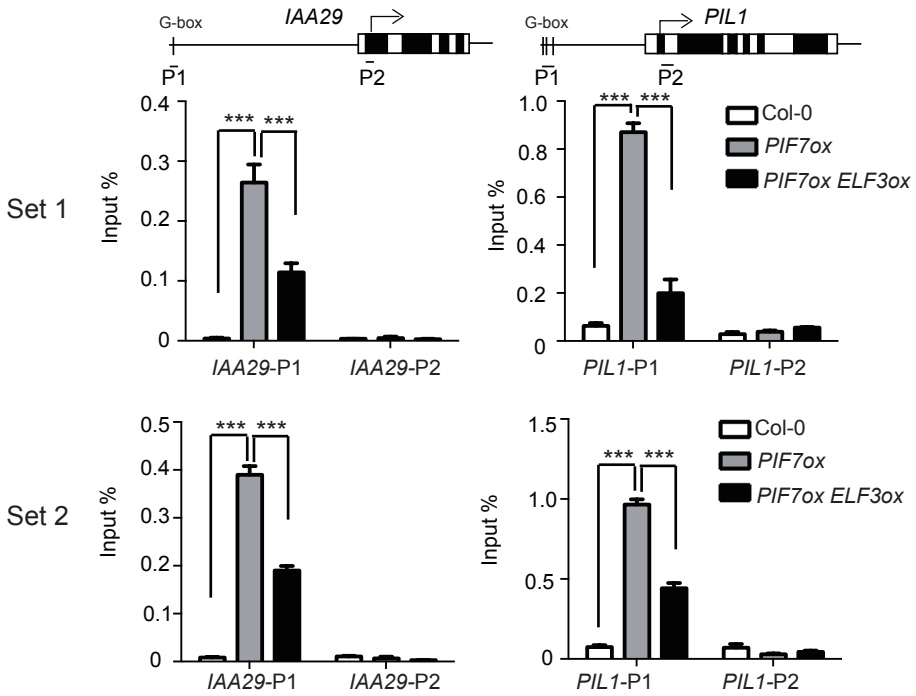

Figure S5. ELF3 suppresses the DNA-binding activity of PIF7 *in vivo*, Related to Figure 4.
ChIP-PCR analysis using anti-Flag agarose at various chromatin regions of *IAA29* and *PIL1* chromatin in Col-0, *PIF7ox*, and *PIF7oxELF3ox* seedlings. ChIP assays were performed via 4-day white light-grown seedlings treated with 1 hour of shade. The top panels show a schematic of the gene structure of *IAA29* and *PIL1*. The back boxes represent coding regions, and the white boxes represent untranslated regions. The G-box within the gene promoter is indicated. The bars labeled with numbers represent regions examined by PCR. The asterisk indicates statistically significant differences between mean values according to Student's t-test (*** $P < 0.001$).

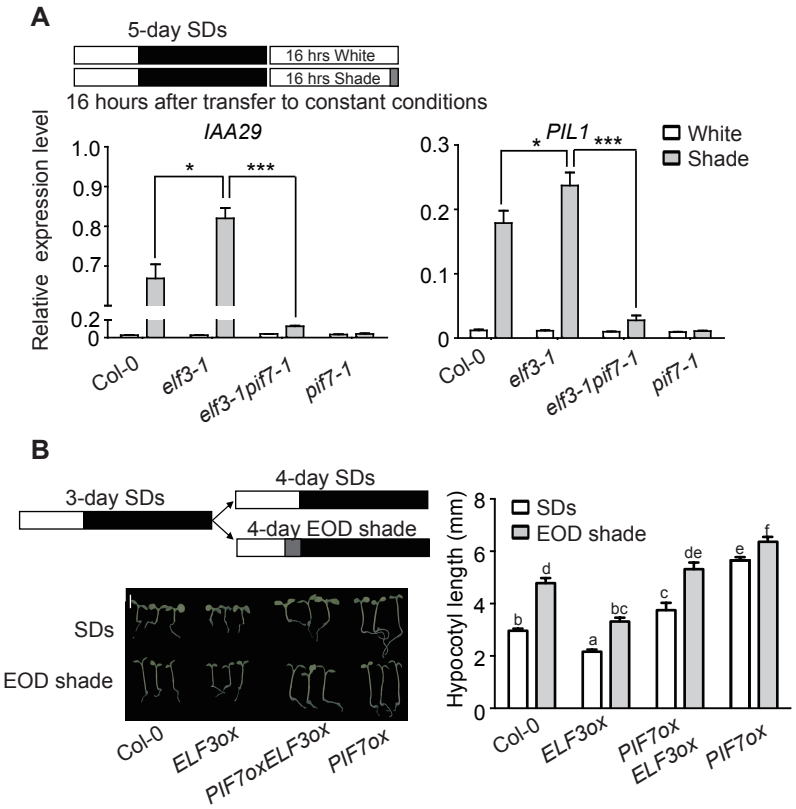

Figure S6. ELF3 acts upstream of PIF7 in shade-induced growth, Related to Figure 5.
(A)Relative expression of *IAA29* and *PIL1* in Col-0, *elf3-1*, *elf3-1pif7-1* and *pif7-1* seedlings at 16 hours after transfer to constant conditions. The top panel represents the light treatment for the detection of shade-induced gene expression. Wild-type seedlings were grown under SD conditions for 5 days and transferred to continuous white light, after which the shade treatment was started or the seedlings were kept under continuous white light for 1 hour. The white, black and gray colors represent white light, darkness and shade, respectively. The bottom panels represent the expression of *IAA29* and *PIL1*. The error bars indicate the SEMs of three independent experiments. The asterisk indicates statistically significant differences between mean values according to Student's t-test (* $P < 0.05$, *** $P < 0.001$). (B) Hypocotyl phenotypes Col-0, *PIF7ox, ELF3ox,* and *ELF3ox-PIF7ox* seedlings under SDs or EOD shade treatment. The top panel represents the light treatment for the hypocotyl measurements of seedlings grown under SDs and EOD shade conditions. The seedlings were grown for 3 days under SDs and either kept under SDs or treated for 2 hours with shade at the end of each day for 4 days. The white, black and gray colors represent white light, darkness and shade, respectively. The bottom panels represent the phenotypes of hypocotyl length. The data are presented as the means with SEMs; more than 20 seedlings were measured. The bars marked with different letters denote significant differences ($P < 0.05$), calculated by Student's t-test. The scale bar represents 2 mm.

Table S1. Primers used in this study, Related to Figure 1, Figure2, Figure3, Figure4, and Figure5.

Primers for genotyping	
pif7-1 LP	CCGTTCATGGTCTAGGCG
pif7-1 RP	CATCCTCTGGTTTATCCTATCACGCCG
elf3-1-F	TGTTGGTCAGTCTTCTCCGA
elf3-1-R	TCCCTACTGTCATTCAAGGG
cca1-1 LP	TGAGATTTCTCCATTTCCGTAGCTTCTGG
cca1-1 RP	ATCCGTTTGGGATCTTTCTGTTCCACATG
lhy LP	CTCTGTTTGGCTGCTGAGAAACTTATAGA
lhy RP	AACCTGACATGACCAAAGAAATGTTCGGA
prr5-11 LP	GTCGGTTTTGTGTTCATATAGA
prr5-11 RP	TCTCAGAAGCATTAGGTCTT
WT *toc1-2*	TCCTTTCAGAGTGTTCTTATCACG
MT *toc1-2*	TCCTTTCAGAGTGTTCTTATCACA
toc1-2 R	TCAAGTTCCCAAAGCATCATC
Primers for clone	
cYFP-ELF3-Kpn1-F	cgg ggtacc ATGAAGAGAGGGAAAGATGAGG
cYFP-ELF3-BamH1-R	ggc ggatcc AAGAGAGGGAAAGATGAGG
nYFP-PIF7-BamH1-F	ggc ggatcc ATGTCGAATTATGGAGTTAA
nYFP-PIF7-Pst1-R	acg ctgcag CTAATCTCTTTTCTCATGAT
nLUC-PIF7-BamH1-F	ggc ggatcc ATGTCGAATTATGGAGTTAA
nLUC-PIF7-SalI-R	aaa gtcgac ATCTCTTTTCTCATGATT
cLUC-ELF3-Kpn1-F	cgg ggtacc ATGAAGAGAGGGAAAGATGAGG
cLUC-ELF3-Sal1-R	aaa gtcgac TTAAGGCTTAGAGGAGTCATAGCG
Pcambia2302-ELF3-Kpn1-F	cgg ggtacc ATGAAGAGAGGGAAAGATGAGG
Pcambia2302-ELF3-BamH1-R	ggc ggatcc AGGCTTAGAGGAGTCATAGCG
pGEX4T-2-ELF3-BamH1-F	ggc ggatcc AAGAGAGGGAAAGATGAGG
pGEX4T-2-ELF3-Sal1-R	acgc gtcgac AGGCTTAGAGGAGTCATAGCG
Primers for qRT-PCR	
YUCCA8-F	TGAAACAAAACAACCCACGA
YUCCA8-R	TTGATTCGCTTTGGGTCTTC
PIL1-F	TGGACTAATTCCAAACACTCCTATCTT
PIL1-R	CACACGAAGGCACCACGA
IAA29-F	TCCGATTTGAACGCCTATCCT
IAA29-R	ACCGTGTGCATATACAAGATGTTTG
Primers for ChIP-PCR	
IAA29-ChIP-1F	GCCATATGGATATGGTCCTTCAAC
IAA29-ChIP-1R	GAAATATCAACGTGAATGTCACGTG
IAA29-ChIP-2F	ATGGAGTTGGATCTTGGTCTATC
IAA29-ChIP-2R	ATTCCCTAACCCAAACGTCG
PIL1-ChIP-1F	TGGATGAATCACGCGGCATT
PIL1-ChIP-1R	GAGCGGAAAGAACCTTCACG
PIL1-ChIP-2F	TGATGTTTCTGCTAAAGGTC
PIL1-ChIP-2R	TTAGATCTCTCGAAGTTCCT