Supporting Information

Liu et al. 10.1073/pnas.1308987110

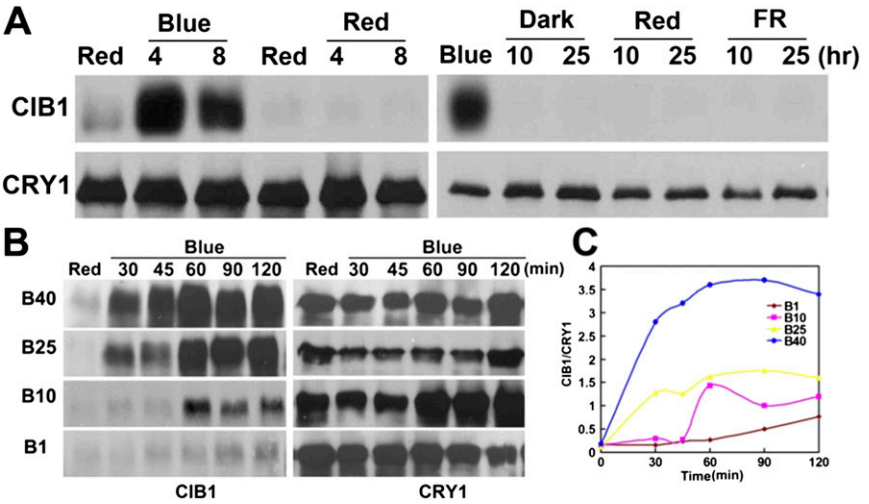

Fig. S1. Immunoblots showing light regulation of CRYPTOCHROME-INTERACTING basic helix–loop–helix 1 (CIB1) protein expression. (*A Left*) Transgenic plants expressing the *35S::Myc–CIB1* transgene were grown in long day (LD) for 3 wk, treated with red light (red; 20 μmol·m^{-2}·s^{-1}) for 16 h, and then transferred to blue light (blue; 35 μmol·m^{-2}·s^{-1}) or kept in red light (red; 20 μmol·m^{-2}·s^{-1}) for the indicated time. (*Right*) Alternatively, the 3-wk-old plants were first treated with blue light (blue; 35 μmol·m^{-2}·s^{-1}) for 16 h and then transferred to dark, red light (20 μmol·m^{-2}·s^{-1}), or far-red light (FR; 5 μmol·m^{-2}·s^{-1}) for the indicated time. (*B*) Three-week-old red-light–grown plants were transferred to blue light of indicated fluence rate (1–40 μmol·m^{-2}·s^{-1}) and time (30–120 min) and analyzed by immunoblot. Samples were fractionated by 10% SDS/PAGE gels, blotted, probed by the anti-Myc antibody, stripped, and reprobed with the anti-cryptochrome 1 (anti-CRY1) antibody to indicate relative loading of the samples. (*C*) A semiquantification of CIB1 expression for the immunoblot shown in *B*. The ECL luminography films shown in *B* were scanned and analyzed by ImageJ software. The relative CIB1 expression (Myc–CIB1/CRY1) was normalized by the CRY1 loading control.

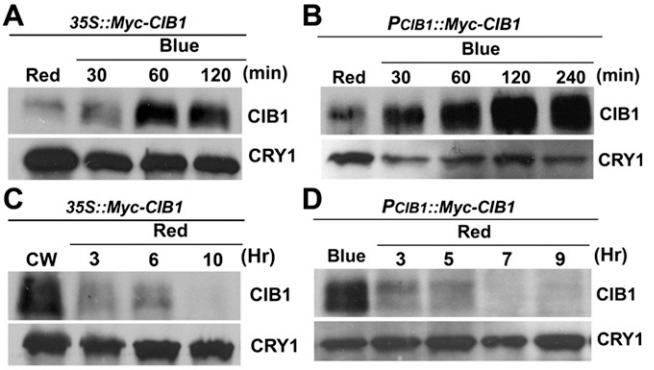

Fig. S2. The *CIB1* promoter is not required for the blue-light–induced CIB1 accumulation in response to blue light. Immunoblots probed with anti-Myc antibody and the control anti-CRY1 antibody are shown for the following samples. (*A*) Eight-day-old etiolated seedlings expressing the *35S::Myc–CIB1* transgene were transferred to red light for 16 h (red; 20 μmol·m^{-2}·s^{-1}) and then transferred to blue light (blue; 35 μmol·m^{-2}·s^{-1}) for the indicated time before sample collection. (*B*) Three-week-old plants expressing the P_{CIB1}*::Myc–CIB1* transgene were grown in LD, exposed to red light (red; 20 μmol·m^{-2}·s^{-1}) for 16 h, and then transferred to blue light (blue; 35 μmol·m^{-2}·s^{-1}) for the indicated time before sample collection. (*C*) Eight-day-old transgenic plants expressing *35S::Myc–CIB1* transgene were grown in continuous white light (CW) and transferred to red light (red; 20 μmol·m^{-2}·s^{-1}) for the indicated time before sample collection. (*D*) Three-week-old plants expressing the P_{CIB1}*::Myc–CIB1* transgene were grown in LD, exposed to blue light (blue; 35 μmol·m^{-2}·s^{-1}) for 16 h, and then transferred to red light (red; 20 μmol·m^{-2}·s^{-1}) for the indicated time before sample collection. Samples were fractionated by a 10% SDS/PAGE, blotted, probed by the anti-Myc antibody (CIB1), stripped, and reprobed with the anti-CRY1 antibody (CRY1).

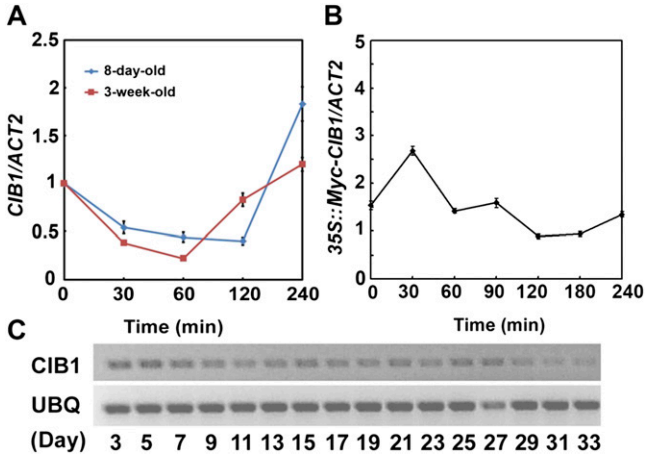

Fig. S3. Analyses of the *CIB1* mRNA expression. (*A*) Quantitative PCR (qPCR) assay showing mRNA expression of the endogenous *CIB1* gene in 8-d-old etiolated wild-type seedlings transferred to blue light (35 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for the indicated time before sample collection (blue) or 3-wk-old LD-grown plants, transferred to dark for 16 h, and then transferred to blue light (35 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for the indicated time before sample collection (red). The first point was set as 1. (*B*) qPCR assay showing mRNA expression of the *35S::Myc–CIB1* transgene in 5-d-old etiolated transgenic seedlings transferred to blue light (35 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at the indicated time before sample collection (red). (*C*) RT-PCR results showing mRNA expression of the *CIB1* gene in plants of different ages. Wild-type plants grown in LD were harvest every 2 d from 3 to 33 d after germination, and the mRNA level were analyzed by conventional RT-PCR. *UBQ* was used as an internal control. The result indicates that the *CIB1* mRNA is relatively low abundant and the expression is not significantly altered during development.

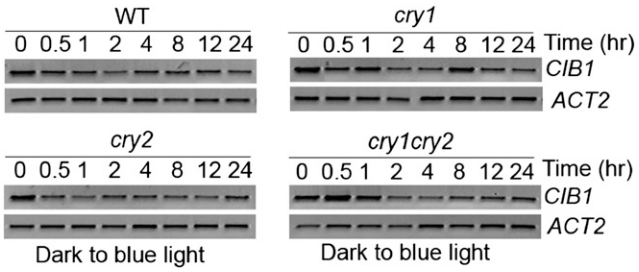

Fig. S4. Lack of significant change of the *CIB1* mRNA expression in the *cry* mutants. Wild-type (WT) and *cry1*, *cry2*, and *cry1cry2* mutant seedlings were grown in dark for 6 d and then transferred into blue light (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for indicated time before sample harvest for RNA analyses. Levels of mRNA expression are shown as the RT-PCR gel images. *ACT2* was used as an internal control.

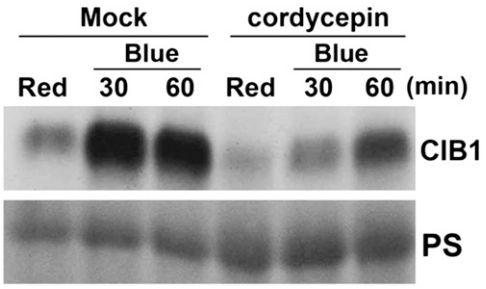

Fig. S5. Immunoblot showing that the transcription inhibitor cordycepin failed to block blue-light–induced increase of CIB1 accumulation. Transgenic plants expressing *35S::Myc–CIB1* were grown in continuous white light (CW) for 3 wk and transferred to red light for 16 h. Leaves were excised and incubated in cordycepin (0.5 mmol/L) or mock solution (0.5% DMSO) in blue light (35 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for the indicated time before sample collection. A Ponceau S-stained band (PS) is used to indicate relative loadings.

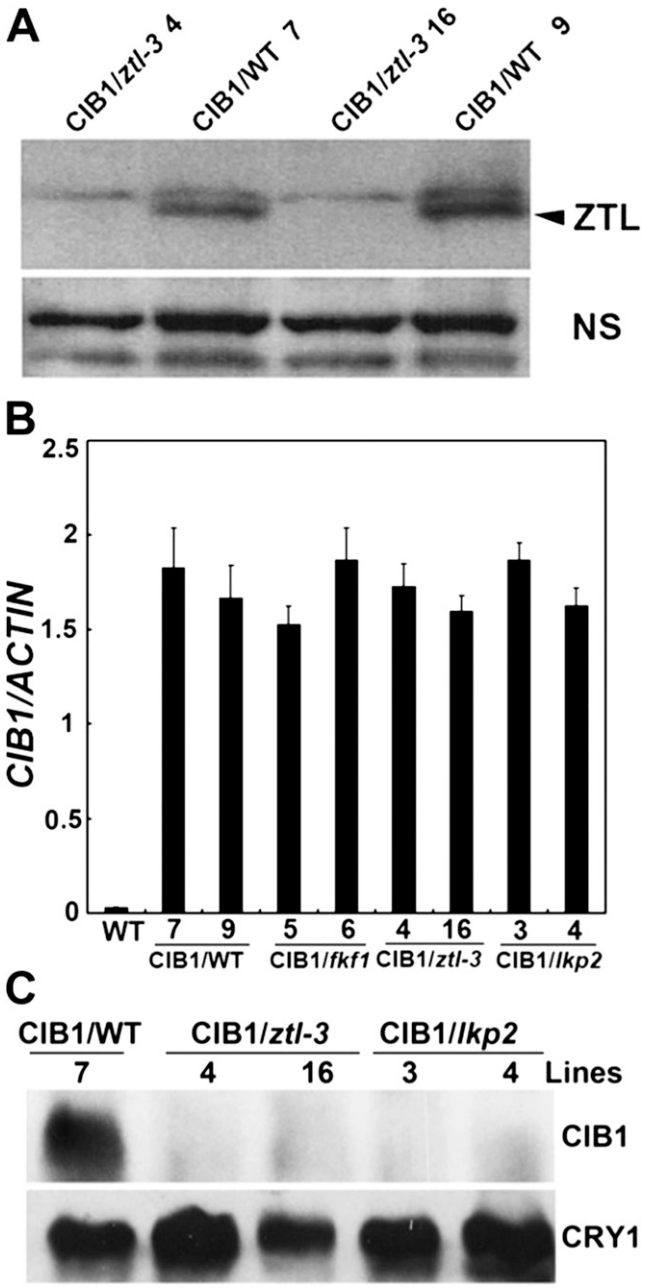

Fig. S6. Expression of the 35S::Myc–CIB1 transgene products in *zeitlupe-3* (*ztl*-3) mutant allele and the *lov kelch protein 2* (*lkp2*) mutant. (*A*) An immunoblot probed with anti-ZTL antibody shows the lack of ZTL protein in the *CIB1*/*ztl-3* line used in *C* and in Figs. 4 and 5. NS refers to nonspecific band, which is used to indicate relative loadings. (*B*) qPCR assay shows the *CIB1* mRNA expression in the transgenic plants expressing the *35S::Myc–CIB1* transgene in the wild-type (WT) or *ztl-3*, *lkp2*, or *flavin-binding kelch repeat 1* (*fkf1*) mutant backgrounds. SDs are shown ($n = 3$). (*C*) Immunoblot showing the lack of CIB1 protein expression in the *ztl-3* or *lkp2* mutant plants grown in white light. Three-week-old plants grown in white light were analyzed by immunoblot as shown. The blot was stripped and reprobed with anti-CRY1 antibody to indicate relative loadings.

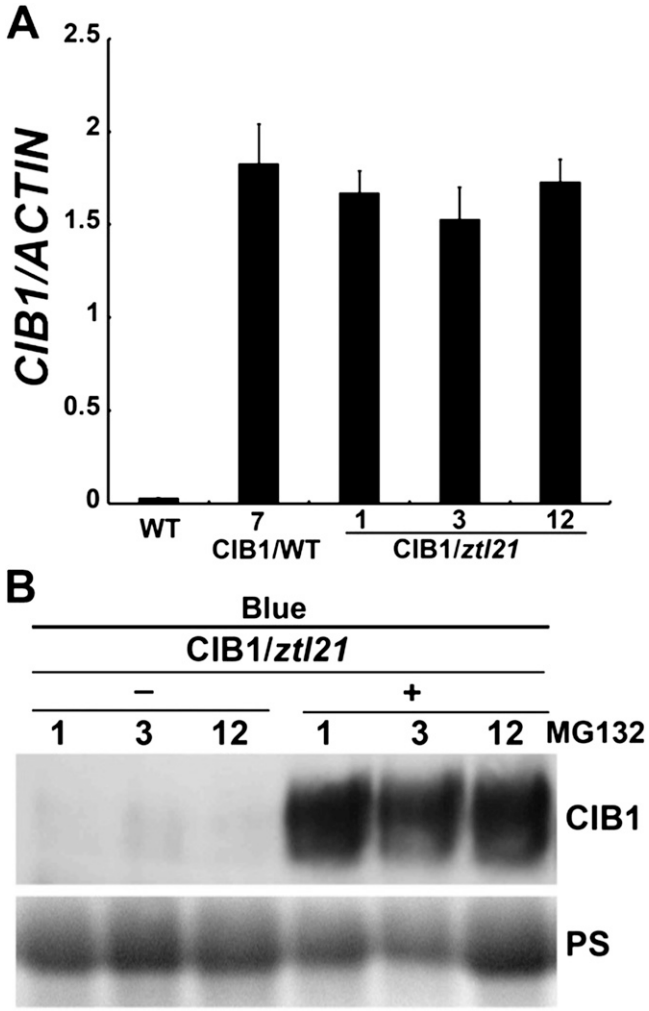

Fig. S7. Expression of the 35S::Myc–CIB1 transgene products in the *ztl21* mutant allele. (*A*) qPCR assay showing similar levels of *CIB1* mRNA expression in the transgenic plants expressing the *35S::Myc–CIB1* transgene in the wild-type (WT) and *ztl21* mutant background. SDs are shown ($n = 3$). (*B*) Immunoblot showing that the lack of CIB1 protein accumulation in the *ztl21* mutant was caused by excessive proteolysis by the 26S proteasome. Three independent lines expressing *35S::Myc–CIB1* in the *ztl21* background were grown in LD (16-h light/8-h dark) for 3 wk and transferred to blue light (35 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 16 h. Leaves were excised and incubated in MG132 (50 μmol/L) or mock solution (0.1% DMSO) in blue light for 3 h, and the samples were analyzed by immunoblot probed with the anti-Myc antibody. A Ponceau S-stained band (PS) is used to indicate relative loadings.

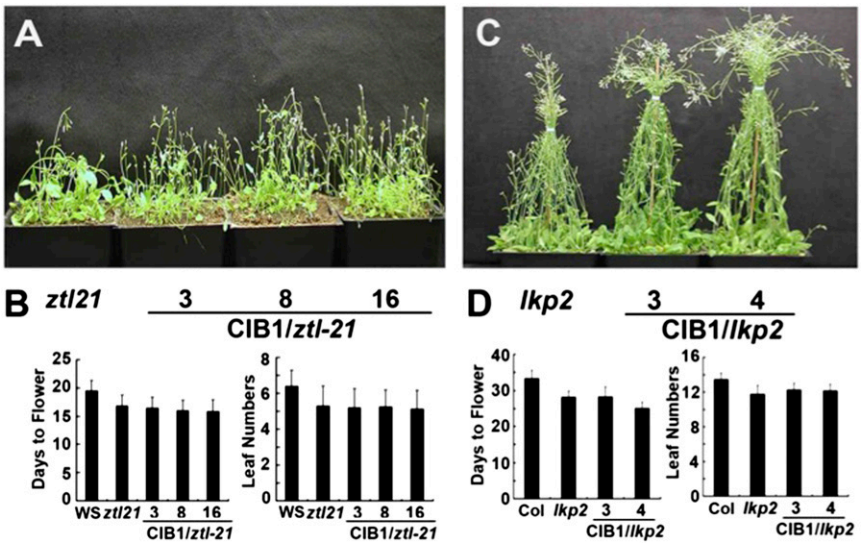

Fig. S8. Overexpression of CIB1 failed to cause strong accelerated flowering in the *ztl* and *lkp2* mutant backgrounds. (*A* and *C*) Images of the indicated genotype grown in LD photoperiods. (*B* and *D*) The flowering times were measured as days to flower and rosette leaf numbers at flowering of the indicated genotypes. SDs are shown ($n > 15$). The expression of the *35S::Myc–CIB1* transgene in the *ztl21* (*CIB1/ztl-21*) and *lkp2* (*CIB1/lkp2*) mutants are shown in Figs. S6 and S7, respectively.

Table S1. Oligonucleotide primers used in this work

Assays	Destination products	Primer names	Primer sequences
qPCR		QACTIN2F	5′-GCTGAGAGATTCAGATGCCCA-3′
		QACTIN2R	5′-GTGGATTCCAGCAGCTTCCAT-3′
		QCIB1F	5′-TGATCCATTGTCATGCTTCAACA-3′
		QCIB1R	5′-CACATGAGAGTCCCACATCGA-3′
Y2H	pEG202-ZTL	ZTL-attr1	5′-AAAAAAGCAGGCTTCATGGAGTGGGACAGTGGT
Co-IP	pEarly201-ZTL	ZTL-attr2	5′-AAGAAAGCTGGGTCTTACGTGAGATAGCTCGC
Y2H	pB42AD-CIB1	CIB1-F	5′-GAATTCATGAATGGAGCTATAGGAG-3′
Protein expression	pCold-CIB1	CIB1-R	5′-CTCGAGTCAAACTCCTAAATTGCC-3′

Co-IP, coimmunoprecipitation. Y2H, yeast two-hybrid.