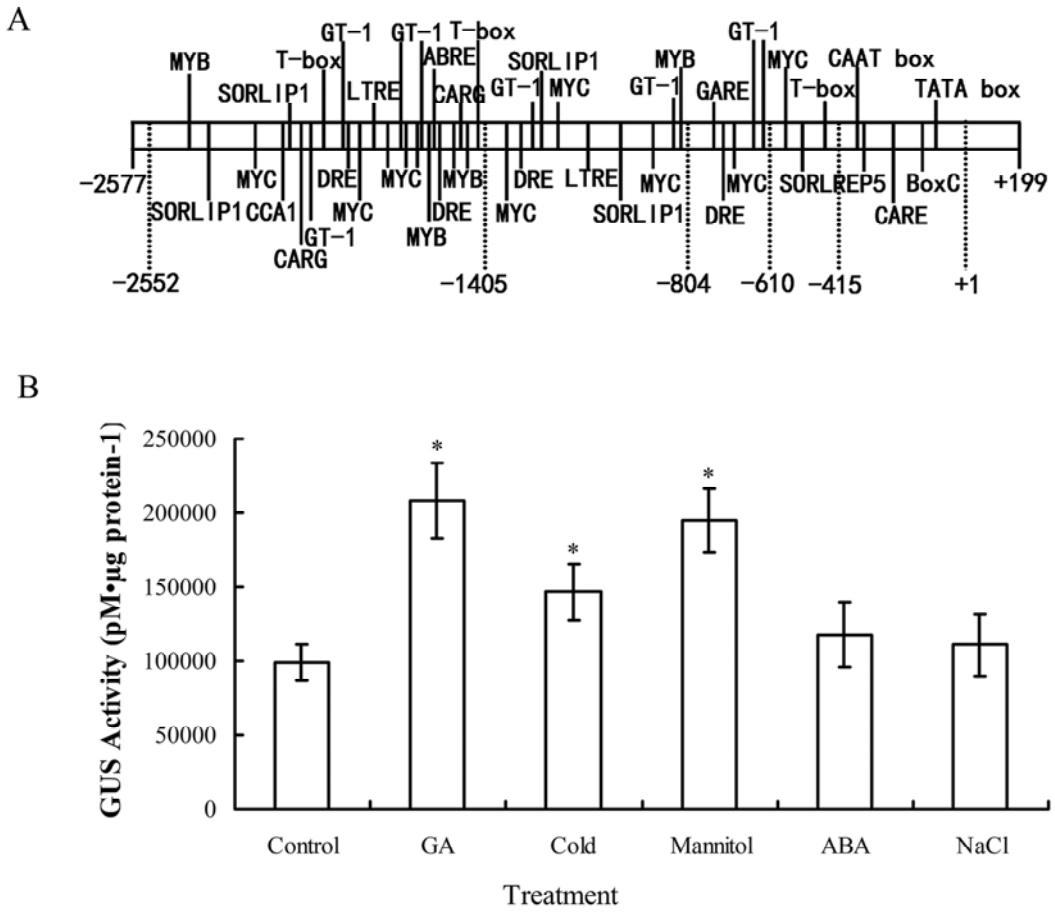
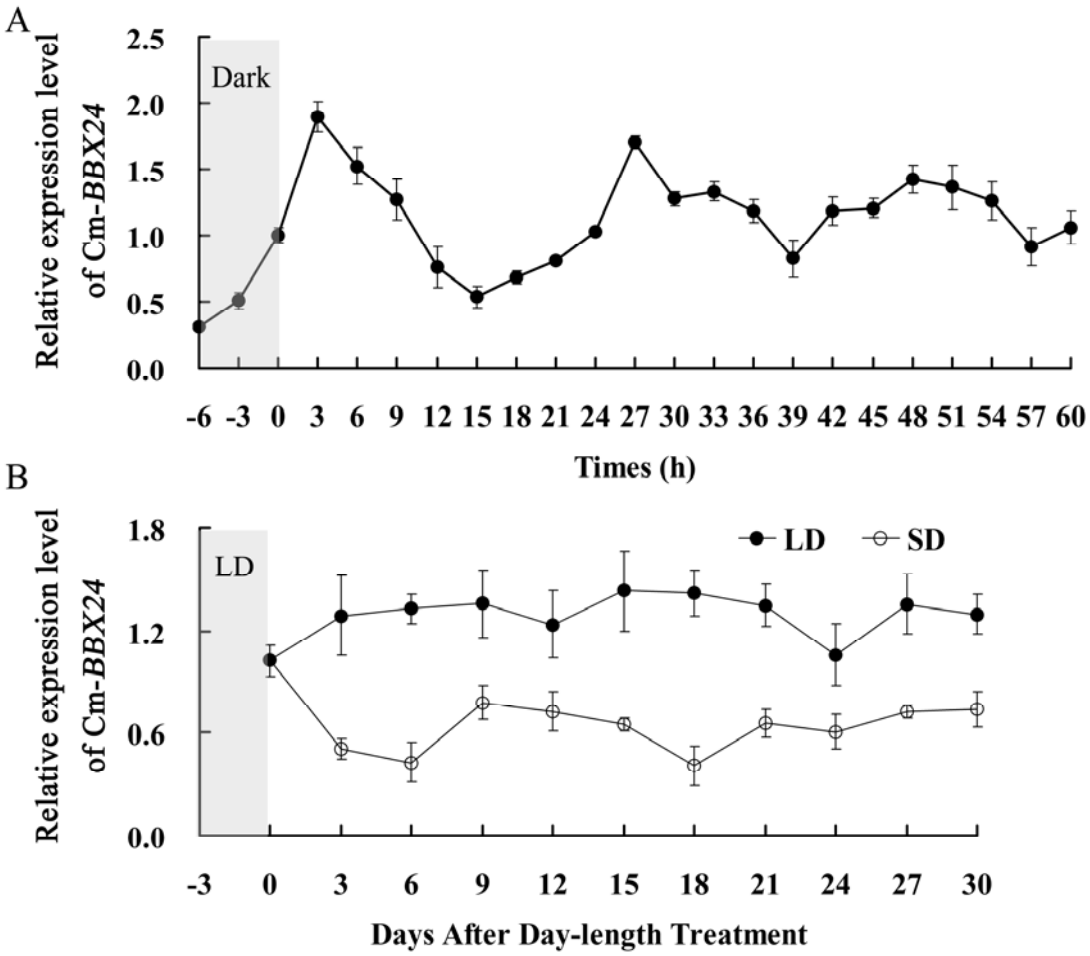


Supplemental Figure 1. Activities of the Cm-*BBX24* promoter in response to hormone treatments and abiotic stresses in transgenic Arabidopsis plants.

(A) *Cis*-element analysis of the Cm-*BBX24* promoter.

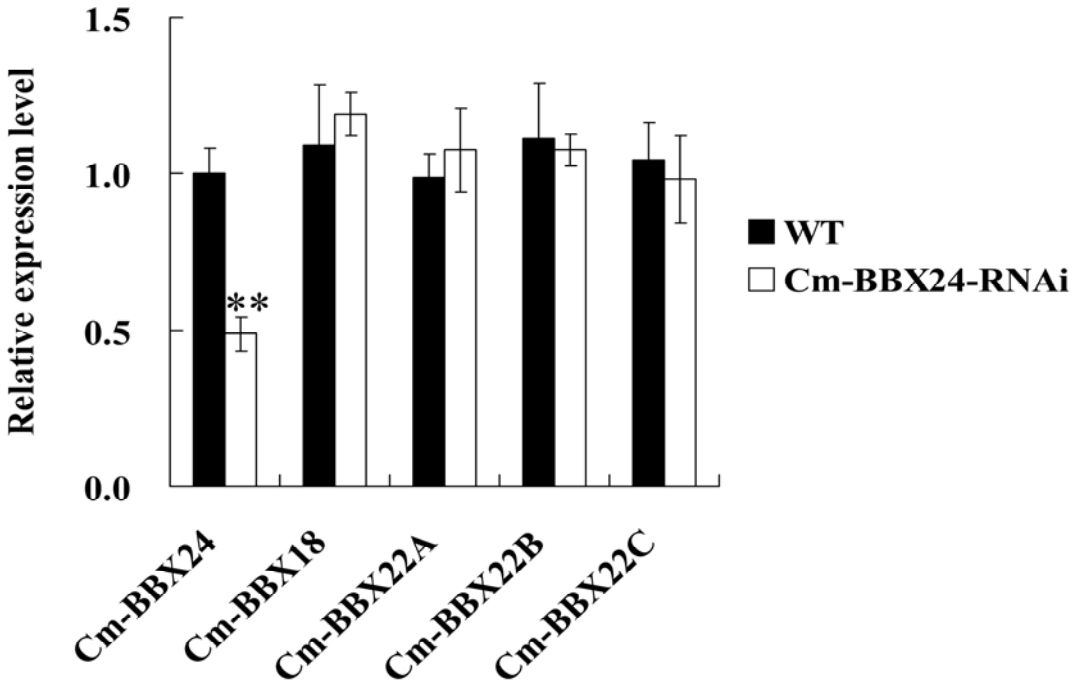
(B) Activities of the Cm-*BBX24* promoter as a consequence of hormone treatments and abiotic stresses. GUS activity was measured immediately after the treatments using 9-day-old plants. Other than the experiments involving cold treatments, plants were transferred onto fresh MS medium containing 80 μM GA_{4+7}, 100 mM mannitol, 400 μM ABA or 150 mM NaCl, and held at 22 ℃ for 4 d. For the cold treatment, plants on fresh MS medium were transferred to a cold chamber maintained at 4 ℃ for 4 days. The control corresponds to plants exposed only to fresh MS medium. Assays were performed on rosettes from 8 transgenic Arabidopsis plants per treatment. The error bars represent the standard deviation of the mean (n = 8). Asterisks represent significant difference between the treatments and untreated controls according to Duncan's multiple range test ($p < 0.05$).



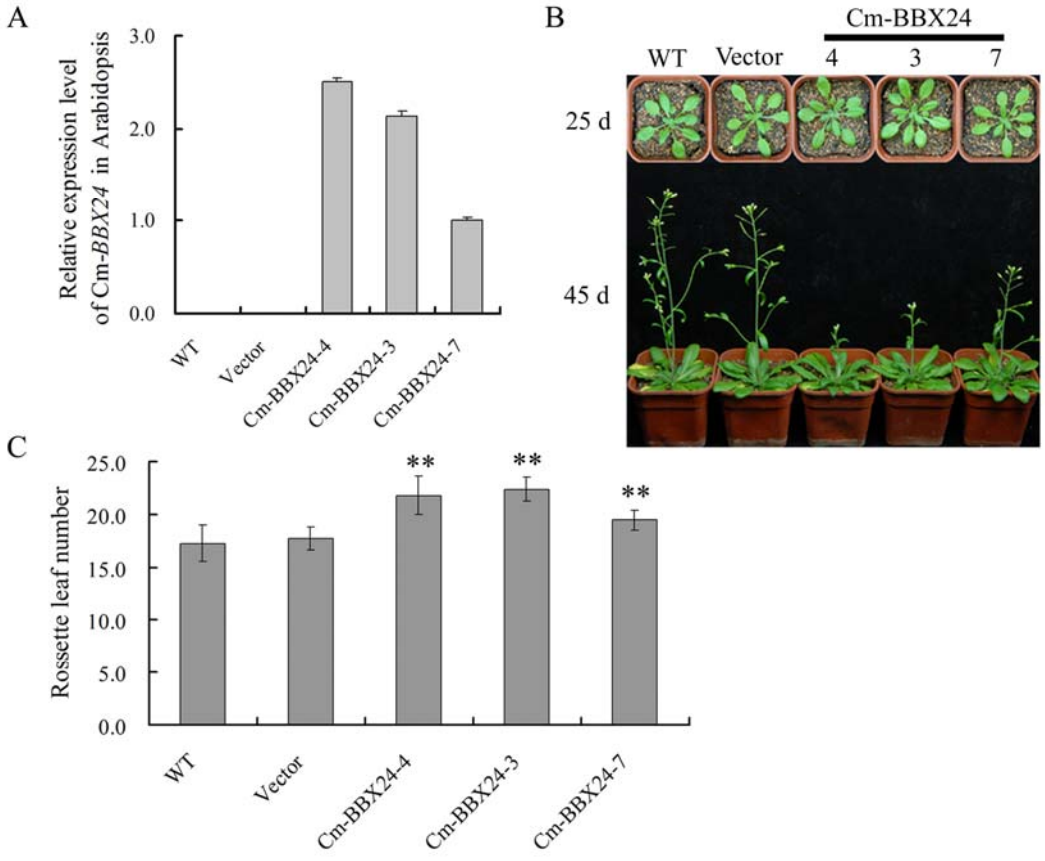
Supplemental Figure 2. Relative expression of Cm-*BBX24* transcripts under the control of the circadian clock and different day lengths.

(A) Wild-type chrysanthemum plants were grown under LD (long day) conditions for 2 weeks and were then exposed to continuous light (LL). Samples were collected at 3 h intervals. -6 and -3 (the gray area) indicate the dark period, and 0 indicates the time at which the plants were transferred to LL. The data were normalized with the expression of the *ubiquitin* gene as an internal reference. Error bars indicate standard deviation (n = 3).

(B) Wild-type chrysanthemum plants were grown under LD for 2 weeks and then exposed to LD or SD (short day) condition. Samples were collected at Zeitgeber Time ZT3 with 3 d intervals. The gray area indicates the LDs, and 0 indicates the time point at which the plants were transferred to SD. The data were normalized with the expression of the *ubiquitin* gene as an internal reference. Error bars indicate standard deviation (n = 3).



Supplemental Figure 3. Comparison of the expression of Cm-*BBX24* and other members of BBX structure group IV in Cm-*BBX24*-RNAi and wild type (WT) chrysanthemum plants. The data reflect three independent replicates and error bars indicate standard deviation. Significant differences were assessed using Duncan's multiple range test ($P < 0.05$).

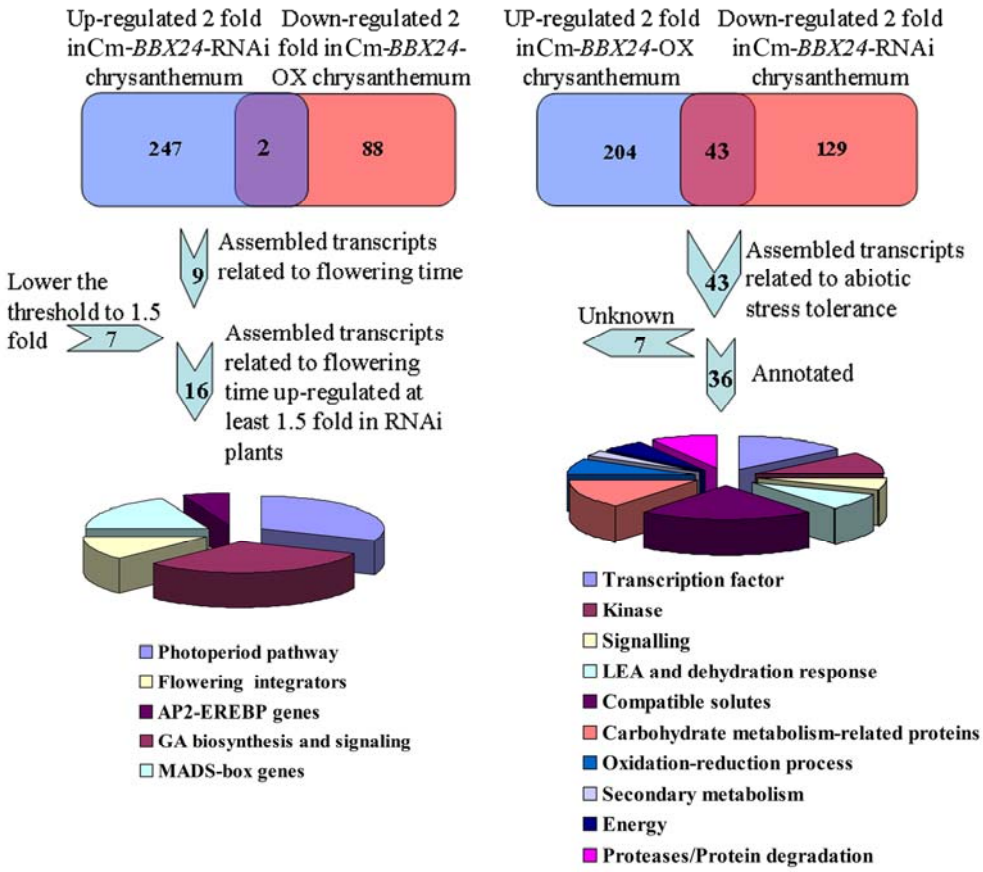


Supplemental Figure 4. Flowering time of Cm-*BBX24*-overexpressing Arabidopsis plants.

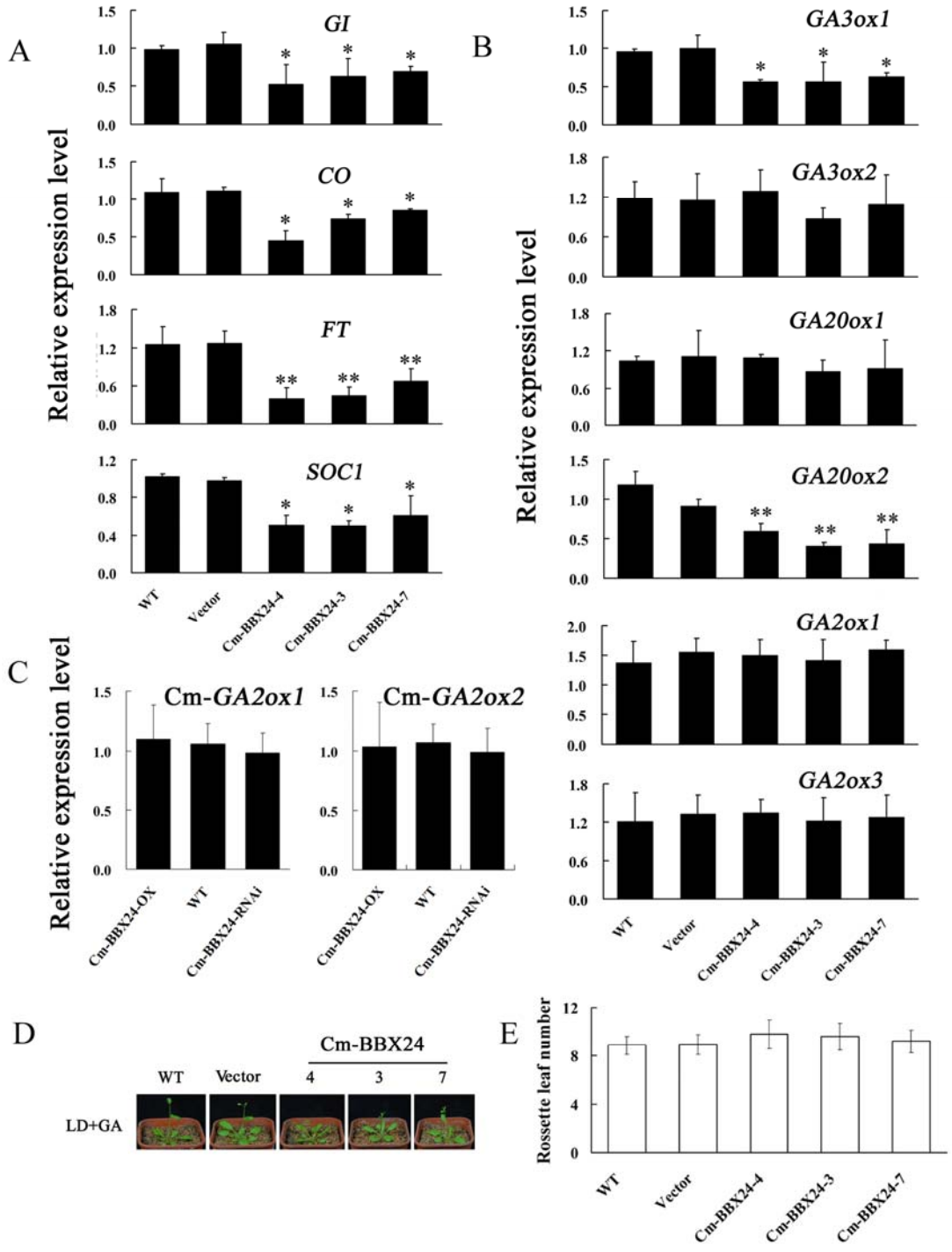
(A) Expression of Cm-*BBX24* in overexpressing lines. Error bars indicate standard deviation (n = 3)

(B) Growth and flowering phenotypes of plants at 25 d and 45 d after sowing. The numbers 3, 4 and 7 represent different transgenic lines. The designation "Vector" corresponds to a vector-only control transgenic line.

(C) Rosette leaf numbers at flowering of Cm-*BBX24*-4, Cm-*BBX24*-3 and Cm-*BBX24*-7, three independent Cm-*BBX24*-overexpressing lines. Error bars shown in each column indicate standard deviation (n = 15). Significant differences assessed using Duncan's multiple range test ($P < 0.05$).



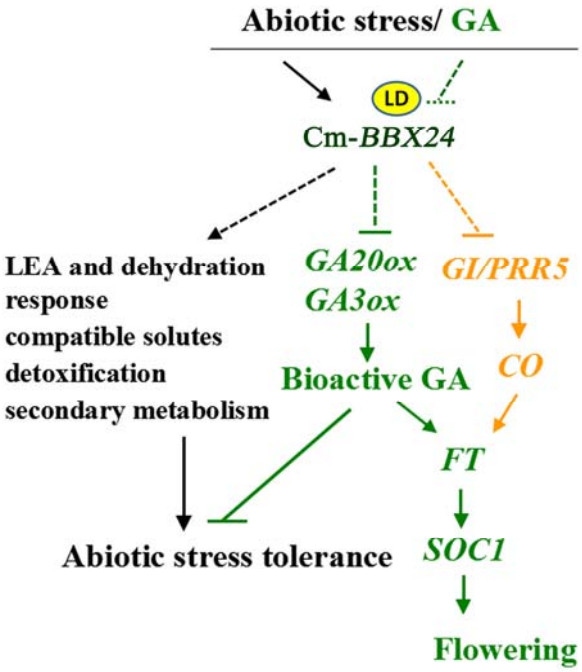
Supplemental Figure 5. The number of assembled transcripts differentially expressed between Cm-*BBX24*-OX, Cm-*BBX24*-RNAi and wild type (WT) chrysanthemum plants. Total RNA was isolated from the aerial parts of 5-week-old transgenic and WT chrysanthemum plants grown under normal conditions. Three independent samples were taken per line. Left: Assembled transcripts up/down-regulated at least 2-fold in Cm-*BBX24*-RNAi/Cm-*BBX24*-OX plants. In addition to the 9 transcripts that were selected, 7 others were designated as differentially expressed flowering-related transcripts using an expression threshold of 1.5-fold. In total, the 16 transcripts were regarded as associated with the early blooming of the RNAi plants and were classified into 5 groups according to known flowering pathways and their protein structures. Right: Transcripts down/up-regulated at least 2-fold in Cm-*BBX24*-RNAi/Cm-*BBX24*-OX plants. 43 assembled transcripts were up-regulated in Cm-*BBX24*-OX plants and down-regulated in Cm-*BBX24*-RNAi plants, of which, 7 transcripts had not assigned function, while annotated transcripts were classified into 10 functional groups. These differentially expressed transcripts are listed in Supplemental Data set 1 online.



Supplemental Figure 6. Expression of the photoperiod pathway- and GA biosynthesis pathway-relevant genes and effect of $GA_{4/7}$ treatment on flowering time in transgenic *Arabidopsis* and chrysanthemum plants.

(A) The expression of flowering-related genes in the photoperiod pathway in transgenic Arabidopsis lines. Error bars shown in each column indicate standard deviation (n =3). Significant differences were assessed using Duncan's multiple range test ($P<0.05$). * presents a 0.05 of significant difference, and ** presents a 0.01 of significant difference between transgenic plants and WT plants.

(B) The expression of GA biosynthesis-related genes in transgenic Arabidopsis lines. Cm-*BBX24*-4, Cm-*BBX24*-3 and Cm-*BBX24*-7: three independent Cm-*BBX24*-OX lines. Error bars indicate standard deviation (n = 3). Significant differences were assessed using Duncan's multiple range test (P<0.05). The expression of *GA20ox3, 4, 5* and *GA2ox2, 4, 6, 7* were also detected without any significant differences.
(C) Expression of Cm-*GA2ox* in Cm-*BBX24*-OX/RNAi and WT chrysanthemum. Cm-*BBX24*-OX and Cm-*BBX24*-OX are independent T0 overexpression and RNAi lines. Error bars indicate standard deviation bars (n = 3).
(D) Effect of $GA_{4/7}$ treatment on flowering time of Cm-*BBX24*-OX *Arabidopsis* plants under LDs. The numbers 3, 4 and 7 represent different transgenic lines.
(E) Rosette leaf number at flowering of Cm-*BBX24*-OX *Arabidopsis* lines. Error bars indicate standard deviation (n = 12). Significant differences assessed using Duncan's multiple range test (P < 0.05), and no significant differences were observed.



Supplemental Figure 7. Schematic model describing the involvement of Cm-*BBX24* in flowering and abiotic stress tolerance in chrysanthemum.
Abiotic stresses cause the induction of Cm-*BBX24*, which can enhances abiotic stress tolerance by up-regulating a series of abiotic stress response genes. Conversely, exogenous GA treatment suppresses the expression of Cm-*BBX24*. In addition, Cm-*BBX24* influences flowering via the classical GI/PRR5-CO-FT-SOC1 photoperiod pathway. Cm-*BBX24* also modulates the content of bioactive GA by regulating the expression of *GA20ox* and *GA3ox*, and the bioactive GA content negatively affects abiotic stress tolerance, but promotes flowering. We propose that GA plays a role in the crosstalk of abiotic stress tolerance and flowering time regulated by Cm-*BBX24*.

Supplemental Table 1. Annotation of genes up-regulated in Cm-*BBX24*-OX plants but down-regulated in Cm-*BBX24*-RNAi plants. Significant differences were corrected with $P<0.05$ and expression ratio ≥ 2.

Gene	Annotation	Fold change	
		OX/WT	RNAi/WT
Signal proteins			
Transcription factor			
UN032230	zinc finger transcription factor	5.40	0.48
UN038273	heat shock transcription factor (HSF)	2.27	0.20
UN063560	WRKY transcription factor	2.13	0.47
UN014605	putative MYB transcription factor	2.71	0.08
UN038337	translation initiation factor eIF-3 subunit 10	3.83	0.13
Kinase			
UN034167	leucine-rich repeat protein kinase family protein	2.02	0.39
UN027925	kinase, putative	2.62	0.46
UN037745	receptor-kinase, putative	2.10	0.42
UN034437	WAK-like kinase	2.89	0.25
Signalling			
UN005929	calmodulin, putative	2.45	0.26
UN005930	calmodulin, putative	2.63	0.26
Function protein			
LEA and dehydration response			
UN040616	late embryogenesis-abundant protein	3.10	0.21
UN037568	dehydration-responsive family protein	2.00	0.39
UN004689	LEA protein group 3	3.25	0.28
Compatible solutes			
UN053280	Lectin	3.63	0.40
UN059247	lectin 3	2.60	0.12
UN012331	agglutinin alpha chain	3.44	0.07
UN059246	tuber agglutinin	2.80	0.12
UN059244	tuber agglutinin	2.43	0.10
UN027223	tuber agglutinin	2.34	0.25
UN012678	mannose/glucose-specific lectin	3.99	0.09
UN012771	tuber agglutinin	6.94	0.33
Carbohydrate metabolism-related proteins			
UN061536	trehalose-6-phosphate synthase, putative	2.15	0.21
UN039943	UDP-glucose glucosyltransferase	4.26	0.49
UN012724	UDP-glycosyltransferase 85C1	2.61	0.32
UN034394	putative glycosyltransferase	2.18	0.24

UN028887	beta-amylase 1	2.97	0.16
Oxidation-reduction process			
UN013137	cytochrome c oxidase subunit III	3.94	0.18
UN065014	L-ascorbate oxidase homolog	2.19	0.33
UN018080	Lipoxygenase	2.22	0.50
Secondary metabolism			
UN050269	sesquiterpene synthase	2.04	0.02
Energy			
UN018725	aminophospholipid ATPase	2.20	0.45
UN018116	H+-transporting two-sector ATPase, alpha/beta subunit, central region, related	5.36	0.22
Proteases/Protein degradation			
UN034196	gamma-glutamyl-gamma-aminobutyrate hydrolase, putative	2.12	0.20
UN025400	26S proteasome non-ATPase regulatory subunit	6.88	0.16
UN016852	Ubiquitin-protein ligase, putative	5.74	0.31

Supplemental Table 2. Differentially expressed genes in Cm-*BBX24*-OX/RNAi and wild type (WT) chrysanthemum plants by qPCR analysis.

Genes	OX/WT		RNAi/WT	
	qRT-PCR	Digital expression	qRT-PCR	Digital expression
Cm-*BBX24*	1.85	1.73	0.49	0.56
Cm-*SOC1*	0.96	1.07	2.01	1.61
Cm-*GA3ox*	0.60	0.62	3.34	2.71
Cm-*GA20ox*	0.32	0.19	2.92	3.36
Cm-*Calmodulin*	3.94	2.45	0.30	0.26
Cm-*FT*	1.27	1.14	1.70	1.51
Cm-*WRKY*	3.02	2.13	0.35	0.47

Supplemental Table 3. Primers used for gene isolation, qRT-PCR analysis and vector construction.

Accession No.	Primer set	
	Forward primer (5'-3')	**Reverse primer (5' -3')**
For vector construction of overexpression in chrysanthemum or *Arabidopsis*		
Cm-*BBX24*-OX (*Xba*I+ *Sma*I)	GTTCTAGAATGAAGATTCAGTGTG ATGTGTGTG	TCCCCGGGTTTAACCAAGATCAG GGACAGTG
For vector construction of RNAi in chrysanthemum		
Cm-*BBX24*-Sense (*Xho*I+ *Eco*RI)	TTGCTCGAGCTTGAAGATAGCTAT GTCCGCC	TTGAATTCGGAATACAATACAAC CAACATTGC
Cm-*BBX24*-Antisense (*Xba*I+ *Hind*III)	TGTCTAGACTTGAAGATAGCTATG TCCGCC	TTTAAGCTTGGAATACAATACAAC CAACATTGC
For vector construction of GUS activation analysis in *Arabidopsis*		
ProCm-*BBX24* (*Hind*III+ *Xba*I)	GTAAAGCTTCAAACTTAAATACAAT GGATGACC	ATTCTAGACTTCAACACACAACTT CTTCACTC
For vector construction of subcellular localization		
Cm-BBX24 –Localization (*Eco*RI+ *Sal*I)	GTGAATTCATGAAGATTCAGTGTGA TGTGTGTGA	CTTGTCGACCAACCAAGATCAGG GACAGTGAAGTG
For vector construction of transcriptional activation analysis		
Cm-BBX24/B-box (*Eco*RI+ *Sal*I)	GTGAATTCATGAAGATTCAGTGTGA TGTGTGTGA	AAGTCGACCTAAGAGCCACACGG ATCCCAG
Cm-BBX24/NB-box (*Eco*RI + *Sal*I)	GTGAATTCATGAGTTCCAGTTCCAC CCAAGAGC	CTGTCGACTTAACCAAGATCAGGG ACAGTGAAG
Cm-BBX24-ORF (*Eco*RI+ *Sal*I)	Same as Cm BBX24/B-box forward	Same as Cm BBX24/NB-box reverse
For gene isolation		
Cm-*BBX24*-Full	GCTTTTCTTTGAGTGAAGAAGTTG	GGAATACAATACAACCAACATTGC
For qRT-PCR analysis		
Cm-*BBX24*	CTGTGTAGAGGACAGAGCCCT	TTTTCGGGCTCTTGGGTGG
Cm-*Ubiquitin*	CTAATGAATGCTTACTGTGACCGAC	AGGCGAATCATCAGTACCAAGTG
Cm-*FT*	GAGCCCAAGGCCATCAATG	TGTAACGTCATGTGCATGGCA
Cm-*GA20ox*	ACTTGGGGTTTCCTTGAGGC	GACCGCGAGTTTCATGGGTA
Cm-*GA3ox*	GACGGTGTTGGGCTCTGTTA	ACTGGTGGTGCATGTAGGTG
Cm-*WRKY*	TTGGTCAAACCTGTGAGTGC	AGTGAGAGCGTCAAGACGAGT
Cm-*Calmodulin*	AGGATGCCGGATACAACGAC	GTTGGGGGAGGAATGGACAC
Cm-*SOC1*	TGGGGTGGATTCACAACTCG	GGCGAACTAAGCGAGCTAGA
Cm-*FTL1*	AATCGTGTGCTATGAGAGCC	GCTTGTAACGTCCTCTTCATGC
Cm-*FTL2*	GAAAGCACGCATATCATAC	CATCGATCAAACACGTACAGTA
Cm-*FTL3*	GAAGGCGTAACAAATAAAAATAGC	CAAGTCTTACAATTTGGTACTGTC G
Cm-*AFT*	CAAGCAAAAAGCAAGGCAATCA	CAACCGGTAACCCCAAGTCATT
Cm-*TFL1*	CCATCATCAAGGCACAATTTCA	TTTCCCTTTGGCAGTTGAAGAA
Cm-*BBX22A*	ACTGTGCCGGAAATGTGATCT	CTGGAACATTCCTTGCTGGC

Cm-*BBX22B*	AATACAACAACCCCGTGCCA	ACGCCAGTAAGCCCAAGAAA
Cm-*BBX22C*	TTGAGCGATCTCAACCAGGG	AACAGTCCGAGTCTCCCATC
Cm-*CmBBX18*	ATGATGGTGCTGGCAGGATG	TGACCATGCATCCGTTGAGG
Cm-*GIGANTEA*	GAACTGTCACTACTCGGAGATTCTT	TCAACTTGTAGAACTCCTTAGAGCC
Cm-*PRR5*	AGCTAACTCTGCATCAACTTCCA	CGACCTCGTGTGAAAGGACA
Cm-*GA2ox2*	AGCGGCTTTCTTGTACTCGT	TGGCTATCTCCGATCCCACA
Cm-*GA2ox1*	TCCGAAGTGTTCCAGCATGT	TCGAGTTCCAACCCTTCAGC
At-*GA2ox1*	AACGTTGGTGACTCTCTCCAGGTG	AACCCTATGCCTCACGCTCTTG
At-*GA2ox2*	AGATGGAAGTTGGGTCGCTGTC	CCCGTTAGTCATAACCTGAAGAGC
At-*GA2ox3*	TCCACCTGACCCGTTTGGTTAC	TACTCAAGCCAGCCAAGGTCAC
At-*GA2ox4*	TTTCGCTCTCAGCTCTCCTTCAGC	TCACGAGCCAACTGCTTAACTGC
At-*GA2ox6*	AGCTCGTCACTCTTCTTTCAGA	TTCCGGTGGTTTGTAACGGT
At-*GA2ox7*	TGATGATCGCAACAACCTCAGAAC	CGAACTCACGTTCACTTGTTTCCC
At-*GA20ox1*	AGATTACTTCTGCGATGCGTTGG	TCTTGATACACCTTCCCAAATGGC
At-*GA20ox2*	CAAGAGTTCGAGCAGTTTGGGAAG	TCGGAAATAGTCTCGGTTTACGC
At-*GA20ox3*	ACATAGGCGACACCTTCATGGC	TCCTTTCTCTCTCGCTGTTCACC
At-*GA20ox4*	GAACATTGGCGACACTTTAATGGC	TGGTGGCTTCACCACTTTGTCC
At-*GA20ox5*	AACGTTGGAGACACCTTCATGGC	ACTGCCCTGTGGTAACAACTCC
At-*GA3ox1*	CCCAACATCACCTCAACTACTGC	GGCCCATTCAATGTCTTCTTCGC
At-*GA3ox2*	CCAGCCACCACCTCAAATACTGTG	ATTAGGCCCGGCCCATTGTATG
At-*GI*	AGCAGTGGTCGACGGTTTATC	ATGGGTATGGAGCTTTGGTTC
At-*CO*	CACTACAACGACAATGGTTCC	GGTCAGGTTGTTGCTCTACTG
At-*FT*	CCCTGCTACAACTGGAACAAC	CACCCTGGTGCATACACTG
At-*SOC1*	AAACGAGAAGCTCTCTGAAAAG	AAGAACAAGGTAACCCAATGAAC

Supplemental Methods

Plant materials and treatments

A. thaliana (Columbia) seeds were surface-sterilized using 2 % NaClO for 10 min, washed with sterile water and stratified at 4 ºC for 3 d, then germinated on MS medium. Nine-day-old seedlings were transplanted to 7-cm diameter pots containing a 1:1 (v/v) mixture of peat and vermiculite, and transferred to a culture room (21 ± 1 ºC with a relative humidity of 60%, 16 h light/8 h dark, and 120 μmol.m^{-2}.s^{-1} illumination).

Genomic DNA extraction and Cm-*BBX24* promoter isolation

Chrysanthemum genomic DNA was extracted from leaves as described in Aljanabi et al. (1997). 2776 bp upstream of the Cm-*BBX24* ATG was isolated from the genomic DNA using reverse PCR and nested PCR techniques. The PCR product from this reaction was then inserted into a pGEM T-Easy vector (Promega, Madison, WI, USA). *Cis*-elements in the Cm-*BBX24* promoter were analyzed using the PLACE (Higo et al. 1999) and PLANTCARE (Lescot et al., 2002) programs.

Arabidopsis transformation

To overexpress Cm-*BBX24* in Arabidopsis, the pBIG-Cm-*BBX24* plasmid was introduced into *A. tumefaciens* strain GV3101, and transformed into Arabidopsis using the floral dip method (Clough and Bent, 1998). To detect the activity of the Cm-*BBX24* promoter, the amplified promoter sequence was digested with *Hind*III and *Xba*I, and inserted into the binary vector pBI121 (Jefferson et al., 1987) to replace the cauliflower mosaic virus 35S promoter upstream of the β-glucuronidase gene, resulting in a Cm-*BBX24* promoter:GUS binary vector. The PCR primers are listed in Supplemental Table 3. The vector described above was introduced into *A. tumefaciens* strain GV3101 and transformed into *Arabidopsis* as above. Independent transformants were screened on MS medium (Murashige and Skoog 1962) containing 50 mg L^{-1} kanamycin. T2 plants were used in this study (Tong et al., 2009).

Phenotypic characterization of transgenic *Arabidopsis*

Homozygous T2 Arabidopsis plants overexpressing the Cm-*BBX24* gene were phenotypically characterized. Transgenic and control Arabidopsis plants were photographed at 25 and 45 d after sowing and leaf numbers per plant were counted 4 d after bolting.

***Arabidopsis* materials for GUS activity analysis of the gene promoter**

For GUS activity measurements of the Cm-*BBX24* promoter in *Arabidopsis*, T2 transgenic *Arabidopsis* seeds were germinated on MS medium at 21 ºC under fluorescent light (16 h light /8h dark). For abiotic stress treatments, 9-day-old plants were transferred to MS medium containing 100 mM mannitol or 150 mM NaCl, or a cold chamber maintained at 4 ºC, for 4 d. For the hormone treatment, 9-day-old plants were transferred to MS medium containing 80 μM $GA_{4/7}$ or 400 μM abscisic acid (ABA) for 4 d. Seedlings grown on MS medium under normal conditions were used as controls. For the GUS analysis, T2 transgenic chrysanthemum plants were incubated in GUS staining solution containing 75.5 mM sodium phosphate (pH 7.0), 0.1% Triton X-100, 0.05 mM K_3/K_4 FeCN, 10 mM EDTA, 20% methanol (v/v) and 50 μg ml^{-1} 5-bromo-4-chloro-3-indolyl glucuronic acid at 37 °C overnight. Tissues were then cleared using 75% ethanol and subsequently used for imaging.

Expression analysis of Cm-*BBX24* under circadian regulation and different day lengths

For the expression analysis of Cm-*BBX24* under circadian regulation, WT plants at the 7-9 leaf stage grown under normal conditions (as described above) were transferred to continuous light conditions for 60 h, and leaf samples were taken every 3 h. The treatments were started at ZT0 (Zeitgeber time 0 from light) and the first sample was taken at 6 h before ZT0. Three replicate samples were taken for qRT-PCR analysis.

For the expression analysis of Cm-*BBX24* under different day lengths, WT plants with the 7-9 leaf stage grown under LD conditions (16 h light/8 h dark) for 2 weeks,

then transferred to SD conditions (8h light/16 h dark) for 30d, with plants grown under LD conditions as control. Samples were collected at ZT3 (Zeitgeber Time 3 from light) with 3 d intervals. Three replicate samples were taken for qRT-PCR analysis.

Quantitative RT-PCR analysis

To assess the specificity of Cm-*BBX24* silencing in the Cm-*BBX24*-RNAi transgenic chrysanthemum lines, 4 additional chrysanthemum *BBX* genes, Cm-*BBX18*, Cm-*BBX22A*, Cm-*BBX22B*, Cm-*BBX22C*, other than Cm-*BBX24* in structure group IV were identified by screening our in-house RNA-seq database. Their expression levels were evaluated in WT and Cm-*BBX24*-RNAi plants using qRT-PCR. Three independent replicates were assessed and the PCR primers used are listed in Supplemental Table 3.

To verify the expression of candidate flowering and abiotic stress related genes identified from the RNA-seq data, Cm-*BBX24*-OX/RNAi and WT plants grown under normal conditions were taken at ZT3 (Zeitgeber time 3 from light). Three replicate samples were taken for qRT-PCR analysis.

Cm-*GA2ox* genes (Cm-*GA2ox1*, Cm-*GA2ox2*) were identified by screening the in-house RNA-seq database and their expression level in WT and Cm-*BBX24*-RNAi plants were determined using qRT-PCR. Three independent replicates were evaluated and the PCR primers used are listed in Supplemental Table 3.

The expression of genes associated with the photoperiod (*GI*, *CO*, *FT*, *SOC1*) and GA biosynthesis (*GA3ox1-2*, *GA20ox1-5*, *GA2ox1-4* and *GA2ox6-7*) pathways that might be regulated by Cm-*BBX24* in the transgenic *Arabidopsis* lines, was assessed in leaves harvested from WT and transgenic *Arabidopsis* plants at a stage immediately prior to flower bud emergence. Three independent replicates were evaluated and the primers used are listed in Supplemental Table 3.

$GA_{4/7}$ treatment of Arabidopsis

WT and Cm-*BBX24*-OX plants were grown under LD conditions. One week after seed germination, the plants were sprayed with 100 μM $GA_{4/7}$ twice per week, and photographs were taken at 20 d after germination, and leaf numbers per plant were counted 4 d after bolting.

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