Supporting Information

Chen et al. 10.1073/pnas.1719455115

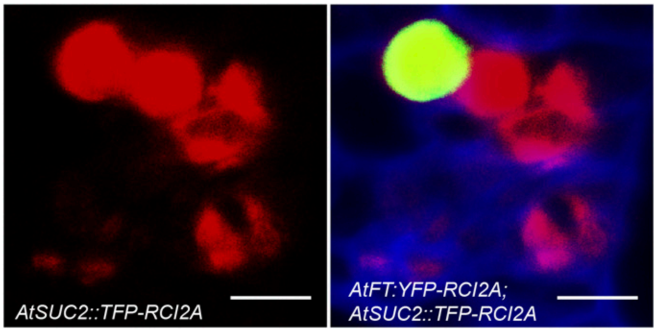

Fig. S1. The *AtFT* and *AtSUC2* promoters are active in the same cell in the minor vein phloem of an *Arabidopsis* leaf. The vein, from a transverse hand section, was also stained with Calcofluor white (*Right*). Red cells are companion cells, as determined by the *AtSUC2:TFP-RCI2A* construct. One companion cell in the top left quadrant of the vein also activated the *AtFT* promoter (*Right*, green fluorescence). Other cell types in the vein (ie., sieve elements and phloem parenchyma cells) cannot be resolved in this image. (Scale bars: 5 μm.)

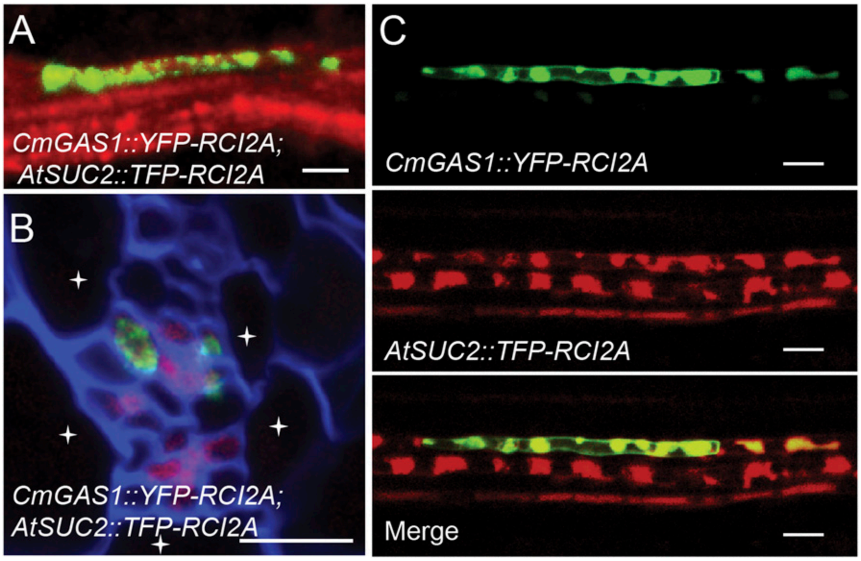

Fig. S2. Localization of *CmGAS1* promoter activities in *Arabidopsis* veins by confocal microscopy in paradermal (*A*), transverse (*B*), and longitudinal (*C*) views. Tissue in *A* was cleared with ClearSee, whereas tissues in *B* and *C* are hand sections. Cell walls are stained blue by Calcofluor white. B, bundle sheath cell. Companion cells express *AtSUC2::TFP-RCI2A* (red), and a subset of companion cells express *CmGAS1::YFP-RCI2A* (green). (Scale bars: 10 μm.)

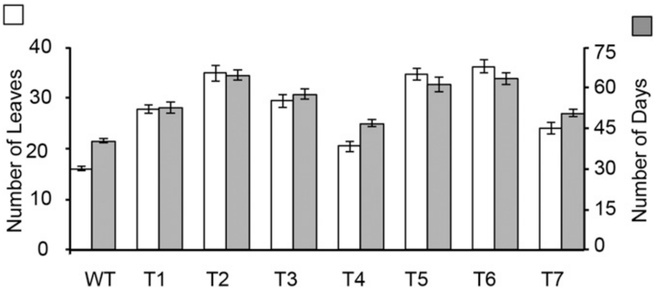

Fig. S3. Plants expressing the diphtheria toxin gene *CmGAS1::DT-A* are late flowering. Phenotype of WT and transgenic (T1–T7) plants expressing the diphtheria toxin gene *CmGAS1::DT-A*. The total number of leaves, and number of days, required for flowering in long days were counted. In every case, means were statistically different from WT based on two-tailed *t* tests with equal variance ($P < 0.05$). Error bars represent SE. n = 13–15 plants.

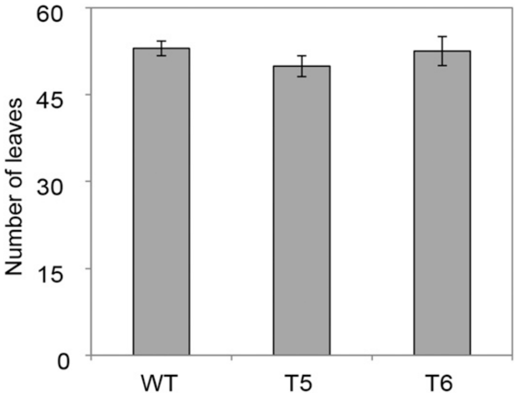

Fig. S4. Number of leaves before flowering in short days. Number of leaves in WT and *CmGAS1::DT-A* plants T5 and T6 at the time of flowering in non-inductive conditions (10 h light, 14 h dark). Error bars represent SE. $n = 6–10$.

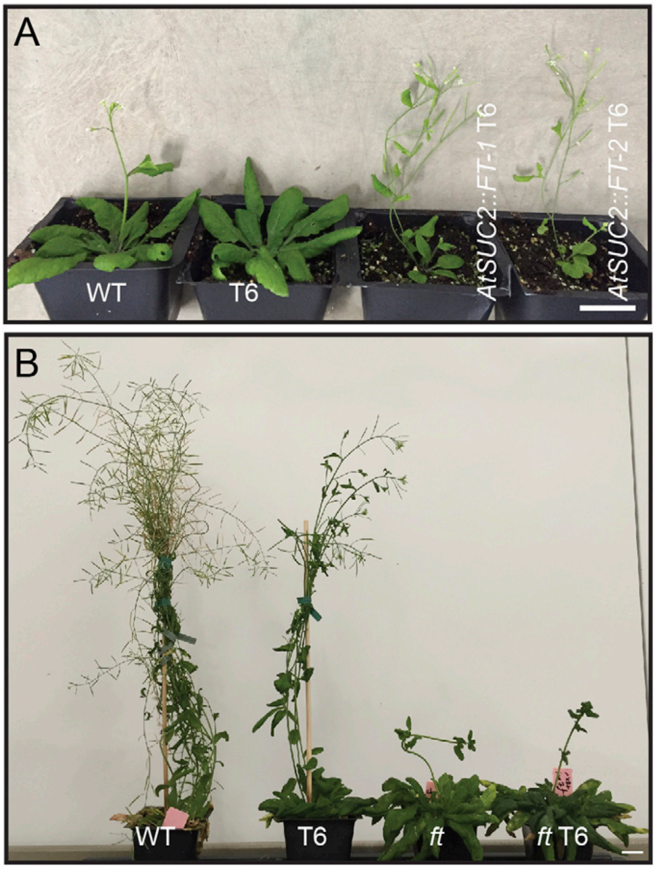

Fig. S5. Additional and reduced *FT* expression results in earlier and later flowering, respectively, in plants with ablated *CmGAS1*-activating cells. (*A*) Two representative independent lines, *AtSUC2::FT-1* and *AtSUC2::FT-2*, in the T6 background were photographed with WT and T6 when WT plants had just started flowering. (*B*) Expression of the diphtheria toxin in *CmGAS1*-activating cells (T6 line) inhibits flowering, but not as strongly as the *ft* mutants or *ft* mutants with ablated *CmGAS1*-activating cells (*ft T6*). (Scale bars: 1 cm.)

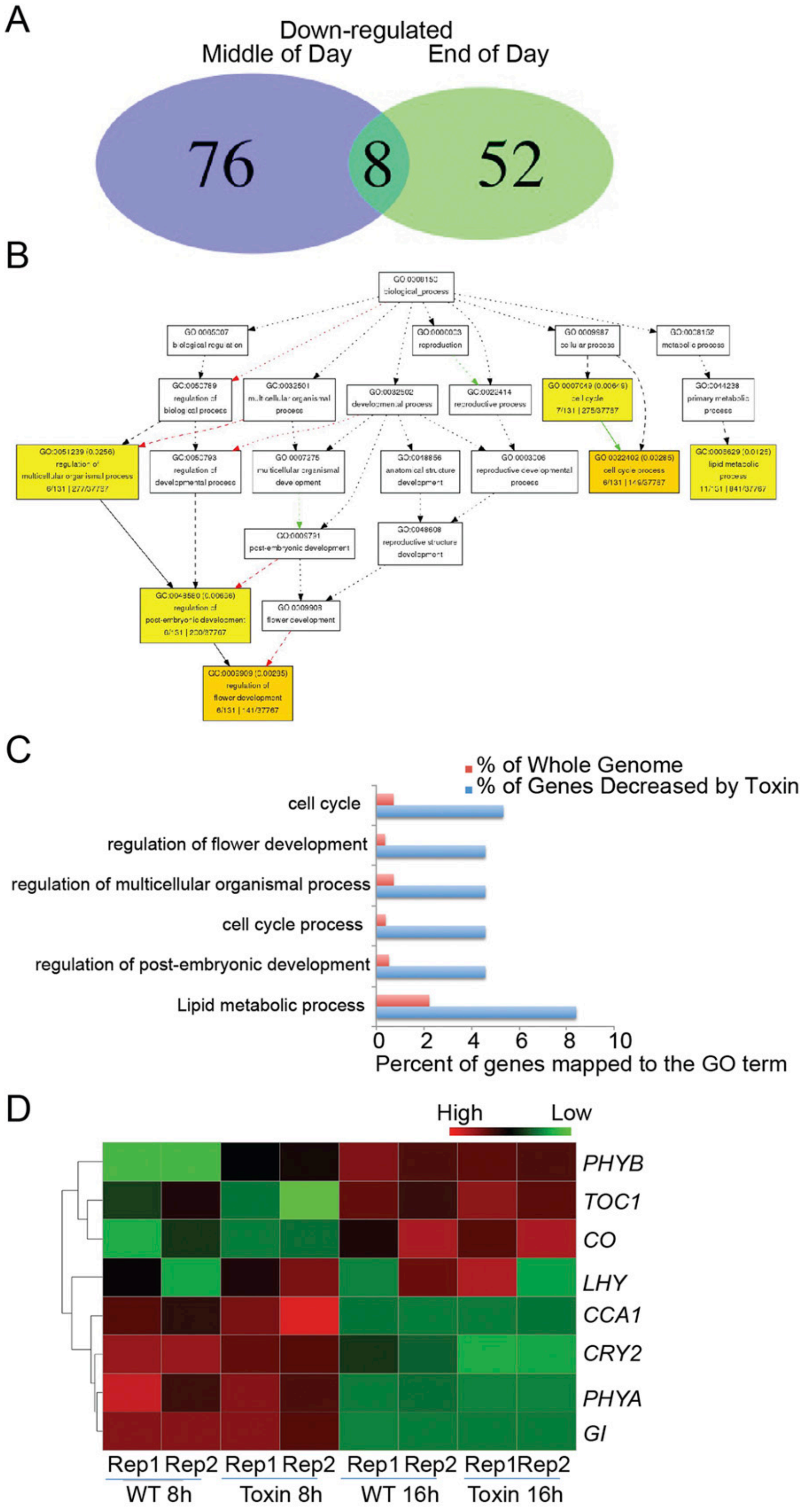

Fig. S6. RNA-seq analysis. (*A*) Venn diagram summarizing the genes down-regulated by *CmGAS1::DT-A* at two time points. Middle of day and end of day represent 8 h and 16 h after lights were turned on, respectively. (*B*) Biological process analysis by agriGO from the combined list in *A*. Six GO terms with significant enrichment are highlighted in yellow. (*C*) Flash chart showing significant enrichment in the six GO terms from *B*. The background/reference for agriGO is *Arabidopsis* gene model TAIR9. (*D*) Gene expression is not changed by *CmGAS1::DT-A* for the flowering regulators that act upstream of *FT* in the photoperiod pathway. Eight representative genes are shown: *PHYA*, *PHYTOCHROME A*; *PHYB*, *PHYTOCHROME B*; *CRY2*, *CRYPTOCHROME 2*; *CCA1*, *CIRCADIAN CLOCK ASSOCIATED 1*; *LHY*, *LATE ELONGATED HYPOCOTYL*; *TOC1*, *TIMING OF CAB EXPRESSION 1*; *GI*, *GIGANTEA*; and *CO*, *CONSTANS*. Toxin indicates *CmGAS1::DT-A*.

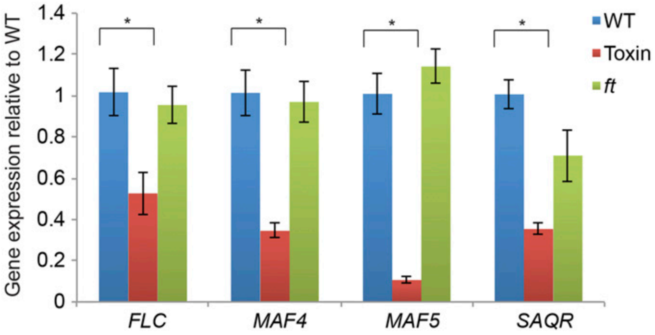

Fig. S7. Fold change in the expression of flowering-related genes in *CmGAS1::DT-A* line T6 and *ft* mutant. *Significant difference ($P < 0.05$). Error bars represent SE ($n = 3$).

Table S1. Primers used in this study

Primer	Sequence
pUC19-GASF	AATGTTCTAGATCTAGATGACTTGGATTAATT
pUC19-GASR	AATGTGAGCTCATTGACTTTGGTGCTTTTGAA
pUC-ToxinF	GACGGTACCATGGATCCTGATGATGTTGTTGATTC
pUC-ToxinR	AGTGAGCTCTCAAGATCGCCTGACACGATTTCCTGC
8xhoGASF	AGCTAGTCGACTCTAGCCTCTAGATGACTTGGATTAATTCTCTAACAAGAA
8speGASR	CTGGGAGGCCTGGATCGACTAGTATTGACTTTGGTGCTTTTGAAACAACAA
o2suc2pxhoF	AATTGGGGCCCAACGTTCCCCATCCCCGACCCTAATGT
suc2pTFP	TTTTCTTACTTTCTTGGTTTGTCAAATATGTACCCATACGATGTTCCAGA
TFPsuc2p	TCTGGAACATCGTATGGGTACATATTTGACAAACCAAGAAAGTAAGAAAA
o2TFPRClpstR	GTAAAAGGTACCGAGCTCCTGCAGCTCATTTGGTGAGGACATAAATGGCG
8FTpxhoF	AGCTAGTCGACTCTAGCCGATTACCTCCCAGCACCAAAGACAAG
8FTpxhoR	GCCTGGGCCCGGCGCGCCTCGAGCATCTTTGATCTTGAACAAACAGGTGGTTTC
AtSAQR-F	TGGCAAAGCCCGTTCTAAT
AtSAQR-R	CTAGTGAGCCTGCATGTCTTC
AtMAF5-F	TGTGTCGGAAGAGTGAAGCCAT
AtMAF5-R	CTGATGATCTTGGCCATGCTGT
AtMAF4-F	GAGAGGAGAACCAGAGTTTGAC
AtMAF4-R	CGTTGGTGATGGTGGTTACT
AtFLC-F	ATCATCATGTGGGAGCAGAAG
AtFLC-R2	GATTTCAACCGCCGATTTAAGG
AtCO-F2	CTTTCAGCTCCATGACCACTAC
AtCO-R2	CAACCTCCTTGGCATCCTTATC
AtActin2-F	TCCCTCAGCACATTCCAGCAGAT
AtActin2-R	AACGATTCCTGGACCTGCCTCATC
NtFT1_2-F	TTGGATCGAGAGGTTGTGAATG
NtFT1-R	GCAACAGGCGAATTGAGATTATG
NtFT2-R	GGCAAATTGAGACCGTGAAAC
NtFT3-F	GAGCACACTTCGGGAATGAA
NtFT3-R	TCTCGAGTCAATTGTCGAAACA
NtFT4-F	TCCCAGCAACTACAGATACAAG
NtFT4-R	GAAACGGGCAAACCAAGATT
NtL25-F	AAAGAAGATTAAGGATGCCGTG
NtL25-R	GCATCATAGTCAGGAGTCAACC

Dataset S1. RNAseq comparison of toxin-deleted and WT leaves at 8 h and 16 h. LogFC, logarithm of fold changes in gene expression; LogCPM, logarithm of counts per million reads; FDR, false discovery rate

Dataset S1