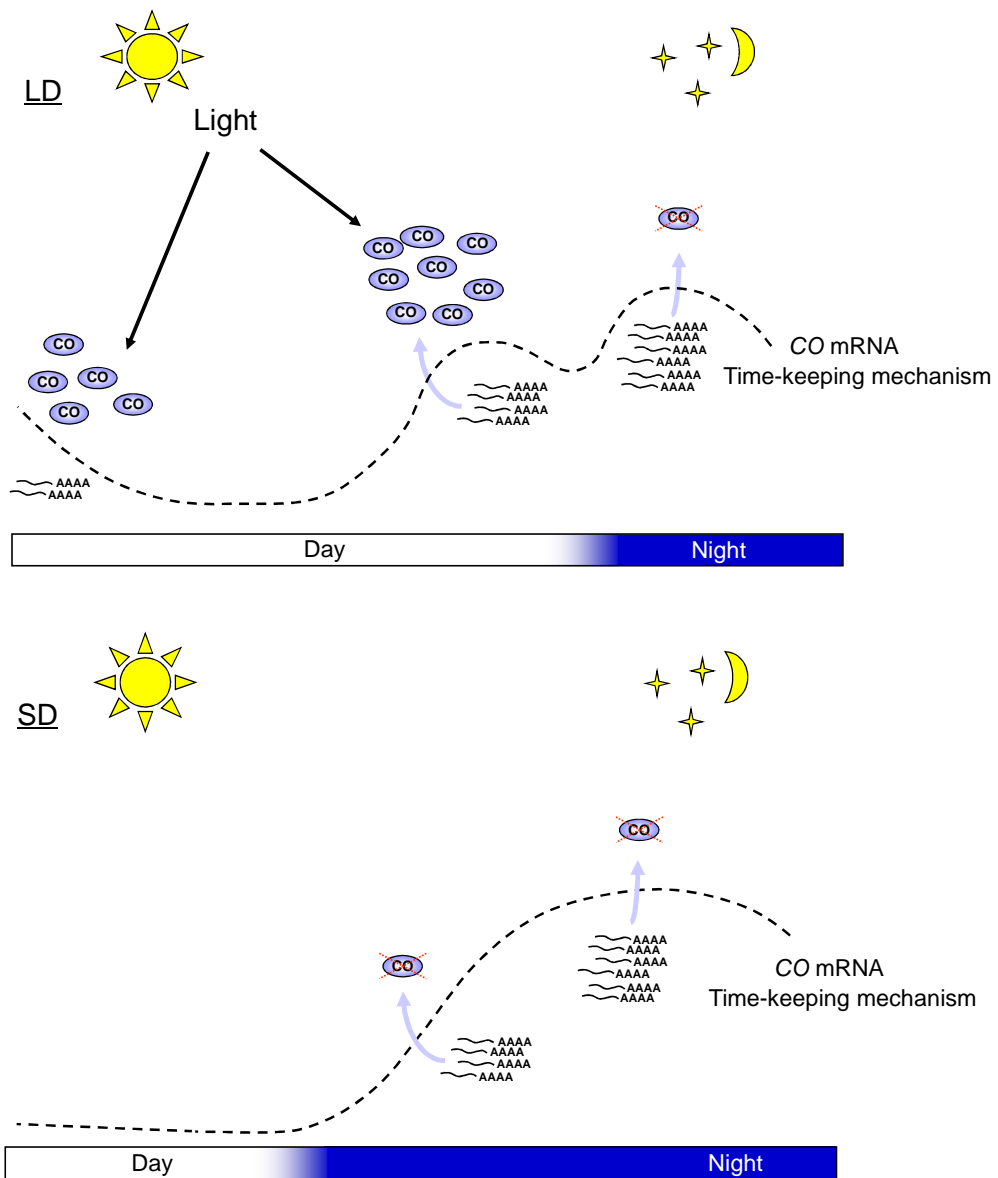


**PSEUDO RESPONSE REGULATORS stabilize CONSTANS protein to promote flowering in response to day length**

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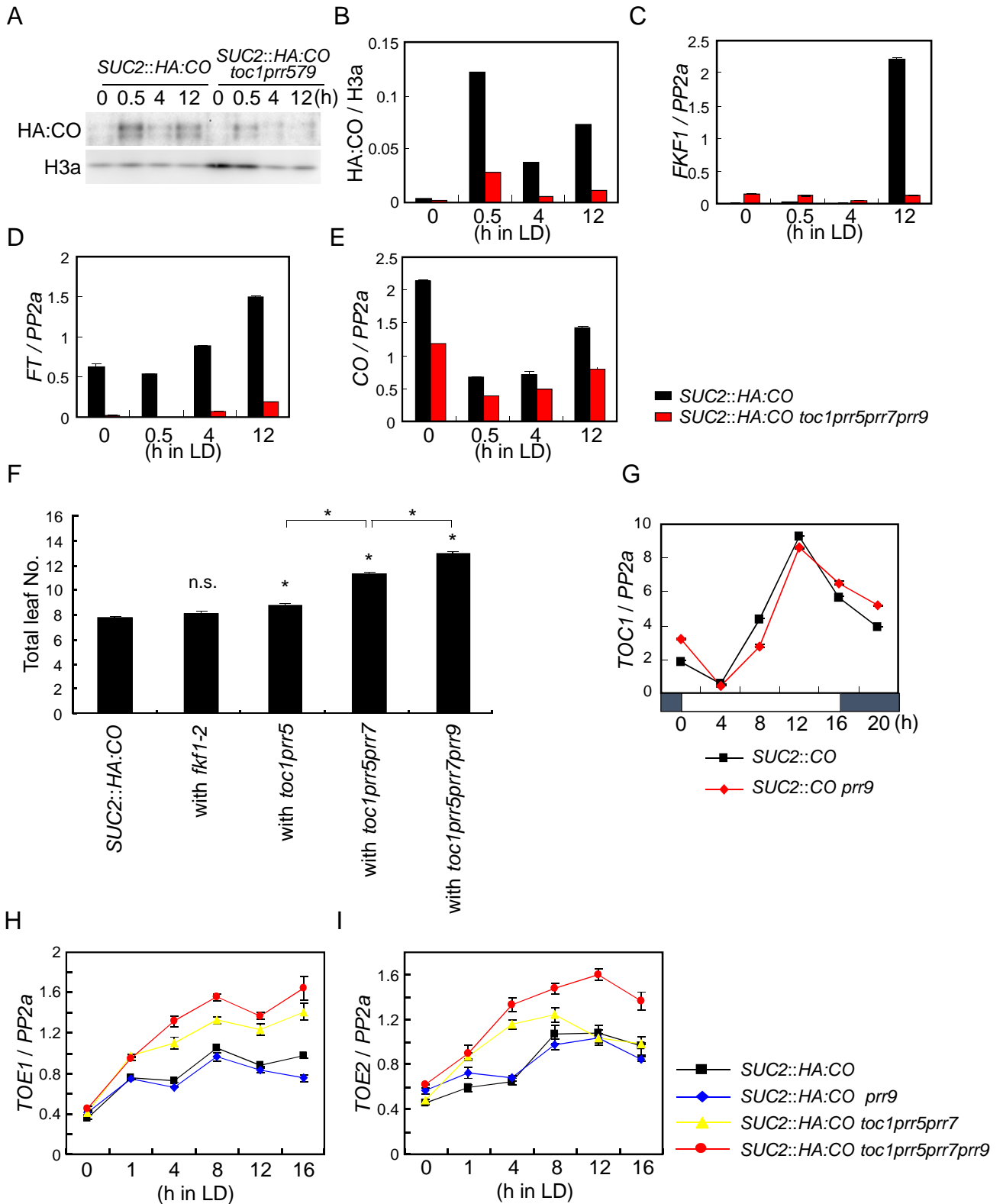
**Appendix Table of Content**

Appendix Figures S1-S4 and legends



### Appendix Figure S1

A model of day-length recognition through *CO*. Accumulation of *CO* transcripts is controlled by the circadian clock, coinciding with exposure of the plant to light in the morning and evening specifically under LD. *CO* protein is stabilized in the light, allowing it to accumulate under LD and promote flowering. By contrast, under SD *CO* is transcribed only in the dark, and under these conditions the protein is rapidly degraded. This model was previously generated by observations that accumulation of *CO* mRNA exhibits particular daily rhythms under LD and SD and that in transgenic plants where *CO* mRNA is constantly expressed *CO* protein accumulates only in the light. The LD-specific accumulation of *CO* protein in wild type is shown in the current paper (Figure 3E and F).

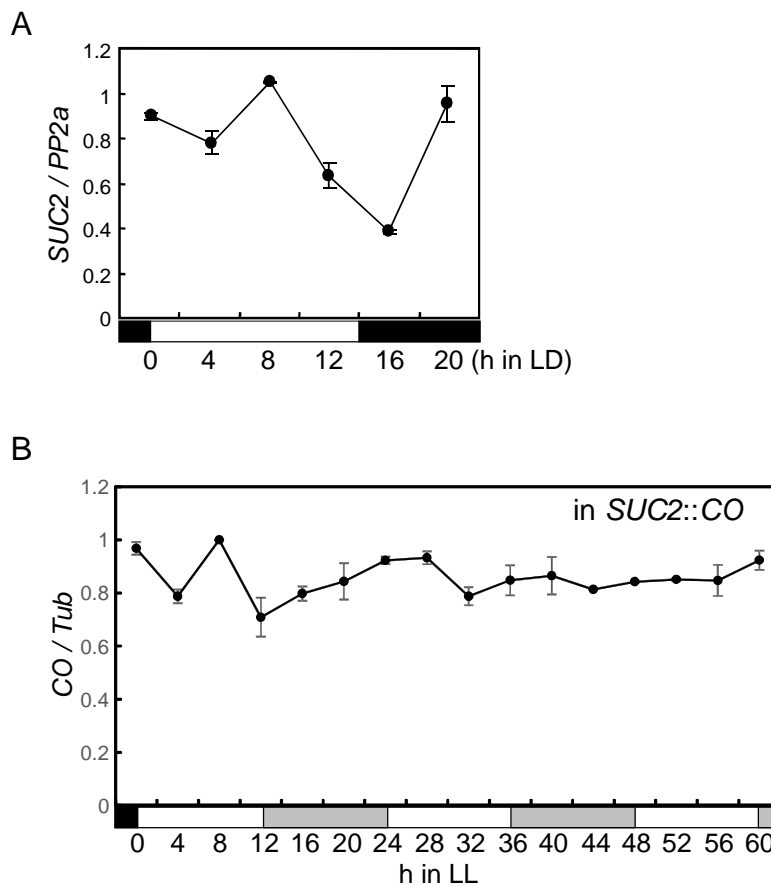


### Appendix Figure S2

Reduced CO protein levels in *prp* mutants are not caused by an indirect effect of impairment of circadian-clock function or a reduction in *FKF1* activity. (**A and B**) Quadruple *toc1 prr5 prr7 prr9* mutants show reduced accumulation of CO protein in the morning in *SUC2::HA:CO*. HA:CO levels were normalized to the Histone 3a levels. (**C-E**) *FKF1*, *FT*, and *CO* mRNA abundance in *prp* quadruple mutant early in the morning under LD.

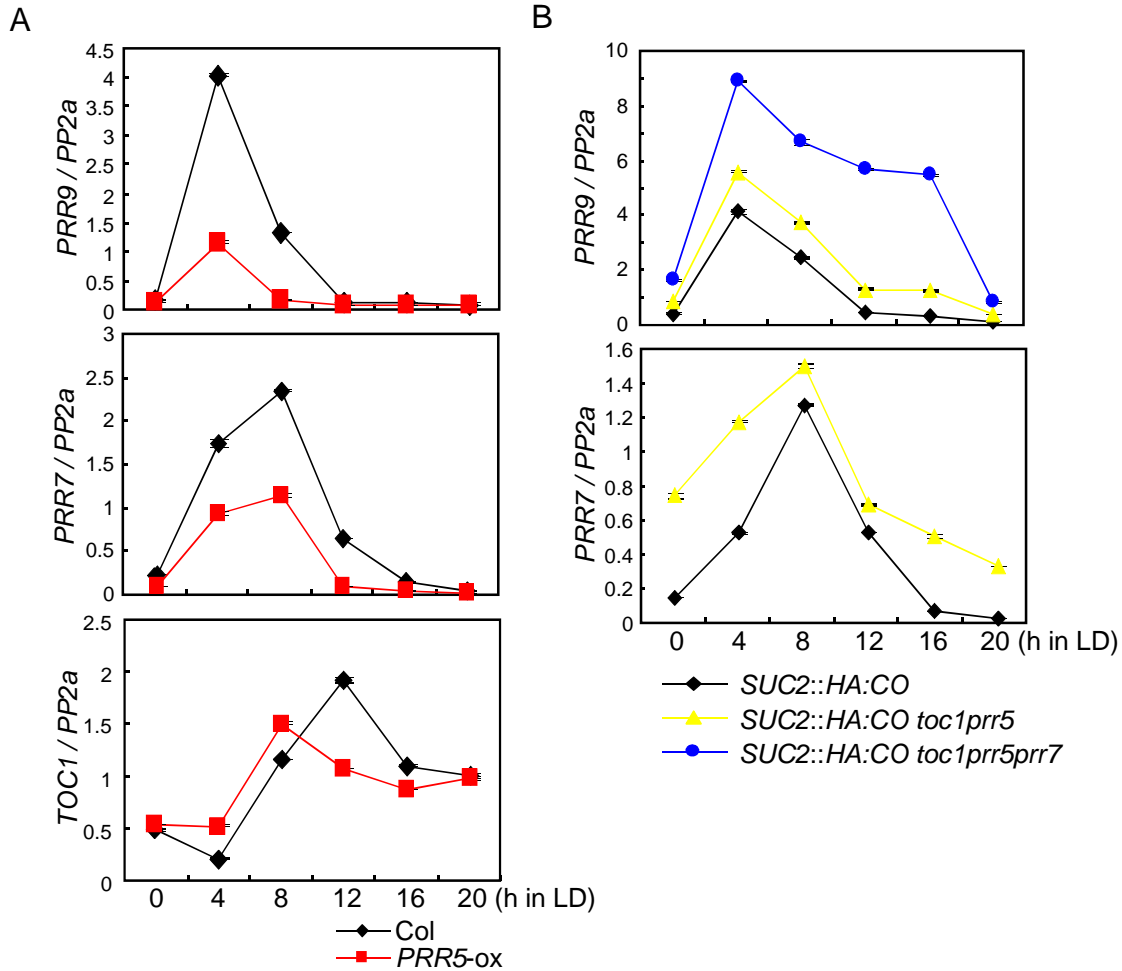
## Appendix Figure S2 (continued)

(F) Effect of *fkf1* mutation on flowering time in the presence of *SUC2::HA:CO*. Approximately 16 plants of each genotype were grown under LDs and the number of total leaves was measured. Error bars indicate standard error. Statistical significance between *SUC2::HA:CO* and each *prr* or *fkf1* mutant, and among multiple *prr* mutants was calculated using Student's t-test; \* P < 0.01; n.s. P > 0.01. (G) *prr9* mutation does not dramatically alter diurnal expression of *TOC1* mRNA under LD. *SUC2::CO* and *prr9 SUC2::CO* were grown under LDs. (H, I) Effect of *prr* mutations on *TOE1* (H) and *TOE2* mRNA (I) abundance in *SUC2::HA:CO* under LD. *SUC2::HA:CO* and the line with *prr* mutations were grown under LDs. In the mRNA expression data the levels of *CO*, *FT*, *TOE1* and *TOE2* were normalized to the *PP2a* levels. Error bars indicate standard error within 2 technical replicates.



## Appendix Figure S3

(A) Expression of *SUC2* mRNA under LD. Col was grown under LDs and harvested every 4 hours. The levels of *SUC2* mRNA were normalized to *PP2a*. Error bars indicate standard error within two technical replicates. (B) Expression of *CO* mRNA in *SUC2::CO* in constant light. These experiments were carried out to check expression of *CO* driven by the *SUC2* promoter and that of the *SUC2* gene. The result shows that the level of *CO* transcripts from the *SUC2* promoter does not exhibit oscillation, consistent with results that the *prr* quadruple mutant was not strongly affected in *CO* or *HA:CO* mRNA levels in *SUC2::CO* or *SUC2::HA:CO*, respectively. Plants were grown under LDs and transferred to continuous light at ZT0, and harvested every 4 hours for 60 hours. In this experiment mRNA from both endogenous and transgenic *CO* were detected. Error bars indicate standard error within two biological replicates. In each replicate two technical replicates were performed.



#### Appendix Figure S4

(A) Expression of *PRRs* in Col and 35S::*PRR5*. Plants were grown under LDs and harvested every 4 hours. The levels of *PRR9*, *PRR7* and *TOC1* mRNA were normalized to those of *PP2a*. This experiment was performed to check the effect of *PRR* overexpression on expression of other *PRRs*. In the *PRR5* overexpressor expression of other *PRRs* is altered. (B) Expression of *PRRs* in *SUC2::HA:CO*, *toc1 prr5 SUC2::HA:CO* and *toc1 prr5 prr7 SUC2::HA:CO*. This experiment was carried out to check daily phases of *PRR* gene expression in *prp* mutants. Results show that peak times of *PRR9* and *PRR7* are not affected in *toc1 prr5 SUC2::HA:CO*. The peak time of *PRR9* expression is not affected in *toc1 prr5 prr7 SUC2::HA:CO*, whereas the level throughout the day/night cycle is increased. The lines were grown under LDs and harvested every 4 hours. The levels of *PRR9* and *PRR7* mRNA were normalized to those of *PP2a*. Error bars indicate standard error within two technical replicates.