

Supplementary Information for

Central clock components modulate plant shade avoidance by directly repressing transcriptional activation activity of PIF proteins

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Supplementary Information Text

Materials and Methods

Comparative Distribution of DNA Binding Sites

To examine the relative distributions of published DNA-binding sites of other transcription factors (TFs) with those of the PIF quartet proteins within the promoters of our 338 PIF-DTGs, we compared the ChIP-defined binding regions for the '25 TFs' (1) with those of the PIFs (2). For this purpose, we used a BED file, kindly provided by K. Heyndrickx, K. Vandepoele and colleagues, containing the boundaries of genomic regions displaying significant binding of the TF specified. We located these binding regions within the promoters of our 338 PIF-DTGs, using the Integrative Genomics Viewer (3, 4), and compared them to the binding peaks of all four PIFs. To quantify the extent and proximity of the binding sites of the '25 TFs' compared to those of the PIFs, across all PIF-DTG promoter regions, we first determined the overlap (if any) of the binding regions of each of the '25 TFs', with the mean of the PIF-bound summits. If the center of a PIF-bound region was located within a '25-TF' binding region, the binding was defined as 'coincident'. The proximity was then defined as the distance between the coincident-TF and PIF binding centers, and was plotted using Mathematica (<http://www.wolfram.com/mathematica/>).

Plant Materials and Growth Conditions

The wild-type and all mutants of *Arabidopsis thaliana* plants used in this study were of the Columbia-0 (Col-0) ecotype. The *35S::PRR5-FLAG (PRR5ox)* (5) and *35S::HA-PRR7 (PRR7ox)* (6) transgenic over-expression lines, as well as the *prp5prp7 (prp57)* (7), *prp5prp7prp9 (prp579)* (7), *pif1pif3pif4pif5 (pifq)* (8), and *pif7-1 (pif7)* (9) mutant lines, were described previously. To generate the *pifqpif7 (pifq7)* quintuple mutant, *pif3pif7* and *pif4pif7* (9) mutant lines were first crossed to generate the *pif3pif4pif7* mutant, and then this triple mutant was crossed with *pifq* to produce the final configuration. The *pifqpif7prp57 (pifprps)* septuple mutant was generated using the CRISPR-cas9 system, as described (10), in the *pifq7* quintuple background. Two 20-nt genomic fragments, 5'-GCGGAGGAAAGTTATCACGT and 5'-GGATGTTCCGACGAGATTTG from the exons of *PRR5* and *PRR7* respectively, were introduced into the same, single construct for producing the small guide RNA for both genes simultaneously.

Arabidopsis seeds were surface sterilized in 70% ethanol and 0.5% Triton X-100 for 10 minutes followed by rinsing five times with sterile water. The imbibed seeds were plated on GM medium without sucrose, and then kept at 4 °C in darkness for 4-5 days. After stratification, the seeds were transferred into continuous white light (WLC) (PAR ~20 $\mu\text{mol/s/m}^2$) at 21 °C for 3 or 5 days. Young seedlings were then either maintained in WLC, or transferred to simulated vegetative shade, consisting of the same WLC plus supplemental far-red light (red to far-red ratio of 0.3), for 1 or 3 hours, or for an additional 4 days. 7-day-old seedlings were photographically recorded, and hypocotyl lengths were determined using the ImageJ program (<https://imagej.nih.gov/ij/>).

Gene Expression Analysis

100 mg of 5-day-old WLC-grown young seedlings, with or without shade treatment, were ground to fine powder in the liquid nitrogen. Total RNA was extracted using the QIAshredder (Qiagen, 79654), RNase-Free DNase Set (Qiagen, 79254), and RNeasy Plus Mini Kit (Qiagen, 74134). First-strand cDNA was synthesized using the SuperScript IV VILO Master Mix with ezDNase Enzyme (Invitrogen, 11766050). Real-time quantitative PCR (qPCR) was performed as described (11), using a CFX96 real-time PCR detection system (Bio-Rad). The relative gene expression in each genotype was normalized to *PP2AA3 (AT1G13320)* as the internal control. Each PCR reaction was repeated at least twice, and the mean value of these technical replicates was recorded as the result of one biological replicate. Data from at least three biological replicates

were collected, and the mean values with standard errors are presented. Primer sequences for qPCR assays are listed in *SI Appendix*, Table S1 online.

In Vitro Co-Immunoprecipitation Assays

The protein coding sequences of all tested genes were fused with an epitope tag at the 3' end using the PCR primers listed in Supplementary Data 1 online. The relevant *AsiSI/PmeI* fragments were cloned into the pF3K WG (BYDV) Flexi Vector (Promega, L5681) to produce expression constructs. The tested proteins were co-expressed in the single reaction, TnT SP6 High-Yield Wheat Germ Protein Expression System (Promega, L3260). 50 μ l of Dynabeads Protein G (Invitrogen, 10004D) were conjugated with 5 μ g of anti-FLAG M2 antibody (Sigma, F1804) in 200 μ l of binding/wash buffer (1 \times PBS, 0.1% IGEPAL CA-630, 0.1 mg/ml BSA) at 4 $^{\circ}$ C for 1 hour, and then incubated with 50 μ l of protein expression reaction mix, in 500 μ l of binding buffer, containing 1 mM PMSF and 1 \times Protease Inhibitor Cocktail (Promega, G6521) at 4 $^{\circ}$ C for 2 hours. HRP-conjugated anti-FLAG (Sigma, A8592) and anti-MYC (Cell Signaling, 2040) antibodies were used to detect the bait and prey proteins, respectively, on immunoblots.

Quasi-In Vivo Co-Immunoprecipitation Assays in *Nicotiana benthamiana*

Full-length coding sequences of *PIF4* and *PIF7* were individually subcloned into the pCR8 Entry vector using a pCR8/GW/TOPO TA Cloning Kit (Invitrogen, K250020), and were then transferred into the pGWB17 binary vector (12) to generate pGWB17-PIF4 (35S::*PIF4*-4xMYC) and pGWB17-PIF7 (35S::*PIF7*-4xMYC) expression constructs using the Gateway LR Clonase II Enzyme mix (Invitrogen, 11791020). To generate a plant expression construct for *PRR5*, a 3xFLAG epitope tag was fused at the C-terminus of the *PIL1* coding sequence, by PCR cloning, and was introduced into the pCambia1302 vector (<https://www.markergene.com/plant-molecular-biology/pcambia-vectors>) at *SpeI* and *PmlI* sites to generate an intermediate construct (pCambia-PIL1-FLAG). The full-length coding sequence of *PRR5* was then subcloned into *SpeI* and *PacI* sites of this intermediate vector to generate the pCambia-PRR5-FLAG clone (35S::*PRR5*-3xFLAG).

Expression constructs were transformed into *Agrobacterium tumefaciens* strain GV3101. Overnight-cultured cells were collected and resuspended in infiltration buffer (10 mM MES, 10 mM MgCl₂, 0.2 mM Acetosyringone). Each construct was diluted to equal cell density (O.D.600 = 0.25) and mixed in the indicated combinations, and incubated for 2 to 4 hours. These *Agrobacterium* cells were then infiltrated into *Nicotiana benthamiana* (tobacco) leaves using 1 ml slip tip syringes. Leaf tissues were harvested after 40 to 48 hours and were quickly frozen in liquid nitrogen.

Co-immunoprecipitation (co-IP) assays were performed as previously described (13) with minor modifications. Each immunoprecipitation was repeated at least twice. The extraction/binding/wash buffer consisted of 100 mM Tris-HCl (pH 7.5), 150 mM NaCl, 10 mM MgCl₂, 1 mM EDTA, 10% glycerol, 0.1% IGEPAL CA-630 (Sigma, I8896), 10 mM NaF, 2 mM Na₃VO₄, 25 mM β -glycerolphosphate, 1 mM PMSF, and 1 \times cOmplete Protease Inhibitor Cocktail (Roche, 4693116001). Total proteins were extracted from 0.5 g fresh-weight of tissue in 1.5 ml of extraction buffer. 5 μ g of anti-FLAG (Sigma, F1804) or anti-MYC (Abcam, ab9132) antibody was pre-conjugated with 50 μ l of Dynabeads Protein G (Invitrogen, 10004D) in 200 μ l of binding buffer at 4 $^{\circ}$ C for 1 hour, and was then incubated with protein extracts at 4 $^{\circ}$ C for 2 hours. HRP-conjugated anti-FLAG (Sigma, A8592) and anti-MYC (Cell Signaling, 2040) antibodies were used in the immunoblot detections.

Bimolecular Fluorescence Complementation (BiFC) Assay

The Bimolecular Fluorescence Complementation (BiFC) system used here was derived from that previously described (14). The coding sequences of *PIF7* and *PRR5* were introduced into pXY106 and pXY104 vectors, to generate 35S::*nYFP-PIF7* (nYFP-PIF7) and 35S::*PRR5-cYFP*

(PRR5-cYFP) constructs, respectively. A nuclear localization signal (NLS) fragment was introduced into the pGWB45 vector (12) to generate the internal control construct, 35S::CFP-NLS (CFP-NLS).

Agrobacterium cultures containing each expression construct were diluted to equal optical density (O.D.600 = 0.25) and mixed in the indicated combinations, together with the CFP-NLS internal control (O.D.600 = 0.1). After 2 to 4 hours pre-incubation, cell mixtures were infiltrated into *Nicotiana* leaf epidermal cells, and incubated for 40 to 48 hours before being analyzed using a confocal microscope (Leica SP8 LIGHTNING system).

The imaging of all construct combinations was recorded at the identical excitation intensity and detection sensitivity using the LAS X program (Leica). The quantification of fluorescence intensity was performed using the ImageJ program (<https://imagej.nih.gov/ij/>), which provided this value as the 'integrated density' of fluorescence in each measured field of the image.

The fluorescence emission intensity for YFP was measured at each spot within a microscope viewing field, and was designated as nuclear-localized if there was a detectable CFP signal at the corresponding location from the CFP-NLS internal control. The mean and standard error for the arbitrary intensity values of the nuclear-localized YFP and CFP fluorescence were then calculated for each field. The ratios of YFP to CFP intensities were calculated for each image field (YFP/CFP), and presented as the mean with standard error. Two to four fields of multiple nuclei were recorded for each construct combination at each infiltration site, and each infiltration was repeated at least twice on separate leaves.

Electrophoretic Mobility Shift Assay (EMSA)

A 165 bp fragment of the *PIL1* promoter was amplified by PCR using the primers listed in the Supplementary Data 1. The promoter fragment was 5' end labelled with digoxigenin (DIG) using a DIG-labelled primer. A 38 bp synthetic G-box probe was produced by the gradient annealing of DIG-labelled primer. DNA binding reactions were performed according to the instructions for the DIG Gel Shift Kit, 2nd Generation (Roche, 03353591910) in a final volume of 20 μ l, containing 1 ng of DIG-labelled probe and 2 μ l of protein product from a TnT SP6 High-Yield Wheat Germ Protein Expression System (Promega). In competition experiments, 50 ng of unlabelled probe was added. The binding reactions were resolved on 6% DNA Retardation Gels (Invitrogen, EC63652BOX) in 1xTBE buffer. Following electrophoresis, electroblotting was performed using positively charged nylon membranes (Roche, 11209299001), and chemiluminescent detection was carried out according to the instructions of the DIG Gel Shift Kit.

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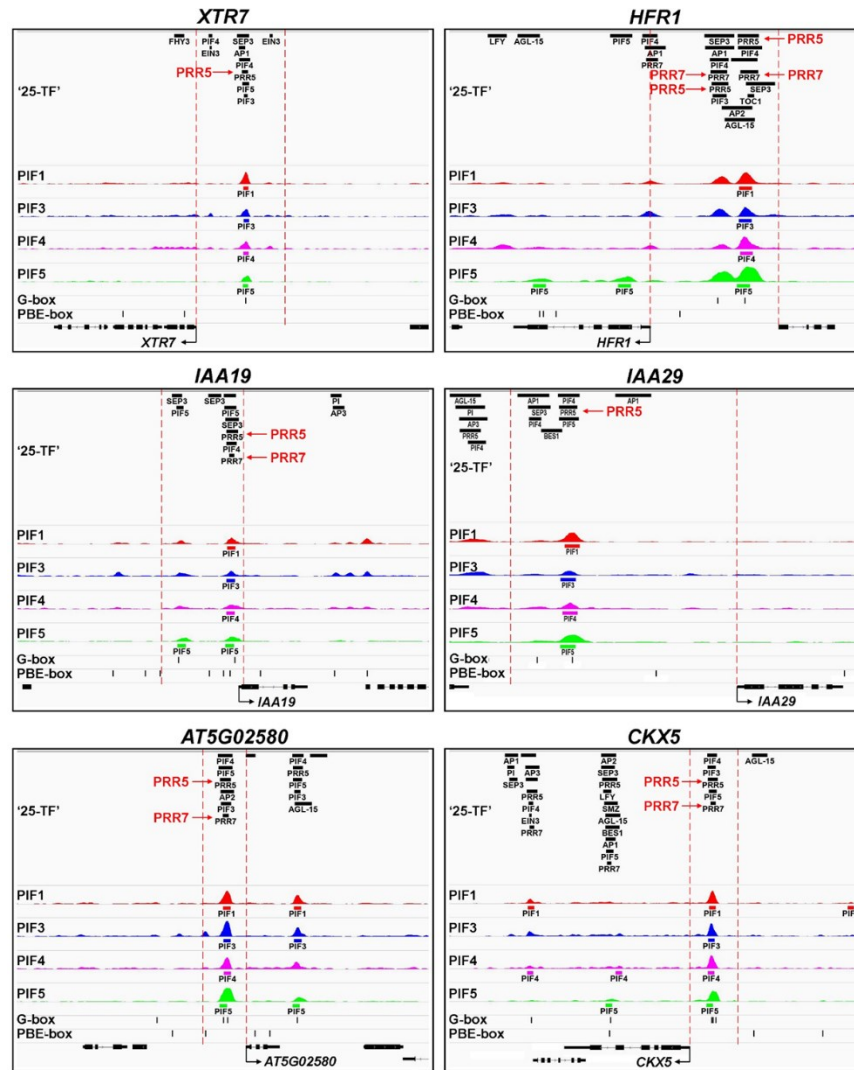


Fig. S1. PRR proteins co-localize with PIF transcription factors on the promoters of additional PIF-DTGs. Browser images of ChIP-seq-determined binding distributions of PIF (lower colored peaks) (2) and '25-TF' (upper short, black horizontal bars; illegible factor names are listed for each gene in Table S1) (1) across the promoter regions (between vertical red dashed lines) of additional selected PIF-DTGs (*XTR7*, *HFR1*, *IAA19*, *IAA29*, *AT5G02580* and *CKX5*). Gene structures depicted below (black boxes linked by lines). Transcription direction indicated by arrows. Locations of G-box (CACGTG) and PBE-box (CATGTG) sequence motifs shown by short, vertical black lines below the ChIP-seq images. PRR5 and PRR7 binding sites highlighted in red.

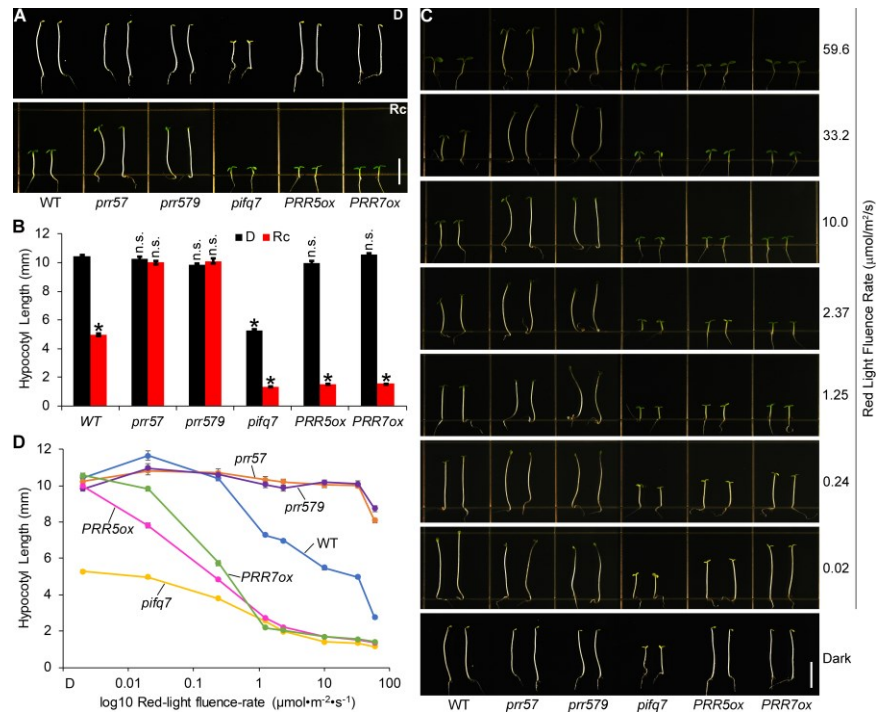


Fig. S2. The PIFs and PRRs have contrasting roles in regulating light-induced seedling development. (A) Phenotypes of wild-type (WT), *prp5prp7* double-mutant (*prp57*), *prp5prp7prp9* triple-mutant (*prp579*), *pifqpif7* quintuple-mutant, PRR5ox and PRR7ox overexpressor seedlings grown at 21 °C, either in darkness (D) or 10 μmole/m²/s continuous red light (Rc) for 4 days. White scale bar represents 5 mm. (B) Hypocotyl lengths of seedlings grown as in (A). (C) Phenotypes of seedlings of the genotypes shown in (A), grown either in the dark or increasing fluence-rates of Rc (as indicated) for 4 days. White scale bar represents 5 mm. (D) Rc-fluence-rate-response curves of hypocotyl length for the seedlings grown as in (C). Data represent the mean and standard error from at least 30 seedlings. Asterisks in (B) indicate that the hypocotyl lengths of Rc-treated seedlings are statistically significantly different from the WT dark-grown control by Student's t-test (P < 1e-10). n.s. indicates not significantly different (P > 1e-5).

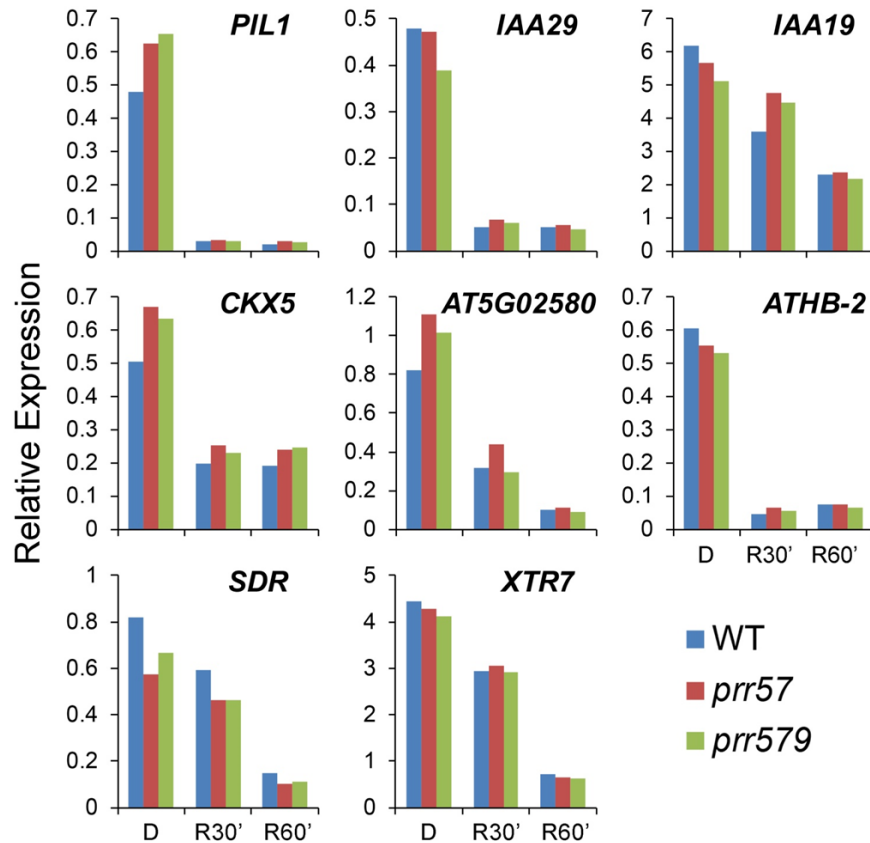


Fig. S3. *prp* mutant seedlings (*prp57* and *prp579*) display no detectable differences from wild-type (WT) in expression of PIF-DTGs when grown in darkness or in response to initial exposure to red light. Time-course analysis of expression of the indicated PIF-DTGs in WT, *prp57* double-mutant, and *prp579* triple-mutant seedlings grown at 21 °C for 4 d in continuous dark (D) followed by 30 (R30') to 60 (R60') minutes red light (10 $\mu\text{mol}/\text{m}^2/\text{s}$) treatment. Transcript levels were determined using RT-qPCR at the times indicated.

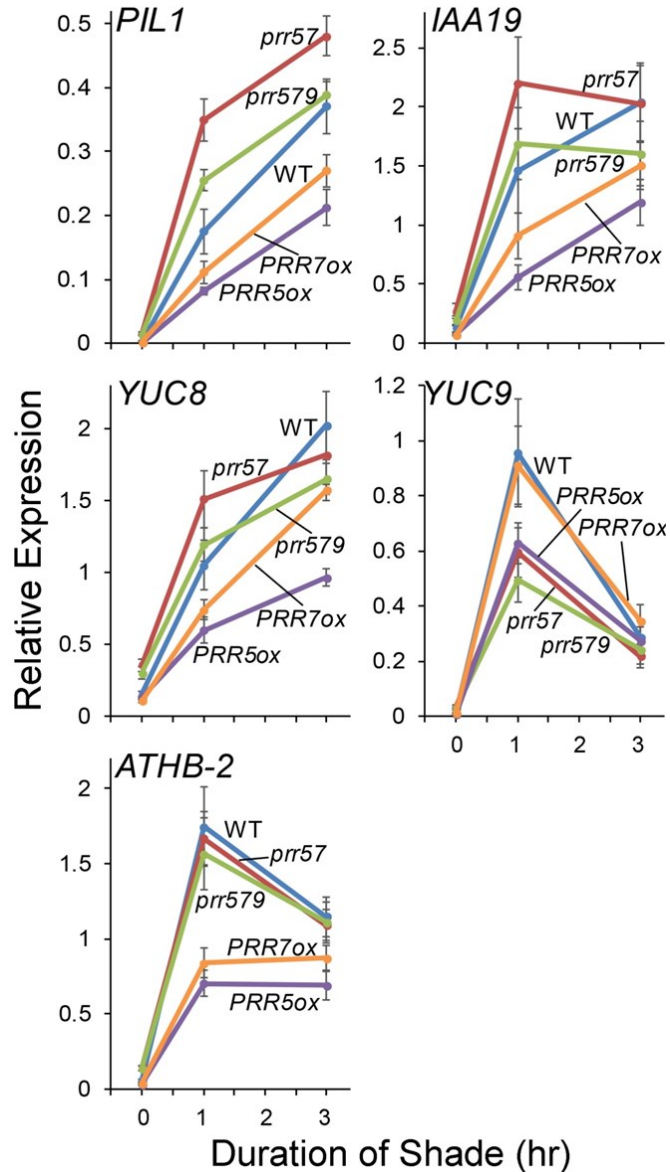


Fig. S4. PRR-imposed repression of shade-induced expression is less robust or apparently absent in a subset of PIF-DTGs. Time-course analysis of expression of the indicated PIF-DTGs in WT, *prr57* double-mutant, *prr579* triple-mutant, *PRR5ox* and *PRR7ox* overexpressor seedlings grown at 21 °C for 5 d in continuous white-light (time zero) followed by 1 to 3 h far-red-light-supplemented white-light (shade; R:FR ratio 0.3). Transcript levels were determined using RT-qPCR at the times indicated. Data represent the mean and standard error for three biological replicates.

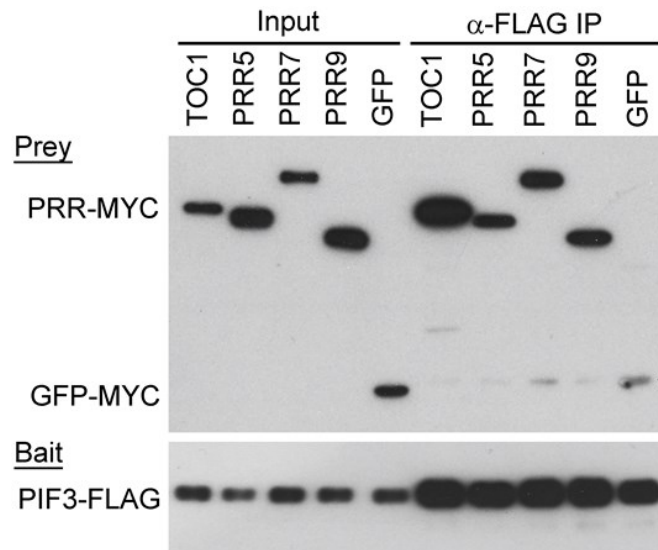


Fig. S5. The PRRs interact with PIF3 in vitro. In vitro Co-IP assays showing interactions of in vitro-synthesized PIF3 with TOC1 and the PRRs. FLAG-tagged PIF3 served as bait. Co-expressed, MYC-tagged TOC1 and PRRs served as prey. GFP-MYC served as the negative control. Asterisk indicates nonspecific cross reacting bands.

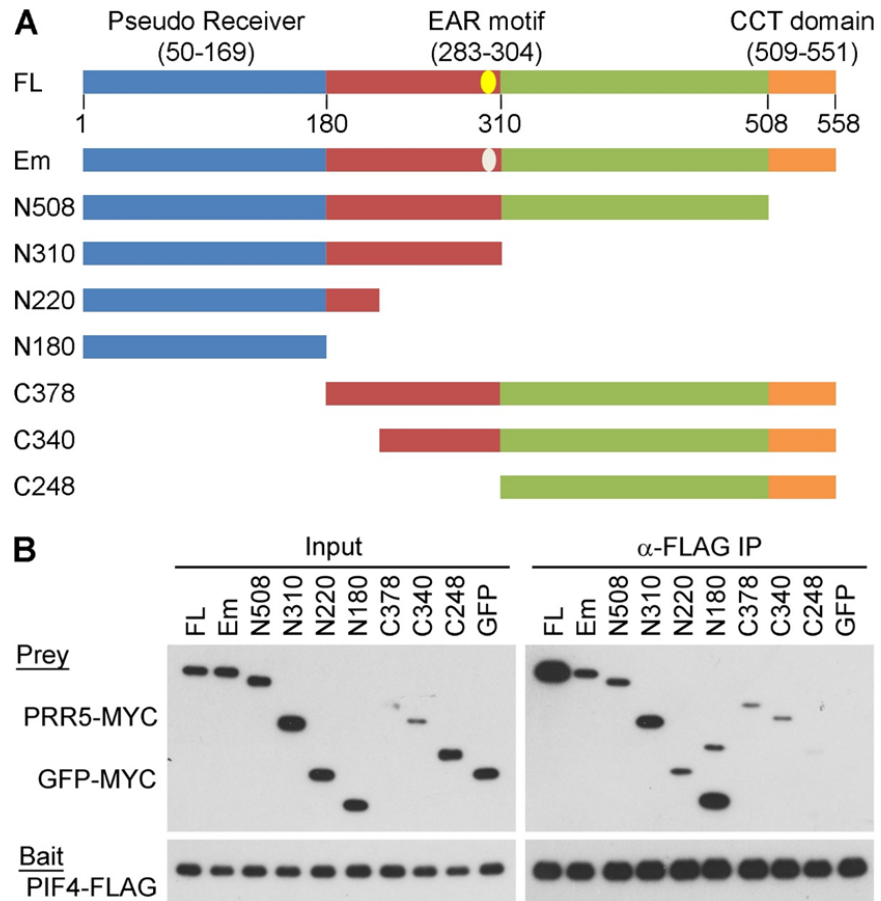


Figure S6. N-terminal pseudo receiver domain of PRR5 mediates interaction with PIF4. (A) Schematic diagram showing the domain structure of full-length PRR5 (FL), with the three conserved motifs labeled, and a series of truncated fragments of the protein, synthesized in vitro and tested for interaction with PIF4 using a Co-IP assay. Em, EAR motif mutation in the FL protein. (B) In vitro Co-IP assays showing interactions of PIF4 with the FL and truncated PRR5 fragments. FLAG-tagged PIF4 served as bait. Co-expressed, MYC-tagged PRR5 fragments served as prey. GFP-MYC served as the negative control.

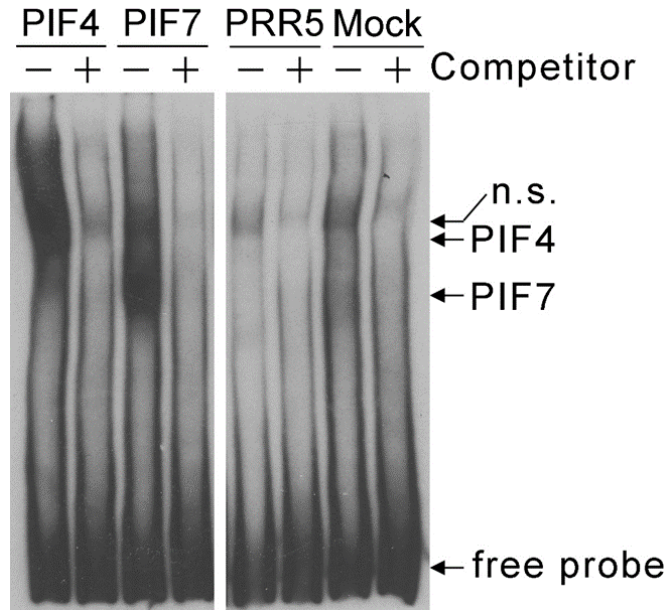


Figure S7. PRR5 does not bind to the PIF-binding sites in the *PIL1* promoter in vitro. Electrophoretic Mobility Shift Assay (EMSA) showing absence of detectable direct binding of PRR5 to a *PIL1* promoter fragment containing the PIF binding sites in this promoter. PIF4, PIF7 or PRR5 proteins were added as indicated. 'Mock' indicates the absence of added protein. '-' indicates the absence of unlabeled competitor probe. '+' indicates addition of unlabeled competitor probe at 50-fold higher concentration than the labeled probe. n.s., non-specific interaction with endogenous proteins in the TnT reactions.

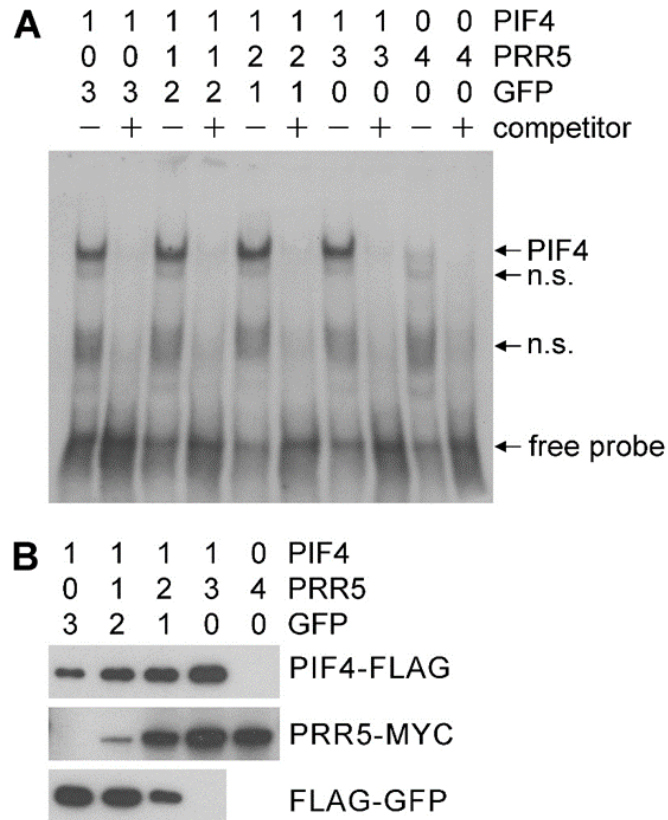


Figure S8. PRR5 does not bind to a G-box motif in vitro. (A) EMSA showing absence of detectable direct binding of PRR5 to a synthetic G-box motif probe, and absence of interference with the direct binding of PIF4 to the same probe. The numbers on the top indicate the relative amount of co-expressed PIF4, PRR5 and/or GFP-control proteins in each reaction. ‘-’ indicates the absence of unlabeled competitor probe. ‘+’ indicates addition of unlabeled competitor probe at 50-fold higher concentration than the labeled probe. n.s., non-specific interaction with endogenous proteins in the TnT reactions. (B) Western blot validation of PIF4 and PRR5 protein levels in each EMSA reaction. The numbers on the top indicate the relative amount of plasmid templates in each co-expression reaction.

Table S1. Transcription Factors Co-bound with PIFs in target gene promoters.

| Gene | Co-bound Factor | | | | | | | | | | | | | | | |
|------------------|-----------------|------|------|------|------|------|------|------|-------|-----|-----|-----|----|------|------|------|
| | PIF1 | PIF3 | PIF4 | PIF5 | PRR5 | PRR7 | TOC1 | SEP3 | AGL15 | AP1 | AP2 | AP3 | PI | FHY3 | EIN3 | BES1 |
| <i>PIL1</i> | + | + | + | + | + | + | | + | + | + | | + | + | | | |
| <i>BIM1</i> | + | + | + | + | + | + | + | + | + | + | | + | + | | | |
| <i>ATHB2</i> | + | + | + | + | + | + | | + | + | + | | + | + | + | | |
| <i>XTR7</i> | + | + | + | + | + | | | + | | + | | | | | + | |
| <i>HFR1</i> | + | + | + | + | + | + | + | + | + | + | + | | | | | |
| <i>IAA19</i> | + | + | + | + | + | + | | + | | | | | | | | |
| <i>IAA29</i> | + | + | + | + | + | | | + | | + | | | | | | + |
| <i>AT5G02580</i> | + | + | + | + | + | + | | | | | + | | | | | |
| <i>CKX5</i> | + | + | + | + | + | + | | | | | | | | | | |

Table S2. List of primers used in this study.

A. List of RT-qPCR primers.

| Gene | Primer | Sequence |
|-----------|-----------|----------------------------|
| AT5G02580 | EP4-QF5 | CATCCATTTGGTGCATCATTTG |
| | EP4-QR5 | CACTCTTCTTTGCCCATGTTGA |
| HFR1 | HFR1-QF1 | GATGCGTAAGCTACAGCAACTCGT |
| | HFR1-QR1 | AGAACCGAAACCTTGCCGTCTTG |
| BBX29 | BBX29-QF3 | CTTCCGGTGGTGAGTTCGTC |
| | BBX29-QR3 | TCTGCTTTTCTCCTCCCTCC |
| IAA29 | IAA29-QF3 | TTGTGCGATCGAGGGTGCTGCGTCT |
| | IAA29-QR3 | TCGTTGGGCTGGCCATTCAAGGCA |
| PIF6 | PIL2-QF5 | TGCACCTTTCATTCCAACGGA |
| | PIL2-QR5 | AGAAGACCGTGAATCCGCAG |
| CKX5 | CKX5-QF5 | GAGGAGCTCAGCCGTGACGC |
| | CKX5-QR5 | TCCTGTGTTGCGTGGTGAGGA |
| PIL1 | PIL1-QF3 | AAATTGCTCTCAGCCATTTCGTGG |
| | PIL1-QR3 | TTCTAAGTTTGAGGCGGACGCAG |
| IAA19 | IAA19-QF1 | TTCCGTGGCATCGGTGTGGC |
| | IAA19-QR1 | GCTGCAGCCCAAACCCGGTAG |
| YUC8 | YUC8-QF1 | GAGGAAAGGGCTCTCAGGTG |
| | YUC8-QR1 | GAAGAGAACCCTTGAGCGT |
| YUC9 | YUC9-QF1 | CTCGCCACCGGTTATCGTAG |
| | YUC9-QR1 | TTCCCTTTCCACGCGTTTG |
| ATHB-2 | HB2-QF5 | GTCGTTGCCGGTCAATGC |
| | HB2-QR5 | CCTAGGACGAAGAGCGTCAAAA |
| PP2AA3 | PP2AA3-QF | TATCGGATGACGATTCTTCGTGCAG |
| | PP2AA3-QR | GCTTGGTCTGACTATCGGAATGAGAG |
| SDR | SDR-QF1 | ATGAGCTCTCCCGTCAGCTTCAGG |
| | SDR-QR1 | CTCCCTTCACACTTGGATGCAGAGC |
| XTR7 | XTR7-QF2 | CGGCTTGACAGCCTCTT |
| | XTR7-QR2 | TCGGTTGCCACTTGCAATT |

B. List of EMSA probe primers.

| Probe | Primer | Sequence |
|---------------|---------------|--|
| PIL1 promoter | PIL1AT-FP-Dig | /5DigN/gatgaatcacgcggcattc |
| | PIL1AT-FP | gatgaatcacgcggcattc |
| | PIL1B-RP-5Dig | /5DigN/gcacaaaaggccCACGTGagcggaagaac |
| | PIL1B-RP | gcacaaaaggccCACGTGagcggaagaac |
| G-box | Gbox-RP-3Dig | TTCGGAAGACGTGTCCACGTGTCCTACTCACCTCGGcc/3Dig_N/ |
| | Gbox-FP-3Dig | ggCCGAGGTGAGTAGGACACGTGGACACGTCTTCCGAA/3Dig_N/ |
| | Gbox-FP | ggCCGAGGTGAGTAGGACACGTGGACACGTCTTCCGAA |
| | Gbox-RP | TTCGGAAGACGTGTCCACGTGTCCTACTCACCTCGGcc |

C. List of cloning primers.

| Construct | Primer | Sequence |
|-------------------|-----------|---|
| pF3K-PIF3-FLAG | PIF3-CF2 | tgaggtaccgcatcgATGCCTCTGTTTGAGCTTTTCAG |
| | PIF3-CR2 | tgagtcgacgtttaaacCTTGTCATCGTCATCCTTGTAGTCCGACGATCCACAAAACCTGATCAG |
| pF3K-PIF4-FLAG | PIF4-CF2 | tgaggtaccgcatcgATGGAACACCAAGGTTGGAGTTTTG |
| | PIF4-CR2 | tgagtcgacgtttaaacCTTGTCATCGTCATCCTTGTAGTCGTGGTCCAAACGAGAACCGTCCG |
| pF3K-PIF7-FLAG | PIF7-CF1 | tcactcgagcgcATGTGCAATTATGGAGTTAAAGAGCTC |
| | PIF7-CR1 | tgagtcgacgtttaaacCTTGTCATCGTCATCCTTGTAGTCATCTCTTTCTCATGATTCGAAGAAC |
| pF3K-TOC1-MYC | TOC1-CF1 | caccgcatcgccATGGATTTGAACGGTGAGTGTAAG |
| | TOC1-CR3 | tgacgtttaaacCAAGTCTTCCTCGGAGATTAGCTTTTGTTTCAGTTCCAAAGCATCATCCTGAG |
| pF3K-PRR5-MYC | PRR5-CF2 | caccgcatcgccATGACTAGTAGCGAGGAAGTAGTTG |
| | PRR5-CR1 | tgacgtttaaacCAAGTCTTCCTCGGAGATTAGCTTTTGTTCTGGAGCTTGTGTGGATTGGACTTGAC |
| pF3K-PRR7-MYC | PRR7-CF1 | caccgcatcgccATGAATGCTAATGAGGAGGGGGAGG |
| | PRR7-CR1 | tgacgtttaaacCAAGTCTTCCTCGGAGATTAGCTTTTGTTTCGCTATCCTCAATGTTTTTTATGTCCG |
| pF3K-PRR9-MYC | PRR9-CF1 | caccgcatcgccATGGGGGAGATTGTGGTTTTAAGTAG |
| | PRR9-CR1 | tgacgtttaaacCAAGTCTTCCTCGGAGATTAGCTTTTGTTCTGATTTGTAGACGCGTCTGAATTC |
| pF3K-GFP-MYC | sGFP-CF2 | tgacgcatcgccATGGTGAGCAAGGGCGAGGAG |
| | sGFP-CR1 | tgacgtttaaacCAAGTCTTCCTCGGAGATTAGCTTTTGTTTCCTGTACAGCTCGTCCATGCCGT |
| pGWB17-PIF4 | PIF4-CF1 | tcagtcgacATGGAACACCAAGGTTGGAGTTTTG |
| | PIF4-CR1 | tcagcggcccggaGTGGTCCAAACGAGAACCGTCCG |
| pGWB17-PIF7 | PIF7-CF3 | atggggcgccATGTCGAATTATGGAGTTAAAGAGCTC |
| | PIF7-CR2 | tccggatcctagggcATCTCTTTTCTCATGATTCGAAGAAC |
| pCambia-PIL1-FLAG | PIL1-CF6 | tgaactagtggatccATGGAAGCAAAACCCTTAGCATC |
| | PIL1-CR6 | tgacacgtgTTACTTGTTCATCGTCATCCTTGTAAATCGATATCATGATCTTTATAATCACCGTCATGGTCTTTGTAGTCggttaattaatgacaGTTTGGCGAGCGATAATAACTAAAAC |
| pCambia-PRR5-FLAG | PRR5-CF2 | caccgcatcgccATGACTAGTAGCGAGGAAGTAGTTG |
| | PRR5-CR6 | actgttaattaaTGGAGCTTGTGTGGATTGGACTTG |
| pXY104-PRR5 | PRR5-CF11 | ggatccATGACTAGTAGCGAGGAAGTAGTTG |
| | PRR5-CR7 | tctagaTGGAGCTTGTGTGGATTGGACTTGAC |
| pXY106-PIF7 | PIF7-CF4 | ggatccATGTCGAATTATGGAGTTAAAGAGCTC |
| | PIF7-CR3 | gtcgactctagaATCTCTTTTCTCATGATTCGAAGAAC |
| pF3K-PRR5-N508 | PRR5-CF7 | caccgcatcgccATGGAACAAAAGCTAATCTCCGAGGAAGACTTGACTAGTAGCGAGGAAGTAGTTG |
| | PRR5-CR3 | tgacgtttaaacTTGAAGAGATTGCTGAATTTTCCCG |
| pF3K-PRR5-N310 | PRR5-CF7 | caccgcatcgccATGGAACAAAAGCTAATCTCCGAGGAAGACTTGACTAGTAGCGAGGAAGTAGTTG |
| | PRR5-CR4 | tgacgtttaaacTCCAGAAGATTGGTTCTCAGAAGC |
| pF3K-PRR5-N220 | PRR5-CF2 | caccgcatcgccATGACTAGTAGCGAGGAAGTAGTTG |
| | PRR5-CR2 | tgacgtttaaacCAAGTCTTCCTCGGAGATTAGCTTTTGTTCTGTACACGAGCTCTGGGCATCACCCAC |
| pF3K-PRR5-N180 | PRR5-CF7 | caccgcatcgccATGGAACAAAAGCTAATCTCCGAGGAAGACTTGACTAGTAGCGAGGAAGTAGTTG |
| | PRR5-CR5 | tgacgtttaaacAAAGCTATCAGGAGCAAGTGAAGTTTG |

| | | |
|--------------------|----------|---|
| pF3K- PRR5-C378 | PRR5-CF4 | caccgcatcgccatgCCATGGAATGAGAGTGTTGGACAG |
| | PRR5-CR1 | tgacgtttaaacCAAGTCTTCCTCGGAGATTAGCTTTTGTCTGG AGCTTGTGTGGATTGGACTTGAC |
| pF3K- PRR5-C340 | PRR5-CF3 | caccgcatcgccatgTGTACAAGACCAGAGATGGAAGGTG |
| | PRR5-CR1 | tgacgtttaaacCAAGTCTTCCTCGGAGATTAGCTTTTGTCTGG AGCTTGTGTGGATTGGACTTGAC |
| pF3K- PRR5-C248 | PRR5-CF5 | caccgcatcgccatgGACCGGCCTTCTCTTCATCCTTC |
| | PRR5-CR1 | tgacgtttaaacCAAGTCTTCCTCGGAGATTAGCTTTTGTCTGG AGCTTGTGTGGATTGGACTTGAC |