Supplemental Information

MATERIALS AND METHODS

Light Source and Growth Conditions

The *Arabidopsis* seeds were surface-sterilized and cold-treated at 4 ℃ for 3 day (d), and then sown on solid half-strength Murashige and Skoog medium supplemented with 1% sucrose. Cold-treated seeds were exposed to white light for 12 to 14 h and then transferred to continuous light conditions (50 $\mu mol \cdot m^{-2} s^{-1}$ for red light, 7 $\mu mol \cdot m^{-2} s^{-1}$ for blue light, and 80 $\mu mol \cdot m^{-2} s^{-1}$ for white light), unless otherwise stated. Monochromatic blue and red light was generated from blue diodes (λ max = 470 nm) and red diodes (λ max = 670 nm) of E-30 LED growth chambers (Percival), respectively. Adult plants were grown at 22 ℃ under 16 hr light/8 hr dark.

Plant Materials and Characterization

Seeds of *bdr1-2* (Salk_057147) were obtained from the Arabidopsis Stock Center (ABRC; Ohio State University; Columbus, OH). The homozygous plants were obtained by PCR using primers BDR1-LP (5’-caacgtccaagagaccaagac-3’) and BDR1-RP (5’-tggtgttttccaatggaaaag-3’). To generate double mutants, the *bdr1-2* homozygous plants were crossed with homozygous *rbd1-2* (Salk_128696C). Homozygous double mutant plants were identified in the F2 progeny by PCR using primers RBD1-LP (5’-tgttgctgtcaaagttcaacg-3’) and RBD1-RP (5’-caagcgtactttgaagttccg-3’).

Pigment Analysis

Total chlorophylls were extracted from the cotyledons using ice-cold acetone at 4 ℃. Supernatants were diluted with ice-cold water to a final acetone concentration of 80%. Chlorophylls and carotenoids were quantified spectrophotometrically as previously described (Lichtenthaler, 1987).

Anthocyanin Measurements

Cotyledons from 7-day-old plants were collected, ground into fine powder in liquid nitrogen, and extracted with 80% methanol containing 5% HCl overnight at 4 ℃. After centrifugation at 14,000g for 20 min, the extracts were transferred to new tubes and the amount of anthocyanins was quantified photometrically (DU800 spectrophotometer; Beckman Instruments).

Plasmid Construction and *Arabidopsis* Transformation

For subcellular localization analysis, RBD1 cDNA was amplified by PCR using primers RBD1-pDF (5'-ggggacaagtttgtacaaaaaagcaggcttcatgagtctggtggttggtca-3') and RBD1-pDR (5'-ggggaccactttgtacaagaaagctgggtctggggatggtgcagaagat-3'). Then the amplified PCR product was cloned into pEarleyGate101 vector using the Gateway® system (Invitrogen, Carlsbad, CA, USA) (Earley et al., 2006). *35Spro:RBD1-YFP-HA* construct was transformed into *Agrobacterium* and then introduced into Col and *bdr1-2* mutant by floral dipping (Clough and Bent, 1998).

Confocal Microscopy

YFP fluorescence images were recorded with a laser scanning confocal microscope (LSM510, Zeiss). An argon laser (25 mW) was used for generating an excitation source at 488 nm. 7-day-old transgenic *p35S:RBD1-YFP* seedlings were examined. YFP and Chl fluorescence were recorded at 525 nm and 660 nm, respectively.

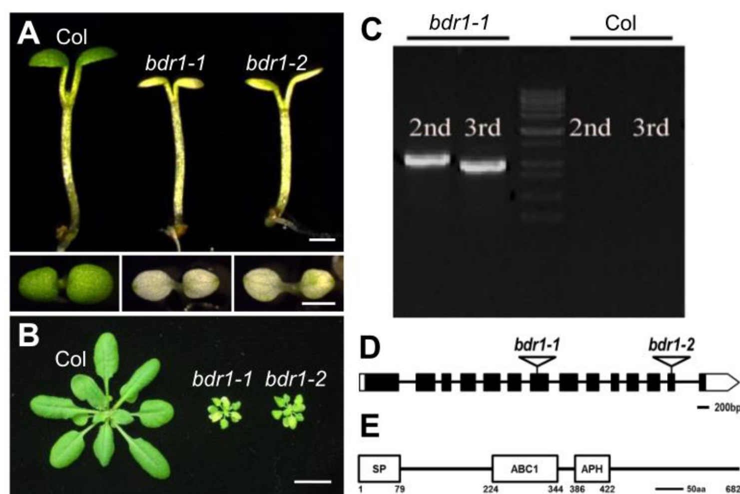
REFERENCES

Clough, S.J., and Bent, A.F. (1998). Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J. **16**:735-743.

Earley, K.W., Haag, J.R., Pontes, O., Opper, K., Juehne, T., Song, K., and Pikaard, C.S. (2006). Gateway-compatible vectors for plant functional genomics and proteomics. Plant J. **45**:616-629.

Lichtenthaler, H. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Meth. Enzymol. **148**:350-382.

Supplemental Figures



Supplemental Figure 1. Characterization of *bdr1-1* and *bdr1-2* mutants.

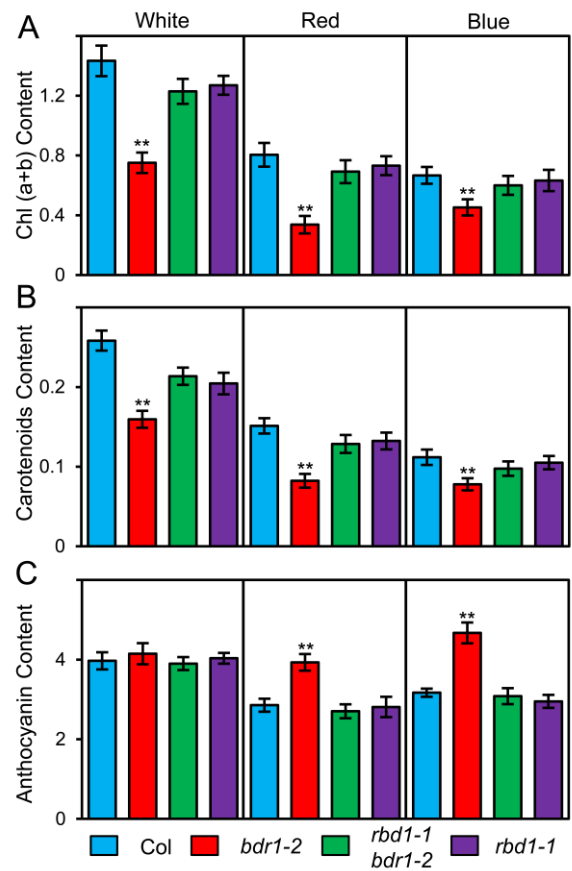
(A) Phenotype of 7-day-old Col, *bdr1-1* and *bdr1-2* seedlings grown under continuous red light (50 $\mu mol \cdot m^{-2} s^{-1}$). Lower panel: Top view of 7-day-old Col, *bdr1-1* and *bdr1-2* seedlings. Bars = 1 mm.

(B) Morphology of Col, *bdr1-1* and *bdr1-2* plants on soil. The photograph shows soil-grown plants 2 weeks after planting. Bars = 10 mm.

(C) The T-DNA insertion site in *bdr1-1* was identified using TAIL-PCR. Three rounds of amplification were performed; the second (2nd) and third (3rd) round products were analyzed using gel electrophoresis. The amplification of Col (wild type) was included as a control.

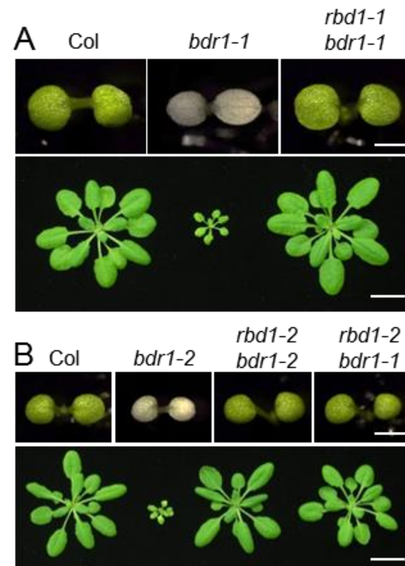
(D) Schematic diagram of the *BDR1* gene. Exons, black filled rectangles; introns, solid lines; UTR (untranslated region), white filled rectangles. The *bdr1-1* and *bdr1-2* (Salk_057147) T-DNA insertions are indicated.

(E) Protein structure of BDR1. SP, signal peptide; ABC1, Activity of bc1 complex; APH, aminoglycoside phosphotransferase; aa, amino acids.



Supplemental Figure 2. The Content of Chlorophylls (A), Carotenoids (B) and Anthocyanin (C) in Col, *bdr1-2*, *rbd1-1 bdr1-2* and *rbd1-1* Cotyledons.

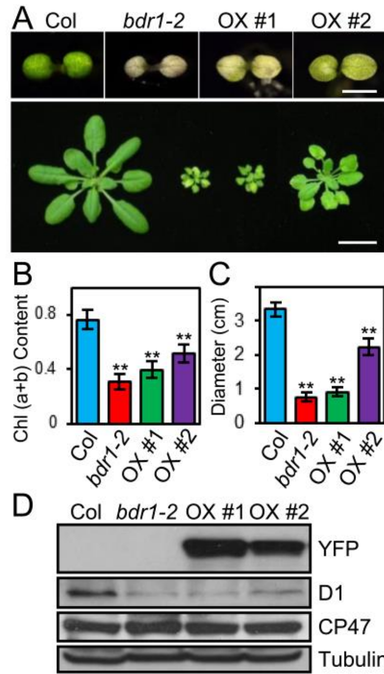
Pigments were extracted with 95% ethanol from the cotyledons of 7-day-old seedlings grown under various light conditions based on fresh weight, and measured by spectrophotometry. Error bars represent ±SD of three replicates. Asterisks indicate a significant difference (P≤0.01, T test).



Supplemental Figure 3. Suppression of *bdr1* Mutant Phenotypes by *rbd1*.

(A) *rbd1-1* suppresses the phenotype of *bdr1-1*. Upper panel: Phenotype of 7-day-old Col, *bdr1-1* and *rbd1-1 bdr1-1* seedlings grown under continuous red light (50 μmol·m^{-2} s^{-1}). Bars = 1 mm. Lower panel: Morphology of Col, *bdr1-1* and *rbd1-1 bdr1-1* plants on soil. The photograph shows soil-grown plants 2 weeks after planting. Bars = 10 mm.

(B) *rbd1-2* (Salk_128696C) suppresses the phenotype of *bdr1-1* and *bdr1-2*. Upper panel: Phenotype of 7-day-old Col, *bdr1-2*, *rbd1-2 bdr1-1* and *rbd1-2 bdr1-2* seedlings grown under continuous red light (50 μmol·m^{-2} s^{-1}). Bars = 1 mm. Lower panel: Morphology of Col, *bdr1-2*, *rbd1-2 bdr1-1* and *rbd1-2 bdr1-2* plants on soil. The photograph shows soil-grown plants 2 weeks after planting. Bars = 10 mm.



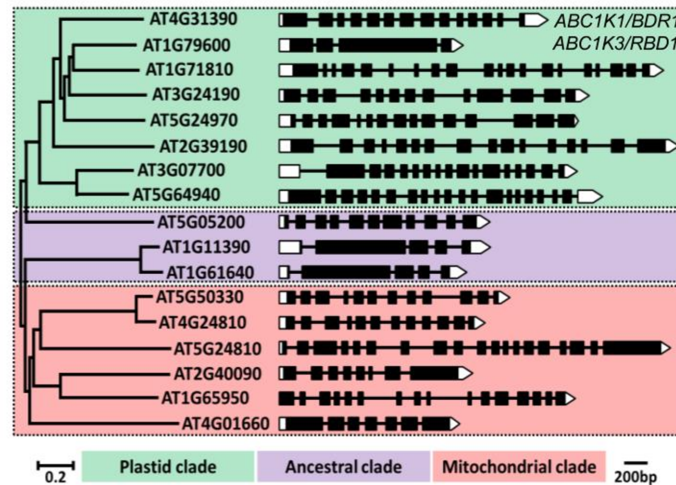
Supplemental Figure 4. *RBD1* Overexpression Seedlings have Similar Phenotype as *bdr1-2* mutants.

(A) Upper panel: Phenotype of 7-day-old seedlings grown under continuous red light (50 μmol m^{-2} s^{-1}). Bars = 1 mm. Lower panel: Morphology of the Col, *bdr1-2* and *p35S:RBD1-YFP-HA* transgenic lines OX#1 and OX#2 on soil. The photograph shows soil-grown plants 2 weeks after planting. Bars = 10 mm.

(B) The chlorophylls content of Col, *bdr1-2* and *p35S:RBD1-YFP-HA* transgenic lines OX#1 and OX#2 cotyledons. Error bars represent ±SD of three replicates. Asterisks indicate a significant difference ($P \leq 0.01$, T test).

(C) Quantification of rosette diameter (average of 48 soil-grown plants). 7-day-old seedlings grown under continuous red light were transferred on soil for 2 weeks and measured respectively. Bars stand for standard deviations. Asterisks indicate a significant difference ($P \leq 0.01$, T test).

(D) Immunoblot analyses of the total proteins from the cotyledons of 7-day-old seedlings grown under continuous red light using anti-YFP, -D1, or -CP47 antibodies. An anti-tubulin immunoblot is shown below to indicate approximately equal loadings.



Supplemental Figure 5. *RBD1* is a Young Retroposed Gene Possibly Derived from a Recent Retrotransposition.

Phylogenetic tree of *Arabidopsis ABC1K* genes. The following parts are shown from left to right. The unrooted trees were constructed based on the alignments of the full-length protein sequences of *ABC1Ks* using the Neighbor-Joining (NJ) method provided by MEGA 5.0. The gene structure is represented by white boxes symbolizing untranslated regions, black boxes symbolizing exons and solid lines between boxes corresponding to introns. The length of the genes can be estimated using the scale bar at the bottom. The three clades can be categorized by their presumptive localizations and origins indicated by color: plastid endosymbiosis (green), mitochondrial endosymbiosis (red), and ancestral (purple). Subcellular localization of each *ABC1K* has been determined by experimental evidence or, in the absence of experimental evidence, by TargetP prediction.