Supporting Information

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SI Materials and Methods

Protein Extraction, Immunoprecipitation, and Protein Gel Blots. Protein extraction, immunoprecipitation, and protein gel blotting were performed as reported previously (1). We used anti-CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1)$^{(13–26)}$ (1), anti-UV RESISTANCE LOCUS 8 (UVR8)$^{(426–440)}$ (1), anti-ELONGATED HYPOCOTYL 5 (HY5) (2), anti–SUPPRESSOR OF PHYA-105 (SPA1) (3), anti-actin (A0480; Sigma-Aldrich), and anti-CHALCONE SYNTHASE (CHS) (sc-12620; Santa Cruz Biotechnology) as primary antibodies. UVR8 homodimers were assayed using SDS-PAGE without sample boiling, as described previously (4). Chemiluminescence signals were generated using Amersham ECL Prime Western Blotting Detection Reagent, and the signals were detected with the Image Quant LAS 4000 mini CCD camera system and were quantified using the Image Quant TL software (GE Healthcare Life Sciences).

Size-Exclusion Chromatography. Total protein extracts were obtained from 7-d-old seedlings of a control line overexpressing *Pro35S::UVR8* (UVR8-Ox), UVR8^{W285A}#3, and UVR8^{W285F}#1, as described previously (1). Gel-filtration experiments were performed on a HiLoad 16/600 Superdex 200-pg column (GE Healthcare) equilibrated with buffer [50 mM Tris (pH7.6), 150 mM NaCl, 2 mM EDTA) and run at a flow rate of 1 mL/min at 4 °C on an Äkta Purifier system (GE Healthcare). Fractions of 2.5 mL were collected and further analyzed by blot. Aliquots (7 μL) of the collected fractions were heat-denatured for 5 min at 95 °C in SDS protein-loading buffer and were loaded on a 26-well Criterion XT Bis-Tris precast 4–12% polyacrylamide gel. SDS-PAGE was run for 50 min at 200 V in MES running buffer. UVR8 Western blot analysis was performed as described above.

Blue Native/SDS-PAGE. Total protein extracts of UVR8-Ox and UVR8^{W285A}#3 were prepared according to the NativePAGE Novex Bis-Tris Gel Electrophoresis protocol and were separated under native conditions over a NativePAGE Novex 4–16% Bis-Tris Gel (Life Technologies). Lanes subsequently were cut out and incubated with denaturing buffer [50 mM Tris (pH 6.8), 66 mM Na_2CO_3, 10% (wt/vol) glycerol, 2% (wt/vol) SDS, 2% β-mercaptoethanol] for 1 h under gentle agitation. Denatured first-dimension lanes were placed onto the stacking of a 12% SDS-PAGE gel, and the proteins were separated in the second dimension using the Mini-PROTEAN II system (BioRad) for 70 min at 180 V. UVR8 Western blot analysis, including UV-B treatment of the gel, was performed as described previously (4).

Yeast Two-Hybrid Growth Assays. Yeast transformation, UV-B irradiation, and His-tagged growth assays were performed as described previously (4). *Arabidopsis UVR8* and *COP1* coding regions were cloned in frame to the GAL4 DNA-binding domain in pGBKT7-GW and to the GAL4 activation domain in the pGADT7-GW vector. The pGBKT7-GW–based plasmids were transformed into Y2HGold yeast cells (Clontech), and pGADT7-GW–based plasmids were transformed into Y187 yeast cells (Clontech). After yeast mating, the transformants were selected on synthetic dropout medium lacking Trp and Leu (SC-Trp-Leu). Interactions between proteins were assayed on SC-Trp-Leu-His medium plates. For UV-B treatment, transformed yeast colonies were placed on SC-Trp-Leu-His medium and were irradiated by narrowband UV-B at 30 °C, as described previously (4).

Quantitative Real-Time PCR. *Arabidopsis* RNA was isolated with the Plant RNeasy kit (Qiagen) according to the manufacturer's instructions. For quantitative real-time PCR analysis, total RNA was treated with DNaseI according to the manufacturer's specifications (Qiagen). Per PCR, cDNA was synthesized from 50 ng RNA with random hexamers and oligo $d(T)_{16}$ in a 1/1 ratio using the TaqMan Reverse Transcription Reagents Kit (Applied Biosystems). Quantitative RT-PCR was carried out using a 7900HT real-time PCR system (Applied Biosystems) and TaqMan probes (Applied Biosystems). PCR reactions were performed using the ABsolute QPCR Rox Mix Kit following the manufacturer's instructions (ABgene). The gene-specific probes and primers were as follows: *HY5* (AT5G11260) probe 6-FAM-TCCAAACTCCGGCACTCGCCGTAT-TAMRA with HY5-forward (5′-AAAGAAGGAATTGAAAGCGATGA-3′) and HY5-reverse (5′-AAGTTTCTTTTCCGACAGCTTCTC-3′); *UVR8* (AT5G63860) probe 6-FAM-AGGATCGACCTTCTCCGACTCAGCTTAGC-TAMRA with UVR8-foward (5′-GGAGAGGATGGACAGTTAGGTCAT-3′) and UVR8-reverse (5′-CACAGGTAACGGAAACAATTTGG-3′); *COP1* (AT2G32950) probe 6-FAM-ACAACGAGCTCGCTTCTGCGTCCA–TAMRA with COP1-forward (5′-AGCCAACCACTTCATGTCTTCA-3′) and COP1-reverse (5′-ATCCCATAAGCGTAGTGTGCTATCT-3′); *EARLY LIGHT-INDUCIBLE PROTEIN 2* (*ELIP2*) (AT4G14690) probe 6-FAM-CGTTTAGCGGTCCAGCACCCGA-TAMRA with ELIP2-forward (5′-GTGAGTACGAAGTTTGGAGATT-TGC-3′) and ELIP2-reverse (5′-TTGCTAGTCTCCCGTTGAT-CCT-3′); *PHOTOLYASE 1* (*PHR1*) (AT1G12370) probe 6-FAM-AACCAACGCGCCGGTGGC-TAMRA with PHR1-forward (5′-GCCGTTGATCTGGCAAACA-3′) and PHR1-reverse (5′-TTGATCGAACAGATTGAAAACGA-3′). Gene-specific probes and primers for *CHS* (AT5G13930), *RUP1* (AT5G52250), and *RUP2* (AT5G23730) were as described previously (5, 6). cDNA concentrations were normalized to the 18S rRNA transcript level using the Eukaryotic 18S rRNA Kit (Applied Biosystems). Expression was determined in biological and technical triplicates.

Anthocyanin and Flavonol Extraction. The photometric determination of anthocyanins in 5-d-old *Arabidopsis* seedlings was performed as described previously (7).

Flavonols were extracted with 400 μL 80% methanol from 50 mg of ground tissue from 5-d-old seedlings. The extracts were shaken for 10 min at 70 °C and then were centrifuged for 5 min at 10,000 rpm in a MiniSpin microcentrifuge (Eppendorf), and the supernatants were evaporated in an Eppendorf Concentrator 5301 at 65 °C. The dried pellet was resuspended in 50 μL 80% methanol. For high-performance thin-layer chromatography (HPTLC), 5 μL of the methanolic extract was loaded onto an HPTLC Silica gel 60 glass plate (Millipore) used as the stationary phase. Adsorption chromatography, diphenylboric acid 2-aminoethylester staining, and documentation of the stained chromatograms were carried out as described previously (8).

Tandem Affinity Purification. Transgenes encoding tandem affinity purification-tag fusions under control of the *CDKA;1* promoter were cloned by multisite Gateway combining the promoter pENL4L3-Pcdka and the coding sequence of the bait (pEN-L1L2-UVR8^{W285A}) into the pKNGSrhino destination vector. In pKNGSrhino, the tobacco etch virus (TEV) protease cleavage sites of the GS tag (pKNGS) (9) were replaced by two rhinovirus 3C protease cleavage sites allowing elution at 4 °C. Transformation of *Arabidopsis* cell-suspension cultures was carried out as described

previously (10). Tandem affinity purification of protein complexes was done based on the GSrhino tag using an adapted downscaled purification protocol similar to the GS protocol described previously (11). In short, cell extracts were made from 2.5 g cell-suspension cells, incubated with 0.1% Benzonase Nuclease (Novagen) for 30 min and cleared by two subsequent centrifugation steps at 36,900 × *g* for 20 min. In the first purification step, a protein input of 25 mg was incubated with 25 μL of IgG-Sepharose 6 Fast Flow beads (GE Healthcare). After washing, protein complexes were eluted with 10 U of rhinovirus 3C protease for 1 h at 4 °C, with a second boost of 10 U applied after 30 min. In the second step, 25 μL of Streptavidin Sepharose High Performance (Amersham) was used. Final elution was done with 40 μL 1× NuPAGE sample buffer containing 20 mM desthiobiotin for 5 min. Beads were separated from eluate in a 1-mL Mobicol column (MoBiTec). Eluted proteins were concentrated in a short run of 7 min on a 4–12% gradient NuPAGE gel (Invitrogen) and visualized with colloidal Coomassie Brilliant Blue staining. Detailed information on MS preparation and protein sequencing on the LTQ Orbitrap Velos is given below. The list of proteins detected by MS was cleaned up based on a list of nonspecific background proteins. This list was assembled by combining our previous background list based on ∼40 tandem affinity purification experiments on wild-type cultures and cultures expressing tandem affinity purification-tagged mock proteins GUS, RFP, and GFP (12), with background proteins identified with LTQ Orbitrap Velos from control GS purifications on wild-type and transgenic cell cultures expressing GS-tagged GUS and GFP. Background proteins were subtracted from the list of MS-identified proteins to obtain the final list of copurified proteins.

LC-MS/MS Analysis. The NuPAGE gel containing concentrated eluted proteins was washed for 2 h in H_2O, polypeptide disulfide bridges were reduced for 40 min in 25 mL of 6.66 mM DTT in 50 mM NH_4HCO_3, and then the thiol groups were alkylated for 30 min in 25 mL 55 mM Iodoacetamide (Sigma Aldrich) in 50 mM NH_4HCO_3. After washing with H_2O, a broad zone containing the proteins was cut from the protein gel, sliced into 24 gel plugs, and collected together in a single Eppendorf tube. Gel plugs were washed twice with H_2O, dehydrated with 95% CH_3CN (vol/vol), rehydrated with H_2O, and dehydrated again with 95% CH_3CN (vol/vol). Dehydrated gel particles were rehydrated in 60 μL digest buffer containing 750 ng trypsin (MS Gold; Promega), 50 mM NH_4HCO_3, and 10% CH_3CN (vol/vol) for 30 min at 4 °C. Proteins were digested at 37 °C for 3.5 h.

The obtained peptide mixtures were introduced into an LC-MS/MS system, the Ultimate 3000 RSLC nano (Dionex) in-line connected to an LTQ Orbitrap Velos (Thermo Fisher Scientific). The sample mixture was loaded on a trapping column made in-house (100-μm i.d. × 20 mm) with 5 μm C18 Reprosil-HD beads (Dr. Maisch GmbH). After back-flushing from the trapping column, the sample was loaded on a reverse-phase column made in-house (75-μm i.d. × 150 mm) with 5 μm C18 Reprosil-HD beads (Dr. Maisch GmbH). Peptides were loaded with solvent A (0.1% trifluoroacetic acid, 2% acetonitrile) and were separated with a linear gradient from 2% solvent A′ (0.1% formic acid) to 50% solvent B′ (0.1% formic acid and 80% acetonitrile) at a flow rate of 300 nL/min, followed by a wash step reaching 100% solvent B′.

The mass spectrometer was operated in data-dependent mode, automatically switching between MS and MS/MS acquisition for the 10 most abundant peaks in a given MS spectrum. In the LTQ Orbitrap Velos, full-scan MS spectra were acquired in the Orbitrap at a target value of 1E6 with a resolution of 60,000. The 10 most intense ions then were isolated for fragmentation in the linear ion trap, with a dynamic exclusion of 20 s. Peptides were fragmented after filling the ion trap at a target value of 1E4 ion counts.

From the MS/MS data in each LC run, Mascot Generic Files were created using the Mascot Distiller software (version 2.4.3.1, Matrix Science, www.matrixscience.com/Distiller.html). When generating these peak lists, grouping of spectra was allowed with a maximum intermediate retention time of 30 s; a maximum intermediate scan count of 5 was used where possible. Grouping was done with a precursor tolerance of 0.005 Da. A peak list was generated only when the MS/MS spectrum contained more than 10 peaks. There was no de-isotoping, and the relative signal-to-noise limit was set to 2. These peak lists then were searched with the Mascot search engine (version 2.4.1, MatrixScience, www.matrixscience.com) using the Mascot Daemon interface (Matrix Science, www.matrixscience.com). Spectra were searched against the TAIR10 database extended (TAIR10-ext) with common Repository of Adventitious Proteins (cRAP) protein sequences (The Global Proteome Machine, www.thegpm.org/crap/) and other non-TAIR10 protein sequences commonly found in tandem affinity purifications, in total containing 35,839 sequence entries. Variable modifications were set to methionine oxidation and methylation of aspartic acid and glutamic acid. Fixed modifications were set to carbamidomethylation of cysteines. Mass tolerance on MS was set to 10 ppm (with Mascot's C13 option set to 1), and the MS/MS tolerance was set at 0.5 Da. The peptide charge was set to 1+, 2+, and 3+, and the instrument setting was ESI-TRAP. Trypsin was set as the protease used, allowing for one missed cleavage; cleavage also was allowed when arginine or lysine was followed by proline. Only high-confidence peptides (ranked one and with scores above the threshold score, set at the 99% confidence interval), were withheld. Only proteins with at least two matched high-confidence peptides were retained.

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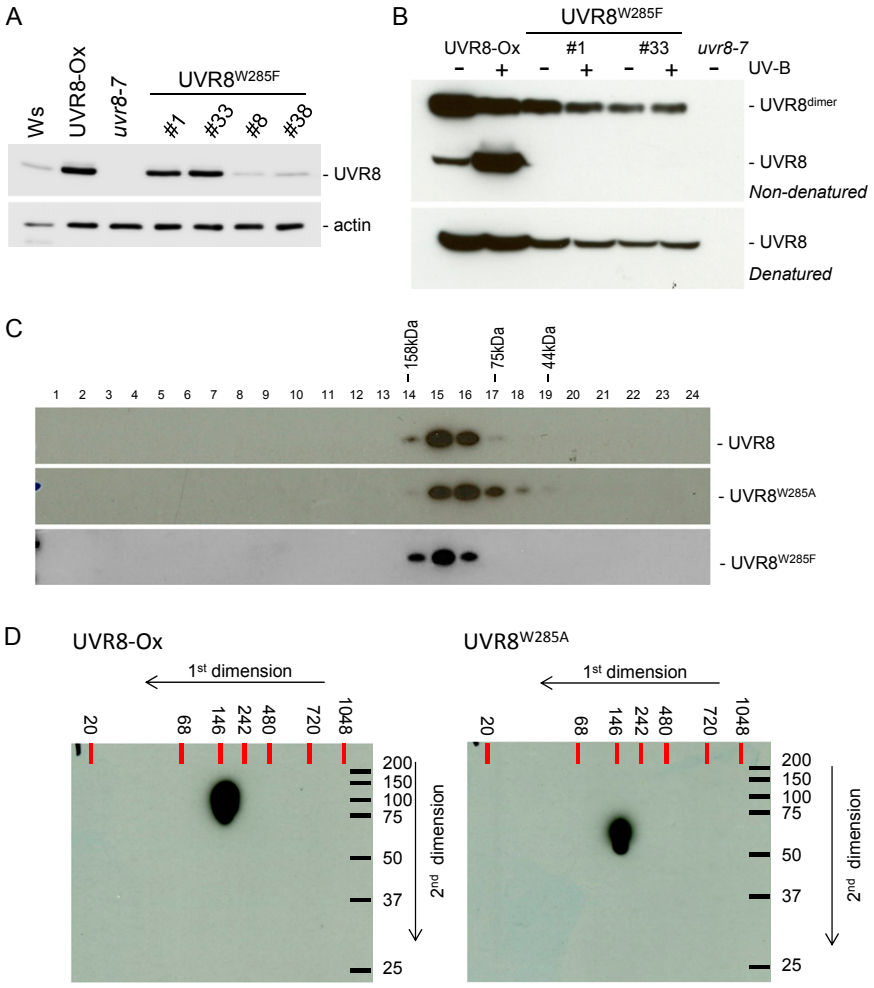

Fig. S1. UVR^{W285F} is a constitutive dimer in transgenic plants, UVR^{W285A} is partially monomeric. (*A*) UVR8 protein levels in wild-type (Ws) plants (compare with levels in *Pro$_{35S}$::UVR8^{W285F}* lines #8 and #38) and in a line overexpressing *Pro$_{35S}$::UVR8* (UVR8-Ox) (compare with levels in *Pro$_{35S}$::UVR8^{W285F}* overexpressor lines #1 and #33). (*B*) Four-day-old seedlings were irradiated for 15 min with (+) or without (–) supplementary broadband UV-B. UVR8 dimers were analyzed on Western blots after SDS-PAGE without sample boiling (*Upper*), and total UVR8 protein was detected in samples denatured by boiling (*Lower*). In *A* and *B*, *uvr8-7* was included as a negative control demonstrating the specificity of the detected bands. (*C*) The gel-filtration profiles of the UVR8, UVR8^{W285A}, and UVR8^{W285F} proteins extracted from transgenic seedlings [UVR8-Ox, *Pro$_{35S}$::UVR8^{W285A}* (line #3) and *Pro$_{35S}$::UVR8^{W285F}* (line #1)] grown under white light devoid of UV-B. The proteins in the fractions were separated by SDS-PAGE and examined by immunoblot analysis using anti-UVR8 antibody. The molecular masses (in kDa) estimated by protein standards are indicated above the appropriate fractions. (*D*) 2D Blue Native/SDS-PAGE on total protein extracts of 7-d-old UVR8-Ox (*Left*) and *Pro$_{35S}$::UVR8^{W285A}* line #3 (*Right*) seedlings grown under white light followed by UVR8 Western blot.

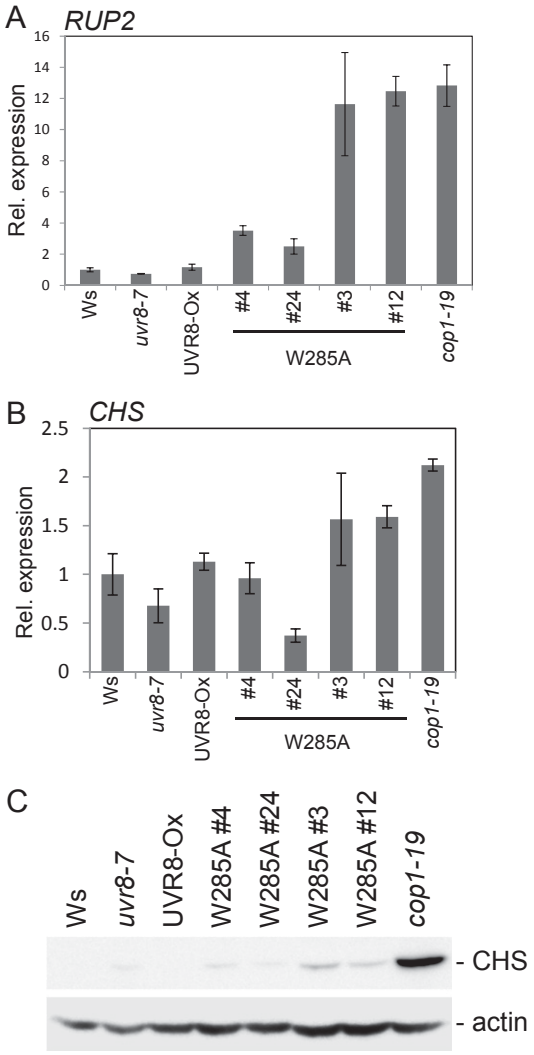

Fig. S2. Results of UVR8^{W285A} expression in constitutive UV-B responses. (*A*) *RUP2* mRNA levels in 4-d-old seedlings grown in the dark. (*B* and *C*) *CHS* mRNA levels (*B*) and CHS protein levels (*C*) in 4-d-old seedlings grown in the dark.

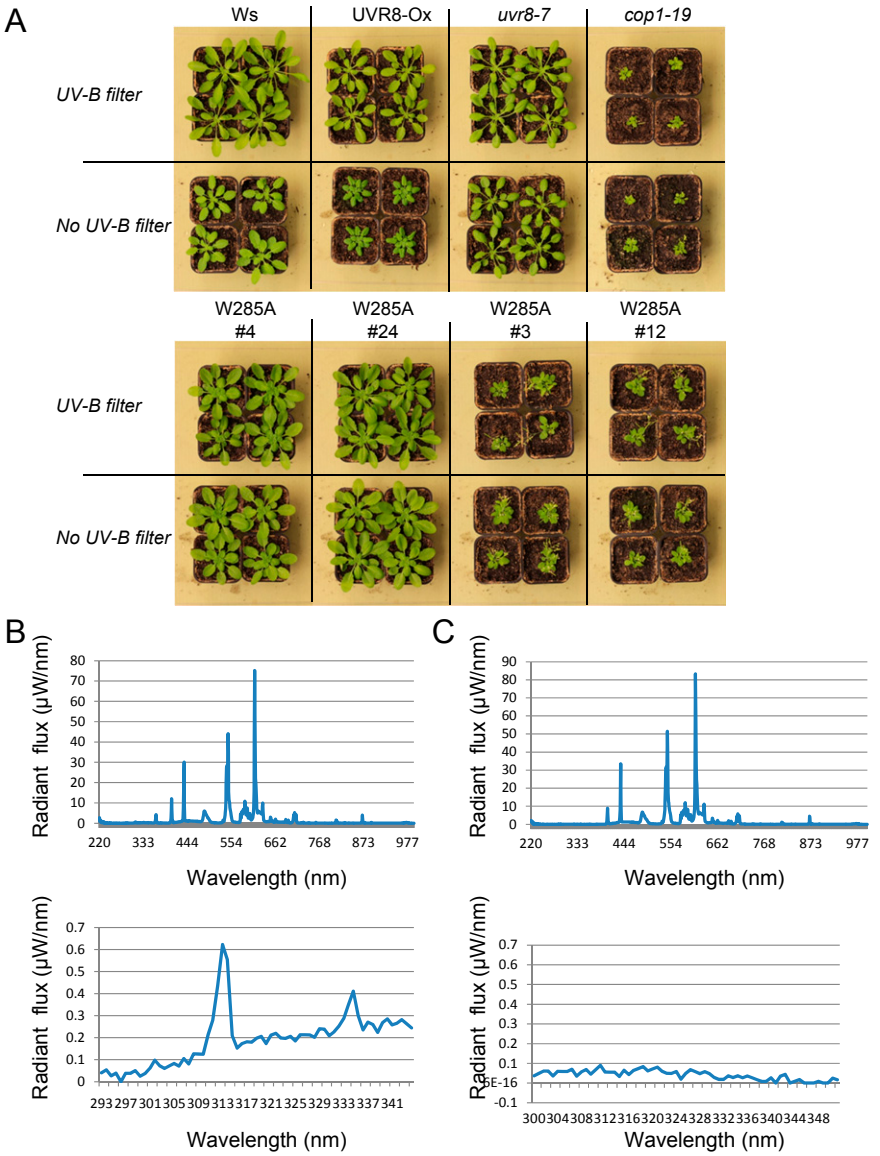

Fig. S3. The UV-B content of the light from standard fluorescent lamps is sufficient to activate UV-B–dependent responses of UVR8. (*A*) Phenotypes of adult wild-type (Ws), *UVR8*-overexpressors (*UVR8-Ox*), and *uvr8-7, cop1-19*, and *UVR8*^{W285A} mutants under standard short-day growing conditions illuminated by fluorescent lamps filtered (UV-B filter) or not (No UV-B filter) with UV-B foil #226. The photographs of plants under the UV-B filter are identical to those shown in Fig. 5; photographs of plants grown with and without filter were taken in parallel. (*B* and *C*) The light spectrum emitted by standard growth fluorescent lamps as measured with an Ocean Optics QE6500 spectrometer without (*B*) and with (*C*) with a UV-B filter #226. (*Upper*) Total spectrum, 200–984 nm. (*Lower*) Spectrum over the UVR8 active range of 280–315 nm.

Other Supporting Information Files

Table S1 (XLSX)