Supplemental Methods

Sequence alignment
Sequence alignment among four SPA proteins was performed with ClustalW program.

Subcellular colocalization study
Protoplasts were isolated from *Arabidopsis* seedlings and transfected using a method described previously (Zhai et al. 2009), and analyzed by confocal microscopy (Leica TCS SP5II).

Yeast two-hybrid assay with GAL4 system
Yeast two-hybrid assay was performed according to manufacturer’s instructions except that the yeast strain SFY526 was used. Transformed colonies were selected on synthetic complete medium lacking Leu and Trp. Twelve independent clones for each pair were used in each performance. Cell cultures were placed under indicated light conditions and incubated at 30°C till the OD600 was between 0.5–0.8. Calculations of relative β-galactosidase activities were performed as described previously (Yang et al. 2001).

HY5 antibody preparation and immunoblot assay
Anti-HY5 antibody was prepared by vaccinating rabbit with synthetic polypeptides corresponding to amino acids of HY5. For probing HY5, immnoblott assay was performed using anti-HY5 antibody. An EMS-mutagenized hy5 mutant allele in Col background (kindly provided by Drs. Y.J. Wu and A.R. Cashmore), which contains a stop codon immediately after the start codon, was used to test the specificity of aniti-HY5 antibody.

Supplemental Figure S1

Supplemental Figure S1. The model of CRY1 signaling pathway. (A) In the previous model of CRY1 signaling pathway, CRY1 negatively and SPA1 positively regulate COP1 by their physical interactions with COP1, respectively. It was unknown whether additional components, such as SPA1, were directly regulated by CRY1 through physical interaction. (B) This study demonstrates that CRY1 directly interacts with SPA1 in a blue light-dependent manner and that light-activated CRY1 promotes the dissociation of COP1 from SPA1, a dissociation that underlies light-controlled dynamics of COP1–SPA1 interaction. Arrows indicate promotion effects and T-bars indicate inhibitory effects. Solid lines represent established regulation and dotted lines represent predicted regulation.
Supplemental Figure S2. CRY interacts with SPA in yeast cells. (A) Yeast two-hybrid bait constructs. All proteins are fused with the LexA DNA-binding domain (LexA). (B) Yeast two-hybrid prey constructs. All proteins are fused with the B42 activation domain (AD). (C) Quantitative yeast two-hybrid analyses of CCT1–SPA1 interactions. (D) Quantitative yeast two-hybrid analyses of CCT1–SCT interactions. (SNT1) N-terminal domain of SPA1; (SCT) C-terminal domain of SPA; (SCC1) coiled-coil domain of SPA1. Data are mean ± SD (n = 10, independent biological replicates).
Supplemental Figure S3. Alignment among SPA1, SPA2, SPA3 and SPA4. Red bars indicate the truncation sites to generate N-terminal (designated SNT) or C-terminal (designated SCT) fragments of four SPA proteins.
Supplemental Figure S4. CRY interacts with SPA1 in a blue light-dependent manner in yeast cells. Quantitative yeast two-hybrid analyses of the CRY–SPA1 interactions under blue light (30 μmol/m²/s) and in darkness for 4h. Data are mean ± SD (n = 12, independent biological replicates). Both the bait and the prey constructs are described in Figure 1.
Supplemental Figure S5. CRY1 and CRY2 colocalize with SPA1 in *Arabidopsis* protoplasts. Protoplasts were isolated from *Arabidopsis* seedlings and co-transformed with indicated DNA constructs by PEG4000. Protoplasts were imaged using CFP and YFP channels of a confocal microscope after incubation for 12-15 h at 22°C. CRY1 and CRY2 localize together with SPA1 to the nuclear speckles in protoplasts. Dic, differential interference contrast in light microscope mode. Bars, 10 μm.
Supplemental Figure S6. Analyses of anti-HY5 antibody specificity and HY5 abundance by Western blotting. (A) HY5 was detected in WT rather than hy5 mutant seedlings grown under continuous white light for 5 days by Western blotting using anti-HY5 antibody on 12% SDS-PAGE gel. (B) HY5 abundance was determined in seedlings with indicated genotypes grown under 30 μmol/m²/s blue light for 5 days by Western blotting using anti-HY5 antibody on 15% SDS-PAGE gel. *Arabidopsis* ACTIN 11 (ACT11) serves as loading control. To note, HY5 protein in *spa1234, cry1spa1234*, and *cop1* mutants showed relatively slower migration on high percentage (15%) SDS-PAGE gel than that in WT and *cry1* mutant, possibly due to protein modifications in these mutants. Asterisk and arrowhead denote nonspecific band and HY5, respectively.
Supplemental Figure S7. CRY1 doesn’t interact with CC1 and SCC1 in either blue light or darkness in yeast cells. (A) Yeast two-hybrid bait constructs. All proteins are fused with the DNA-binding domain of GAL4 (BD). (CC1) coiled-coil domain of COP1; (SCC1) coiled-coil domain of SPA1. (B) Yeast two-hybrid prey constructs. All proteins are fused with the activation domain of GAL4 (AD). (C) Quantitative yeast two-hybrid analyses of the CRY1–CC1 and CRY1–SCC1 interactions under blue light (50 μmol/m²/s) and in darkness. Data are mean ± SD (n = 12, independent biological replicates).
Supplemental Figure S8. Specificity analysis of anti-COP1 antibody by Western blotting and coimmunoprecipitation assay showing SPA1–COP1 interaction. (A) COP1 was detected in transgenic Myc–SPA1/spa1-3 (Myc–SPA1) rather than cop1-4 mutant seedlings by Western blotting using anti-COP1 antibody. (B) Coimmunoprecipitation using anti-Myc agarose beads in the extracts from Myc–SPA1/spa1-3 and spa1-3 (spa1) mutant seedlings grown under continuous white light for 5 days before being transferred to darkness for another 3 days. 1 mg or 3 mg of total protein were used for coimmunoprecipitation assay, respectively. The immunoprecipitates were probed with anti-COP1 and anti-Myc antibodies, respectively. Arrowhead indicates COP1. Asterisks indicate non-specific bands recognized by anti-COP1 antibody. Arrowhead indicates COP1.