

Supplemental Figure S1. Spatial expression pattern of *BBX32* in 5-day old dark and light-grown seedlings

A and B, Tissue-specific GUS expression pattern observed in 5-day old *ProBBX32:GUS* seedlings grown in dark (A) and in white light (WL) (B) at 80 μ mol m⁻² s⁻¹ fluence (long day, 16h/8h). The seedlings were treated with X-Gluc, the substrate for GUS, and the staining was observed. **a** and **b** in **B** indicate Shoot Apical Meristem (SAM) and root-hypocotyl junction respectively. The scale bar indicates 200 μ m.



Supplemental Figure S2. Relative expression levels and cotyledon and hypocotyl phenotypes of loss-of- and gain-of-function mutants of *BBX32*

A, RT-qPCR data showing relative expression levels of *BBX32* in *bbx32* and overexpressor lines 35S:BBX32 #13 and 35S:BBX32 #15 grown for 4 days in cycling white light (WL) (16h/8h). n=2, *UBQ10* was used for normalization. **B**, Representative images of seedlings of Col-0, *bbx32*, 35S:BBX32 #13 and 35S:BBX32 #15 grown in 80 µmol m⁻² s⁻¹ cycling WL (16h/8h) for 4 days. Scale bar = 5 mm. Representative seedling images of each genotype were spliced together to prepare the composite image for comparison purpose. **C** and **D**, Graphical representation of cotyledon opening angle (**C**) and hypocotyl lengths (**D**) of the indicated genotypes corresponding to **B**. The data represent mean of three biological replicates each with ~20 seedlings, n=3. (**A**, **C** and **D**) Error bars indicate SEM. Asterisks represent statistically significant difference (***, P < 0.001) as determined by one-way ANOVA followed by Dunnett's test; ns, non-significant. In the box plots **C** and **D**, centre line denotes median; box limits denote upper and lower quartiles; whiskers denote 1.5x interquartile range; points denote outliers in panels.



Supplemental Figure S3. The hypocotyl elongation phenotype of *35S:BBX32* is suppressed by BRZ application in a dose-dependent manner

A, Representative images of Col-0, *bbx32*, *35S:BBX32* #13, *35S:BBX32* #15 treated with brassinazole (BRZ). The seedlings were grown on MS plates containing different concentrations (0.5 μ M, 1 μ M, 2 μ M) of BRZ in dark for 7 days. The plate without BRZ was used as control. The scale bar represents 2.5 mm. Representative seedling images of each genotype were spliced together to prepare the composite image for comparison purpose. **B**, Graphical representation of average hypocotyl length (mm) of indicated genotypes at various concentrations of BRZ (0.5 μ M, 1 μ M, 2 μ M) in dark. The data represents mean of 30 seedlings. Error bar=SEM. Significance was assessed by two-way ANOVA followed by Tukey's *post hoc* test, Letters denote statistical groups (P<0.05).



Supplemental Figure S4. BR regulates *BBX32* expression, and BBX32 does not interact with BES1

A and B, Representative GUS stained seedling images showing *BBX32* expression in brassinosteroids (BR) **(A)** and brassinazole (BRZ) **(B)** treated conditions in dark. **A**, The *ProBBX32:GUS* seedlings were grown in dark for 5 days with or without BR (1 μ M) in the medium. The scale bar represents 200 μ m. **B**, The seedlings were grown in dark for 5 days with or without BRZ (1 μ M). The scale bar measures 200 μ m. The mock used as control in both **A** and **B** was ethanol. **C**, Relative expression of *BBX32* in cotyledons and hypocotyls of *bzr1-1D* compared to Col-0. n=2, *UBQ10* was used for normalization. Error bar = SEM, two-way ANOVA and Tukey's *post hoc* test was performed to assess the significance, Letters denote the different statistical groups (P<0.01). **D**, Yeast two hybrid assay showing the interaction between BBX32 and BES1. AD and BD represents GAL4 activation domain and binding domain respectively. BBX32 fused with BD and BES1 fused with AD were tested for their interaction. DDO (double-dropout) denotes the lack of leucine and tryptophan in the medium. TDO (triple-dropout) denotes the lack of histidine additionally.



Supplemental Figure S5. Relative expression level, cotyledon, and hypocotyl phenotypes of loss-of- and gain-of-function mutants of *PIF3* and interactions of BBX32 with PIF3 and its role in affecting transcriptional activation potential

A, Representative images of Col-0, pif3-3, 35S:PIF3 #1 and 35S:PIF3 #2 seedlings grown for 4 days in 80 μ mol m⁻² s⁻¹ cycling white light (WL, long day, 16h/8h). Scale bar = 5 mm. **B**, RT-gPCR data showing relative expression levels of PIF3 in pif3-3, 35S:PIF3 #1 and 35S:PIF3 #2 grown for 4 days in cycling WL (16h/8h) compared to Col-0. n=2. UBQ10 was used for normalization. C and D, Graphical representation of cotyledon opening angle (C) and hypocotyl lengths (D) of the Col-0, pif3-3, 35S:PIF3 #1 and 35S:PIF3 #2 grown for 4 days in cycling WL (16h/8h), n=3, Error bar denotes SEM. Asterisks indicate statistically significant differences (***, P < 0.001, *, P<0.05) calculated by one-way ANOVA and Dunnett's test; ns, non-significant. In the box plots **C** and **D**, centre line denotes median; box limits denote upper and lower quartiles; whiskers denote 1.5x interquartile range; points denote outliers in panels. E, Representative images demonstrating the effect of different concentrations of BRZ on cotyledon opening response of 7 days old, dark-grown Col-0, bbx32, pif3-3, bbx32pif3-3 seedlings and their corresponding cotyledon opening angles. Scale bar = 2.5 mm. The data is the mean of 30 seedlings. Error bar represents SEM, Twoway ANOVA followed by Tukey's post hoc test was performed to determine the statistical groups indicated by letters (P<0.05). In A and E, Representative seedling images of each genotype were spliced together to prepare the composite image for comparison purpose. F,

Luciferase assay showing the effect of BBX32, PIF3, and BZR1 on the *CHS* promoter. The genes of the indicated proteins cloned in *35S* vectors and *CHS* promoter cloned in luciferase vector were co-transfected into the *Arabidopsis* protoplasts. The relative luciferase activity was measured after 16h incubation, n=2. Error bar represents SEM. One-way ANOVA followed by Dunnett's test was performed to determine the statistical significance indicated by asterisks (***, P<0.0001, **, P<0.001).





Supplemental Figure S6. Transcriptional regulation of genes involved in opening and closing of cotyledons by BBX32 during Dark to Light transition.

RT-qPCR data showing relative expression levels of *SAUR16*, *SAUR50*, *LAX3* and *ACS5* in isolated cotyledons of Col-0, *bbx32* and *35S:BBX32* #15 seedlings grown in dark for 3 days and moved to white light (80 μ mol m⁻² s⁻¹) for 6 hr. n=2, Error bar = SEM. *UBQ10* was used for normalization. Letters denote the statistically different groups determined by two-way ANOVA followed by Tukey's *post hoc* test (P<0.01).

Supplemental Table S1. List of Primers used in the study

Primers	Sequence (5' to3')
BBX32 SALK_059534	AACTCCACCGCTCTTTCTCTC
LP	
BBX32 SALK_059534	TTGGATTACCATTATTCCGTTTC
RP	
BBX32 attB1	GGGGACAAGTTTGTACAAAAAGCAGGCTGG ATGGTGAGCTTTTGCGAGC
BBX32 attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTGTCAAACGTTGTCGTTTTCAG
BZR1 attB1	GGGGACAAGTTTGTACAAAAAGCAGGCTGG ATGACTTCGGATGGAGCTACG
BZR1 attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTGTCAACCACGAGCCTTCCCATT
BES1 attB1	GGGGACAAGTTTGTACAAAAAGCAGGCTGG ATGAAAAGATTCTTCTATAAT
BES1 attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTGTCAACTATGAGCTTTACCATT
PIF1attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGATGCATCATTTTGTCCCT
PIF1 attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTGTTAACCTGTTGTGTGGT
PIF3 attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGATGCCTCTGTTTGAGCTTT
PIF3 attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTGTCACGACGATCCACAAAACT
PIF4 attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGATGGAACACCAAGGTTGG
PIF4 attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTG CTAGTGGTCCAAACGAGAA
PIF5 attB1	GGGGACAAGTTTGTACAAAAAGCAGGCTGG ATGGAACAAGTGTTTGCTG
PIF5 attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTGTCAGCCTATTTTACCCATA
LB1.3	ATTTTGCCGATTTCGGAAC
35SEnd pro	CGCAAGACCCTTCCTCTAT
BBX32pro attB1	GGGGACAAGTTTGTACAAAAAGCAGGCTGG ACTGGCTGGGGAGAATTATT
BBX32pro attB2	GGGGACCACTTTGTACAAGAAAGCTGGGTGTGAGTCGATCTCTCTGATC
BBX32 qFP	GAGCTTTTGCGAGCTTTGTGG
BBX32 qRP	GGGCAGATGACACGCCGGAA
PIF3 qFP	CTGAAAGGAGACGGCGTGATAG
PIF3 qRP	CAGATAGTAACCAGACGCCATTGAC
UBQ10 qFP	GGCCTTGTATAATCCCTGATGAATAAG
UBQ10 qRP	AAAGAGATAACAGGAACGGAAACATAGT
WAG2 qFP	GCCGTTTCACCGCGACGGA
WAG2 qRP	GCGTTTGCGACTCGCGTAG
LAX3 qFP	CATCCCCGCATTGGCTCATA
LAX3 qRP	AACCCGAACCCAACTACGAA
ACS5 qFP	CGGGTTGGTTTAGGGTTTGTT
ACS5 qRP	CCCAGTTAGAGACTGTCTTCT
SAUR14 qFP	GATTCTCCGACAAGCCAAACT
SAUR14 qRP	TCTTGAAATGAAGGCTGGTCC
SAUR16 qFP	TGCTACGACGAGGAAGGTCT
SAUR16 qRP	ACCTTGTACGCTTTTCGCCT
SAUR50 qFP	CACTTCCCTGTCTATGTCGGA
SAUR50 qRP	TCTTCCTCGGCTCGTTGTAA
BBX32 CDS BamH1	CGC <mark>GGATCC</mark> ATGGTGAGCTTTTGCGAGC
FP	
BBX32 CDS ECoRI RP	CCGGAATTCTCAAACGTTGTCGTTTTCAG
BZR1 CDS BamHI FP	CGCGGATCCCGCGGATCCATGACTTCGGATGGAGCTACG
BZR1 CDS ECoRI RP	CCGGAATTCCCGGAATTCTCAACCACGAGCCTTCCCATT
PIF3 CDS BamHI FP	CGCGGATCCCGCGGATCCATGCCTCTGTTTGAGCTTTTC
PIF3 CDS ECoRI RP	CCGGAATTCCCGGAATTCTCACGACGATCCACAAAACTG
CHSpro KpnI FP	CGGGGTACCGGGATGTTAATATGGGCCAG
CHSpro Pstl RP	AACTGCAGTATAGTATACACCAACTTGGGTT

Gateway sequence (attB1 or attB2) / Restriction sites (5' to 3') are indicated in red colour.