Supporting Information

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SI Materials and Methods

Yeast 2-Hybrid Assays. Yeast two-hybrid (Y2H) assays were performed using the AH109 yeast strain and pGBKT7 (encoding the Gal4-BD) and pGADT7 (encoding Gal4-AD) plasmids (Clontech). Positive control plasmids encoded the Gal4 DNA-BD fused with murine p53 (+BD) and the Gal4-AD fused with SV40 large T-antigen (+AD) (Clontech). UVR8–Gal4-BD and COP1–Gal4-AD fusions have been described previously (1, 2). For the COP1–Gal4-BD fusion, the full-length coding sequence of *COP1* was amplified from WT Col-0 cDNA using the COP1F and COP1R primers (Table S1). The resulting PCR product was cloned into the EcoRI, SalI sites of the MCS of pGBKT7. For the PIF3–Gal4-BD fusion, the full-length coding sequence of *PIF3* was amplified from cDNA of heat-treated (6 h, 37 °C) Col-0 plants using the PIF3F and PIF3R primers (Table S1). The resulting PCR product was cloned into the EcoRI, SalI sites of the MCS of pGBKT7. For the fusions of *PIF4*, *PIF5*, and *PIF7* with Gal4-AD, partial-length coding sequences of *PIF4* (lacking the first two amino acids) and *PIF5* (lacking the first 10 amino acids), and the full-length coding sequence of *PIF7* were amplified from cDNA of heat-treated (6 h, 37 °C) Col-0 plants. The primer pairs were PIF4F + PIF4R, PIF5F +PIF5R, and PIF7F + PIF7R (Table S1). The resulting PCR products were cloned into the NdeI, ClaI sites (*PIF4*) and the EcoRI, BamHI sites (*PIF5* and *PIF7*) of the MCS of pGADT7. All constructs were verified by sequencing.

The preparation and transformation of yeast-competent cells was carried out as described previously (3) with the following exceptions: between five and seven colonies of transformed yeast were pooled together for spotting onto the selective media, all washing steps were repeated twice, and the final resuspension of cells before spotting was done in 1× TE instead of in 0.8% NaCl. Cells were spotted on plates containing SD medium (63041; Clontech) with minus leucine (L), minus tryptophan (W), minus histidine (H), and minus adenine (A) dropout supplements. Nonselective SD-L-W plates served as a viability control. Low-stringency SD-L-W-H plates were used to detect weak interactions, and high-stringency SD-L-W-H-A plates were used for detection of stronger interactions. Plates were left for 3 d at 30 °C in darkness or 0.1 μmol $m^{-2}s^{-1}$ UV-B (supplied by a Philips TL20W/01RS tube). Photographs were taken after 48 h for SD-L-W, after 72 h for SD-L-W-H, and after 96 h for SD-L-W-H-A.

1. Cloix C, et al. (2012) C-terminal region of the UV-B photoreceptor UVR8 initiates signaling through interaction with the COP1 protein. *Proc Natl Acad Sci USA* 109(40): 16366–16370.
2. Holm M, Hardtke CS, Gaudet R, Deng XW (2001) Identification of a structural motif that confers specific interaction with the WD40 repeat domain of *Arabidopsis* COP1. *EMBO J* 20(1-2):118–127.
3. O'Hara A, Jenkin GI (2012) In vivo function of tryptophans in the *Arabidopsis* UV-B photoreceptor UVR8. *Plant Cell* 24:3755–3766.

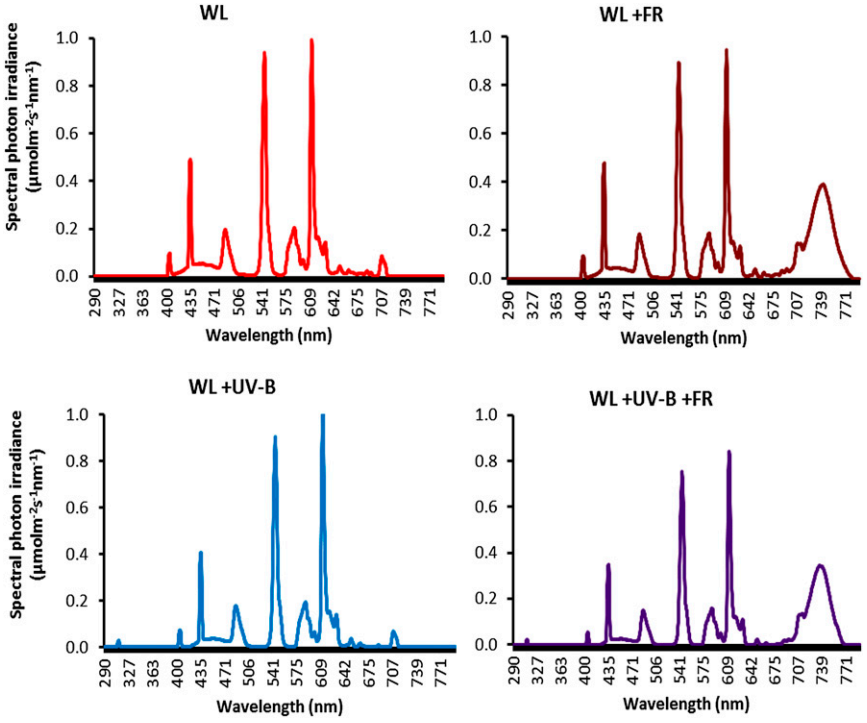

Fig. S1. Spectra of experimental conditions used for low R:FR shade avoidance experiments. 90 μmol $m^{-2}s^{-1}$ white light (WL) was supplemented with FR to reduce the R:FR to 0.05. Supplementary UV-B was given at 400 mWm^{-2} (~1 μmol $m^{-2}s^{-1}$).

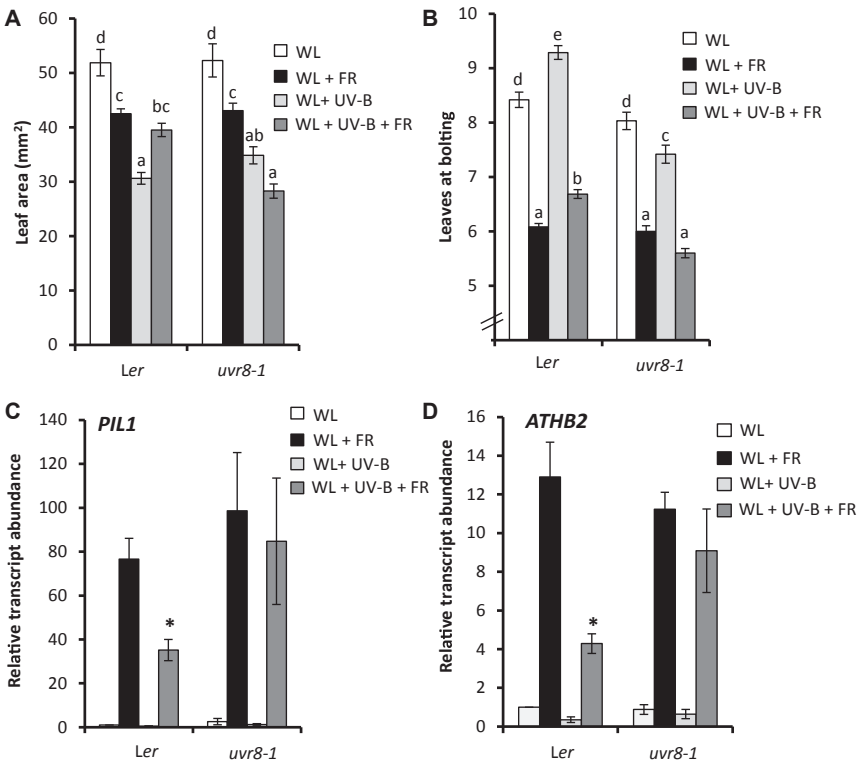

Fig. S2. UV-B decreases leaf area, delays flowering, and inhibits the expression of shade avoidance marker genes *PIL1* and *ATHB2*. (*A*) Leaf area of WT (L*er*) and *uvr8-1* rosettes grown in WL) for 10 d before transfer to WL, +FR, +UV-B, and +UV-B +FR for a further 9 d. (*B*) Flowering times (as measured by rosette leaf number) of plants grown as in *A*. $n \geq 22 \pm$ SE. Different letters indicate statistically significant differences ($P < 0.05$) between means. (*C* and *D*) Relative transcript abundance of *PIL1* (*C*) and *ATHB2* (*D*) in 7-d-old WT (L*er*) and *uvr8-1* seedlings grown for 7 d in WL before transfer to WL, +FR, +UV-B, and +FR+UV-B for 4 h. $n = 3 \pm$ SE. *Significant difference in transcript abundance compared with +FR ($P < 0.05$).

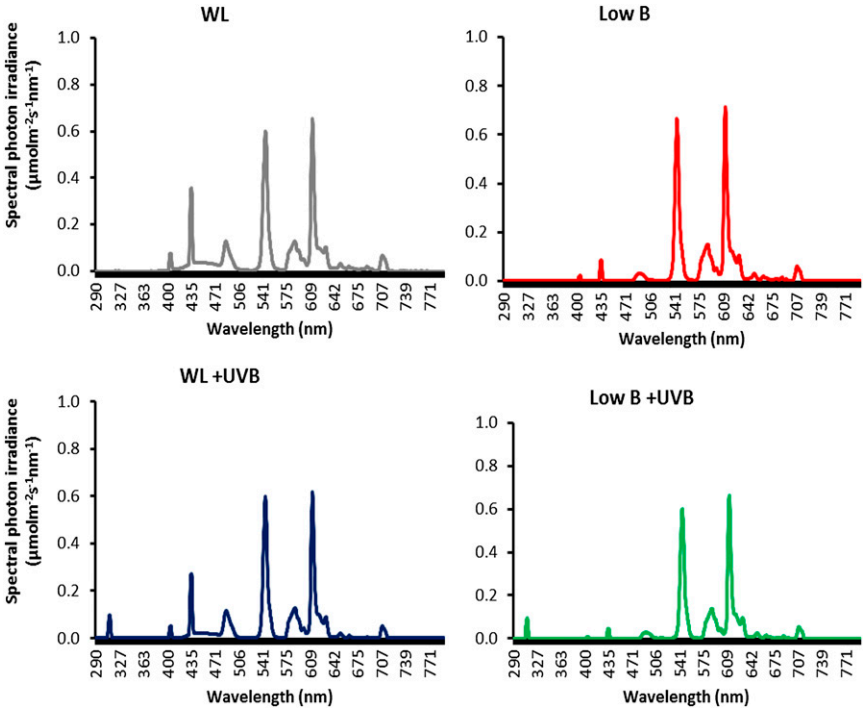

Fig. S3. Spectra of experimental conditions used for low blue light shade avoidance experiments. Here 90 µmol $m^{-2}s^{-1}$ WL was filtered through a neutral density filter to reduce PAR to 65 µmol $m^{-2}s^{-1}$, or through a yellow filter to reduce blue light (400–500 nm) from ~12 µmol $m^{-2}s^{-1}$ to ~2 µmol $m^{-2}s^{-1}$ (total PAR, 65 µmol $m^{-2}s^{-1}$). Supplementary UV-B was given at 400 mWm^{-2}.

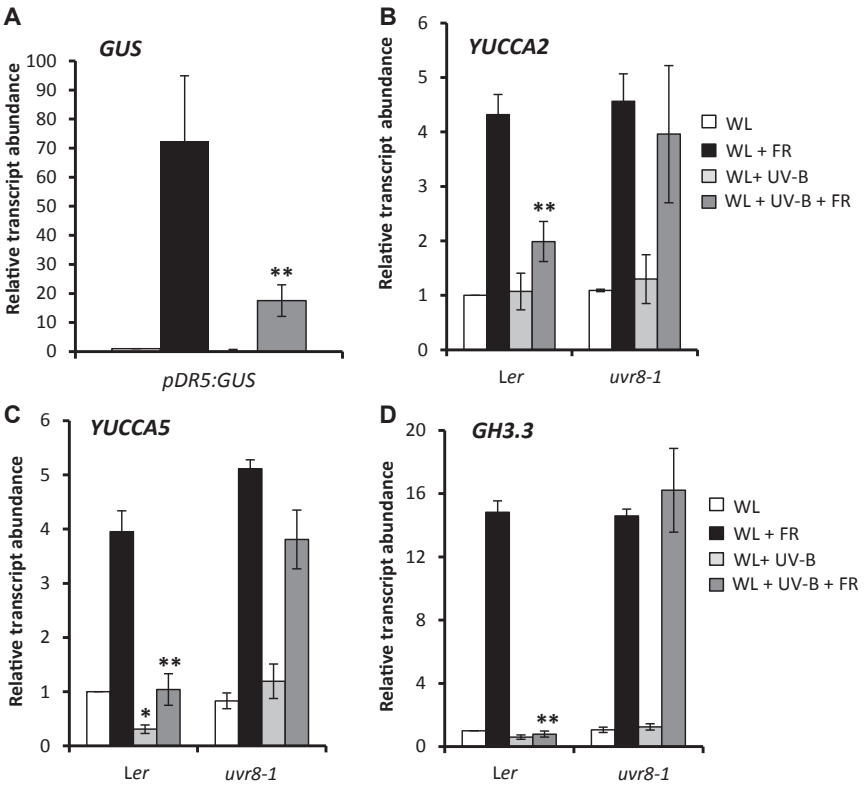

Fig. S4. UV-B inhibits auxin activity. (*A*) *GUS* transcript abundance in *pDR5:GUS* seedlings grown for 7 d in continuous WL before transfer to WL, +FR, +UV-B, and +FR+UV-B for 2 h. $n = 3 \pm$ SE. *Difference in transcript abundance compared with +FR ($P = 0.055$). (*B–D*) Relative transcript abundance of *YUCCA2* (*B*), *YUCCA5* (*C*), and *GH3.3* (*D*) in 7-d-old WT (L*er*) and *uvr8-1* seedlings transferred to experimental conditions for 4 h. $n = 3 \pm$ SE. *Significant difference in transcript abundance compared with WL ($P < 0.05$). **Significant difference in transcript abundance compared with +FR ($P < 0.05$).

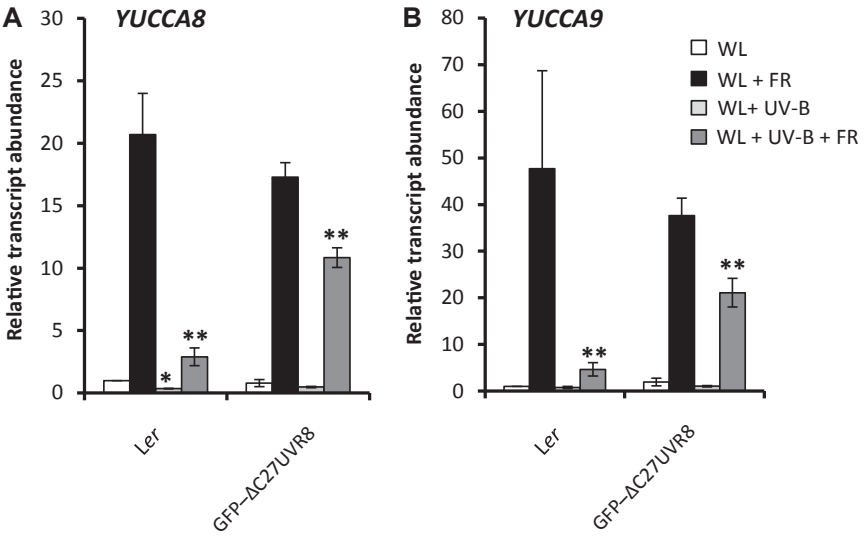

Fig. S5. UV-B–mediated inhibition of low R:FR-induced auxin biosynthesis genes occurs in GFP–ΔC27UVR8 plants. Relative transcript abundance of *YUCCA8* (*A*) and *YUCCA9* (*B*) in 7-d-old WT (L*er*) and GFP–ΔC27UVR8 seedlings transferred to experimental conditions for 4 h. $n = 3 \pm$ SE. *Significant difference in transcript abundance compared with WL ($P < 0.05$). **Significant difference in transcript abundance compared with +FR ($P < 0.05$).

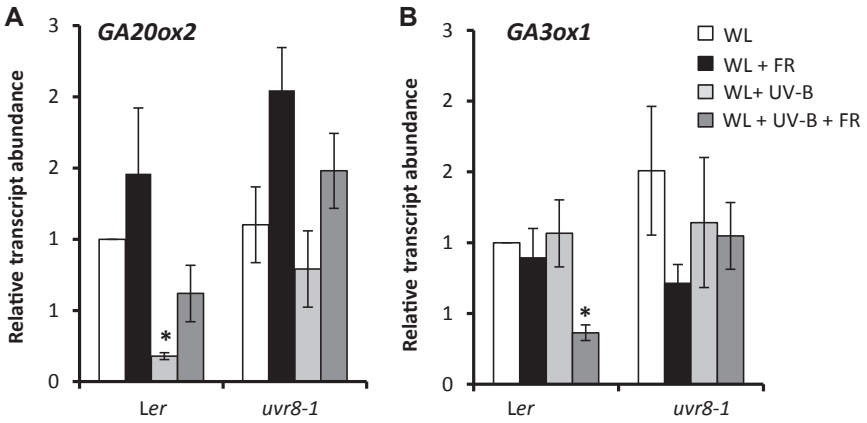

Fig. S6. GA biosynthesis genes do not display increased transcript abundance in UV-B. Relative transcript abundance of *GA20ox2* (*A*) and *GA3ox1* (*B*) in 7-d-old WT (L*er*) and *uvr8-1* seedlings transferred to experimental conditions for 4 h. $n = 3 \pm$ SE. *Significant difference in transcript abundance compared with WL ($P < 0.05$).

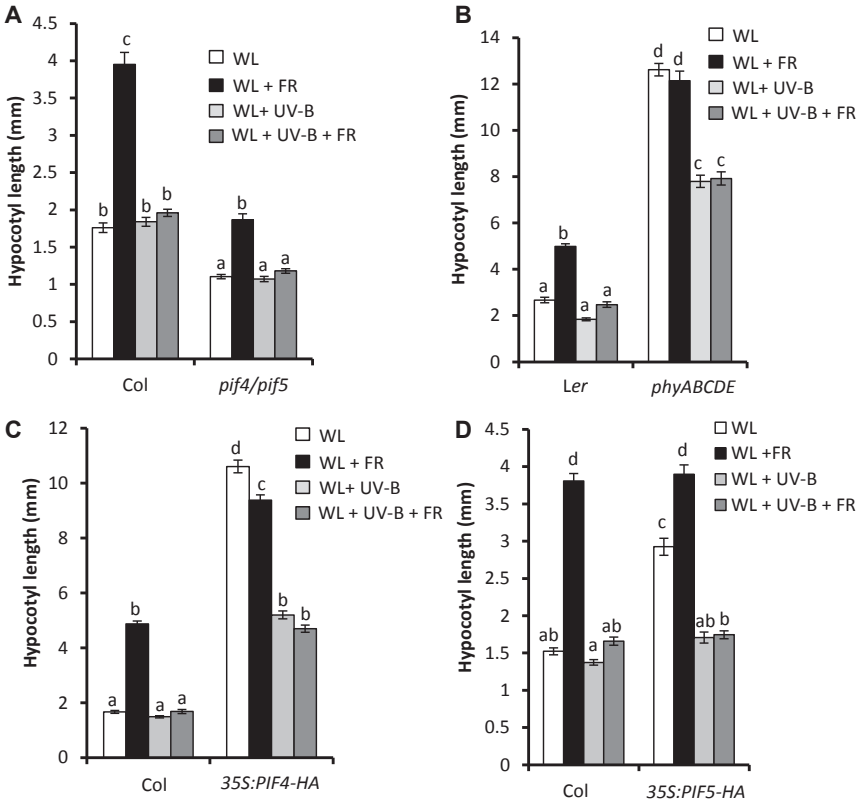

Fig. S7. UV-B–mediated inhibition of low R:FR-induced hypocotyl elongation occurs independent of phytochrome and involves PIF4 and PIF5. (*A*) Hypocotyl lengths of WT (Col-0) and *pif4/5* seedlings grown for 3 d in WL before transfer to WL, +FR, +UV-B, and +FR+UV-B for a further 4 d. (*B*) Hypocotyl lengths of WT (L*er*) and *phyABCDE* seedlings grown as in *A*. (*C* and *D*) Hypocotyl lengths of WT (Col-0), PIF4ox (*35S:PIF4-HA*) (*C*), and PIF5ox (*35S:PIF5-HA*) (*D*) grown as in *A*. $n \geq 34 \pm$ SE. Different letters indicate statistically significant differences between means ($P < 0.05$).

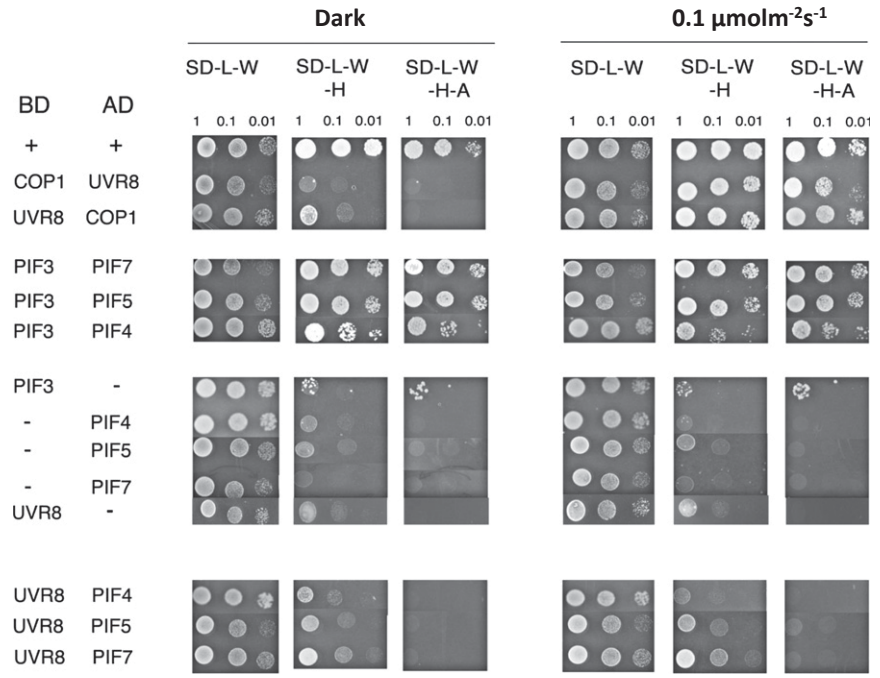

Fig. S8. UVR8 does not interact with PIFs in a UV-B–dependent manner. Yeast two-hybrid plasmids containing a DNA binding domain (BD) and activation domain (AD) fused to the proteins indicated were cotransformed in yeast and spotted on to nonselective (SD-L-W), partially selective (SD-L-W-H), and fully selective (SD-L-W-H-A) media plates. Three dilutions (decreasing from left to right) are shown for each treatment.

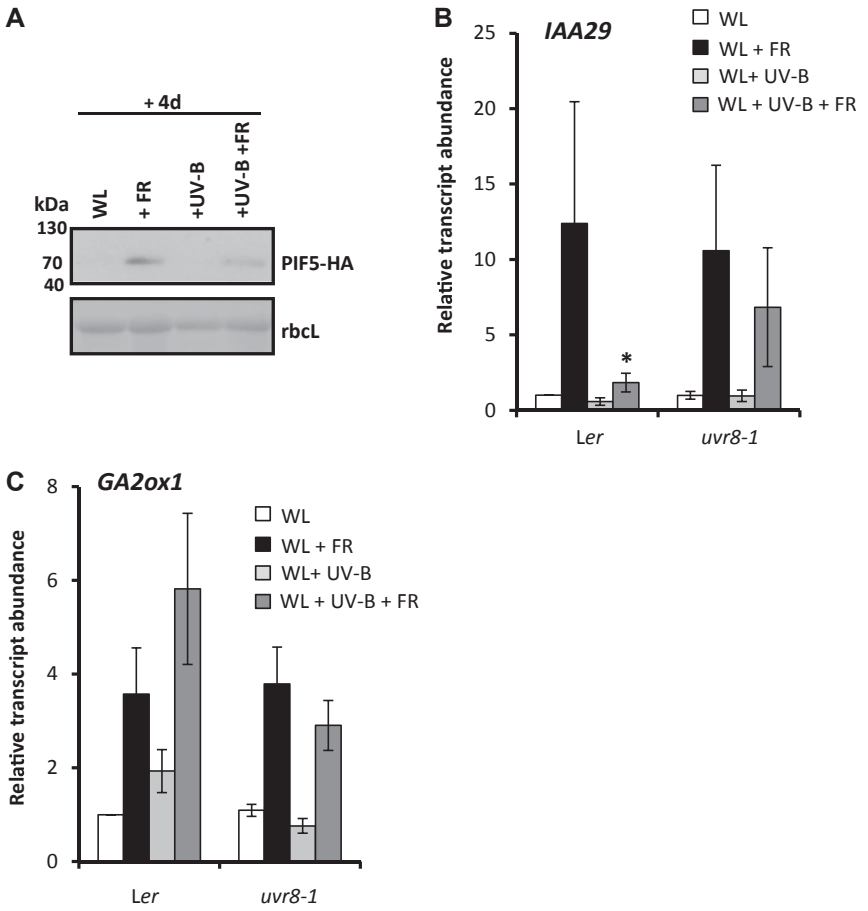

Fig. S9. UV-B inhibits low R:FR-mediated PIF5 protein accumulation and *IAA29* transcript abundance, but does not promote *GA2ox1* transcript abundance during prolonged treatment. (*A*) Western blot showing PIF5-HA abundance in *35S:PIF5-HA* seedlings grown for 3 d in continuous WL and transferred to indicated conditions for 4 d. A Ponceau stain of Rubisco large subunit (rbcL) is shown below as a loading control. Similar results were achieved in two biological repeats. (*B* and *C*) Relative transcript abundance of *IAA29* (*B*) and *GA2ox1* (*C*) in 7-d-old WT (L*er*) and *uvr8-1* seedlings treated in experimental conditions for 4 d. $n = 3 \pm$ SE. *Difference in transcript abundance compared with +FR ($P = 0.095$). Although *IAA29* error bars are larger than responses at 4 h (Fig. 3*D*), the same trends were observed over three biological repeats.

Table S1. Primer sequences used for cloning and qPCR

Primer	Sequence
COP1F	ATATTGAATTCATGGAAGAGATTTCGACGGA
COP1R	ATTGTCGACCTACTAGAATCACGCAGCGAGT
PIF3F	ATAGAATTCATGCCTCTGTTTGAGCTTTTCAGG
PIF3R	ATAGTCGACATGTCACGACGATCCACAAAACTG
PIF4F	AAACATATGCACCAAGGTTGGAGTTTTGAGGAGAAT
PIF4R	AAAATCGATACCTAGTGGTCCAAACGAGAACCGTC
PIF5F	TTTGAATTCGAAGATAATTTTCACATGTCCACTA
PIF5R	ATTGGATCCTCAGCCTATTTTACCCATATGAAGAC
PIF7F	AAAGAATTCATGTCGAATTATGGAGTTAAAGAGC
PIF7R	AATGGATCCCAATAATACTAGGTCGCTAGACTAATC
ActinF	tcagatgcccagaagtgttgttcc
ActinR	ccgtacagatccttcctgatatcc
UBC21F	GAATGCTTGGAGTCCTGCTTG
UBC21R	CTCAGGATGAGCCATCAATGC
HY5F	CGGAGAAAGTCAAAGGAAG
HY5R	CCAACTCGCTCAAGTAAG
HYHF	GGAAGAAACCCTGTTGATAAAGA
HYHR	GCATTGTGTTCTCGTTCGT
YUCCA2F	GGTGACACGGATCGGTTAGGGT
YUCCA2R	TGCCGAATAATGCATTACCCGT
YUCCA5F	TTCAACGAGTGTGTCCAGTCTGCT
YUCCA5R	TCTCTGGAACAACTTTCTCCGCGT
YUCCA8F	ATCAACCCTAAGTTCAACGAGTG
YUCCA8R	CTCCCGTAGCCACCACAAG
YUCCA9F	Gtcccattcgttgtggtcg
YUCCA9R	Ttgccacagtgacgctatgc
IAA29F	ATCACCATCATTGCCCGTAT
IAA29R	ATTGCCACACCATCCATCTT
GH3.3F	GGTCGGGAAAGAGTACGAGC
GH3.3R	CTTCCTCCGCACAAACTTGA
GA2ox1F	CCTTCGGATACGGGAACAGTAAGATTG
GA2ox1R	GTGTACTCTTCCAATGCGTTTCTGAAAG
GA20ox2F	ATGCTCACCGTTTGATGG
GA20ox2R	CCTTCCCAAACTGCTCG
GA3ox1F	ACGTTGGTGACCTCTTCCAC
GA3ox1R	CCCCAAAGGAATGCTACAGA
GUSF	CCCTTACGCTGAAGAGATGC
GUSR	gaggttaaagccgacagcag
PIL1F	AAATTGCTCTCAGCCATTCGTGG
PIL1R	TTCTAAGTTTGAGGCGGACGCAG
ATHB2F	GAGGTAGACTGCGAGTTCTTACG
ATHB2R	GCATGTAGAACTGAGGAGAGAGC