Supporting Information

Andrés et al. 10.1073/pnas.1409567111

SI Materials and Methods

ChIP Experiments and Primers Used in This Work. For ChIP experiments, plants were grown in SD for 2 wk and collected at Zeitgeber 8 (ZT8). A line expressing SVP fused to GFP under its own promoter in the *svp-41* mutant [*SVP::SVP:GFP svp-41* (1)] was compared with the control line, in our case *35S::GFP*. After cross-linking the tissue, the ChIP was performed as in ref. 2 with minor changes. Before proteinase K treatment, samples were treated with RNase for 1 h at 37 °C, purified with MinElute Reaction Cleanup kit (Qiagen) and eluted in 15 µL. Polyclonal antibody against GFP from Abcam (Ab290) was used to immunoprecipitate chromatin. The eluted chromatin was then diluted to a final volume of 100 µL of water, and 3 µL were used for quantitative PCR (qPCR) experiments in a LightCycler 480 Instrument (Roche). Enrichment was evaluated as the percentage of input for each sample with primers expanding the locus of the gene *GA20ox2* (Fig. S2 *A* and *B*; also see list of primers below). The *SEP3* gene was used as a positive control (Fig. S2*C*).

The following primers were used for ChIP-qPCR:

Primers Used for ChIP-qPCR

Primer	Sequence (5′→3′)
xGA20ox2fwA	CTTGTCCTTTAGATTGAGACCAAAG
xGA20ox2reA	GCAAGCTTCAAGTCATGTTAGTGT
xGA20ox2fwD	AACCTTCCATTGACATTCCAG
xGA20ox2reD	ATTGACGACGAGGAAGAAGC
xGA20ox2fwE	GACCATCCTTCATCAAGACC
xGA20ox2reE	CAATATTGACAACGAAAGCC
xGA20ox2_fwF	TTATGATGACTCTTGTTACACTACATGT
xGA20ox2_reF	TGTTAAGTACTATCTACGTGTCATG
xGA20ox2_fwG	AGATGGGATTGTTGTTGGTAATAAC
xGA20ox2_reG	AATACCCTGACTTCACTTGGTC
xGA20ox2_fwH	CGGTATAACAAGCCGGTTTAT
xGA20ox2_reH	GAGTTCGAGCAGTTTGGGTAC
xGA20ox2_fwK	GTGTTGTTTTGGATTCTGATTAG
xGA20ox2_reK	GCTTACATAAATTTGATAAATGAAAG
xSEP3reA	AGATGAGAATCGGACGGCT

The following primers were used for qRT-PCR:

Primers Used for qRT-PCR

Primer	Sequence (5′→3′)
GA20ox2-F	ATGGCGTTTTTCTTGTGTCC
GA20ox2-R	CCAATTCGAAAAGGAATCGA

Quantification of Gibberellins. Aliquots (about 100–200 mg fresh weight) of frozen material were extracted with 80% methanol–1% acetic acid, and the extracts were passed consecutively through hydrophilic–lipophilic Balance (reverse phase), mixed-mode cation exchange (cationic exchange), and mixed-mode weak anion-exchange (ionic exchange) columns (Oasis 30 mg, Waters) to purify the GAs, as described (3). The final residue was dissolved in aqueous 5% (vol/vol) acetonitrile–1% (vol/vol) acetic acid, and the gibberellins (GAs) were separated using an autosampler and reverse-phase UPHL chromatography (2.6 µm Accucore RP-MS column, 50-mm length × 2.1-mm inner diameter; ThermoFisher Scientific) with an aqueous 5–50% (vol/vol) acetonitrile gradient containing 0.05% (vol/vol) acetic acid at 400 µL/min over 14 min. The GAs were analyzed by electrospray ionization (negative mode, spray voltage 3.0 kV, heater temperature 150 °C, sheath gas flow rate 40 µL/min, auxiliary gas flow rate 10 µL/min) and targeted selected ion monitoring (capillary temperature 300 °C, S-lens RF level 70, resolution 70.000) using a Q-Exactive spectrometer (Orbitrap detector; ThermoFisher Scientific). [17,17-^{2}H]GAs (GA_4 purchased from L. Mander, Canberra, Australia, and GA_{12}, GA_{24}, GA_9, and GA_{51} from OlChemIm, Olomouc, Czech Republic) were added to the extracts as internal standards for quantification. The concentrations of GAs in the extracts were determined using embedded calibration curves and the Xcalibur program 2.2 SP1 build 48.

1. Gregis V, Sessa A, Dorca-Fornell C, Kater MM (2009) The Arabidopsis floral meristem identity genes AP1, AGL24 and SVP directly repress class B and C floral homeotic genes. *Plant J* 60(4):626–637.
2. Gendrel AV, Lippman Z, Yordan C, Colot V, Martienssen RA (2002) Dependence of heterochromatic histone H3 methylation patterns on the Arabidopsis gene DDM1. *Science* 297(5588):1871–1873.
3. Seo M, Jikumaru Y, Kamiya Y (2011) Profiling of hormones and related metabolites in seed dormancy and germination studies. *Methods Mol Biol* 773:99–111.

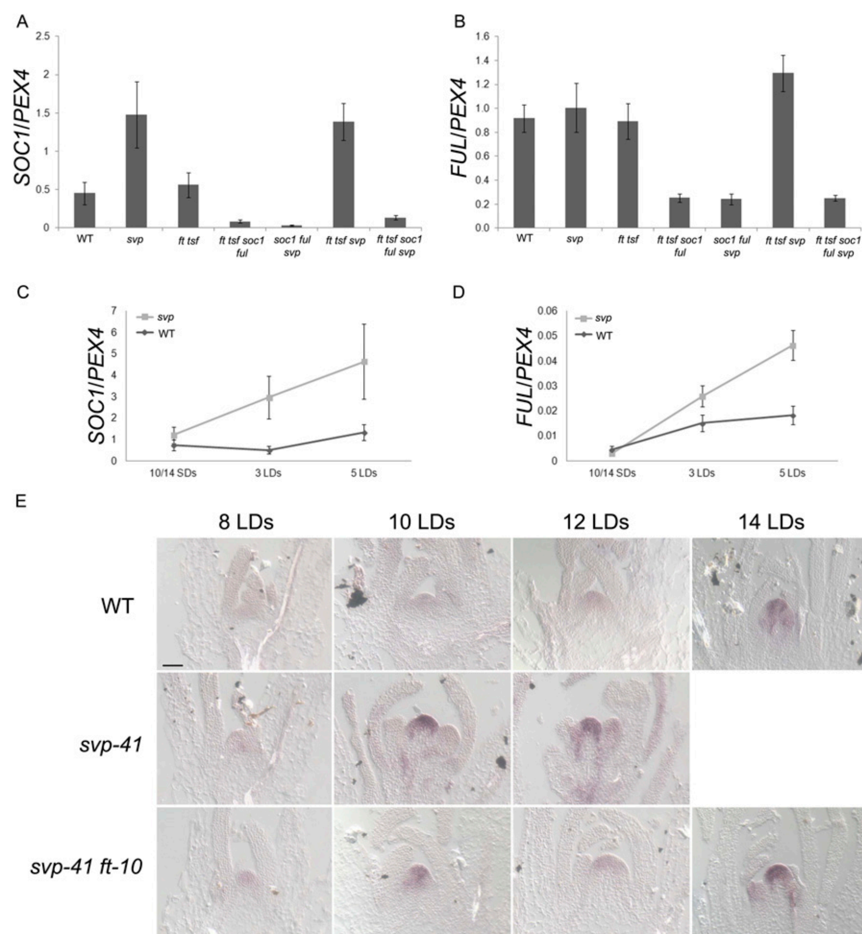

Fig. S1. Transcriptional control of SVP downstream targets. Expression levels of *SOC1* (*A* and *C*) and *FUL* (*B* and *D*) in different genetic background (*A* and *B*) and in a shift experiment (*C* and *D*). In *A* and *B*, the plants were grown for 2 wk under SDs and the seedlings were harvested at ZT8. In *C* and *D*, the wild type and *svp-41* plants were grown while they were still at vegetative stage for 14 and 10 SDs, respectively, and then transferred to LDs for 3 and 5 additional days. The apices of these plants were harvested at ZT8. (*E*) The spatial pattern of *FUL* mRNA during a time course under LDs in wild type, *svp-41*, and the *ft-10 svp-41* plants grown for 8, 10, 12, and 14 LDs. (Scale bar: 50 μm.)

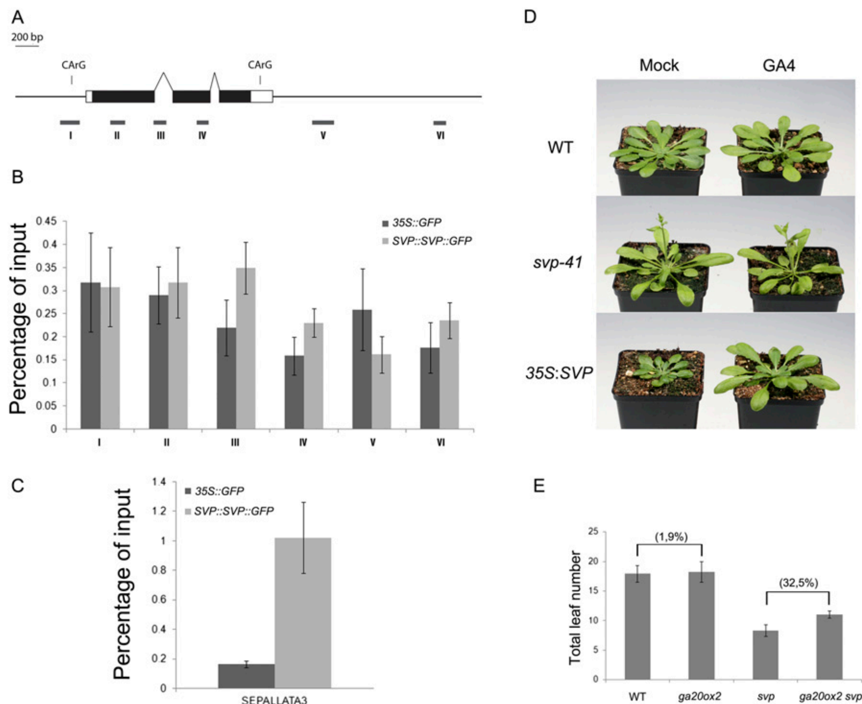

Fig. S2. ChIP analysis of SVP:GFP at the *GA20ox2* locus, response to GA treatments in SVP mutants and overexpressors, and flowering time of *svp-41* compared with *svp-41 ga20ox2-1* plants in LDs. SVP direct-binding analysis to *GA20ox2* by ChIP-PCR. (*A*) Schematic diagram showing the *GA20ox2* genomic region. Exons are represented by black boxes, introns by the black line, and 3′ and 5′ UTR regions are represented white boxes. Consensus-binding sequences (CArG box) of MADS domain proteins are depicted. Gray boxes denote fragments spanning the locus examined by the ChIP enrichment test. (*B*) ChIP analysis of SVP-GFP binding to different regulatory regions of *GA20ox2* described in *A*. (*C*) A *SEP3* fragment of the promoter was amplified as a positive control for ChIP experiments. Results are represented as the percentage of input. Error bars represent SD. (*D*) Phenotype of wild type (*Top*), *svp-41* (*Middle*), and *35S::SVP* (*Bottom*) plants after GA4 treatment under SD conditions. GA4 was applied two times per week at ZT8. (*E*) Flowering time of *svp-41* mutant compared with *svp-41 ga20ox2-1* plants grown under LDs. Wild-type and *ga20ox2-1* mutant plants were used as control. The numbers in parentheses indicate the differences in flowering time expressed as a percentage. The ANOVA analysis showed that this difference is statistically significant (Holm–Sidak test, $P = 0.022$).

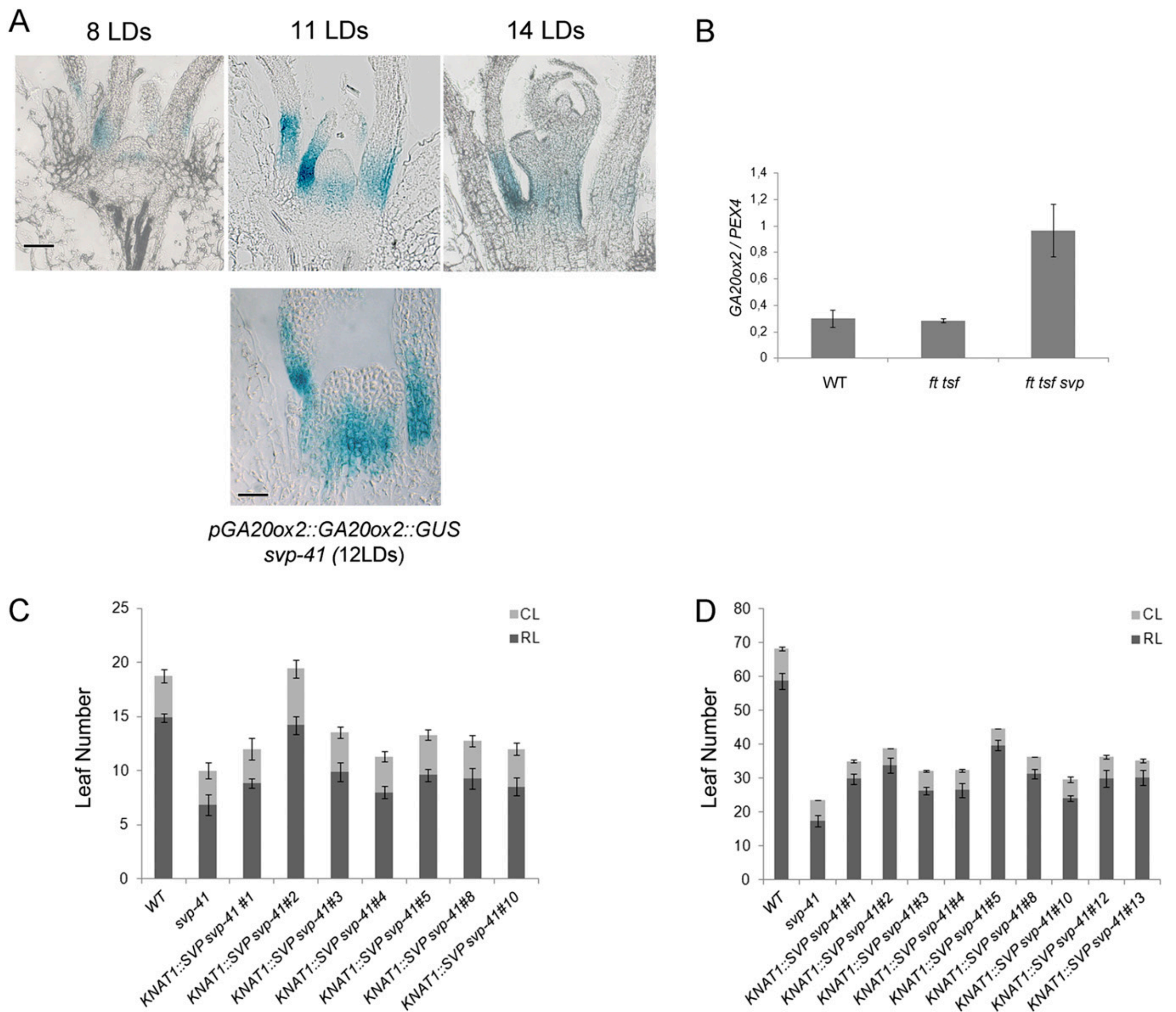

Fig. S3. Spatial expression studies of *GA20ox2:GUS* and effect of misexpression of *SVP* in the SAM. (*A*) Histochemical localization of GUS activity at SAM of *pGA20ox2::GA20ox2:GUS* seedlings harvested at 8, 11, and 14 LDs (see also Fig. 5 legend) and of *pGA20ox2::GA20ox2:GUS svp-41* seedlings harvested during the transition to flowering (12 LDs). (Scale bar: 50 μm.) (*B*) Levels of *GA20ox2* mRNA in apices of wild-type, *ft-10 tsf-1*, and *ft-10 tsf-1 svp-41* mutant plants grown for 2 wk in SDs. (*C* and *D*) Flowering time of p*KNAT1::SVP svp-41* T2 lines: (*C*) under LDs and (*D*) under SDs.

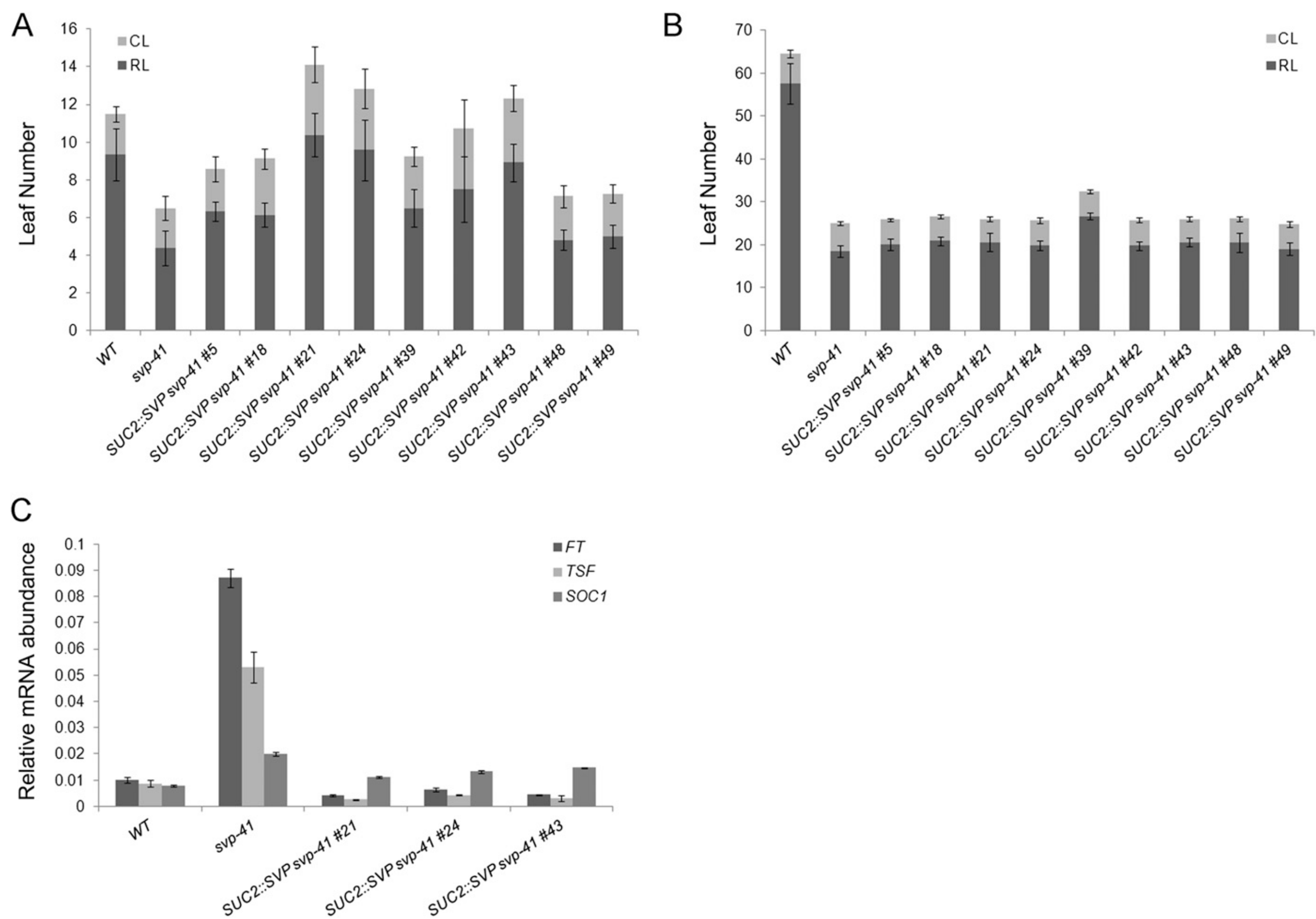

Fig. S4. Effect of misexpression of *SVP* in leaves. Flowering time of transgenic plants misexpressing *SVP* from the phloem-specific promoter *pSUC2* in *svp-41* plants under LDs (*A*) and SDs (*B*). (*C*) Expression levels of *FT*, *TSF*, and *SOC1* in p*SUC2::SVP svp-41* plants (lines #21, #24, and #43) grown for 10 d under LDs.

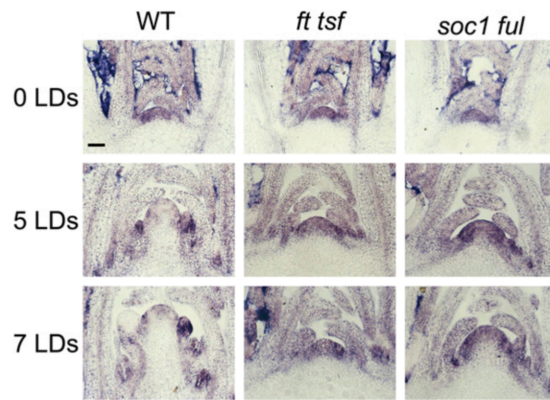

Fig. S5. Photoperiodic control of *SVP* expression involves *FT TSF* and *SOC1FUL*. Temporal and spatial expression patterns of *SVP* at the meristem of *ft-10 tsf-1* and *soc1-2 ful-2* double mutants plants grown for 3 wk in SDs (0 LD) and then transferred to LDs (7 LDs). (Scale bar: 50 μm.)

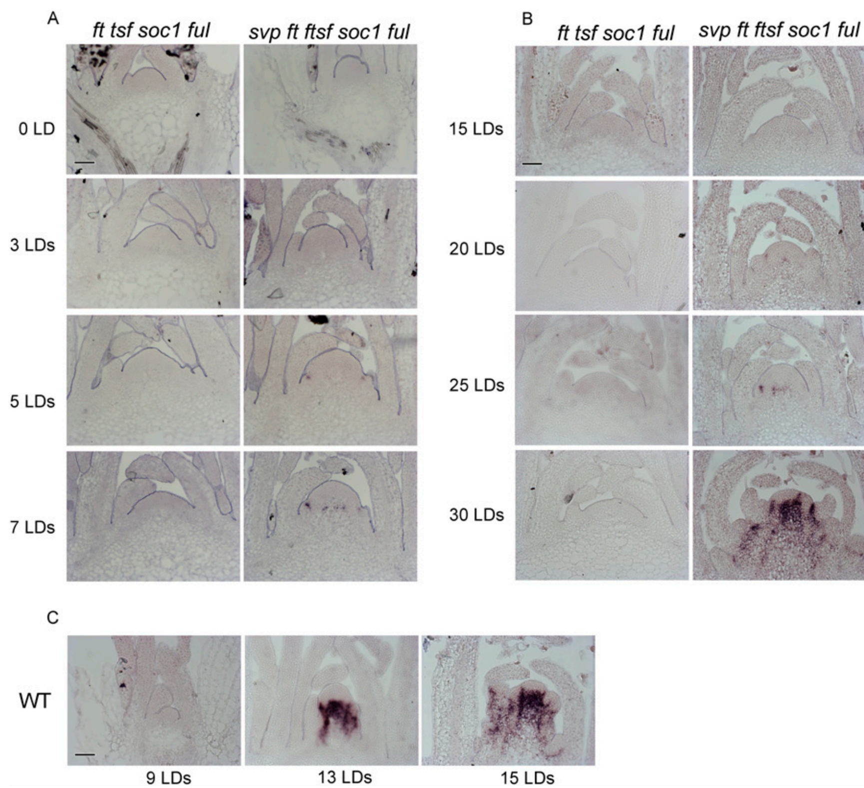

Fig. S6. SVP regulates *SPL4* expression downstream of the photoperiod pathway. Temporal and spatial expression patterns of *SPL4* at the meristem of *ft-10 tsf-1soc1-2 ful-2* and *svp-41 ft-10 tsf-1 soc1-2 ful-2* mutant plants grown for 3 wk in SDs (0 LD) and then transferred to LDs (3, 5, and 7 LDs) (*A*) and for 15, 20, 25, and 30 LDs (*B*). (*C*) Pattern of *SPL4* mRNA expression at the meristem of wild-type control under LDs (9, 13, and 15 LDs). (Scale bar: 50 μm.)

Table S1. Phenotypic characterization of *svp* and *svp ga20ox2* double mutants

Genotype	Chlorophyll content (μmol/m^2)	Height (cm)	Radius (cm)
WT	210.0 ± 11.9^{a,b}	45.0 ± 3.4	2.6 ± 0.3^{a}
svp	183.6 ± 16.4	51.9 ± 5.0	3.2 ± 0.6
ga20ox2	218.5 ± 15.7^{a}	33.8 ± 6.4	1.7 ± 0.2
svp ga20ox2	200.9 ± 11.9^{b}	39.7 ± 3.4	2.3 ± 0.3^{a}

Mean values among the treatment groups show statistical differences ($P < 0.001$). Mean values among the treatment groups indicated with the same letter do not show statistically significant difference. Leaf radius and chlorophyll content were estimated in 14-old-day plants grown in SDs, and the stem elongation measurement was carried out just before senescence started. $n = 10$.