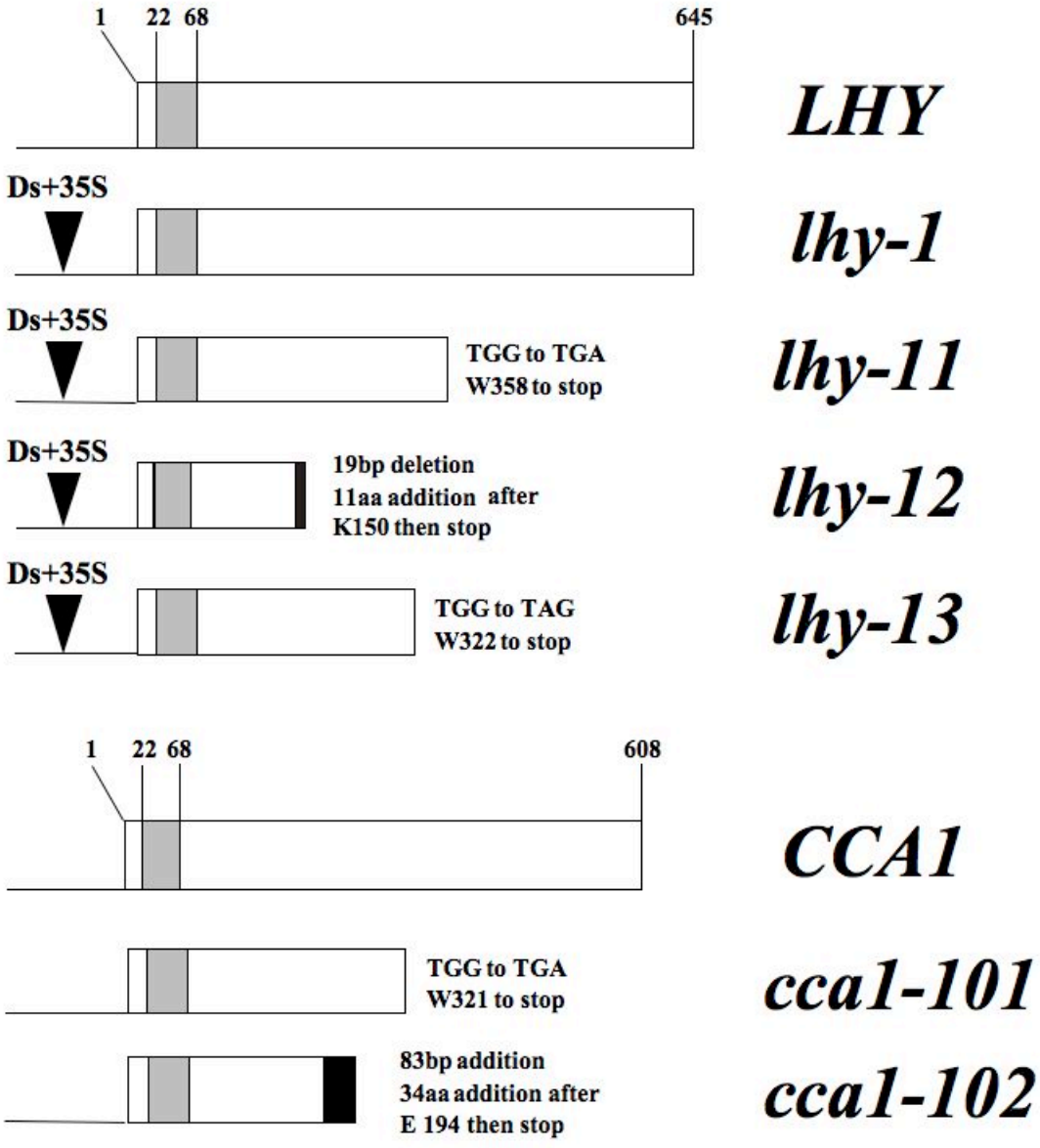
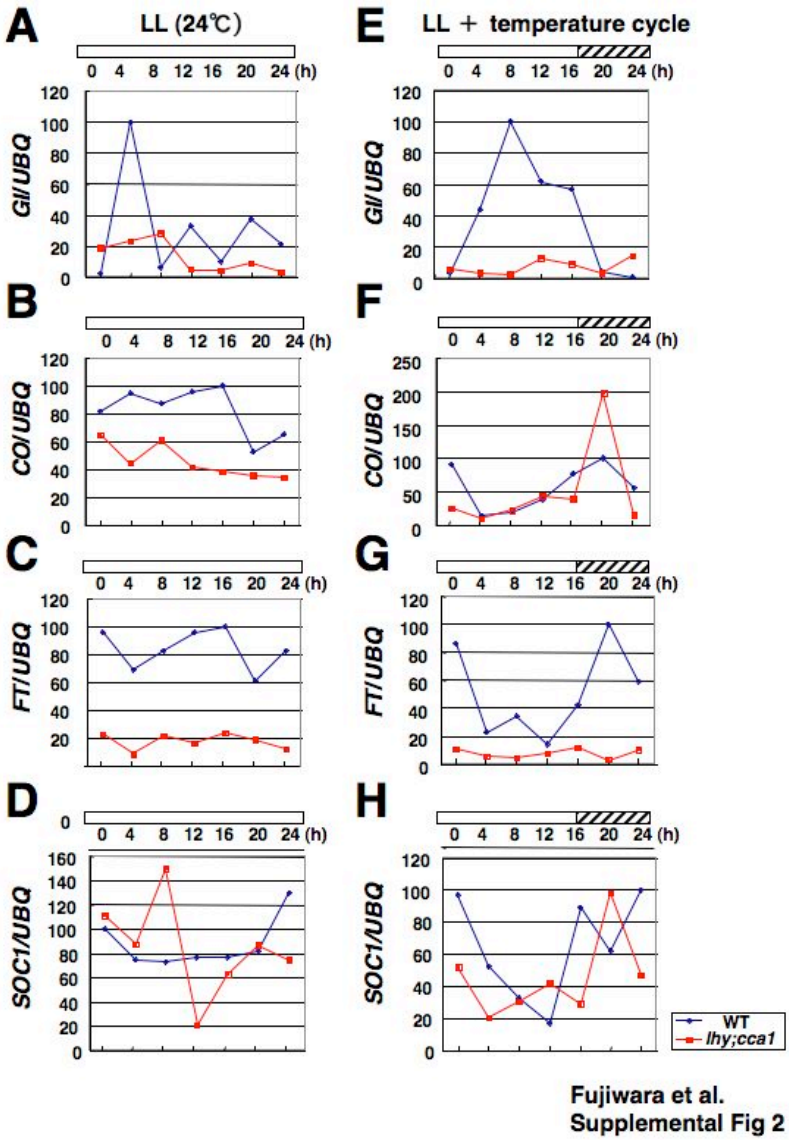
Supplemental Data. Fujiwara et al. (2008). Circadian Clock Proteins LHY and CCA1 Regulate SVP Protein Accumulation to Control Flowering in *Arabidopsis*.

Supplemental Figures



Fujiwara et al. Supplemental Fig 1

Supplemental Figure 1. Summary of *lhy* and *cca1* mutations.

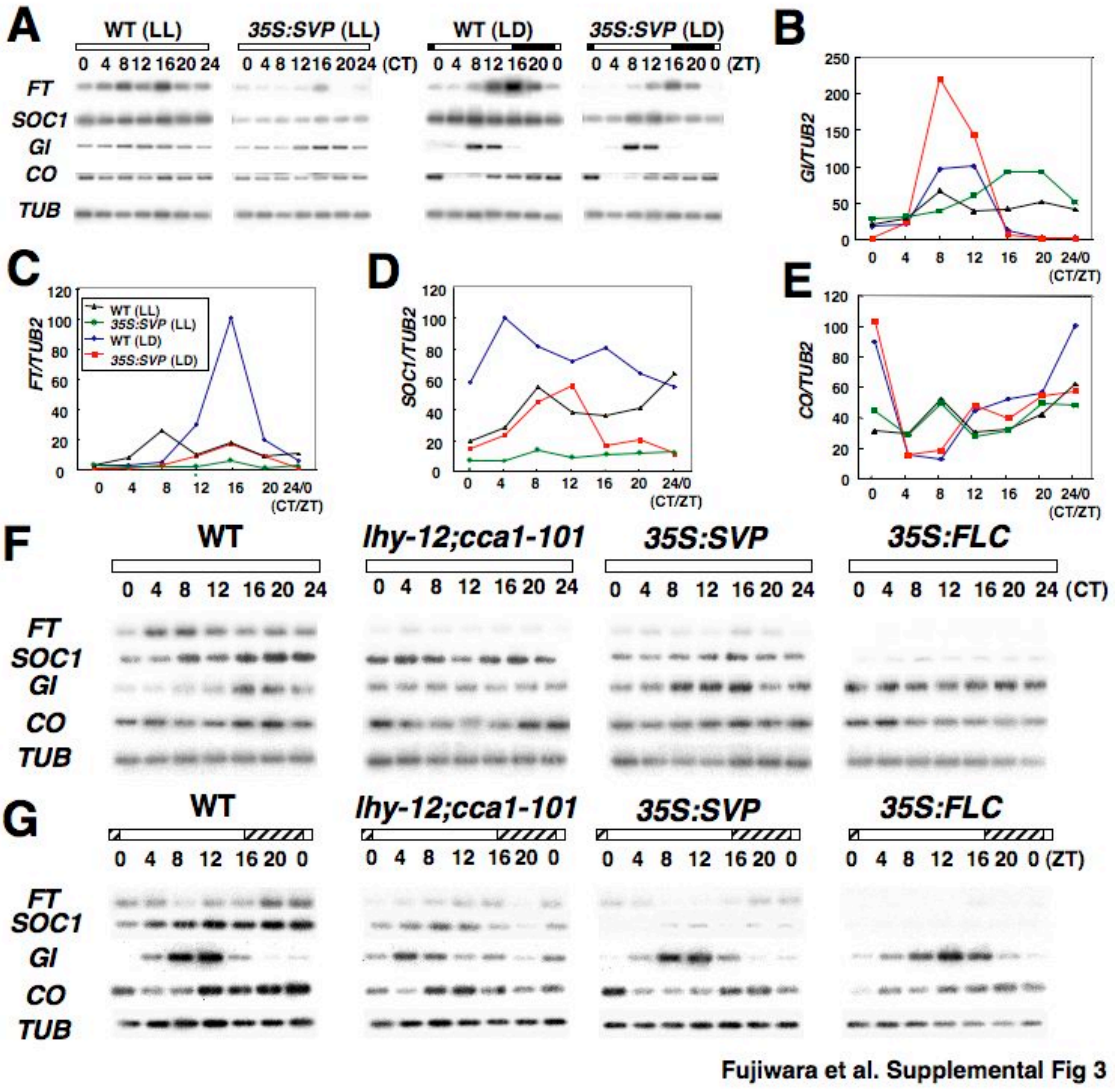


Supplemental Figure 2. Quantitative RT-PCR Analysis of *GI*, *CO*, *FT* and *SOC1* under LL with and without Temperature Cycles.

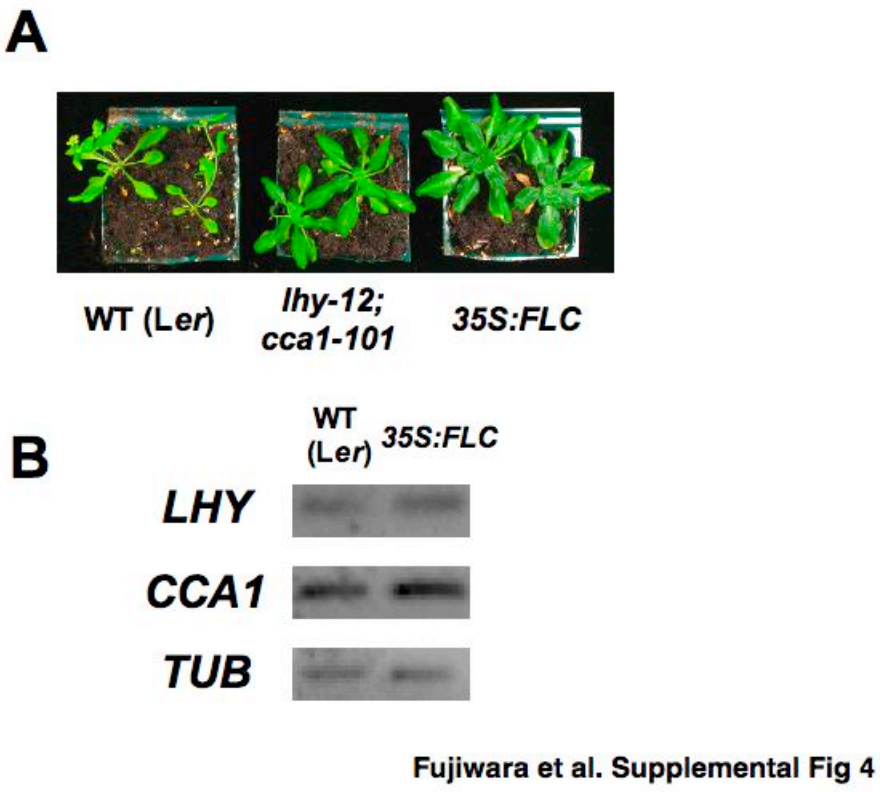
(**A** to **D**) Expression of *GI* (**A**), *CO* (**B**), *FT* (**C**) and *SOC1* (**D**) in wild type (WT) and *lhy-12;cca1-101* grown under LL (24°C). Open boxes represent continuous light conditions and hours from the first sampling were shown above the boxes.

(**E** to **H**) Expression of *GI* (**E**), *CO* (**F**), *FT* (**G**) and *SOC1* (**H**) in wild type (WT) and *lhy-12;cca1-101* grown under temperature cycles (16 h 24°C / 8 h 20°C, LL) for 12 days. Open and striped bars along the horizontal axis represent warm and cold periods, respectively. Hours from dawn (zeitgeber time; ZT) were shown above the bars.

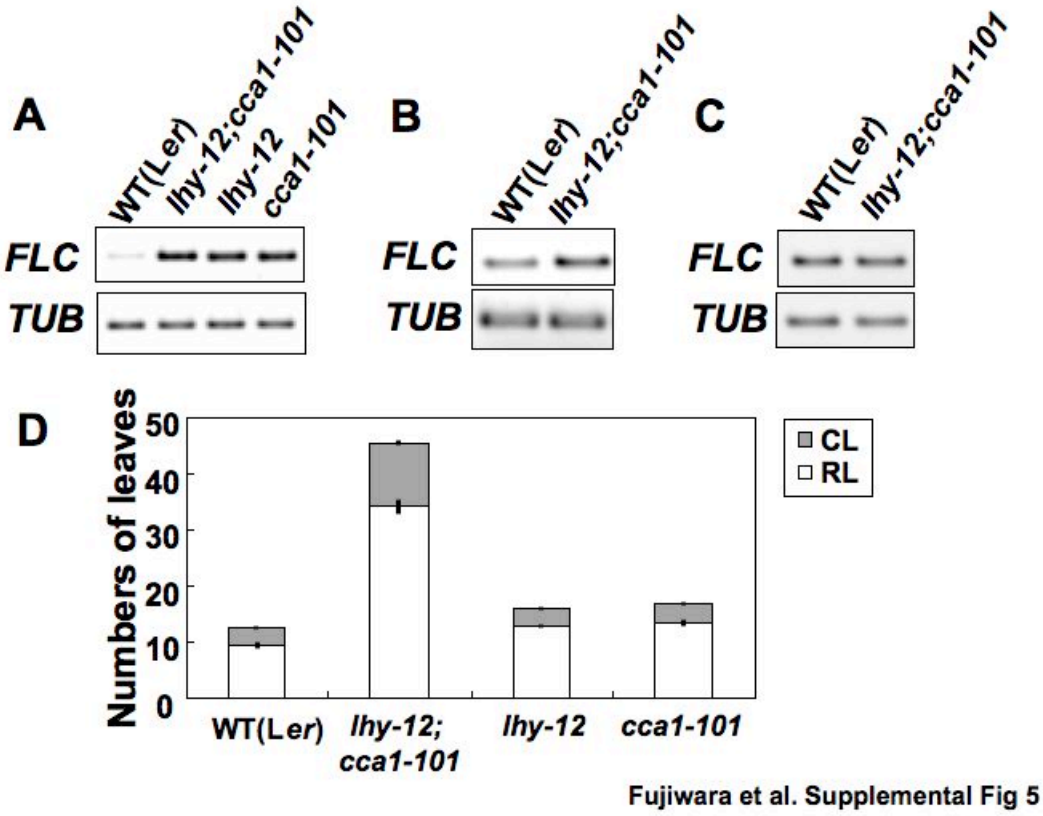
All the experiments in this figure were done at least twice with similar results.



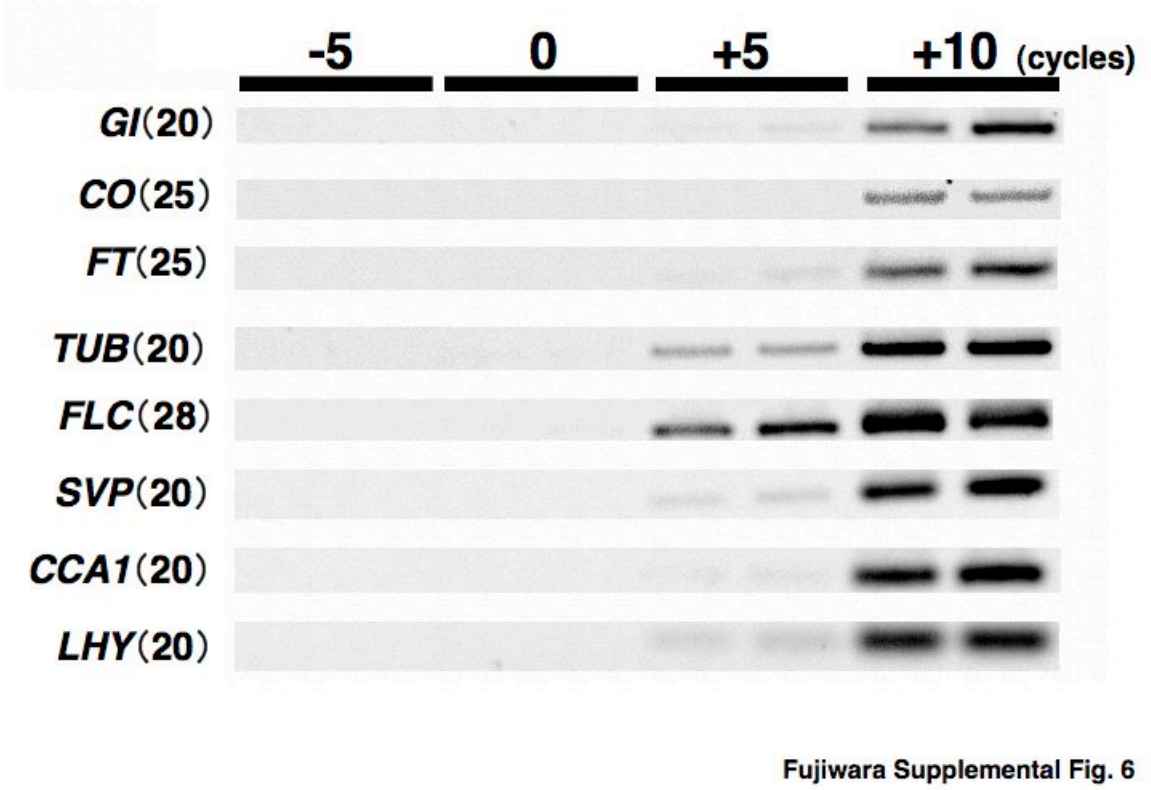
Supplemental Figure 3. Partial redundant functions of SVP and FLC in the control of flowering. (**A**-**E**) Expression of *FT* (**A** and **C**), *SOC1* (**A** and **D**), *GI* (**A** and **B**), *CO* (**A** and **E**) and *TUB* (**A**-**E**) in L*er* WT and *35S:SVP* plants grown under LL and LD for 12 days. Open and dark bars along the horizontal axis represent light and dark periods, respectively and hours from first sampling (LL) and from dawn (LD, zeitgeber time; ZT) were shown below the bars (**A**). (**F** and **G**) Expression of *FT*, *SOC1*, *GI*, *CO* and *TUB* in L*er* WT, *lhy-12;cca1-101*, 35S:*SVP* and 35S:*FLC* plants grown under LL with or without temperature cycles for 12 days. Open bars represent LL and hours from the first sampling were shown (**F**). Open and striped bars along the horizontal axis represent warm (24°C) and cold (20°C) periods, respectively and hours from the beginning of warm period were shown (zeitgeber time; ZT) (**G**).



Supplemental Figure 4. Characterization of *35S:FLC* in LL. (**A**) Wild type *Arabidopsis*, *lhy-12;cca1-101* and *35S:FLC* plants 30 days after sowing in LL. (**B**) Expression of *LHY* and *CCA1* genes in wild type (WT) and *35S:FLC* plants in LL.



Supplemental Figure 5. *FLC* expression in wild type (WT), *lhy-12*, *cca1-101* and *lhy-12;cca1-101* plants under LL. Higher (**A**) and slightly higher (**B**) *FLC* expression in *lhy-12;cca1-101* than that in wild type plants under LL. (**C**) Similar *FLC* expression in *lhy-12;cca1-101* to that in wild type under LL. (**D**) Flowering times of WT, *lhy-12*, *cca1-101* and *lhy-12;cca1-101* plants in LL. CL and RL represent cauline and rosette leaves, respectively. Data are presented as mean +/- SE.



Supplemental Figure 6. PCR cycles for RT-PCR used in Figures 2, 3, 5, Supplemental Figures 3 and 5. Gene names and numbers of cycles used for RT-PCR and Southern blots in this work are shown. L*er* wild type plants were grown under LL for 10 days, RNAs were prepared and cDNAs were synthesized as described in Materials and Methods. PCR was performed using two independent samples with four different PCR cycles: the cycle used for this work (0) and minus 5 (-5), plus 5 (+5) and plus 10 (+10) cycles. Gels were stained with Ethidium bromide and analyzed by Molecular Imager (Bio-Rad, CA, USA).

Supplemental Methods

RT-PCR Analysis of Gene Expression

Plants were grown on soil for 10 days or 14 days and above ground tissue was used for RNA preparation. RT-PCR was performed with *GI*, *CO*, *FT*, *TUB* (Mizoguchi et al., 2005), *SOC1*(Blazquez et al., 2002), FLC (Ratcliffe et al., 2003), *LHY*, *CCA1* and *SVP* (this work) specific primers. RT-PCR procedures were as described in (Fujiwara et al., 2005) with slight modifications. Sequences of primers and PCR conditions (cycles and Tm) are as follows: *GI* (F: CTGTCTTTCTCCGTTGTTTCACTGT, R: TCATTCCGTTCTTCTCTGTTGTTGG, Tm: 55°C, cycles: 20, Mizoguchi et al., 2005), *CO* (F: ACGCCATCAGCGAGTTCC, R: AAATGTATGCGTTATGGTTAATGG, Tm: 60°C, cycles: 25, Mizoguchi et al., 2005), *FT* (F: ACAACTGGAACAACCTTTGGCAATG, R: ACTATATAGGCATCATCACCGTTCGTTACTCG, Tm: 58°C, cycles: 25, Mizoguchi et al., 2005), *TUB* (F: CTCAAGAGGTTCTCAGCAGTA, R: TCACCTTCTTCATCCGCAGTT, Tm: 60°C, cycles: 20, Mizoguchi et al., 2005), *FLC* (F: TTAGTATCTCCGGCGACTTGAACCCAAACC, R: AGATTCTCAACAAGCTTCAACATGAGTTCG, Tm: 58°C, cycles: 28, Ratcliffe et al., 2003), *SOC1* (F: GGATCGAGTCAGCACCAAACC, R: CCCAATGAACAATTGCGTCTC, Tm: 58°C, cycles: 22), LHY (F: GCCTGGGAACAACGGTACA, R: GGTCTTACTTGTTTCAATGTCG, Tm: 56°C, cycles: 20), CCA1 (F: CGTGAAAGGTGGACTGAGGAAGAAC, R: GCGGAAAGTGCTTGCGTTTGATGTC3, Tm: 58, cycles: 20) and *SVP* (F: GGAGAGGAACTTCAAGGACT, R: CCATAGGCAGAAACTTACAC, Tm: 58°C, cycles: 20, this work). Signals were detected by Southern blot. We confirmed that the PCR amplification was not saturated with the number of PCR cycles used for the experiments (Supplemental Figure 6). All RT-PCR analyses were performed at least twice with independent RNA samples.

Quantitative Real-Time RT-PCR Analysis of Gene Expression

Quantitative analysis of gene expression was performed by Realtime RT-PCR using Roche Light Cycler apparatus and SYBR Green I detection (Supplemental Figure 2). Ubiquitin (UBQ) was used as a control to normalize the amount of cDNA. For the quantification of gene expression the following primers were used. *FT*: 5' GGAACAACCTTTGGCAATGAGAT 3' and 5' CTGCCAAGCTGTCGAAACAA 3'. *GI*: 5' CCCAAGTAGTGAGAATGACT 3' and 5' CACCACTACACCATCGGAAA 3'. *CO*: 5' AACTGCAGCGTACCACAGAC 3' and 5' GGATGAAATGTATGCGTTATGG 3'. *SOC1*: 5' GGATCGAGTCAGCACCAAACC 3' and 5' CCCAATGAACAATTGCGTCTC 3'. *UBQ*: 5' GAATACCTCCTTGTCCTGGATCT 3' and 5' GTACTTTGGCGGATTACAACATC 3'.

Supplemental References

Fujiwara, S., Nakagawa, M., Kamada, H., Coupland, G., and Mizoguchi,T. (2005) Circadian clock components in *Arabidopsis* I. The *terminal flower 1* enhances the early flowering phenotype of a circadian clock mutant, *lhy cca1*. Plant Biotech. **22:** 311-317.

Mizoguchi, T., Wright, L., Fujiwara, S., Cremer, F., Lee, K., Onouchi, H., Mouradov, A., Fowler, S., Kamada, H., Putterill, J., and Coupland, G. (2005) Distinct roles of *GIGANTEA* in promoting flowering and regulating circadian rhythms in Arabidopsis. Plant Cell **17:** 2255-2270.