Two E3 Ligases Antagonistically Regulate the UV-B Response in Arabidopsis

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Supporting Information

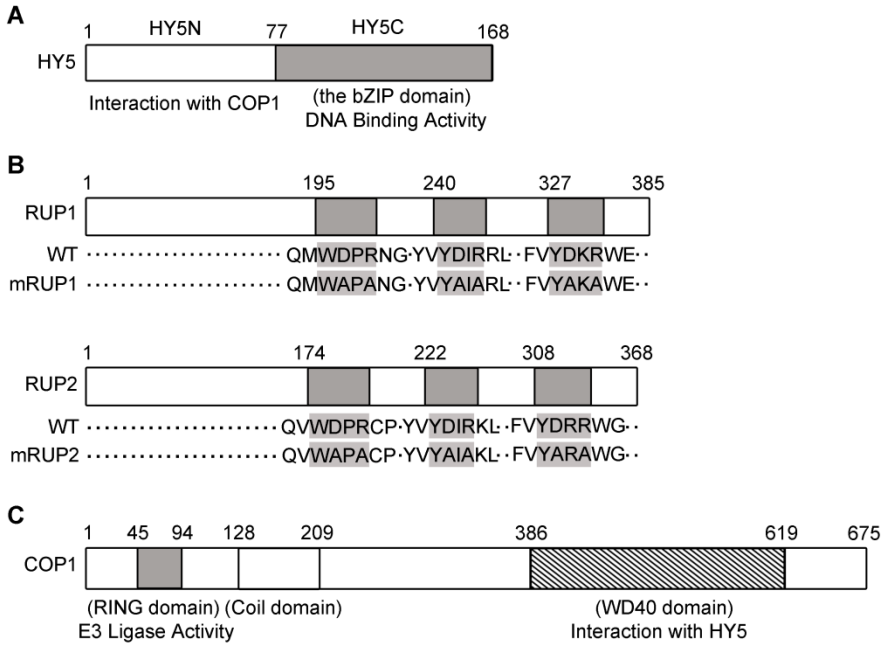

Fig. S1. Schematic Diagrams of HY5, RUP1, RUP2 and COP1 Proteins.

(A) Schematic diagram of the functional domains of HY5 protein. HY5N, the N-terminal domain of HY5. HY5C, the C-terminal domain of HY5. Numbers indicate the positions of amino acids.

(B) Schematic diagrams of RUP1 and RUP2 proteins. WT, RUP1/RUP2 with wild-type DWD motifs. mRUP1/mRUP2, RUP1/RUP2 with mutated DWD motifs. Numbers indicate the positions of amino acids.

(C) Schematic diagram of the functional domains of COP1 protein. Numbers indicate the positions of amino acids.

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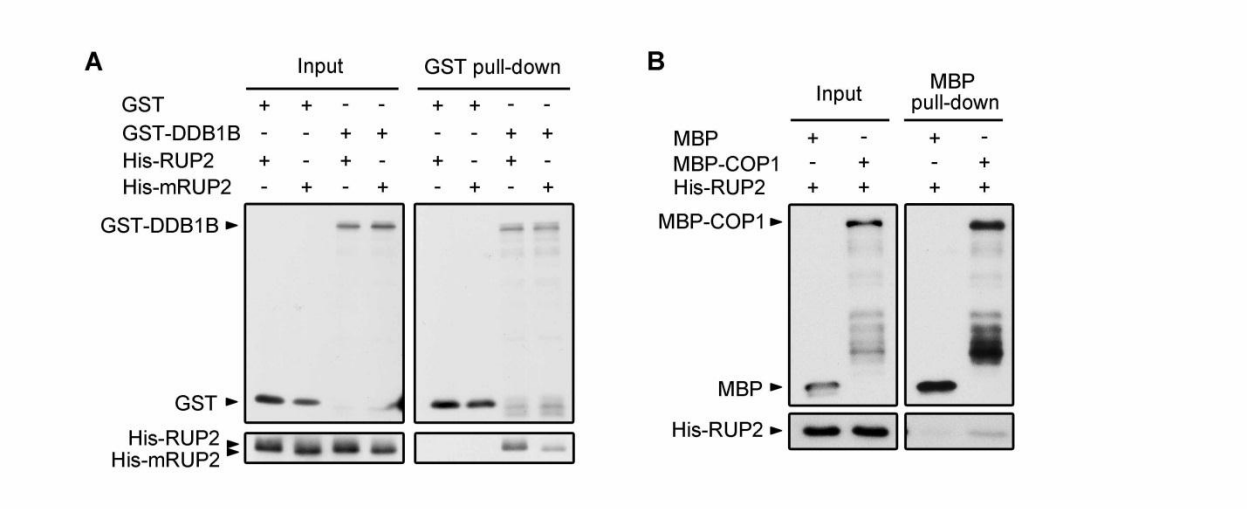

Fig. S2. In Vitro Pull-down Assays of GST-DDB1B/MBP-COP1 and His-RUP2.

(A) DDB1B interacts with RUP2 in vitro. The purified GST or GST-DDB1B was incubated with His-RUP2 or His-mRUP2 before being pulled down by Glutathione Sepharose. His-RUP2 and His-mRUP2 were detected by anti-RUP2 antibodies.

(B) COP1 interacts with RUP2 in vitro. The purified MBP or MBP-COP1 was incubated with His-RUP2 before being pulled down by Amylose Resin. His-RUP2 was detected by anti-RUP2 antibodies.

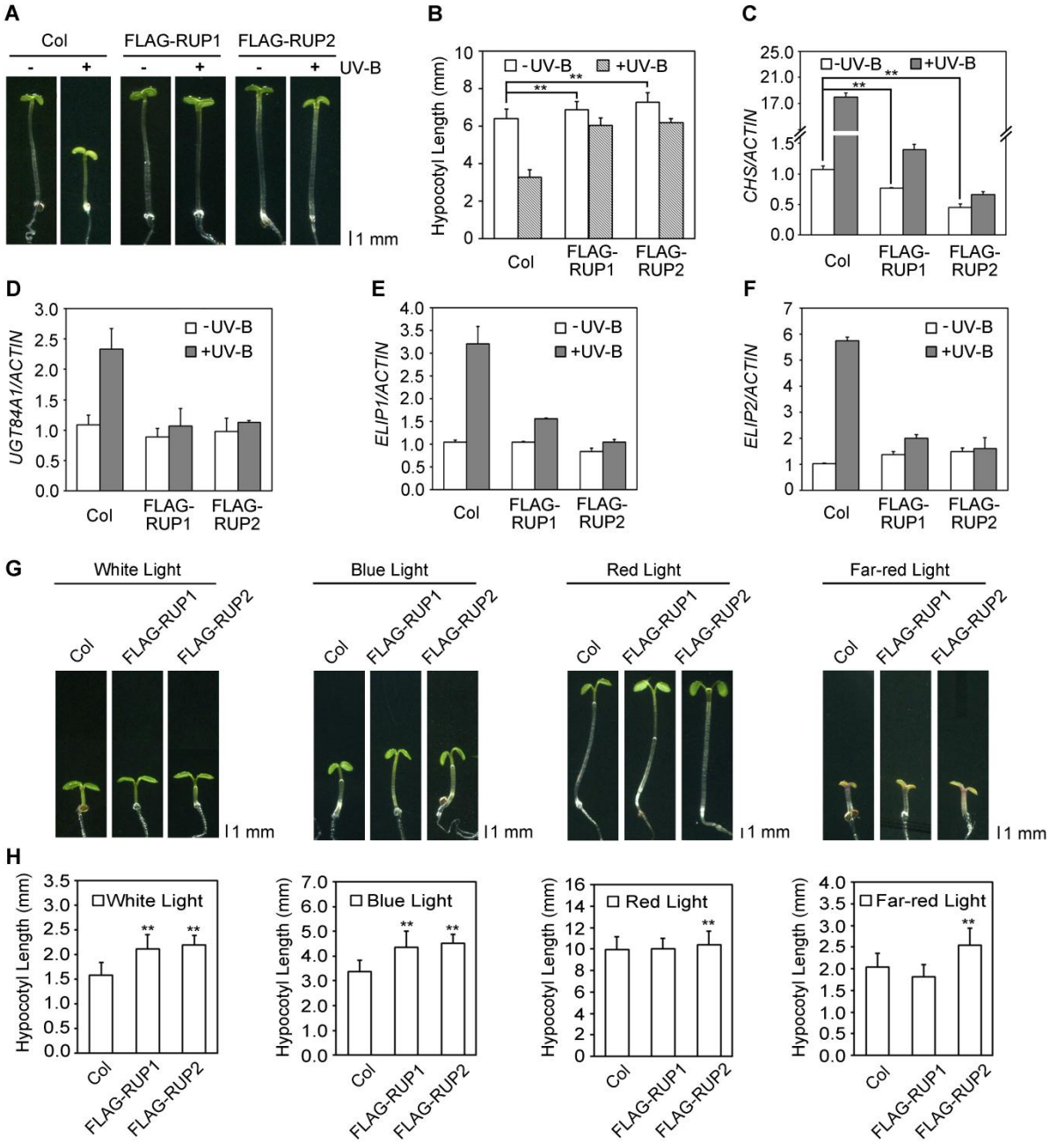

Fig. S3. FLAG-RUP1 and FLAG-RUP2 Exhibit Hyposensitivity to Photomorphogenic UV-B.

(A) Phenotypes of 4-day-old Col, FLAG-RUP1, and FLAG-RUP2 seedlings grown under –UV-B and +UV-B.

(B) Hypocotyl length of the seedlings shown in (A). Mean ± SD, n≥30. The asterisks indicate significant differences by Student's t test (**p<0.01).

(C-F) *CHS* (C), *UGT84A1* (D), *ELIP1* (E), and *ELIP2* (F) mRNA levels in the seedlings shown in (A). Mean ± SD, n=3. The asterisks indicate significant differences by Student's t test (**p<0.01).

(G) Phenotypes of 4-day-old Col, FLAG-RUP1 and FLAG-RUP2 seedlings grown under white light, blue light, red light and far-red light conditions.

(H) Hypocotyl length of the seedlings shown in (G). Mean ± SD, n≥30. The asterisks indicate significant differences by Student's t test (**p<0.01) compared to Col under each light condition.

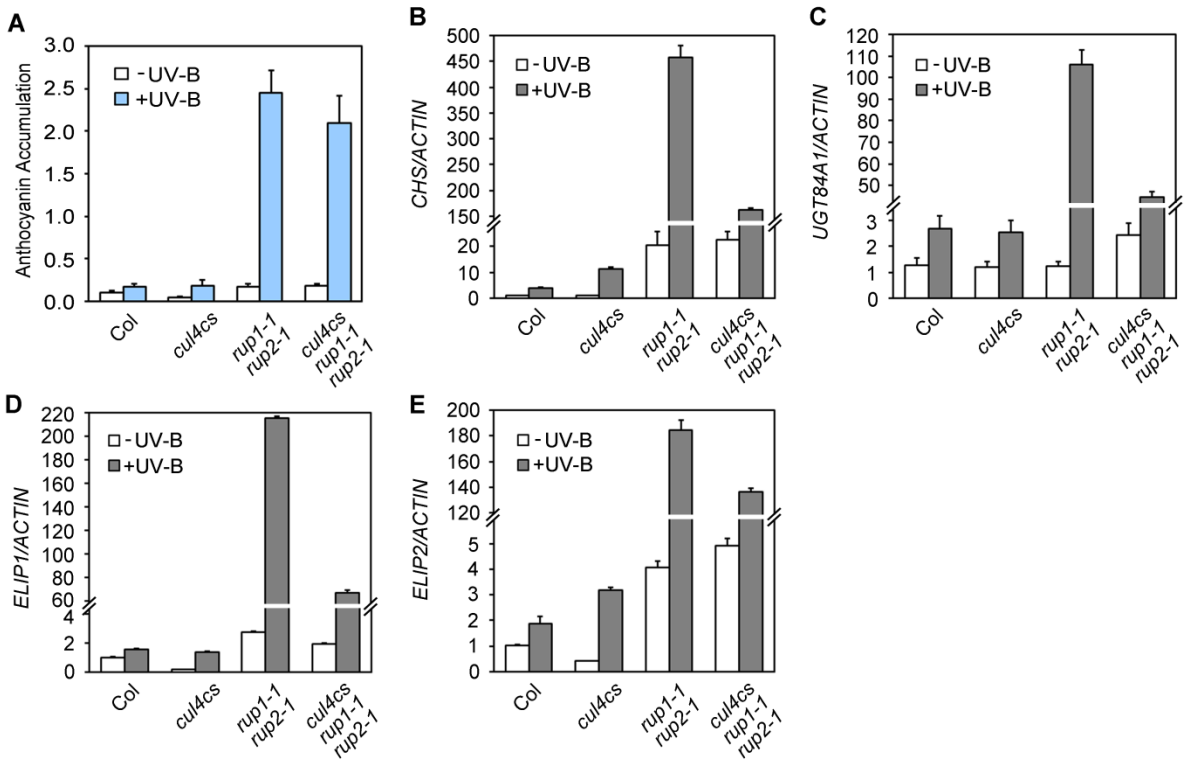

Fig. S4. CUL4 and RUP1/RUP2 Genetically Interact to Influence UV-B-induced Anthocyanin Accumulation and Gene Expression.

(A) Anthocyanin content of 4-day-old Col, *cul4cs*, *rup1-1 rup2-1*, and *cul4cs rup1-1 rup2-1* seedlings grown under –UV-B and +UV-B. Mean ± SD, n=3.

(B-E) *CHS* (B), *UGT84A1* (C), *ELIP1* (D), and *ELIP2* (E) mRNA levels in the seedlings shown in (A). Mean ± SD, n=3.

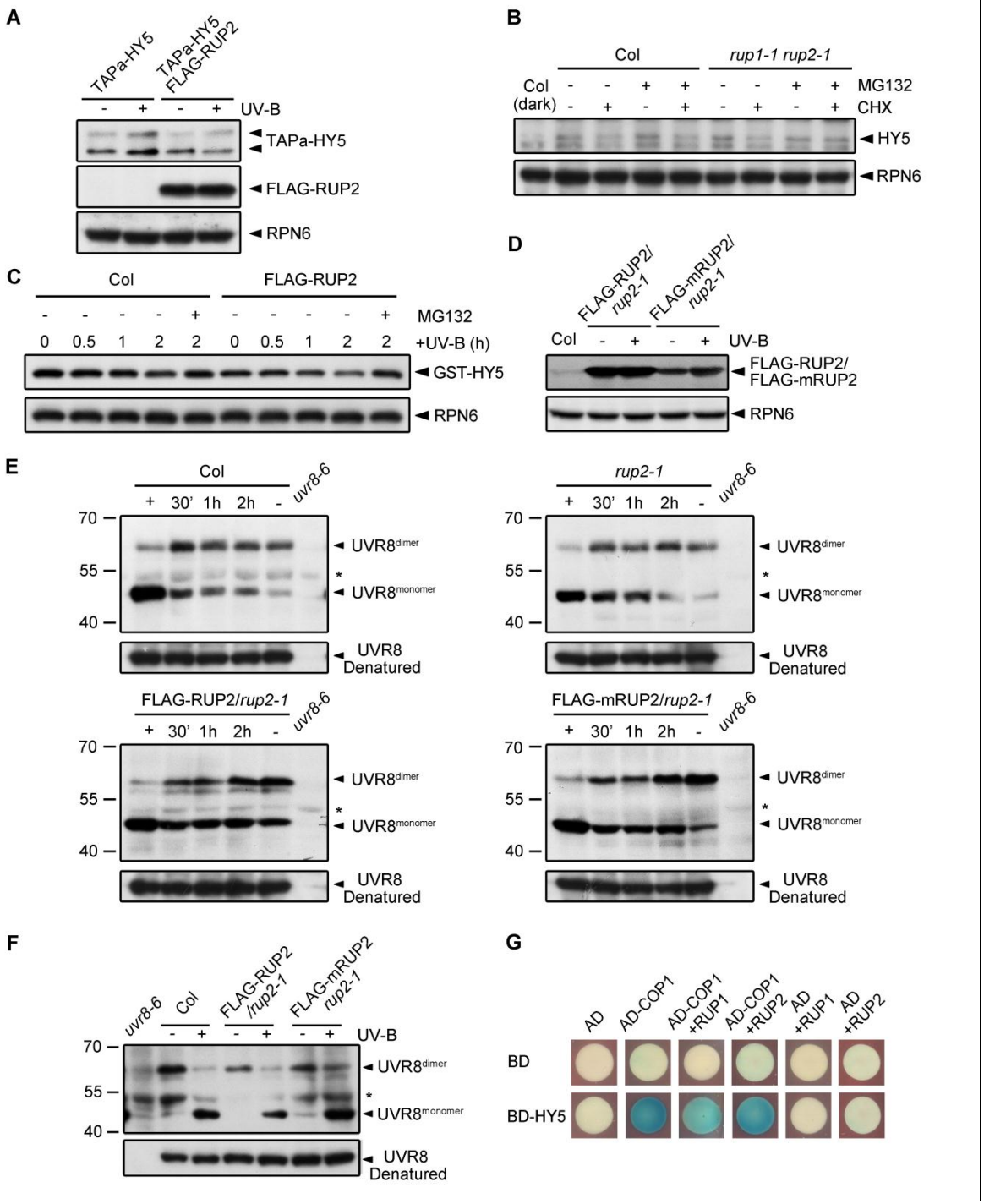

Fig. S5. The Effects of RUP1/RUP2 on HY5 Stability, UVR8 Conformation and the COP1-HY5 Interaction.

(A) TAPa-HY5 protein levels in 4-day-old TAPa-HY5 and TAPa-HY5 FLAG-RUP2 seedlings grown under −UV-B and +UV-B. Proteins were analyzed by immunoblotting with anti-c-Myc, anti-FLAG, and anti-RPN6 antibodies. RPN6 was used as a loading control.

(B) Immunoblot analysis of HY5 proteins in 4-day-old Col and *rup1-1 rup2-1* seedlings grown under –UV-B and treated with 500 μM CHX and/or 50 μM MG132 for 3 hours. HY5 was detected with anti-HY5 antibodies. RPN6 was used as a loading and negative control.

(C) The effect of FLAG-RUP2 on HY5 stability in vitro, as analyzed by cell-free degradation assays. Purified GST-HY5 was incubated with total proteins extracted from 4-day-old UV-B-grown Col and FLAG-RUP2 seedlings for 2 hours. The degradation mixture was treated with or without 50 μM MG132. GST-HY5 was detected with anti-GST antibody. RPN6 was used as a loading and negative control.

(D) FLAG-RUP2/FLAG-mRUP2 protein levels in 4-day-old FLAG-RUP2/*rup2-1* and FLAG-mRUP2/*rup2-1* seedlings grown under −UV-B and +UV-B. Proteins were analyzed by immunoblotting with anti-FLAG and anti-RPN6 antibodies. RPN6 was used as a loading control.

(E) Immunoblot analysis of UVR8 conformation in 4-day-old Col, *rup2-1*, FLAG-RUP2/*rup2-1* and FLAG-mRUP2/*rup2-1* seedlings grown under +UV-B and then transferred to –UV-B for 2 hours or grown under continuous –UV-B. Denatured UVR8 was used as a loading control. UVR8 was detected with anti-UVR8 antibodies. The asterisks indicate nonspecific bands.

(F) Immunoblot analysis of UVR8 conformation in 4-day-old Col, FLAG-RUP2/*rup2-1* and FLAG-mRUP2/*rup2-1* seedlings grown under continuous –UV-Band +UV-B. Denatured UVR8 was used as a loading control. UVR8 was detected with anti-UVR8 antibodies. The asterisks indicate nonspecific bands.

(G) The effect of RUP1/RUP2 on the COP1-HY5 interaction in yeast. Co-transformed yeast cells were selected and then transferred to induction medium supplemented with X-β-gal for blue color development.

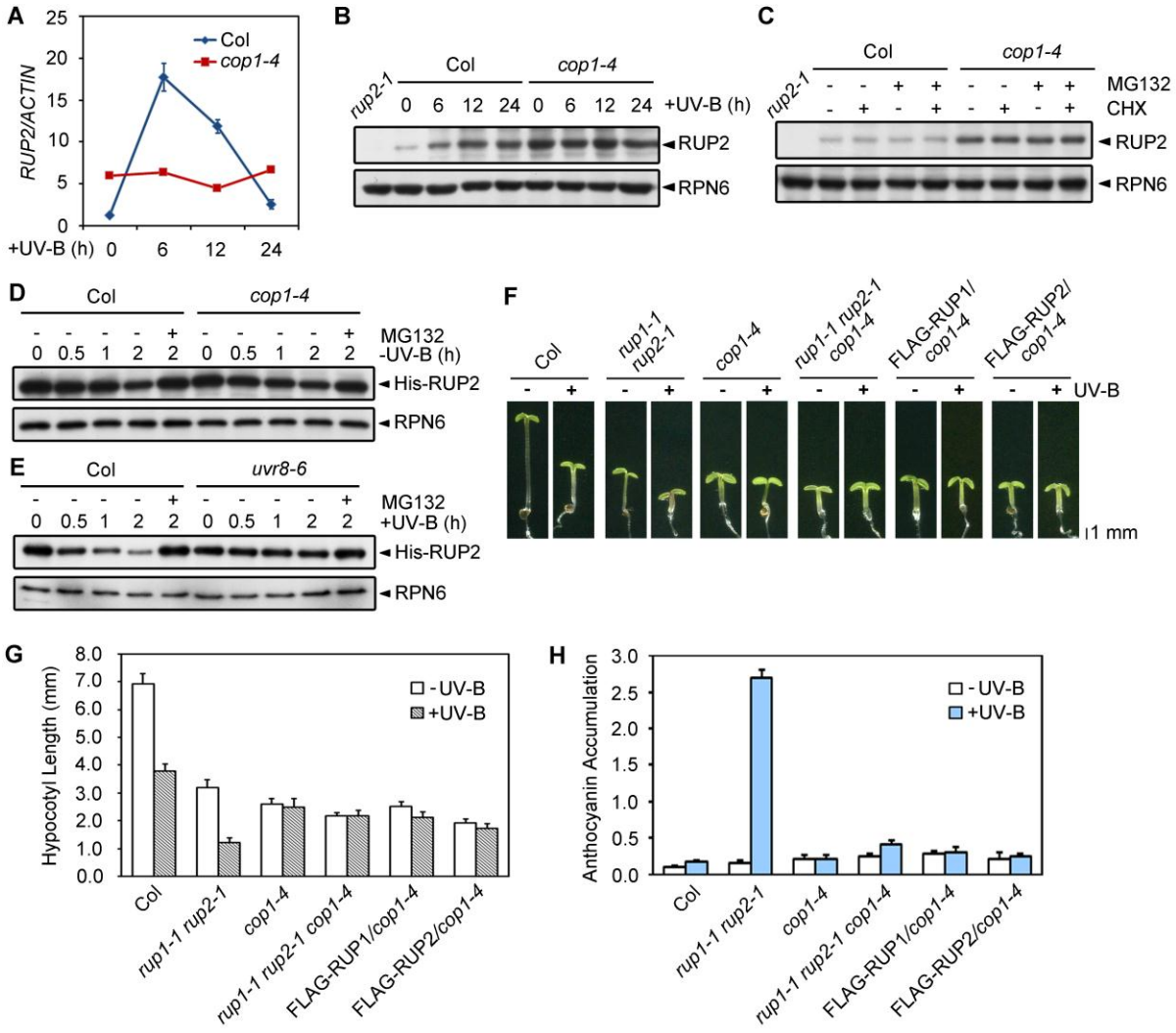

Fig. S6. The Effects of COP1 on the Expression, Stability, and Function of RUP1/RUP2.

(A) The effect of *cop1-4* mutation on the UV-B-induced expression of *RUP2* in *Arabidopsis*. Four-day-old −UV-B-grown Col and *cop1-4* seedlings were transferred to +UV-B for the indicated time periods. Mean ± SD, n=3.

(B) RUP2 protein levels in 4-day-old Col and *cop1-4* seedlings grown under −UV-B and transferred to +UV-B for the indicated time periods. Proteins were analyzed by immunoblotting with anti-RUP2 and anti-RPN6 antibodies. RPN6 was used as a loading control.

(C) Immunoblot analysis of RUP2 proteins in 4-day-old Col and *cop1-4* seedlings grown under –UV-B and treated with 500 μM CHX and/or 50 μM MG132 for 3 hours. RUP2 was detected with anti-RUP2 antibodies. RPN6 was used as a loading and negative control.

(D) The effect of COP1 on RUP2 stability in vitro under –UV-B, as analyzed by cell-free degradation assays. Purified His-RUP2 was incubated with total proteins extracted from 4-day-old Col and *cop1-4* seedlings grown under –UV-B for 2 hours. The degradation mixture was treated with or without 50 μM MG132. His-RUP2 was detected with anti-RUP2 antibodies. RPN6 was used as a loading and negative control.

(E) The effect of UVR8 on RUP2 stability in vitro under +UV-B, as analyzed by cell-free degradation assays. Purified His-RUP2 was incubated with total proteins extracted from 4-day-old Col and *uvr8-6* seedlings grown under +UV-B for 2 hours. The degradation mixture was treated with or without 50 μM MG132. His-RUP2 was detected with anti-RUP2 antibodies. RPN6 was used as a loading and negative control.

(F) Phenotypes of 4-day-old Col, *rup1-1 rup2-1*, *cop1-4*, *rup1-1 rup2-1 cop1-4*, FLAG-RUP1/*cop1-4*, and FLAG-RUP2/*cop1-4* seedlings grown under –UV-B and +UV-B.

(G) Hypocotyl length of the seedlings shown in (E). Mean ± SD, n≥30.

(H) Anthocyanin content of the seedlings shown in (E). Mean ± SD, n=3.

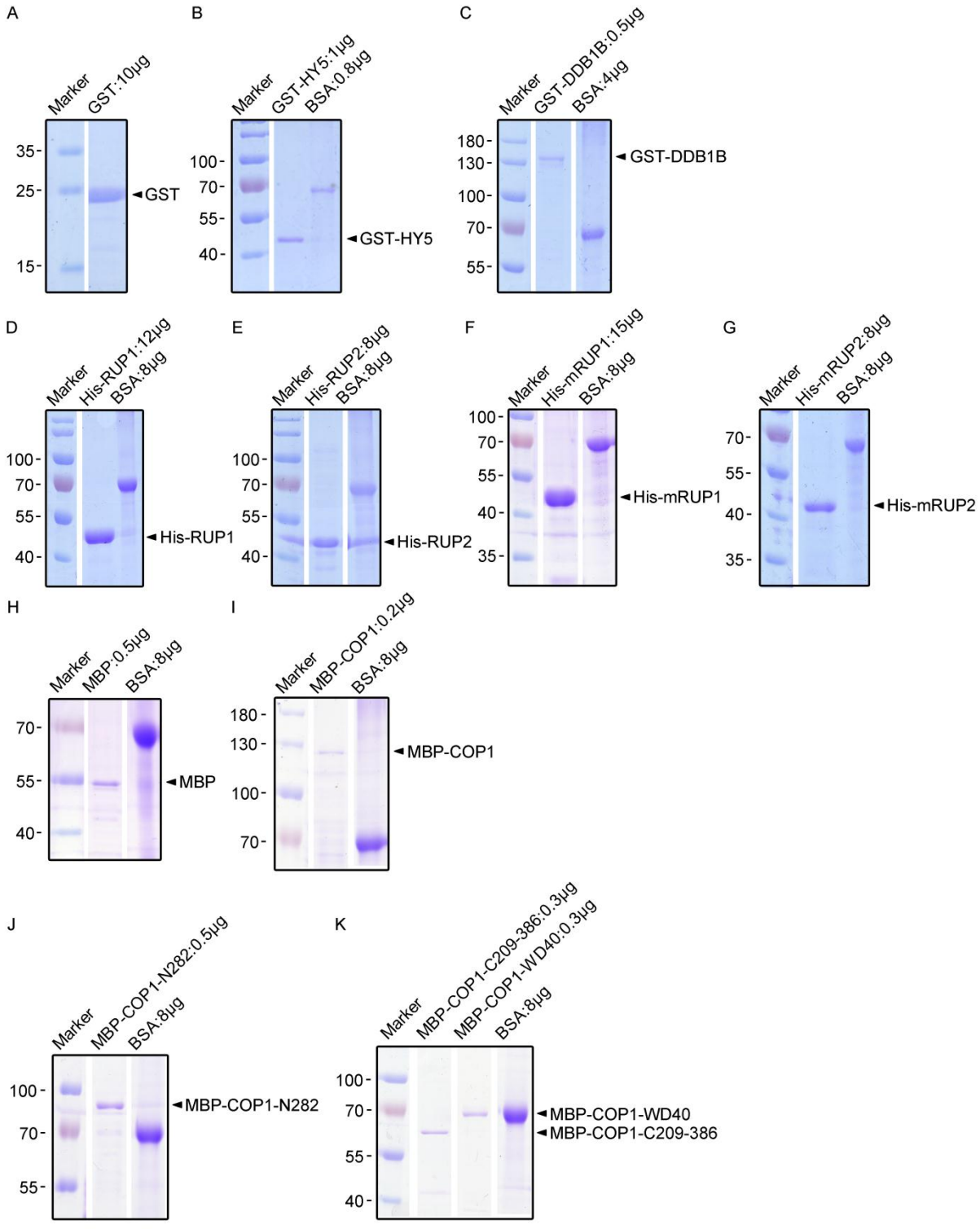

Fig. S7. The Recombinant Proteins Used in This Study.

Table S1. Primers Used in This Study.

Gene name	Primers
Primers used for plant transformation	
RUP1-F	ATAC ggtacc ATGGAGGCTT TGTTCTGCTC
RUP1-R	ATAC ctcgag TTAGCTTTGT TTGCCCGAGA
RUP2-F	ATAC gtcgac ATGAACACTC TTCATCCTCA
RUP2-R	ATAC gagctc CTATGGTTTT CTTTTGCCCA
FLAG-mRUP2-F	ATAC ctcgag CAGGATCCCCCGGGCTGCAG
FLAG-mRUP2-R	ATAC actagt CTATGGTTTTCTTTTGCCCA
Primers used for yeast two-hybrid assays	
AD/BD-RUP1-F	ATAC caattg ATGGAGGCTT TGTTCTGCTC
AD/BD-RUP1-R	ATAC ctcgag TTAGCTTTGT TTGCCCGAGA
AD-RUP2-F	ATAC caattg ATGAACACTC TTCATCCTCA
AD-RUP2-R	ATAC ctcgag CTATGGTTTT CTTTTGCCCA
Primers used for *in vitro* pull-down assays	
His-RUP1-F	ATAC caattg ATGGAGGCTT TGTTCTGCTC
His-RUP1-R	ATAC ctcgag TTAGCTTTGT TTGCCCGAGA
His-RUP2-F	ATAC caattg ATGAACACTC TTCATCCTCA
His-RUP2-R	ATAC ctcgag CTATGGTTTT CTTTTGCCCA
MBP-COP1-F	ATAC gtcgac ATGGAAGAGATTTCGACGGA
MBP-COP1-R	ATAC ctgcag TCACGCAGCGAGTACCAGAA
MBP-COP1-	ATAC gaattc ATGGAAGAGATTTCGACGGA

N282-F	
MBP-COP1-N282-R	ATAC ctgcag ctcgag TCACGAATCTGACCCACTCAGCG
MBP-COP1-C209-386-F	ATAC gaattc ATGGCTAGGGACAGATATTCTGTAAAGTT
MBP-COP1-C209-386-R	ATAC ctgcag ctcgag TCAAAACAGCTCATCATCACGATCA
MBP-COP1-WD40-F	ATAC gaattc ATGGCCACTGCTGGTGTTTCTAGAT
MBP-COP1-WD40-R	ATAC ctcgag TCACGCAGCGAGTACCAGAA

Primers used for site-directed mutagenesis

mRUP1 R198A-F	*D196A*	GAACGGTTCAAATGTGGGCTCCAGCTAACGGAGGGACGTTAG
mRUP1 R198A-R	*D196A*	CTAACGTCCCTCCGTTAGCTGGAGCCCACATTTGAACCGTTC
mRUP1 R243A-F	*D241A*	CCGGAATGCCTACGTGTATGCCATCGCAAGACTAGTGGACCC
mRUP1 R243A-R	*D241A*	GGGTCCACTAGTCTTGCGATGGCATACACGTAGGCATTCCGG
mRUP1 R330A-F	*D328A*	CAAGTGTTTGTGTACGCTAAGGCGTGGGAGGAACCGGTTTGG
mRUP1	*D328A*	CCAAACCGGTTCCTCCCACGCCTTAGCGTACACAAACACTTG

R330A-R	
mRUP2 D175A R177A-F	GACTATGCAAGTATGGGCTCCGGCGTGTCCGCCGGAAGAATC
mRUP2 D175A R177A-R	GATTCTTCCGGCGGACACGCCGGAGCCCATACTTGCATAGTC
mRUP2 D223A R225A-F	CGGAAAGGGTACGTTTACGCTATAGCGAAACTCGTTGACCCG
mRUP2 D223A R225A-R	CGGGTCAACGAGTTTCGCTATAGCGTAAACGTACCCTTTCCG
mRUP2 D309A R311A-F	AGGGTGTTTGTGTACGCTAGGGCATGGGGGAAGCCGGTTTGG
mRUP2 D309A R311A-R	CCAAACCGGCTTCCCCCATGCCCTAGCGTACACAAACACCCT

Primers used for firefly luciferase complementation imaging

RUP1-nLUC-F	ATAC ggtacc ATGGAGGCTTTGTTCTGCTC
RUP1-nLUC-R	ATAC gtcgac GCTTTGT TTGCCCGAGA
RUP2-nLUC-F	AGAC agatct ATGAACACTCTTCATCCTC
RUP2-nLUC-R	ATAC gtcgac TGGTTTTCTTTTGCCC
cLUC-RUP1-F	ATAC ggtacc ATGGAGGCTTTGTTCTGCTC
cLUC-RUP1-R	ATAC gtcgac TTAGCTTTGT TTGCCCGAGA
cLUC-RUP2-F	AGAC agatct GATGAACACTCTTCATCCTC
cLUC-RUP2-R	ATAC gtcgac CTATGGTTTTCTTTTGCCC
cLUC-COP1-F	ATAC ggtacc ATGGAAGAGA TTTCGACGGA

cLUC-COP1-R	ATAC gtcgac TCACGCAGCGAGTACCAGAA
cLUC-COP1-N282-F	CGGTACCCGGGATCCAATGGAAGAGATTTCGACGGA
cLUC-COP1-N282-R	GCTCTGCAGGTCGACTCACGAATCTGACCCACTCAGCG
cLUC-COP1-C209-386-F	CGGTACCCGGGATCCAATGGCTAGGGACAGATATTCTGTAAAG
cLUC-COP1-C209-386-R	GCTCTGCAGGTCGACTCAAAACAGCTCATCATCACGATCA
cLUC-COP1-WD40-F	CGGTACCCGGGATCCAATGGCCACTGCTGGTGTTTCTAGAT
cLUC-COP1-WD40-R	GCTCTGCAGGTCGACTCACGCAGCGAGTACCAGAACT
cLUC-COP1ΔRING-F1	CGGTACCCGGGATCCAATGGAAGAGATTTCGACGGA
cLUC-COP1ΔRING-R1	ACCGCCGCTTCCTCCGTCGTCA
cLUC-COP1ΔRING-F2	GGAGGAAGCGGCGGTCTCGATAAGCTATTGAAGAAAACTT
cLUC-COP1ΔRING-R2	GCTCTGCAGGTCGACTCACGCAGCGAGTACCAGAACT
cLUC-COP1ΔCoil-F1	CGGTACCCGGGATCCAATGGAAGAGATTTCGACGGA

cLUC-COP1⊿Coil-R1	CCGAAACTGATCCAAGGGCGAT
cLUC-COP1⊿Coil-F2	TTGGATCAGTTTCGGAAGTTGCGGATGCTCGGAGATGA
cLUC-COP1⊿Coil-R2	GCTCTGCAGGTCGACTCACGCAGCGAGTACCAGAACT
cLUC-COP1⊿R⊿C-F1	CGGTACCCGGGATCCAATGGAAGAGATTTCGACGGA
cLUC-COP1⊿R⊿C-R1	CCGAAACTGATCCAAGGGCGAT
cLUC-COP1⊿R⊿C-F2	TTGGATCAGTTTCGGAAGTTGCGGATGCTCGGAGATGA
cLUC-COP1⊿R⊿C-R2	GCTCTGCAGGTCGACTCACGCAGCGAGTACCAGAACT
Primers used for ubiquitination assays in HEK293T cells	
FLAG-COP1-F	AGAGAATTC ggtacc ATGGAAGAGATTTCG
FLAG-COP1-R	CTTCCATGG ctcgag TCACGCAGCGAGTAC
RUP2-HA-F	AGAGAATTC ggatcc ATGAACACTC TTCAT
RUP2-HA-R	CTTCCATGG ctcgag TGGTTTTCTTTTGCC
Primers used for yeast three-hybrid assays	
RUP1 (Y3H)-F	ATAC ggtacc ATGGAGGCTTTGTTCTGCTCT
RUP1 (Y3H)-R	ATAC gaattc TTAGCTTTGTTTGCCCGAGA
RUP2 (Y3H)-F	GTCGAATTGGGTACCATGAACACTCTTCATCCTCACAA

RUP2 (Y3H)-R	ACCCGGGTGGAATTCCTATGGTTTTCTTTTGCCCAC
Primers used for qRT-PCR	
Actin-F	CAAGGCCGAGTATGATGAGG
Actin-R	GAAACGCAGACGTAAGTAAAAAC
RUP2-F	TCGGATGACGGGACT
RUP2-R	GACGCAACAAACAGCA
CHS-F	ACGTCACGTGTTGAGCGAGTATGG
CHS-R	GAGGAACGCTGTGCAAGACGACTG
UGT84A1-F	AGTCGGGTTTATCGTTCT
UGT84A1-R	ATCCCTTTACCTTTAGCAC
ELIP1-F	CGTTGCCGAAGTCACCAT
ELIP1-R	AATCCAACCATCGCTAAACG
ELIP2-F	CACCACAAATGCCACAGTCT
ELIP2-R	TGCTAGTCTCCCGTTGATCC