Expanded View Figures

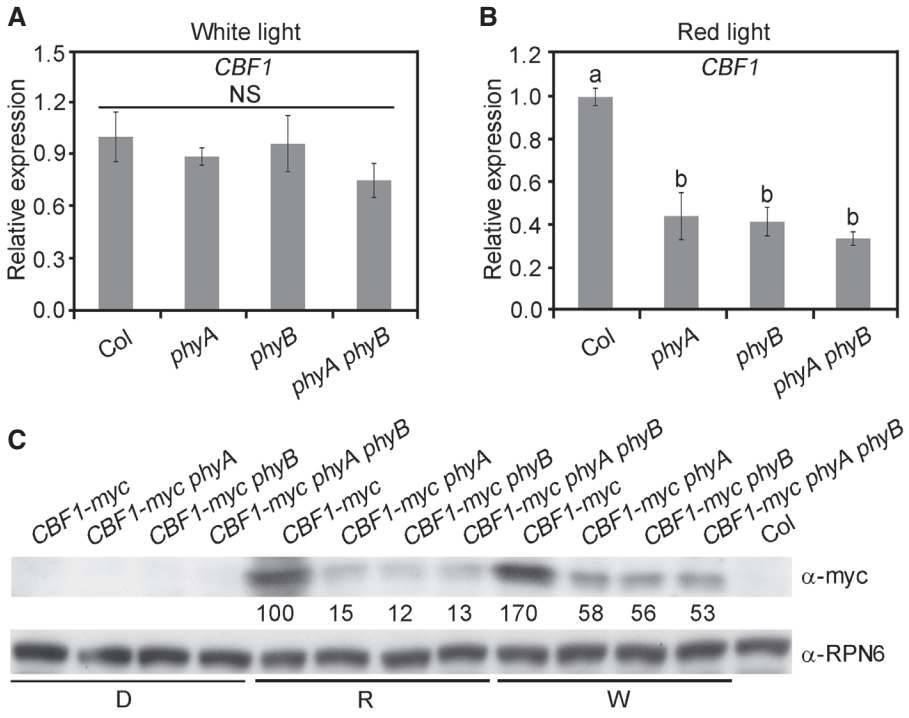

Figure EV1. *CBF1* transcript and protein levels are induced by phyA and phyB in red light.

A, B qRT–PCR assays showing the expression of *CBF1* in 4-day-old Col, *phyA*, *phyB*, and *phyA phyB* seedlings grown at 22°C in continuous W (A) or R light (B). Error bars represent SD of three technical replicates. Different letters represent significant differences by one-way ANOVA with Duncan's *post hoc* test ($P < 0.05$). NS, not significant.

C Immunoblots showing the levels of CBF1-myc proteins in 4-day-old *CBF1-myc*, *CBF1-myc phyA*, *CBF1-myc phyB*, and *CBF1-myc phyA phyB* seedlings grown at 22°C in darkness (D) or continuous R or W light. Anti-RPN6 was used as a sample loading control. Numbers below the immunoblot indicate the relative band intensities of CBF1-myc normalized to those of loading control, respectively. The ratio of the first clear band was set to 100.

Source data are available online for this figure.

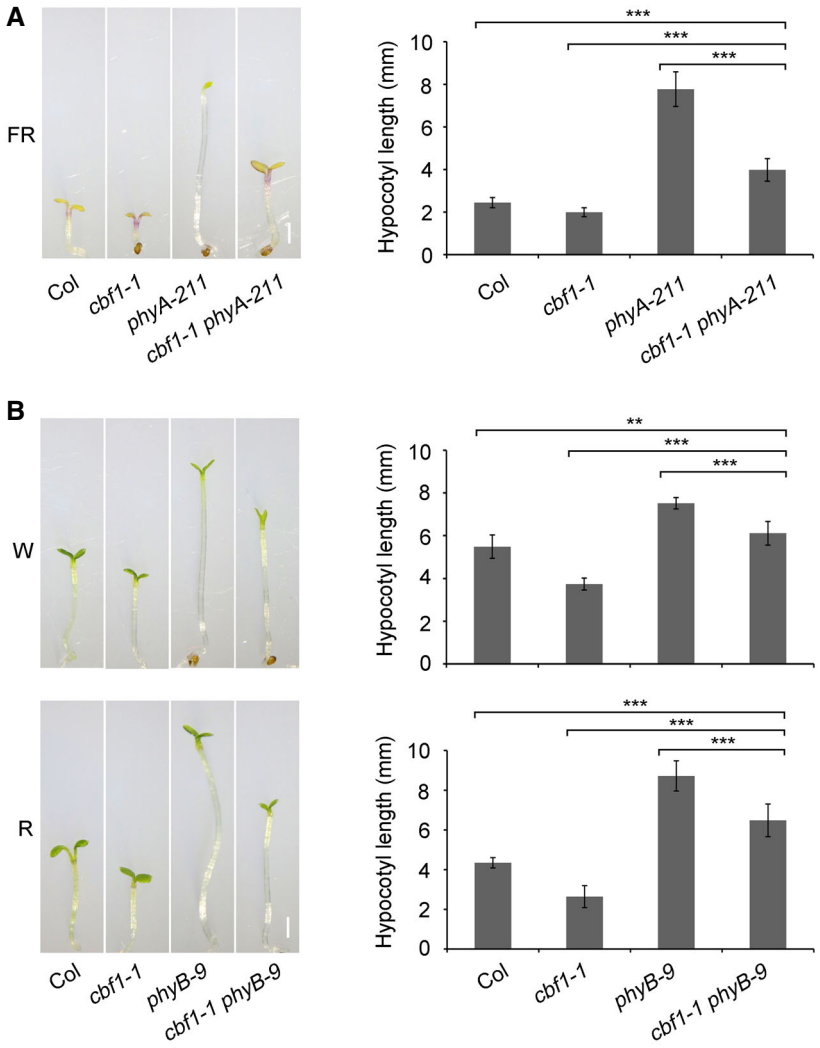

Figure EV2. Genetic relationship between CBF1 and phyA/phyB.

A Phenotypes and hypocotyl lengths of 4-day-old Col, *cbf1-1*, *phyA-211*, and *cbf1-1 phyA-211* seedlings grown at 22°C in continuous FR light.

B Phenotypes and hypocotyl lengths of 4-day-old Col, *cbf1-1*, *phyB-9*, and *cbf1-1 phyB-9* seedlings grown at 22°C in continuous W or R light.

Data information: In (A) and (B), error bars represent SD from 20 seedlings. $^{**}P < 0.01$ and $^{***}P < 0.001$ (two-tailed *t*-test) for the indicated pairs of seedlings. Scale bar = 1 mm.

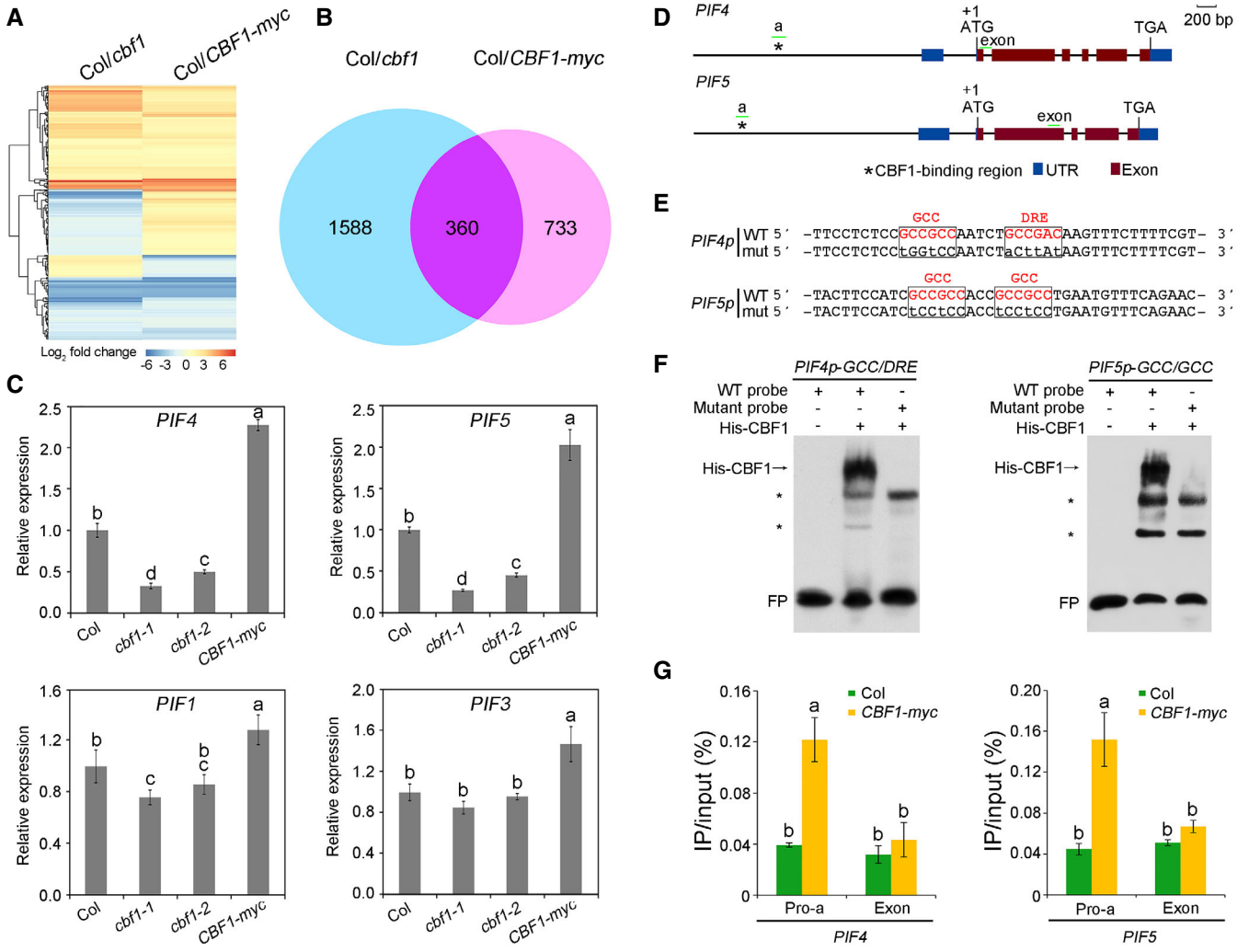

Figure EV3. CBF1 positively regulates *PIF4* and *PIF5* expression by directly binding to their promoters.

A Cluster analysis of genes whose expression was changed in *cbf1* and *CBF1-myc* seedlings compared with Col. The bar represents the $\log_2$ of the ratio.

B Venn diagram showing the numbers and overlaps of genes whose expression was changed in *cbf1* and *CBF1-myc* seedlings.

C qRT–PCR assays showing the expression levels of *PIF4*, *PIF5*, *PIF1*, and *PIF3* in 4-day-old Col, *cbf1-1*, *cbf1-2*, and *CBF1-myc* seedlings grown at 22°C in continuous W light. Error bars represent SD of three technical replicates. Different letters represent significant differences by one-way ANOVA with Duncan's *post hoc* test ($P < 0.05$).

D Schematic illustration of the exon–intron structures of *PIF4* and *PIF5*. Asterisks indicate the CBF1-binding regions in the *PIF4* and *PIF5* promoters, and the sequences are shown in (E). The short green lines depict the location of amplicons used for ChIP-qPCR shown in (G). UTR, untranslated region.

E Wild-type (WT) and mutated (mut) EMSA probes according to the sequences of CBF1-binding regions in the *PIF4* and *PIF5* promoters. Wild-type GCC or DRE elements are shown in red, and the nucleotide substitutions in the mutant fragments are shown as lowercase letters.

F EMSAs showing that His-CBF1 directly binds the wild-type, but not mutant probes of the *PIF4* and *PIF5* promoter fragments. Asterisks indicate nonspecific binding. FP, free probe.

G ChIP assays showing that CBF1 directly binds to *PIF4* and *PIF5* promoters *in vivo*. Four-day-old Col and *CBF1-myc* seedlings grown at 22°C in continuous white light were harvested and subjected to ChIP analysis using anti-c-myc Affinity Gel (Sigma-Aldrich), and the precipitated DNA was recovered and analyzed by qPCR assays. The locations of amplicons used for ChIP-qPCR assays are shown in (D). Error bars represent SD of three technical replicates. Different letters represent significant differences by one-way ANOVA with Duncan's *post hoc* test ($P < 0.05$).

Source data are available online for this figure.

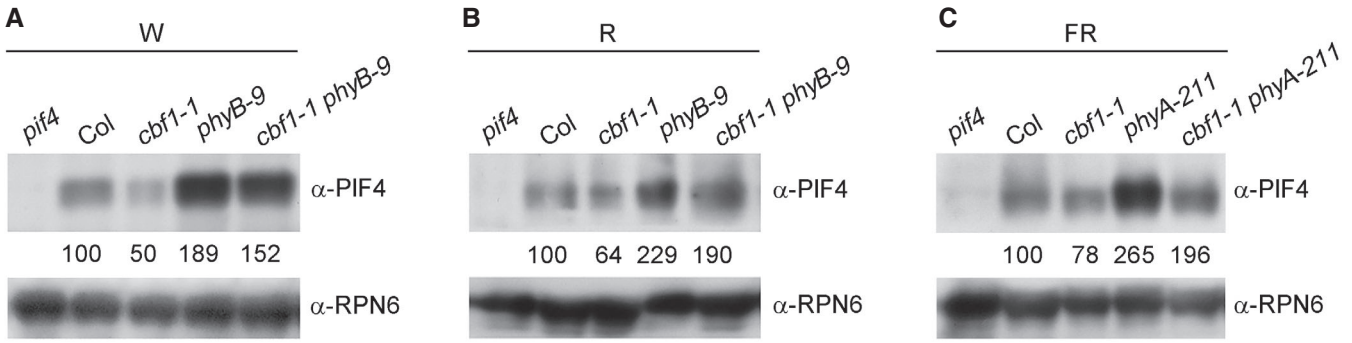

Figure EV4. PIF4 protein levels.

A–C Immunoblots showing the levels of PIF4 proteins in 4-day-old Col, *cbf1-1*, *phyB-9*, and *cbf1-1 phyB-9* seedlings grown at 22°C in continuous W (A) or R light (B), and in 4-day-old Col, *cbf1-1*, *phyA-211*, and *cbf1-1 phyA-211* seedlings grown at 22°C in continuous FR light (C). Numbers below the immunoblots indicate the relative band intensities of PIF4 normalized to those of RPN6, respectively. The ratio was set to 100 for the respective band in Col. The ratio of the first clear band was set to 100 for each blot.

Source data are available online for this figure.