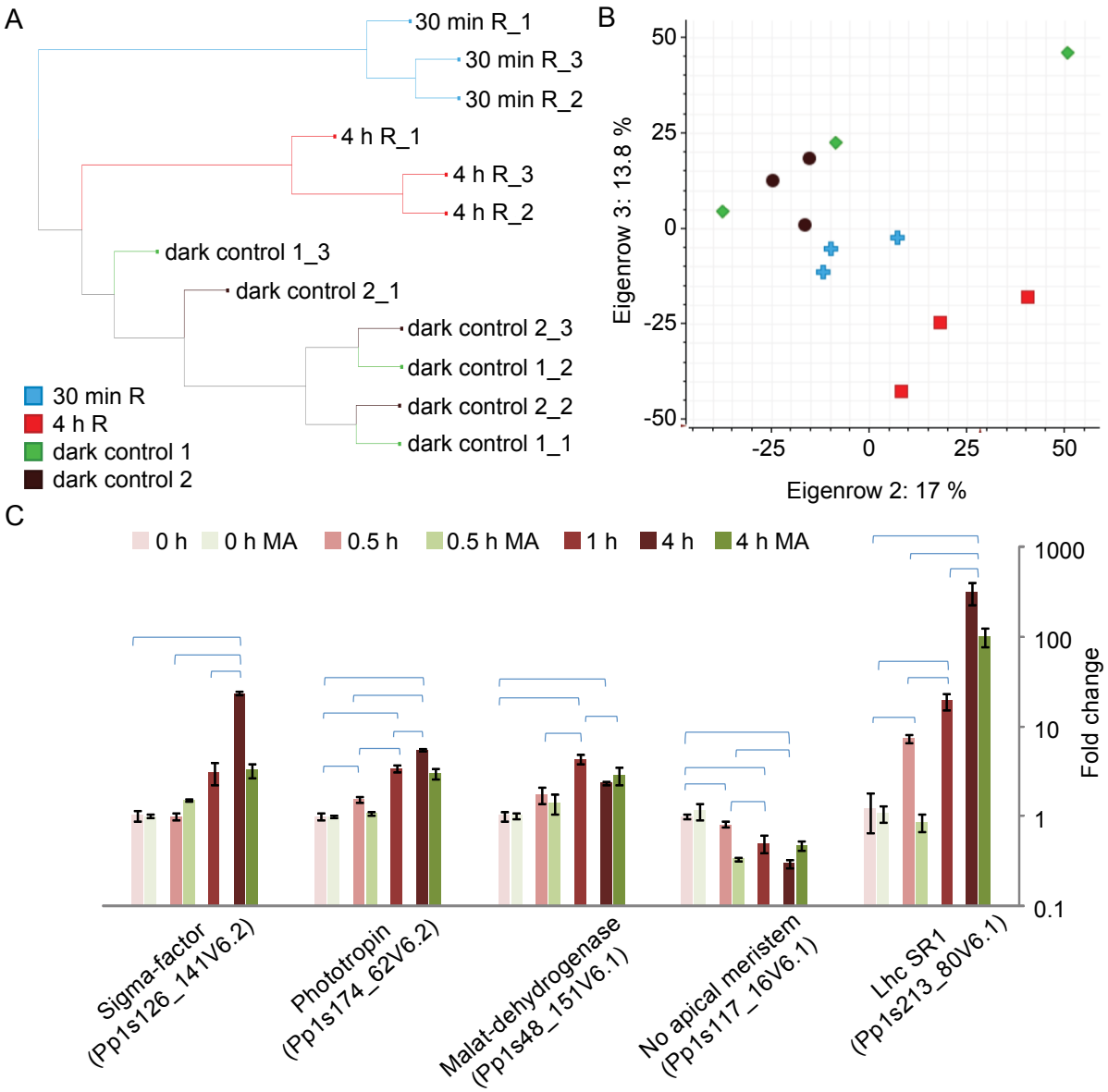


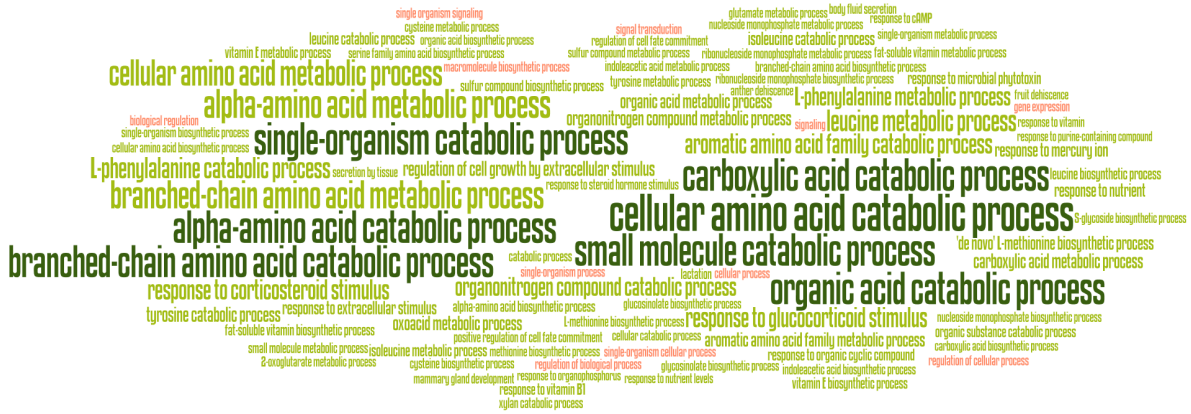
Supplemental Figure 1. Global review and validation of microarray data.
(Supports Figure 1.)
(A) Hierarchical Clustering of microarray experiments (biological triplicates are indicated as 1 - 3). Prior to light treatment plants were adapted to darkness for 2 weeks (dark control 1 - 2 weeks). Also shown is an additional dark control (dark control 2 - 3 weeks) (Hiss *et al.*, 2014). 30 min R samples are shown in red, 4 h R samples in blue, dark controls in black and green.
(B) Principal Component Analysis of microarray experiments. Color code same as in (A).
(C) Validation of fold changes of selected genes by qPCR. Cp-values of genes of interest were normalized to Pp1s545_10V6.1 (Thioredoxin) (Hiss *et al.*, 2014) except for Pp1s213_80V6.1 (Lhc SR1), that was normalized with Pp1s215_36V6.1 (pectinesterase family protein), by applying the DDCt method. Reference genes were selected by their constant expression in this treatment as well as for their little variation over a wide range of treatments. Final fold changes were calculated in comparison to 0 h. Error bars represent standard error of biological triplicates. qPCR results are shown in red and microarray (MA) results in green graduation from 0 h up to 4 h, respectively. Significant (student's t-test: one-tailed, heteroscedastic) differences between time points in qPCR are shown as brackets above the respective bars.

A

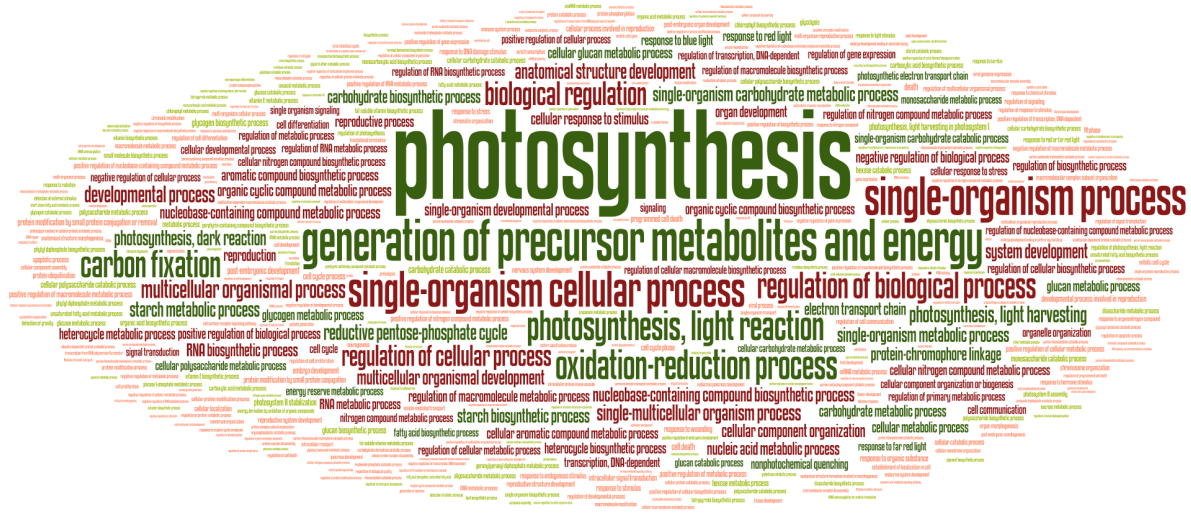
B

Supplemental Figure 2. Word cloud visualization of DEGs after 30 min R light according to Gene Ontology (GO) terms.
(Supports Figure 1.)
(A) Word cloud visualization of DEGs that were down-regulated after 30 min R light compared to darkness. Word size is proportional to the log10(q value). Over-represented GO terms are colored dark green if q ≤ 0.0001 and light green if q > 0.0001. Under-represented GO terms are colored dark red if q ≤ 0.0001 and light red if q > 0.0001.
(B) Word cloud visualization of DEGs that were up-regulated after 30 min R light. Color scheme like in (A).

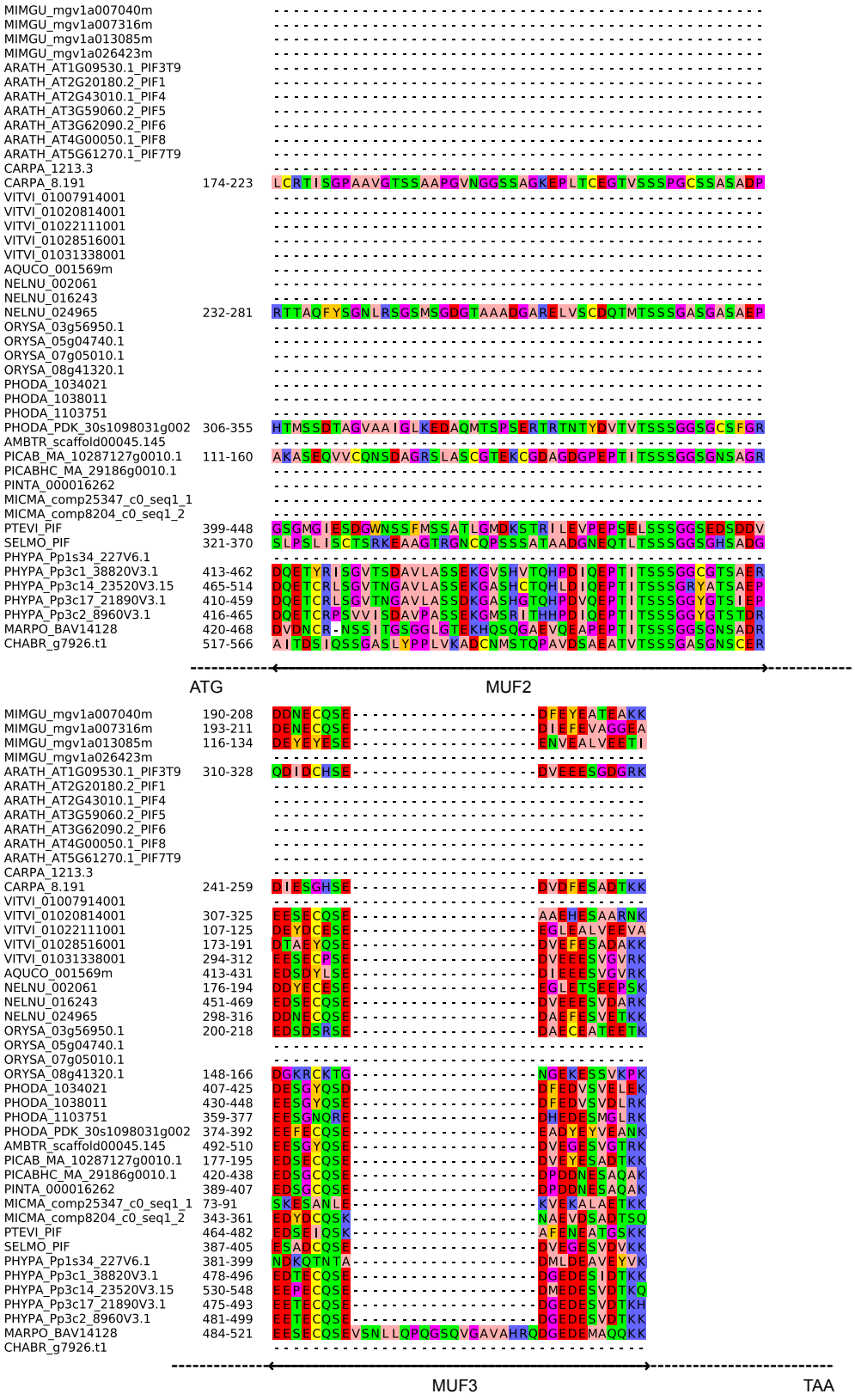
A



B



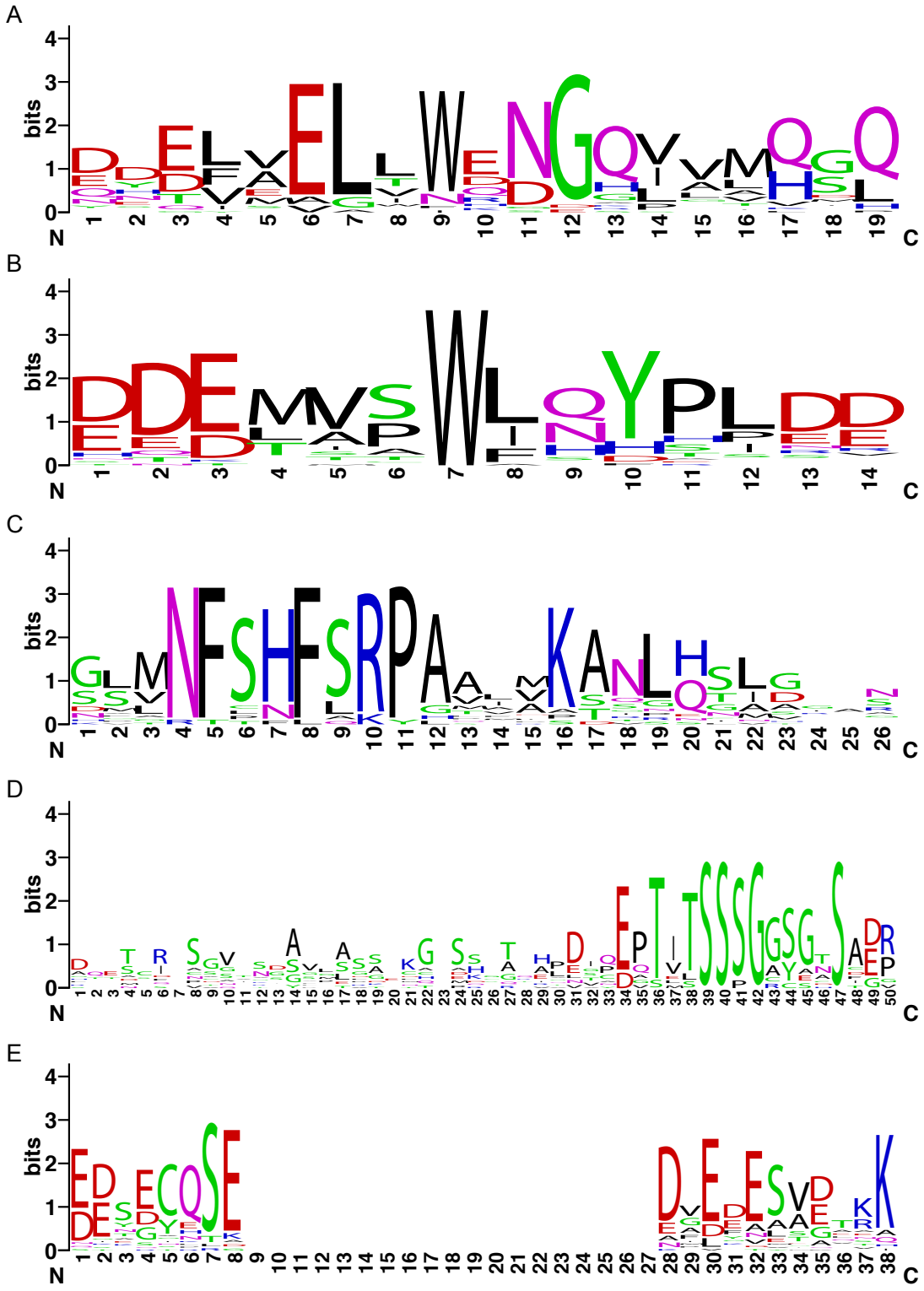
Supplemental Figure 3. Word cloud visualization of DEGs after 4 h R light according to Gene Ontology (GO) terms.
(Supports Figure 1.)
(A) Word cloud visualization of DEGs that were down-regulated after 4 h R light compared to darkness. Word size is proportional to the log10(q value). Over-represented GO terms are colored dark green if q ≤ 0.0001 and light green if q > 0.0001. Under-represented GO terms are colored dark red if q ≤ 0.0001 and light red if q > 0.0001.
(B) Word cloud visualization of DEGs that were up-regulated after 4 h R light. Color scheme like in (A).



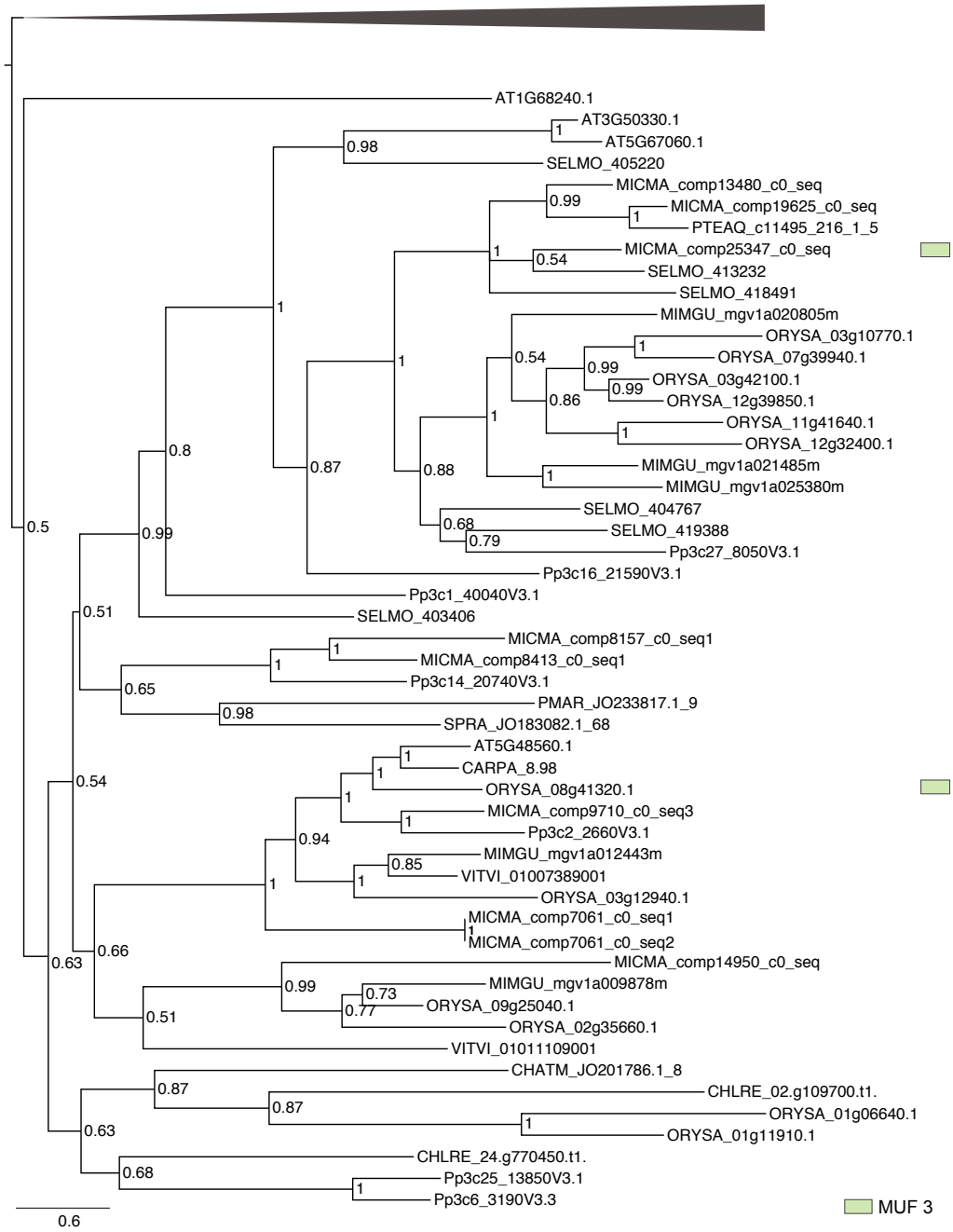
Supplemental Figure 4. Sequence alignment of motifs of unknown function (MUF) 2 and 3 detected in PIF bHLH proteins.

(Supports Figure 2.)

The dashed line indicates intercalated regions. Species abbreviations are in a five letter code, where the first three letters represent the genus and the trailing two the species (e.g., ORYza SAtiva). For each motif the respective positions in the protein sequence are indicated. See Supplemental Table 1 for list of species and Supplemental File 1 for full-length alignment.

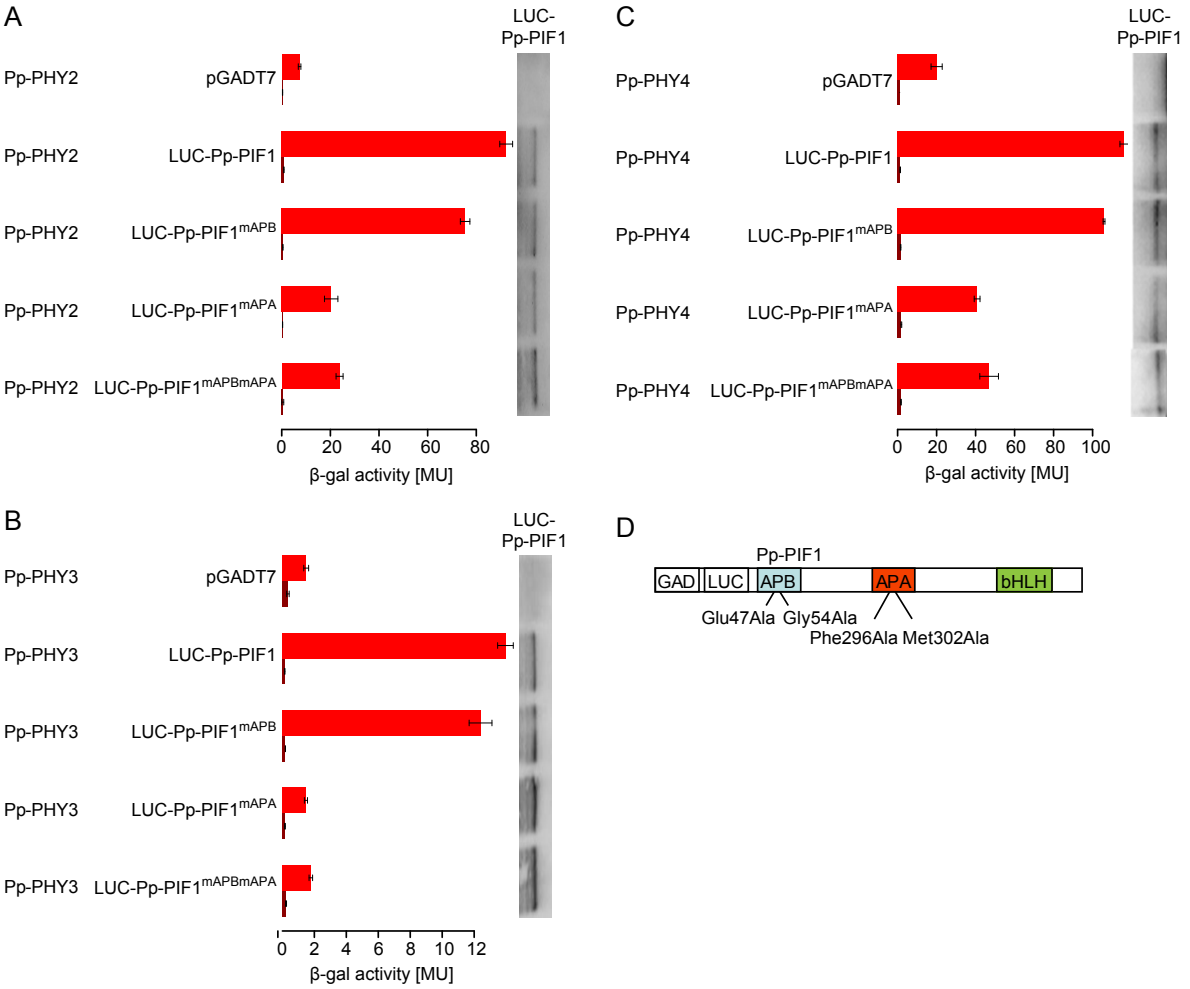


Supplemental Figure 5. Sequence logos of the PIF APB motif, the motifs of unknown function (MUF) and the APA motif.
(Supports Figure 2.)
(A) Sequence logo of the APB motif that was detected in bHLH proteins used for phylogenetic analyses. Motif detection was done with MEME. Logos were generated using weblogo (UC Berkeley, CA, USA).
(B) Sequence logo of the MUF1 detected in bHLH proteins as described in (A).
(C) Sequence logo of the APA motif detected in bHLH proteins as described in (A).
(D) Sequence logo of the MUF2 detected in bHLH proteins as described in (A).
(E) Sequence logo of the MUF3 detected in bHLH proteins as described in (A).



Supplemental Figure 6. Excerpt of phylogenetic tree of plant bHLH TFs - clade not containing canonical PIFs. (Supports Figure 3.)

The phylogeny is based on Bayesian inference; support values (BI posterior probabilities) are shown at the nodes of the tree. Species abbreviations for all organisms except *P. patens* (Pp) and *A. thaliana* (At) are in a five letter code, where the first three letters represent the genus and the trailing two the species (e.g., ORYza SAtiva) (see Supplemental Data Set 3 for list of species and Supplemental File 1 for full-length alignment). The sequence names contain the accession number. The presence of five motifs (APA, APB and three motifs of unknown function (MUF) as inferred by MEME *de novo* motif detection are depicted as boxes. The clade containing all canonical PIFs (indicated by the triangular shape at the top) has been collapsed to enhance readability; for expansion of this clade, refer to Figure 3.

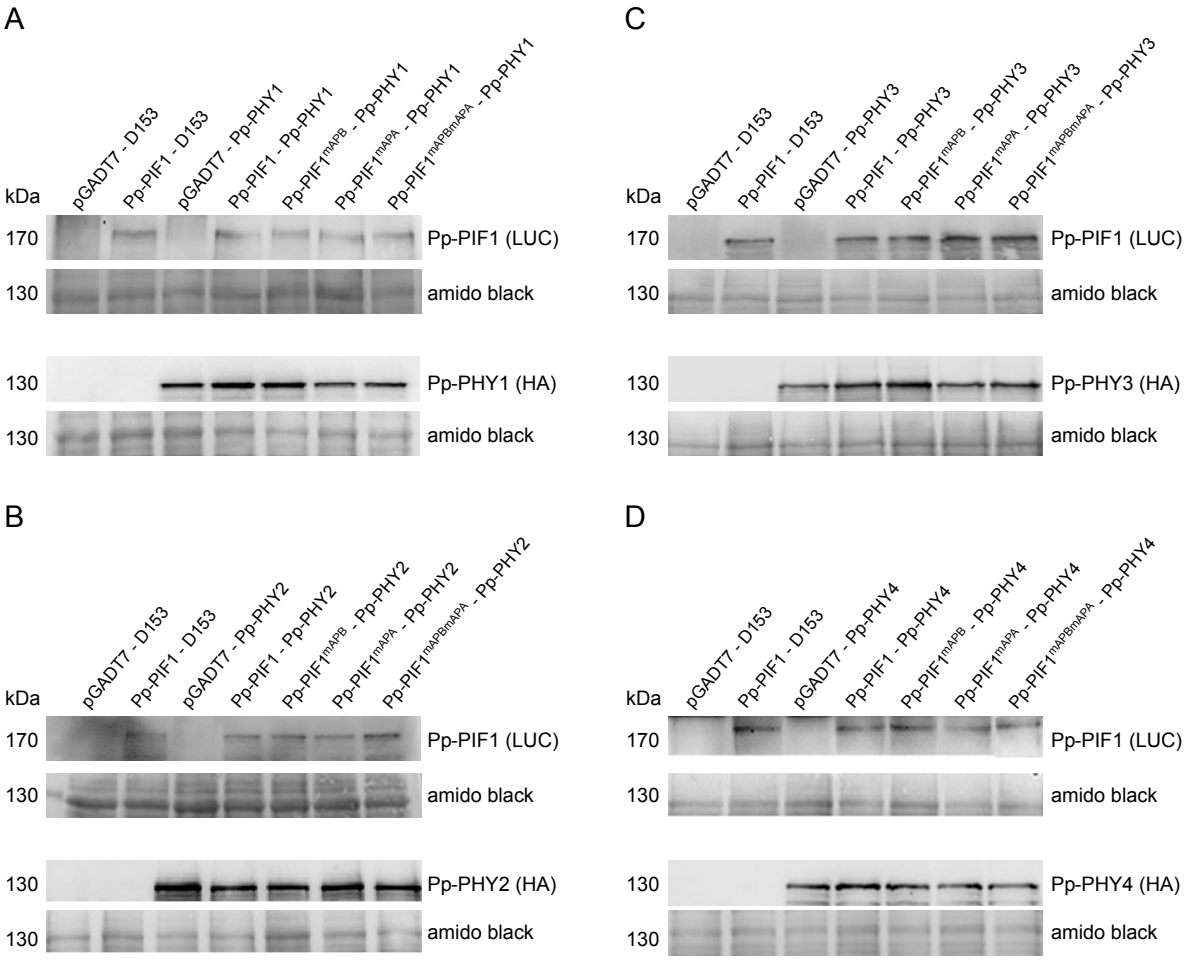


Supplemental Figure 7. Interaction of LUC-Pp-PIF1 with light-activated Pp-PHY2-4 in yeast requires APA motif.

(Supports Figure 4.)

(A-C) The light-induced interaction of full-length Pp-PIF1 fused to LUC with Pp-PHY2, 3 and 4 is impaired by mutations in the APA motif. GAD plasmids (pGADT7) containing the coding sequence for Pp-PIF1 and mutated versions of Pp-PIF1 (Pp-PIF1^{mAPB}, Pp-PIF1^{mAPA}, Pp-PIF1^{mAPBmAPA}), respectively, fused to the GAL4 activation domain (GAD) and the coding sequence of Luciferase (LUC) were used in yeast-two-hybrid assays with GBD plasmids (D153) containing the coding sequence for Pp-PHY2, Pp-PHY3 and Pp-PHY4, respectively, fused to the GAL4 DNA binding domain (GBD). Phytochromes were converted into the Pfr or Pr form by irradiating yeast cultures for 5 min with R (12 µmol m^{-2} s^{-1}) or FR (12 µmol m^{-2} s^{-1}) light. The β–galactosidase activity was measured after an additional incubation in the dark for 4 h. MU, Miller Units. Bars indicate the mean of three biological replicates (i.e., three independent cultures were grown; each culture was measured in triplicate); error bars represent 95 % confidence interval.The expression of wild-type and mutated Pp-PIF1 in yeast was analyzed by immunoblot analysis using an antibody specific to LUC. For complete immunoblot analyses of LUC-Pp-PIF1 and Pp-PHY protein abundance refer to Supplemental Figure 8.

(D) Mutations inserted in Pp-PIF1 APB and APA motifs are shown schematically.

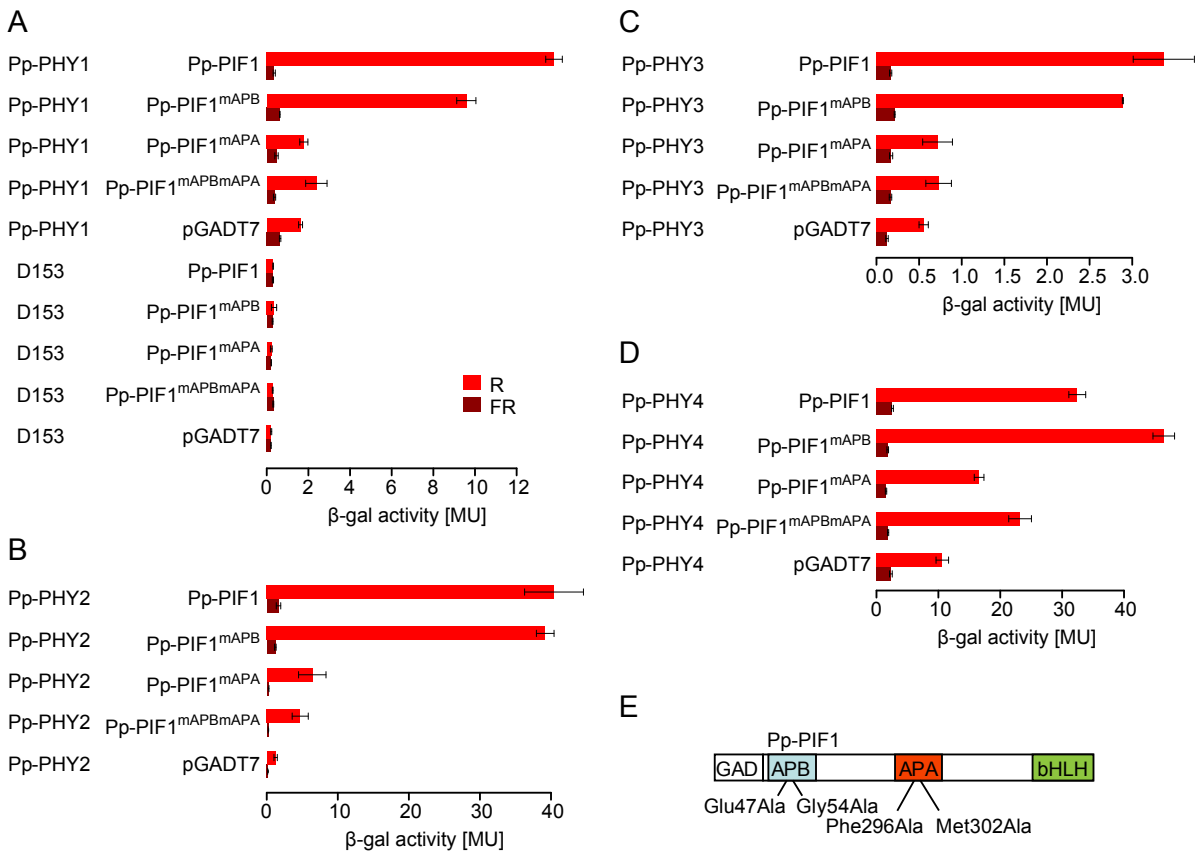


Supplemental Figure 8. Immunoblot analysis of Pp-PIF1 and Pp-PHY protein abundance in yeast.

(Supports Figure 4.)

(A-D) Wild-type and mutated Pp-PIF1 were expressed to similar levels in yeast. Yeast cells were transformed with combinations of plasmids as depicted. To monitor protein abundance, wild-type and mutated Pp-PIF1 fused to Luciferase (LUC) (pGADT7 plasmids) and Pp-PHYs fused to an HA tag (HA) (D153 plasmids) were detected in total protein extracts by Immunoblot analysis using antibodies either specific to LUC or to the HA tag. Amido black stainings of the membranes before immuno analyses are shown as loading controls.

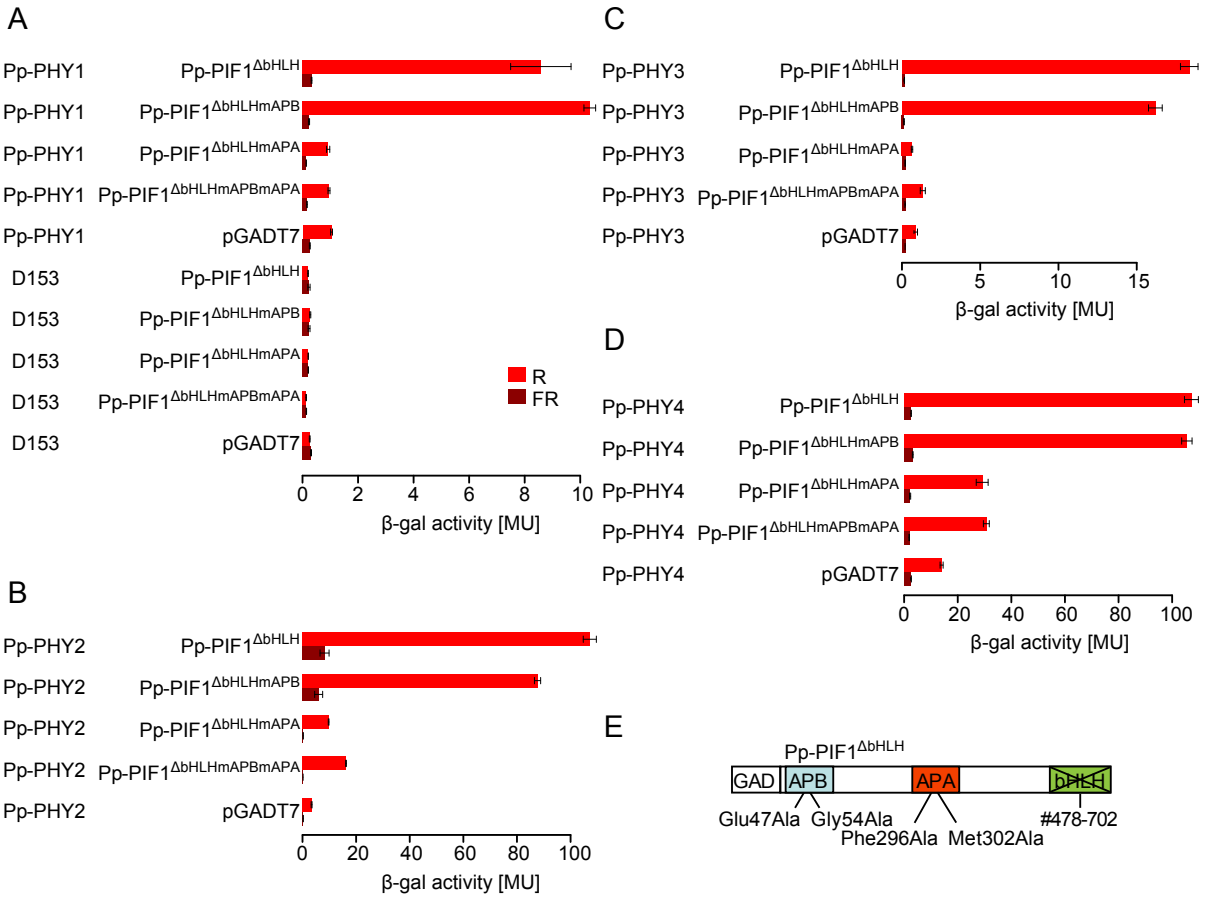
For corresponding measurements of β–galactosidase activity refer to Figure 4 and Supplemental Figure 7. The Pp-PIF1-LUC panels are duplicated in Figure 4 and Supplemental Figure 7.



Supplemental Figure 9. Interaction of Pp-PIF1 with light-activated phytochromes.
(Supports Figure 4.)

(A-D) Interaction of full-length Pp-PIF1 with Pp-PHYs requires APA motif similar to LUC-Pp-PIF1 (Figure 4). GAD plasmids (pGADT7) containing the coding sequence for Pp-PIF1 and its mutated versions (Pp-PIF1^{mAPB}, Pp-PIF1^{mAPA}, Pp-PIF1^{mAPBmAPA}), respectively, fused to the GAL4 activation domain (GAD) were used in yeast-two-hybrid assays with GBD plasmids (D153) containing the coding sequence for Pp-PHY1 to 4 fused to the GAL4 DNA binding domain (GBD). PHYs were converted into the Pfr or Pr form by irradiating yeast cultures for 5 min with R (12 µmol m^{-2} s^{-1}) or FR (12 µmol m^{-2} s^{-1}) light. The β–galactosidase activity was measured after an additional incubation in the dark for 4 h. MU, Miller Units. Bars indicate the mean of three biological replicates (i.e., three independent cultures were grown; each culture was measured in triplicate); error bars represent 95 % confidence interval.

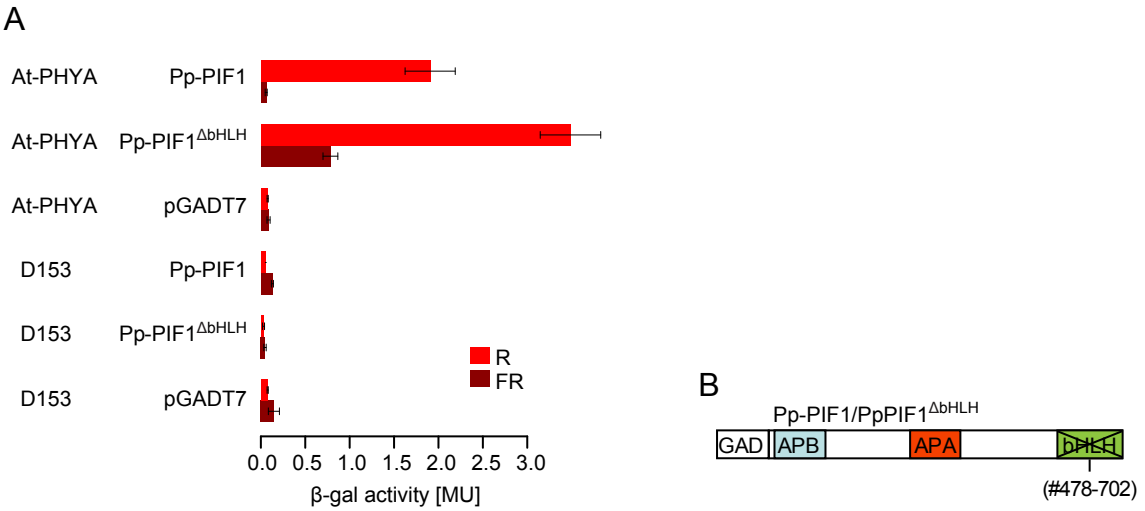
(E) Mutations inserted in Pp-PIF1 are shown schematically.



Supplemental Figure 10. Interaction of Pp-PIF1$^{\Delta bHLH}$ with light-activated phytochromes.
(Supports Figure 4.)

(A-D) Pp-PIF1$^{\Delta bHLH}$ interacts with Pp-PHYs in a light-dependent manner. This interaction requires the APA motif. GAD plasmids (pGADT7) containing the coding sequence for Pp-PIF1$^{\Delta bHLH}$ and its mutated versions (Pp-PIF1$^{\Delta bHLHmAPB}$, Pp-PIF1$^{\Delta bHLHmAPA}$, Pp-PIF1$^{\Delta bHLHmAPBmAPA}$), respectively, fused to the GAL4 activation domain (GAD) were used in yeast-two-hybrid assays with GBD plasmids (D153) containing the coding sequence for Pp-PHYs fused to the GAL4 DNA binding domain (GBD). Phytochromes were converted into the Pfr or Pr form by irradiating yeast cultures for 5 min with R (12 µmol m^{-2} s^{-1}) or FR (12 µmol m^{-2} s^{-1}) light. The β–galactosidase activity was measured after an additional incubation in the dark for 4 h. MU, Miller Units. Bars indicate the mean of three biological replicates (i.e., three independent cultures were grown; each culture was measured in triplicate); error bars represent 95 % confidence interval.

(E) Mutations inserted in Pp-PIF1 APB and APA motifs as well as the deletion of the bHLH domain are shown schematically.

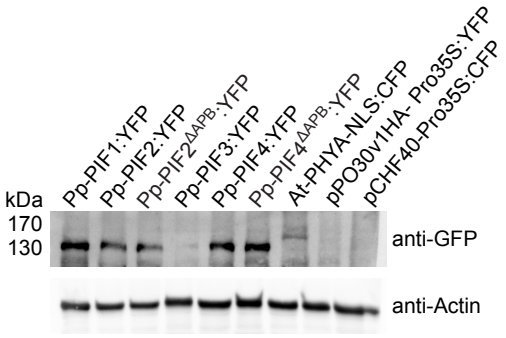


Supplemental Figure 11. Interaction of Pp-PIF1 and Pp-PIF1$^{\Delta bHLH}$ with light-activated At-PHYA.

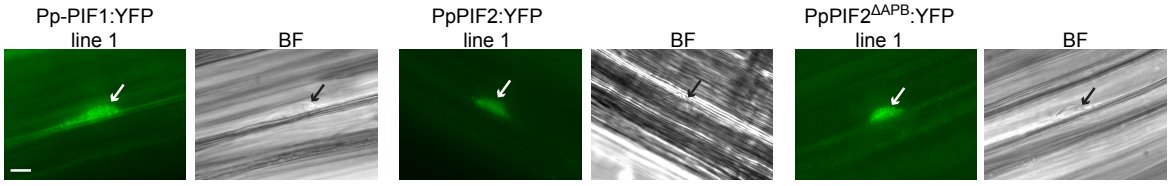
(Supports Figure 4.)

(A) Pp-PIF1 and Pp-PIF1$^{\Delta bHLH}$ interact with At-PHYA. GAD-Pp-PIF1/Pp-PIF1$^{\Delta bHLH}$ plasmids (pGADT7) and At-PHYA-GBD plasmid (D153) were used in yeast-two-hybrid assays. At-PHYA was converted into the Pfr or Pr form by irradiating yeast cultures for 5 min with R (12 µmol m^{-2} s^{-1}) or FR (12 µmol m^{-2} s^{-1}) light. The β–galactosidase activity was measured after an additional incubation in the dark for 4 h. MU, Miller Units. Bars indicate the mean of three biological replicates (i.e., three independent cultures were grown and measured); error bars represent 95% confidence interval.

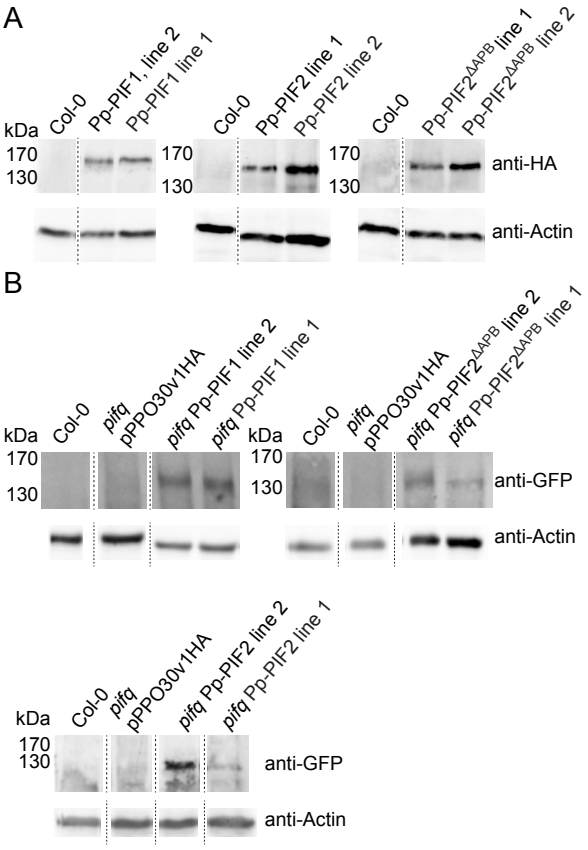
(B) The deletion of the bHLH domain inserted in Pp-PIF1 is shown schematically.



Supplemental Figure 12. Immunoblot analysis of Pp-PIF expression in *N. benthamiana*.
(Supports Figure 6.)
Pp-PIFs and At-PHYA-NLS were expressed as full-length proteins in *N. benthamiana*. *N. benthamiana* leaves were transformed with plasmids as depicted. To monitor protein abundance, Pp-PIFs fused to an YFP-tag (pPO30v1HA vector) and At-PHYA fused to an CFP-tag (pCHF40 vector) were detected in total protein extracts by protein gel blot analysis using an GFP-antibody (anti-GFP). Protein extracts from leaves transformed with empty vectors were used as negative controls. The detection of Actin using an Actin-antibody (anti-Actin) is shown as loading control.
For corresponding localization analyses in epidermal leave cells refer to Figure 6.



Supplemental Figure 13. Localization of PIFs from *P. patens* in *A. thaliana* seedlings.
(Supports Figure 7.)
Pp-PIF1, Pp-PIF2 and Pp-PIF2$^{\Delta APB}$ localize to the nucleus in *A. thaliana*. Col-0 seedlings expressing Pro35S-driven Pp-PIF1:YFP, Pp-PIF2:YFP or Pp-PIF2$^{\Delta APB}$:YFP were grown for 4 to 5 d in darkness before epifluorescence microscopic analysis. Bar = 10 µm. Arrows indicate nuclei. BF = bright field.

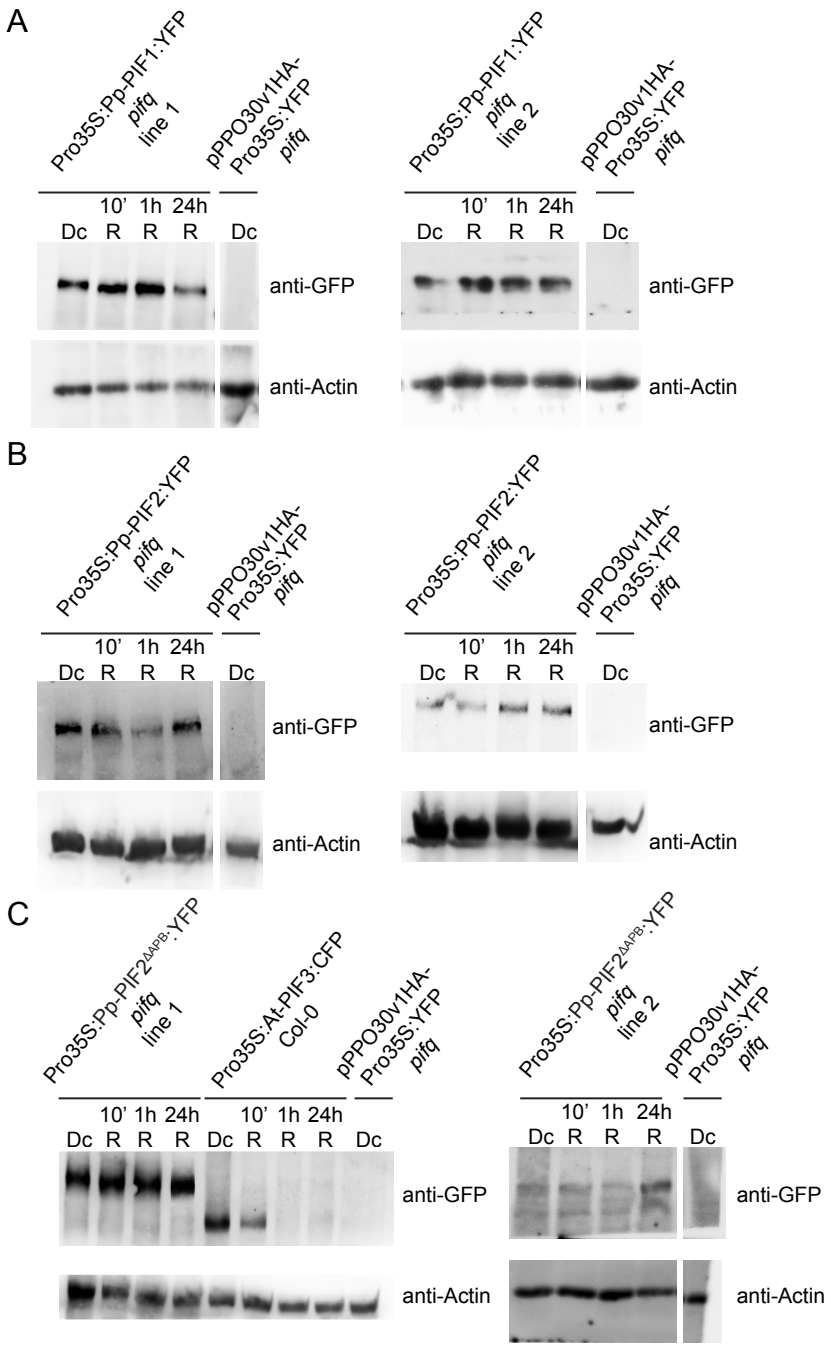


Supplemental Figure 14. Immunoblot analysis of Pp-PIF expression in *A. thaliana* Columbia-0 (Col-0) and *pifq* mutant seedlings.

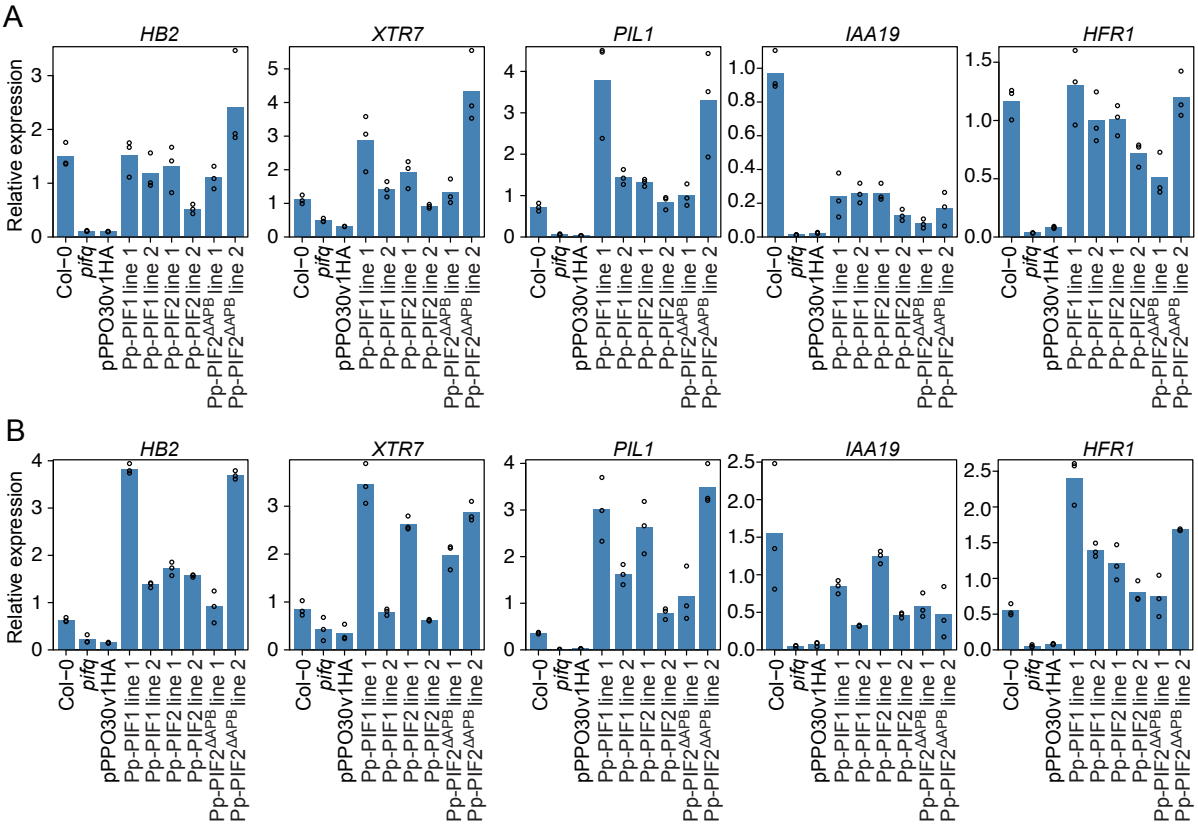
(Supports Figures 7 and 8.)

(A) Pp-PIF1, Pp-PIF2 and Pp-PIF2$^{\Delta APB}$ are expressed as full-length proteins in corresponding Pp-PIF overexpressor lines. *A. thaliana* Col-0 seedlings expressing Pro35S-driven Pp-PIF1:YFP, Pp-PIF2:YFP or Pp-PIF2$^{\Delta APB}$:YFP fused to an HA tag (HA) were grown in darkness for 4 days before harvesting. Pp-PIFs were detected in total protein extracts by immunoblot analysis using an antibody specific to the HA-tag (anti-HA). The detection of Actin protein using an Actin-specific antibody (anti-Actin) is shown as loading control.

(B) Pp-PIF1, Pp-PIF2 and Pp-PIF2$^{\Delta APB}$ are expressed as full-length proteins in corresponding Pp-PIF overexpressor lines. *A. thaliana pifq* seedlings expressing Pro35S-driven Pp-PIF1:YFP, Pp-PIF2:YFP or Pp-PIF2$^{\Delta APB}$:YFP fused to an GFP tag (GFP) were grown in darkness for 4 days before harvesting. Pp-PIFs were detected in total protein extracts by immunoblot analysis using an antibody specific to the GFP-tag (anti-GFP). The detection of Actin protein using an Actin-specific antibody (anti-Actin) is shown as loading control.



Supplemental Figure 15. Pp-PIF protein stability in *A. thaliana pifq* mutants.
(Supports Figure 8.)
(A) Pp-PIF protein stability in response to red light (R) was analysed by immunoblot. After 4 days in darkness (Dc), *pifq* seedlings expressing Pro35S:Pp-PIF1 were exposed to R. Pp-PIF1 fused to a YFP-tag (pPPO30v1HA vector) was detected in total protein extracts by immnuoblot using a GFP-antibody (anti-GFP). The detection of Actin using an Actin-antibody (anti-Actin) is shown as loading control.
(B,C) *A. thaliana pifq* seedlings expressing Pro35S:Pp-PIF2 and Pro35S:Pp-PIF2$^{\Delta APB}$, respectively, were analyzed as described in (A). Col-0 seedlings expressing Pro35S:At-PIF3 were used to monitor R-dependent degradation (C).



Supplemental Figure 16. Complementation of expression phenotypes in *A. thaliana pifq* mutants through expression of Pp-PIFs.

(Supports Figure 8.)

(A, B) Biological replicates of the expression analysis of PIF-dependent genes in *A. thaliana pifq* mutants expressing Pp-PIFs as shown in Figure 8C. The expression of the genes *HB2*, *XTR7*, *PIL1*, *IAA19* and *HFR1* was analysed by quantitative real-time PCR in 4 days dark-grown Col-0, *pifq* and *pifq* seedlings expressing Pro35S-YFP (pPPO30v1HA, empty vector control) or Pro35S-Pp-PIF1:YFP, Pro35S-Pp-PIF2:YFP and Pro35S-Pp-PIF2$^{\Delta APB}$:YFP, respectively. Data were normalized to *PP2AA3* mRNA. Technical replicates (repeats within an experiment) are shown as circles; bars represent the mean. Y-axis scale was adjusted for maximal resolution in each plot. Gene accession numbers are listed in the Supplemental Methods.

Name	Sequence (5' à 3')	Restriction Sites
ah010	TTA CAC CAT CCG GAG GTC AG	-
ah042	CGC GGA TCC CGC TCT AGA ATG GTG AGC AAG GGC GAG G	BamHI
ah094	GGA CTA GTT ATC TAG AGC CCT AGG ATC CGC CTT GTA CAG CTCGTC CAT G	SpeI
ah127	CGC GGA TCC AAA AAT GCC TCT GTT TGA GCT TTT CA	BamHI
ah224	GAA GAT CTA AAA ATG GAA GAC GCC AAA AAC A	BglI
ah225	GGA CTA GTT ATC TAG AGC TTA CCT AGG ATC CGC CAC GGC GAT CTT TCC GCC C	BamHI, AvrII XbaI, SpeI
ah385	GGA CTA GTT GCG GCC GCT CCT CCA ACC T	SpeI
ah708	GGA CTA GTT TGA AGT GGA CCT CCA CCC A	SpeI
ah772	CGC GGA TCC TAG GAG ATC TTG AGT GTT GTT CCA GTT TG	BamHI, AvrII, BglII
ah773	GCT CTA GAA CTA GTG CGC AAC GCA ATT AAT GTG	SpeI, XbaI
ah787	GTA CGT CGT ATG GGT AGC TAG CCT TGT ACA GCT CGT CCA TG	-
ah788	GGA CTA GTT TAA GCG TAA TCT GGT ACG TCG TAT GGG TAG C	SpeI
ah791	AGC TGA TCT CAG AGG AGG ACC TGG CTA GCA TGG TGA GCA AGG GCG AG	-
ah792	GAA GAT CTA AAA ATG GCC GAG GAG CAG AAG CTG ATC TCA GAGGAG G	BglII
ah839	CGC GGA TCC AAA AAT GAG TCT CTA TGT GCC A	BamHI
ah840	GGA CTA GTG AAA AAA TGA GTC TCT GTG TGC CAG	SpeI
ah841	GGA CTA GTA TGA GGT GGG ACC CCA CTC	SpeI
ah842	GCT CTA GAA AAA TGA GCG AGG TGT TGG AGG	XbaI
ah843	GCT CTA GAA TGA GAT GGA CCC CCA TTC	XbaI
ah844	GAA GAT CTA AAA ATG AAT CGT CTT GTG CCA G	BglII
ah845	GCG CCT AGG ATG AGG AGG ACC GCC ATT C	AvrII
ah849	GTG GTT CAA GTT TAT TAG CG	-
ah852	GGA CTA GTT GGA AGT AAC AGA GGG ACC AC	SpeI
ah867.2	CGC GGA TCC TAG GCG ACG ATC CAC AAA ACT GAT CA	BamHI, AvrII
ah994	GGA CTA GTT GTA ATA GGT TTA TGT TTT GTG TCT ACC	SpeI
ah995	GGA CTA GTT GTT GCA GGT TTT TGT TTG GTG	SpeI
p079	GGT TTC CTA GGG CTA GCA AAA ATG TCG ACT CCC AAG AAG A	AvrII, NheI
p080	GGT TTC CTA GGG CTA GCC ATT TGA CTT GAA GCA TCA TCC C	AvrII, NheI
p081	GGT TTC CTA GGG CTA GCA AAA ATG TCG ACC ACC AAG TTG G	AvrII, NheI
p083	GGT TTC CTA GGG CTA GCT CTC ACA CTG CCT GCA TCA	AvrII, NheI
p084	GGT TTC CTA GGG CTA GCA AAA ATG TCG GCT CCG AAG AAG A	AvrII, NheI
p085	GGT TTC CTA GGG CTA GCC ATT TGA CTT GCA GCA TCA TCC T	AvrII, NheI
p086	GGT TTC CTA GGG CTA GCA AAA ATG TCG ACC CCC AAG TTG	AvrII, NheI
p087	GGT TTC CTA GGG CTA GCG ACA ACA GTA GAT CTC ACA CTA CCT	SpeI
Pp-PHY1 F	AAA CTC GAG ATG TCG ACT CCC AAG	XhoI
Pp-PHY1 R	AAA GGT ACC TCA CAT TTG ACT TGA AGC	KpnI
Pp-PHY2 F	AAA CTC GAG ATG TCG ACC CCC AAG	XhoI
Pp-PHY2 R	AAA GGT ACC TCA GAC AAC AGT AGA TCT C	KpnI
Pp-PHY3 F	AAA GTC GAC ATG TCG GCT CCG AAG	SalI
Pp-PHY3 R	AAA GGT ACC TCA CAT TTG ACT TGC AGC	KpnI
Pp-PHY4 F	AAA CTC GAG ATG TCG ACC ACC AAG	XhoI
Pp-PHY4 R	AAA GGT ACC TCA TCT CAC ACT GCC TGC	KpnI
Pp-PIF2 F	AAA ACT AGT TAT GAG TCT CTG TGT GC	SpeI
Pp-PIF2 R	AAA CTC GAG TCA ATG AGG TGG GAC CCC AC	XhoI
Pp-PIF3 F	GCT AAG CTT ATG AAT CGT CTT GTG CCA GAG	HindIII
Pp-PIF3 R	AAA GCG GCC GCT TAA TGA GGA GGA CCG CCA TT	NotI
Pp-PIF4 F	GCT AAG CTT ATG AGC GAG GTG TTG GAG GGT	HindIII
Pp-PIF4 R	AAA GCG GCC GCT TAA TGA GAT GGA CCC CCA TT	NotI
Pp-PIF4$^{\Delta APB}$F	GCT AAG CTT ATG GCC ATG GAG GCC AGT GAA	HindIII
Pp-PIF4$^{\Delta APB}$R	AAA GCG GCC GCT TAA TGA GAT GGA CCC CCA TT	NotI
tx020 R	TGC ATT ATC ATT CCA AGC CAC TGC CAA AGT ATC CTG ATC AGG C	-
tx021 F	GCA GTG GCT TGG AAT GAT AAT GCA AAA ATA GAC ATC CAA GGA C	-
tx022 R	CGC CGC CGC TGG CCT CGA AGC GTG AGA GAA ATT CAT GGA ACC	-
tx023 F	GCT TCG AGG CCA GCG GCG GCG GTT AAA GCT AAT TTG CAT AGT C	-
tx024 R	CGC GGC TGC AGG CCT CGA AGC GTG AGA GAA ATT CAT GGA TC	-
tx025 F	GCT TCG AGG CCT GCA GCC GCG GTC AAA GCA AAT TTG CAT AG	-
tx040 F	GAA GAT CTA AAA ATG CAG GGG CAA AGT AGT AAA TG	BglII

gBlock fragment gB-ah003 (HindIII and BamHI sites are underlined; the silent T90A nucleotide exchange is indicated in lower case)

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TGATGCTTTACTAGAGGCTGCGGTGAATGAAGCACCCACGGAAGTACATCCTGGGCATCACATCGATGTTGCTCATGACG
AAATGGTGTCTTGGTTGCAATATCCACTGGATGATACGTTGGAGAGGAACTACTGCTCCGATTTTTTTGGAGAGCTTCCAG
ATTCCCACACTCAGTTATTGAGAGAGTCTTTTGGGCATGGATCCACCAAGACAG

Supplemental Table 1. List of primers used for cloning. Restriction sites are underlined; mutated nucleotides are highlighted in red.

Gene identifier	Sequence (5' à 3')	Gene description
Pp1s545_10V6.1	TGC CCT CTT TTC AAT TCC AC	Thioredoxin
Pp1s545_10V6.1	ACA AAG TGC CGG TTT ACG TC	Thioredoxin
Pp1s174_62V6.2	TGA GTG GGT ACA GTG CGA AG	Phototropin
Pp1s174_62V6.2	TCA GCA AAC GAC CAC AGA AG	Phototropin
Pp1s213_80V6.1	CCT TGA GGG ACG ACT ACG AG	Lhc SR1
Pp1s213_80V6.1	GAT CTC TTC ACC CGA GAC CA	Lhc SR1
Pp1s126_141V6.2	CAC AGA GCG AAA GGT CAC AA	sigma factor
Pp1s126_141V6.2	CTT CGT CAG CTT CCC TTC AC	sigma factor
Pp1s117_16V6.1	CCT GTC ATT GCT GAG GTG AA	no apical meristem
Pp1s117_16V6.1	TGT GGA TGG GTT TGT CTG TG	no apical meristem
Pp1s48_151V6.1	GAG GAG GTT TTT GCT GAT GC	malat-dehydrogenase
Pp1s48_151V6.1	CGA TCA GAG CAT TCG TGT TG	malat-dehydrogenase
Pp1s215_36V6.1	ACG AGT GAA CTG GTC CAA GG	pectinesterase family
Pp1s215_36V6.1	CGG TAG TGG CTC AGT GCA TA	pectinesterase family
AT3G15540	TTC CGT GGC ATC GGT GTG GC	IAA19
AT3G15540	GCT GCA GCC CAA ACC CGG TAG	IAA19
AT1G02340	ATT GGC CAT TAC CAC CGT TTA C	HFR1
AT1G02340	TGA GGA GAA GAA GCT GGT GAT G	HFR1
AT2G46970	AAA TTG CTC TCA GCC ATT CGT GG	PIL1
AT2G46970	TTC TAA GTT TGA GGC GGA CGC AG	PIL1
AT4G14130	CGG CTT GCA CAG CCT CTT	XTR7
AT4G14130	TCG GTT GCC ACT TGC AAT T	XTR7
AT4G16780	GTC GTT GCC GGT CAA TGC	ATHB-2
AT4G16780	CCT AGG ACG AAG AGC GTC AAA A	ATHB-2
AT1g13320	TAT CGG ATG ACG ATT CTT CGT GCA G	PP2AA3
AT1g13320	GCT TGG TCG ACT ATC GGA ATG AGA G	PP2AA3

Supplemental Table 2. List of primers used for quantitative real-time PCR.

SUPPLEMENTAL METHODS

Cloning of constructs

A list including all primers used for the generation of DNA constructs can be found in the lists at the end of this document.

The coding sequences of Pp-PIFs were amplified by PCR on *P. patens* cDNA using the primers ah839/ah708 for Pp-PIF1 and ah840/ah841 for Pp-PIF2 and Pp-PIF2$^{\Delta APB}$, ah844/ah845 for Pp-PIF3, and tx040/ah843 for Pp-PIF4$^{\Delta APB}$; the PCR products were introduced into pJET1.2 (Thermo Fischer Scientific/Fermentas) via blunt end ligation. The coding sequence for Pp-PIF4 (corresponing to Pp1s147_126V6.1) was obtained as follows. A PCR fragment was amplified from total cDNA using the primers ah842/ah843 and blunt end ligated into pJET1.2. This fragment was similar to Pp1s147_126V6.1 but contained genomic DNA inserted between nucleotide 126 and 127 of Pp1s147_126V6.1. To obtain the coding sequence of Pp1s147_126V6.1 we ordered the gBlock fragment gb-ah003 (IDT) coding for the first 651 nucleotides of Pp1s147_126V6.1 and containing a silent mutation (T90A) to disrupt the a BglII site in Pp1s147_126V6.1. The gBlock fragment was cut with HindIII and BamHI and ligated into the HindIII/BamHI site of pJET containing the initial PCR fragment.

The coding sequences of Pp-PHYs were amplified by PCR on *P. patens* cDNA using the primers p079/p080 for Pp-PHY1, p086/p087 for Pp-PHY2, p084/p085 for Pp-PHY3 and p081/p083 for Pp-PHY4; the PCR products for Pp-PHY1, Pp-PHY2 and Pp-PHY3 were introduced into pJET1.2 and the PCR product for Pp-PHY4 into the TOPO vector (life technologies/Invitrogen) via blunt end ligation.

D153ah:At-PHYA is a yeast two hybrid vector coding for At-PHYA:GAL4 BD, which has been described previously (Hiltbrunner et al., 2006).

D153ah:At-FHY1 is a yeast two hybrid vector containing a BamHI-XbaI-At-FHY1-SpeI cassette. It was obtained by cutting At-FHY1 from pGADT7-FHY1(Hiltbrunner et al., 2005) using BamHI/SpeI and ligating it into the BamHI/SpeI site of D153ah:At-PHYA to replace At-PHYA.

To generate yeast two hybrid vectors coding for Pp-PHY:GAL4 BD we cut Pp-PHY1 and Pp-PHY3 from the corresponding pJET constructs using AvrII and ligated them into D153ah:At-FHY1 digested with XbaI/SpeI. Pp-PHY2 and Pp-PHY4 were cut from the corresponding pJET and TOPO constructs, respectively, using NheI and ligated into D153ah:At-FHY1 digested with XbaI/SpeI, thereby replacing At-FHY1.

Yeast two hybrid vectors coding for Pp-PIF:GAL4 AD were generated as follows. Pp-PIF1 was cut from pJET:Pp-PIF1 using BamHI/SpeI and ligated into pGADT7:At-FHY1 (Hiltbrunner et al., 2005) digested with BamHI/SpeI. Pp-PIF2 was cut from the corresponding pJET construct with SpeI and ligated into pGADT7:At-FHY1 digested with XbaI/SpeI, thereby replacing At-FHY1. Pp-PIF3 was cut from pJET:Pp-PIF3 using BglII/AvrII and ligated into pGADT7:At-FHY1 digested with BamHI/SpeI. Pp-PIF4 and Pp-PIF4$^{\Delta APB}$ were cut from pJET:Pp-PIF4 and pJET:Pp-pJET:Pp-PIF4$^{\Delta APB}$, respectively, using BglII/XbaI and ligated into pGADT7:At-FHY1 digested with BamHI/SpeI. To obtain a truncated version of Pp-PIF1 lacking the bHLH domain, we amplified a fragment using the primers ah849/ah994 and the corresponding pGADT7:Pp-PIF construct as template. The PCR fragment was

digested with XbaI/SpeI and ligated into the corresponding pGADT7:Pp-PIF construct digested with XbaI/SpeI, thereby removing the bHLH domains.

To generate Pp-PIF mutants (Pp-PIF1^{mAPB}, Pp-PIF1^{mAPA}, Pp-PIF1^{mAPAmAPB}, Pp-PIF2^{mAPA}, Pp-PIF2$^{\Delta APBmAPA}$), we performed site directed mutagenesis using overlap extension PCR. Four primer pairs were designed in order to introduce the corresponding mutated sites into Pp-PIF sequences. For Pp-PIF1^{mAPB}, an overlap-PCR product was obtained using the primers ah839/tx020 and tx021/ah708 and pJET:Pp-PIF1 as template. Pp-PIF1^{mAPA} was obtained using the primers ah839/tx022 and tx023/ah708 and pJET:Pp-PIF1 as template. The primers ah839/tx022 and tx023/ah708 were also used for the generation of Pp-PIF1^{mAPBmAPA}, using the construct Pp-PIF1^{mAPB} as template. Overlap PCR products were obtained with the primers ah840/tx024 and tx025/ah841 and the templates pJET:Pp-PIF2 and pJET:Pp-PIF2$^{\Delta APB}$ in order to generate Pp-PIF2^{mAPA} and Pp-PIF2$^{\Delta APBmAPA}$, respectively. Mismatched nucleotides are highlighted in primer sequences; mutations were confirmed by DNA sequencing.

All Pp-PIF1 mutants were cut from corresponding pJET constructs using BamHI/SpeI and ligated into the BamI/SpeI site of pGADT7:At-FHY1 (Hiltbrunner et al., 2005) to replace At-FHY1. For Pp-PIF2^{mAPA}, Pp-PIF2$^{\Delta APBmAPA}$, SpeI digested products were ligated into the plasmid pGADT7:At-FHY1 digested with XbaI/SpeI. To obtain Pp-PIF1-Luciferase fusions, all pGADT7:Pp-PIF1 constructs were digested with BamHI and SpeI and ligated into the BamHI/SpeI site of pGADT7:Luc.

pGADT7:Luc is a yeast two hybrid vector containing an AD-Luc-BamHI-AvrII-XbaI-SpeI cassette. It was obtained as follows: The Luc coding sequence was PCR amplified from pCHF91 (Sheerin et al., 2015) using the primers ah224 and ah225, cut with BglII/SpeI and ligated into the BamHI/SpeI site of pGADT7-At-FHY1 to replace At-FHY1.

For *in vitro* co-immunoprecipitation (co-IP) assays *P. patens* phytochromes (Pp-PHY1 to 4) coding sequences were PCR amplified using the respective plasmids described above and the following primers: Pp-PHY1 F/Pp-PHY1 R for Pp-PHY1, Pp-PHY2 F/Pp-PHY2 R for Pp-PHY2, Pp-PHY3 F/Pp-PHY3 R for Pp-PHY3 and Pp-PHY4 F/Pp-PHY4 R for Pp-PHY4. The PCR products were cloned into pTNT vector (Promega, Madison, WI). The coding sequences for Pp-PIF2, Pp-PIF2^{mAPA}, Pp-PIF2$^{\Delta APB}$, Pp-PIF2$^{mAPA\Delta APB}$, Pp-PIF3, Pp-PIF4 and Pp-PIF4$^{\Delta APB}$ were PCR amplified using the respective plasmids described above and primers Pp-PIF2 F/Pp-PIF2 R, Pp-PIF3 F/Pp-PIF3 R, Pp-PIF4 F/Pp-PIF4 R and Pp-PIF4$^{\Delta APB}$ F/Pp-PIF4$^{\Delta APB}$ R, respectively. The PCR products were cloned into pET17GAD vector (EMD Biosciences Inc., Madison, WI). All the vectors were verified by sequencing and expression in *in vitro* TNT expression system (Promega, Madison, WI).

pPPO30v1HA is a T-DNA vector containing a Pro35S:BamHI-XbaI-YFP-HA:TerRbcS cassette and a Butafenacil/Inspire resistance gene for selection of transgenic plants (Rausenberger et al., 2011). It was obtained as follows: YFP was PCR amplified from pCHF30 (Hiltbrunner et al., 2006) using the primers ah042 and ah787. The resulting PCR fragment was then used as template for another PCR with primers ah042 and ah788. This PCR fragment was digested with BamHI/SpeI and ligated into the BamHI/XbaI site of pPPO5v1 (Sheerin et al., 2015). Using the primers ah772 and ah773 the beta-Gal alpha fragment of pBS II KS (Stratagene) was amplified, cut with BamH/XbaI and ligated into the BamHI/XbaI site of pPPO30v1HA resulting in pPPO30v1-HA-beta-Gal alpha

For transformation of tobacco and *Arabidopsis*, respectively, we cloned Pp-PIF coding sequences into pPPO30v1HA. Pp-PIF1 was cut from pJET:Pp-PIF1 using BamHI/SpeI and ligated into the BamHI/XbaI site of pPPO30v1HA. Pp-PIF2 and Pp-PIF2$^{\Delta APB}$, respectively, were cut from the corresponding pJET constructs using SpeI and ligated into pPPO30v1HA-beta-Gal alpha digested with AvrII/XbaI to replace the beta-Gal alpha fragment. Pp-PIF3 was cut from pJET:Pp-PIF3 using BglII/AvrII and ligated into pPPO30v1HA digested with BamHI/XbaI. Pp-PIF4 and Pp-PIF4$^{\Delta APB}$ were cut from pJET:Pp-PIF4 and pJET:Pp-pJET:Pp-PIF4$^{\Delta APB}$, respectively, using BglII/XbaI and ligated into pPPO30v1HA digested with BamHI/XbaI.

A T-DNA vector containing a Pro35S:myc:mCerulean: TerRbcS cassette was obtained as follows: First, a fragment coding for mCerulean and part of the myc tag was amplified from mCerulean cDNA (Koushik et al., 2006) using the primers ah791/ah094. This fragment was then used as template for a second PCR with primers ah792/ah094 to add the remaining part of the myc tag. Finally, the PCR fragment was cut with BglII/SpeI and ligated into BamHI/XbaI site of pCHF5 (Hiltbrunner et al., 2005). D153ah:At-PHYA:NLS is a Y2H BD vector containing At-PHYA-NLS-BD. A PHYA fragment containing the NLS was PCR-amplified from ProPHYA:PHYA:NLS:GFP5 (Genoud et al., 2008) using the primers ah010/ah385, cut with XbaI/SpeI and ligated into D153ah:At-PHYA digested with XbaI/SpeI. The cloning of the Pro35S:At-PHYA:NLS:CFP:TerRbcS construct is described in Sheerin et al. (2015).

pUC1930 contains a Pro35S:BamHI:XbaI:YFP:TerRbcS cassette and has been described previously (Possart and Hiltbrunner, 2013). To generate constructs coding for Pro35S:Pp-PIF:YFP:TerRbcS that were used for particle bombardment we cut Pp-PIF1 with BamHI/SpeI, Pp-PIF2 and Pp-PIF2$^{\Delta APB}$, respectively, with SpeI, PpPIF3 with BglII/AvrII, Pp-PIF4 and Pp-PIF4$^{\Delta APB}$, respectively, with BglII/XbaI from the pJET:Pp-PIF constructs. The obtained fragments were ligated into pUC1930 digested with BamHI/XbaI and XbaI, respectively.

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