Supplementary Fig. 1 Characterization of CRISPR/Cas9-day mutant lines and analyses of the phenotypes after knockdown of DAY. a, The overview of CRISPR/Cas9-mediated day mutant (Cas9-day) #1 and #2 lines. The panel shows the sequence chromatograms taken from Benchling. b, Seeds counting of CRISPR/Cas9-day mutants. The left panel shows representative pictures of siliques after clearing with Hoyer’s solution. Scale bar: 1 cm. The right panel shows quantification of seed numbers per siliques. Dots represent individual observations. Twenty siliques were examined for each genotype. Statistical significance was determined by one-way ANOVA, Dunnett’s multiple comparisons test ****, P < 0.0001. c, Progeny analysis of CRISPR/Cas9-day mutants. Notably, the day/day genotype was missing among the progeny. d, Representative phenotypes of the DAY/day heterozygous mutant plants, compared with the DAY/DAY phenotypes. e, The abundance of DAY/DAY, DAY/day, and perished seedlings in the progeny of Cas9-day (DAY/day) was quantified as a percentage of their total numbers. The % values in the middle of the graph are the total of the percentages of the DAY/day and perished seedlings. f, g, Arabidopsis Dexamethasone (DEX)-inducible DAY RNAi plants (DAYi) exhibit growth retardation, leaf yellowing, short petioles, and retarded silique growth upon DEX treatment. Plants were grown in soil for 10 days (f) or until bolting (g), and were then sprayed with either ethanol or 1 μM DEX for two (f) or three weeks (g). Scale bar, 3 cm. h, Morphology of silique growth in DAYi plants mimics those of BR biosynthesis (det2-1) and receptor (bri1-301) mutants. Scale bar: 1 cm.
Supplementary Fig. 2 Reduction of DAY in Col-0 and bri1-301, but not bri1-9, caused growth retardation and leaf yellowing. a. Phenotypes of DAY VIGS in Arabidopsis. Arabidopsis WT (Col-0; 6-leaf stage), bri1-9 (4-leaf stage) and bri1-301 (4-leaf stage) plants were subjected to VIGS. The bri1-9 allele carries a mutation in the BRI1 extracellular domain (S662F), and bri1-301 is a weak mutant allele with a mutation in the kinase domain (G989I). VIGS-mediated knockdown of DAY in the WT and bri1-301 backgrounds, but not in bri1-9, caused growth retardation, including extremely short petioles and leaf yellowing, when compared with the VIGS vector control (TRV2). For VIGS, the DAY cDNA fragment (436 bp; nucleotide residues 401–837) was cloned into the VIGS vector pTRV2 and Arabidopsis plants were infiltrated with Agrobacterium containing the TRV2 or TRV2:DAY constructs. Plants were photographed 2 weeks after infiltration. Scale bar, 1 cm. b, c, RT-qPCR analysis of DAY transcript levels (b) and BR-related genes (c) in DAY VIGS plants. The expression levels of the BR-induced genes PRE1, PRE2, and PRE6 decreased, while expression of the BR-repressed genes ROT3 and SAUR36 increased. Transcript levels were normalized to PP2A A3 mRNA and expressed relative to those of the TRV2 control. Error bars indicate SD from three technical replicates. Asterisks denote statistical significance of the differences between TRV2 and TRV2:DAY based on unpaired, two-tailed Student’s t-test. *, P ≤ 0.05 and **, P ≤ 0.01.


Supplementary Fig. 3 DAY promotes BR signaling in the dark. Seedlings were grown in the dark for 5 days on medium containing ethanol (EtOH) or 1 µM DEX (DEX) with 30 µM bikinin, 50 nM BL, or 1 µM BR biosynthesis inhibitor brassinazole (Brz). Scale bar for all pictures, 2 cm.
Supplementary Fig. 4 The DAY homolog, CDF-RELATED GENE RESPONSIVE TO SENESCENCE (CRS), is not required for light-induced cotyledon greening. a, The crs mutant and CRS overexpression (OE) plants (line #6 and #8) show normal cotyledon greening during the dark-to-light transition. Seedlings were grown on ½ MS medium in the dark for 5 days (D), and then transferred to the light for 6 or 24 h (L). Scale bar for all pictures, 0.1 cm. b, crs mutant and CRS OE plants show normal growth in the dark (left panel) and in the light (right panel). Seedlings were grown on ½ MS medium in the dark for 5 days or in the light for 9 days. Scale bar, 2 cm. c, DEX-treated DAYi, but not the crs mutation or CRS OE, show a decrease in POR protein accumulation. DAYi seedlings (line #1 and #2) were grown in the dark for 5 days on ½ MS medium containing either ethanol (-) or 1 μM DEX (+), and subjected to immunoblotting using antibodies against cpHSP70 and POR (left panel). The crs and CRS OE seedlings were grown on ½ MS medium in the dark for 5 days before immunoblotting (right panel).
Supplementary Fig. 5 Mutations in BR signaling or biosynthesis do not affect cotyledon greening and POR protein accumulation in the light and dark, respectively. a, Seedlings of the WT (Ws-2 and Col-0), BR receptor mutants (bri1-5, bri1-301, and bri1-116), and BR biosynthetic mutant (det2-1) were grown on ½ MS medium in the dark for 5 days (D), and then exposed to light (L) for 6 or 24 h. Scale bar, 0.1 cm. b, Seedlings of the indicated genotypes were grown on ½ MS medium in the dark for 5 days (left panel), and then subjected to immunoblotting with anti-cpHSP70 and anti-POR antibodies (right panel). POR protein levels were mostly consistent across all genotypes. bzar1-1D, brassinazole-resistant 1-1D. Scale bar, 2 cm.
Supplementary Fig. 6 DAY is localized to chloroplasts, ER, TGN/EE and plasma membrane. a, mRFP and GFP fusion proteins were transiently co-expressed in N. benthamiana leaves by agroinfiltration and epidermal layers were observed by CLSM. ER-GFP or TGN/EE-GFP were used as an ER or TGN/EE marker, respectively. Scale bar, 50 µm. b, DAY-mRFP co-localizes with ER-GFP and chlorophyll auto-fluorescence in N. benthamiana mesophyll protoplasts. Scale bar, 10 µm. c, GFP signals from in transgenic Arabidopsis plants expressing DAY-GFP under the CaMV35S promoter (35S::DAY-GFP). DAY-GFP is localized to the plasma membrane, mesh-like structures, and inner cell speckles in dark-grown cotyledons and light-grown root cells. Scale bar, 20 µm (cotyledon) or 50 µm (root). d, DAY mRNA levels are significantly higher than other chloroplast-related genes in roots. RT-qPCR was performed to determine transcript levels of chloroplast-related genes and DAY in aerial tissues and roots. Transcript levels were normalized to PP2A A3 mRNA. Error bars indicate SD from three technical replicates. Statistical significance was determined by one-way ANOVA, Dunnett's multiple comparisons test ****, P < 0.0001.
Supplementary Fig. 7 The cTP and TM1 are important for targeting of DAY to chloroplasts and the endomembrane system, respectively. Green fluorescence of the indicated DAY-GFP variants was merged with chloroplast autofluorescence (Chlorophyll) in epidermal cells. Constructs were expressed in N. benthamiana leaves via agroinfiltration. ∆cTP, deletion of cTP; ∆TM1, deletion of TM1; ∆TM1/2, deletion of TM1 and TM2; ∆TM3/4, deletion of TM3 and TM4; ∆TM4, deletion of TM4. Scale bar, 50 µm.
Supplementary Fig. 8 The cTP, TM3, and TM4 are not required for DAY localization to the endomembrane system. Green fluorescence of the indicated DAY-GFP variants was merged with ER-mRFP or SYP61-mRFP in leaf protoplasts. Constructs were expressed in \textit{N. benthamiana} leaves via agroinfiltration, and leaf protoplasts were generated for observation under CLSM. ΔcTP, deletion of cTP; ΔTM3/4, deletion of TM3 and TM4; ΔTM4, deletion of TM4. Scale bar, 10 µm.
Supplementary Fig. 9 Reduction of Day causes leaf yellowing and growth retardation in N. benthamiana. Related to Fig. 3a. a, 6-leaf stage N. benthamiana plants were subjected to VIGS using the tobacco rattle virus (TRV) system. VIGS of NbDay (TRV:NbDAY) and TRV control plants were photographed 4 weeks after infiltration. Scale bar, 5 cm. b, Immunoblot analyses of TRV:NbDAY and TRV control plants indicating that Day promotes the accumulation of POR in the light.
Supplementary Fig. 10 DAY interacts with neither chloroplast Hsp70 (cpHsp70) nor cytosolic Hsp70 (Hsp70). a, b, BiFC assays between DAY and cpHsp70 (a) or Hsp70 (b). YFP signals were examined by CLSM in mesophyll cells (a) or epidermal cells (b) after agroinfiltration. Interactions between cpHsp70 and Hsc90 in chloroplasts (a), and between Hsp70 and Hsp101 in the cytosol (b) were observed as positive controls. Scale bar, 20 μm. c, Immunoblotting using anti-Myc and anti-HA antibodies to examine protein expression in (a) and (b).
DAY deficiency affects neither endocytosis nor VHA-A1 distribution in Arabidopsis roots. DAYi (a) and DAYi x pVHA-A1::VHA-A1-RFP (b) seedlings were grown in the light for 9 days in the presence of ethanol or 1 µM DEX and root tips were observed by CLSM. To visualize endocytic vesicles, roots were stained with FM4-64 dye for 30 min before CLSM. To visualize TGN/EE, DAYi plants were crossed with transgenic plants expressing an RFP fusion protein of VHA-A1 (pVHA-A1::VHA-A1-RFP). Enlarged views of the boxed areas are shown (En). VHA-A1, VACUOLAR H⁺ ATPASE A1 SUBUNIT. Scale bar, 50 µm.
Supplementary Fig. 12 DAY does not enhance BRI1 kinase activity in vitro. In vitro kinase assays were performed with various forms of the cytosolic domain of BRI1 using the BR13 synthetic peptide as a substrate, in the presence or absence of MBP or MBP-DAY. Two technical replicates are shown for the kinase assays. mBRI1, the kinase dead form of BRI1 (K911E); bBRI1, basal state of BRI1 (Low phosphorylated BRI1;[3]); CPM, counts per minute.

Supplementary Fig. 13 Biological replicate of Fig. 4b. Nine-day-old seedlings from WT (Col-0), bri1 mutant (bri1-116), and DAYi plants grown in the presence of ethanol (-) or 1 μM DEX (+) were subjected to immunoblotting with anti-BRI1 and anti-α-tubulin (TUB) antibodies.
Supplementary Fig. 14 bzar1-1D rescues BR-deficient phenotypes in DAY-silenced seedlings. a, Seedlings from DAYi and DAYi x bzar1-1D lines were grown on ½ MS medium for 5 days at 22°C and then transferred to medium containing ethanol or 1 µM DEX at 28°C for 7 days. Scale bar for all pictures, 2 cm. b, RT-qPCR analysis of DAY and BR-related gene expression in DAYi and DAYi x bzar1-1D lines following ethanol or DEX treatment. Transcript levels were normalized to PP2A A3 mRNA and expressed relative to EtOH-treated seedlings. (n = 2; technical replicates)
Supplementary Fig. 15 DAY enhances BRI1 accumulation in Arabidopsis. a, b, Overexpression of DAY increases the amount of BRI1 protein. Seven day old Col-0 and 35S::DAY-GFP seedlings grown on ½ MS medium were subjected to immunoblotting with anti-GFP (a) and anti-BRI1 (b) antibodies. For loading control, immunoblotting was performed with anti-α-tubulin (TUB) antibody. Numbers on the immunoblot of BRI1 indicate the relative amount of BRI1 compared to TUB. c, Biological replication of (b) with bri1-116 mutation. ND; not detected.
Supplementary Fig. 16 Hypocotyl elongation of *DAY* overexpression plants is less sensitive to BR biosynthesis inhibitor BRZ. *DAY* overexpression seedlings developed longer hypocotyls than those of WT upon Brz treatment. Seedlings were grown on medium with or without 250 nM Brz in the dark for 5 days. *n* denotes numbers of biologically independent hypocotyls, Brz- (left number) and mock-treated (right number). Statistical significance was determined by one-way ANOVA, Dunnett's multiple comparisons test **, *P* ≤ 0.01; ****, *P* < 0.0001. Scale bar, 1 cm.
Supplementary Fig. 17 Transcriptome of **DAYi** seedlings significantly overlaps with the **bri1-116** mutant. **a**, Gene ontology (GO) term enrichment analyses for differentially expressed genes (DEGs) in **DAYi** seedlings in the dark (left panel) and in the light (right panel). In the dark, **DAY-**responsive genes primarily promoted growth, with genes classified in the response to the growth-promoting hormone auxin as the most significant; in the light, the most significant **DAY**-responsive genes were related to photosynthesis. In both the light and dark, the second most enriched group of **DAY** DEGs were those involved in oxidation-reduction processes, suggesting that **DAY** mainly regulates chloroplast development. During the transition of etioplasts to chloroplasts, most expressed proteins are involved in oxidation-reduction processes. For each analysis, the top 25 enriched terms are presented based on their p-values from Fisher’s exact test. The x-axis depicts the –log(p-value), for which a higher value represents more significant enrichment. **b**, Venn diagrams show a significant overlap between the **DAYi** and **bri1-116** transcriptomes in the dark and light. Of the 4397 DEGs in **DAYi** and 4064 in **bri1-116** identified in dark-grown seedlings, 1765 overlapped. Most were regulated in the same direction, showing either an increase or decrease in both mutants (correlated DEGs). In the light-grown seedlings, of the 5956 **DAYi** DEGs and 2799 **bri1-116** DEGs, 1539 were coregulated and 1311 of these were correlated. Numbers indicate DEGs. Pink and light blue circles represent the transcripts expressed in **DAYi** seedlings and the **bri1-116** mutant, respectively. Percentages in black and red colors (in parentheses) represent the % of DEGs from **DAYi** and **bri1-116**, respectively. **c**, To confirm the transcriptome data from **DAYi** plants, transcript levels of BR-activated and BR-repressed genes were determined by RT-qPCR analysis. **DAYi** seedlings were grown in the light for 9 days in the presence of ethanol or 1 µM DEX. (n = 2; technical replicates)

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**DAYi GO enrichment – dark significant, bri1-116 correlated**

Supplementary Fig. 18 GO term enrichment analyses of DAYi DEGs in dark conditions. DAYi DEGs that were significantly correlated with those of bri1-116 (p ≤ 0.01; Fisher’s exact test) in the dark are arranged in decreasing order based on their –log(p-value). Black bars represent enrichment in the dark, while gray bars represent enrichment in the light. Red dotted-line indicates the p-value threshold of 0.01. Correlated genes in dark conditions are enriched for GO terms related to plant growth promotion.
Supplementary Fig. 19 GO term enrichment analyses of DAYi DEGs in light conditions. DAYi DEGs that were significantly correlated with those of bri1-116 (p ≤ 0.01; Fisher’s exact test) in the light are arranged in decreasing order based on their $-\log(p$-value). Black bars represent enrichment in the dark, while gray bars represent enrichment in the light. Red dotted-line indicates the p-value threshold of 0.01. Correlated genes in light conditions are enriched for GO terms related to photosynthesis.
Supplementary Fig. 20 DAY directly interacts with the kinase domain of BRI1 in vitro. 

**a**. DAY interacts with the cytosolic domain of BRI1. MBP, MBP-DAY lacking the cTP (MBP-ΔcTP), and MBP-DAY were immunoprecipitated with the Flag-tagged cytosolic domain of BRI1 (Flag-BRI1:CD) immobilized on an anti-Flag affinity gel. Eluates were analyzed by immunoblotting using an anti-MBP antibody. Flag-BRI1:CD was visualized by Ponceau S staining.

**b**. DAY binds to the cytosolic domain of BRI1-LIKE 1 (BRL1:CD). MBP and MBP-DAY were pulled down by Flag-BRL1:CD immobilized on anti-Flag affinity gel. Eluates were examined by immunoblotting using an anti-MBP antibody. Flag-BRL1:CD was visualized by Ponceau S staining.

**c**. DAY interacts with the kinase domain of BRI1. MBP and MBP-DAY were pulled down by various forms of Flag-BRI1 proteins immobilized on anti-Flag affinity gel. Eluates were analyzed by immunoblotting using an anti-MBP antibody. Amounts of the different Flag-BRI1 proteins were visualized by Ponceau S staining. JM, juxta-membrane domain; KD, kinase domain; CT, C-terminal tail.