

Supplemental Figure 1. Transgenic $35S_{pro}$:*PIL1-FLAG* Plants Display Inhibited Hypocotyl Elongation Phenotypes under Light Conditions. (A) Photograph of representative seedlings of indicated genotypes. Seedlings were grown under continuous indicated conditions for 4 d. Bars, 2 mm. DK, darkness; BL, blue light; RL, red light; FRL, far-red light. #6, #19 and #22, three independent lines of $35S_{pro}$:*PIL1-FLAG*/WT transgenic plants. (B) Quantification of hypocotyl lengths of the seedlings shown in (A). Error bars represent \pm SD (n = 30). The letters "a" to "c" indicate statistically significant differences between means for hypocotyl lengths of indicated genotypes determined by Tukey's LSD test ($P \le 0.01$).



Supplemental Figure 2. Expression of *P1L1-FLAG* under the Control of the *P1L1* Native Promoter in the *pil1* Mutant Rescues the Elongated Hypocotyl Phenotype. (A) Photograph of representative seedlings of indicated the genotypes. Seedlings grown under continuous indicated conditions for 4 d. Bars, 2 mm. DK, darkness; BL, blue light; RL, red light; FRL, far-red light. #15, #26 and #27, three independent lines of *P1L1*_{pro}:*P1L1-FLAG/pil1* transgenic plants. (B) Quantification of hypocotyl lengths of the seedlings shown in (A). Error bars represent \pm SD (n = 30). The letters "a" and "b" indicate statistically significant differences between means for hypocotyl length of indicated genotypes determined by Tukey's LSD test ($P \le 0.01$).



Supplemental Figure 3. Immunoblot Analysis of the Protein Levels of COP1 and PIL1 Fragments in Yeast Cells. (A) and (B) Total protein extracted from transformed yeast cells was subjected to immunoblot analysis with anti-LexA (A) and anti-HA (B) antibodies, respectively. Yeast cells expressing the indicated combination of constructs are numbered as (A) 1, COP1/PIL1; 2, COP1 \triangle 283-675/PIL1; 3, COP1 \triangle 1-282/PIL1; 4, COP1 \triangle 1-386/PIL1; 5, COP1 \triangle 1-282 \triangle 387-675/PIL1; and (B) 1, COP1/PIL1 \triangle 1-283; 2, AD/BD; 3, COP1/PIL1; 4, COP1/PIL1 \triangle 283-416. Asterisks denote degraded protein.



Supplemental Figure 4. qRT-PCR Analysis Showing the Expression of *PIL1* in *PIL1_{pro}:PIL1-FLAG/pil1* Seedlings. The seedlings were grown in darkness for 4 d (DK), and then transferred to red light for 60 min (RL). Expression levels were normalized to an internal *ACTIN2* control and presented relative to the seedlings grown in darkness set at unity. Error bars represent \pm SD (n = 3). WT represents wild type; #15, #16, and #27 represent three independent lines of *PIL1_{pro}:PIL1-FLAG/pil1* transgenic plants.



Supplemental Figure 5. Immunoblot Analysis Showing PIL1-FLAG Accumulation in $PIL1_{pro}$: PIL1-FLAG#15/pil1 Seedlings. Total protein from $PIL1_{pro}$: PIL1-FLAG#15/pil1 seedlings grown in continuous darkness (DK) and the indicated light conditions for 4 d were subjected to immunoblot analysis with anti-FLAG and anti-ACTIN antibodies. RL, red light; FRL, far-red light; BL, blue light.



Supplemental Figure 6. PIL1 Preferentially Interacts with the Pfr Form of phyB.

Yeast cells coexpressing the constructs of indicated combinations, were grown on nonselective (SD-T-L) or selective media with 25 μ M PCB (SD-T-L-H+PCB) in continuous red light (RL= 3 μ mol/s·m²) or darkness (DK) for 6 d.



Supplemental Figure 7. PIL1 and phyA Localize Together to NBs in Tobacco Cells. Dic, differential interference contrast. Bars, 5 μm.



Supplemental Figure 8. Immunoblot Analysis Showing Specificity of Anti-phyB Antibody. phyB is detected in WT and *phyB* by immunoblotting used anti-phyB antibody. The asterisks show non-specific bands.



Supplemental Figure 9. PIL1 Accumulation is Promoted by phyB in Continuous Red Light Conditions. Total protein from $35S_{pro}$: *PIL1-FLAG*#19/WT and $35S_{pro}$: *PIL1-FLAG*#19/phyB seedlings grown in continuous darkness (DK) and red light (RL) for 4 d was subjected to immunoblot analysis with anti-FLAG and anti-ACTIN antibodies. PIL1/ACT indicates the band intensities of PIL1-FLAG normalized to ACTIN and is presented relative to that in darkness set at unity.



Supplemental Figure 10. Negative Controls Showing No Colocalization of CFP with YFP-PIF1, YFP-PIF3, YFP-PIF5, or YFP-PIL1, and YFP with PIL1-CFP or PIF4-CFP in Vivo. Dic, differential interference contrast. Bars, 5 μm.



Supplemental Figure 11. qRT-PCR Analysis Showing the Expression of PIF Direct-Target Genes in Multiple Genotypes. (A), (B) and (C) Expression levels were determined by qRT-PCR, normalized to an internal *ACTIN2* control and presented relative to the wild type set at unity. 1, Wild type; 2, *hfr1*; 3, *pil1*; 4, *pil1 hfr1*; 5, *pifq*. Data represent the mean of biological triplicates \pm SD (n = 3). The seedlings were grown in darkness for 3 d (C), and then exposed to red light for 10 min (A), 30 min (B), respectively.



Supplemental Figure 12. Expression of PIF Direct-Target Genes is Suppressed in Transgenic Plants Overexpressing *PIL1* and *HFR1*. Expression levels were determined by qRT-PCR, normalized to an internal *ACTIN2* control and presented relative to the wild type set at unity. 1, WT; 2, $35S_{pro}$:*PIL1-FLAG*#19/WT; 3, $35S_{pro}$:*GFP-HFR1/hfr1*; 4, *pifq*. Data are represented as the mean of biological triplicates \pm SD (n = 3). The seedlings were grown in darkness for 3 d and exposed to red light for 20 min.



Supplemental Figure 13. PIL1 and HFR1 Suppress *ST2A* and *IAA19* Expression in the Dual-Luciferase Assay. (A) Schematic presentation of the reporter construct expressing *LUC* under the control of *ST2A* and *IAA19* promoters (*ST2A_{pro}* and *IAA19_{pro}*), respectively. (B) and (C) Tobacco leaves were infiltrated with the strains harboring the *ST2A_{pro}:LUC* (B) or *IAA19_{pro}:LUC* (C) reporter and effectors in the indicated combinations. The values represent the ratio of LUC activities to REN activities (LUC/REN). Error bars represent \pm SD (n = 3).

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Supplemental Ta	able 1. List of	Vectors and	Primers	Used in	This Work	•
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Assays	Destination	Templa	Primer	Primer sequence
	products	te	name	
Yeast	pB42AD-PI	PIL1	PIL1F1	5'-GCCGGATCCATGGAAGCAAAACCCT
brid	L1		PIL1R1	TAGCATCA-3' 5'-GGACTAGTTTAGTTTGGCGAGCGATA ATAACT-3'
	pB42AD-PI L1∆284-41 6		PIL1F1 PIL849R 1	5'-GGACTAGTCTGAACTTGAAGTTGAA GGG TCCG-3'
	6 pB42AD-PI L1△1-283 pBrige-PIL 1 pBrige-PIL 1△APB LexA- COP1 LexA- COP1△283 -675	PIL1 COP1	PIL1 850F1 PIL1R1 PIL1F2 PIL1F2 PIL1F2 PIL181R 1 PIL1 139F1 PIL1 R2 COP1F1 COP1R1 COP1R1 COP1N 282R COP1C T1F COP1R1	5'-GCCGGATCCATGATGAGTATGGGAAA TGGA TTA-3' 5'-GCCGGATCCGGATGGAAGCAAAACC CTTAGCATCA-3' 5'-GGGGTCGACTTAGTTTGGCGAGCGA TAATAACT-3' 5'-TCTTTGGTCTTTTTAATTTTGGTTTAA TGT TTGA-3' 5'-AAAATTAAAAAGACCAAAGAACAA CGGTTCTTTT-3' 5'-CGGAATTCATGGAAGAGATTTCGAC GGATCCG-3' 5'-CCCCTCGAGTCACGCAGCGAGTACC AGAACTTT-3'
	COP1∆283 -675		COP1C T2F COP1R1 COP1C	5'-CGGAATTCCAAAGTTTGAATCAGTC AACTGTC-3'

	LexA-COP 1∆1-282 LexA- COP1∆1-3 86 LexA-∆1-2 82∆387-67		T2F COP1R1 COP1C T1F COP1C T3R HFR1F1 HFR1F1 HFR1R1 PIF5F1	5'-CGGAATTCGCCACTGCTGGTGTTTCT AGATGT-3' 5'-CGGAATTCGCCACTGCTGGTGTTTCT AGATGT-3'
	J LexA-HFR	HFR1	PIF5R1	5'-CCCCTCGAGAAACAGCTCATCATCA CGATCAAA-3' 5'-CGGAATTCATGTCGAATAATCAAGCT
	1		phyBF1 phyBR1	
	pGADT7-P	PIF5	F	5'-CCCCTCGAGTCATAGTCTTCTCATCG CATGGGA-3 5'-CGGAATTCATGGAACAAGTGTTTGC
	IF5			TGATTGG-3'
	pGADT7-p hyB	phyB		5'-CCCCTCGAGTCAGCCTATTTTACCCA TATGAAGA-3' 5'-GGCCAATTGATGGTTTCCGGAGTCG GGGGTAGT-3'
				5'-CCCCCCGGGCTAATATGGCATCATCA GCATCAT-3'
Colocal	pHB-CFP	CFP	CFPF1	5'-GGACTAGTATGGTGAGCAAGGGCGA
ization			CFPR2	GGAGCTG-3'
				5'-CGG
				AGCTCTTACTTGTACAGCTCGTCCATGC
				C-3'
	pHB-PIL1-	PIL1	PIL1F1	
	CFP		PIL1R3	5'-GGACTAGTGTTTGGCGAGCGATAATA ACTAAA-3'
1	1	1	1	

pEarleyGat		PIL1attr	5'-CACCATGGAAGCAAAACCCTTAGCA
e104-PIL1		1	TCA-3'
		PIL1attr	5'-TTAGTTTGGCGAGCGATAATAACT-3'
		2	
pEarleyGat	COP1	COP1att	5'-CACCATGGAAGAGATTTCGACGGAT
e104-COP1		r1	CCG-3'
		COP1att	5'-TCACGCAGCGAGTACCAGAACTTT-3
		r2	,
pHB-PIF4-	PIF4	PIF4infu	
CFP		sionF	5'-TCCTGCAGCCCGGGGGGATCCAATGG
		PIF4infu	AACACCAAGGTTGGAGTTTT-3'
		sionR	5'-GCCCTTGCTCACCATACTAGTGTGGT
pEarleyGat	HFR1	HFR1att	CCAAACGAGAACCGTCGGT-3'
e101-HFR1		r1	5'-CACCATGTCGAATAATCAAGCTTTCA
		HFR1att	TG-3'
		r2	5'-TAGTCTT CTCATCGCATGGGAAGA-3'
pEarleyGat	phyB	phyBattr	
e101-phyB		1	5'-CACCATGGTTTCCGGAGTCGGGGGT
		phyBattr	AGT-3'
		2	5'-ATATGGCATCATCAGCATCATGTC-3'
pEarleyGat	PIF1	PIF1attr	
e104-PIF1		1	5'-CACCATGGATCCTCAGCAGCAACCT
		PIF1attr	TCT-3'
		2	5'-TTAACCTGTTGTGTGGGTTTCCGTG-3'
pEarleyGat		PIF3attr	
e104-PIF3		1	5'-CACCATGCCTCTGTTTGAGCTTTTCA
		PIF3attr	GG-3'
		2	5'-TCACGACGATCCACAAAACTGATC-3
			,

	pEarleyGat	PIF5	PIF5attr	
	e104-PIF5		1	
			PIF5attr	5'-CACCATGGAACAAGTGTTTGCTGAT
			2	TGG-3'
	pHB-YFP	YFP	YFPF1	5'-
				GCCTATTTTACCCATATGAAGACT-3'
			YFPR1	
				5'-CCCAAAGCTTATGGTGAGCAAGGGC
				GAGGAGCTG-3'
				5'-CGG
				AGCTCTTACTTGTACAGCTCGTCCATGC
				C-3'
BiFC	pXY104-PI	PIL1	PIL1F1	
	L1		PIL1R4	5'-GTCGACGTTTGGCGAGCGATAATAA
				CTAAA-3'
	pXY106-PI		PIL1F1	
	L1		PIL1R5	5'GGGGTCGACTTAGTTTGGCGAGCGA
				ТААТ-3'
	pXY106-PI	PIL1∆A	PIL1F1	
	L1 APB	PB	PIL1R4	
	рХҮ106-Н	HFR1	HFR1F2	5'-GCCGGATCCATGTCGAATAATCAAG
	FR1		HFR1R2	CTTTCATG-3'
			PIF1F1	5'-GGACTAGTTAGTCTTCTCATCGCAT
				GGGAAGA-3'
	pXY106-PI	PIF1	PIF1R1	5'-GCTTCTAGAATGGAACAAGTGTTTG
	F1			CTGATTGG-3'
			PIF3F1	5'-GGGGTCGACGCCTATTTTACCCATA
				TGAAGACT-3'
	pXY106-PI	PIF3	PIF3R1	5'-GCCGGATCCATGCCTCTGTTTGAGC

	F3			TTTTCAGG-3'
			PIF4F1	5'-GGGGTCGACTCACGACGATCCACAA
				AACTGATC-3'
	pXY106-PI	PIF4	PIF4R2	5'-GCTTCTAGAATGGAACACCAAGGTT
	F4			GGAGTTTT-3'
			PIF5F2	5'-GGGGTCGACCTAGTGGTCCAAACGA
				GAACCGTC-3'
	pXY104-PI	PIF5	PIF5R2	5'-GCCGGATCCATGGAACAAGTGTTTG
	F5			CTGATTGG-3'
			phyBF2	5'-GGGGTCGACGCCTATTTTACCCATA
			phyBR2	TGAAGA-3'
	pXY104-ph	phyB		5'-CCCCCGGGGGGATGGTTTCCGGAGTC
	yВ			GGGGGTAGT-3'
				5'-GCTTCTAGAACTACCCCCGACTCCG
				GAAACCAT-3'
Dual-L	PGreen_08	PIL1 _{pro}	PIL1 _{pro} F	5'-GCCGGATCCCGTATTCGTATAGAAT
UC	00-PIL1 _{pro} -		1	AGTTTATT-3'
	LUC		PIL1 _{pro} R	5'-GCTTCTAGATGAAGTAAACTGAACA
		IAA19 _{pro}	1	AAGCTTTC-3'
	PGreen_08		IAA19 _{pro}	5'-GCCGGATCCGAGTTCTAAATTTTGA
	00-IAA19 _{pro}		F1	CTTAACTA-3'
	-LUC	ST2A _{pro}	IAA19 _{pro}	5'-GCTTCTAGATTCTTGAACTTCTTTT
			R1	ТТССТСТ-3'
	PGreen_08		ST2A _{pro} F	5'-GCCGGATCCTAAAGATTAAGAAAAA
	00-ST2Apro-	HFR1	1	GGATAAAC-3'
	LUC		ST2A _{pro}	5'-GCTTCTAGATGTTTTTTTTTTTGTTATAG
			R1	ATATAT-3'
	pHB-HFR1		HFR1F2	

	-CFP	GFP	HFR1R3	
			GFPF1	5'-GGACTAGTTCTTCTCATCGCATGGG
				AAGA-3'
	pBs-GFP-PI		GFPR1	5'-CCCCTCGAGATGGTGAGCAAGGGCG
	F5	PIF5		AGGAGCTG-3'
			PIF5F2	5'-CCCAAAGCTTTTACTTGTACAGCTCG
			PIF5R3	TCCATGCC-3'
		MYC		
			MYCF1	5'-GGACTAGTTCAGCCTATTTTACCCA
				TATGAAGA-3'
	pKL71-MY		MYCR1	5'-CCCCTCGAGCGGTATCGATTTAAA
	C-GUS		GUSF1	GCTATGGA-3'
		GUS		5'-CGG
			GUSR1	AGCTCCCCCGGGCTGCAGGAATTCAA
				GT-3'
				5'-CGGAGCTCATGTTACGTCCTGTAG
				AAACCCCA-3'
				GCTTCTAGATCATTGTTTGCCTCCCT
				GCTGCGG-3'
Transfo	pCAMBIA	NOS	NOSF1	5'-GCCGGATCCGAATTCCATGGGCCCA
rmation	1300-NOS			CTAGTTCGTTCAAACATTTGGCAATAA
			NOSR1	AG-3'
				5'-GGCCAATTGGCTGTCGAGGGGGGGAT
	pCAMBIA	PIL1 _{pro}	PIL1 _{pro} F	CAATTCCC-3'
	1300- <i>pPIL1</i>		2	5'-GCTTCTAGACGTATTCGTATAGAAT
	-NOS		PIL1 _{pro} R	AUTITATI-5
		PIL1	2	5'GCCGGATCCTGAAGTAAACTGAACA
	pBS-PIL1-F		PIL1F1	3'
	LA		PIL1R2	

G	pBS-PIL		
pCAMBIA	1-FLAG		
1300- <i>pPIL1</i>		PIL1F1	
-PIL1FLAG	GFP	pBSR1	
-NOS			5'-GCTTCTAGATAAAGGGAACAAAAG
pBS-PIL1-		GFPF2	CTGGAGCTC-3'
GFP			5'-GCCGGATCCATGGTGAGCAAGGGCG
	PIL1	GFPR2	AGGAGCTG-3'
			5'-GGACTAGTTTACTTGTACAGCTCGTC
		PIL1F3	CATGCC-3'
			5'-GGGGTCGACATGGAAGCAAAACCCT
		PIL1R6	TAGCATCA-3'
			5'-GCCGGATCCGTTTGGCGAGCGATAAT
			AACTAAA-3'

Supplemental Methods

Supplemental Method 1. Growth Conditions

Seeds were sown on Murashige and Skoog (MS) medium and were cold treated for 3 d at 4 °C. After exposure to white light for 12 h to stimulate germination, plates with seeds were transferred to appropriate light conditions for 3 to 4 d at 22 °C. Blue, red, and far-red light were supplied by LED light sources, with irradiance fluence rates of: $15 \,\mu$ mol/m²/s, 8 μ mol/m²/s, and 2 μ mol/m²/s, respectively, unless otherwise indicated. Light intensity was measured with an ILT1400-A radiometric photometer (ILT). For MG132 and DMSO treatments, seedlings were first grown on solid MS medium for 4 d, and then transferred to liquid MS medium containing MG132 (50 μ M, dissolved in DMSO) or 0.5% DMSO under the indicated light conditions.

Supplemental Method 2. Plasmid Construction

PCR-amplified fragments encoding GFP and full-length PIL1 without terminator were ligated into *Bam*HI-*Spe*I and *XhoI-Bam*HI sites of pBluescript SKt (abbreviated as pBS), respectively, resulting in pBS-PIL1-GFP, and fragment encoding full-length PIL1 without terminator cloned into the *Bam*HI-*Spe*I sites of pBS-3×FLAG (Liu et al., 2008), resulting in pBS-PIL1-FLAG. Fragments encoding PIL1-GFP and PIL1-3×FLAG, were excised by *SalI-SacI* and cloned into the plant expression vector pKYL71 (Schardl et al., 1987) to generate pKYL71-PIL1-GFP and pKYL71-PIL1-3×FLAG, respectively.

To make constructs expressing BD-PIL1 \triangle APB and nYFP-PIL1 \triangle APB, the fragment encoding PIL1 \triangle APB (PIL1 lacking 28-46 amino acid) was obtained by overlapping PCR with primers listed in Supplemental Table 1 online, excised by *Bam*HI-*Sal*I or *Bam*HI-*Spe*I, and cloned into the pBbrige (Clontech) and pXY106 vectors, respectively, to generate BD-PIL1 \triangle APB and nYFP-PIL1 \triangle APB. To make constructs for the Dual-LUC assay, PCR-amplified fragments encoding GFP without terminator and full-length PIF5 were cloned into *XhoI-Hind*III and *Bam*HI -*Spe*I sites of pBS, respectively, resulting in pBS-GFP-PIF5. Fragment encoding GFP-PIF5, was

excised by *XhoI-SacI* and cloned into pKYL71 to generate pKYL71-GFP-PIF5. Fragment encoding 6×MYC and full-length GUS were cloned into *XhoI-SacI* and *SacI-XbaI* sites of pKYL71, respectively, resulting in pKYL71-MYC-GUS. All of the constructs used were confirmed by DNA sequencing. Other constructs were generated through PCR and subsequent cloning into the corresponding vectors listed in Supplemental Table 1 online. All the primers used in the study are also listed in Supplemental Table 1 online.

Supplemental Method 3. Construction of Double, Triple, and Quintuple Mutants

To generate *pill hfr1* double mutants, *pil1-2* was crossed with *hfr1-201*. F2 seeds were plated and then grown on soil to the adult stage. PCR was used to confirm the double mutants. For the construction of the cop1 pil1, cop1 hfr1 and cop1 pil1 hfr1 mutants, we firstly crossed cop1-4 with pil1-2 and hfr1-201 respectively. F2 seeds were plated and grown under darkness for 3 d. The seedlings with short hypocotyls and expanded cotyledons were chosen and grown on soil and the double mutants were confirmed by PCR. Then we crossed *cop1 pil1* with *cop1 hfr1*, and identified the *cop1 pill hfr1* triple mutant in F2. For the construction of the *pill pifq* quintuple mutant, *pifq* was crossed with *pil1-2*. F2 seeds were plated and grown under blue light for 4 d, and the seedlings with taller hypocotyls were chosen and grown on soil, and the seedlings containing homozygous *pil1-2* mutations were confirmed by PCR. The F3 seeds were plated and grown under blue light for 4 d, and seedlings with shorter hypocotyls were identified. The final confirmation of the *pill pifq* quintuple mutant was performed by PCR. All the primers used in PCR were described previously (Soh, 2000; Yamashino et al., 2003; Hug et al., 2004; Monte et al., 2004; Khanna et al., 2007; Leivar et al., 2008).

Supplemental Method 4. Hypocotyl Length Measurements

Thirty seedlings grown for 4 d under the indicated light conditions were photographed and the resulting digital photographs were subjected to measurements of hypocotyl lengths using ImageJ software (http://rsbweb.nih.gov/ij/).

Supplemental Method 5. Fluorescence Microscopy

Root cells of the 4-d-old $35S_{pro}$:*PIL1-GFP*#5/WT and $35S_{pro}$:*PIL1-GFP*#5/cop1 seedlings grown on MS medium under different treatments were analyzed using a confocal microscope with GFP filter sets. All fluorescence images were taken with identical exposure.

Supplemental Method 6. Antibodies

Rabbits were vaccinated with synthesized peptide (CVPRKRPLSTASGSG) to generate anti-phyB antiserum. The anti-phyB antibody was further purified from the antiserum using protein A. The anti-ACTIN, -HA, and -LexA antibodies were purchased from Abmart Inc. and Merck Inc.

Supplemental Method 7. Yeast Protein Extraction

All yeast cells were harvested when OD600 was at 0.4~0.6. Equal amounts of yeast cells for each sample were calculated from OD600. The total yeast protein was extracted using A Yeast Protein Extraction kit according to the manufacture's manual (CWbiotech Inc. Cat#CW0890).

Supplemental Method 8. qRT-PCR

Total RNA (500 ng) was reverse-transcribed using the PrimeScript RT Reagent Kit (Takara). Amplification was carried out with SYBR Premix Ex Taq II (Takara). Real-time PCR was performed in a CFX96 Real time system (Bio-Rad). The thermal profile for real-time PCR was 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s. The expression of PIF direct-target genes was normalized against the expression of the endogenous control gene *ACTIN2*. All experiments were performed with three independent biological replicates in three technical repetitions each. The RT-PCR primers were described previously (Zhang et al., 2013).

Supplemental References

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