Supporting Information - Figures

A CRY-BIC negative feedback circuitry regulating blue light sensitivity of Arabidopsis

Xu Wang[1,7,8], Qin Wang[1,7,8], Yun-Jeong Han[2,8], Qing Liu[1], Liangfeng Gu[1], Zhaohe Yang[1], Jun Su[1], Bobin Liu[1], Zecheng Zuo[1], Wenjin He[3,7], Jian Wang[4], Bin Liu[5], Minami Matsui[6], Jeong-Il Kim[2*], Yoshito Oka[1*], and Chentao Lin[7*]

Figure S1


GaBIC1(EPS56384)
GmBIC5(XP_003536631)
GmBIC4(ACU19002)
GmBIC3(XP_003542603)
GmBIC2(XP_003524435)
GmBIC1(XP_003524464)
LjBIC2(AFK44733)
LjBIC1(BT144939)
CaBIC2(XP_004494175)
MtBIC2(XP_003625814)
LaBIC1(CCH47172)
MnBIC1(EXB88708)
SlBIC1(XP_004246546)
TcBIC1(EOY08236)
PrpBIC1(EMJ03889)
VvBIC1(CAN69280)
VvBIC2(CAN80332)
PotBIC1(XP_002312818)
PotBIC2(XP_002328254)
RcBIC2(XP_002523802)
AlBIC2(XP_002877340)
AtBIC2
CrBIC2(EOA24961)
GmBIC6(XP_003540248)
GmBIC7(XP_001238535)
MtBIC2(XP_003596820)
CaBIC3(XP_004487496)
RcBIC1(XP_002528742)
CrBIC1(EOA26109)
AlBIC1(XP_002877881)
AtBIC1
CsBIC2(AC435272)
CsBIC1(XP_004161241)
CaBIC1(XP_004494053)
CsBIC3(XP_004149256)
MnBIC2(EXB38438)
GaBIC2(EPS67524)
GaBIC3(EPS70666)
PrpBIC2(EMJ08035)
PotBIC3(XP_002328255)
PotBIC4(XP_002312817)
RcBIC3(XP_002523801)
RcBIC4(XP_002523800)
TcBIC2(EOY33440)
VvBIC3(XP_002275909)
SlBIC2(XP_004954965)
ZmBIC2(NP_001142587)
OsBIC2(NP_001047026)
SbBIC2(XP_002452201)
BdBIC2(XP_003575071)
HvBIC1(BAJ89837)
BdBIC1(XP_003581139)
OsBIC1(NP_001052731)
SiBIC1(XP_004975560)
SbBIC1(XP_002446410)
ZmBIC1(NP_001143077)
PpBIC1(ABK22013)
PtBIC1(AFG64787)
PtBIC1(AEW98096)
PtrpBIC1(XP_001752727)
PtrpBIC2(XP_001765197)
0.1
Moss
Php: Physcomitrella patens
Gymnosperm
Pr: Pinus radiata
Ps: Picea sitchensis
Pit: Pinus taeda
Angiosperm(Dicot)
Al: Arabidopsis lyrata
At: Arabidopsis thaliana
Ca: Cicer arietinum
Cr: Capsella rubella
Cs: Cucumis sativus
Ga: Genlisea aurea
Gm: Glycine max
La: Lupinus angustifolius
Lj: Lotus japonicus
Mn: Morus notabilis
Mt: Medicago truncatula
Prp: Prunus persica
Pot: Populus trichocarpa
Rc: Ricinus communis
Sl: Solanum lycopersicum
Tc: Theobroma cacao
Vv: Vitis vinifera
Angiosperm(Monocot)
Bd: Brachypodium distachyon
Hv: Hordeum vulgare
Os: Oryza sativa
Sb: Sorghum bicolor
Si: Setaria italica
Zm: Zea mays

Figure S2

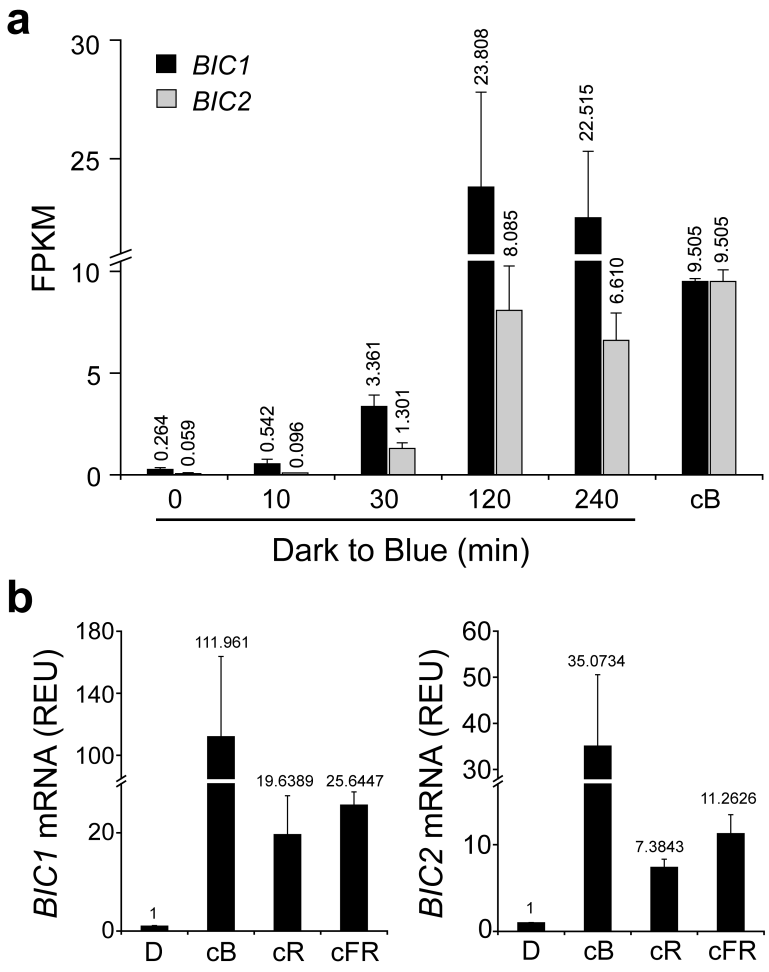

a
BIC1
BIC2
FPKM
30
25
10
5
0
0.264
0.059
0.542
0.096
3.361
1.301
23.808
8.085
22.515
6.610
9.505
9.505
0
10
30
120
240
cB
Dark to Blue (min)
b
BIC1 mRNA (REU)
180
140
100
20
0
1
111.961
19.6389
25.6447
D
cB
cR
cFR
BIC2 mRNA (REU)
60
50
40
30
10
0
1
35.0734
7.3843
11.2626
D
cB
cR
cFR

Figure S3

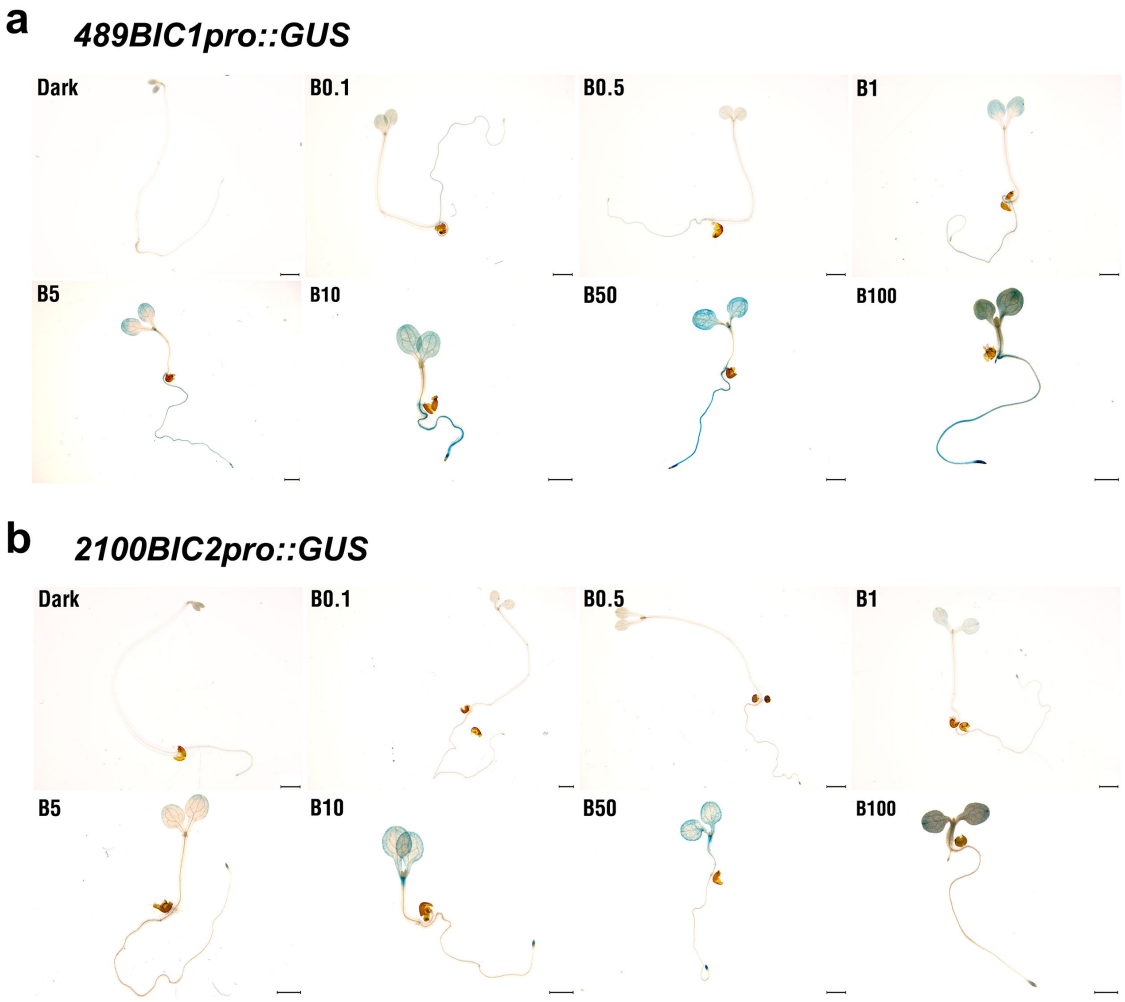
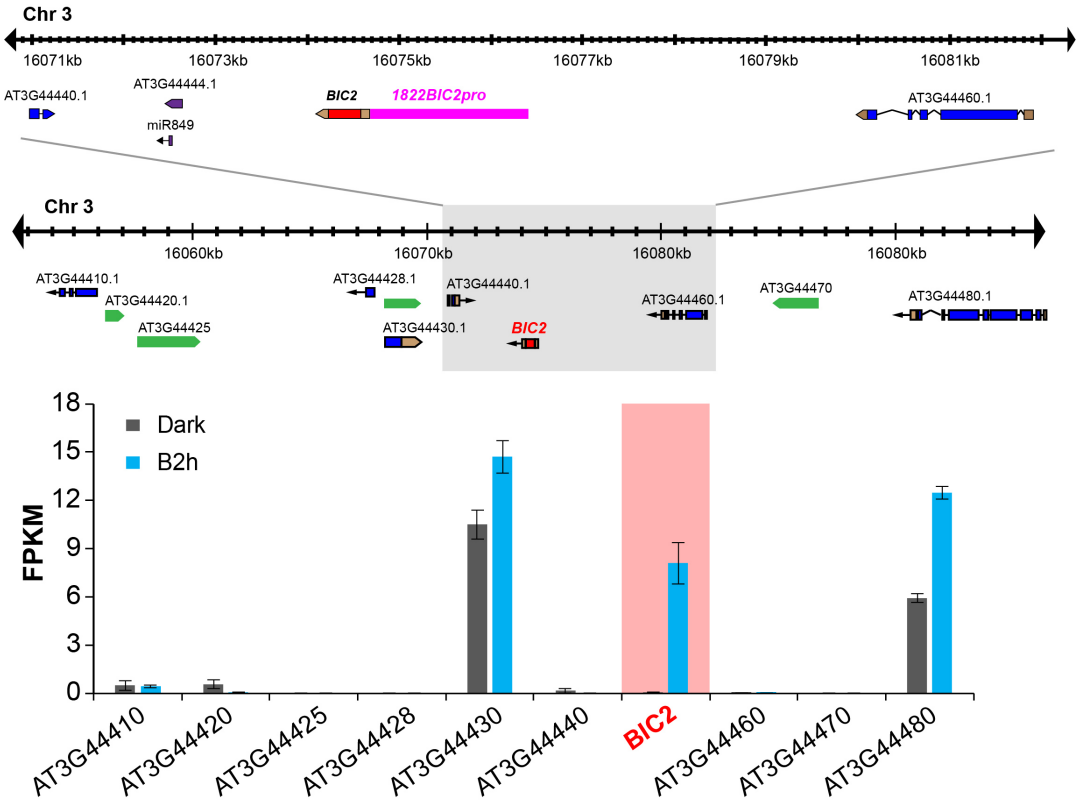

a
489BIC1pro::GUS
Dark
B0.1
B0.5
B1
B5
B10
B50
B100
b
2100BIC2pro::GUS
Dark
B0.1
B0.5
B1
B5
B10
B50
B100

Figure S4


Chr 3
16071kb
16073kb
16075kb
16077kb
16079kb
16081kb
AT3G44440.1
AT3G44444.1
miR849
BIC2
1822BIC2pro
AT3G44460.1
Chr 3
16060kb
16070kb
16080kb
16080kb
AT3G44410.1
AT3G44420.1
AT3G44425
AT3G44428.1
AT3G44440.1
AT3G44430.1
BIC2
AT3G44460.1
AT3G44470
AT3G44480.1
FPKM
Dark
B2h
18
15
12
9
6
3
0
AT3G44410
AT3G44420
AT3G44425
AT3G44428
AT3G44430
AT3G44440
BIC2
AT3G44460
AT3G44470
AT3G44480

.

Figure S5

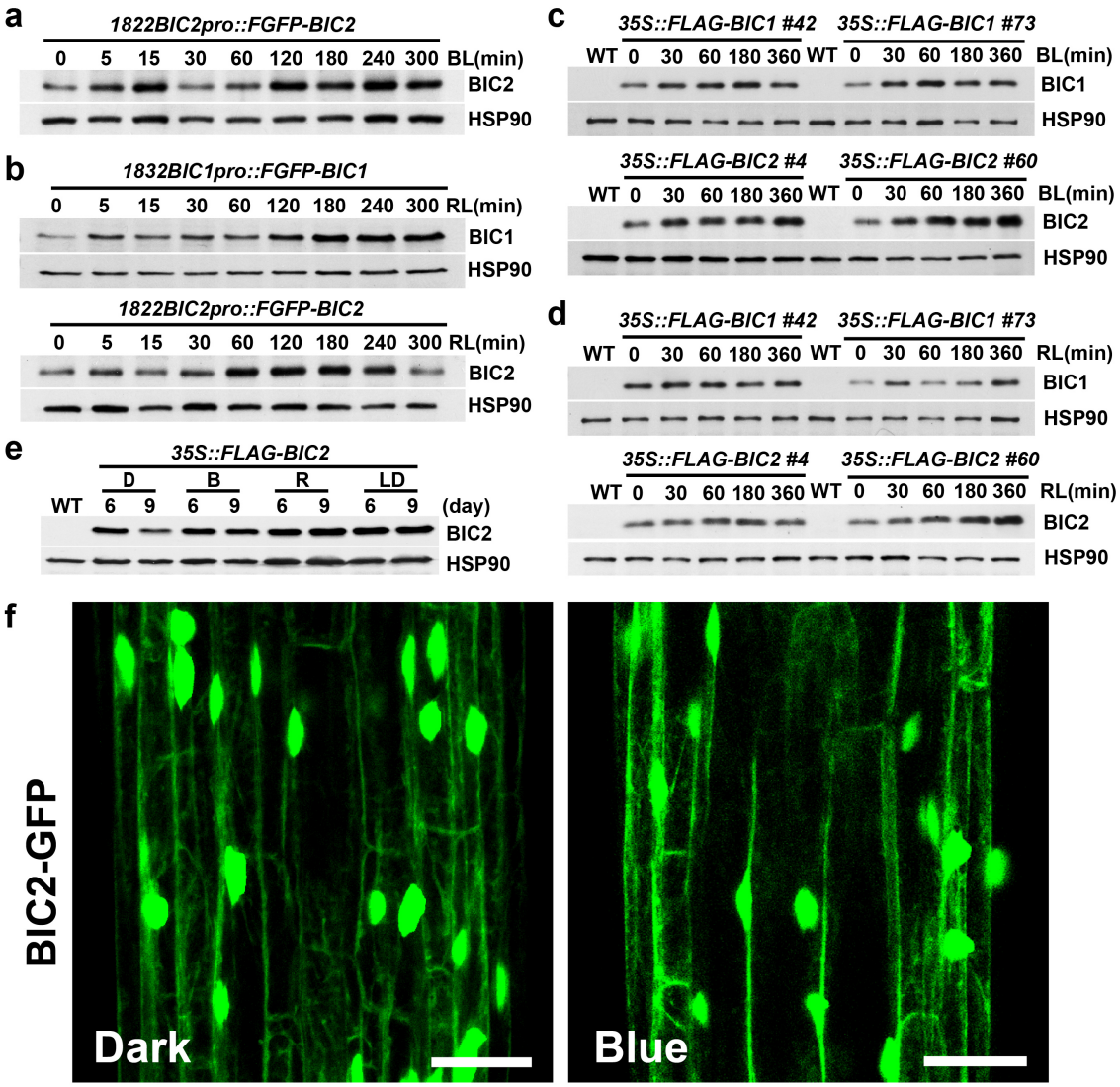
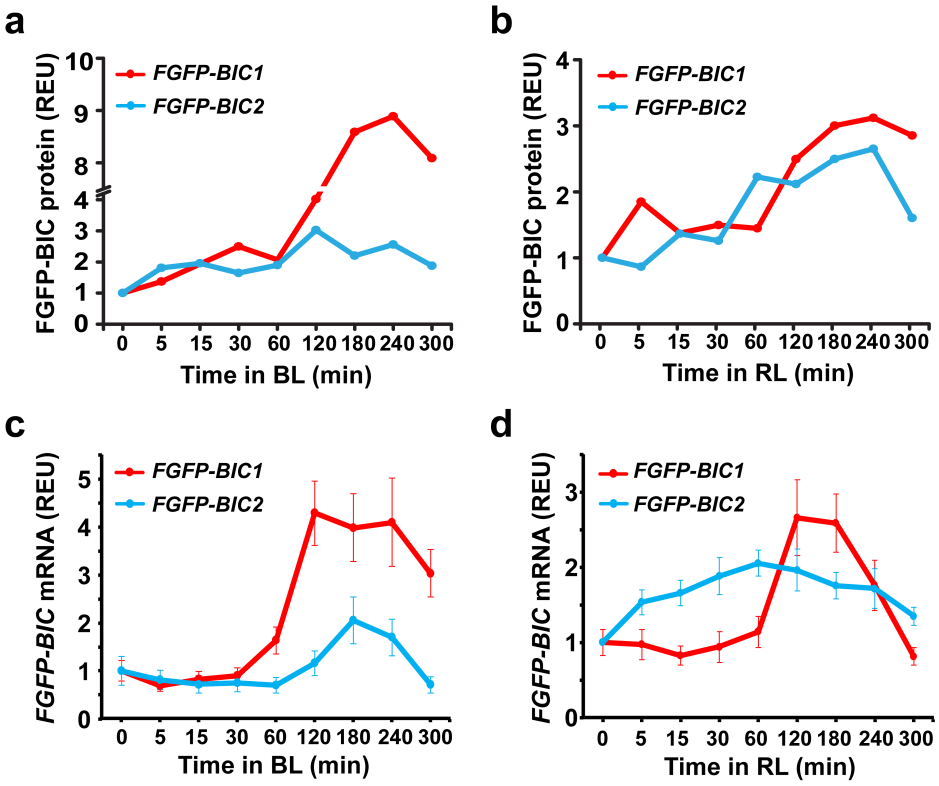

a
1822BIC2pro::FGFP-BIC2
0 5 15 30 60 120 180 240 300 BL(min)
BIC2
HSP90
b
1832BIC1pro::FGFP-BIC1
0 5 15 30 60 120 180 240 300 RL(min)
BIC1
HSP90
1822BIC2pro::FGFP-BIC2
0 5 15 30 60 120 180 240 300 RL(min)
BIC2
HSP90
c
35S::FLAG-BIC1 #42
35S::FLAG-BIC1 #73
WT 0 30 60 180 360 WT 0 30 60 180 360 BL(min)
BIC1
HSP90
35S::FLAG-BIC2 #4
35S::FLAG-BIC2 #60
WT 0 30 60 180 360 WT 0 30 60 180 360 BL(min)
BIC2
HSP90
d
35S::FLAG-BIC1 #42
35S::FLAG-BIC1 #73
WT 0 30 60 180 360 WT 0 30 60 180 360 RL(min)
BIC1
HSP90
35S::FLAG-BIC2 #4
35S::FLAG-BIC2 #60
WT 0 30 60 180 360 WT 0 30 60 180 360 RL(min)
BIC2
HSP90
e
35S::FLAG-BIC2
D B R LD
WT 6 9 6 9 6 9 6 9 (day)
BIC2
HSP90
f
BIC2-GFP
Dark
Blue

Figure S6


a
FGFP-BIC protein (REU)
FGFP-BIC1
FGFP-BIC2
0 5 15 30 60 120 180 240 300
Time in BL (min)
b
FGFP-BIC protein (REU)
FGFP-BIC1
FGFP-BIC2
0 5 15 30 60 120 180 240 300
Time in RL (min)
c
FGFP-BIC mRNA (REU)
FGFP-BIC1
FGFP-BIC2
0 5 15 30 60 120 180 240 300
Time in BL (min)
d
FGFP-BIC mRNA (REU)
FGFP-BIC1
FGFP-BIC2
0 5 15 30 60 120 180 240 300
Time in RL (min)

Supporting Information - Table

A CRY-BIC negative feedback circuitry regulating blue light sensitivity of Arabidopsis

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Table S1. The relative expression levels of *BIC1* and *BIC2* in response to blue light shown in Figure 2.

Time (min)	Fluence rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	*BIC1* REU	S.E.	*BIC2* REU	S.E.
0	0.1	1	0.23526	1	0.18915
20	0.1	4.65893	0.97559	2.38392	0.47806
40	0.1	42.84001	5.93908	9.06307	1.82179
60	0.1	47.61464	4.27414	18.85227	3.39669
120	0.1	273.7408	60.58904	26.35491	4.91537
0	0.5	1	0.23526	1	0.18915
20	0.5	4.78991	1.04816	2.89454	0.48194
40	0.5	40.91272	7.71456	11.3924	3.65014
60	0.5	61.67726	5.44981	22.05958	3.59029
120	0.5	507.28991	95.7389	55.71524	7.94898
0	1	1	0.23526	1	0.18915
20	1	5.61778	1.0917	4	0.67555
40	1	39.48775	7.05874	11.2876	2.08133
60	1	51.50593	4.59981	23.53387	3.61337
120	1	514.37142	94.28539	116.16245	16.9215
0	5	1	0.23526	1	0.18915
20	5	7.04533	1.54067	9.38268	1.59526
40	5	50.06651	9.33013	15.49073	2.31373
60	5	50.0975	4.66786	27.66519	5.24691
120	5	588.13356	100.023	133.74428	21.5093
0	10	1	0.23526	1	0.18915
20	10	16.37398	3.22283	18.63574	2.93425
40	10	43.41134	7.91556	18.9834	3.80151
60	10	78.9755	7.49194	36.9286	6.23327
120	10	636.19858	109.4778	137.187	28.6800
0	50	1	0.23526	1	0.18915
20	50	15.67072	3.16481	16.91229	3.29628
40	50	52.34573	10.79796	32.37182	6.24525
60	50	154.3434	12.18343	56.62367	10.9685
120	50	805.27254	135.5645	244.41618	24.8442
0	100	1	0.23526	1	0.18915
20	100	12.52437	2.53298	16.99062	2.94425
40	100	124.21251	25.29764	32.50475	6.84529
60	100	217.26822	36.6526	67.80564	9.69295
120	100	1392.37619	250.31219	271.22255	43.7270

Supporting Information Legends

Figure S1. Phylogenetic analysis of BIC proteins.
The unrooted phylogenetic tree was generated by the neighbor-joining method. Genus/species abbreviations are shown in the figure. The scale bar indicates substitution per site**.**

Figure S2. *BIC* mRNA expression in response to lights.
a, RNA-seq results showing the absolute expression levels of *BIC* genes in response to blue light. 5-day-old wild-type seedlings grown under continuous blue light (cB; 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 5-day-old wild type etiolated seedlings exposed to blue light (20 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for indicated time (min) were used for RNA-seq. The mean FPKM (Fragments Per Kilobase of transcript per Million mapped reads) values of three repeats for each sample are indicated on the top of each column. Standard deviations (n=3 biological replicates) are shown.
b, qPCR showing the levels of mRNA expression of *BIC1* and *BIC2* under different light conditions. RNA was isolated from the wild-type seedlings grown in the dark (D), blue light (cB; 8 $\mu mol \cdot m^{-2} \cdot s^{-1}$), red light (cR; 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and far-red light (cFR; 6 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 5 days. The relative expression unit (REU) was calculated by re-normalization of the normalized qPCR signal in plants exposed to light against the normalized qPCR signal in plants kept in the dark. *PP2A* (At1g69960) was used as the reference gene for qPCR normalization. The mean REU values of three repeats for each sample are revealed on the top of each column. Standard deviations (n=3) are shown.

Figure S3. Blue light-dependent activity of the *BIC1* and *BIC2* promoters.
Histochemical images of GUS stain of transgenic seedlings expressing the *489BIC1pro::GUS* or *2100BIC2pro::GUS* reporters. *Arabidopsis* seedlings expressing the *489BIC1pro::GUS* (**a**) or *2100BIC2pro::GUS* (**b**) were grown in the dark or in blue

light (0.1, 0.5, 1, 5, 10, 50 or 100 μmol·m^{-2}·s^{-1}) for 5 days and stained with X-gluc. Scale bar=1 mm.

Figure S4. The chromatin segments where *BIC2* gene resides in is not transcription-repressed region in the dark.

Genome browser view of the *BIC2* locus on chromosome 3 in *A. thaliana* accession Col. The 10kb region (top) enlarged from the ~40kb (middle) areas around the *BIC2* gene and all genes within this ~40kb region are shown. Blue and golden boxes represent exons and untranslated regions are shown. *BIC2* has no intron and it is highlighted by red color. Green boxes represent non-protein-coding RNAs genes. Purple boxes represent microRNA genes. Arrows indicate the direction of transcription. The promoter sequence used for making transgenic lines in Figure. 3f and Figure S5a-b (*1822BIC2pro::FGFP-BIC2*) are represented by a pink box. The relative levels of mRNA expression derived from a RNA-seq experiment for all the genes in the ~40kb region are shown (bottom). In this RNA-seq experiment, etiolated wild-type seedlings were kept in the dark (Dark) or exposed to blue light (20 μmol·m^{-2}·s^{-1}) for 2 hr (B2h) were analyzed. FPKM stands for Fragments Per Kilobase of transcript per Million mapped reads. Three biological repeats were performed for RNA-seq analysis. The error bar indicates the standard deviation of FPKM values of each gene for three repeats. Levels of *BIC1* mRNA expression is highlighted, the absolute values of *BIC1* mRNA expression is included in Figure S2a.

Figure S5. Light slightly enhances BICs protein stabilities without affecting subcellular localization of BIC2.

a and **b**, Immunoblots showing that levels of FGFP-BIC1 and FGFP-BIC2 recombinant proteins in transgenic plants expressing the *1832BIC1pro::FGFP-BIC1* or *1822BIC2pro::FGFP-BIC2* transgene driven by the *BIC* promoters. 7-day-old etiolated seedlings kept in the dark or exposed to blue (a; BL; 30 μmol·m^{-2}·s^{-1}) and red light (b; RL; 30 μmol·m^{-2}·s^{-1}) for indicated time (min) before sample collection. The recombinant FGFP-BIC1 (Flag-GFP-BIC1) and BIC2 (Flag-GFP-BIC2) protein were analyzed by

immunoblot probed with the anti-Flag antibody. HSP90 was used as the loading control. The upstream regions of *BIC1* (1832 bp including 5'-UTR) and *BIC2* (1822 bp including 5'-UTR) genes were used as the *BIC* promoters.

c and **d**, Expression of the constitutively transcribed recombinant BICs protein in response to blue (c) and red light (d). 7-day-old etiolated seedlings of wild type (WT), *35S::Flag-BIC1* (#42 and #73) and *35S::Flag-BIC2* (#4 and #60) were exposed to blue (c; BL; 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and red light (d; RL; 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for the indicated time (min). The recombinant BIC1 and BIC2 proteins were detected by immunoblot using anti-Flag antibody. HSP90 was used as the loading control.

e, Expression of the constitutively transcribed recombinant BIC2 protein in 6-day-old or 9-day-old seedlings. Seedlings of *35S::Flag-BIC2* were grown in the dark, continuous blue light (30 $\mu mol \cdot m^{-2} \cdot s^{-1}$), continuous red light (30 $\mu mol \cdot m^{-2} \cdot s^{-1}$), or long day conditions for 6 or 9 days. The recombinant Flag-BIC2 protein were detected by immunoblot using anti-Flag antibodies. HSP90 was used as the loading control.

f, Lack of light effect on BIC2 subcellular localization. 3-day-old etiolated seedlings of *35S::BIC2-GFP* were kept either in the dark (Dark) or blue light (10 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 3 hr (Blue). BIC2-GFP subcellular distributions in hypocotyl were observed under a confocal microscope. Scale bar= 50 μm.

Figure S6. Protein and mRNA expression of the *1832BIC1pro::FGFP-BIC1 and 1822BIC2pro::FGFP-BIC1* transgenes in response to light.

a and **b**, Densitometry analysis of the immunoblots shown in Figure. 3c (for the *1832BIC1pro::FGFP-BIC1* transgene), and Figure S5 a-b (for the *1822BIC2pro::FGFP-BIC2* transgene). The band intensities of FGFP-BIC1 and FGFP-BIC2 were digitized, normalized against that of HSP90, then re-normalized to the value of time 0 to obtain the Relative expression units (REU). Two independent protein extracts were used for the immunoblot analysis. The mean value of the REU from two repeats were shown in the Figure S6 a and b.

c and **d**, qPCR showing the mRNA levels of *FGFP-BIC1* and *FGFP-BIC2* transgenes in response to blue (**c**) or red light (**d**). The RNAs isolated from the same tissue samples used for the immunoblot analyses shown in Figure 3c (for the *1832BIC1pro::FGFP-*

BIC1 transgene) or Figure S5a-b (for the *1822BIC2pro::FGFP-BIC1* transgene) were used in this experiment. The relative expression unit (REU) was calculated by re-normalization of the normalized qPCR signal of all the samples to the normalized qPCR signals of the sample kept in the dark. *PP2A* (At1g69960) was used as the reference gene for qPCR normalization. Three biological repeats were used for each experiment and the error bars indicate the standard error of three repeats.

Table S1. The relative expression levels of *BIC1* and *BIC2* in response to blue light shown in Figure 2.