Supporting Information

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SI Materials and Methods

PCR Genotyping of Mutants and Isolation of Double Mutants. Single mutants were crossed, and the double mutants were identified by PCR genotyping in the F2 generation. Plant genomic DNA for PCR analysis was prepared as described previously (1). The left-border–specific primer commonly used to genotype SALK insertion lines is LBa1 (5′-TGGTTCACGTAGTGGGCC-3′) (2).

- *hy5-215* (3): HY5_for3 (5′-TAA GAA AAA TGC AGG AAC-3′) + HY5_rev4 (5′-CTC ATC GCT TTC AAT TCC-3′) = 0,34kb WT; HY5_for3 + *hy5-215*_rev (5′-CTC ATC GCT TTC AAT TCT-3′) = 0,34kb for *hy5-215*.
- *rup1-1* (SALK_060638): At5g52250_for1 (5′-CGG GAT CCA TTT AAA TCT CTC TCT TTC CGC CG-3′) + At5-g52250_rev1 (5′-GGT CTA GAG GCG CGC CCA CAT TTG AAC CGT TCC-3′) = 0,4kb WT; At5g52250_rev1 + LBa1 = 0,71kb for *rup1-1*.
- *rup2-1* (SALK_108846): S_108846_LP (5′-CCG GCG AAA CTT AGT AGT C-3′) + S_108846_RP (5′-CTT GAA GAA AGT CAT TCC CA-3′) = 1,1kb WT; S_108846_LP + LBa1 = 0,69kb for *rup2-1*.
- *uvr8-6* (SALK_033468) (4): UVR8_for5 (5′-AGG AGT GAT ATG CAT TC-3′) + UVR8_rev6 (5′-TCC CAA ACT AGA CAG ACG-3′) = 1,26kb WT; UVR8_for5 + LBa1 = 0,67kb for *uvr8-6*.

Cloning of *RUP1* and *RUP2* Genes and Generation of Transgenic *Arabidopsis* Lines. The WT *RUP2* genomic fragment and *RUP1* and *RUP2* ORFs were cloned into pDONR207 and sequenced to check the integrity of the cloned fragment; the primers are described below. Gateway-based cloning was then used to insert the ORF into the binary destination vectors pB2GW7 and pB7WGY2 (5) and insert the *RUP2* genomic clone into pMDC107 (6). The constructs were verified by sequencing, and *Arabidopsis* plants were transformed using *Agrobacterium*. The resulting transgenic lines described in this work have the transgene integrated at a single locus.

The following primers were used to amplify and clone *RUP1* and *RUP2* coding sequences into the pDONR207 vector (Invitrogen):

- *RUP1* full-length −STOP (1,145 bp): RUP1-BP_for (5′-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC AAT GGA GGC TTT GTT CTG C-3′) and RUP1-BP_rev (5′-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC GCT TTG TTT GCC CGA GAA-3′)
- *RUP1* full-length +STOP (1,148 bp): RUP1-BP_for (5′-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC AAT GGA GGC TTT GTT CTG C-3′) and RUP1-BPwD_rev+Stop (5′-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TTA GCT TTG TTT GCC CGA-3′)
- *RUP2* full-length −STOP (1,102 bp): RUP2-BP_for (5′-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC AAT GAA CAC TCT TCA TCC T-3′) and RUP2-BP_rev (5′-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TGG TTT TCT TTT GCC CAC-3′)
- *RUP2* full-length +STOP (1,105 bp): RUP2-BP_for (5′-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC AAT GAA CAC TCT TCA TCC T-3′) and RUP2-BP_rev+Stop (5′-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC CTA TGG TTT TCT TTT GCC-3′).

The following primers were used to amplify and clone the *RUP2* genomic fragment:

- *RUP2* genomic −STOP (2,671 bp): RUP2gen-BP_for (5′-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT TCA CGT ATG ACT CGT CCT TAC TTT GC-3′) and RUP2gen-BP_rev (5′-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC CTA TGG TTT TCT TTT GCC CAC GTA A-3′).

Nuclear Protein Extraction. Total protein was extracted from 12-d-old seedlings after a 6-h exposure to supplemental narrowband UV-B in extraction buffer [20 mM MOPS (pH 7), 0.5 M hexylene glycol, 10 mM $MgCl_2$, 5 mM β-mercaptoethanol, and one Complete Mini Protease Inhibitor Mixture Tablet (Roche) added per 10 mL of buffer]. The homogenate was passed through four layers of Miracloth (Calbiochem), after which Triton X-100 was added until a 0.5% final concentration was achieved. The total extract was centrifuged for 10 min at 1,000 × g at 4 °C. The supernatant was collected as cytosolic fraction and the pellet as nuclear fraction. The nuclear fraction was washed three times with extraction buffer. The protein concentrations of the total, nuclear, and cytosolic fractions were determined by the Amido black method, and equal amounts of protein were loaded onto an 8% SDS/PAGE gel.

Phylogeny. The evolutionary history based on the WD40-repeats of the 85 *Arabidopsis* DWD proteins was inferred using the neighbor-joining method (7). The optimal tree with the sum of branch length 66.52398388 is shown in Fig. S3. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and represent the number of amino acid substitutions per site. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (pairwise deletion option). There were a total of 492 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (8).

1. Edwards K, Johnstone C, Thompson C (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Res* 19:1349.
2. Alonso JM, et al. (2003) Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* 301:653–657.
3. Oyama T, Shimura Y, Okada K (1997) The *Arabidopsis HY5* gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. *Genes Dev* 11: 2983–2995.
4. Favory JJ, et al. (2009) Interaction of COP1 and UVR8 regulates UV-B–induced photomorphogenesis and stress acclimation in *Arabidopsis*. *EMBO J* 28:591–601.
5. Karimi M, Inzé D, Depicker A (2002) GATEWAY vectors for *Agrobacterium*-mediated plant transformation. *Trends Plant Sci* 7:193–195.
6. Curtis MD, Grossniklaus U (2003) A gateway cloning vector set for high-throughput functional analysis of genes in planta. *Plant Physiol* 133:462–469.
7. Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425.
8. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599.

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RUP1 MEALFCSEIPNNNIRSSINDLSSSSSYTWPMIMTSSSSSSSSPTIMNIEN  50
RUP2 --------------------------------MNTLHPHKQQQEQAQQQE  18

RUP1 IPRCDWDLSLSAVVSSAS-TGSDAIGAIEFDPTGEIIATGGIARKIRSYR  99
RUP2 EARYEWDLSLSTVVSSSSSASDVIGAIEFDPTDNIVATAGISRKIRFYG  68

RUP1 LSSLLE---------SRDDHVTASESYICTPAKLSSLKWRPDFSGRVIGS 140
RUP2 LPSLLRNNAVSGTGVSFVDQATACEYYICTPAKLSSLRWRPGSGGRVIGS 118

RUP1 GDYDGVVTEYDVEKQVPVSERDEHGGRRIWSVDYTLYNG-SLIGASGSDD 189
RUP2 GDYDGVVMEYDLEKRTPVFERDEHGGRRVWSVDYTRHGGASTVGASGSDD 168

RUP1 GTVQMWDPRNG-GTLEETVRPGG--GAAICSVEFDPFGGSSIAVGCADRN 236
RUP2 GTMQVWDPRCPPEESVGVVRPAGICRSAVCCVEFDPSGGPAVAVGCADRK 218

RUP1 AYVYDIRRLVDPLIVLDGHTKTVTYARFMDSHTIVTGSTDGSLKQWDIDN 286
RUP2 GYVYDIRKLVDPALTLQGHTKTVSYVRFLDGGTVVTAGTDGCLKLWSVED 268

RUP1 GRRVVRTYRGHVNSRNFVGLSVWRHGGLVVSGSENNQVFVYDKRWEEPVW 336
RUP2 GR-VIRTYEGHVNNRNFVGLSVWRNGALFGCGSENNRVFVYDRRWGKPVW 317

RUP1 VCGLGHTNR-FGSDRRFVSSVCLRQVDEDWCTLVAGGSDGALEIFSGKQS 385
RUP2 VDGFEPVGMNSGSDKRFVSSVCWRQSGVDQCTLVAGGSDGVLQVYVGKRK 367

RUP1 -
RUP2 P 368
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Fig. S1. Amino acid sequence alignment of *Arabidopsis* RUP1 and RUP2 proteins. Identical and similar amino acids are highlighted in black and gray, respectively. Dashes indicate gaps in the sequence to optimize the alignment.

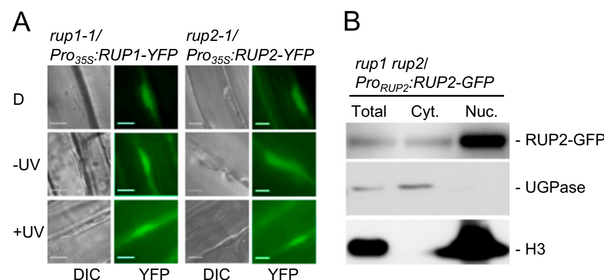

Fig. S2. Fluorescent protein–tagged RUP1 and RUP2 proteins show nuclear and cytosolic localization. (*A*) Subcellular localization of RUP1-YFP and RUP2-YFP in stably transformed *Arabidopsis* plants. Representative cells of 4-d-old seedlings grown in darkness (D), continuous white light (−UV), and continuous white light with supplementary narrowband UV-B (+UV) are shown. (Scale bar: 10 μm.) (*B*) Protein gel blot of total protein, and cytosolic (cyt.) and nuclear (nuc.) fractions of *rup1 rup2*/*Pro$_{RUP2}$:RUP2-GFP* plants grown in white light supplemented with narrowband UV-B probed with anti-GFP, anti-UGPase (cytosolic control), and anti-histone H3 (nuclear control) antibodies.

Fig. S3. A neighbor-joining phylogenetic tree constructed from 85 *Arabidopsis* DWD proteins containing WD40-repeats (according to ref. 1).

1. Lee JH, et al. (2008) Characterization of *Arabidopsis* and rice DWD proteins and their roles as substrate receptors for CUL4-RING E3 ubiquitin ligases. *Plant Cell* 20:152–167.

Fig. S4. Additional controls for BiFC assays with empty vectors YC-RUP1 and YC-RUP2.

Fig. S5. (*A*) Structure of *RUP1* and *RUP2*, and the location of T-DNA insertions in *rup1-1* and *rup2-1*. (*B*) RNA gel blot analysis of WT (Col), *rup1-1* and *rup2-1* single mutants, and *rup1 rup2* double mutants. Total RNA was isolated from 4-d-old seedlings irradiated with UV-B for the indicated times before harvesting. Blots were sequentially hybridized with specific probes for *RUP1* and *RUP2*. Ethidium bromide–stained rRNA is shown as a loading control. (*C*) Seedlings were grown in darkness, under white light (–UV-B), or under white light supplemented with UV-B (+UV-B). The extracts used for anthocyanin measurements are shown below the pictures of the seedlings. (*D* and *E*) Quantitative RT-PCR analysis of *HY5* (*D*) and *CHS* (*E*) gene activation in response to UV-B in *rup1 rup2* double mutants compared with WT Col. Four-day-old seedlings were irradiated with UV-B for the indicated times before harvesting. Error bars represent the SD of three biological repetitions.

Fig. S6. *RUP1* and *RUP2* genes are red light (R)-, far-red light (FR)-, and blue light (B)-inducible, but *rup1 rup2* double mutants are not affected in hypocotyl growth inhibition under these light qualities. (*A*) Quantitative RT-PCR analysis of *RUP1* and *RUP2* gene activation in response to R, FR, and B light by WT Col. Four-day-old etiolated seedlings grown in darkness were irradiated for 1 and 6 h with the indicated light qualities and compared with the dark control. (*B–D*) Hypocotyl length measurements of WT and *rup1 rup2* double mutants grown for 4 d under different FR (*B*), R (*C*), and B (*D*) photonfluence rates compared with *phyA*, *phyB*, and *cry1 cry2* mutants, respectively ($n = 30$). (*E*) Quantitative RT-PCR of *HY5* gene activation in response to 1 h of B, R, and FR compared with control samples that were kept in the dark (*D*). (*F*) Quantitative RT-PCR of *CHS* gene activation in response to 6 h of B, R, and FR. In *E* and *F*, 4-d-old etiolated seedlings of WT, *rup1 rup2* double mutant, and *rup2-1/Pro$_{35S}$:RUP2* overexpression lines were irradiated with B (38.5 μmol m^{-2} s^{-1}), R (38.9 μmol m^{-2} s^{-1}), and FR (20.0 μmol m^{-2} s^{-1}) for the indicated time. Data from control seedlings kept in the dark (D) were set at 1. Error bars represent SD of technical triplicates.

Fig. S7. Model of the RUP-mediated feedback that represses the UV-B photoregulatory pathway. Under supplementary UV-B (WL + UVB), the specific perception by a UV-B photoreceptor results in rapid UVR8–COP1 interaction. UV-B perception results in stabilization of the HY5 bZIP transcription factor and the COP1/UVR8-mediated activation of *HY5* gene expression. Activation of *RUP1* and *RUP2* gene expression in response to UV-B requires UVR8, COP1, and HY5 proteins. RUP1 and RUP2 protein accumulation then provides a negative feedback regulation of the UV-B photoregulatory pathway through direct interaction with UVR8.

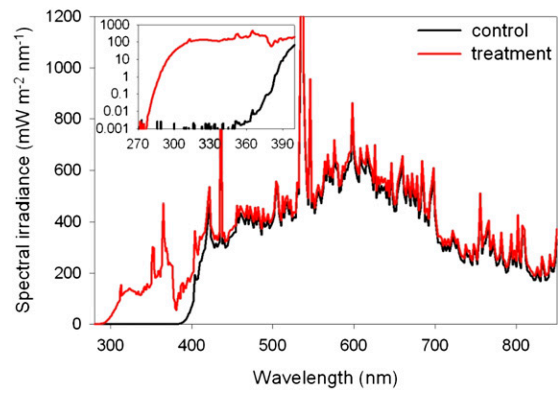

Fig. S8. Spectral irradiances of the study in the sun simulator for control and UV treatment. (*Insert*) UV range of 270–400 nm in logarithmic scale.

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