

Supplemental Information

Figure S1. CRY photooligomerization *ex vivo* or *in vivo*.
(Related to Figure 1, Figure 2 and Table 1)

Figure S2. The dark reversion analyses of CRY2 photooligomerization at different temperature.
(Related to Figure 1 and Table 1)

Figure S3. Homooligomerization analyses of plant and animal CRYs.
Homooligomerization analyses of plant and animal CRYs.
(Related to Figure 2, Figure 4)

Figure S4. Photooligomerization analyses of soybean CRYs (GmCRY) in HEK293.
(Related to Figure 4)

Figure S5. *CRY-PHR* transgenic plants reduce sensitivity to blue light.
(Related to Figure 5)

Figure S6. The subcellular localization of PHR1-NLS-GFP and PHR2-NLS-GFP recombinant proteins in *Arabidopsis*.
(Related to Figure 5, Figure 6)

Figure S7. No late-flowering phenotypes are observed in *CRY-PHR* overexpression plants, due to low dosage of CRY2 needed in controlling flowering time.
(Related to Figure 5)

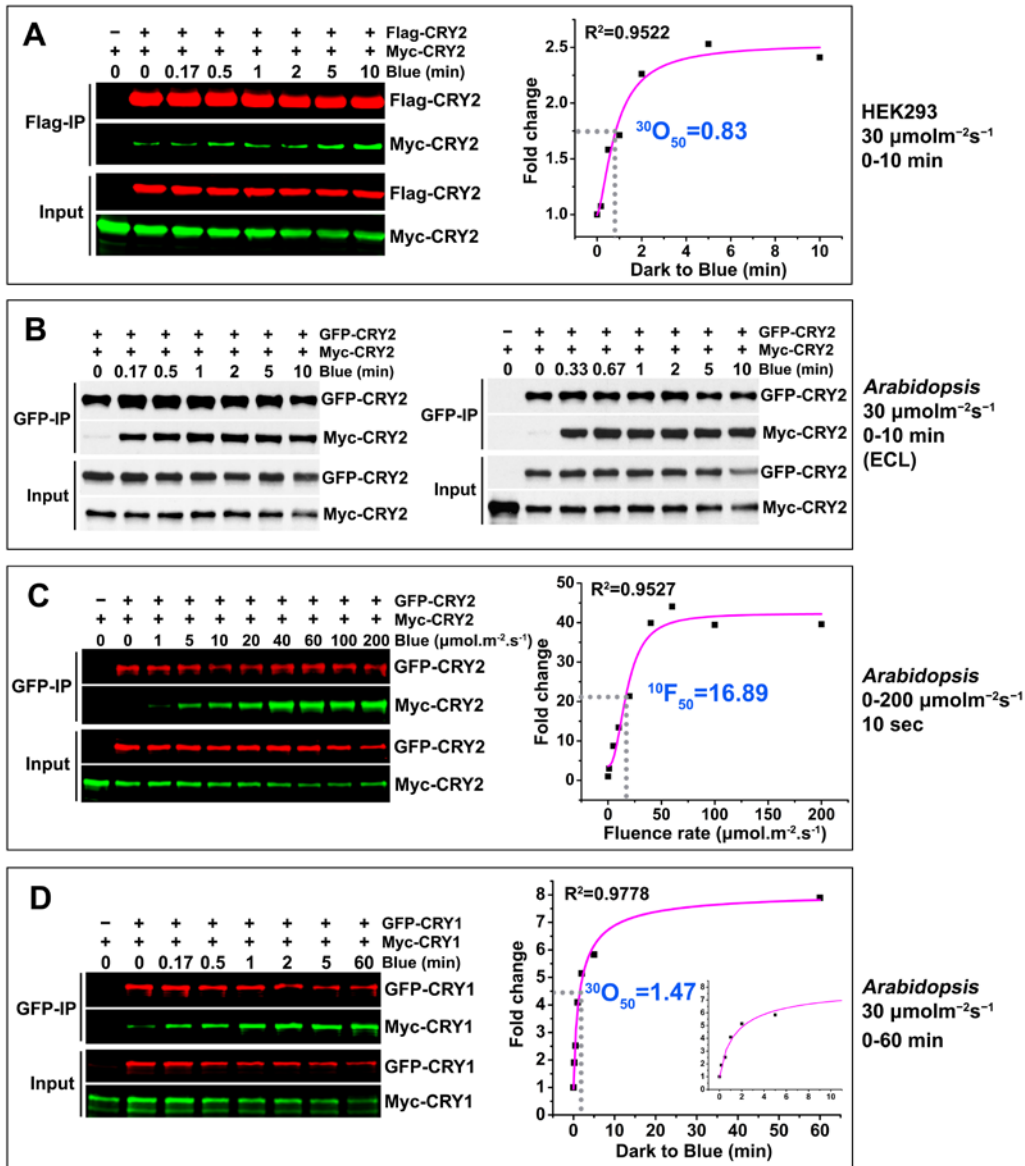


Figure S1. CRY photooligomerization ex vivo or in vivo.

(A) The kinetics analyses of CRY2 photooligomerization in response to blue light in HEK293 cells. Cells were treated with $30 \mu\text{molm}^{-2}\text{s}^{-1}$ blue light for indicated time.

(B) The kinetics analyses of CRY2 photooligomerization in plants observed by conventional ECL method. Two independent Co-IP experiments were carried out and analysed by enhanced chemiluminescence (ECL) method. Seven-day-old etiolated *Arabidopsis* seedlings co-expressing GFP-CRY2 and Myc-CRY2 were exposed to $30 \mu\text{molm}^{-2}\text{s}^{-1}$ blue light for indicated time.

(C) The fluence-rate response analyses of CRY2 photooligomerization in plants. Seven-day-old *Arabidopsis* seedlings co-expressing GFP-CRY2 and Myc-CRY2 were exposed to blue light of the indicated fluence rates for 10 seconds.

(D) The kinetics analyses of CRY1 photooligomerization in response to blue light in plants. Seven-day-old etiolated seedlings co-expressing GFP-CRY1 and Myc-CRY1 were grown on petri dishes, exposed to blue light of $30 \mu\text{molm}^{-2}\text{s}^{-1}$, and tissues were collected at the indicated time. The insert small graph shows the first 10 minutes in the curve.

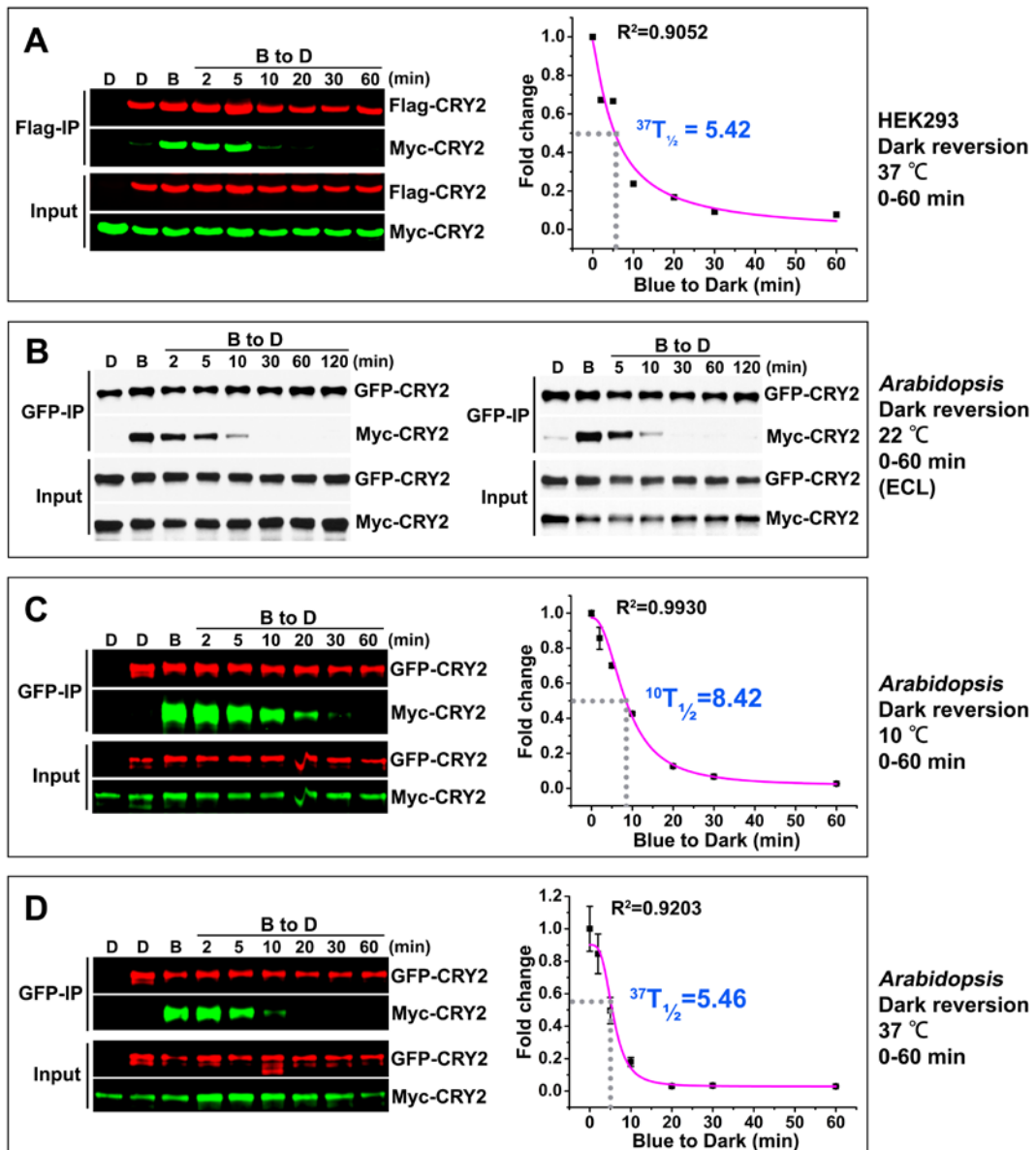


Figure S2. The dark reversion analyses of CRY2 photooligomerization at different temperature.

(A) The dark reversion analyses of CRY2 photooligomerization in HEK 293 cells at 37°C. HEK293 cells co-transfected with Flag-CRY2 or Myc-CRY2 were irradiated with $100 \mu\text{molm}^{-2}\text{s}^{-1}$ blue light for 10 min to allow CRY2 oligomerization, and then cells were subjected to the dark for the indicated time (2 to 60 min) at 37°C.

(B) The dark reversion analysis of CRY2 photooligomerization at 22°C in plants observed by conventional ECL method. Two independent Co-IP experiments were carried out and analysed by enhanced chemiluminescence (ECL) method. Seven-day-old seedlings co-expressing GFP-CRY2 and Myc-CRY2 were irradiated with $30 \mu\text{molm}^{-2}\text{s}^{-1}$ blue light for 5 min before transferring to darkness (at 22°C) for the indicated times.

(C-D) The dark reversion analyses of CRY2 photooligomerization at 10°C (C) or 37°C (D) in plants. Seven-day-old seedlings co-expressing GFP-CRY2 and Myc-CRY2 were irradiated with $30 \mu\text{molm}^{-2}\text{s}^{-1}$ blue light for 5 min before transferring to darkness (at 10°C or 37°C) for the indicated times.

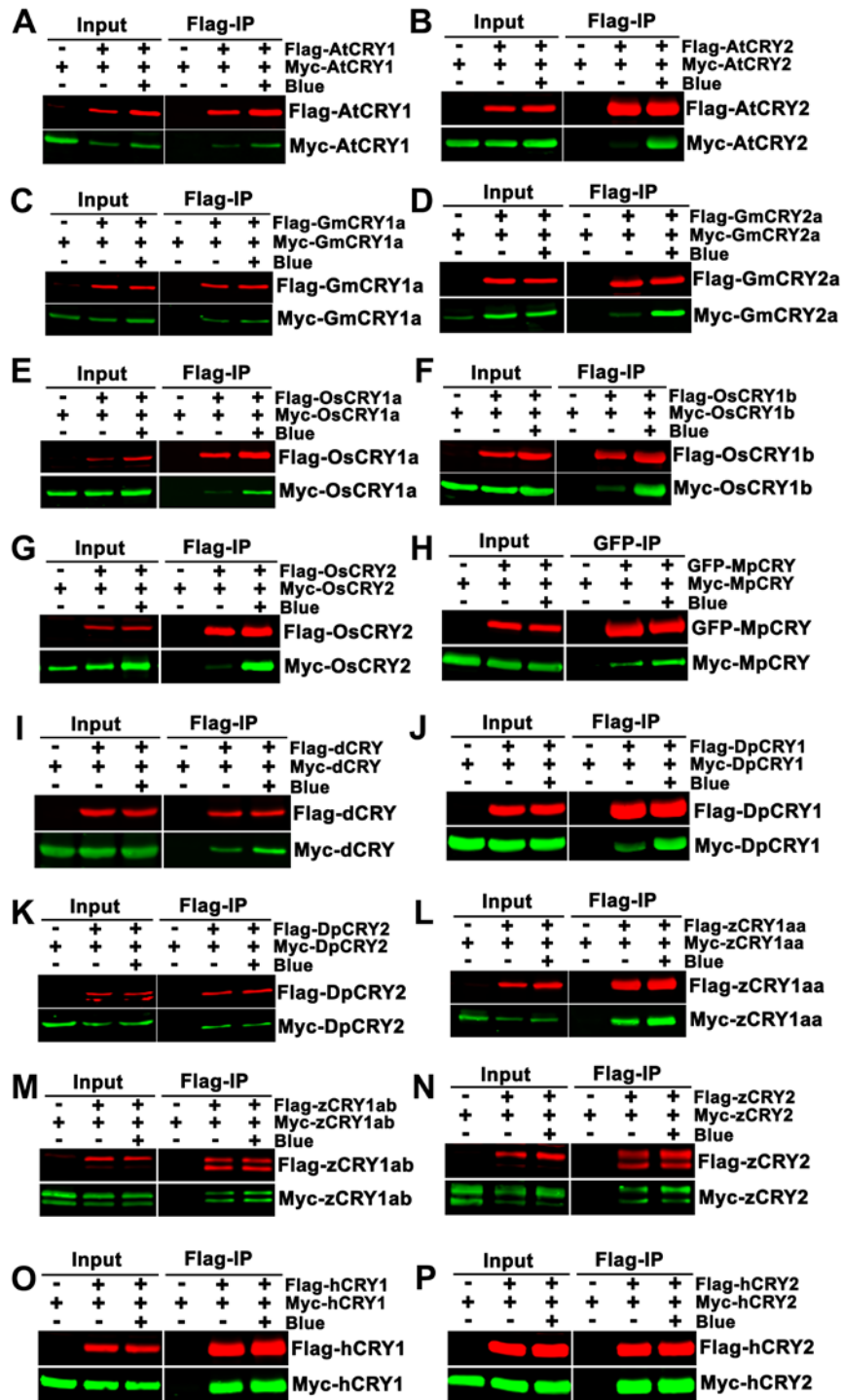


Figure S3. Homooligomerization analyses of plant and animal CRYs.

HEK293 cells were transfected with equal amount of two plasmids encoding Flag-CRY or Myc-CRY from different species: AtCRYs of *Arabidopsis*, GmCRYs of soybean (*Glycine max*), OsCRYs of rice (*Oryza sativa*), MpCRY of liverwort (*Marchantia polymorpha*), dCRY of *Drosophila*, DpCRYs of Monarch butterfly (*Danaus plexippus*), zCRYs of Zebrafish, and hCRYs of human (*Homo sapien*). After transfection, cells were exposed to $100 \mu\text{molm}^{-2}\text{s}^{-1}$ for 2 hours. Flag-CRYs were co-immunoprecipitated by Flag-agarose gels. The immunoblots were probed by the anti-Flag antibody to detect IP product and anti-Myc antibody to detect Co-IP product.

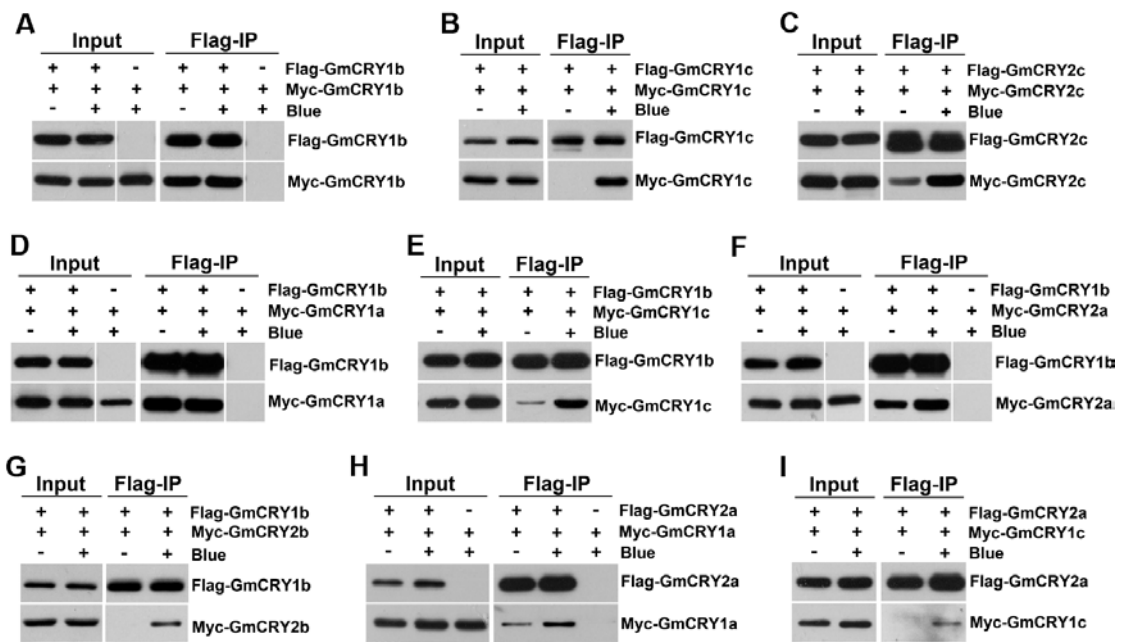


Figure S4. Photooligomerization analyses of soybean CRYs (GmCRY) in HEK293.

HEK293 cells were transfected with equal amount of Flag-GmCRY and Myc-GmCRY plasmids. 40 hours after transfection, cells were exposed to $100 \mu\text{molm}^{-2}\text{s}^{-1}$ for 2 hours or kept in the dark at 22°C . Flag-GmCRYs were immunoprecipitated by Flag-agarose gels. The proteins were analyzed by immunoblotting with Flag and Myc antibodies. (A-C) shows the homooligomerization of GmCRYs. (D-I) shows the heterooligomerization between GmCRYs.

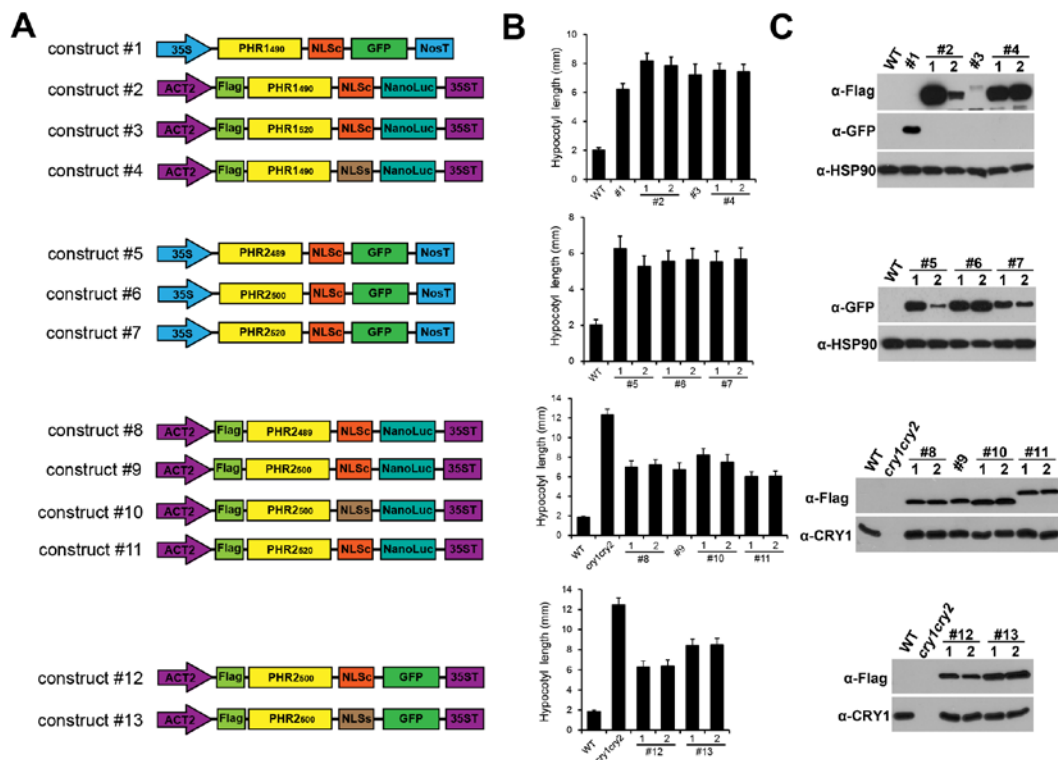


Figure S5. *CRY-PHR* transgenic plants reduce sensitivity to blue light.

(A) The structures of 13 different DNA constructs used for generating *CRY-PHR* transgenic plants. 35S, cauliflower mosaic virus 35S promoter; ACT2, actin2 promoter; Flag, Flag epitope tag; PHR1₄₉₀, CRY1 1-490 aa; PHR1₅₂₀, CRY1 1-520 aa; PHR2₄₈₉, CRY2 1-489 aa; PHR2₅₀₀, CRY2 1-500 aa; PHR2₅₂₀, CRY2 1-520 aa; NLSs, SV40 nuclear localization signal; NLS_c, nuclear localization signal from CRY2 (541-557 aa); GFP, green fluorescence protein; NanoLuc, nano luciferase; 35ST, 35S terminator.

(B) Hypocotyl phenotypes of *PHR* transgenic lines. 13 constructs described in (A) were all transformed into wild type background. Seedlings were grown in $30 \mu\text{molm}^{-2}\text{s}^{-1}$ continuous blue light for six days before measurement. Hypocotyls of 1 to 2 independent transgenic *CRY-PHR* lines were shown. Hypocotyl length with standard deviations is shown ($n \geq 20$).

(C) Immunoblots showing *PHR* recombinant protein expression levels in transgenic lines. *PHR* fusion proteins were detected with anti-Flag or anti-GFP antibodies. *CRY1* and *HSP90* are used as loading controls.

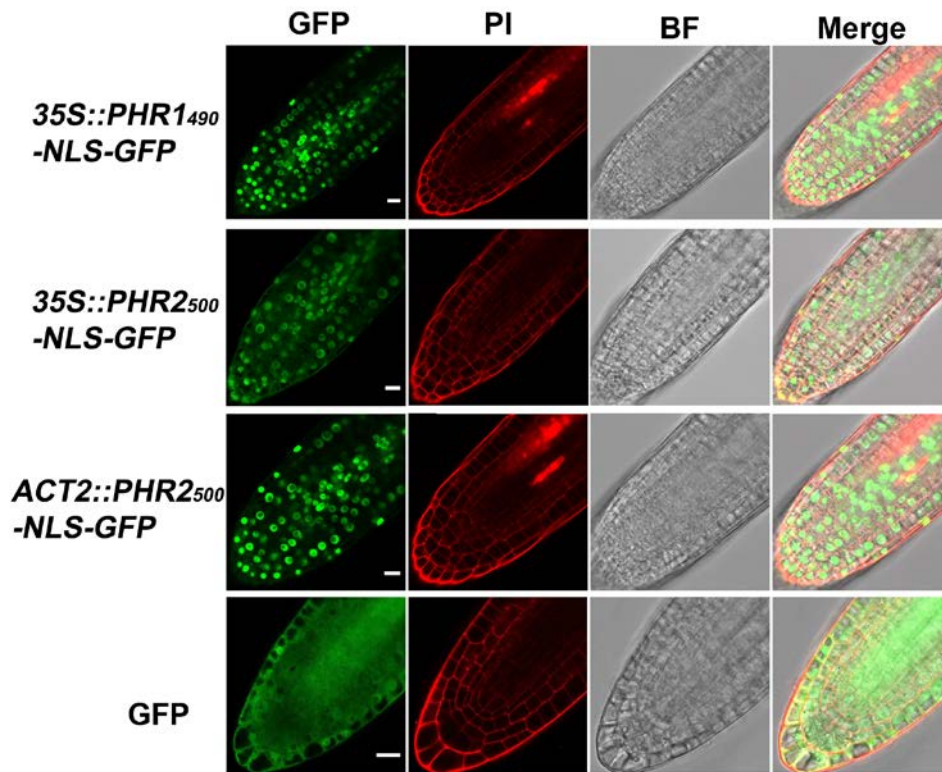


Figure S6. The subcellular localization of PHR1-NLS-GFP and PHR2-NLS-GFP recombinant proteins in *Arabidopsis*.

Six-day-old PHR transgenic lines (*PHR1-NLS-GFP* and *PHR2-NLS-GFP*) were analyzed by confocal microscope. PI stain is used to show the cell boundaries. Scale bar: 10 μ m.

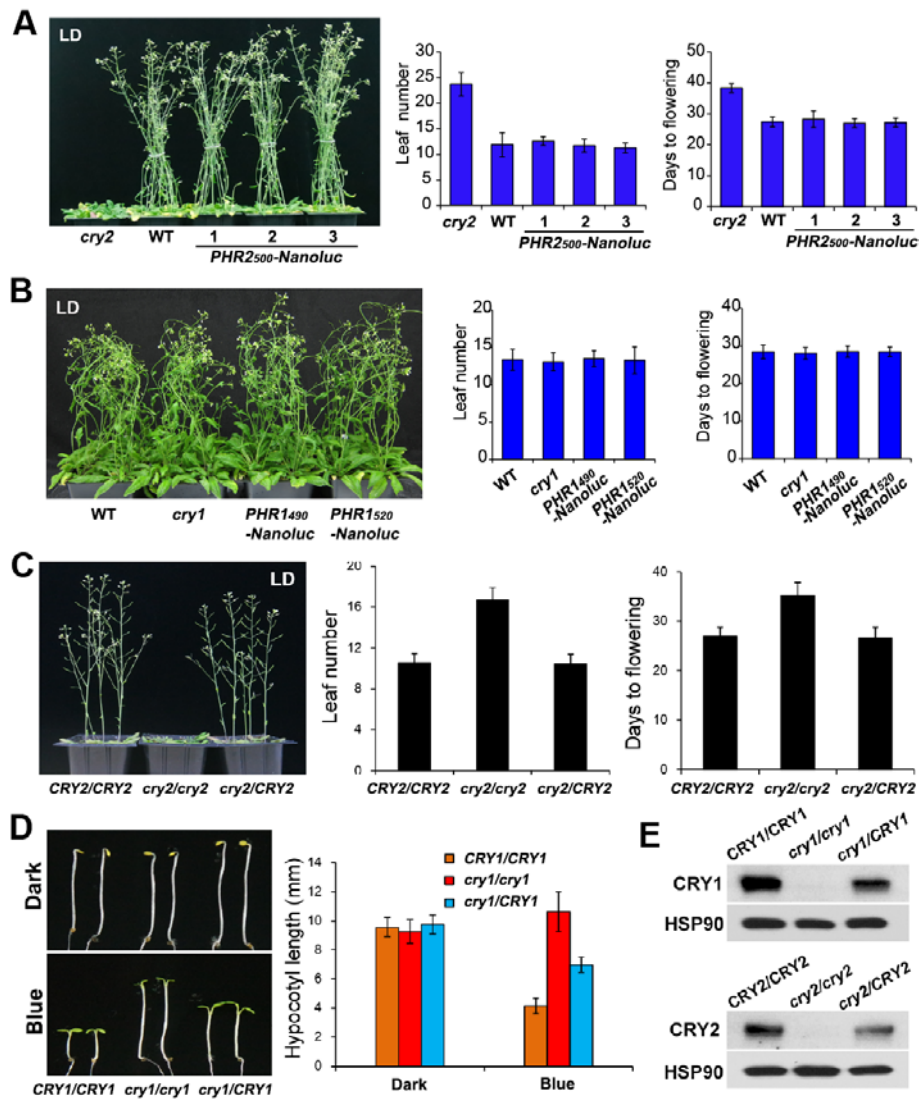


Figure S7. No late-flowering phenotypes are observed in *CRY-PHR* overexpression plants, due to low dosage of *CRY2* protein needed in controlling flowering time.

(A-B) Flowering phenotype analysis of *CRY-PHR* transgenic plants. Plants with *PHR2* (A) and *PHR1* (B) overexpressed in wild-type background were grown under long day conditions (16 hour light / 8 hour dark). Leaf number and days to flowering with standard deviations are shown ($n \geq 20$).

(C) Flowering phenotypes of *CRY2* homozygous mutant (*cry2/cry2*) and heterozygous mutant (*cry2/CRY2*) plants. *CRY2/CRY2* stands for wild type. Plants of indicated genotypes were grown in long day conditions. Leaf number and days to flowering with standard deviations are shown ($n \geq 20$).

(D) Hypocotyl phenotypes of *CRY1* homozygous mutant (*cry1/cry1*) and heterozygous mutant (*cry1/CRY1*) seedlings. *CRY1/CRY1* stands for wild type. Seedlings were grown under darkness or continuous blue light ($15 \mu\text{molm}^{-2}\text{s}^{-1}$) for 5 days. Hypocotyl length with standard deviations is shown ($n \geq 20$).

(E) Immunoblots showing the CRY protein expression levels in *CRY* homozygous (*cry/cry*) or heterozygous (*cry/CRY*) mutant plants. Endogenous *CRY1* and *CRY2* proteins were blotted with *CRY1* or *CRY2* antibody, respectively. HSP90 is used as loading controls.