

Supplemental Figure 1. Expression of BBX25 and HY5 Proteins in Yeast.

(A) to (C) Immunoblots showing similar expression of proteins in the wild-type and point-mutated versions of BBX25 (A), HY5 full length (B) and HY5 truncated versions (C) in yeast.



Supplemental Figure 2. Absence of FRET between Unfused CFP/ YFP.

(A) Absence of FRET between unfused CFP/ YFP using the same microscopic settings as in Figure 1E.

(B) Relative intensities of both YFP and CFP inside the nucleus measured once before and twice after the bleaching. Bars indicate SD (n = 7).



Supplemental Figure 3. *BBX25* Transcript Accumulation in Mutant and Overexpression Lines.

(A) Quantitative RT-PCR analysis of *BBX25* expression in Col-0 and *bbx25-1* seedlings grown in WL (80 μ mol m⁻² s⁻¹) for fivedays. Error bars represent SE (n= 3).

(B) Quantitative RT-PCR analysis of *BBX25* expression in BBX25 overexpressing lines grown in WL (80 μ mol m⁻² s⁻¹) for six-days. Error bars represent SE (n= 3).



Supplemental Figure 4. Physiological Characterization of *bbx25* Mutants and Overexpression Lines in Monochromatic Lights.

(A) to (C) Hypocotyl length of six-day-old seedlings grown in R , FR and BL, respectively. Error bars represent SE ($n \ge 25$). Asterisks show genotypes that differ significantly from Col-0 (Student's t test, *** P \le 0.001).

(D) to (F) Photographs of seedlings from genotypes showed in A to C. Scale Bar = 1 mm.



Supplemental Figure 5. Molecular Characterization of *BBX24* T-DNA Insertion Mutant Alleles.

(A) Schematic representations of *BBX24* (At1g06040) gene. The arrow indicates the position of the start site Met, T indicates the T-DNA insertion positions. Black rectangular boxes depict exons and lines in between these boxes represent introns.

(B) RNA gel blot showing BBX24 transcript accumulation just before dawn in Col-0 and bbx24-1 mutant.

Supplemental Data. Gangappa et al. (2013). Plant Cell 10.1105/tpc.113.109751



Supplemental Figure 6. *bbx25-2* and *bbx24-1* Additively Suppress *hy5* Phenotype in Monochromatic Lights.

Hypocotyl phenotype of five-days-old constant R (30 µmol m⁻² s⁻¹), FR (1 µmol m⁻² s⁻¹) and BL (10 µmol m⁻² s⁻¹) grown seedlings. Error bars indicate SE ($n \ge 30$ seedlings). Asterisks indicate genotypes either significantly differs from *hy5-215* (Student's t test, ** P ≤ 0.01) or significantly differ from *hy5-215 bbx25-2* (Student's t test, *** P ≤ 0.001). Error bars represents SE ($n \ge 30$). All the experiments were performed at least twice with similar results. The graph depicts a representative of one of those experiments.





Anthocyanin content in five-day-old seedlings of indicated genotypes grown in R (60 μ mol m⁻² s⁻¹), FR (0.1 μ mol m⁻² s⁻¹) and BL (20 μ mol m⁻² s⁻¹). Error bars represent SE (n= 3).



Supplemental Figure 8. Elevated Expression of CHS and CHI Genes in *bbx25 bbx24* Mutant is HY5 Dependent in Monochromatic Lights. Quantitative RT-PCR analysis of CHS (A, C, E) and CHI (B, D, F) genes in four-day-old seedlings grown in dark and then irradiated for 0, 1 or 2 h with R (100 μ mol m⁻² s⁻¹), FR (10 μ mol m⁻² s⁻¹) and BL (30 μ mol m⁻² s⁻¹). Error bars represent SE (n= 3).





(A) Photos of Col-0, single, double and triple mutant seedlings grown in dark for five days Scale Bar = 1 mm.

(B) Hypocotyl length of seedlings from indicated genotypes grown in R (15 μ mol m⁻² s⁻¹), FR (0.1 μ mol m⁻² s⁻¹) and BL (10 μ mol m⁻² s⁻¹) for five-days. Error bars represent SE (n \geq 30). Asterisks show pairs of genotypes that differ significantly between them (Student's t test, ** P \leq 0.01).



Supplemental Figure 10. Overexpression of GFP-BBX25 in Col-0 and cop1-6 Mutant Backgrounds.

Hypocotyl length of GFP-BBX25 overexpressing lines in Col-0 and *cop1-6* seedlings grown in dark for five days. Error bars represent SE ($n \ge 35$). Asterisks show pair of genotypes that differ significantly from *cop1-6* (Student's t test, ** P ≤ 0.01).



Supplemental Figure 11. Expression Analysis of Double B-Box (*DBB*), *HY5* and *HYH* Genes.

Q-RT PCR analysis of *BBX18-BBX21*, *HY5* and *HYH* genes in Col-0 and *bbx25-2 bbx24-1* seedlings grown in dark for four days and then irradiated with WL (80 μ mol m⁻² s⁻¹) for 0, 2 and 4 h. Error bars represent SE (n= 3). Asterisks show that the gene expression differs significantly between Col-0 and *bbx25-2 bbx24-1* at the same time point (Student's t test, ** P \leq 0.01).

Supplemental Table 1: List of Primers Used in This Study.

Primer name	Direction (5'3')
Yeast two-hybrid assay	
BBX25FL-FP	GGAATTCCATATGACTGATTACCGGCTACAACCAAC
BBX25FL-RP	CCATC GATTTAACCGATTTTTGAAATCAAAC
BBX25B1-FP	TGTTGTGCTGCTGCAGCTGCTCT
BBX25B1-RP	AGAGCAGCTGCAGCACAACA
BBX25B2-FP	TTCTGTGTAGCGGCTAGGGCTCT
BBX25B2-RP	AGAGCCCTAGCCGCTACACAGAA
FRET assay	
CFP-BBX25-FP	AAGGTACCATGAAGATACAATGTGATGTGT
CFP-BBX25-RP	CGTCTAGATTAGCCTAGGTCGGGGGAC
RNA gel blots	
BBX24 FP	GCCACTGGGATCAAAGTAGCTCTG
BBX24 RP	GCCAAGATCAGGGACAATGAAGTG
BBX25FP	GAAGGCAGCTTTCATATTCTGTG
BBX25RP	GCCTAGGTCGGGGGACTAGGAAG
18srRNA-FP	AATCCTGACACGGGGGGGGGGGGGGGGGGGGGGGGGGGG
18srRNA-RP	TGAAGGGATGCCTCCACGTAGCTAG
Overexpression of	
BBX25	
BBX25OEFP	CATGCCATGGTCCTCTGATATTCAGATTCTTACTTCTC
BBX25OERP	GGAAGATCTTTAGCCTAGGTCGGGGACTAGGAAGTGC
Quantitative Real time	
PCR	
ARR7FP	ATTGTGGATCGTAAAGTCATCGAG
ARR7RP	TCGCTTTACATCTGCTAGCTTCAC
HVA22cFP	CTATATGCATCAGTGAAAGCAATAG
HVA22cRP	TAGCTCTTTGAGGATCTCTGTAG
FHLFP	ATAGTTGCTGTGGAATCTCTAGAC
FHLRP	ATTGTTCTATGCTTGATGTAGCAG
ERECTAFP	GTCCTACAATCAGCTAACTGGTG
ERECTARP	GTAAGCTTCCCAAGCTCTGGTG
BBX20FP	CGGTGTTTTGTTGTGCGGAT
BBX20RP	AATAATGCACGCCTCTCCCC
BBX21FP	CCAAGTCCACCACGCTAACA
BBX21RP	TTTGTGTGTTCGTTCGCAGC
BBX22FP	TGGATCATCTAAGGCGGATACT
BBX22RP	GACGCTGTAGGTGGAGACTG
BBX23FP	AAAAGATGCAGCCTCAGCCA
BBX23RP	TGCACAGCATTGCTCTATCC

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BBX25FP	GTGTGAGAAAGCTCCGGCCACG
BBX25RP	AGTATTTGGCGCATGGGTCGCC
HY5FP	CCATCAAGCAGCGAGAGGTCATCAA
HY5RP	CGCCGATCCAGATTCTCTACCGGAA
HYHFP	CCCACAAGAAGCACAAAACTGAGGAAA
HYHRP	CTTCCACGGCGGCGTTTAGCTGTAGAGA
Actin2-FP	GCCAGTGGTCGTACAACCGGTATTG
Actin2-RP	TTCCCGCTCTGCTGTTGTGGTG
Protoplast Assay	
BBX 24FP	CATGCCATGGCAAATCCTTATCTTCTCTCTTTCAC
BBX24RP	GGAAGATCTTTAGCCAAGATCAGGGACAATGAAG
BBX25 FP	CATGCCATGGCCAAAATCCTCTTAAAATCTCATAAAG
BBX 25RP	GGAAGATCTTTAGCCTAGGTCGGGGACTAGGAAG
HY5FP	CATGCCATGGCAGAGATCTGACGGCGGTAGCCAG
HY5RP	GGAAGATCTTCAAAGGCTTGCATCAGCATTAG
BBX21 promo FP	TCATTTCCGTGCAAAATGTTAATTTTG
BBX21 promo RP	CTTCTCTTATCTCAAACTCATCCAG
Genotyping of T-DNA	
mutants	
LB3	TAGCATCTGAATTTCATAACCAATCTCGATACAC
BBX25LP	AGGCCAGAAAGAGAACTGAGG
BBX25 RP	GCCTTTTGTTTCTTTCCCTTG
Spm1	CTTATTTCAGTAAGAGTGTGGGGGTTTTGG
BBX25LP1	ACTGCTTTTGTCTCTCAAGTCATTTGTCT
BBX25RP1	CCTGCAAGTCAACAAAGATGAACACAAGA
LBb1	GCGTGGACCGCTTGCTGCAACT
BBX24LP	TTGCTGCTGGCTTGTGGATTT
BBX24RP	AAAGAGTGGGACCCGCAAGAG