Plant Communications, Volume *3*

Supplemental information

A Phytochrome B-PIF4-MYC2/MYC4 module inhibits secondary cell wall thickening in response to shaded light

Fang Luo, Qian Zhang, Hu Xin, Hongtao Liu, Hongquan Yang, Monika S. Doblin, Antony Bacic, and Laigeng Li

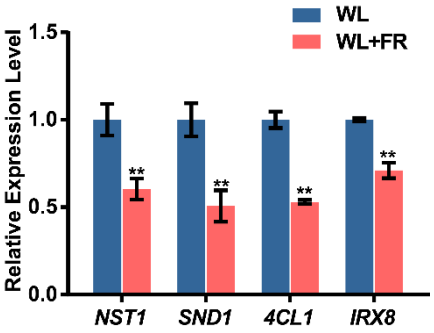
Supplemental information

A Phytochrome B-PIF4-MYC2/MYC4 Module Inhibits Secondary Cell Wall Thickening in Response to Shaded Light

Fang Luo, Qian Zhang, Hu Xin, Hongtao Liu, Hongquan Yang, Monika S Doblin, Antony Bacic and Laigeng Li

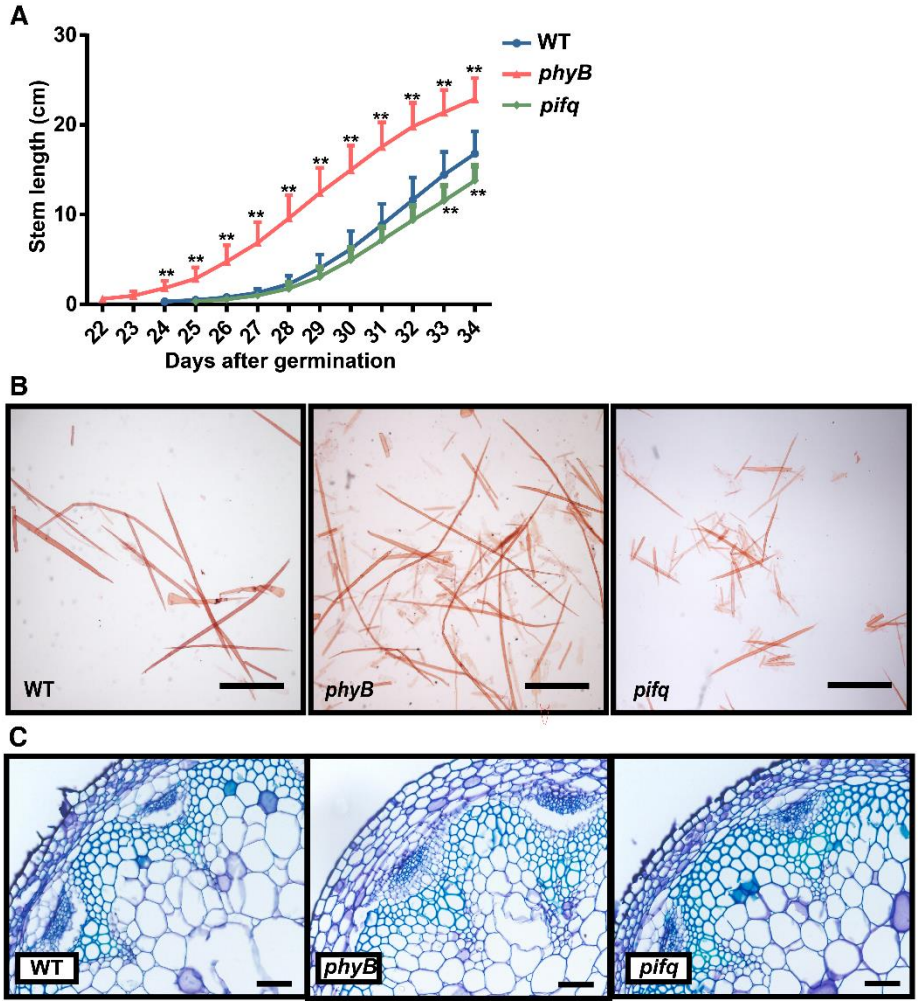
Supplemental Figures 1~9;

Supplemental Table 2



Supplemental Figure 1. Expression of SCW-related genes under different light conditions.

Expression of SCW regulatory (*NST1* & *SND1*) and biosynthesis-related (*4CL1* & *IRX8*) genes in stems of *Arabidopsis* grown under different light conditions. Three biological repeats were performed. Student's t test (**P < 0.01) was used for statistical analysis, mean ± SD.

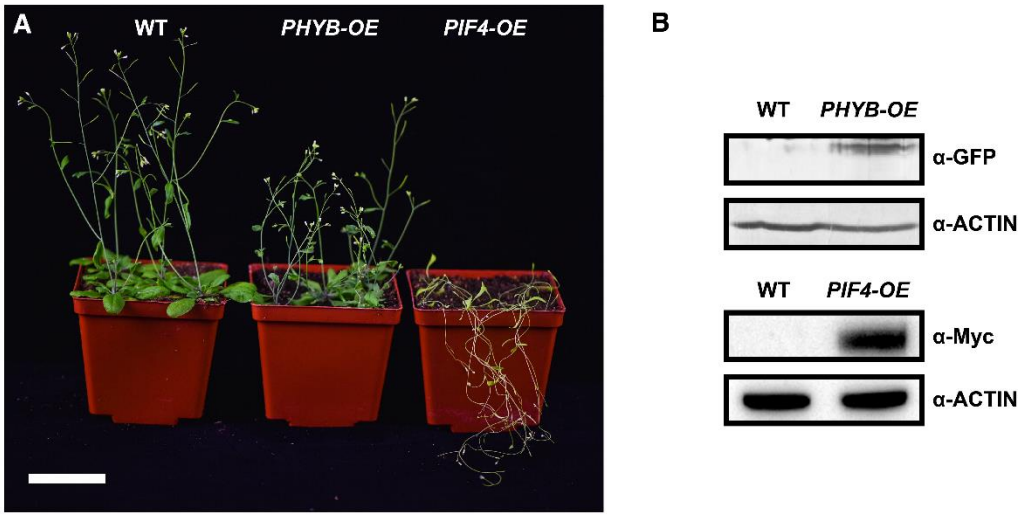


Supplemental Figure 2. *PHYB* and *PIF*s affect inflorescence stem properties.

(A) Length of inflorescence stems was recorded in WT, *phyB* and *pifq* plants grown in white light. Student's t test (**P < 0.01) was used for statistical analyses, n = 15, mean ± SD.

(B) Disaggregated fiber cells of basal inflorescence stems stained with Safranine T. Scale bar = 0.5 mm.

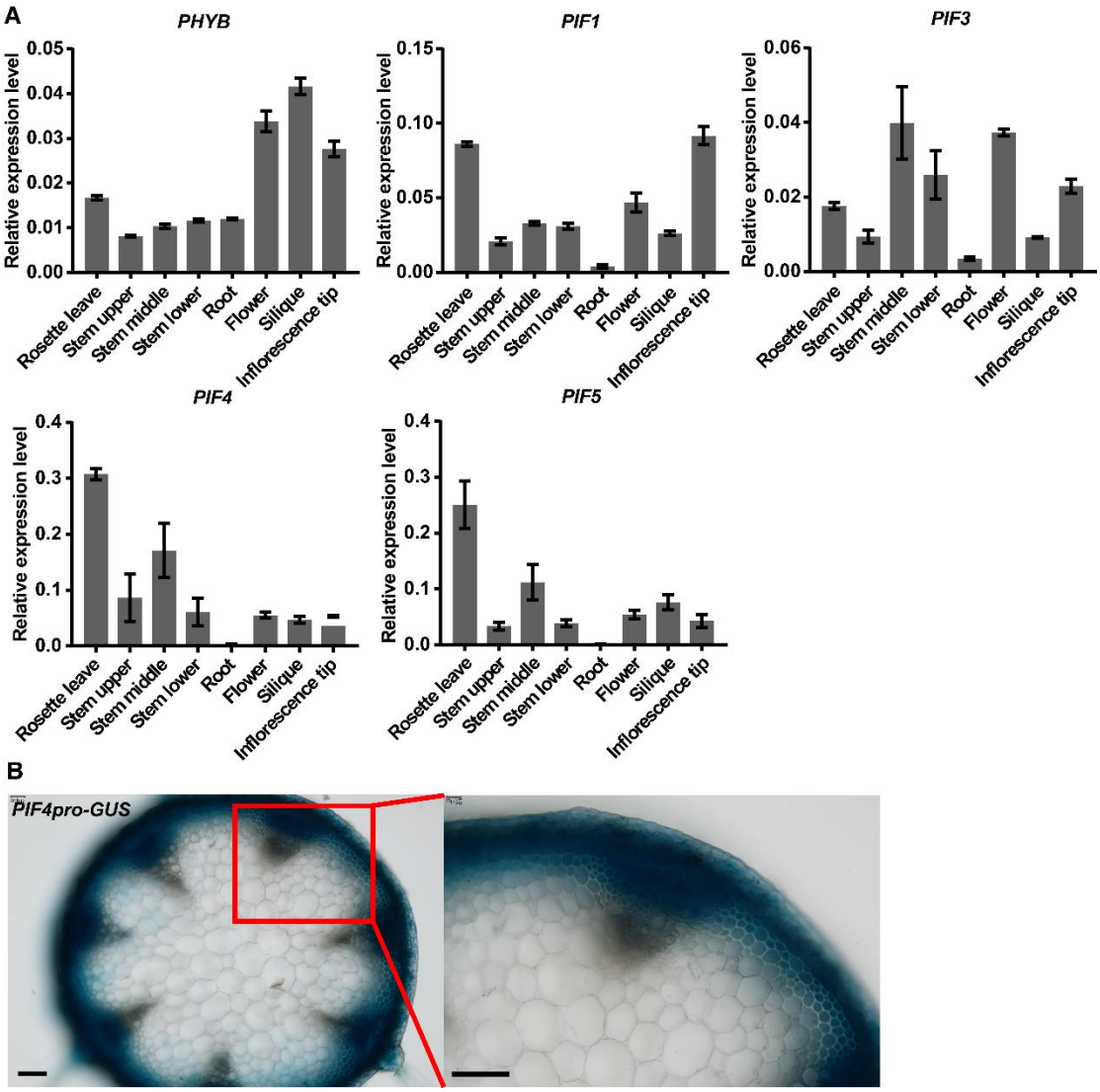
(C) Light micrographs of stem cross-sections stained with Toluidine blue. Scale bar = 50 μm.



Supplemental Figure 3. Phenotypes of *PHYB-OE* and *PIF4-OE* plants.

(A) Inflorescence stem phenotypes of *PHYB-OE* and *PIF4-OE* plants relative to WT. Scale bar = 5 cm.

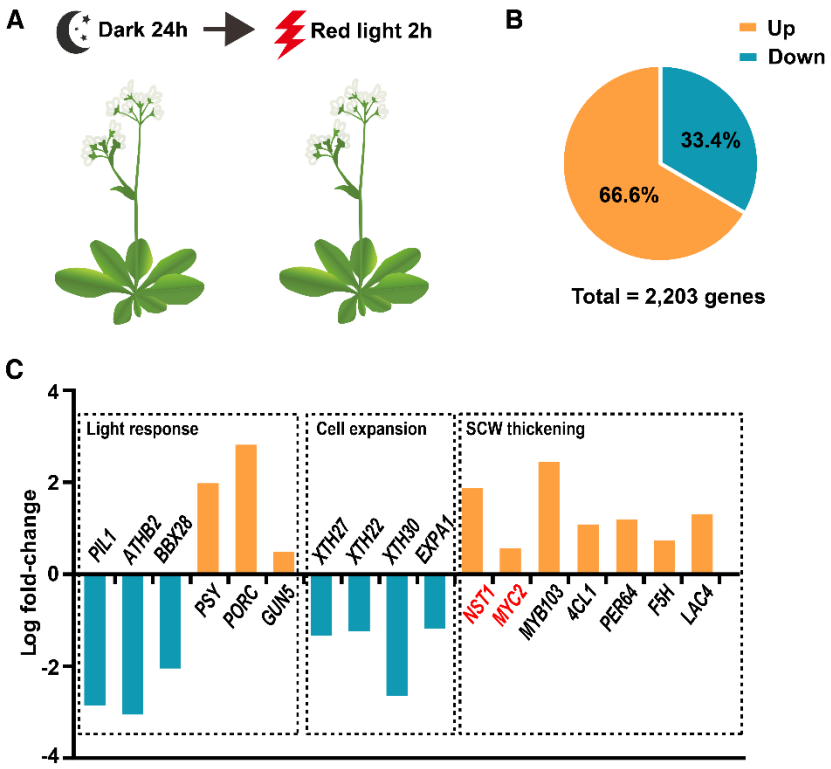
(B) Western blot detection of PHYB-YFP and PIF4-TAP proteins in the transgenic plants. ACTIN was used as an internal control.



Supplemental Figure 4. Expression pattern of *phyB* and *PIF* genes.

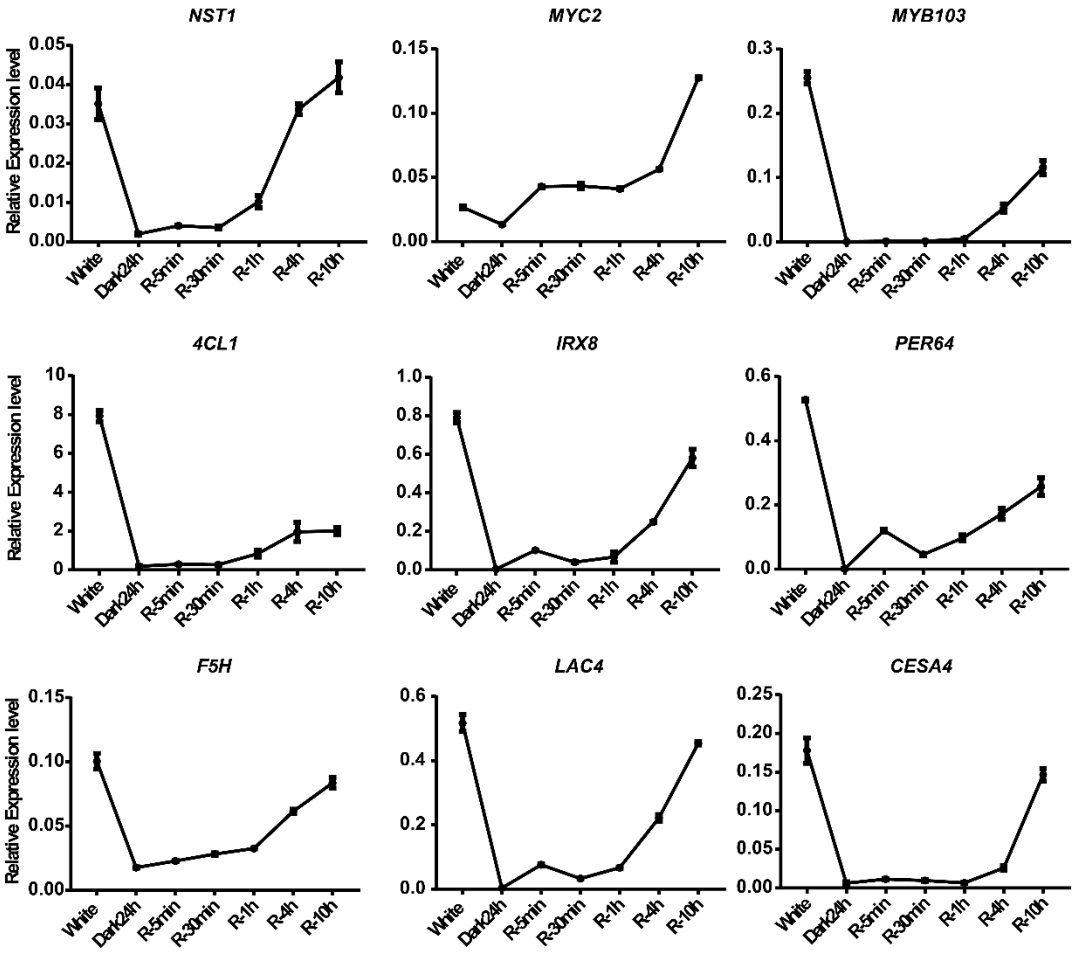
(A) Expression of *PHYB*, *PIF1*, *PIF3*, *PIF4*, and *PIF5* in different tissues of 5-week old *Arabidopsis* plants. The y-axis range of *PIF4*/*PIF5* showing their expression level is one order of magnitude larger than that of *PIF1*/*PIF3*. Mean ± SD.

(B) *PIF4* promoter activity in *Arabidopsis* inflorescence stems. GUS activity was stained in hand-cut cross-sections of inflorescence stem. Scale bars = 200 μm.



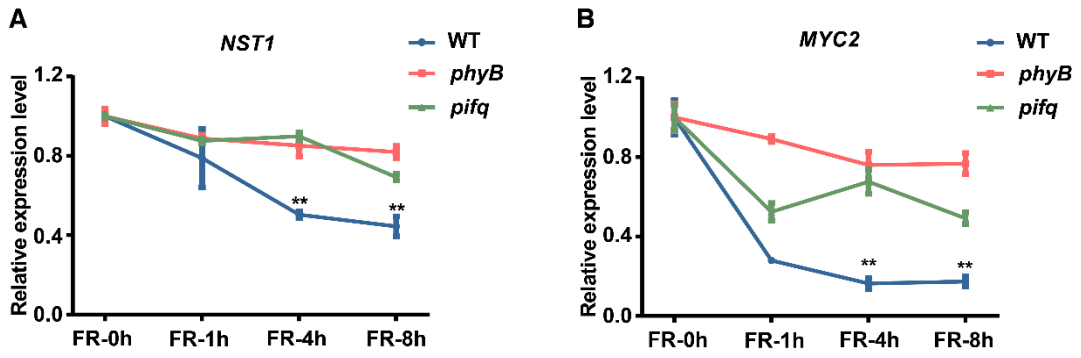
Supplemental Figure 5. Transcriptional analysis of *Arabidopsis* inflorescence stem treated with red light.

(A) Schematic showing sampling for RNA-sequencing. *Arabidopsis* plants at 5-weeks old were transferred to the dark for 24 h to shut-down expression of the light-induced genes. Plants were then treated with red light for 2 h and the inflorescence stem harvested to examine gene expression.
(B) Pie chart of up-regulated and down-regulated genes in response to red light.
(C) Log2 value of differentially expressed light-response genes, cell expansion genes and SCW thickening-related genes.



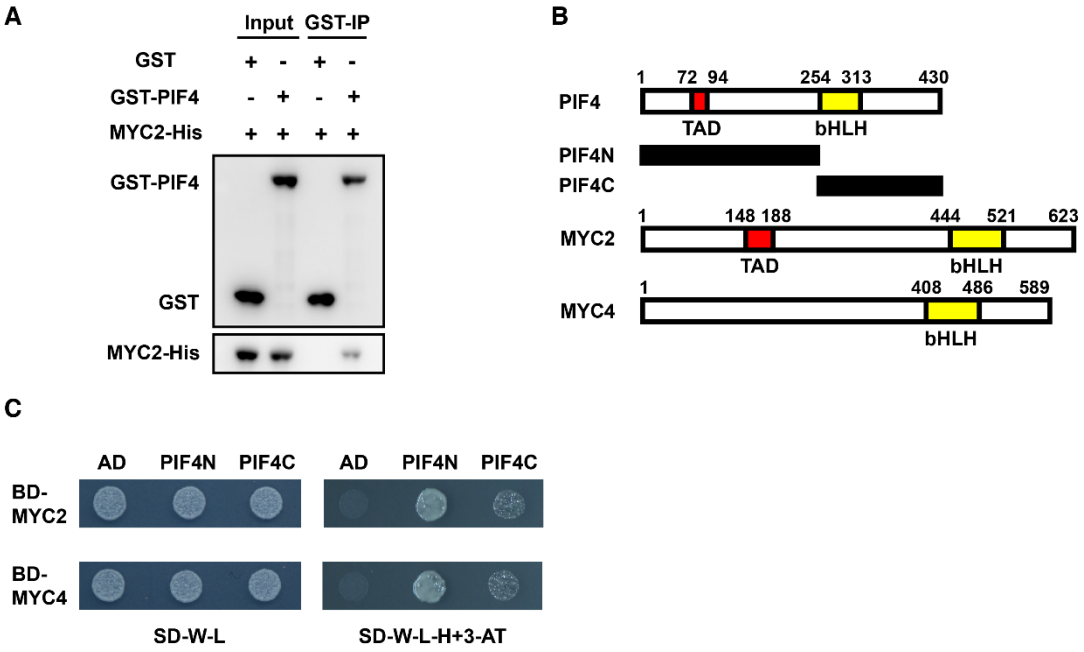
Supplemental Figure 6. Expression of *MYC2* and SCW formation-related genes is induced by red light.

Arabidopsis plants at 5-weeks old were transferred to the dark for 24 h to shut-down expression of light-induced genes. Plants were then treated with red light to examine the red-light induction of gene expression in inflorescence stem. R: red light. Three biological repeats were performed. Mean ± SD.



Supplemental Figure 7. Inhibition of MYC2 expression in response to far-red light treatment is dependent upon PHYB and PIFs.

Expression of *NST1* and *MYC2* in FR light. *Arabidopsis* inflorescence stem was treated with far-red light for 0, 1, 4 and 8 h to examine gene expression. Three biological replicates were performed. Student's t test (**P < 0.01) was used for statistical analysis, mean ± SD.

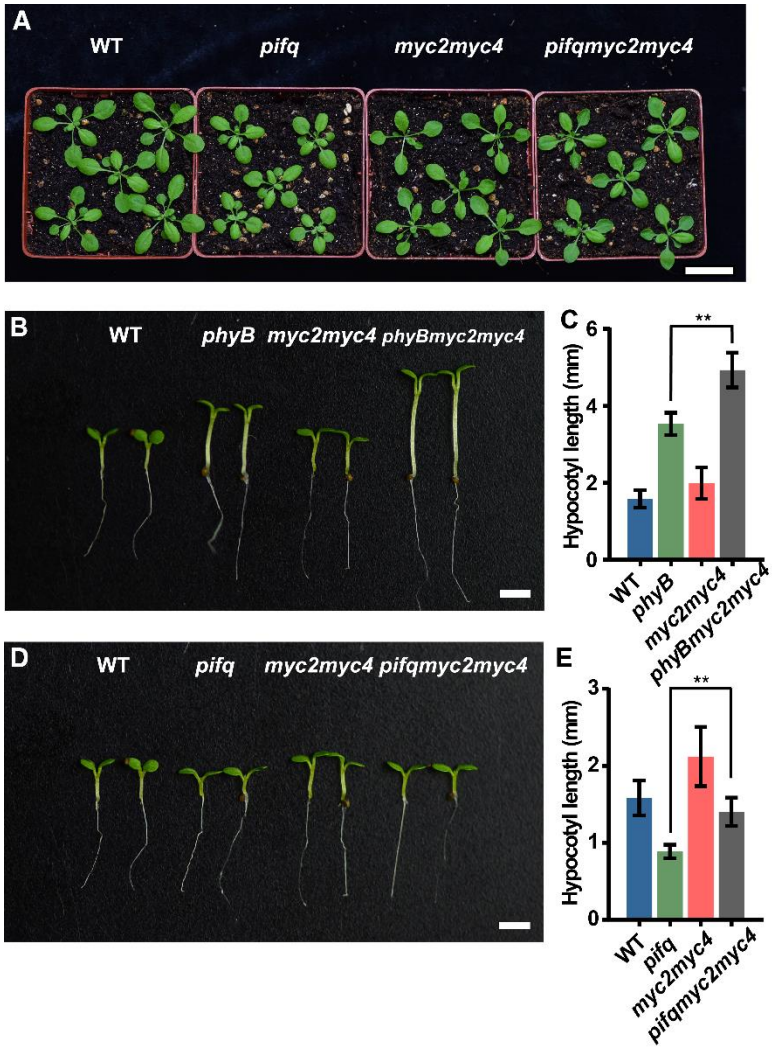


Supplemental Figure 8. PIF4 physically interacts with MYC2.

(A) Pull-down assay of PIF4 and MYC2. His-tagged MYC2 was incubated with purified GST-PIF4 or GST proteins and GST agarose. Bound proteins were detected by immunoblotting using anti-GST and anti-His.

(B) Schematic representation of the constructs of PIF4, MYC2 and MYC4 and truncated fragments of PIF4 in the yeast two-hybrid assay.

(C) Interaction of the PIF4 fragments with MYC2 and MYC4. AD: Gal4 activation domain. BD: Gal4 DNA binding domain.



Supplemental Figure 9. *myc2myc4* mutation rescued the phenotype of *pifq* mutant.

(A) Mutation of *myc2myc4* in *pifq* partially restored its phenotype in rosette leaves. Scale bar = 3 cm.

(B) Mutation of *myc2myc4* in *phyB* enhanced its phenotype in hypocotyl elongation. Scale bar = 2 mm.

(C) Measurements of hypocotyl length in (**B**). Tukey HSD test (**P < 0.01) was used for statistical analysis, n = 6, mean ± SD.

(D) Mutation of *myc2myc4* in *pifq* restored its hypocotyl elongation phenotype. Scale bar = 2 mm.

(E) Measurements of hypocotyl length in (**D**). Tukey HSD test (**P < 0.01) was used for statistical analysis, n = 6, mean ± SD.

Supplemental Table 2: Primer sequences used in this study

qRT-PCR	*PHYB*	Forward:TTGGAGGCCACAGACTTGAACG
		Reverse:TCCCTCTTTAGCACAAATGAACCG
	PIF1	Forward:CACGGATCCATATCAGCAGTTCC
		Reverse:TGGGTACGATGTTGCTTGATTCTG
	PIF3	Forward:AACGGGTTTGGGTTCAAAGAGAAG
		Reverse:TTGATCCTATCACGCCGTCTCC
	PIF4	Forward:CCGACCGGTTTGCTAGATACATCG
		Reverse:ATCTCCATCGGCTGCATCTGAGTC
	PIF5	Forward:ACTCATACCTCACTGCAGCAGAAC
		Reverse:CACTTCCCATCCACATCACTTGG
	MYC2	Forward:AAACCACGTCGAAGCAGAGAGAC
		Reverse:TTGGTACAACCGCTCGTAACGC
	NST1	Forward:TGGAAAGCAACTGGCCGCGA
		Reverse:GGGAAGCTCCTCCGACGGGA
	SND1	Forward:TGCATGCCCGAGAGCCAAACA
		Reverse:GCCAAGCTACGAGCCGGTCA
	CESA4	Forward:AGATGCGGAGTGGAAAGAACGTG
		Reverse:GGTTGTCTTGCTTCAGCATCTAGG
	4CL1	Forward:AGGCTTTGCTCATCGGTCATCC
		Reverse:CCAGCTGCTTCTTCTTTCATTGCG
	IRX8	Forward:CGACCTAGCGGCTTGGAGGA
		Reverse:GCGGCACTTTCAGCATCGGC
	LAC4	Forward:TGCATTGGTCATCCTTCCCAAAC
		Reverse:CCACCATTCACCTAGAACGATGAC
	F5H	Forward:GGTCTCTTGTAACGTTGGTAAGCC
		Reverse:GGTAAGTTATGTTGCGGGTCAGTG
	PER64	Forward:TTTCCACGACTGTTTCGTCAGAGG
		Reverse:GGAGGTCCATCTTTCTCTGCTTTG
	MYB103	Forward:ATGGAGTTGTGGGAAACAGGTG
		Reverse:TGACGGTTGATGACGACTGTAATG
	ACTIN2	Forward:AACCGGTATTGTGCTGGATTC
		Reverse:AGGTTTCCATCTCCTGCTCG
Genotyping	*PHYB*	Forward:GCAGAACCGTGTCCGAATGATAG
		Reverse:GATTCGCAAGCAACCACTCC
	MYC2	Forward:GACCCGATTGGAACACCTGGA
		Reverse:GCTCTGAGCTGTTCTTGCGTA
	MYC4	Forward:GACGAATGTTCAAGTAACCGA
		Reverse:CCATTCTCAATCCCATTCTTG
	PIF1	Forward:CTCTTTTGGATCTTTCTGGGG
		Reverse:GACTTGCGCACGATAGCTAAC
	PIF3	Forward:CACATGTAGTATACCATCTTG
		Reverse:GGCCAAGAAAAACTTGCCAG

	PIF4	Forward:ACCTCCTCAAGTCATGGTTAAGCCTAAGCC
		Middle:TCCAAACGAGAACCGTCGGT
		Reverse:TAGCATCTGAATTTCATAACCAATCTCGATACAC
	PIF5	Forward:TTCTTGTTTGTGGGTTTGGAC
		Reverse:TGAAAGAGAAGCATAAGAGGGG