

***New Phytologist* Supporting Information**

Article title: **Cool nighttime temperatures induce the expression of *CONSTANS* and *FLOWERING LOCUS T* to regulate flowering in *Arabidopsis***

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The following Supporting Information is available for this article:

Fig. S1 Transcript accumulation levels of *ISOPENTENYL PYROPHOSPHATE/DIMETHYLALLYL PYROPHOSPHATE ISOMERASE (IPP2)* remain unaltered over the day across three temperature treatments.

Fig. S2 Growth is delayed and *FLOWERING LOCUS T (FT)* level is depressed in seedlings exposed to constant 12°C temperatures.

Fig. S3 Growth is delayed and *FLOWERING LOCUS T (FT)* level is reduced in seedlings exposed to constant 17°C temperatures.

Fig. S4 *CONSTANS (CO)* protein stabilization is not altered during the cool night.

Fig. S5 Proposed mechanism for the transcriptional regulation of *FLOWERING LOCUS T (FT)* in long days with temperature changes.

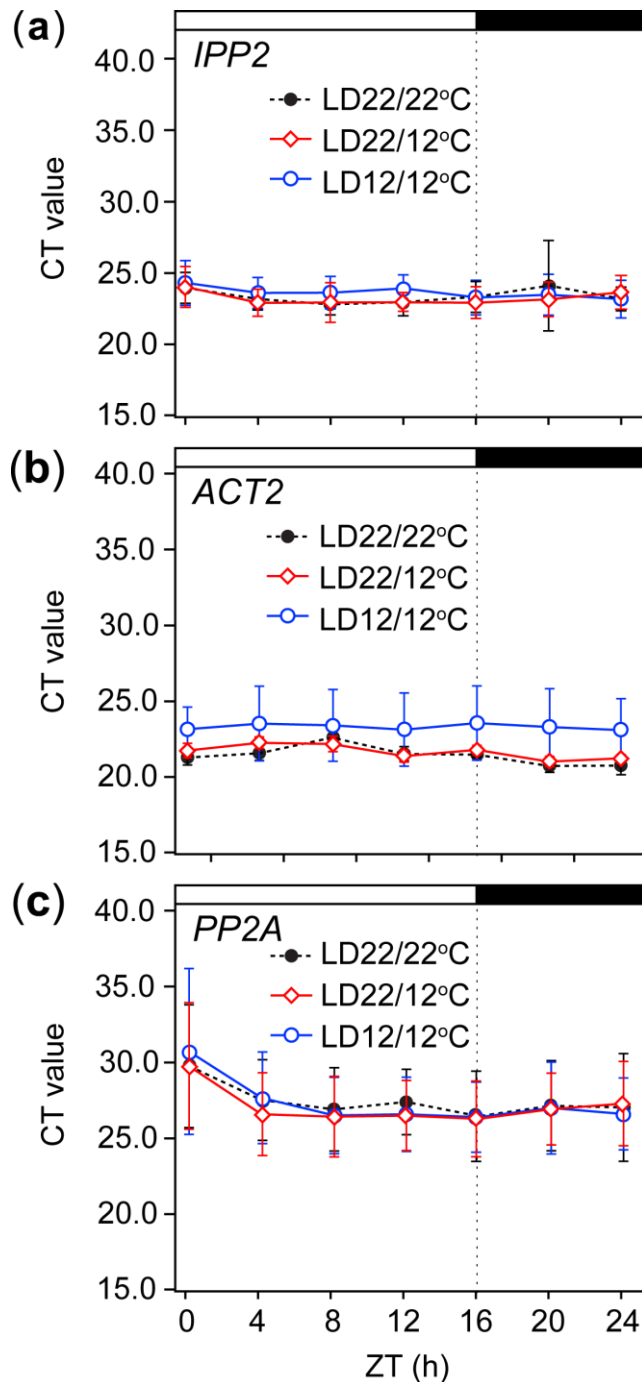


Fig. S1 Transcript accumulation levels of *ISOPENTENYL PYROPHOSPHATE/DIMETHYLALLYL PYROPHOSPHATE ISOMERASE* (*IPP2*) remain unaltered over the day across three temperature treatments. (a, b, c) Transcript accumulation patterns of *IPP2*, *ACTIN 2* (*ACT2*), and *SERINE/THREONINE PROTEIN PHOSPHATASE 2A* (*PP2A*) in 11-d-old Col-0 plants in LD22/22°C (long days with day : night temperatures = 22°C : 22°C), LD22/12°C, and LD12/12°C conditions. Seedlings were grown for 7 d in short-day conditions before being transferred to LD temperature treatments. Data represent means \pm SEM derived from three biological replicates. Topmost white and black bars designate day and night, respectively. Dotted vertical lines are times of lights off.

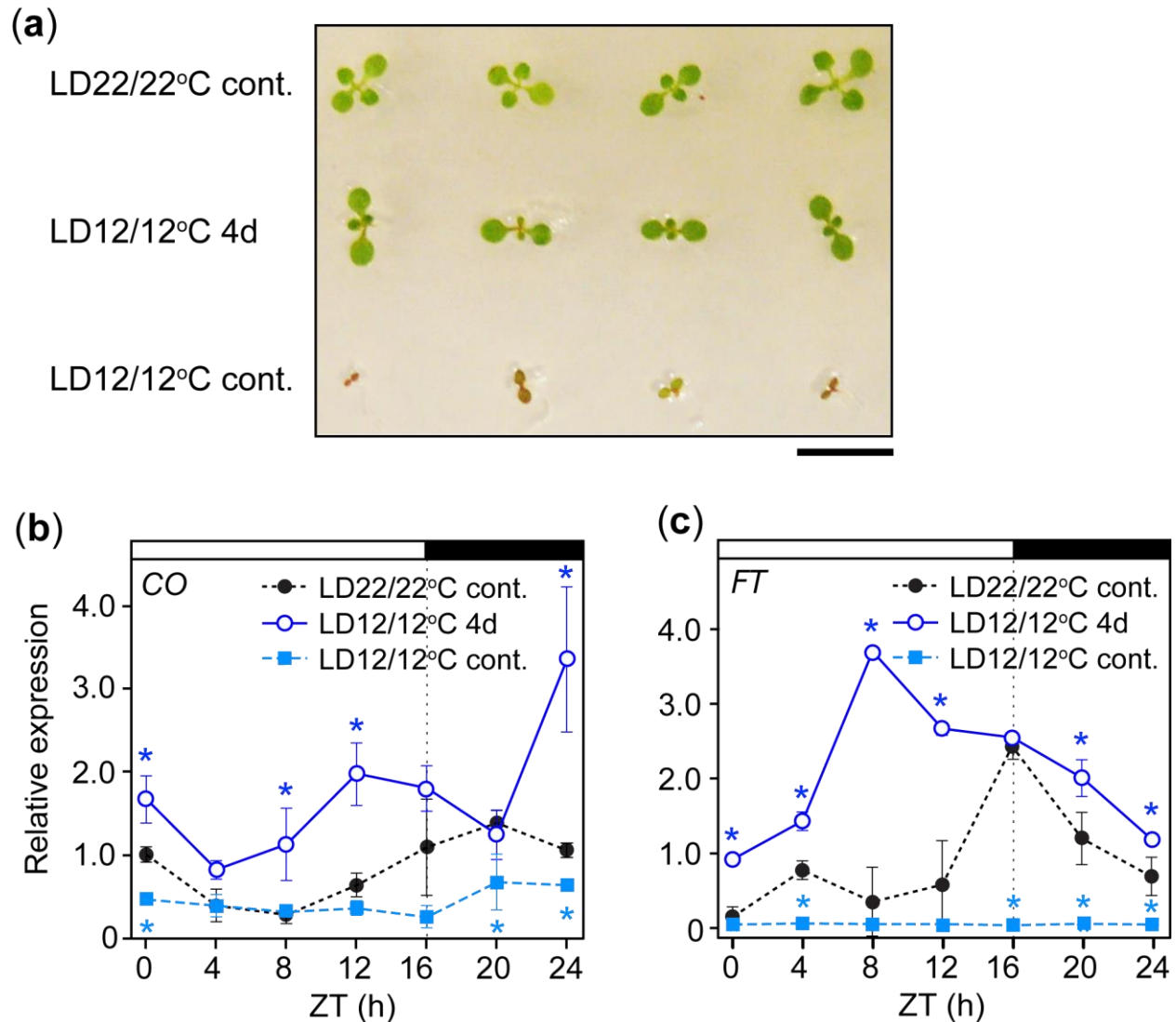


Fig. S2 Growth is delayed and *FLOWERING LOCUS T* (*FT*) level is depressed in seedlings exposed to constant 12°C temperatures. Transcript accumulation patterns of *FT* and *CONSTANS* (*CO*) are very low in seedlings exposed to cool (12°C) temperatures from seed. (a) Visual comparison of individuals grown from seed in LD22/22°C (long days with day : night temperatures = 22°C : 22°C) and LD12/12°C conditions (cont.), or in LD22/22°C for 7 d then transferred to LD12/12°C for 4 d (4d). Photos taken when seedlings were 12 d old. Scale bar, 1 cm. (b, c) Transcript accumulation patterns of *FT* and *CO* in these conditions. Harvest began at dawn when seedlings were 11 d old. Data represent means \pm SEM derived from three biological replicates. Asterisks indicate $P < 0.05$ and 95% CI of difference between pair did not contain zero in statistical comparisons made to the LD22/22°C control. Topmost white and black bars designate day and night, respectively. Dotted vertical lines are times of lights off.

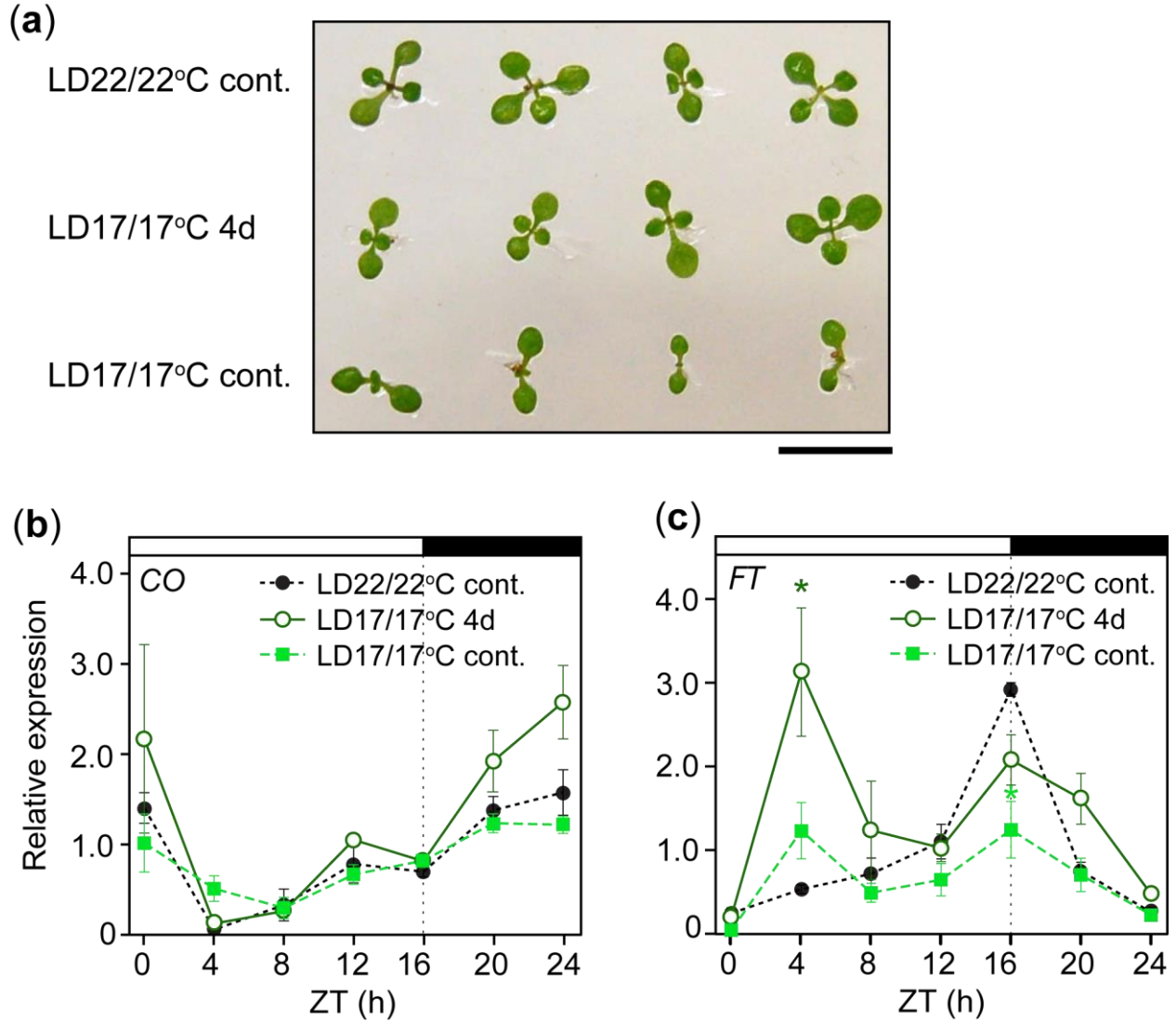


Fig. S3 Growth is delayed and *FLOWERING LOCUS T* (*FT*) level is reduced in seedlings exposed to constant 17°C temperatures. Transcript accumulation patterns of *FT* is reduced in seedlings exposed to cool (17°C) temperatures from seed. (a) Visual comparison of individuals grown from seed in LD22/22°C (long days with day : night temperatures = 22°C : 22°C) and LD17/17°C conditions (cont.), or in LD22/22°C for 7 d then transferred to LD17/17°C for 4 d (4d). Photos taken when seedlings were 12 days old. Scale bar, 1 cm. (b, c) Transcript accumulation patterns of *FT* and *CONSTANS* (*CO*) in these conditions. Harvest began at dawn when seedlings were 11 d old. Data represent means \pm SEM derived from three biological replicates. Asterisks indicate $P < 0.05$ and 95% CI of difference between pair did not contain zero in statistical comparisons made to the LD22/22°C control. Topmost white and black bars designate day and night, respectively. Dotted vertical lines are times of lights off.

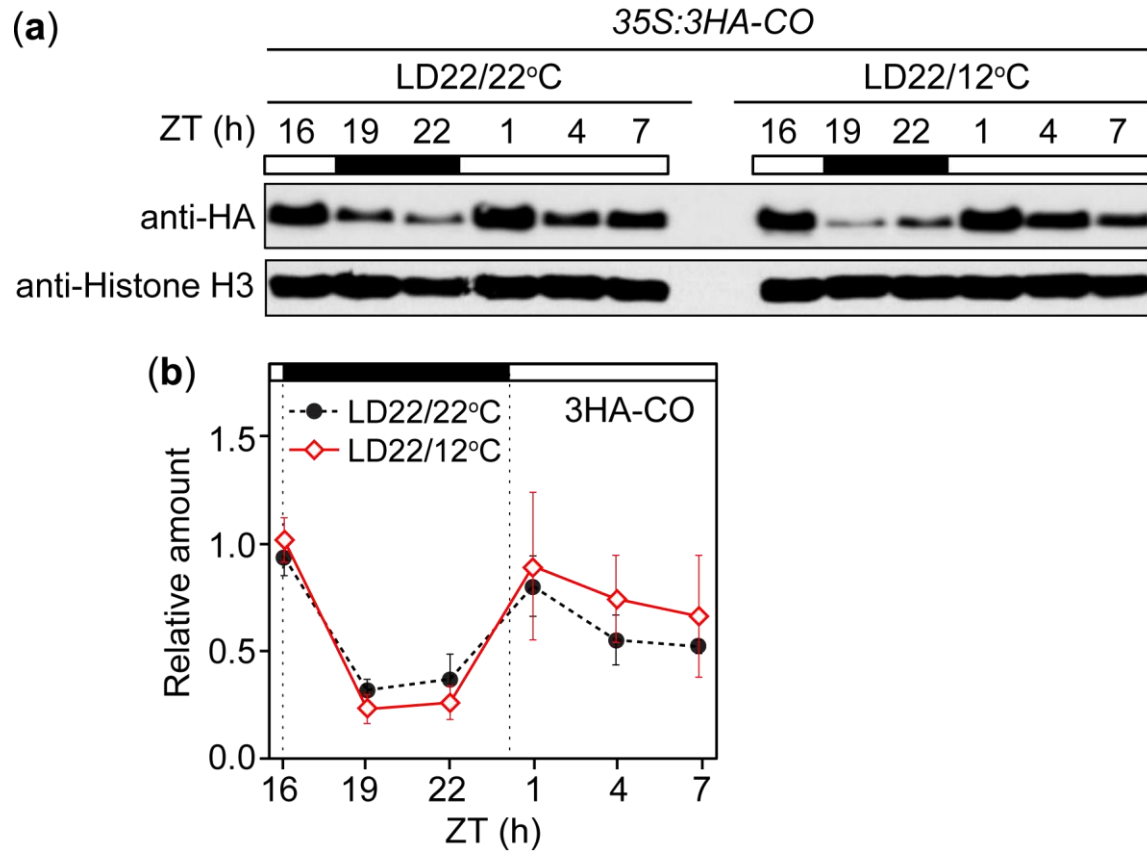


Fig. S4 CONSTANS (CO) protein stabilization is not altered during the cool night. (a, b) Accumulation patterns (a) and quantification (b) of 3HA-CO proteins in *35S:HA-CO* plants grown in LD22/22°C (long days with day : night temperatures = 22°C : 22°C) and LD22/12°C conditions. Histone H3 serves as a loading control. Data represent means \pm SEM derived from three biological replicates. Topmost white and black bars designate day and night, respectively. Dotted vertical lines are times of lights off.

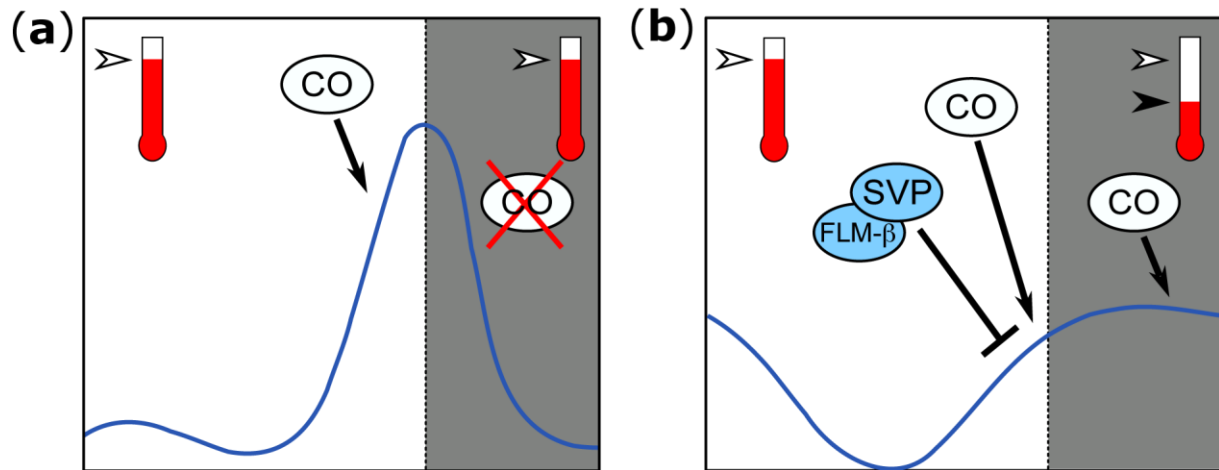


Fig. S5 Proposed mechanism for the transcriptional regulation of *FLOWERING LOCUS T* (*FT*) in long days with temperature changes. (a) As previously shown, *CONSTANS* (*CO*) protein is stabilized in the afternoon of long days and induces *FT* transcript accumulation. (b) When warm/cool temperature cycles are imposed over long days, *SHORT VEGITATIVE PHASE* (*SVP*) in conjunction with *FLOWERING LOCUS M* (*FLM*)- β suppresses *FT* at the end of the light period, whereas more *CO* protein is present at night (gray box) and induces *FT* transcript accumulation.