Supporting Information

Materials and Methods

Plant Materials and Growth Conditions

The wild-type (WT) *Arabidopsis thaliana* seeds used in this study are Columbia-0 (Col-0) ecotype. All the *ein3eil1 (ee)*, *pif1pif3*, *pif1pif3pif4pif5* (*pifQ*), *cop1-4*, *5xEBS-GUS*, *PIF1p-GUS*, *PIF3p-GUS*, *PIF4p-GUS*, and *PIF5p-GUS* are in the Col-0 background and previously reported (1-4). To generate multiple mutants, we crossed the parental plants and confirmed the homozygous lines by genotyping. The seeds were sterilized by using 75% ethanol with 0.1% Triton X-100 and grown on a half-strength MS medium (2.2 g/L MS salts, 5 g/L sucrose, and 8 g/L agar, pH=5.7). The Image J software was used in phenotype analysis. For hypocotyl elongation analysis, seedlings were grown in the dark or continuous red light for 4 days, and the hypocotyl lengths were measured from the join point of two cotyledons to the point of hypocotyl-root junction. For cotyledon opening and expansion, the angle between two cotyledons and areas of cotyledons were measured. More than 20 seedlings were recorded in each set of experiment.

Immunoblot Assays

Plant seedlings were grown in the dark or continuous red light for 4 days, and harvested and ground to powder in liquid nitrogen. Total proteins were extracted by

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adding the protein extraction buffer (50 mM Tris-HCl, pH=7.5, 150 mM NaCl, 10 mM $MgCl_2$, 0.1% Tween 20, 1 mM PMSF, and one Roche complete EDTA-free Protease Inhibitor Cocktail Tablet/50ml). The protein samples were boiled in SDS loading buffer and separated on 10% SDS-PAGE gels. The endogenous anti-HY5 (1:1000 dilution) antibodies were used for immunoblot detection of HY5 protein abundance (5). HSP90 protein was used as a sample loading control and was detected by anti-HSP90 (Cell Signaling Technology, 1:4000 dilution) antibodies.

Chromatin Immunoprecipitation analysis

Plant seedlings were grown in the dark for 4 days on 1/2 MS medium, or with 10μM β-estrogen to induce expression of *PIF* genes, or with 10μM ACC to elevate EIN3 protein levels. The chromatin immunoprecipitation (ChIP) assay was performed as previously described (6). To avoid light irradiation, all the procedures of sample harvesting, cross-linking, protein extraction, and immunoprecipitation were performed in the dark room with a dim-green safe light. The immunoprecipitation was performed by using anti-Myc polyclonal antibody (Sigma). After ChIP, quantitative PCR (qPCR) was used to examine the enrichment of certain DNA fragments. For ChIP-Seq analysis, the data were originally from the previous studies. We visualized the sequence peaks by using the tool of Integrative Genomics Viewer (http://software.broadinstitute.org/software/igv/).

RNA Extraction and qRT-PCR analysis

Plant seedlings were harvested and ground to powder in liquid nitrogen under a dim-green safe light in the dark room. Total RNA was extracted by using the Spectrum Plant Total RNA Kit (Sigma), and On-Column DNase I (Sigma) was used to degrade DNA. The quality of RNA samples were detected by using spectrophotometric and gel electrophoretic analysis. One microgram RNA was used to synthesize cDNA by using the ReverTra Ace qPCR RT Master Mix (TOYOBO). Real-time quantitative PCR (RT-qPCR) was performed in 7500 Fast Real-Time PCR System (Applied Biosystems) by using SYBR Green Mix (Takara). The shown data are representative results of three independent biological repeats.

Transcriptome Assays

Total RNA was extracted by using the same procedures as that of qRT-PCR analysis. Sequencing was carried out with the Illumina HiSeq 2000 sequencer and the resulting reads were assembled by mapped to the reference genome of Arabidopsis thaliana with TopHat v2.1.0 (http://tophat.cbcb.umd.edu) (7). Transcript expression was evaluated by cuffdiff v2.2.1 (http://cufflinks.cbcb.umd.edu) (8), and transcript abundance was estimated by fragments per kilobase of exon model per million mapped fragments (FPKM). The genes which fold change was higher than two and q–value was lower than 0.01 were characterized as differentially expressed genes (DEGs). Among the DEGs, genes identified as "UP" were up-regulated simultaneously in 4-day old dark grown (D4) *pifQ*, *pifQee*, *HY5*ox/*pifQee* seedlings and red light-grown (R4) WT seedlings compared with D4 WT seedlings. Those

simultaneously down-regulated genes were identified as "Down" genes, and the remaining were named "Other" genes. Statistical analyses and visualizing data were performed by R version 3.3.2 and the DEGs were compared with the online tool Venn (http://bioinfogp.cnb.csic.es/tools/venny/index.html). Heatmap was achieved depending on Z-score by the heatmap. 2 package and the default complete linkage method in R. GO enrichment was performed by using the tool agriGO (http://bioinfo.cau.edu.cn/agriGO/) (9).

Histochemical GUS staining assays

Seedlings for GUS staining assays were grown in the dark for 4 days on 1/2 MS medium, and treated with 90% (vol/vol) acetone at -20℃ for 30min. 1 X PBS buffer was used to wash the acetone away. Then the treated seedlings were submerged in the substrate buffer (1 X PBS, 1 mM $K_3Fe(III)(CN)_6$, 0.5 mM $K_4Fe(II)(CN)_6$, 1 mM EDTA, 1 % Triton X-100, and 1 mg/mL X-gluc) and incubated at 37℃. A graded series of ethanol (30%, 70% and 95% vol/vol) was used for dehydration after the incubation, then stored in 95% (vol/vol) ethanol at 4℃. More than 10 seedlings were analyzed in the assay and representative images are shown.

Supplementary Figures

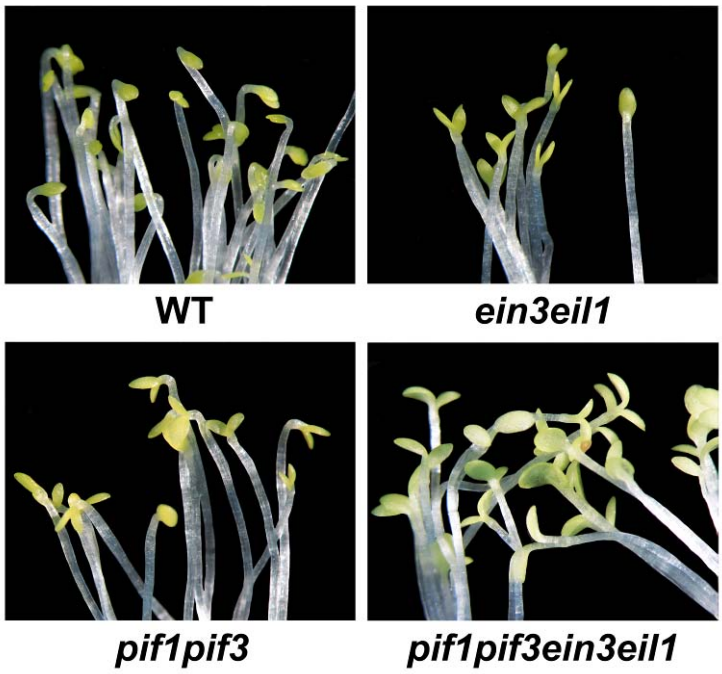

Fig. S1. EIN3/EIL1 and PIFs function additively in repressing cotyledon development. Cotyledon images of 10-day old etiolated seedlings grown in darkness.

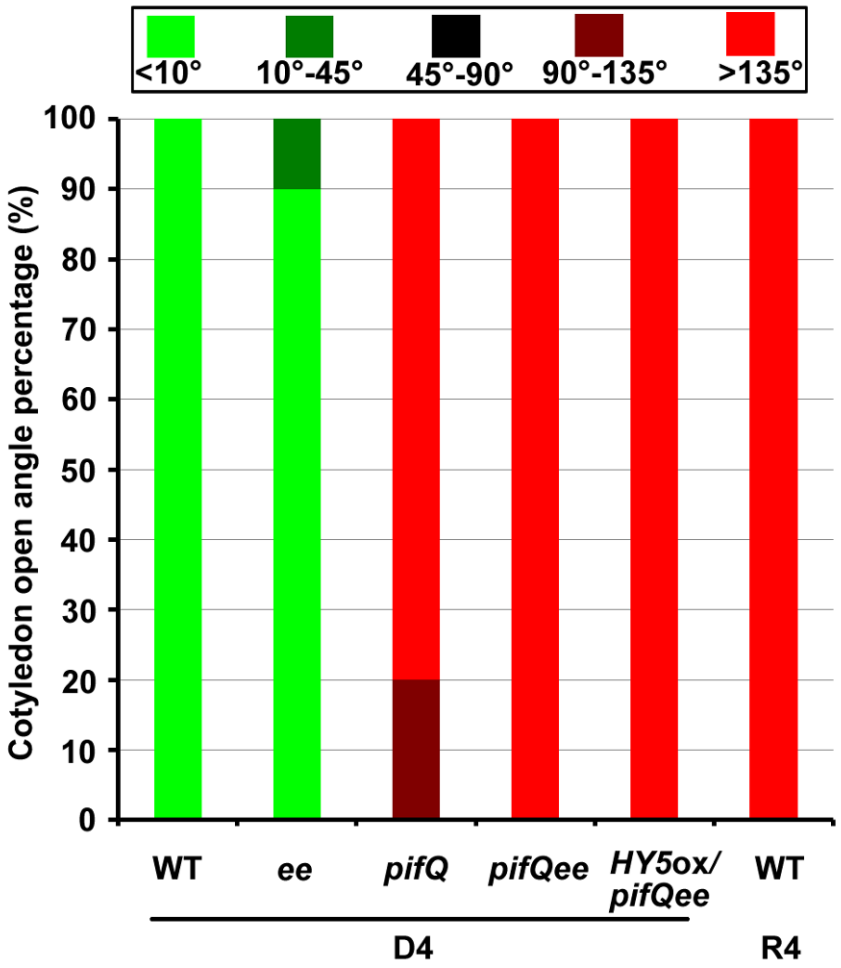

Fig. S2. EIN3/EIL1 and PIFs collectively repress cotyledon opening in the dark. Cotyledon open angles of 4-day old seedlings grown in darkness (D4) or continuous red light (R4) were measured.

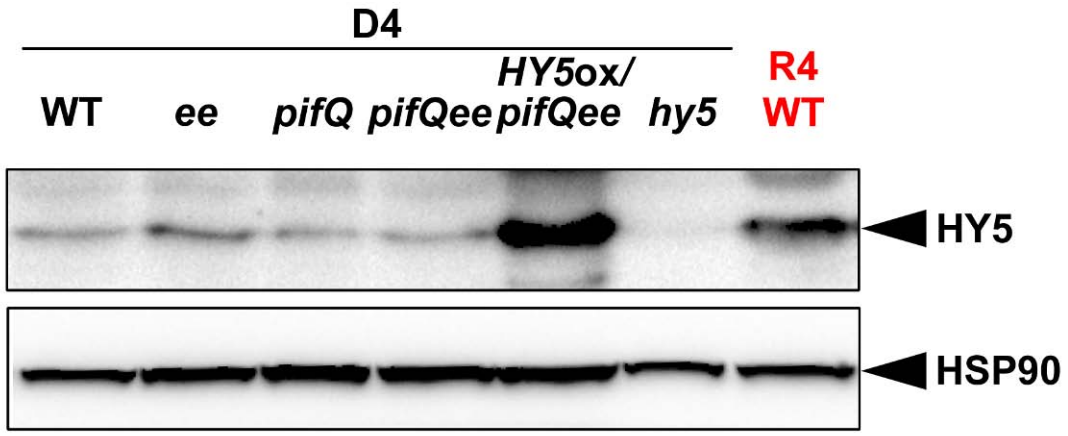

Fig. S3. EIN3/EIL1 or PIFs do not alter the protein levels of HY5. Western blot results for determination of the endogenous HY5 protein levels in 4-day old seedlings grown in darkness (D4) or continuous red light (R4). HSP90 was used as a loading control. Anti-HY5 and anti-HSP90 antibodies were used for immunoblot detection.

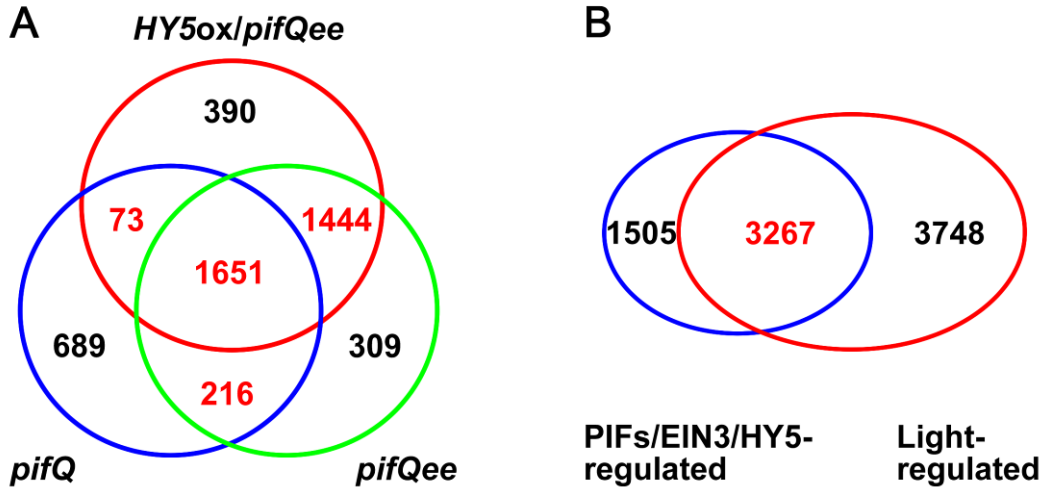

Fig. S4. PIFs, EIN3/EIL1, and HY5 direct half of the light-regulated transcriptomic changes. Venn diagram showing the overlaps of *pifQ*-, *pifQee*-, and *HY5*ox/*pifQee*-regulated genes (A), and the overlaps of PIFs/EIN3/HY5- and light-regulated genes (B). A union set of *pifQ*-, *pifQee*-, and *HY5*ox/*pifQee*-regulated genes was designated as PIFs/EIN3/HY5-regulated genes.

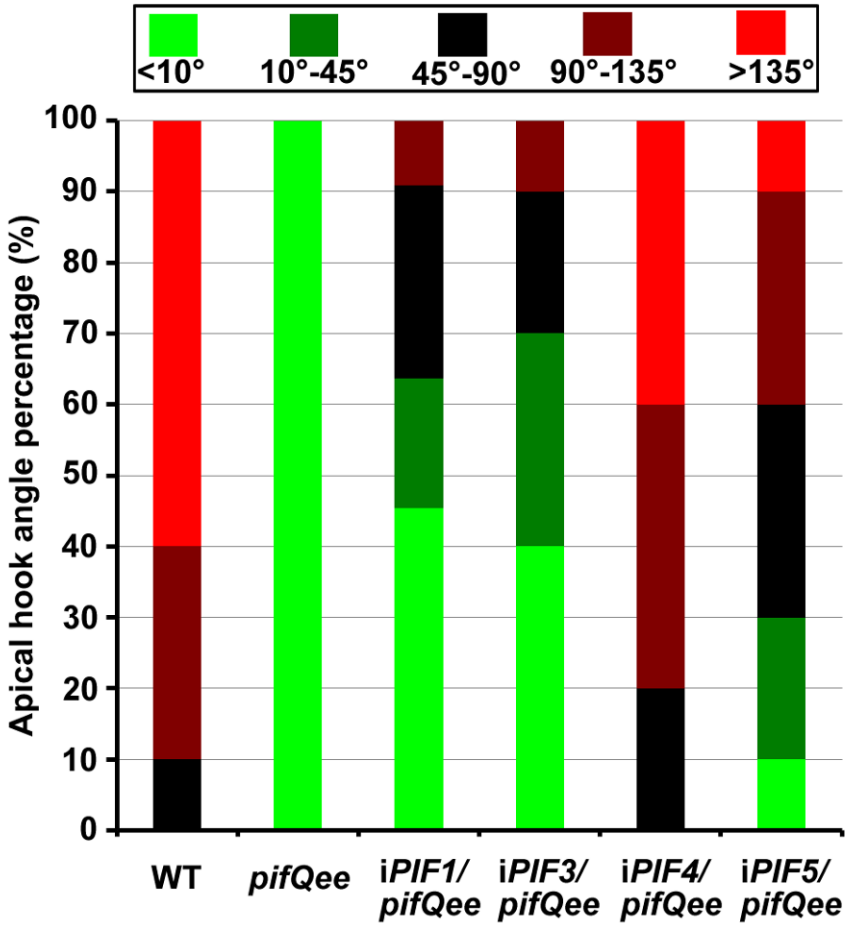

Fig. S5. PIFs promote apical hook formation independently of EIN3 in the dark. Apical hook angles of 4-day old etiolated seedlings grown on 1/2 MS medium supplemented with 10 μM β-estradiol.

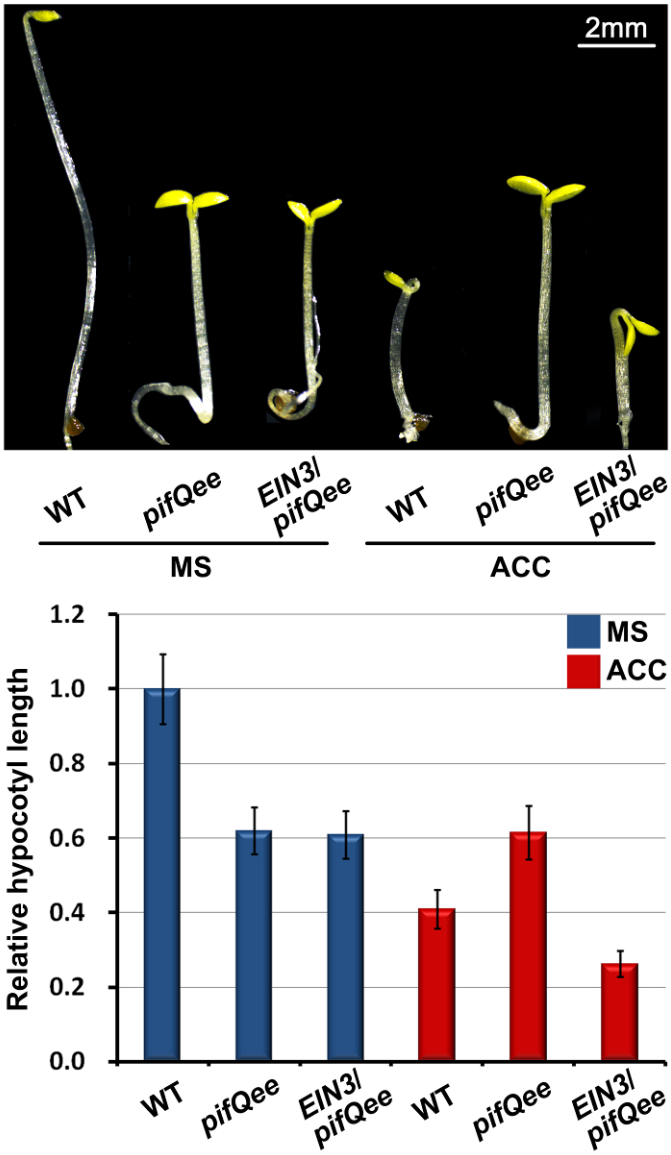

Fig. S6. EIN3/EIL1 represses hypocotyl elongation independently of PIFs in darkness. Representative images (Top) and hypocotyl lengths (Bottom) of 4-day old etiolated seedling grown on 1/2 MS medium supplemented without (MS) or with (ACC) 10 μM ACC. The wild-type (WT) value was set as 1, and the relative hypocotyl lengths were calculated. Error bars represent SD (n=20).

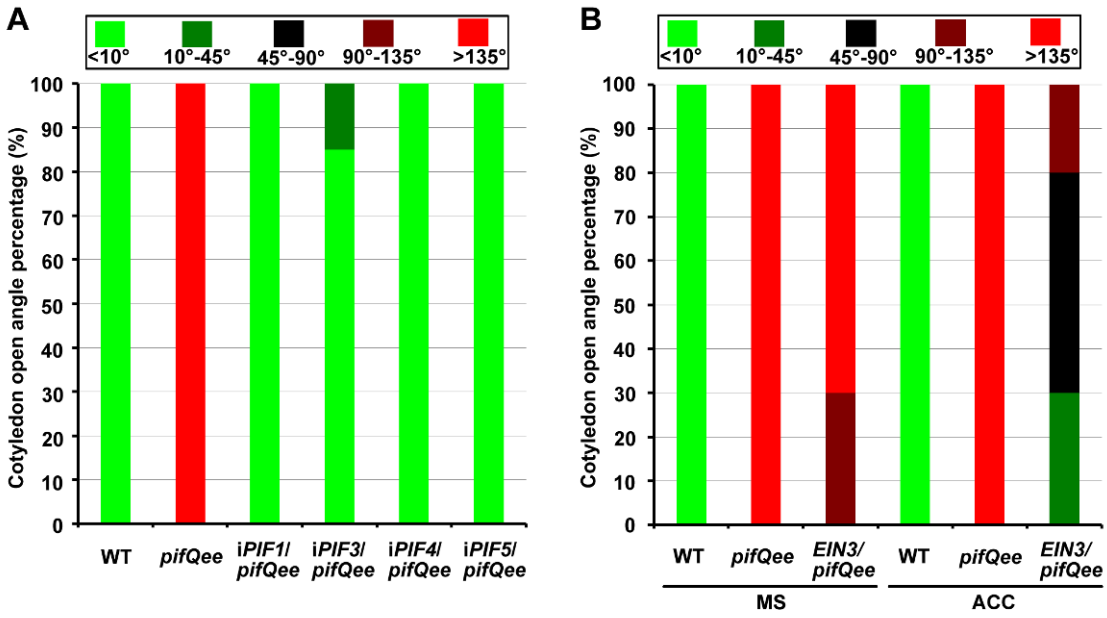

Fig. S7. EIN3/EIL1 and PIFs repress cotyledon opening in a way independently of each other. Cotyledon open angles of 4-day-old etiolated seedlings grown on 1/2 MS medium supplemented with 10 μM β-estradiol (A), without (MS) or with (ACC) 10 μM ACC (B). The wild-type (WT) value was set as 1, and the relative cotyledon open angles were calculated. Error bars represent SD (n=20).

Other Supporting Information Files

Dataset S1

Dataset S2

References

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