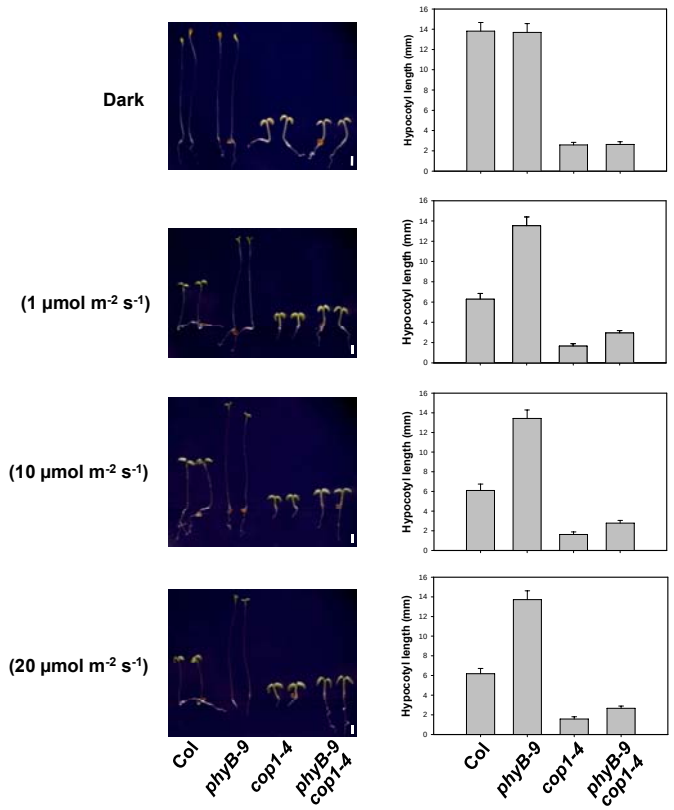
Supplemental Data. Jang et al. Plant Cell. (2010). 10.1105/tpc.109.072520



Supplemental Figure 1. Phenotypes of WT (Col), *phyB-9*, *cop1-4*, and *phyB-9cop1-4* under R light.

(Left panel) Four-day old seedlings grown in darkness or under R light (1, 10, and 20 μmol m^{-2} s^{-1}). Scale bar, 1 mm. (Right panel) data were presented as average hypocotyl length ± standard deviation (SD; n > 80).

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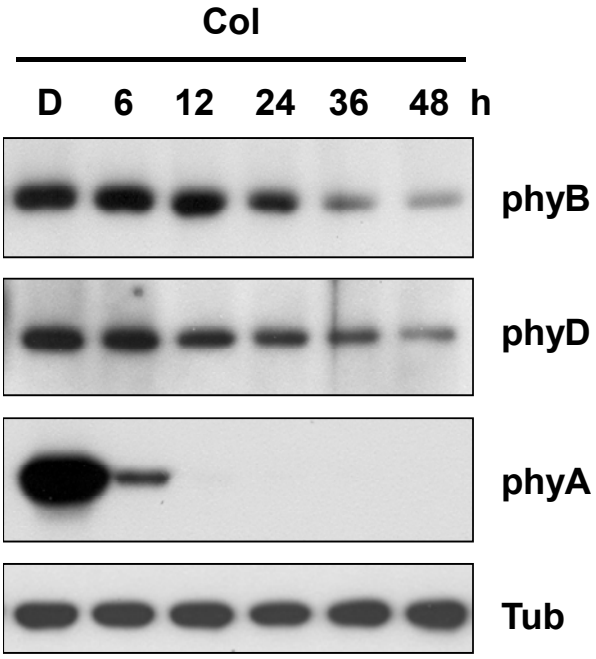

Supplemental Figure 2. phyA, phyB, and phyD degradation in R light.

Four-day old etiolated seedlings were exposed to R light (10 μmol m^{-2} s^{-1}) for the mentioned time points. The anti-phyA monoclonal antibody was described (Shinomura et al. 1996).

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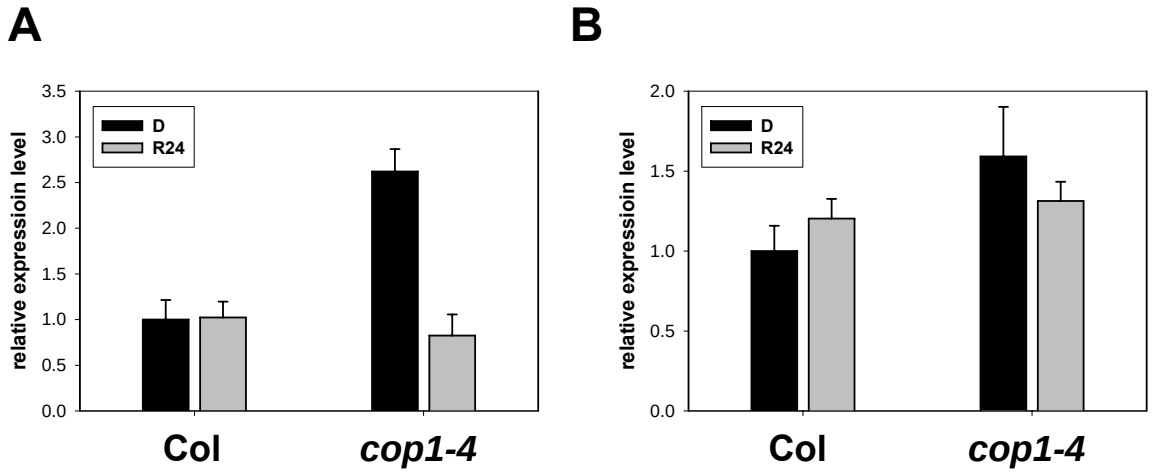

Supplemental Figure 3. Relative expression levels of *phyB* (A) and *phyD* (B) in Col and *cop1-4*.

The expression levels were measured by Q-PCR and the values were normalized using the signal from the *Actin2* gene. Four-day old etiolated seedlings (D) were exposed to R light (10 μmol m^{-2} s^{-1}) for 24 h. Data show average value ± standard deviations (SD; n=3).

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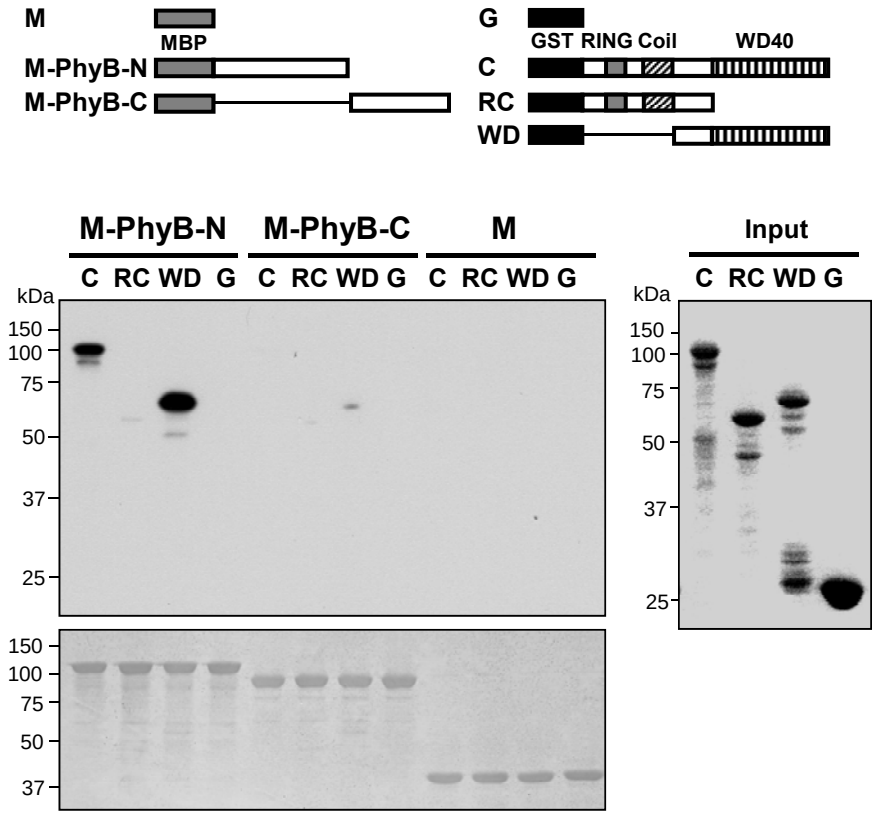

Supplemental Figure 4. Definition of the COP1 domain that binds to PhyB-N.

(Upper panel) Schematic diagrams of bait proteins (M-PhyB-N, M-PhyB-C, and M) and target proteins (G, GST; C, GST-COP1; RC, GST-RING+Coil; WD, GST-WD40). (Lower left) About 500 ng of target proteins were pulled-down with 1 μg of bait proteins and detected by anti-GST antibody. Membrane staining after pull-down assay was used to monitor amounts of bait proteins. Lower right, purified target proteins used in pull-down assays (Input).

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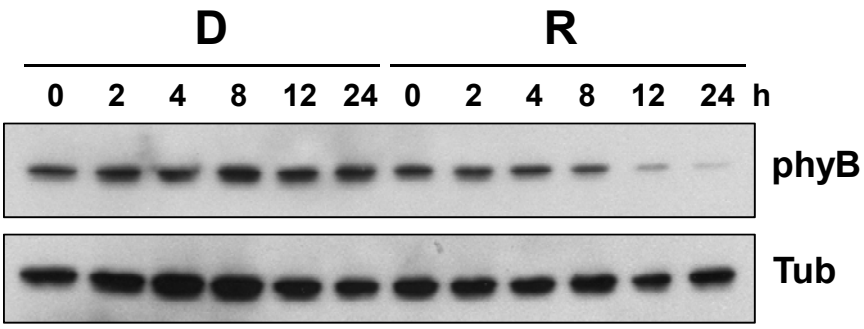

Supplemental Figure 5. Half life of phyB in darkness and under R light.

For protein decay experiment, four-day-old etiolated seedlings were transferred to liquid MS medium containing 100 μM cycloheximide, kept in the dark for 30 min, and then exposed to R light (10 μmol m^{-2} s^{-1}) or further incubated in the dark (D). Samples were harvested as indicated and analyzed by Western blot analysis using anti-phyB and anti-tub antibodies.

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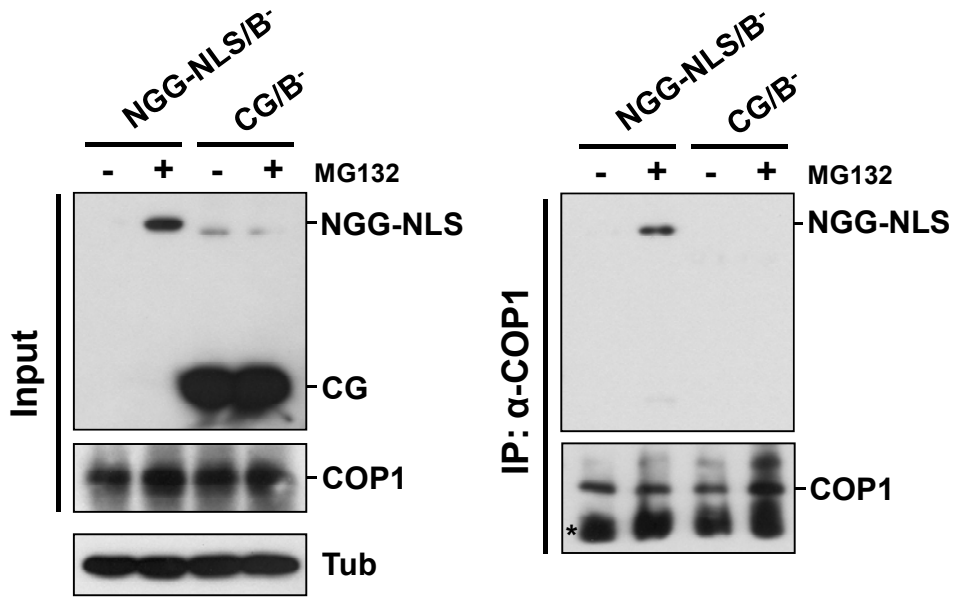

Supplemental Figure 6. Co-immunoprecipitation of NGG-NLS with COP1.

Protein extracts of transgenic *Arabidopsis* seedlings (NGG-NLS/B- and CG/B-) treated with or without MG132 (25 μM) under R light (10 μmol m^{-2} s^{-1}) for 12 h were immunoprecipitated with anti-COP1 antibody. Input proteins and immunoprecipitates were detected by Western blot analyses using anti-COP1 and anti-GFP antibodies. Input refers to the starting amount in extracts used for immunoprecipitation reactions. Asterisk indicates the cross-reaction with the heavy chain of the protein A-conjugated antibody.

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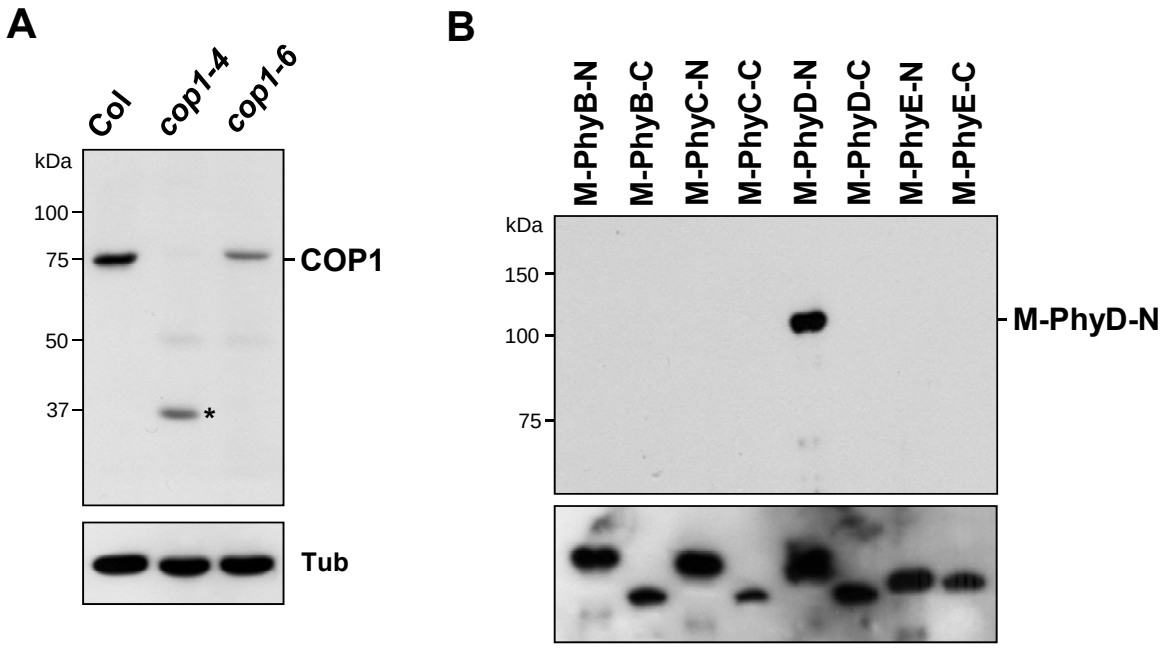
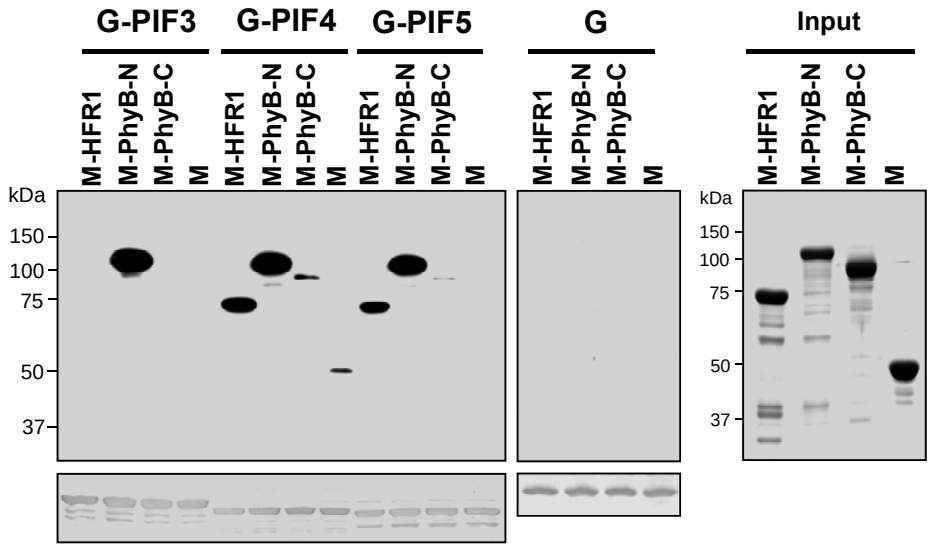


Supplemental Figure 7. The specificity of monoclonal anti-COP1 and polyclonal anti-phyD antibodies.

(A) Four-day old dark-grown seedlings (WT, *cop1-4*, and *cop1-6*) were used to detect COP1. Asterisk indicates the truncated COP1 protein (approximately 33 kDa) in *cop1-4* (McNellis et al., 1994). Note that *cop1-6* generates a protein with 5 additional amino acids (CLVLW) inserted between Glu-301 and Phe-302 of the WT COP1 (McNellis et al., 1994).

(B) The recombinant proteins were detected using anti-phyD (upper panel) and anti-MBP (Lower panel) antibodies.

Supplemental Data. Jang et al. Plant Cell. (2010). 10.1105/tpc.109.072520



Supplemental Figure 8. In vitro interaction between PhyB-N and PIFs.

In vitro pull-down assays were done with 500 ng of target proteins (M-HFR1, M-PhyB-N, M-PhyB-C, and MBP) that were pulled-down with 1 μg of bait proteins (G-PIF3, G-PIF4, G-PIF5, and GST) and detected by anti-MBP antibody. PhyB-N and PhyB-C are apoproteins. Membrane staining after pull-down assays was used to monitor amounts of bait proteins. Purified target proteins used in pull-down assay were resolved on SDS-PAGE and indicated as Input.

Supplemental Data. Jang et al. Plant Cell. (2010). 10.1105/tpc.109.072520

Supplemental Table 1. Primer sequences for cloning

Name	Forward primer	Reverse primer
PhyB-N 5’ – 3’	caccATGGTTTCCGGAGTCGGGGGTA	ctaCATATCCCTACATGGCTGAACCACAC
PhyB-C 5’ – 3’	caccATGGCGGGGGAACAGGGGATTGATG’	ctaATATGGCATCATCAGCATCATGTCA
PhyC-N 5’ – 3’	caccATGTCATCGAACACTTCACGAAGC	ctaATCCACAAGTGGGACATCCACA
PhyC-C 5’ – 3’	caccATGAATAGGGTTCAGAAGGTAGATGAA	tcaAATCAAGGGAAATTCTGTGAGGAT
PhyD-N 5’ – 3’	aagaattcATGGTCTCCGGAGGTGGTAGCAAAAC	aatctagatcaATCATCTCCATGTGGCTGAACCGCC
PhyD-C 5’ – 3’	caccATGGTACAGCAAGGGATGCAGGAG	TCATGAAGAGGGCATCATCATCATTAG
PhyE-N 5’ – 3’	aagaattcATGGGATTCGAGAGTTCAAGCTCAGC	aatctagatcaATCTCTTGCTACGCCATTACCAGACAAAACT
PhyE-C 5’ – 3’	caccATGGCTAATGAGCTTACTTCTTTTGTG	CTACTTTATGCTTGAACTACCCTCTGT
PIF3 5’ – 3’	caccATGCCTCTGTTTGAGCTTTTCAGG	TCACGACGATCCACAAAACTGATCAGA
PIF4 5’ – 3’	caccATGGAACACCAAGGTTGGAGTTTTGA	CTAGTGGTCCAAACGAGAACCGTCG
PIF5 5’ – 3’	caccATGGAACAAGTGTTTGCTGATTGGA	TCAGCCTATTTTACCCATATGAAGACT

* Unmatched DNA sequences to template plasmid DNA are indicated as lowercase letters and the lined sequences in PhyB-N and PhyE-N are for the restriction enzymes, *Eco*RI and *Xba*I.

Supplemental Data. Jang et al. Plant Cell. (2010). 10.1105/tpc.109.072520

Supplemental Table 2. Primer sequences for site-directed mutagenesis

Name	Primer sequence
PIF5 (E31A/G37A) 5'-3'	Forward
	TGAATTAGTG**GcG**CTATTGTGGAGAGAT**GcT**CAAGTGGTTTTACA
	Reverse
	TGTAAAACCACTTG**AgC**ATCTCTCCACAATAG**CgC**CACTAATTCA

* Unmatched DNA sequences to template plasmid DNA are indicated as lowercase letters.

Supplemental Data. Jang et al. Plant Cell. (2010). 10.1105/tpc.109.072520

Supplemental References

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Shinomura, T., Nagatani, A., Hanzawa, H., Kubota, M., Watanabe, M., and Furuya, M. (1996). Action spectra for phytochrome A- and B-specific photoinduction of seed germination in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **93:** 8129-8133.