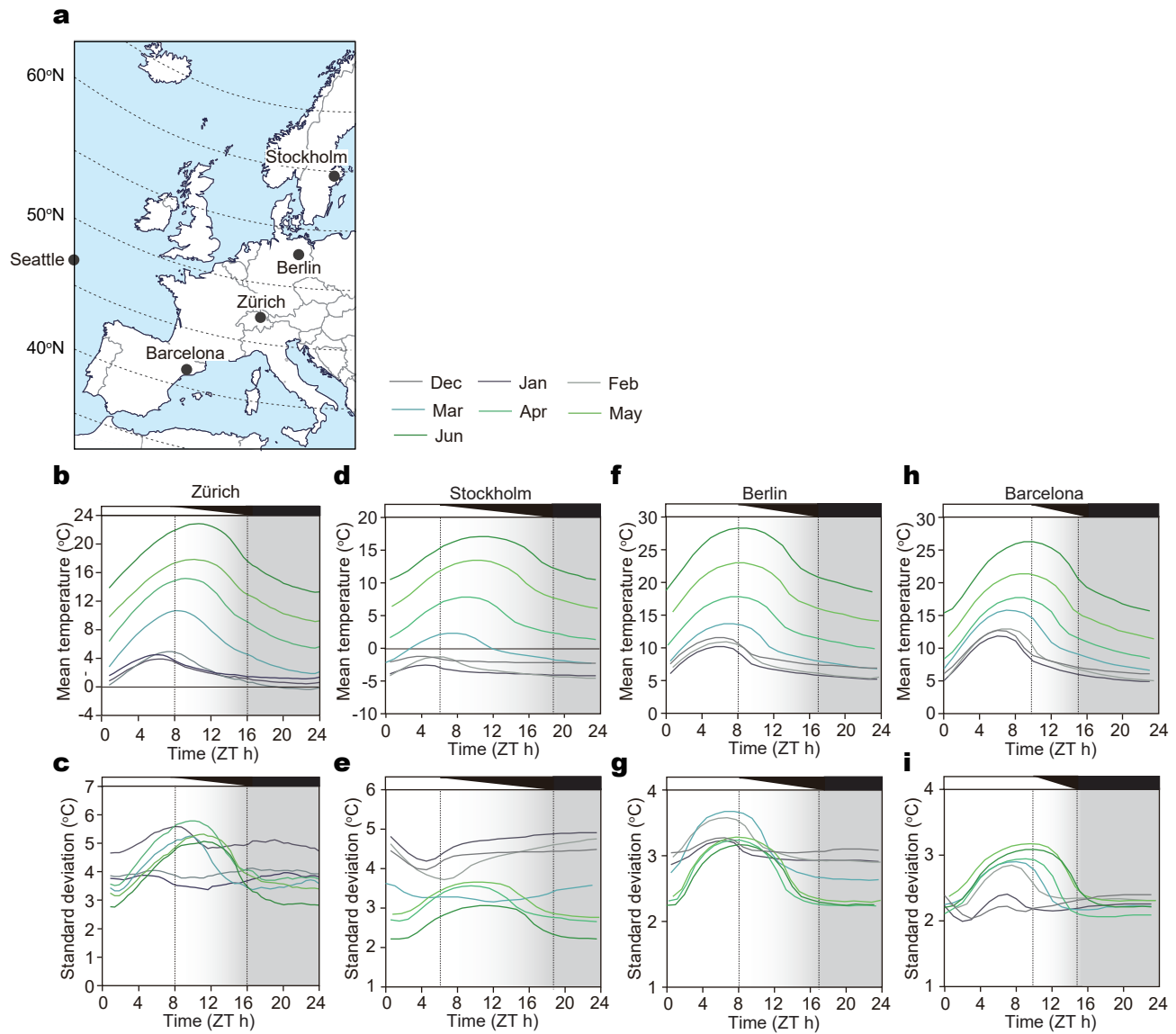
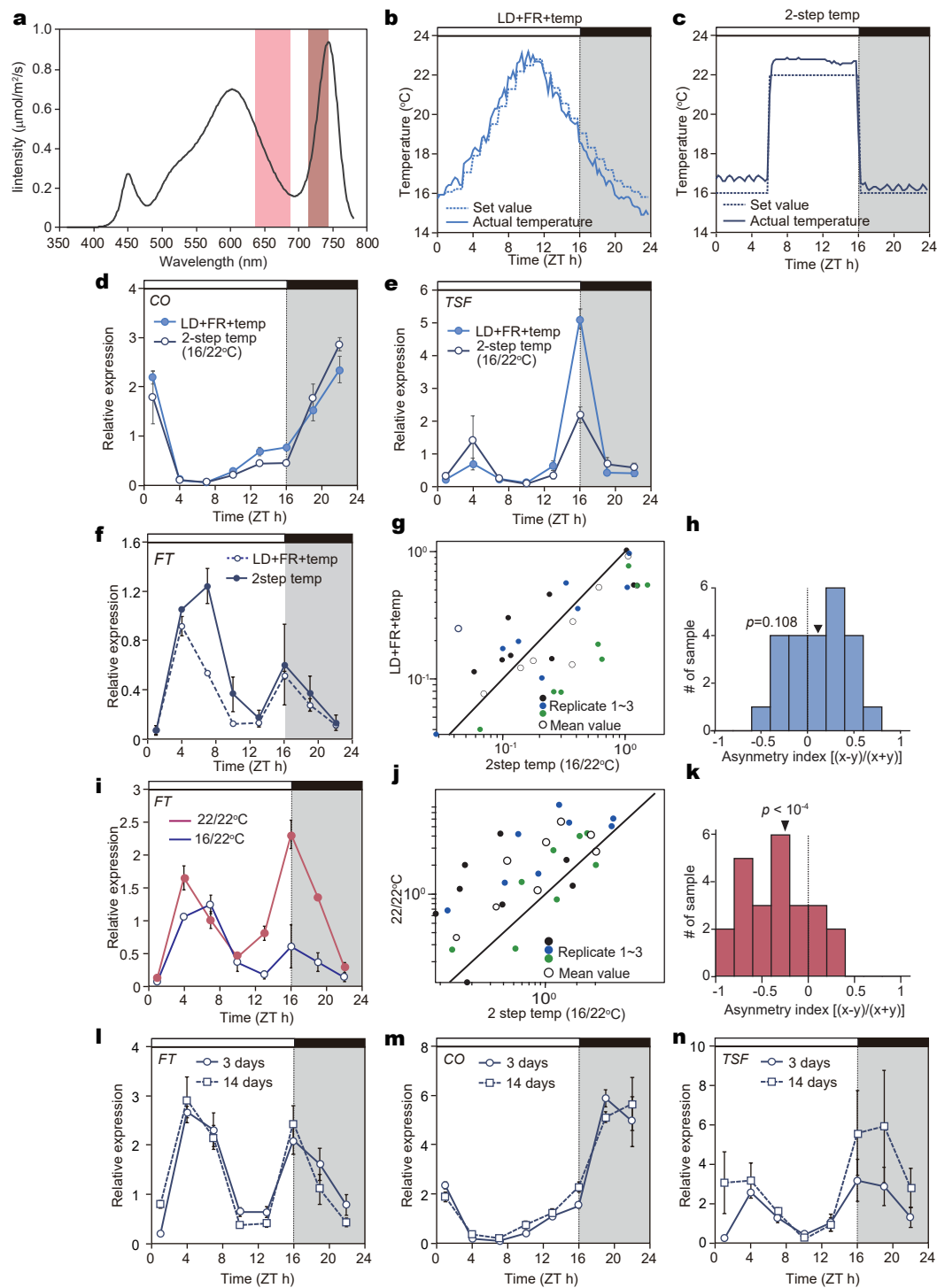




Supplementary Fig. 1

Monthly temperature records from European cities from winter to spring months.

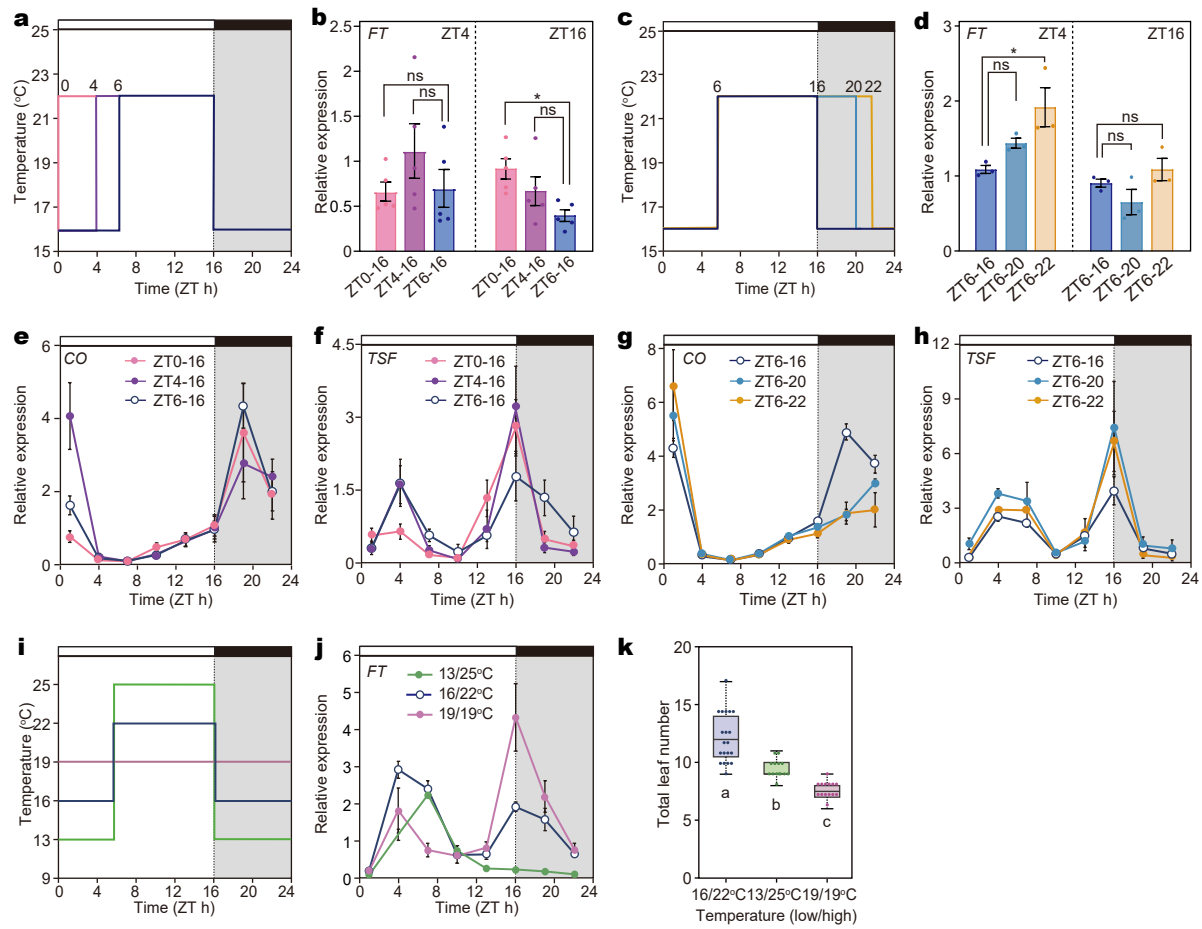
a, The geographical locations of European cities used for the climate data analyses. The latitude of Seattle, USA, was indicated on the left outline. **b-i**, Climate records of 2012 to 2019 in Zürich (**b**, **c**), Stockholm (**d**, **e**), Berlin (**f**, **g**), and Barcelona (**h**, **i**) were used for analyses. Hourly temperature record (**b**, **d**, **f**, **h**), and standard deviation (**c**, **e**, **g**, **i**) of air temperature within 24-hour window. Zeitgeber time (ZT) 0 was set as the sunrise time.



Supplementary Fig. 2

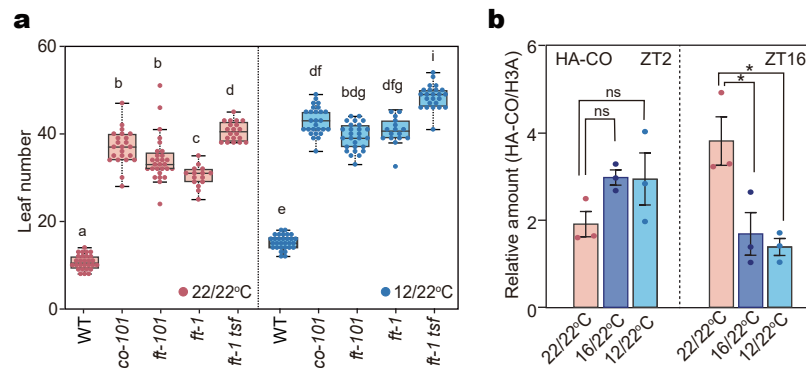
Experimental lab LD conditions used for this study and gene expression profiles of *FT*, *CO*, and *TSF*.

a, Light spectrum of white LED supplemented with dim FR LED used in this study. Light intensities of wavelength used to calculate R/FR ratio (660 ± 20 and 730 ± 15) are highlighted, based on the peak absorption of Pr/Pfr forms of phytochrome. **b-c**, Temperature records of the chambers used in this paper. Gradual change from 16°C to 22°C (**b**, LD+FR+temp, Song et al., 2018) were simplified to 2-step scheme (**c**, 16/22°C). Set values and actual records of chamber temperature were shown in broken and solid lines, respectively. **d-e**, Results of Q-PCR analysis showing *CO* (**d**) and *TSF* (**e**) expression under LD+FR+temp (**b**) and 2-step temperature change (**c**). The geometric mean value from gradual temperature conditions was set to 1. **f**, Expression profiles of *FT* between LD+FR+temp (**b**) and 16/22°C (**c**). The same figure from **Fig. 1e** was used. **g**, A scatter plot showing the correlation of *FT* values at each time point obtained in (**f**). The solid line indicates a reference line where *FT* values between the two conditions are identical. **h**, Distribution of asymmetry index calculated by the formula $(FT_{LD+FR+temp} - FT_{16/22^\circ C}) / (FT_{LD+FR+temp} + FT_{16/22^\circ C})$ obtained from each time point. Significant difference from normal distribution were tested by one-sample T-test ($n = 24$). **i-k**, Comparison of *FT* expression profiles between 22/22°C and 16/22°C (**i**), a scattered plot to test correlation (**j**), a distribution of asymmetry index (**k**) are shown. **l-n**, Expression profiles of *FT* (**l**), *CO* (**m**), and *TSF* (**n**) in the plants entrained under 16/22°C for 3 or 14 days. All gene expression results (means $\pm$ s.e.m.) in this paper were normalized against *IPP2* and *PP2A* from three biologically independent samples.



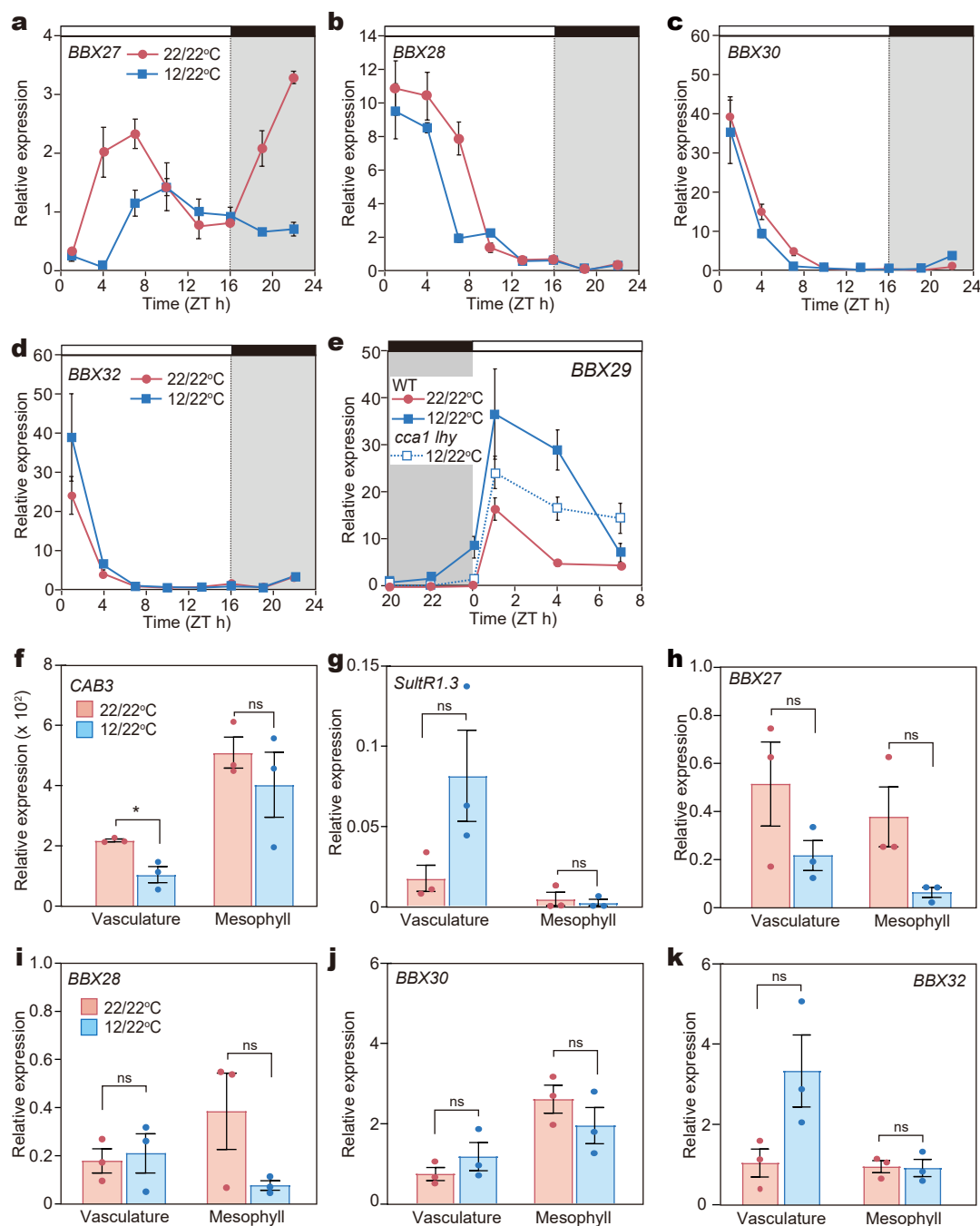
Supplementary Fig. 3 Temperature regulates *FT* expression in time-dependent manner.

a, c, i, Comparison of 2-step temperature conditions with different transition timing (**a**; low-to-high, **c**; high-to-low), or different amplitudes (**i**). **b, d**, Effect of the temperature transition timings on the expression levels of *FT* on ZT4 and ZT16. Asterisks indicate significantly different groups and ns indicates no significance compared to ZT16-6 samples ($p < 0.05$, Dunnett's test, $n = 5$). **e-h**, Expression profiles *CO* (**e** and **g**) and *TSF* (**f** and **h**) under the conditions described in (**a**) or (**c**). Gene expression values are all relative to the geometric mean value from 16/22°C, ZT6-16 (as indicated in solid blue line with open circles). **i-k**, Effect of the day/night temperature difference on amplitude on daily expression profiles of *FT* (**j**) and flowering time (**k**). Each box is located between the upper and the lower quartiles and the whiskers indicate the 1.5-times interquartile ranges. The horizontal lines in the boxes represent the median. Letters indicate significantly different groups ($p < 0.05$) based on Tukey's HSD test. $14 \leq n \leq 20$.



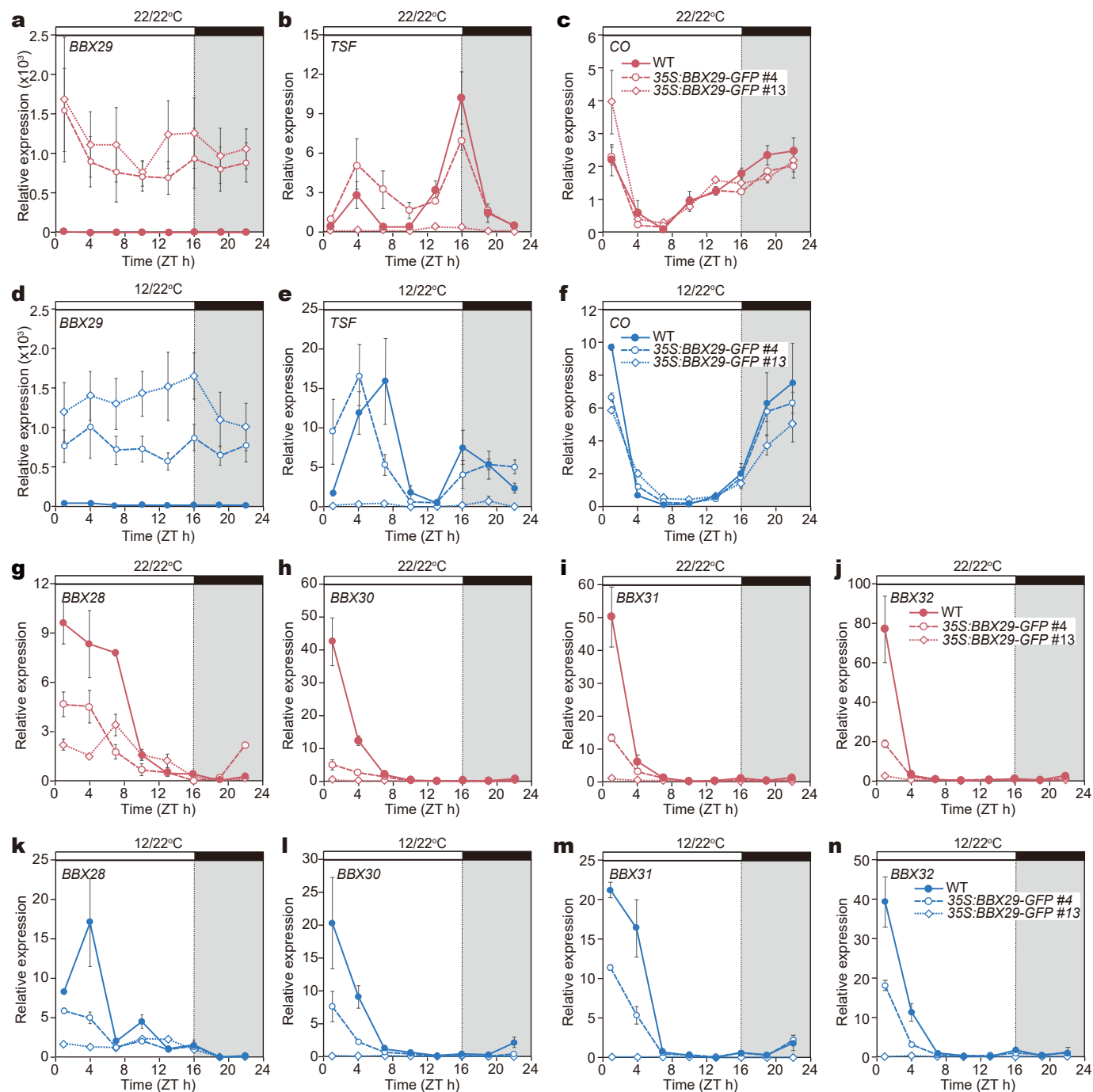
Supplementary Fig. 4 Involvement of *CO-FT* module in flowering repression under cooler night-to-morning temperature.

a, Flowering time results of late-flowering mutants under 22/22°C and 12/22°C. Each box is located between the upper and the lower quartiles and the whiskers indicate the 1.5-times interquartile ranges. The horizontal lines in the boxes represent the median. Letters indicate significantly different groups ($p < 0.05$) based on Tukey's HSD test. $12 \leq n \leq 18$. **b**, Effect of night-to-morning temperature reduction on the accumulation of CO protein on ZT2 and ZT16. Asterisks indicate significantly different groups and ns indicates no significance compared to 22/22°C conditions ($p < 0.05$, Dunnet's test, $n = 3$).



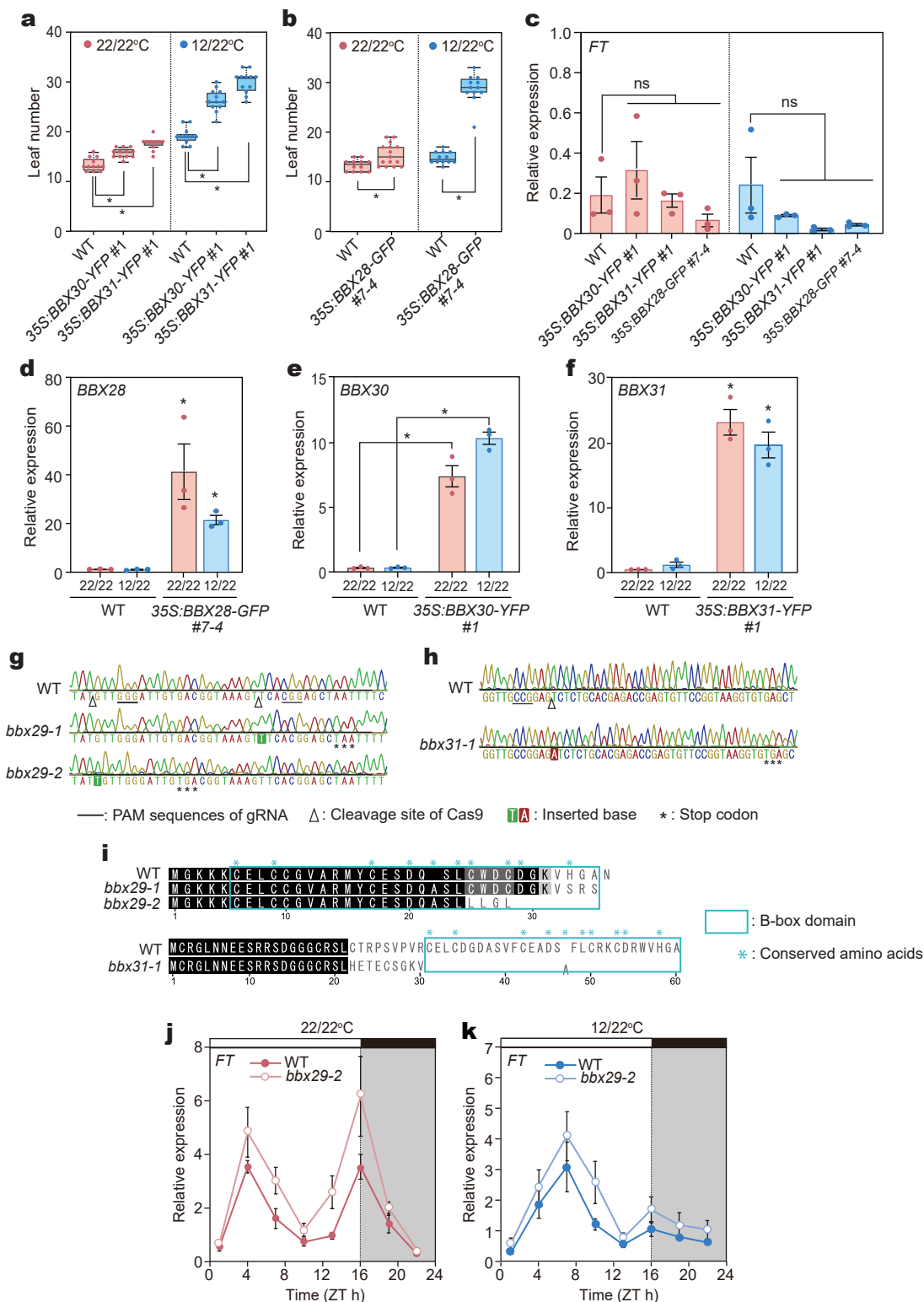
Supplementary Fig. 5 Expression of Group V *BBX* genes in response to the reduction of night-to-morning temperature.

a-d, Expression profiles of *BBX27* (**a**), *BBX28* (**b**), *BBX30* (**c**), *BBX32* (**d**) under 22/22°C and 12/22°C. **e**, Detailed expression profiles of *BBX29* from ZT20 to ZT4 under 22/22°C and 12/22°C. WT expression is shown in solid line, whereas *cca1 lhy* under 12/22°C is shown in dotted line. All expression values are relative to the geometric mean value of wild-type expression under 22/22°C. **f-k**, Expression of marker genes and Group V *BBX* genes in mesophyll and vascular tissues isolated from WT plants on ZT1. *CAB3* (**f**) and *SultR1.3* (**g**) were used as mesophyll and vasculature marker to confirm successful isolation. Bars represent the mean, error bars represent the s. e. m. ($n = 3$). Astarisks indicate significantly different groups ($p < 0.05$) and ns indicates no significance based on Welch's T-test or Student's T-test.



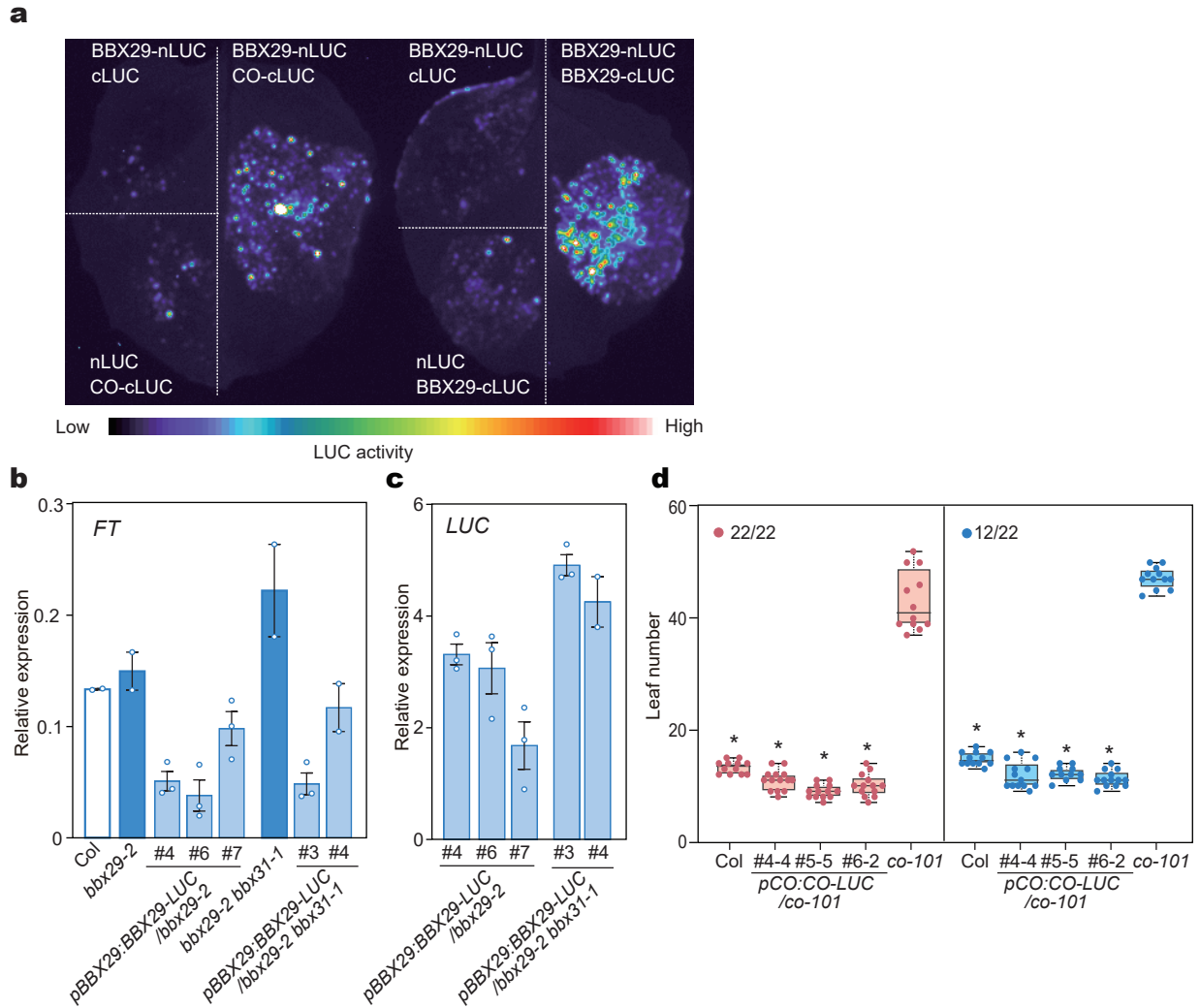
Supplementary Fig. 6 Overexpression of *BBX29* alter the expression patterns of flowering regulators and other Group V *BBX* genes.

a-n, Expression profiles of *BBX29* (**a**, **d**), *TSF* (**b**, **e**), *CO* (**c**, **f**), *BBX28* (**g**, **k**), *BBX30* (**h**, **l**), *BBX31* (**i**, **m**), and *BBX32* (**j**, **n**) under 22/22°C (**a-c**, **g-j**) and 12/22°C (**d-f**, **k-n**). All expression values are relative to the geometric mean value of wild-type expression under 22/22°C.



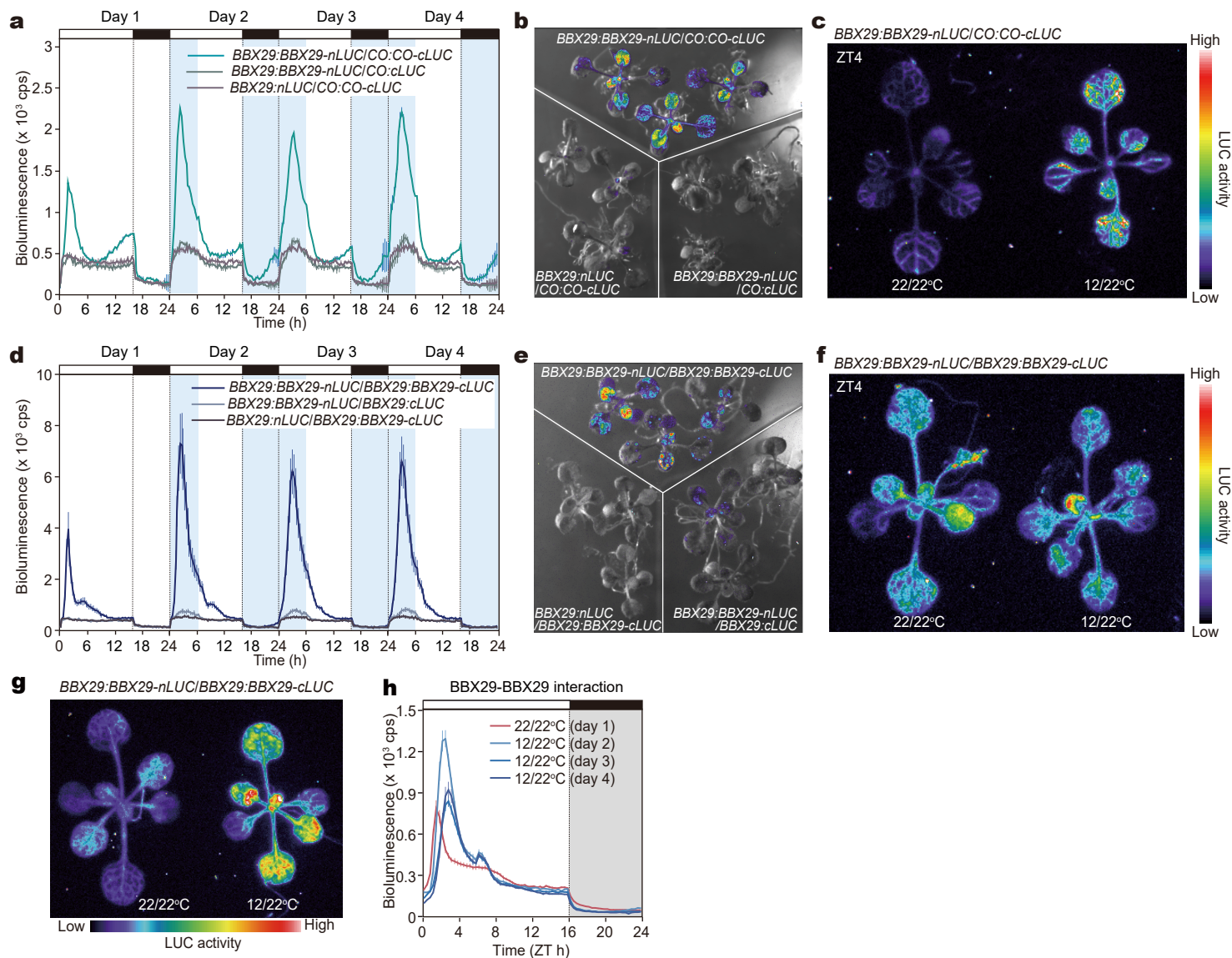
Supplementary Fig. 7 Group V members of *BBX* genes act as flowering repressors.

a-b, Flowering time of plants overexpressing *BBX28* (**a**) and *BBX30*, *31* (**b**) under 22/22°C and 12/22°C. Box plots display the upper and the lower quartiles and the whiskers indicate the 1.5-times interquartile ranges. The horizontal lines in the boxes represent the median. Asterisks indicate significantly different groups based on Student's T-test (**a**) or Dunnet's test (**b**). $p < 0.05$, $n = 12$. **c**, *FT* expression on ZT4 in the overexpression lines described in (**a**, **b**). **d-f**, Expression level of *BBX28* (**d**), *BBX30* (**e**), and *BBX31* (**f**) in corresponding overexpression lines. The expression values are relative to the mean value of wild-type gene expression. Bars represent the mean, error bars represent the s. e. m. ($n = 3$). Asterisks indicate significant difference and ns indicates no significance based on Dunnet's test (**c**, $p < 0.05$) or Student's T-test (**d-f**, $p < 0.05$). **g-h**, DNA sequences of *BBX29* (**g**) and *BBX31* (**h**) from WT and knock-out mutants. The open arrowheads indicate the cleavage sites of Cas9 and underlined bases are the PAM sequences for the gRNA, and inserted bases are shown as outline characters on filled backgrounds. Asterisks indicated stop codons from altered reading frame. **i**, Amino acid alignment of B-box domain from WT and mutants of *bbx29* or *bbx31*. Zinc-binding amino acids conserved among the B-box domain are marked. **k-l**, Expression profiles of *FT* in WT and *bbx29-2* under 22/22°C (**k**) and 12/22°C (**l**). Expression values are relative to the geometric mean value of wild-type *FT* expression.



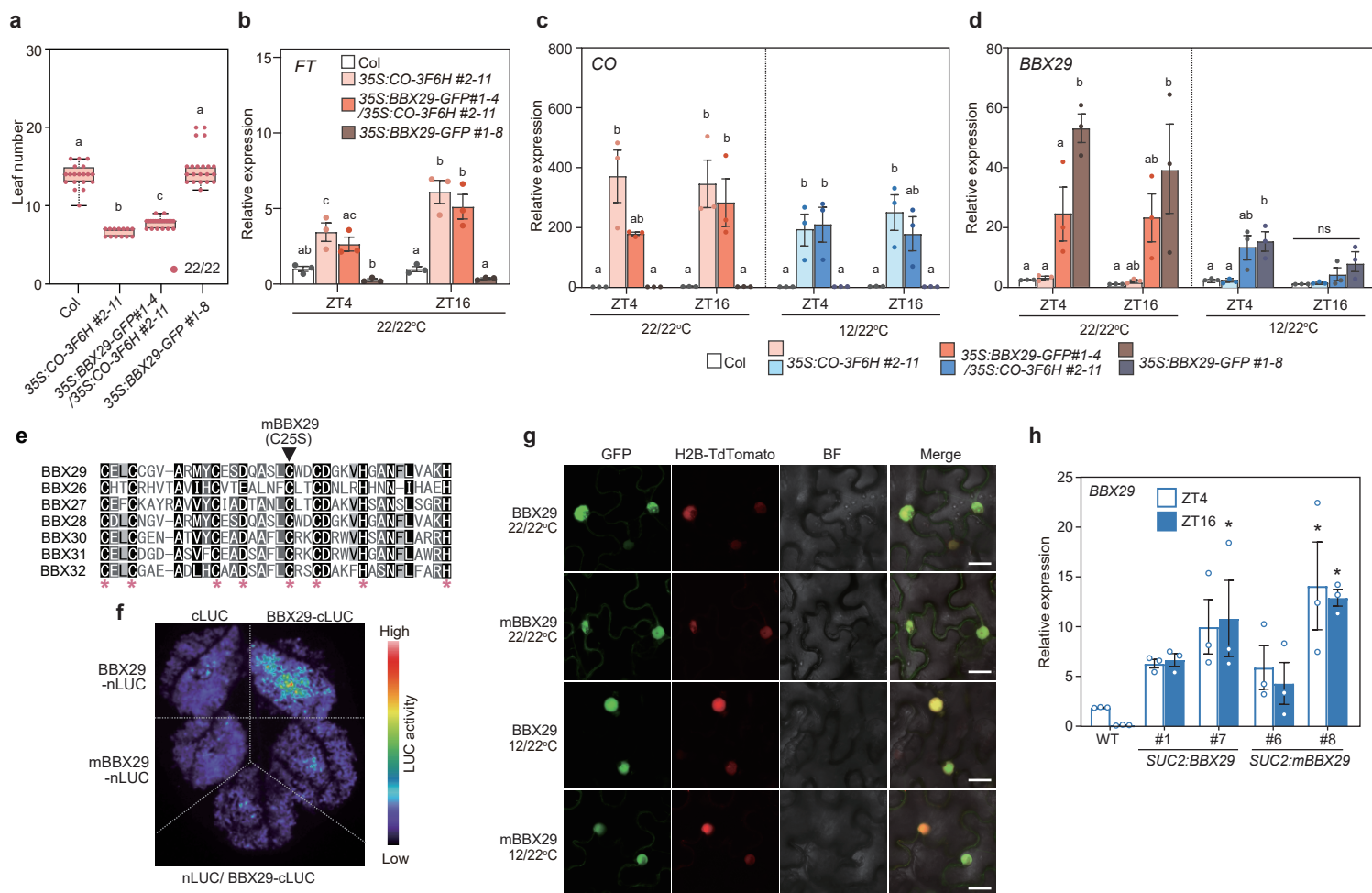
Supplementary Fig. 8 BBX29 and CO form a protein complex and regulate flowering.

a, Results of split-LUC assay showing the heterodimerization and homodimerization of BBX29 and CO in *N. benthamiana* leaves. **b-c**, Expression level of *FT* (**b**) and *LUC* (**c**) in the mutants of *bbx29* and *bbx29 bbx31* complemented by *BBX29:BBX29-LUC*. Plants were harvested on ZT4 of 12/22°C. **d**, Flowering time of the *co* mutant complemented by *CO:CO-LUC* under 22/22°C and 12/22°C. Box plots display the upper and the lower quartiles and the whiskers indicate the 1.5-times interquartile ranges. The horizontal lines in the boxes represent the median. Asterisks indicate significant difference against *co-101* based on Dunnett's test. $p < 0.05$, $n = 12$.



Supplementary Fig. 9 Cool temperature enhances morning interaction of BBX29 and CO in the vasculature.

a-h, *In vivo* split-LUC assay from *BBX29:BBX29-nLUC/CO:CO-cLUC* (**a-c**) and *BBX29:BBX29-nLUC/BBX29:BBX29-cLUC* (**d-h**). Graphs represent the real-time monitoring of bioluminescence from interactions between BBX29-CO (**a**) or BBX29-BBX29 (**d**) with corresponding negative controls (means $\pm$ s.e.m., $n = 5$). N- or C-terminal half of luciferase driven by either *BBX29* or *CO* promoter were used as negative controls. Temperature transition from 22/22°C to 12/22°C was done on ZT0 of Day2, as highlighted by light blue. Experiments were repeated 3 times with similar results. Bioluminescence images of plants from ZT2 of Day 2 are shown in (**b**) and (**e**). Close images of *BBX29:BBX29-nLUC/CO:CO-cLUC* (**c**) and *BBX29:BBX29-nLUC/BBX29:BBX29-cLUC* (**f**) are taken from ZT4 of 22/22°C or 12/22°C conditions. **g-h**, Results from split-LUC assay testing homodimerization of BBX29. Images were taken on ZT2 of 22/22°C and 12/22°C (**g**). Graphs represent real-time monitoring of bioluminescence from *BBX29:BBX29-LUC* (**h**) during the transition from 22/22°C (Day 1) to 12/22°C (Day 2 to Day 4). Data are presented in mean $\pm$ s.e. m. ($n = 10$). cps; counts per second.



Supplementary Fig. 10 *BBX29* partially suppress the effect of *CO* to promote flowering.

a, Flowering time of plants overexpressing *CO-3F6H* and/or *BBX29-GFP* under 22/22°C. Box plots display the upper and the lower quartiles and the whiskers indicate the 1.5-times interquartile ranges. The horizontal lines in the boxes represent the median. Letters indicate significant difference based on Dunnett's T3 multiple comparison test ($p < 0.05$, $13 \leq n \leq 20$).

b-d, Expression of *FT* (**b**), *CO* (**c**), and *BBX29* (**d**) on ZT4 and ZT16 of 12/22°C and 22/22°C conditions among the lines represented in (**a**). The expression values are relative to the mean value of wild-type gene expression under 22/22°C. Bars represent the mean, error bars represent the s. e. m. ($n = 3$).

e, An amino acid alignment of B-box domain from Group V of BBX family proteins in Arabidopsis. Cys-to-Ser mutation (C25S) introduced in mBBX29 is shown by a black triangle. Asterisks indicate conserved amino acids essential for Zinc binding.

f, Results of split-LUC assay showing the effect of C25S mutation on BBX29 homodimerization in *N. benthamiana* leaves.

g, Subcellular localizations of BBX29-GFP and mBBX29-GFP observed in *N. benthamiana* leaves. Confocal images were taken on ZT4 of 22/22°C and 12/22°C. Scale bars, 10 μ m.

h, Expression of *BBX29* on ZT4 and ZT16 of 12/22°C conditions among the lines harboring *SUC2:BBX29-GFP* and *SUC2:mBBX29C25S-GFP*. Asterisks indicate significant difference based on Dunnett's T3 multiple comparison test against WT ($p < 0.05$, $n = 3$).