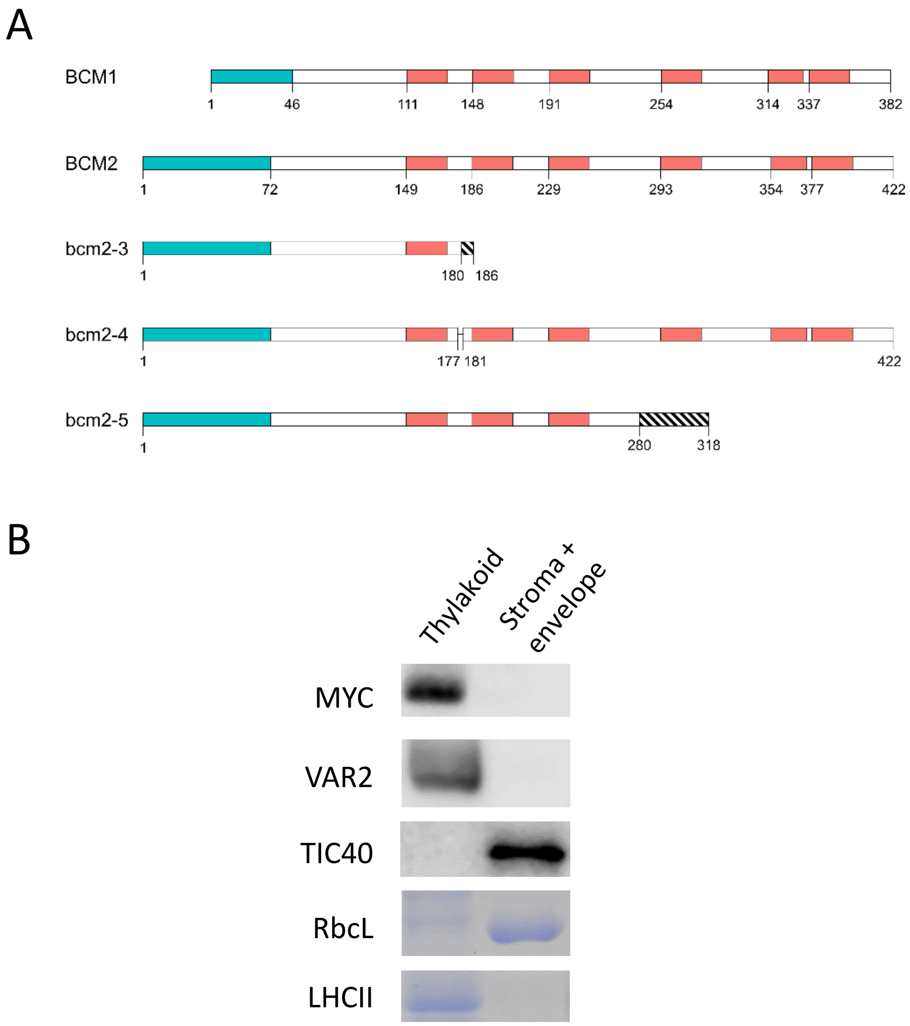


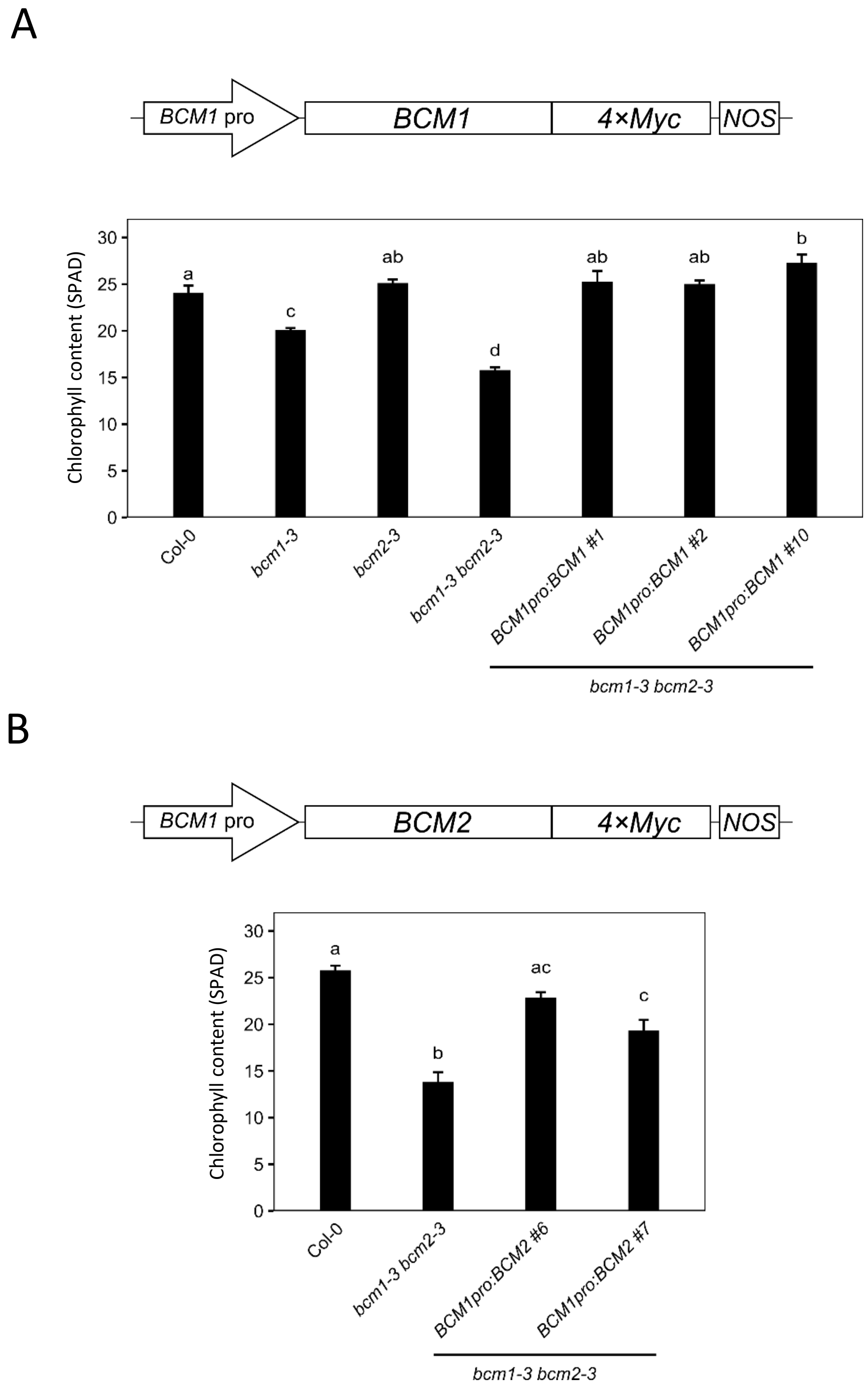
Supplemental Figure S1. Protein structures and subcellular localization of BCM1 and 2. A, Predicted protein structures of the BCMs and their mutants. 'Blue' and 'red' regions represent plastid transit peptides and transmembrane regions, respectively. Hatched regions indicate regions in which frameshifts occurred. *bcm2-4* contains a deletion corresponding to the 178-180th amino acid region. B, Subcellular fractionation of the BCM1-4 × MYC protein in *bcm1-3 bcm2-3* plants. 'MYC' indicates the results of Western blot analysis of BCM1 using anti-MYC antibody. VAR2 and TIC40 were used as references for thylakoid and inner envelope localization. RbcL and LHCII were visualized by SDS-PAGE gel staining with Coomassie Brilliant Blue.

A

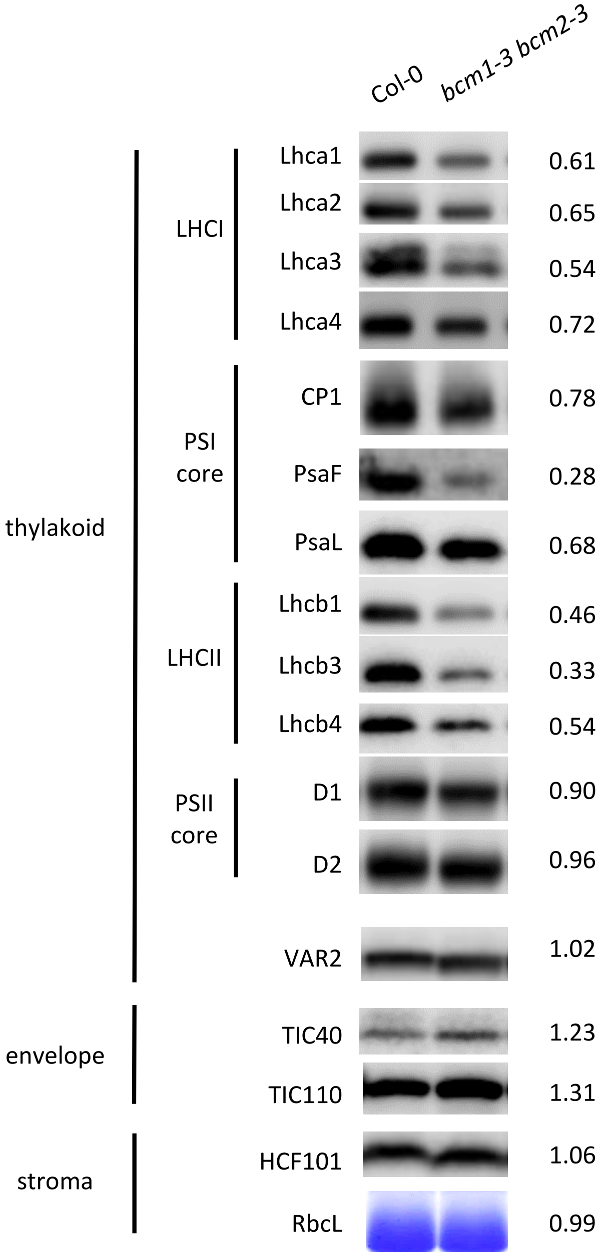
BCM2	523 bp	GCA	GGA	ATA	GAT	TAC	ACA	GGA	GGT	TTT	AAT	GCT	TCT	TTA	GAT
	175 aa	A	G	I	D	Y	T	G	G	F	N	A	S	L	D
bcm2-3	523 bp	GCA	GGA	ATA	GAT	TAC	A-AG	GAG	GTT	TTA	ATG	CTT	CTT	TAG	AT
	175 aa	A	G	I	D	Y	K	E	V	L	M	L	L	*	
bcm2-4	523 bp	GCA	GGA	ATA	---	---	---	GGA	GGT	TTT	AAT	GCT	TCT	TTA	GAT
	175 aa	A	G	I	-	-	-	G	G	F	N	A	S	L	D
BCM2	825 bp	TCT	CGA	GGA	ATG	GTT	GCT	TTG	ACT	GGA	CTC	TTG	CCA	CCA	TTT
	279 aa	S	R	G	M	V	A	L	T	G	L	L	P	P	F
	877 bp	GTA	CCA	TTT	GCT	CAA	GTA	TTT	GCA	GCC	ACT	ATT	ACA	GCC	GCT
	293 aa	V	P	F	A	Q	V	F	A	A	T	I	T	A	A
	919 bp	CTC	ACT	GGT	TCT	CTC	TAC	TAC	ATA	GCT	GCT	TCC	CCT	AAA	GAT
	307 aa	L	T	G	S	L	Y	Y	I	A	A	S	P	K	D
bcm2-5	825 bp	TCA	TCG	AGG	AAT	GGT	TGC	TTT	GAC	TGG	ACT	CTT	GCC	ACC	ATT
	279 aa	S	S	R	N	G	C	F	D	W	T	L	A	T	I
	877 bp	TGT	ACC	ATT	TGC	TCA	AGT	ATT	TGC	AGC	CAC	TAT	TAC	AGC	CGC
	293 aa	C	T	I	C	S	S	I	C	S	H	Y	Y	S	R
	919 bp	TCT	CAC	TGG	TTC	TCT	CTA	CTA	CAT	AGC	TGC	TTC	CCC	TAA	AGA
	307 aa	S	H	W	F	S	L	L	H	S	C	F	P	*	

B

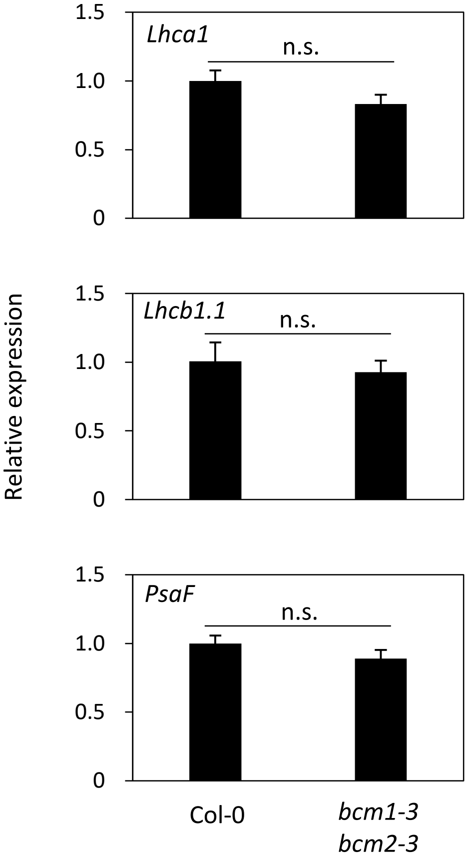
Supplemental Figure S2. Mutations and phenotypes of the *bcm* mutants. A, Alignment of *bcm2-3*, *bcm2-4*, and *bcm2-5* genes / proteins. *bcm2-3* and *bcm2-5* contain a 1-bp deletion and 1-bp insertion, respectively, resulting in frameshifts and the generation of premature stop codons. *bcm2-4* contains a 9-bp deletion, resulting in a three-amino-acid deletion. Nucleotide mutations are shown in red. B, Phenotypes of the *bcm1 bcm2* double mutants. Plants were grown at 22°C for 23 days under long-day conditions. Scale bar: 1 cm.



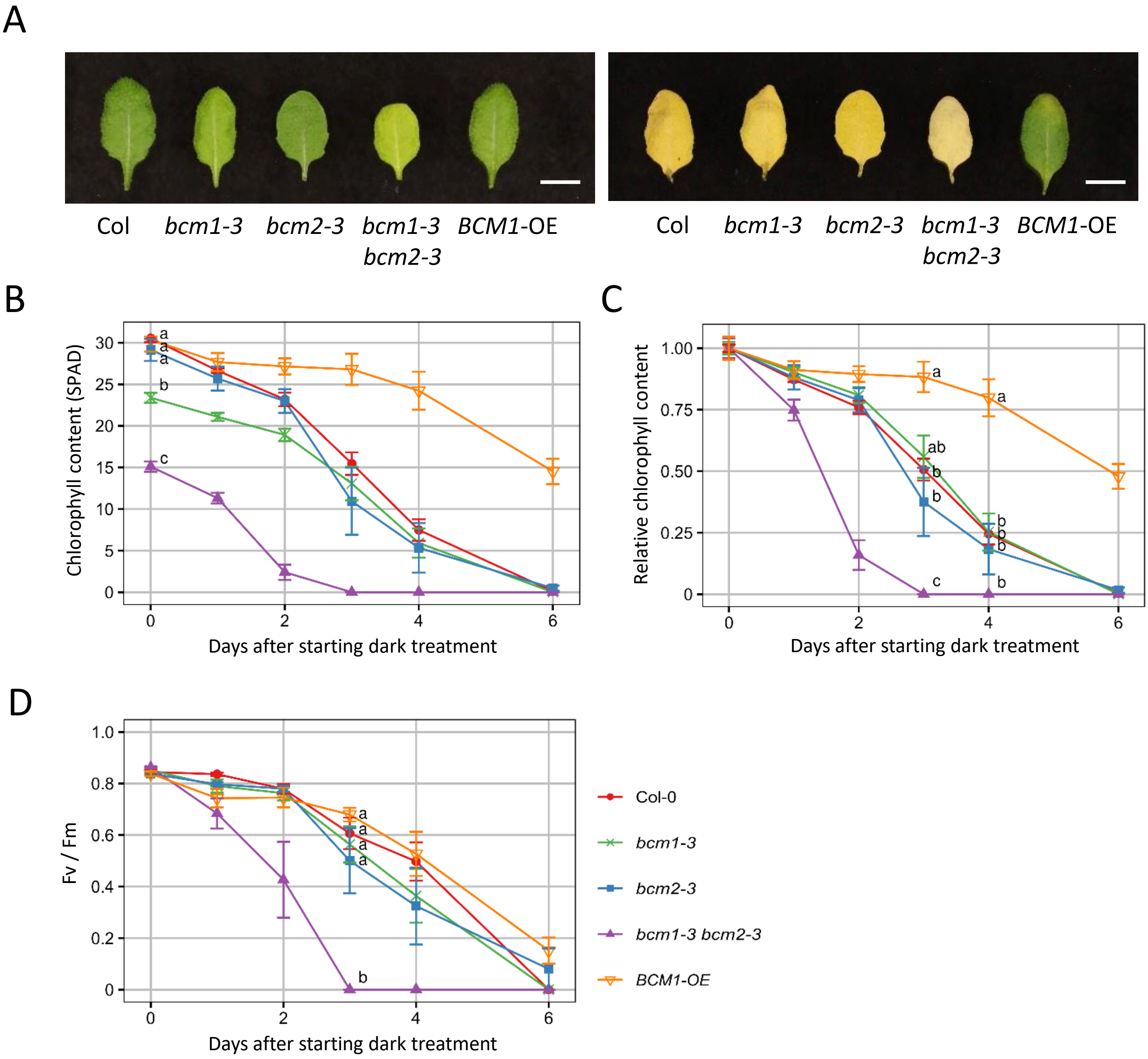
Supplemental Figure S3. Complementation of the *bcm1 bcm2* phenotype by *BCM1* and *BCM2.* The low chlorophyll content phenotype of *bcm1-3 bcm2-3* was complemented by *BCM1*-promoter-driven *BCM1* (A) and *BCM2* (B) transgenes. Data were statistically analyzed using Tukey's multiple comparison method ($p < 0.05$). Error bars indicate standard errors ($n = 4$).



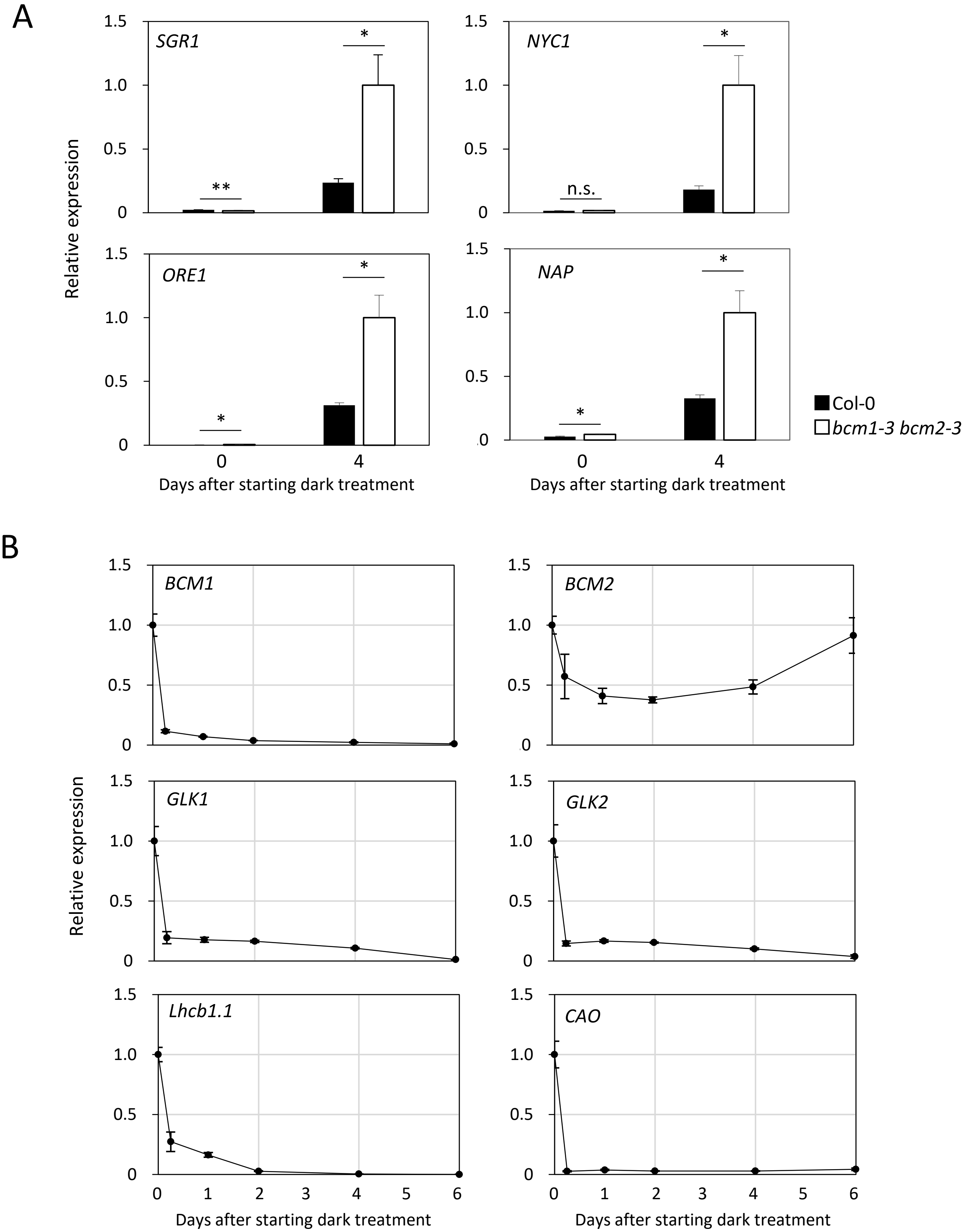
Supplemental Figure S4. Western blot analysis of chloroplast proteins in presenescent leaves of *bcm1 bcm2*. Proteins were extracted from the same fresh weight of pre-senescent leaves and subjected to SDS-PAGE analysis followed by Western blot analysis. RbcL was visualized by SDS-PAGE gel staining with Coomassie Brilliant Blue. Values shown to the right of the panel indicate relative band intensities (*bcm1-3 bcm2-3* / Col-0).



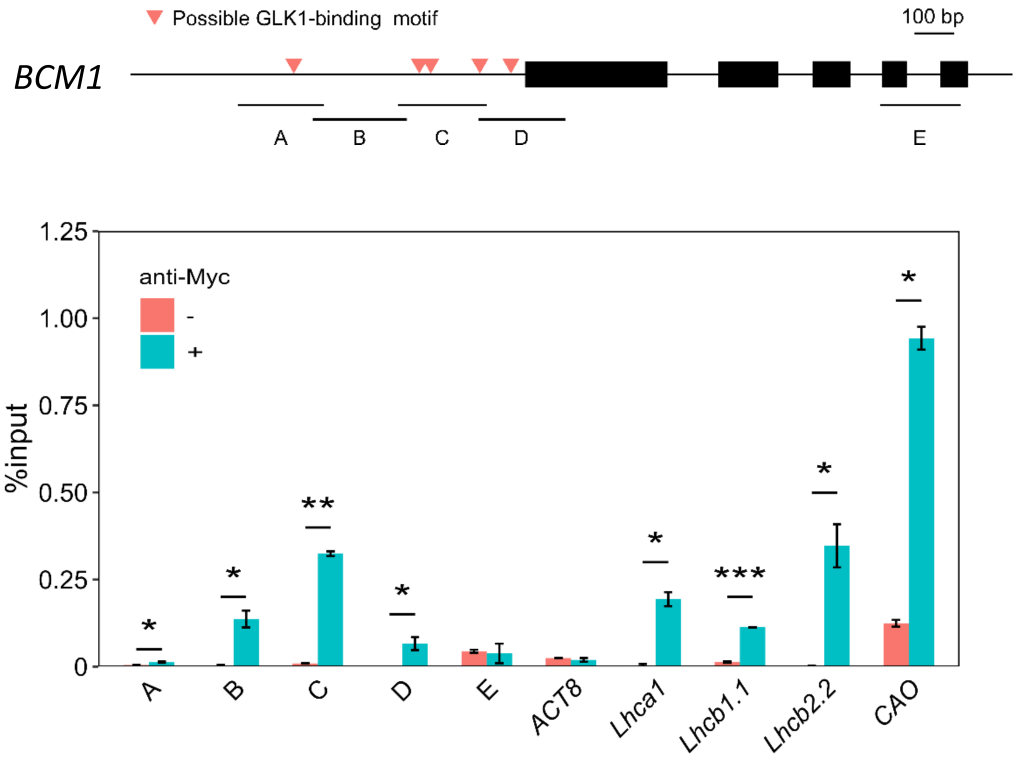
Supplemental Figure S5. Expression of photosynthesis-related genes in presenescent leaves of *bcm1 bcm2*. RT-qPCR analysis of photosystem-related genes was performed using *bcm1-3 bcm2-3* pre-senescent leaves. Error bars indicate standard errors (n = 4). 'n.s.': not significant (Student's t test).



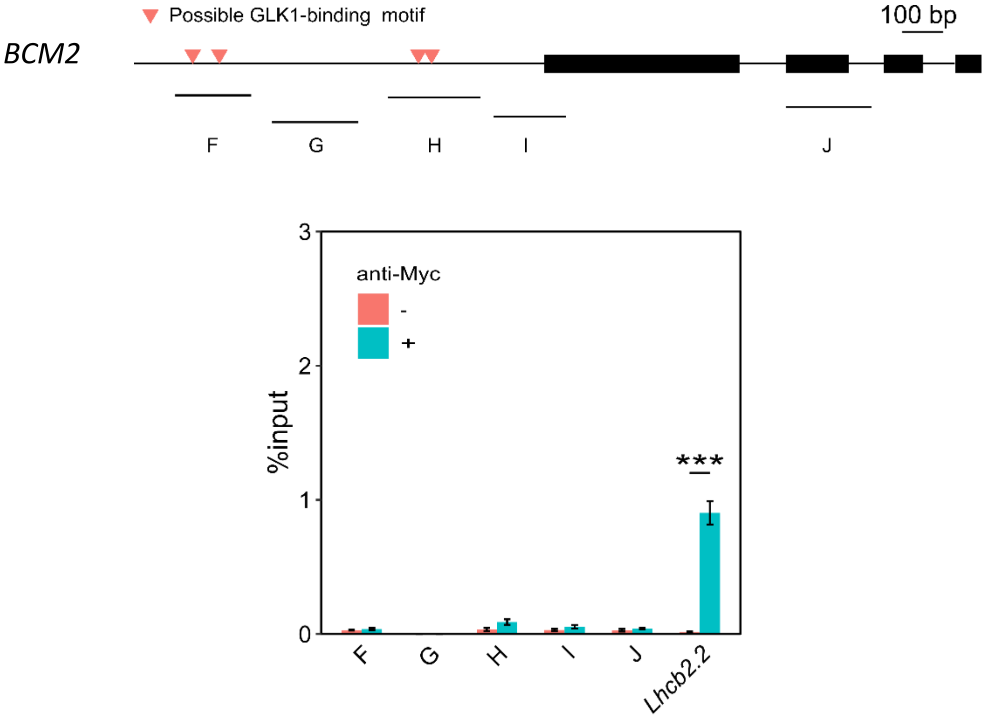
Supplemental Figure S6. Chlorophyll degradation in *bcm* mutants and *BCM1* overexpressing plants during leaf senescence. A, Leaves of *bcm* mutants and BCM-OE plants before (left) and (right) six days after starting dark incubation. Seventh leaves of 28-day-old plants grown at 22°C under short-day conditions were detached and incubated in the dark at 22°C. Scale bar: 1cm. B and C, Changes in chlorophyll content with time during dark incubation. B and C indicate SPAD values and relative values to pre-senescent leaves. D, Changes in Fv / Fm values with time during dark incubation. Detached leaves were incubated at 22°C in the dark. SPAD value is non-destructive chlorophyll measurement. Data were statistically analyzed using Tukey's multiple comparison method ($p < 0.05$). Error bars indicate standard errors (n = 4).



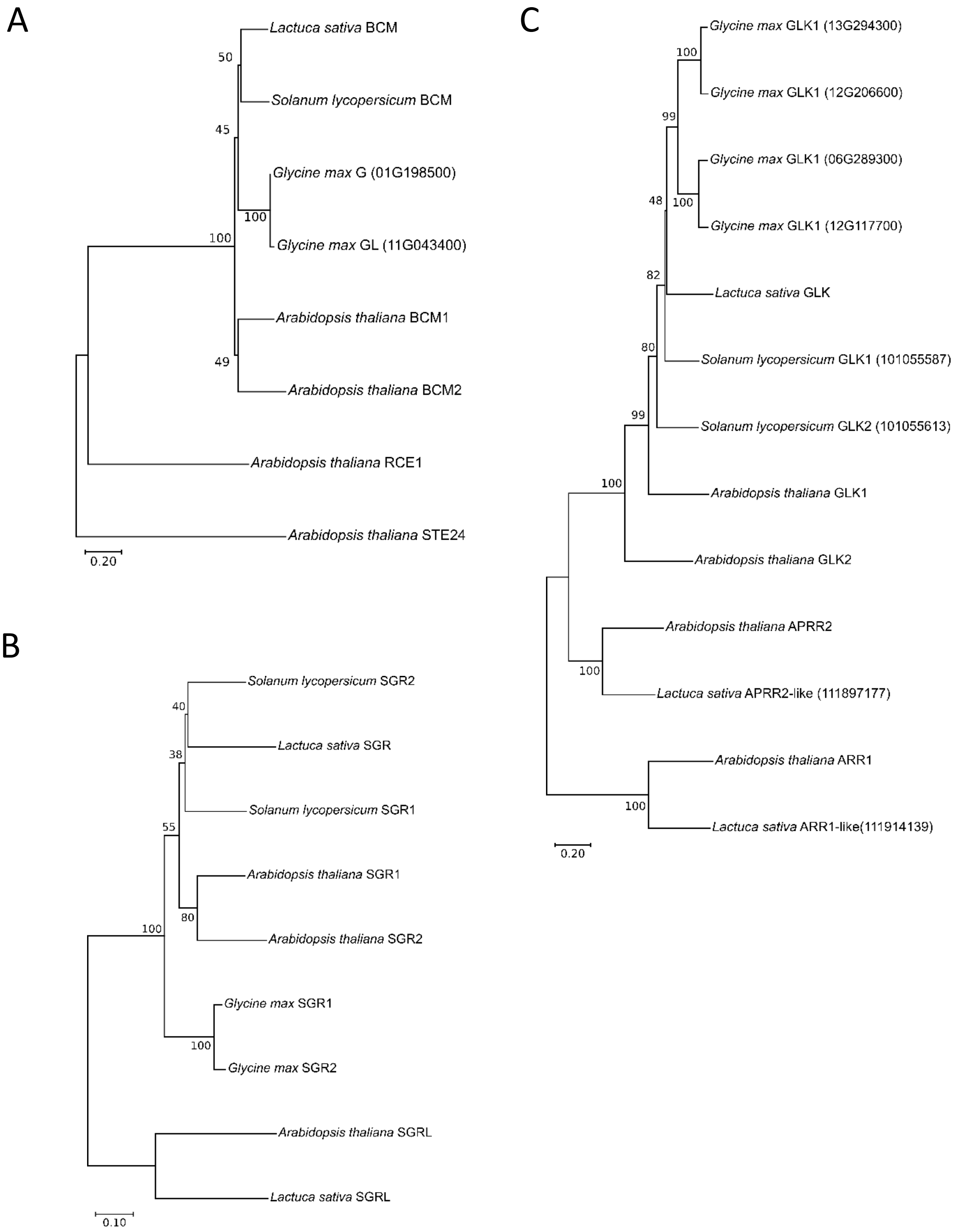
Supplemental Figure S7. Expression of senescence- and photosynthesis-related genes during dark incubation. A, RT-qPCR analysis of senescence-inducible genes in *bcm1 bcm2* before and four days after starting dark incubation. Eighth leaves of 25-day-old plants grown at 22°C under short-day conditions were detached and incubated in the dark at 22°C. B, Changes in expression of *BCM*s, *GLK*s and GLK-targeted genes with time during dark incubation. Error bars indicate standard errors (n=4 in A, n=5 in B). * $p < 0.05$. ‘n.s.’: not significant (Student’s t test).



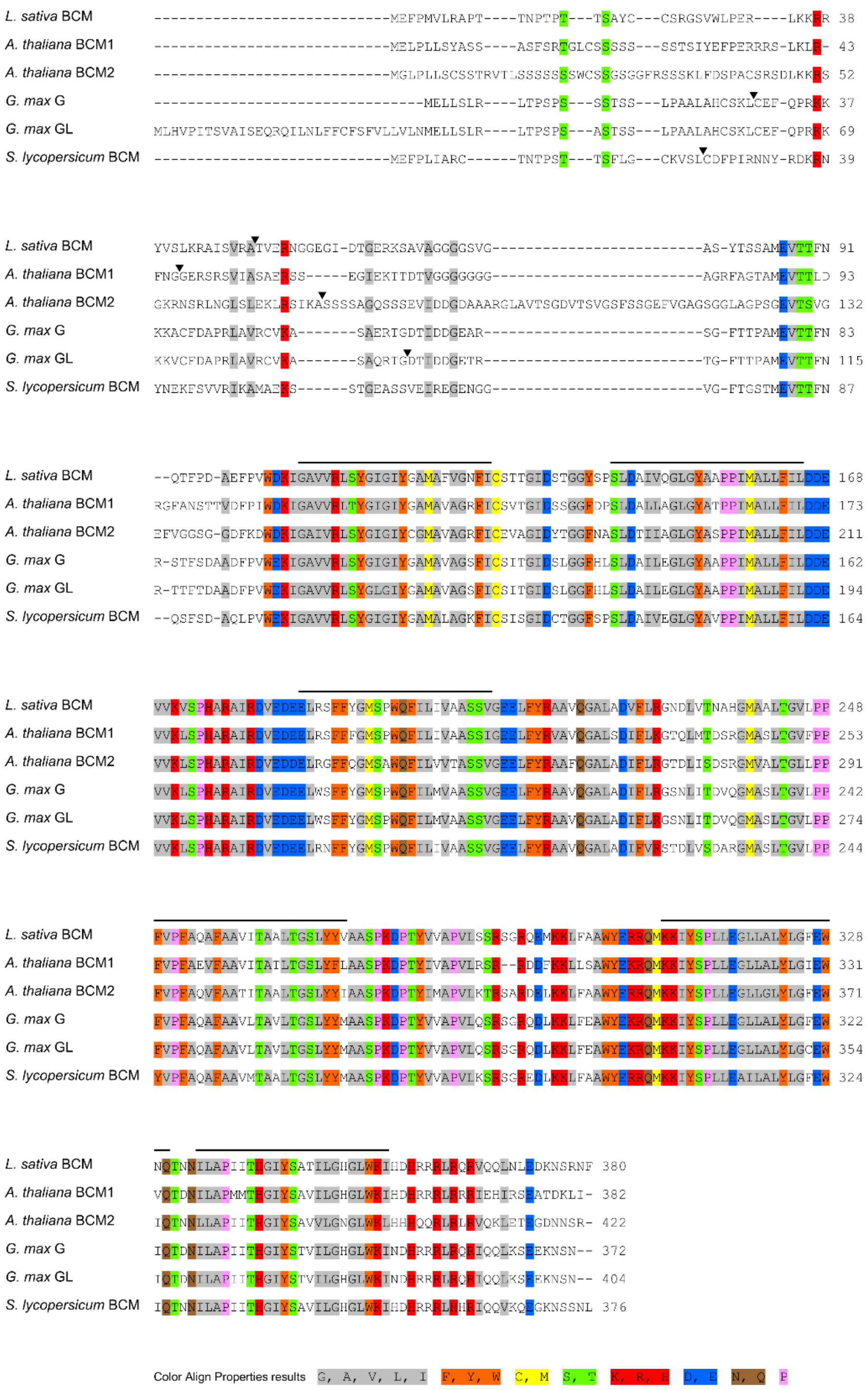
Supplemental Figure S8. ChIP-qPCR analysis of GLK1 binding to the *BCM1* promoter. ChIP-qPCR analysis was performed as described in Fig.3. Fold enrichment is indicated as the % input. ** and *** $p < 0.01$ and $p < 0.001$, respectively (Student's t test). Error bars indicate standard errors (n=3) .



Supplemental Figure S9. ChIP-qPCR analysis of GLK1 binding to the *BCM2* promoter. ChIP-qPCR analysis of GLK1 binding to the *BCM2* promoter was performed using GLK1-4 × MYC overexpressing plants. The upper panel shows the gene structure of *BCM1*. Red triangles indicate the positions of possible GLK1-binding sequences. Black boxes represent exons. F-J represent DNA regions used in ChIP-qPCR analysis. The lower panel shows the results of ChIP-qPCR analysis. Fold enrichment is indicated as the % input. *** indicate $p < 0.001$ (Student's t test). Error bars indicate standard errors ($n = 4$).



Supplemental Figure S10. Neighbor-joining trees of BCM, SGR, and GLK families. A, A phylogenetic tree of the BCM family. B, A phylogenetic tree of the SGR family. C, A phylogenetic tree of GLK family. RCE1 and STE24 in Arabidopsis (A), SGRLs in Arabidopsis and lettuce (B), and APRR2s and ARR1s in Arabidopsis and lettuce (C) were used as outgroups, respectively. Bars indicate genetic distances (the number of amino acid substitutions per site). Numbers above branches indicate bootstrap values from 1000 bootstrap analyses.



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L. sativa BCM            ----------------------------MEFPMVLRAPT----TNPTPT---TSAYC---CSRGSVWLPER----LKKRR 38
A. thaliana BCM1         ----------------------------MELPLLSYASS----ASFSRTGLCSSSSS---SSTSIYEFPERRRS-LKLR- 43
A. thaliana BCM2         ----------------------------MGLPLLSCSSTRVTLSSSSSSSWCSSGSGGFRSSSKLFDSPACSRSDLKKRS 52
G. max G                 --------------------------------MELLSLR----LTPSPS---SSTSS---LPAALAHCSKLCEF-QPRKK 37
G. max GL                MLHVPITSVAISEQRQILNLFFCFSFVLLVLNMELLSLR----LTPSPS---ASTSS---LPAALAHCSKLCEF-QPRKK 69
S. lycopersicum BCM      ----------------------------MEFPLIARC------TNTPST---TSFLG---CKVSLCDFPIRNNY-RDKRN 39

L. sativa BCM            YVSLKRAISVRATVERNGGEGI-DTGERKSAVAGGGGSVG-------------------------AS-YTSSAMEVTTFN 91
A. thaliana BCM1         FNGGERSRSVIASAERSS-----EGIEKTTDTVGGGGGGG-------------------------AGRFAGTAMEVTTLD 93
A. thaliana BCM2         GKRNSRLNGLSLEKLRSIKASSSSAGQSSSEVIDDGDAAARGLAVTSGDVTSVGSFSSGEFVGAGSGGLAGPSGEVTSVG 132
G. max G                 KKACFDAPRLAVRCVKA-------SAERTGDTIDDGEAR--------------------------SG-FTTPAMEVTTFN 83
G. max GL                KKVCFDAPRLAVRCVKA-------SAQRTGDTIDDGETR--------------------------TG-FTTPAMEVTTFN 115
S. lycopersicum BCM      YNEKFSVVRIKAMAEKS------STGEASSVEIREGENGG-------------------------VG-FTGSTMEVTTFN 87

L. sativa BCM            --QTFPD-AEFPVWDKIGAVVRLSYGIGIYGAMAFVGNFICSTTGIDSTGGYSPSLDAIVQGLGYAAPPIMALLFILDDE 168
A. thaliana BCM1         RGFANSTTVDFPIWDKIGAVVRLTYGIGIYGAMAVAGRFICSVTGIDSSGGFDPSLDALLAGLGYATPPIMALLFILDDE 173
A. thaliana BCM2         EFVGGSG-GDFKDWDKIGAIVRLSYGIGIYCGMAVAGRFICEVAGIDYTGGFNASLDTIIAGLGYASPPIMALLFILDDE 211
G. max G                 R-STFSDAADFPVWEKIGAVVRLSYGIGIYGAMAVAGSFICSITGIDSLGGFHLSLDAILEGLGYAAPPIMALLFILDDE 162
G. max GL                R-TTFTDAADFPVWEKIGAVVRLSYGLGIYGAMAVAGSFICSITGIDSLGGFHLSLDAILEGLGYAAPPIMALLFILDDE 194
S. lycopersicum BCM      --QSFSD-AQLPVWEKIGAVVRLSYGIGIYGAMALAGKFICSISGIDCTGGFSPSLDAIVEGLGYAVPPIMALLFILDDE 164

L. sativa BCM            VVKVSPHARAIRDVEDEELRSFFYGMSPWQFILIVAASSVGEELFYRAAVQGALADVFLRGNDLVTNAHGMAALTGVLPP 248
A. thaliana BCM1         VVKLSPHARAIRDVEDEELRSFFFGMSPWQFILIVAASSIGEELFYRVAVQGALSDIFLKGTQLMTDSRGMASLTGVFPP 253
A. thaliana BCM2         VVKLSPHARAIRDVEDDELRGFFQGMSAWQFILVVTASSVGEELFYRAAFQGALADIFLRGTDLISDSRGMVALTGLLPP 291
G. max G                 VVKLSPHARAIRDVEDEELWSFFYGMSPWQFILMVAASSVGEELFYRAAVQGALADIFLRGSNLITDVQGMASLTGVLPP 242
G. max GL                VVKLSPHARAIRDVEDEELWSFFYGMSPWQFILMVAASSVGEELFYRAAVQGALADIFLRGSNLITDVQGMASLTGVLPP 274
S. lycopersicum BCM      VVKLSPHARAIRDVEDEELRNFFYGMSPWQFILIVAASSVGEELFYRAAVQGALADIFVRSTDLVSDARGMASLTGVLPP 244

L. sativa BCM            FVPFAQAFAAVITAALTGSLYYVAASPKDPTYVVAPVLSSRSGRQEMKKLFAAWYERRQMKKIYSPLLEGLLALYLGFEW 328
A. thaliana BCM1         FVPFAEVFAAVITATLTGSLYFLAASPKDPTYIVAPVLRSR--RDDFKKLLSAWYEKRQMKKIYSPLLEGLLALYLGIEW 331
A. thaliana BCM2         FVPFAQVFAATITAALTGSLYYIAASPKDPTYIMAPVLKTRSARDELKKLFAAWYERRQMKKIYSPLLEGLLGLYLGFEW 371
G. max G                 FVPFAQAFAAVLTAVLTGSLYYMAASPKDPTYVVAPVLQSRSGRQDLKKLFEAWYEKRQMKKIYSPLLEGLLALYLGFEW 322
G. max GL                FVPFAQAFAAVLTAVLTGSLYYMAASPKDPTYVVAPVLQSRSGRQDLKKLFEAWYEKRQMKKIYSPLLEGLLALYLGCEW 354
S. lycopersicum BCM      YVPFAQAFAAVMTAALTGSLYYMAASPKDPTYVVAPVLKSRSGREDLKKLFAAWYERRQMKKIYSPLLEAILALYLGFEW 324

L. sativa BCM            NQTNNILAPIITHGIYSATILGHGLWKIHDHRRRLRQRVQQLNLEDKNSRNF 380
A. thaliana BCM1         VQTDNILAPMMTHGIYSAVILGHGLWKIHDHRRRLRRRIEHIRSEATDKLI- 382
A. thaliana BCM2         IQTNNLLAPIITHGIYSAVVLGNGLWKLHHHQQRLRLRVQKLETEGDNNSR- 422
G. max G                 IQTDNILAPIITHGIYSTVILGHGLWKINDHRRRLRQRIQQLKSEEKNSN-- 372
G. max GL                IQTDNILAPIITHGIYSTVILGHGLWKINDHRRRLRQRIQQLKSEEKNSN-- 404
S. lycopersicum BCM      IQTNNILAPIITHGIYSAVILGHGLWKIHDHRRRLHHRIQQVKQEGKNSSNL 376
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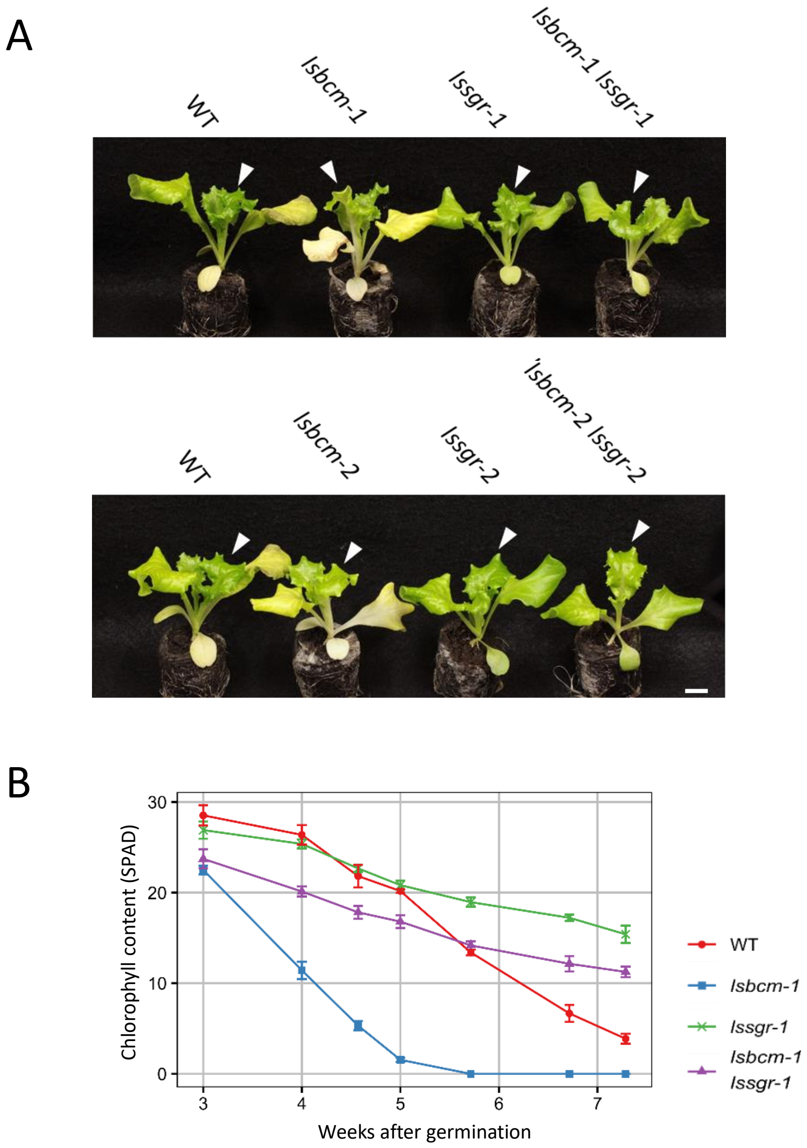
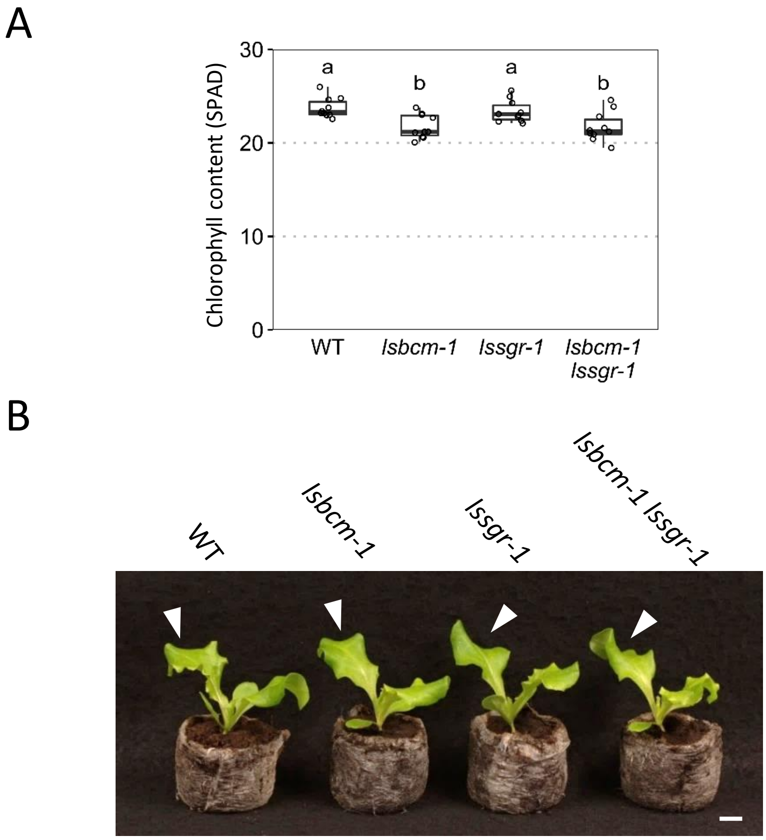
Color Align Properties results G, A, V, L, I F, Y, W C, M S, T K, R, H D, E N, Q P

Supplemental Figure S11. Amino acid sequence alignment of BCM proteins in lettuce, Arabidopsis, soybean and tomato. Triangles and bars indicate predicted cleavage sites of transit peptides and transmembrane regions, respectively. Amino acid residues with similar properties are indicated by the same color.



Supplemental Figure S12. Natural senescence of *lsbcm*, *lssgr*, and *lsbcm lssgr* mutants in lettuce. A, Four-week-old lettuce mutant plants. Scale bar: 1cm. B, Changes in chlorophyll contents of the *lsbcm*, *lssgr*, and *lsbcm lssgr* mutants with time until eight weeks after germination. The third true leaves indicated by an arrowhead in (A) were used for measurement of SPAD values. SPAD value is non-destructive chlorophyll measurement. Error bars indicate standard errors (n = 4).



Supplemental Figure S13. Characterization of *lsbcm* and *lssgr* mutants. A, Box plot of the chlorophyll contents of pre-senescent leaves of the *lsbcm* and *lssgr* mutants. Median and first and third quartiles are shown. Whiskers represent the minimum and maximum values. Data were statistically analyzed using Tukey's multiple comparison method ($p < 0.05$) ($n = 10$). B, Phenotypes of two-week-old lettuce mutants used in (A). Chlorophyll contents of the second true leaves indicated by arrowheads were measured. Scale bar: 1cm. SPAD value is non-destructive chlorophyll measurement.

Supplemental Table S1. Primers used in genome editing and qPCR

Purpose of use	Primer Name	Sequence (5' to 3')
gRNA (Arabidopsis)	BCM2_gRNA1_F	GATTGCAGGAATAGATTACACAGG
	BCM2_gRNA1_R	AAACCCTGTGTAATCTATTCCTGC
	BCM2_gRNA2_F	ATTGGATCTTATCTCGGATTCTCG
	BCM2_gRNA2_R	AAACCGAGAATCCGAGATAAGATC
gRNA (lettuce)	LsBCM_gRNA1_F	ATTGAGCGCTGTCGCTGGCGGCGG
	LsBCM_gRNA1_R	AAACCCGCCGCCAGCGACAGCGCT
	LsBCM_gRNA2_F	ATTGACAGGAATCGATAGCACAGG
	LsBCM_gRNA2_R	AAACCCTGTGCTATCGATTCCTGT
	LsSGR_gRNA1_F	AAACTCCATCGTCTTCGTTTCTAC
	LsSGR_gRNA1_R	ATTGGTAGAAACGAAGACGATGGA
	LsSGR_gRNA2_F	ATTGGTTGACGAGAAGAAGCATCC
	LsSGR_gRNA2_R	AAACGGATGCTTCTTCTCGTCAAC
RT-qPCR (Arabidopsis)	qRT_ACT8_F1	AGCACTTTCCAGCAGATGTG
	qRT_ACT8_R1	GAAATGTGATCCCGTCATGG
	qRT_BCM1_F2	GAATGGGTTCAGACGGATAAT
	qRT_BCM1_R2	GTGGCCTCCGATCTAATGTG
	qRT_BCM2_F1	CGTTTCAGGGTGCACTAGCT
	qRT_BCM2_R1	CCAGTGAGAGCGGCTGTAAT
	qRT_CAO1_F1	ACACCAACAGGTTCATATAT
	qRT_CAO1_R1	ACAGCAGAAGCAGTGGTTGA
	qRT_GLK1_F1	TGGCATCCAATGCATAACGG
	qRT_GLK1_R1	CGCTCTCTTTTGACGGATGT
	qRT_GLK2_F1	AAACCTAAGGTGGATTGGAC
	qRT_GLK2_R1	TCTGAAGATGGCTAGCAACG
	qRT_Lhca1_F1	TTCTGCTCCTGGTGACTTT
	qRT_Lhca1_R1	AGCATAGCCCATCTACAGT
	qRT_Lhcb1.1_F1	GTACTTGGGTCCATTCTCTG
	qRT_Lhcb1.1_R1	GCCAAAAGCTCAGGGAAGA
	qRT_NAP_F1	CATGAGGTTAGATGAATGGGTACT
	qRT_NAP_R1	TCATCCTCCATTAGTACTTCGTCC
	qRT_NYC1_F1	TCTCTTCGGTTGCTGTTCAA
	qRT_NYC1_R1	CCAAAACCATGTTAGCACCA
	qRT_ORE1_F1	ACAGCTAAGAACGAATGGGT
	qRT_ORE1_R1	CCATTCGGTTAATGTGTGGA
	qRT_PsaF_F1	AAACGCAGGTTCGACAACTA
	qRT_PsaF_R1	GGTCTCCGTTCACTATCAAG
	qRT_SGR1_F1	GATTGTTCCCGTTGCAAGGT
	qRT_SGR1_R1	AAGTCCTAGGGAGCGTTGAA
RT-qPCR (lettuce)	qRT_LsACT7_F1	CAATGGAACTGGAATGGTGAAGG
	qRT_LsACT7_R1	CTTTTCCATGTCATCCCAGTTGC
	qRT_LsBCM_F1	CGACGAAGTGGTGAAGGTATCG
	qRT_LsBCM_R1	CAGCTAATGCACCCTGAACTGC
	qRT_LsGLK_F1	TGGCATCATCATCACCAGAGAG
	qRT_LsGLK_R1	GCTCTCCTTGGATGGATGTAGG
	qRT_LsLhca1_F1	GATTTTGGGTTTGACCCACTT
	qRT_LsLhca1_R1	CATAGCCCATCTGCAGTGAAT
	qRT_LsSGR_F1	AGATCGAAGGCCCAAATAATC
	qRT_LsSGR_R1	CAGGATGCTTCTTCTCGTCAA
ChIP-qPCR	ChIP_ACT8_F1	CAGGACTTGTTTACGTTTTCAGG
	ChIP_ACT8_R1	GGATTGACTGTGATAGGGTCG
	ChIP_CAO_F1	TGGACAACCAAACCAGGAAAA
	ChIP_CAO_R1	ATCTGAGAATGCCTTCGGGAA
	ChIP_BCM1-A_F1	TCATACGTATCTTTCAATAAAGAGATTCC
	ChIP_BCM1-A_R1	CGAAAAACAGTAAAATTAACACTTTAAGC
	ChIP_BCM1-B_F1	GCTTAAAGTGTTAATTTTACTGTTTTTCG
	ChIP_BCM1-B_R1	GGGTAACTGCCATCTCATAAAC
	ChIP_BCM1-C_F1	GTTTATGAGATGGCAGTTACCC
	ChIP_BCM1-C_R1	GTGCTTGTGCTTTTTGTTTGG
	ChIP_BCM1-D_F1	CCAAACAAAAAGCACAAGCAC
	ChIP_BCM1-D_R1	CGGAAACTCATAGATACTAGTTGAGG
	ChIP_BCM1-E_F1	CTCCAGTTTATACTCATTGTCGC
	ChIP_BCM1-E_R1	GAATCTGTCATCAACTGTGTTCC
	ChIP_Lhca1_F1	TGCAGTGGATAAATGGCAAA
	ChIP_Lhca1_R1	GTGGACGAGAGGTGTTTGGT
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	ChIP_Lhcb2.2_F1	CACCACCACAACCATTACCA
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