Supplementary Materials

Light-induced phosphorylation and degradation of the negative regulator PIF1 depends upon its direct physical interactions with photoactivated phytochromes

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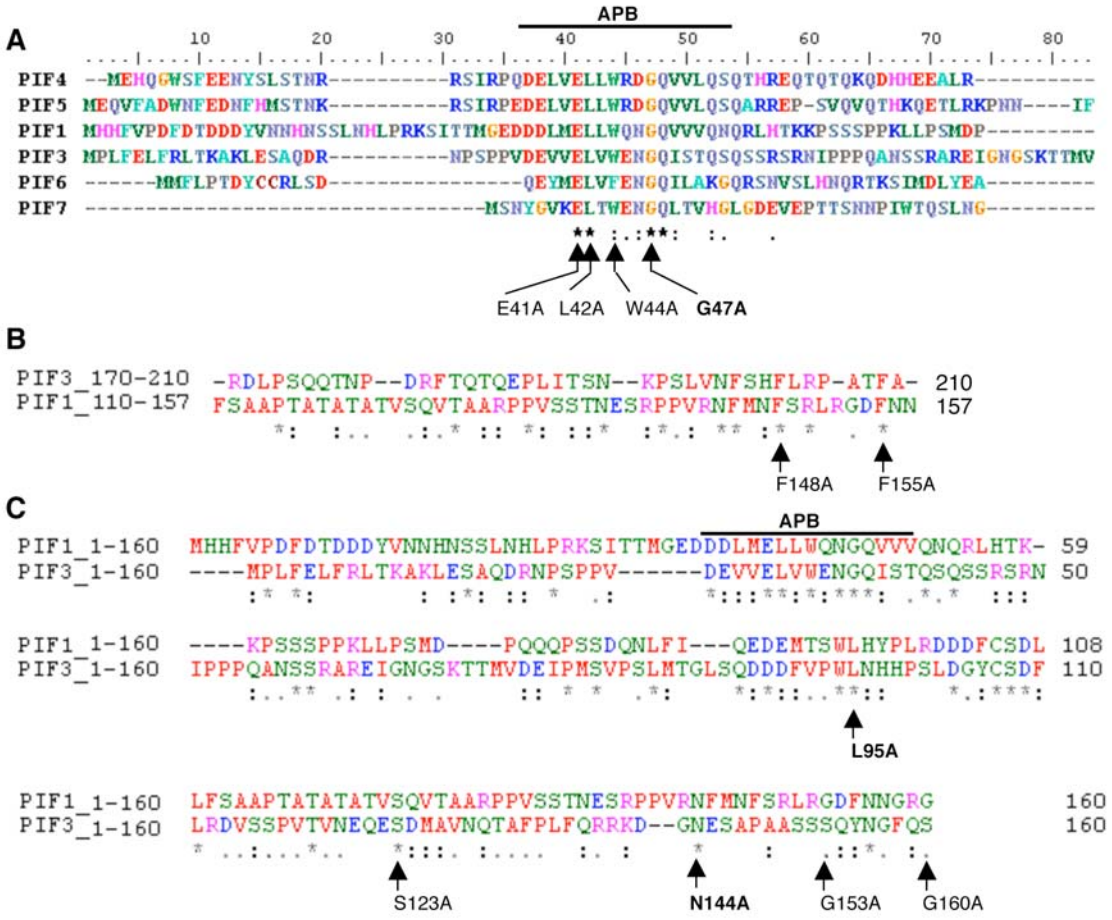
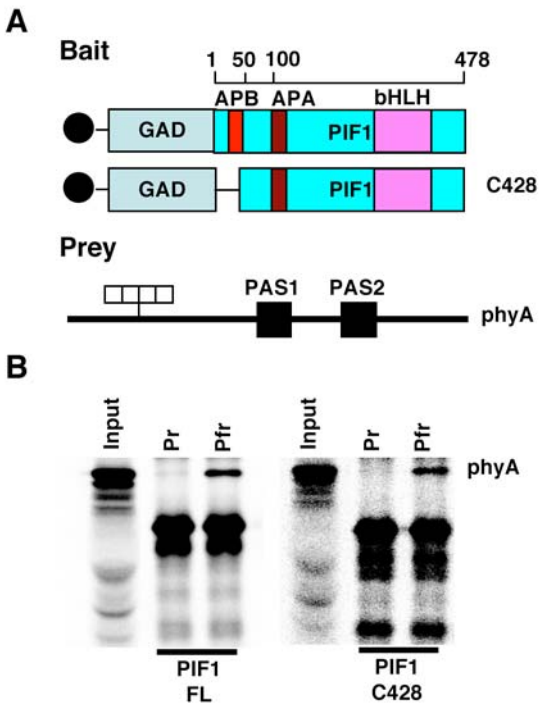
Supplementary Table 1: Primer sequences used in experiments described in text

Gene	Forward Primer	Reverse Primer
Cloning		
PIF1N150	CCAATCGATGTTCAAAACCAGAGACTTCA	AGATATTACATCTCTTGGTAGATGTTGT
PIF1C151-478	GATATCGATCTGAGAGGGGATTTTAAT	GTGGTCGACTCACCTGTTGTGTGGTTTC
PIF1-NT-bHLH	GTCATCGATAAGAGATCTCGTGCTGCT	CTGGTCGACATCATCTGTATTTGAA
PHYA-N100	CGACATATGGAGAATGCATCTGAGCTGTTG	CTACTTGTTTGCTGCAGCGAGCTCGAGTC
PHYA-NT	CGACATATGATGTCAGGCTCTAGGCCGACTC	GACTCGAGCTAACTATCCTTGAAAGCATTCCT
PHYA-CT	CGACATATGGAAACTACTGATGTGAATACA	CTACTTGTTTGCTGCAGCGAGCTCGAGTC
PHYA-C712	CGACATATGATGTCAGGCTCTAGGCCGACTC	GACTCGAGCTAAACTAAACTTATTGGCCCAGC
PHYA-C812	CGACATATGATGTCAGGCTCTAGGCCGACTC	GACTCGAGCTATAGACGACAACATGACTTCTG
PHYA-C1022	CGACATATGATGTCAGGCTCTAGGCCGACTC	GACTCGAGCTACATCAGCATGAAATCTGCCAA
PHYA-C1072	CGACATATGATGTCAGGCTCTAGGCCGACTC	GACTCGAGCTACATTTGGTTTAGTAAAAACTCAGG
Site-directed mutagenesis		
PIF1E293D	CATAATCTCTCTGACAGAAAACGGAGAGATAG	CTATCTCTCCGTTTTCTGTCAGAGAGATTATG
PIF1E41A	AGATGATGATCTTATGGCGCTTTTATGGCAGAACG	CGTTCTGCCATAAAAGCGCCATAAGATCATCATCT
PIF1L42A	ATGATGATCTTATGGAGGCTTTATGGCAGAACGGT	ACCGTTCTGCCATAAAGCCTCCATAAGATCATCAT
PIF1W44A	ATCTTATGGAGCTTTTAGCGCAGAACGGTCAAGTT	AACTTGACCGTTCTGCGCTAAAAGCTCCATAAGAT
PIF1G47A	GCTTTTATGGCAGAACGCTCAAGTTGTTGTTCAA	TTGAACAACAACTTGAGCGTTCTGCCATAAAAGC
PIF1N144A	CCGCCGGTGAGGGCCTTCATGAATTTCTC	GAGAAATTCATGAAGGCCCTCACCGGCGG
PIF1S123A	ACCGCGACGGTGGCTCAAGTCACCGCC	GGCGGTGACTTGAGCCACCGTCGCGGT
PIF1G160A	GATTTTAATAACGGTAGAGCTGGTGAATCTGGACC	GGTCCAGATTCACCAGCTCTACCGTTATTAAAATC
PIF1G153A	TTCTCGAGGCTGAGAGCGGATTTTAATAACGG	CCGTTATTAAAATCCGCTCTCAGCCTCGAGAA
PIF1L95A	GAAATGACTTCTTGGGCTCATTATCCTCTCCG	CGGAGAGGATAATGAGCCCAAGAAGTCATTTC
PIF1F148A	GAGGAACTTCATGAATGCCTCGAGGCTGAGAGGGG	CCCCTCTCAGCCTCGAGGCATTCATGAAGTTCCTC
PIF1F155A	GAGGCTGAGAGGGGATGCTAATAACGGTAGAGGTG	CACCTCTACCGTTATTAGCATCCCCTCTCAGCCTC
PIF1del11	CTTCAGATCAGAATCTTCATTATCCTCTCCGTGA	TCACGGAGAGGATAATGAAGATTCTGATCTGAAG
PIF1del34	CCTTCTTCAGATCAGAATGCTACCGCGACGGTGAG	CTCACCGTCGCGGTAGCATTCTGATCTGAAGAAGG
PIF1del43	CCGCACCTACTGCGACGGGTGAATCTGGACCGTT	AACGGTCCAGATTCACCCGTCGCAGTAGGTGCGG

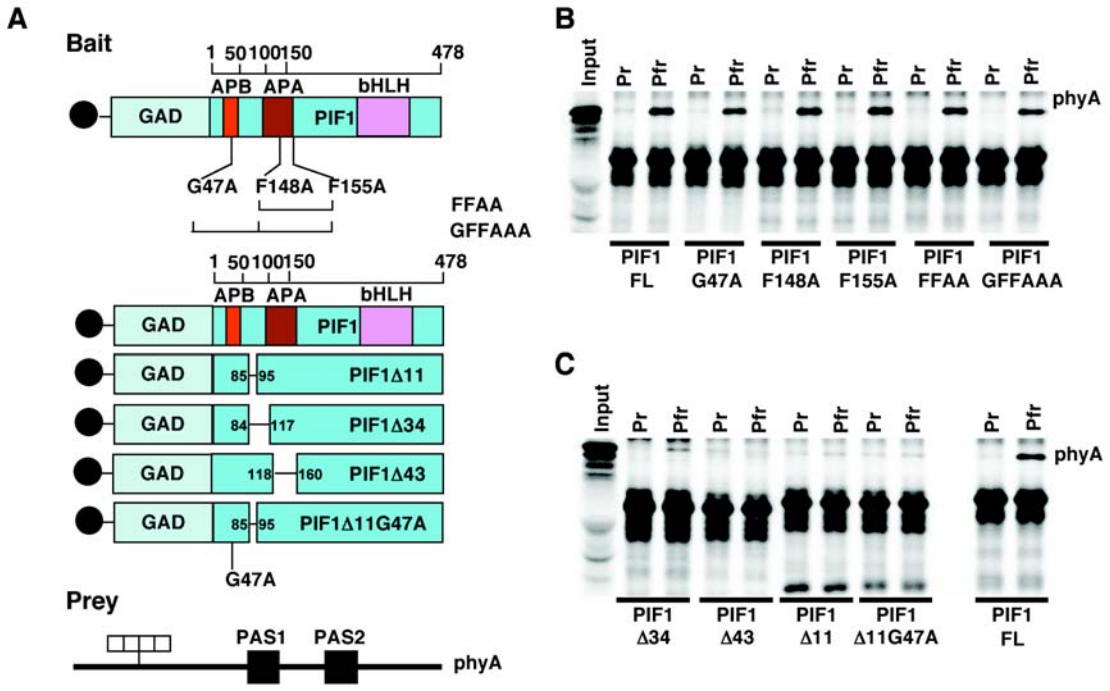


Supplementary Figure 1: Sequence alignments of the APB and APA motifs in PIFs.

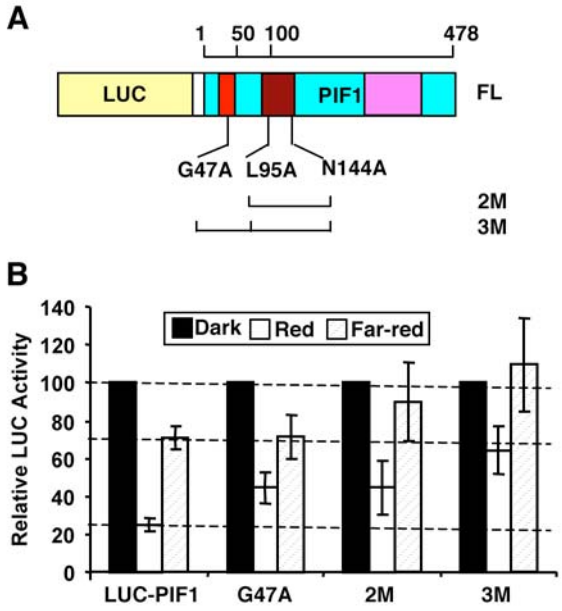
A) Alignment of the predicted amino acid sequences from the N-terminal regions of PIF1, and PIF3-PIF7. The putative APB motif is indicated by a thick line on the top. The amino acid residues mutated in PIF1 are shown at the bottom. Asterisks, colons and periods below the alignments indicate identical, conserved and semi-conserved amino acid residues, respectively. B) Sequence alignment of the putative APA motif present in PIF1 and PIF3. Amino acid regions in PIF1 (110-157) and PIF3 (170-210) are aligned. The phenylalanine residues critical for interaction between PIF3 and phyA are indicated by arrows. C) Alignment of the predicted amino acid sequences from the N-terminal 160 amino acid regions of PIF1 and PIF3. The amino acid residues (Leucine 95 and Asparagine 144) responsible for interaction between PIF1 and phyA are shown in bold beneath the alignment.



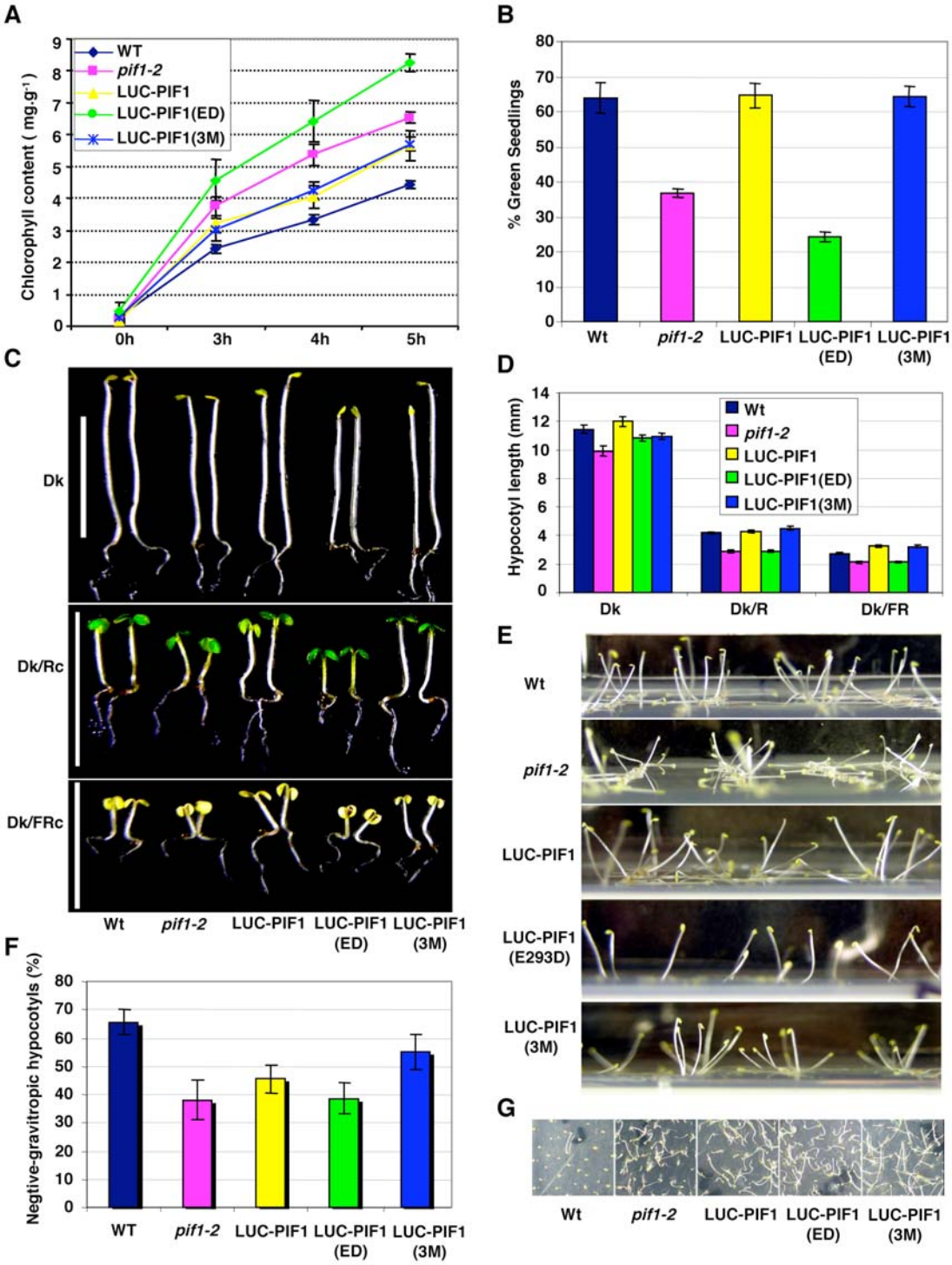
Supplementary Figure 2: The APB motif is not responsible for the interaction of PIF1 with phyA. A) Schematic diagram of the bait and prey used for the co-immunoprecipitation assays. The amino acid region deleted in the experimental construct C428 is indicated. B) Autoradiograph showing interactions of wild type PIF1, or a truncated PIF1 with the first 50 amino acids deleted (PIF1C428) with the Pr and Pfr forms of full length phyA. The leftmost lane of each set shows the input and the right two lanes show the pellet fractions from the *in vitro* co-immunoprecipitation assays.



Supplementary Figure 3: The putative APA motif present in PIF3 is not responsible for the Pfr-specific interaction of PIF1 with phyA. A) Schematic diagram of the bait and prey used for the co-immunoprecipitation assays. The amino acid residues mutated or deleted in each construct are marked. B) Autoradiograph showing interactions of wild type PIF1, PIF1G47A, PIF1F148A, PIF1F155A, PIF1FFAA and PIF1GFFAAA with the Pr and Pfr forms of phyA. The leftmost lane shows the input and the others show the pellet fractions from the *in vitro* co-immunoprecipitation assays. C) Autoradiograph showing interactions of wild type and various deletion mutants of PIF1 with the Pr and Pfr forms of phyA. The leftmost lane shows the input and the others show the pellet fractions from the *in vitro* co-immunoprecipitation assays.



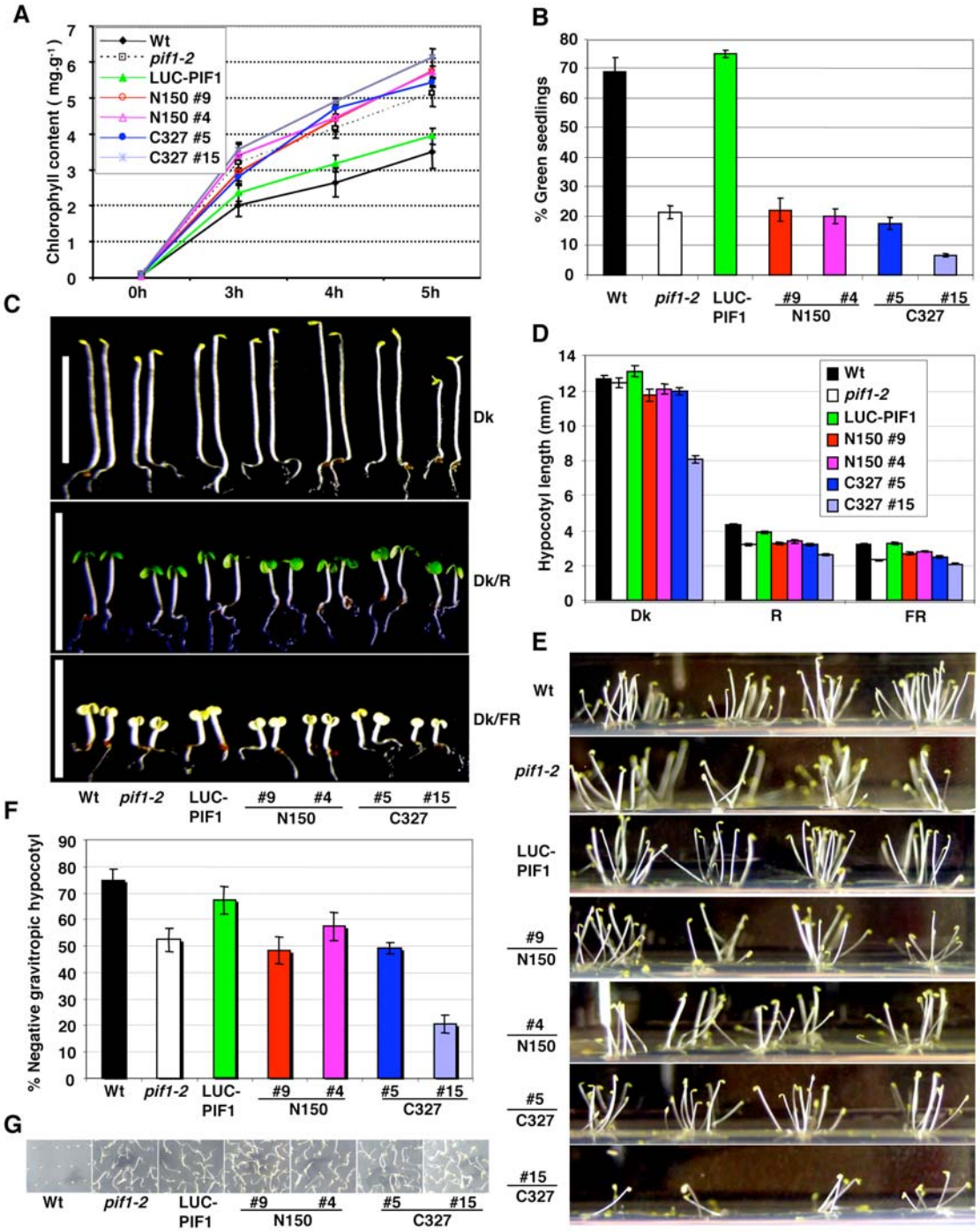
Supplementary Figure 4: Direct interactions of PIF1 with phyA and/or phyB are necessary for the light-induced degradation of PIF1. A) The amino acid residues mutated in the LUC-PIF1 constructs are shown. B) LUC activity was measured from 4-day-old dark-grown seedlings transferred to continuous R (10 μmol$m^{-2}s^{-1}$) or FR (10 μmol$m^{-2}s^{-1}$) light for 1 h as described (Shen et al., 2005). Means ± SE of five biological replicates are shown.



Supplementary Figure 5: Rescue of the *pif1-2* chlorophyll biosynthetic phenotypes in the transgenic seedlings expressing wt and point mutant versions of PIF1.

A) Chlorophyll content in transgenic seedlings. Transgenic lines: *LUC-PIF1* (LP-FL), *LUC-PIF1-E293D* (LP-ED) and *LUC-PIF1-3M(G47A,N144A,L95A)* (LP-3M) in the *pif1-2* background were

grown with wild type and *pif1-2* mutants for 2.5 days in the dark and then transferred to 80 $\mu molm^{-2}s^{-1}$ white light for 3, 4 or 5 h. Total chlorophyll content was determined as in Huq *et al.*, (2004). Means ± SE of three biological replicates are shown. B) Transgenic, wild type and *pif1-2* mutant seedlings were grown for 5 days in the dark and then transferred to white light for two days. Green seedlings were counted and expressed as percentage of green seedlings/genotype. Means ± SE of three biological replicates are shown ($n \geq 30$). C) Visible phenotypes of the transgenic, wild type and *pif1-2* seedlings grown under 12h red light (Rc, 15 $\mu molm^{-2}s^{-1}$) or far-red light (FRc, 12 $\mu molm^{-2}s^{-1}$) /12h dark cycles for 4 days. White bar = 10 mm. D) Bar-graph showing hypocotyl lengths of the transgenic, wild type, and *pif1-2* seedlings grown as described in (C). Means ± SE of three biological replicates are shown ($n \geq 30$). E) Visible gravitropic phenotypes of the transgenic, wild type and *pif1-2* seedlings grown in the dark. F) Percentage of hypocotyls in each genotype that displayed negative gravitropism in the dark. Means ± SE of three biological replicates are shown ($n \geq 30$). G) Visible phenotype of seed germination in the transgenic, wild type and *pif1-2* lines exposed to FR (3.2 $\mu molm^{-2}s^{-1}$) light for 5 min and then incubated in the dark for 6 days. Wild type seeds do not complete germination in FR light conditions.



Supplementary Figure 6: Rescue of the *pif1-2* chlorophyll biosynthetic phenotypes in the transgenic seedlings expressing wild type and truncated versions of PIF1.

A) Chlorophyll content in wild type, *pif1-2* and the transgenic seedlings. Two independent *LUC-PIF1* N-terminal (N150 line #9 and line #4) and C-terminal (C327 line #5 and line #15) truncated transgenic lines created in the *pif1-2* background were grown with the wild type, *pif1-2* mutant and LP line for chlorophyll measurements as described (Huq *et al.*, 2004). Means ± SE of three biological

replicates are shown. B) Transgenic, wild type and *pif1-2* mutant seedlings were grown for 6 days in the dark and then transferred to white light for two days. Green seedlings were counted and expressed as percentage of green seedlings/genotype. Means ± SE of three biological replicates are shown (n≥30). C) Visible phenotypes of the transgenic, wild type and *pif1-2* seedlings grown under 12h red- (Rc, 15 $\mu molm^{-2}s^{-1}$) or far-red-light (FRc, 12 $\mu molm^{-2}s^{-1}$)/12h dark cycles for 4.5 days. White bar = 10 mm. D) Bar-graph showing hypocotyl lengths of the transgenic, wild type, and *pif1-2* seedlings grown as described in (C). Means ± SE of three biological replicates are shown (n≥30). E) Visible gravitropic phenotypes of the transgenic, wild type and *pif1-2* seedlings grown in the dark. F) Percentage of hypocotyls in each genotype that displayed negative gravitropism in the dark. Means ± SE of three biological replicates are shown (n≥30). G) Visible phenotype of seed germination in transgenic, wild type and *pif1-2* lines exposed to FR (3.2 $\mu molm^{-2}s^{-1}$) light for 5 min and then incubated in the dark for 6 days. Wild type seeds do not complete germination after FR light exposure.