Supporting Information

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SI Text

Plant Material. The *Arabidopsis* (*Arabidopsis thaliana* L.) mutant lines used are in the Columbia background. The *pif3* mutant used in this study is an independently isolated T-DNA insertion allele obtained from SALK (030753; http://signal.salk.edu/) and is identical to *pif3–1* (1). The *pif1* mutant is a new allele from the Syngenta *Arabidopsis* Insertion Library (2) that has an insertion in the third exon, 5′ of the bHLH domain (Fig. S1*A*). This *pif1* allele is therefore similar, but not identical, to *pif1–1* (3) and has been designated *pif1–101*. Northern blots probed with a 5′ gene fragment confirmed that *pif1–101* does not accumulate any full length *PIF1* transcript. Hypocotyl measurements in continuous R and FR light demonstrated that both *pif1–101* and the new *pif3–1* were hypersensitive to red (Fig. S1*B*) and far-red (Fig. S1*C*) light with the *pif1pif3* double mutant showing an additive phenotype. These phenotypes are consistent with previous studies on *pif1* (4, 5) and *pif3* (1, 6) including the *pif1pif3* double mutant (4). The *pif1–2* and *pif3–3* mutants were kindly provided by P. Quail (University of California, Berkeley).

Light Sources. Broad band WL was provided directly at 110 or 310 μmol·m^{-2}·s^{-1}, and at 10 μmol·m^{-2}·s^{-1} through 2 layers of neutral density filter [#211; Lee Filters (Andover, UK)]. Narrow waveband light sources were provided by LED displays as described in ref. 7 with the following exception: Far-red light (FR) from the LEDs was passed through a single filter (#116; Lee Filters) to remove <700 nm to give a final fluence rate of 10 μmol·m^{-2}·s^{-1}. Red light (R) was given at a fluence rate of 80 μmol·m^{-2}·s^{-1}.

Real-Time PCR. RNA was extracted as described in ref. 8 with the following exception: Approximately 500 μg of seedling material was snap frozen in liquid N_2 before homogenization in 150 μL of phenol (pH 4.8) and 500 μL of "RNA Miniprep" buffer (100 mM NaCl; 10 mM Tris, pH 7.0; 1 mM EDTA; and 1% SDS) using an Ultra-Turrax T8 hand held homogenizer (IKA Labortechnik). The homogenizer was washed once in 70% ethanol (vol/vol) and twice in distilled water between extractions.

The forward (FOR) and reverse (REV) primers used were as follows: *CHLH* (At5g13630), FOR 5′-CTGGTCGTGACCCTAGAACAG-3′, REV 5′-GATTGCCAGCTTCTTCTCTG-3′; *GUN4* (At3g59400), FOR 5′-CTCCATTGCCAATCTCAC-3′, REV 5′-CCGAATCTACCATCACTGTG-3′; *HEMA1* (At1g58290), FOR 5′-CAAGAACTCTGCAGCTGATC-3′, REV 5′- CCATTCAGCTTCAGGTATAGC-3′; *PIF1* (At2g20180), FOR 5′-GCTAGATGAAGCTATTGAGTACATGA-3′, REV 5′-CTGCTGGTTCGGTACAAAGA-3′; *PIF3* (At1g 09530), FOR 5′-GAATCTGCTCAAGACAGGAAC-3′, REV 5′-CTCGTTGACAGTAACAGGAGAC-3′; *CCA1* (At2g46830), FOR 5′-GATGATGTTGAGGCGGATG-3′, REV 5′-TGGTGTTAACTGAGCTGTGAAG-3′; *LHY* (At1g01060), FOR 5′-GAGAGCGATGGACTGAGGA-3′, REV 5′-CAATGTCGCCACTTACCTG-3′; *TOC1* (At5g61380) FOR 5′-TCTTCGCAGAATCCCTGTGAT-3′, REV 5′-GCTGCACCTAGCTTCAAGCA-3′; *CAX1* (At2g38170) FOR 5′-AGCGGTTTTGCATGGTTGGTTG-3′, REV 5′-CCCTTCATGTAGTGAGAACACC-3′; *YLS8* (At5g08290) FOR 5′-TTACTGTTTCGGTTGTTCTCCA-3′, REV 5′-CACTGAATCATGTTCGAAGCAA-3′.

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2. Sessions A, et al. (2002) A high-throughput *Arabidopsis* reverse genetics system. *Plant Cell* 14:2985–2994.
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5. Shen H, Moon J, Huq E (2005) PIF1 is regulated by light-mediated degradation through the ubiquitin-26S proteasome pathway to optimize photomorphogenesis of seedlings in *Arabidopsis*. *Plant J* 44:1023–1035.
6. Halliday KJ, Hudon M, Ni M, Qin M, Quail PH (1999) *poc1*: An *Arabidopsis* mutant perturbed in phytochrome signaling because of a T DNA insertion in the promoter of *PIF3*, a gene encoding a phytochrome-interacting bHLH protein. *Proc Natl Acad Sci USA* 96:5832–5837.
7. McCormac AC, Fischer A, Kumar AM, Söll D, Terry MJ (2001) Regulation of *HEMA1* expression by phytochrome and a plastid signal during de-etiolation in *Arabidopsis thaliana*. *Plant J* 25:549–561.
8. Stephenson PG, Terry MJ (2008) Light signalling pathways regulating the Mg-chelatase branchpoint of chlorophyll synthesis during de-etiolation in *Arabidopsis thaliana*. *Photochem Photobiol Sci* 7:1243–1252.

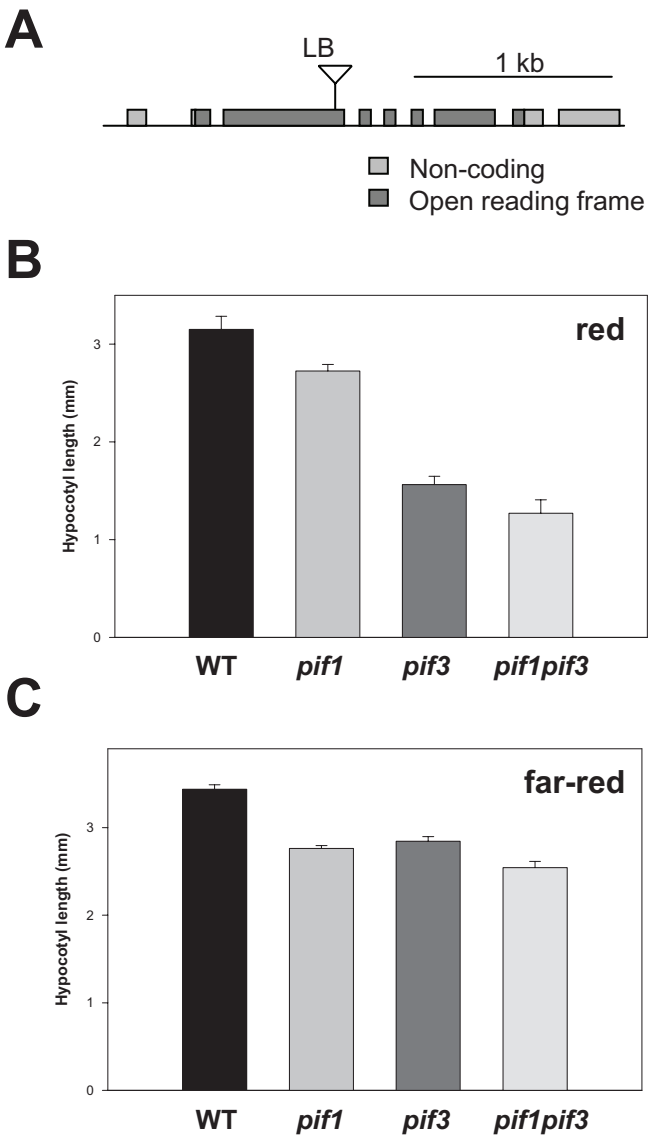

Fig. S1. Characterization of new *pif1* and *pif3* mutants. (*A*) The *PIF1* gene and position of *pif1–101* insertion. (*B* and *C*) Hypocotyl lengths of *pif1–101*, *pif3–1*, and *pif1pif3* double mutants grown in 80 μmol·m^{-2}·s^{-1} red (*B*) or 10 μmol·m^{-2}·s^{-1} far-red (*C*) light for 5 d after 1 d in the dark. Values shown are the mean ± SE of 3 independent experiments.

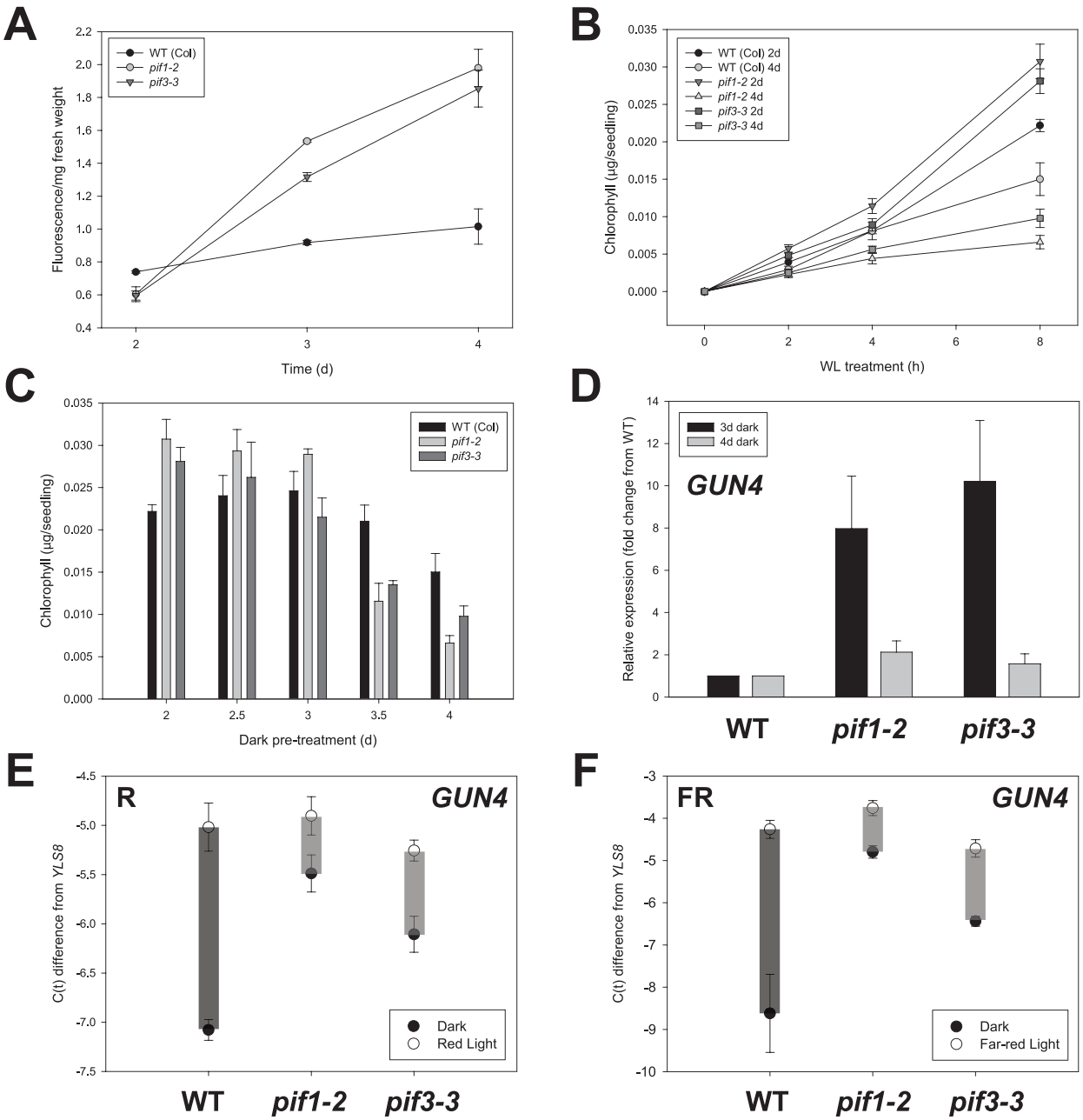

Fig. S2. Phenotype of *pif1–2* and *pif3–3* alleles. (*A*) Pchlide accumulation in WT and *pif* mutant seedlings in darkness (*B*) Chlorophyll accumulation in WT and *pif* mutants after transfer to 110 μmol· m^{-2}·s^{-1} WL after 2 or 4 d in the dark. (*C*) Chlorophyll levels in WT and *pif* mutant seedlings after 8 h of WL after different periods of darkness. (*D*) Real-time PCR data showing expression of the *GUN4* gene in WT and *pif* mutants after 3 or 4 d in the dark. Data are presented as the fold difference from WT after normalizing to the control gene *YLS8*. (*E* and *F*) Real-time PCR data showing expression of *GUN4* in WT and *pif* mutants after either 2 d in the dark or 2 d in the dark followed by 8 h of 80 μmol·m^{-2}·s^{-1} R light (*E*) or 4 h of 10 μmol·m^{-2}·s^{-1} FR light (*F*). Vertical bars indicate the level of light induction, and time points correspond to those of maximum light induction in WT seedlings (8). Data are presented as the *C*(*t*) difference from *YLS8*. Values shown are the mean ± SE of ≥3 independent experiments.

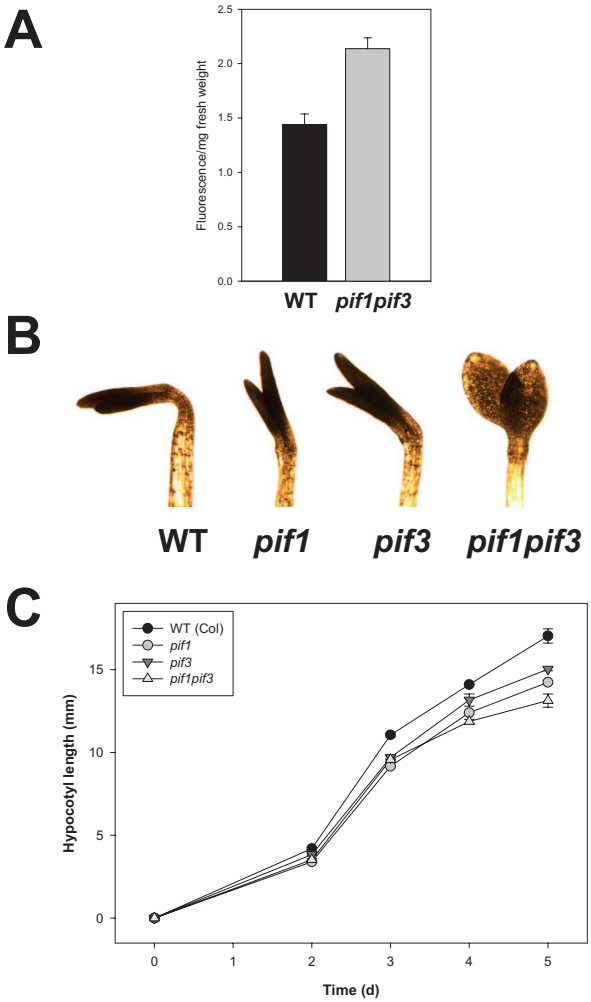

Fig. S3. Phenotype of *pif* mutant seedlings grown in complete darkness. Seedlings were grown as in Fig. 1 except that no 2-h WL pretreatment was given. (*A*) Pchlide accumulation in WT and *pif1pif3* double mutant seedlings after 5 d in the dark. (*B*) Cotyledons of WT and *pif* mutant seedlings after 4 d in the dark. (*C*) Hypocotyl growth of WT and *pif* mutant seedlings in darkness. Values shown in (*A*) and (*C*) are the mean ± SE of 4 and 3 independent experiments, respectively. Photographs shown in *B* are representative and at the same scale.

Fig. S4. Seedling phenotype of WT, *pif1*, *pif3*, and *pif1pif3* double mutants after 4 d in the dark and 1 d of WL (110 μmol·m^{-2}·s^{-1}).

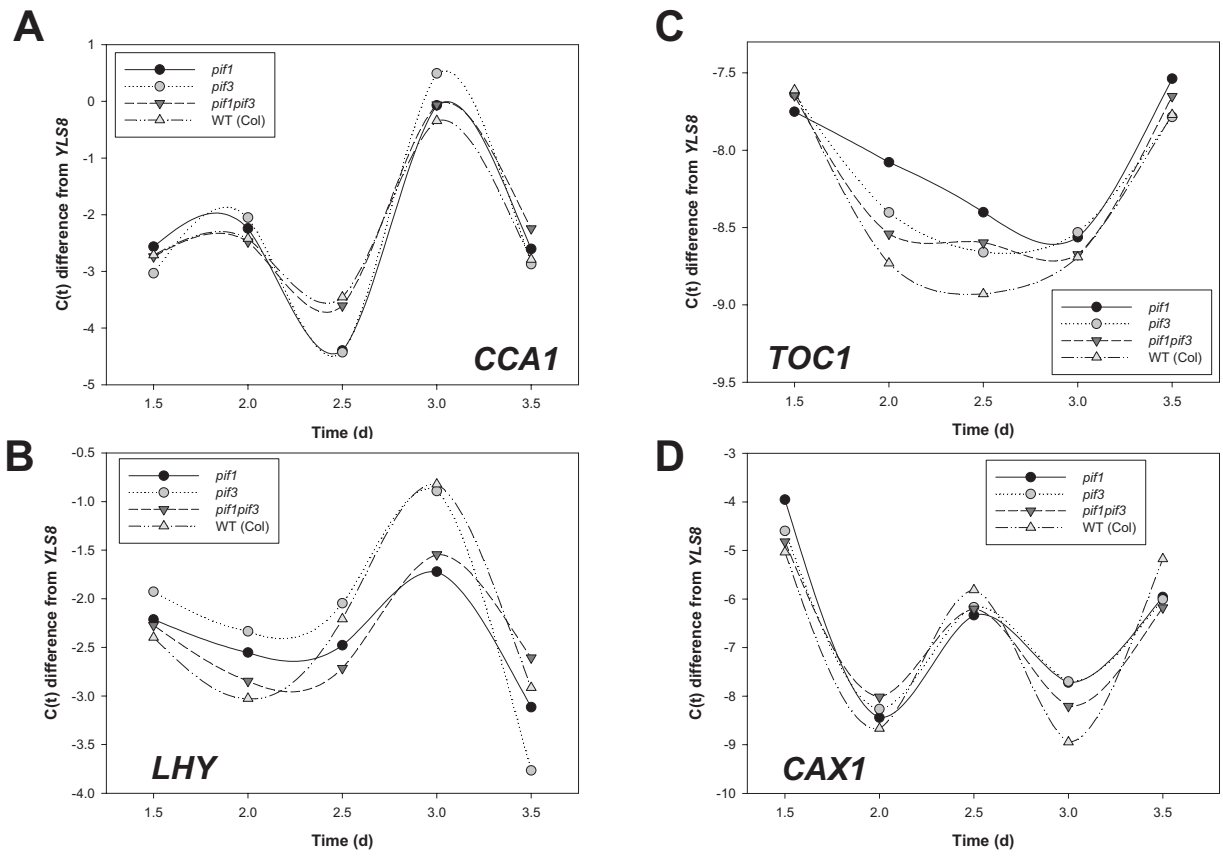

Fig. S5. The *pif1*, *pif3*, and *pif1pif3* mutations do not affect circadian expression of clock genes. Real-time PCR data showing expression of the *CCA1* (*A*), *LHY* (*B*), *TOC1* (*C*), and *CAX1* (*D*) genes in WT and the *pif1*, *pif3*, and *pif1pif3* mutants for up to 3.5 d in the dark. Data are presented as the *C*(*t*) difference to the control gene *YLS8*. Gene expression values shown are the mean ± SE of 3 independent experiments.

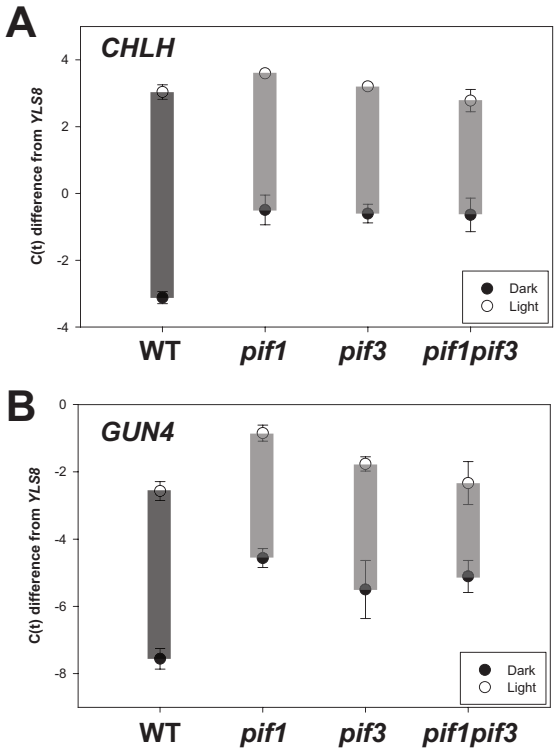

Fig. S6. Expression of tetrapyrrole synthesis genes in *pif* mutant seedlings after light treatment. Expression of the *CHLH* (*A*) and *GUN4* (*B*) genes in WT, *pif1*, *pif3*, and *pif1pif3* mutants after either 3 d in the dark (filled symbols) or 2 d in the dark followed by 1 d of WL (110 μmol·m^{-2}·s^{-1}; open symbols). Vertical bars indicate the level of light induction. Data were determined by real-time PCR and presented as *C*(*t*) difference from the *YLS8* control gene. Values shown are the mean ± SE of ≥3 independent experiments.