Supporting Information

Wang et al. 10.1073/pnas.1406635111

SI Materials and Methods

Complementation Analysis. For complementation of *cdkc;2–3*, a 5.9-kb genomic DNA fragment containing the cyclin-dependent kinase C (*CDKC;2*) gene was amplified from cosmid clone JAtY49B16 by PCR. This fragment was cloned into the BamHI/SacI sites of the pCambia1300 vector alone or as a C-terminal translational fusion to generate pCambia-*pCDKC;2::CDKC;2-HA* and pCambia-*pCDKC;2::CDKC;2-FLAG*. Mutant plants were transformed using *Agrobacterium tumefaciens* strain GV3101 carrying various *CDKC;2* constructs via the standard floral dipping method. Primary transformants were selected on germination media (GM) plates containing 25 μg/mL hygromycin (Sigma).

***FLOWERING LOCUS C*-Terminator Exchange Construct.** For cloning of *FLOWERING LOCUS C (FLC)*-terminator exchange (*TEX*), *FLC* was cloned as a genomic SacI fragment (~12 kb) that comprises ~6 kb of the genomic sequence of the FLC gene with its flanking natural 5′ (~3.5 kb) and 3′ (~2.6 kb) sequences. Using PCR, an EheI site was introduced into the *FLC* sequence TAGCCACC containing the *FLC* translational stop TAG codon. An SspI-SspI fragment containing the *Arabidopsis rbcs3B* (At5G38410) terminator (706 bp) was PCR amplified and cloned in the sense direction between the EheI and SwaI restriction sites. This fragment replaced the *FLC* 3′ terminator region using the endogenous SwaI site located 741 bp downstream of the TAG translation stop of *FLC*. Therefore, the *FLC-TEX* construct contains a 12-kb *FLC* genomic DNA fragment composed of the promoter region, gene body, and replacement of the 3′ region by *rbcs3B* terminator (Fig. 4*B*). The *FLC-TEX* construct was transformed into the *FRI flc-2* genotype carrying a rearrangement/deletion allele of *FLC*. A representative *FLC-TEX* line (no. *577*) was selected and crossed in *cdkc;2–2* with endogenous active *FLC*.

Expression Analysis Using Real-Time RT-PCR. Samples from plants grown in long days (16/8 h photoperiod) were harvested, and total RNA was extracted. Then, 2.5 μg of RNA was treated with TURBO DNase (Invitrogen) and used for cDNA synthesis (SUPERSCRIPT first STRAND RT-PCR SYSTEM; Invitrogen). cDNA was diluted and used for quantitative PCR using a Roche Lightcycler 480 and SYBR Green Master Mix. *UBC21* (AT5G25760) was used as a reference gene for normalization. Values are means derived from three biological repeats ± SEM. All of the primers used in the RT-PCR are listed in Table S1.

Measurement of the *COOLAIR* Levels in Different Genetic Backgrounds. The expression levels of polyadenylated *COOLAIR* were measured as previously reported (1). Briefly, the total RNA was reverse transcribed using a mixture of oligo d(T), set 6 new LP, polyA set1 LP, and polyA set4 LP primers (Table S1) (polyA set1 LP and polyA set4 LP are specific for certain proximal and distal polyadenylation sites). The cDNA was then used to determine the level of proximal polyadenylated, distal polyadenylated and total *FLC* antisense through qPCR. The proximal and distal polyadenylated *COOLAIR* are presented as relative to the total *COOLAIR* (Fig. 4) or as normalized to *UBC21* (Fig S6). To measure the total *FLC* antisense levels (polyadenylation and splicing independent) in *FLC-TEX* and the corresponding control (Col, *flc-2*), total RNA was reverse transcribed using UBC21 RP and DTA RT primer (Table S1), which is specific to the antisense *FLC* region in both endogenous *FLC* and *FLC-TEX*. The cDNA was then used for qPCR with primer DTA LP + RP (Table S1) and *UBC21* RP + LP. *UBC21* was used as a reference gene for normalization.

ChIP Assays. Seedlings were grown on GM plates at 23–25 °C with a photoperiod of 16 h light and 8 h dark for 2 wk before collecting. Two grams of plant tissue was cross-linked with formaldehyde for 15 min by vacuum infiltration, followed by the addition of glycine to 125 mM with another 5 min of vacuum infiltration. ChIP was performed as described in ref. 2 with minor modifications. After being ground into fine powder, plants were suspended in 30 mL of buffer [20 mM Hepes, 0.44 M sucrose, 1.25% (wt/vol) ficoll, 2.5% (wt/vol) Dextran T40, 10 mM $MgCl_2$, 0.5% Triton X-100, 5 mM DTT, 1× protease inhibitor mixture (Roche Applied Science)], filtered twice through two layers of Miracloth, and centrifuged at 3,200 × *g* for 15 min. For histone ChIP and CDKC2-HA ChIP, nuclear pellets were resuspended in Nuclei Lysis Buffer [50 mM Tris·HCl (pH 8.0), 10 mM EDTA, 1% SDS, 1× protease inhibitor mixture] and sonicated with ten 30-s-long pulses (30-s intervals) using a Diagenode Bioruptor (high setting). Immunoprecipitation was performed using 30 μL of Dynabeads protein A (Invitrogen) and the appropriate antibody at 4 °C overnight. Immunoprecipitated DNA was eluted after reverse cross-linking by boiling at 95 °C for 10 min followed by treatment with 40 μg of proteinase K for 1 h at 48 °C. Samples were treated with StrataClean resin (Agilent Technologies). Then, 5 μL of anti-Histone H3 antibody (ab1791; Abcam), H3K4me2 antibody (07–030; Millipore), and H3Ac antibody (06–599; Millipore) or 5 μL of anti-HA antibody (ab9110; Abcam) was used for each immunoprecipitation (IP) reaction. Values are means ± SEM from three biological repeats; data are presented as a ratio of (modified histone level at *FLC*/H3 *FLC*) to (modified histone level at *Actin*/ H3 *Actin*) to minimize the error generated when handling different tubes (Actin levels at position +55 post-transcription start site are used for normalization). *Actin*, *STM*, and *IGN5* are used as the internal controls and demonstrate the dynamic range of the ChIP assay. ChIP efficiencies ranged from 2% to 30% of input.

In the polymerase II (Pol II) ChIP, the nuclear pellet was resuspended in 1 mL of TAP buffer [100 mM NaCl, 20 mM Tris (pH 7.5), 2.5 mM EDTA, 10% (vol/vol) Glycerol, 1% Triton X-100, 1× protease inhibitor mixture] and given 20 strokes in a Dounce homogenizer. The resulting solution was sonicated 40× (15 s on/45 s off, low setting; Diagenode Bioruptor). Then, 250 μL of the supernatant were used for each IP together with 50 μL of Dynabeads protein G and 10 μg of anti-Ser^2 P C-terminal domain (CTD) (3E10; Active Motif) or anti-CTD (4H8, ab5408; Abcam). The IP reaction was performed with rotation for 4 h at 4 °C, and beads were washed 2 × 15 min with low salt wash buffer [150 mM NaCl, 20 mM Tris (pH 7.5), 2.5 mM EDTA, 0.05% Na-deoxycholate, 1% Triton X-100, 1× protease inhibitor], 2 × 15 min with high salt wash buffer [500 mM Nacl, 20 mM Tris (pH 7.5), 2.5 mM EDTA, 0.05% Na-deoxycholate, 1% Triton X-100, 1× protease inhibitor], 1 × 15 min with LiCl Wash buffer [250 mM LiCl, 20 mM Tris (pH 7.5), 2.5 mM EDTA, 0.5% Na-deoxycholate, 0.5% Nonidet P-40, 1× protease inhibitor] (for 4H8 only), and 1 × 15 min with TE buffer [10 mM Tris (pH 7.5), 1 mM EDTA]. The resulting DNA was recovered in the same way as for histone ChIP. Values are means ± SEM from two biological repeats; data are presented as ration of (Pol II *FLC*/input *FLC*) to (Pol II *Actin*/ input *Actin*) to remove between-tube variation (Actin level at position +55 post-transcription start site was used for the

normalization). *Actin*, *STM*, and *IGN5* are used as the internal controls and demonstrate the dynamic range of the ChIP assay. ChIP efficiencies ranged from 0.3% of Input (3E10) to 2% of Input (4H8).

Western Blotting. Seedlings were grown on GM agar plates at 23–25 °C with a 16-h photoperiod for 2 wk. One gram of seedlings from wild-type, mutants, or transgenic plants was harvested and ground to fine power in liquid nitrogen. Total protein was extracted by protein extraction buffer [50 mM Tris·HCl (pH 8.0), 50 mM NaCl, 10 mM EDTA, 2% (wt/vol) SDS, 1× protease inhibitor mixture (Roche Applied Science)]. The samples were centrifuged twice at 14,000 × *g* at 4 °C for 20 min. Protein samples were separated by SDS/PAGE and transferred onto a PVDF (polyvinylidenedifluoride) membrane (GE Healthcare). The samples were analyzed by Western blotting using anti-FLAG (F3165; Sigma), anti-HA (ab9110; Abcam), anti-Ser^2 P CTD (3E10; Active Motif), anti-CTD [8WG16] (ab817; Abcam), anti-Histone H3 (ab1791; Abcam), and anti-FCA (3), and visualized by chemiluminescence using Immobilon Chemiluminescent HRP substrate (Thermo Fisher Scientific).

CDKC;2 Purification for Proteomic Analysis. Seedlings (10 g, fresh weight) grown in GM media for 2 wk under long day conditions were frozen and ground using liquid nitrogen in a mortar, and thawed into 10 mL of TAP buffer containing 150 mM NaCl, 20 mM Tris·HCl (pH 8), 2.5 mM EDTA, 0.5% Triton X-100, 5% (vol/vol) Glycerol, and 1× protease inhibitor mixture (Roche Applied Science). Proteins were extracted on a rotating platform for 30 min at 4 °C and centrifuged twice at 20,000 × *g* for 15 min at 4 °C. Centrifuged extracts were incubated with 70 μL of ANTI-FLAG M2 Magnetic Beads (M8823; Sigma) for 2 h at 4 °C and washed two times with TAP buffer without Triton X-100. They were then eluted with 100 μL of 1× FLAG (F3290; Sigma) solution (200 μg/mL) according to the manufacturer's instructions.

Liquid Chromatography-Tandem Mass Spectrometry Analysis. CDKC;2-FLAG and control samples were cut from SDS/PAGE gels and washed, reduced, and alkylated, and treated with trypsin according to standard procedures. Peptides were extracted with 5% formic acid/50% acetonitrile, dried down, and redissolved in 0.1% TFA. For liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis (4), a sample aliquot was applied via a nanoAcquity (Waters) UPLC-system running at a flow rate of 250 $nL \cdot min^{-1}$ to an LTQ-Orbitrap mass spectrometer (Thermo Fisher). Peptides were trapped using a precolumn (Symmetry C18, 5 μm, 180 μm × 20 mm; Waters), which was then switched in-line to an analytical column (BEH C18, 1.7 μm, 75 μm × 250 mm; Waters) for separation. Peptides were eluted with a gradient of 3–37% (vol/vol) acetonitrile in water/0.1% formic acid at a rate of 0.5% min^{-1}. The column was connected to a 10-μm SilicaTip nanospray emitter (New Objective) attached to a nanospray interface (Proxeon) for infusion into the mass spectrometer. The mass spectrometer was operated in positive ion mode at a capillary temperature of 200 °C. The source voltage and focusing voltages were tuned for the transmission of MRFA peptide (*m/z* 524) (Sigma-Aldrich). Data-dependent analysis was carried out in orbitrap-IT parallel mode using CID fragmentation on the five most abundant ions in each cycle. The orbitrap was run with a resolution of 30,000 over the MS range from *m/z* 350 to *m/z* 1,800 and an MS target of 10^6 and 1 s maximum scan time. Collision energy was 35, and an isolation width of 2 was used. Only monoisotopic 2^+ and 3^+ charged precursors were selected for MS2. The MS2 was triggered by a minimal signal of 1,000 with an AGC target of 3×10^4 ions and 150-ms scan time using the chromatography function for peak apex detection. Dynamic exclusion was set to 1 count and 30 s exclusion with an exclusion mass window of ±20 ppm. MS scans were saved in profile mode whereas MSMS scans were saved in centroid mode.

Alternatively, samples were analyzed by LC-MS/MS using a Synapt G2 HDMS mass spectrometer (Waters). The LC conditions were the same as described above, except that a longer gradient was used (0.33% min^{-1} acetonitrile from 3% to 37%). The mass spectrometer was run in positive sensitivity mode using data-independent MS^E ion mobility TOF. The collision energy (transfer cell) was optimized using a lookup table. The mass spectrometer was calibrated with sodium iodide, and the lock mass option was used with glufibrinogen peptide (Sigma) as calibrant.

Raw files from the orbitrap were processed with MaxQuant version 1.3.0.5 (4) (http://maxquant.org) to generate recalibrated peaklist files whereas raw files from the Synapt G2 were processed in PLGS (Waters). All generated peaklist files were used for database searches using an in-house Mascot 2.4 Server (Matrix Science Limited). The searches were performed on the TAIR_10_pep_20101214.fasta database and on a common contaminants database using trypsin/P with two missed cleavages, carbamidomethylation (C) as fixed, and oxidation (M), acetylation (protein N terminus), and phosphorylation (STY) as variable modifications. Mass tolerances were 6 ppm for parent ions and 0.6 Da for fragment ions for Orbitrap data, and 20 ppm (parent) and 0.1 Da (fragments) for Synapt data. Mascot search results were imported and evaluated in Scaffold 3.6.4 (www.proteomesoftware.com), resulting in a false discovery rate of 0% for both peptides and proteins.

1. Liu FQ, et al. (2007) The *Arabidopsis* RNA-binding protein FCA requires a lysine-specific demethylase 1 homolog to downregulate *FLC*. *Mol Cell* 28(3):398–407.
2. Wierzbicki AT, Haag JR, Pikaard CS (2008) Noncoding transcription by RNA polymerase Pol IVb/Pol V mediates transcriptional silencing of overlapping and adjacent genes. *Cell* 135(4):635–648.
3. Quesada V, Macknight R, Dean C, Simpson GG (2003) Autoregulation of *FCA* pre-mRNA processing controls *Arabidopsis* flowering time. *EMBO J* 22(12):3142–3152.
4. Cox J, Mann M (2008) MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat Biotechnol* 26(12):1367–1372.

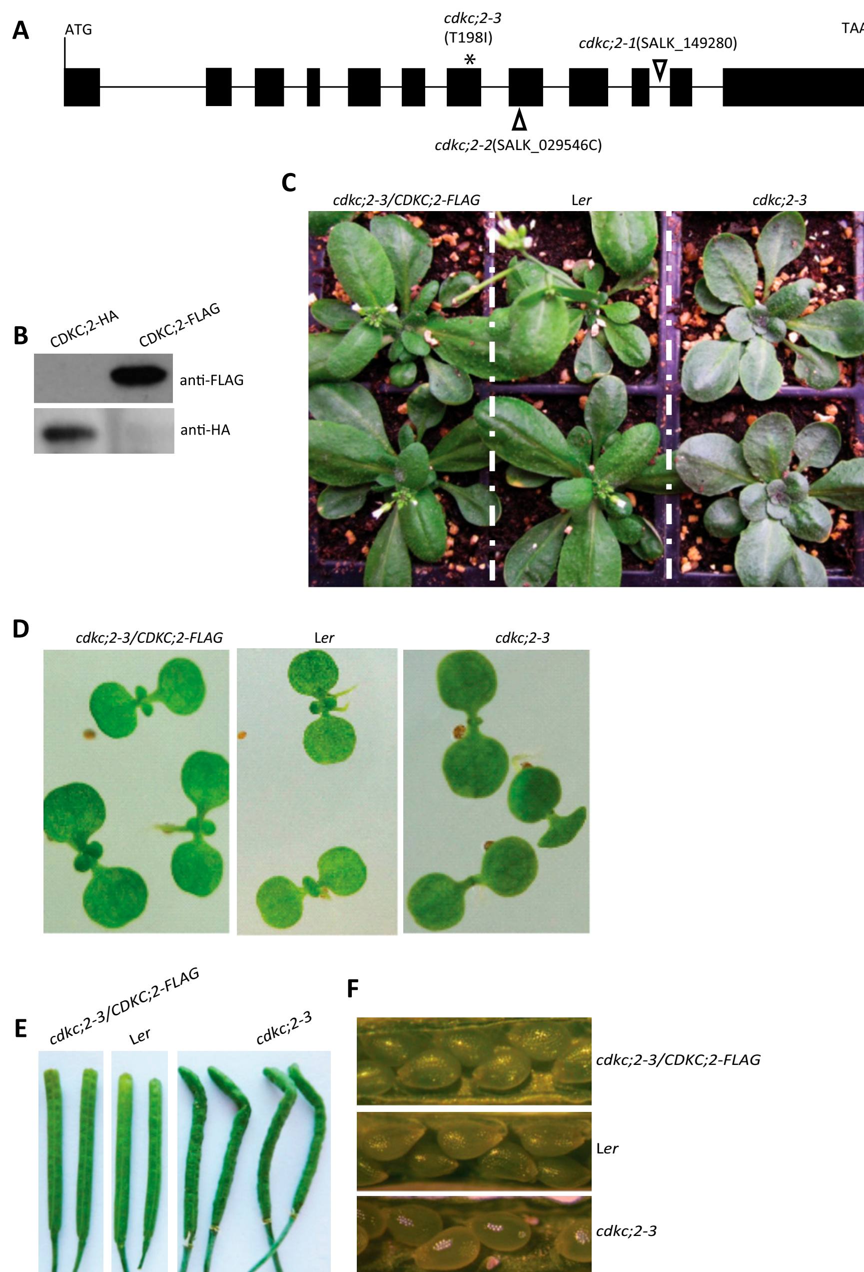

Fig. S1. Characterization of *cdkc;2*. (*A*) Schematic diagram showing the positions of the transferred DNA insertions or nucleotide change at the *CDKC;2* locus. Black rectangles represent exons. (*B*) Detection of CDKC;2-FLAG and CDKC;2-HA proteins in transgenic *Arabidopsis* plants. CDKC;2 proteins were detected by Western blotting of samples immunoprecipitated by anti-FLAG and anti-HA antibody from extracts from the transgenic plants. (*C*) *cdkc;2–3* has gray leaves compared with wild-type L*er*. (*D*) *cdkc;2–3* grows slightly slower than wild type (L*er*). (*E*) *cdkc;2–3* has curved siliques compared with wild type (L*er*) in ~85% of homozygous progeny. (*F*) *cdkc;2–3* causes abortion in a small number of seeds. The phenotype could be the result of defective male or female gametes or defective embryo development.

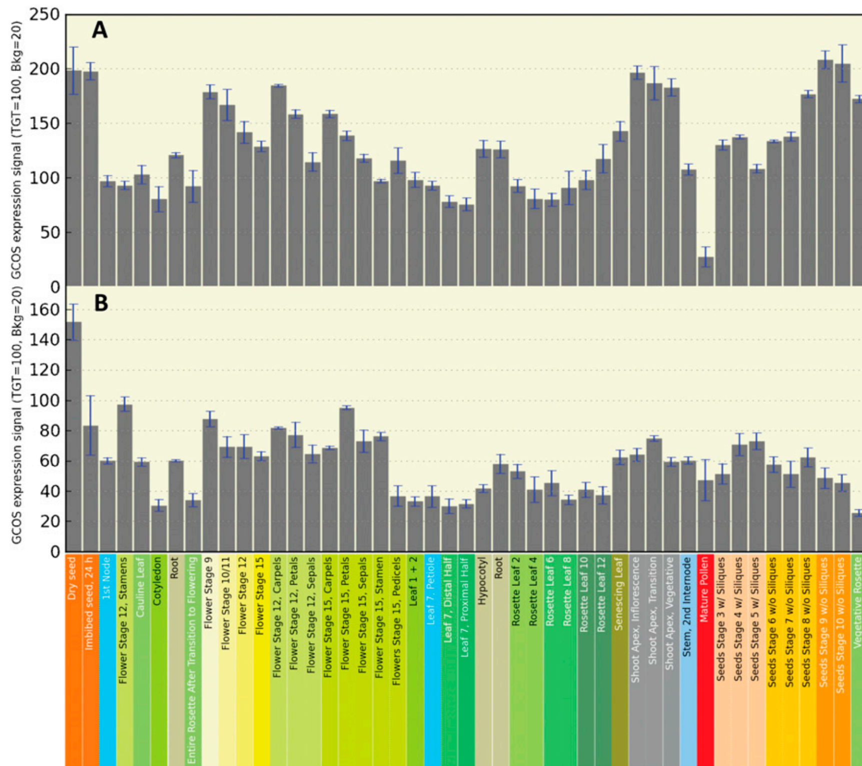

Fig. S2. Expression patterns for *CDKC;2* (*A*) and *CDKC;1* (*B*). Gene-expression data were downloaded from The Arabidopsis Information Resource (TAIR) Web site (*CDKC;2*; http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi?primaryGene=AT5G64960&modeInput=Absolute; *CDKC;1*; http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi?primaryGene=AT5G10270&modeInput=Absolute).

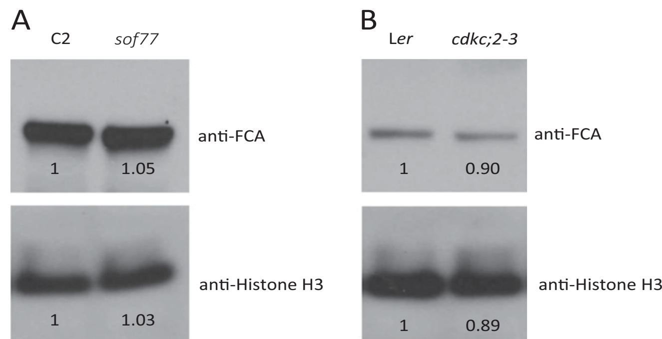

Fig. S3. (*A*) Detection of the FCA protein in C2 progenitor line and *sof77*. (*B*) Detection of the FCA protein in L*er* and *cdkc;2-3*. FCA was detected by Western blotting of samples immunoprecipitated by anti-FCA antibody from extracts from leaves. Histone H3 was used as loading control.

A

```
CDKC;2 and CDKC;1(Arabidopsis thaliana)  GNLTNRVITLWYRPPELLLGAT
    CDKC;2 and CDKC;1(Oryza sativa)      GNLTNRVITLWYRPPELLLGST
   CTK1 (Saccharomyces cerevisiae)       ADYTNRVITLWYRPPELLLGTT
              BUR1 (S. cerevisiae)       AKYTSVVVTRWYRAPELVLGDK
             CDK12 (Homo sapiens)        RPYTNKVITLWYRPPELLLGEE
                CDK9 (H. sapiens)        NRYTNRVVTLWYRPPELLLGER
```

B

```
CDKC;1   1   MAMASFGQLNLEEPPPIWGSRSVDCFEKLEQIGEGTYGQVYMAKEIKTGEIVALKKIRMD  60
CDKC;2   1   MAAAAFGQLNLEEPPPIWGSRSVDCFEKLEQIGEGTYGQVYMAKEIKTGEIVALKKIRMD  60

CDKC;1   61  NEREGFPITAIREIKILKKLHHENVIQLKEIVTSPGRDRDDQGKPDNNKYKGGIYMVFEY 120
CDKC;2   61  NEREGFPITAIREIKILKKLHHENVIHLKEIVTSPGRDRDDQGKPDNNKYKGGIYMVFEY 120

CDKC;1   121 MDHDLTGLADRPGLRFTVPQIKCYMKQLLTGLHYCHVNQVLHRDIKGSNLLIDNEGNLKL 180
CDKC;2   121 MDHDLTGLADRPGLRFTVPQIKCYMKQLLTGLHYCHVNQVLHRDIKGSNLLIDNEGNLKL 180

CDKC;1   181 ADFGLARSYSHDHTGNLTNRVITLWYRPPELLLGATKYGPAIDMWSVGCIFAELLHAKPI 240
CDKC;2   181 ADFGLARSYSHDHTGNLTNRVITLWYRPPELLLGATKYGPAIDMWSVGCIFAELLNGKPI 240

CDKC;1   241 LPGKNEQEQLNKIFELCGSPDEKLWPGVSKMPWFNNFKPARPLKRRVREFFRHFDRHALE 300
CDKC;2   241 LPGKTENEQLNKIYELCGSPDESNWPGVSKMPWYNQMKSSRPLKRRVREIYRHFDRHALE 300

CDKC;1   301 LLEKMLVLDPAQRISAKDALDAEYFWTDPLPCDPKSLPTYESSHEFQTKKKRQQQRQNEE 360
CDKC;2   301 LLEKMLVLDPSQRICAKDALDAEYFWTDPLPCDPKSLPTYESSHEFQTKKKRQQMRHNEE 360

CDKC;1   361 AAKRQKLQHPPLQHSRLPPLQHG-GQSHAAPHWPAGPNHPTNNA--PPQVPAGPSHNFYG 417
CDKC;2   361 AAKKQKLQHPQQQHSRLPPQQHGVGQSHAAPLWPAGPNHPMNNNAPPPQIPAG-GHYYGG 419

CDKC;1   418 KPR-GPPGPNRYPPSGNQSGGY-NQSRGGYSSGSYPPQGRGAPYVAGPRGPSGGPYGVGP 475
CDKC;2   420 KPRGGAPVPNRYPPSGNQTGGYNNQSRGGYSSGAYPPQGRGAPYGAGPRGPSGG-YGVGP 478

CDKC;1   476 PNYTQGGQYGGSGSSGRGQNQ-----RNQQYGWQ 504
CDKC;2   479 PNYSQGGGQYGGSGGSGRGQNPMGGARNQQYGWQ 512
```

Fig. S4. Amino acid sequence alignments. (*A*) Alignments of CDK9 homologs. Conserved residues are highlighted in yellow. (*B*) Alignment of CDKC;2 and CDKC;1 from *Arabidopsis*. Identical residues are highlighted in red.

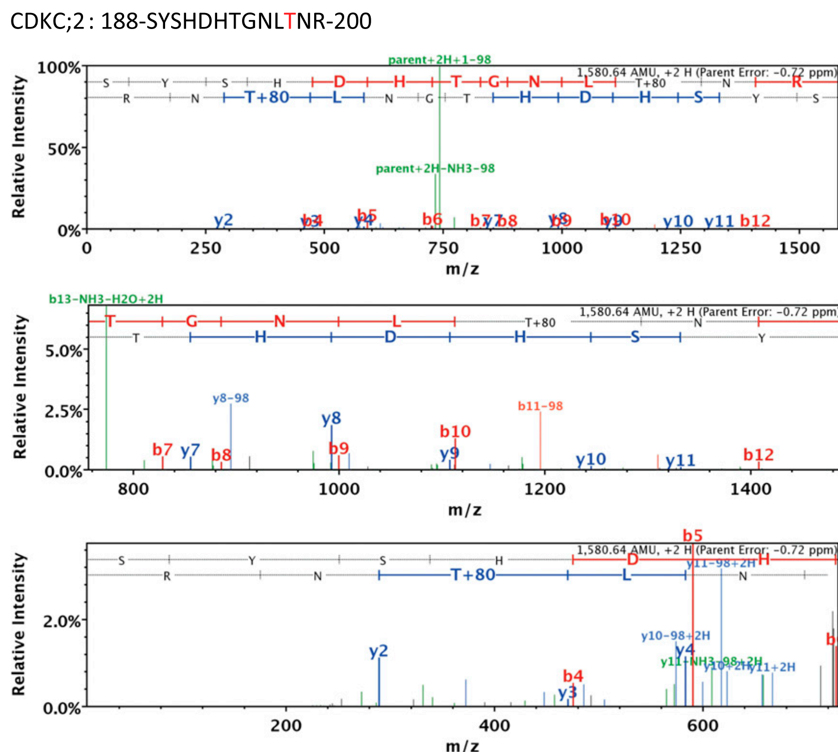

Fig. S5. T198 was found to be phosphorylated. The peak b11-98 shows clearly that the T is phosphorylated. The differences b12 – b10 and y3 – y2 also show that the T is phosphorylated.

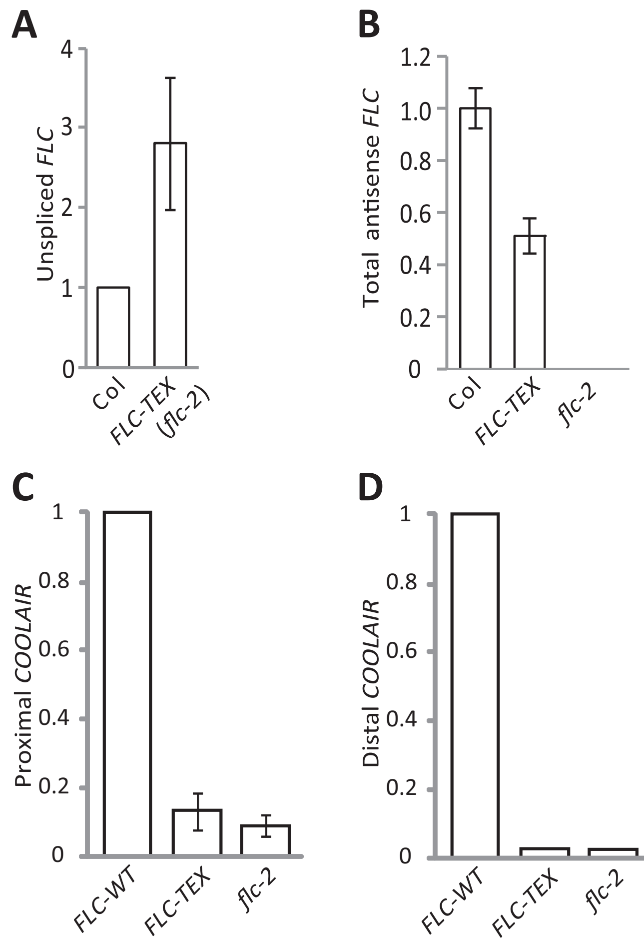

Fig. S6. (*A*) Unspliced *FLC* analysis by qRT-PCR. (*B*) Total antisense *FLC* level in wild type, *FLC-TEX flc-2*, and *flc-2*. (*C*) Proximal polyadenylated *COOLAIR* level in wild type, *FLC-TEX flc-2*, and *flc-2* by qRT-PCR analysis. (*D*) Distal polyadenylated *COOLAIR* level in wild type, *FLC-TEX flc-2*, and *flc-2* by qRT-PCR analysis. (*C* and *D*) These three genotypes contain an active *FRIGIDA* allele. Error bars represent SEM derived from three biological repeats.

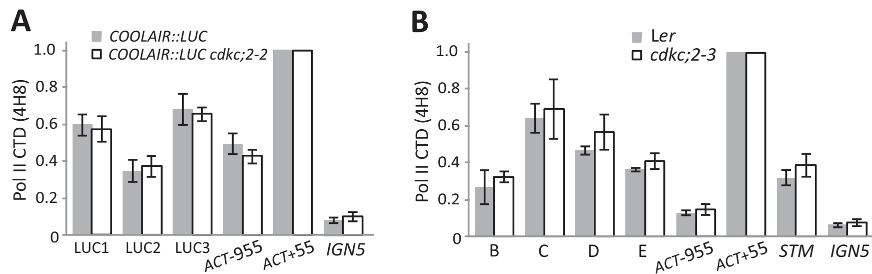

Fig. S7. (*A*) ChIP experiments assaying RNA Pol II (using anti-CTD-4H8) in *COOLAIR::LUC* regions in *cdkc;2–2* and Col. (*B*) ChIP experiments assaying RNA Pol II (using anti-CTD-4H8) in *FLC* regions in *cdkc;2–3* and L*er*. Values are means ± SEM from two biological repeats; data were presented as ratio of (Pol II *FLC*/input *FLC*) to (Pol II *Actin*/input *Actin*). *Actin*, *STM*, and *IGN5* were used as the internal control for the ChIP experiments.

Table S1. List of primers

Primer name	Sequence	Purpose
RLfca9	TCTTTGGCTCAGCAAACC	dCAPS marker for *fca-9* (StyI)
GSO379	TGTTGAGATGGTGAAACTGTG	
FLD-4.5	AGCTAGGCAACTGATGAG	Genotyping wild type *FLD*
FLD-4.3	AGAAACCTGCCTGAATGTC	
SALK_029546CL	CGTTGGAGTCCACGTTCTTT	Genotyping *fld-4*
fld-4–5-2	TTATATCCAAATGGCGAGAAAAT	
(FY-) F17	AAACAGCCTCTTCCAGGTTCC	Genotyping wild type *FY*
(FY-) FY4	CTGTTGGAAAGGGTTGTTGTAGCCTGGAATC	
BL3(RDR2-1.3)14-E5	TAGCATCTGAATTTCATAACCAATCTCGATACAC	Genotyping *fy-2*
(FY-) FY4	CTGTTGGAAAGGGTTGTTGTAGCCTGGAATC	
GSO420	GCGACCAAAACCTTGTTCACTAAAGC	dCAPS marker for *fy-1* (HindIII)
GSO421	AACTCAGACCCAAGTACTCGG	
sof77PsiI L	ATATTCTCATGATCATACTGGAAACCTTA	dCAPS marker for *cdkc;2–3* (PsiI)
sof77PsiI R	TCAATCGCTGGTCCATATTTAGT	
CDKC;2 3 F	TGCTTATACGTGCAGCTTTTTC	Genotyping wild type *CDKC;2*
CDKC;2 3 R	TAACCTCCACGGCTCTGATT	
SALK_029546CL	CGTTGGAGTCCACGTTCTTT	Genotyping wild type *cdkc;2–2*
SALK_029546C R	CAGGTGATCCACAAAGCTCA	
TE-CHECK-F	TCATCATGTGGGAGCAGAAG	Genotyping *FLC-TEX*
RBCS3-S-AII	GTTAATATTAACCTAGGAACTACACGC	
LUC 2F	GAGGTTCCATCTGCCAGGTA	Genotyping *LUC*
LUC 2R	CGCTTCCGGATTGTTTACAT	
UJ43	GAAGATCATCGAATTGGC	Genotyping transgene *FRI* in L*er* background
CLFRI-10	CATGTCGTAATCATGCAAC	
RLFCA3	CCTATTCCGAACAGCATGG	Genotyping transgene *FCA*
RLFCA9	TCTTTGGCTCAGCAAACC	
Sense FLC ex3 LP	CGCAATTTTCATAGCCCTTG	Real-time PCR for nascent *FLC*
Sense FLC ex3 RP	CTTTGTAATCAAAGGTGGAGAGC	
FLC cDNA 393F	AGCCAAGAAGACCGAACTCA	Real-time PCR for *FLC* mRNA
FLC cDNA 550R	TTTGTCCAGCAGGTGACATC	
polyA set1 LP	TTTTTTTTTTTTTTTACTGCTTCCA	Real-time PCR for proximal pA from antisense *FLC*
set1 RP	CACACCACCAAATAACAACCA	
polyA set4 LP	TTTTTTTTTTTTTTTGCGGTACAC	Real-time PCR for distal pA from antisense *FLC*
set4 RP	GGGGTAAACGAGAGTGATGC	
set6 new LP	TGTATGTGTTCTTCACTTCTGTCAA	Real-time PCR for total antisense *FLC*
set6 new RP	GCCGTAGGCTTCTTCACTGT	
DTA-LP	GTATCTCCGGCGACTTGAAC	Real-time PCR for measure total antisense *FLC in FLC-TEX*
DTA-RP	GGATGCGTCACAGAGAACAG	
DTA-RT	AATATCTGGCCCGACGAAG	RT primer for measure total antisense *FLC in FLC-TEX*
Q RBCS R-2	ACAATAGGAAATGAAATGAGCAG	RT for transgenic *FLC* in *FLC-TEX*
Q Un FLC endor F	TTAATGGTTGTTATTTGGTGG	Real-time PCR for transgenic *FLC* in *FLC-TEX*
Q RBCS R-1	TCATAAGAATGTTACCGGTTC	
VC139-UJ26-FRI	AGATTTGCTGGATTTGATAAGG	Genotyping active *FRI* in Col background
VC140-UJ34-FRI	ATATTTGATGTGCTCTCC	
FLC -4875 F	AAGCATGAGGGACCAATCTG	Genotyping *flc-2*
FLC -4651 R	CCAAACCAGAACTGACAACG	
Q PCR LUC F	TTCCATCTGCCAGGTATCAG	Real-time PCR for *LUC*
Q PCR LUC R	ATCCAGATCCACAACCTTCG	
cstf64.f7	ATTCAGATTAGTTACGGATAGAGA	dCAPS marker for *cstf64-1* (Hpy188I digests wild type)
cstf64.R2	ACGGGTTTTGTCAGTGC	
FLC -501 F	ACTATGTAGGCACGACTTTGGTAAC	ChIP primers for the *FLC* gene region A
FLC -381 R	TGCAGAAAGAACCTCCACTCTAC	
FLC 157 F	CGACAAGTCACCTTCTCCAAA	ChIP primers for the *FLC* gene region B
FLC 314 R	AGGGGGAACAAATGAAAACC	
FLC 438 F	GGCGGATCTCTTGTTGTTTC	ChIP primers for the *FLC* gene region C
FLC 502 R	CTTCTTCACGACATTGTTCTTCC	
FLC 1533 F	TTGACAATCCACAACCTCAATC	ChIP primers for the *FLC* gene region D
FLC 1670 R	TCAATTTCCTAGAGGCACCAA	
FLC 1884 F	AGCCTTTTAGAACGTGGAACC	ChIP primers for the *FLC* gene region E
FLC 2062 R	TCTTCCATAGAAGGAAGCGACT	

Table S1. Cont.

Primer name	Sequence	Purpose
FLC 3088 F	GGGGCTGCGTTTACATTTTA	ChIP primers for the *FLC* gene region F
FLC 3224 R	GTGATAGCGCTGGCTTTGAT	
FLC 3899 F	CTTTTTCATGGGCAGGATCA	ChIP primers for the *FLC* gene region G
FLC 4069 R	TGACATTTGATCCCACAAGC	
FLC set1 polyA.aL	AGATTATAGATACTGCTTCCAAACT	ChIP primers for the *FLC* gene region H
FLC set1 polyA.aR	TTCACACCACCAAATAACAAC	
FLC 5534F	TGGTTGTTATTTGGTGGTGTG	ChIP primers for the *FLC* gene region J
FLC 5649 R	ATCTCCATCTCAGCTTCTGCTC	
FLC 5948 F	CGTGTGAGAATTGCATCGAG	ChIP primers for the *FLC* gene region U
FLC 6066 R	AAAAACGCGCAGAGAGAGAG	
FLC 6768 F	TTGTAAAGTCCGATGGAGACG	ChIP primers for the *FLC* gene region V
FLC 6838 R	ACTCGGCGAGAAAGTTTGTG	
LUC1-F	TCAACCGCCGATTTAAGGT	ChIP primers for the *COOLAIR::LUC* gene region LUC1
LUC1-R	CGCTGATCAATTCCACAGTTT	
LUC2-F	AGAGATACGCCCTGGTTCCT	ChIP primers for the *COOLAIR::LUC* gene region LUC2
LUC2-R	ATAAATAACGCGCCCAACAC	
LUC3-F	GTTCCATCTGCCAGGTATCA	ChIP primers for the *COOLAIR::LUC* gene region LUC3
LUC3-R	CCGGTATCCAGATCCACAAC	
Actin -995-F	TGGGTCTCATATAGAACACTCACAAAGGT	ChIP primers measure promoter of Actin7 as low expression reference
Actin -832-R	GACCAAAACCCGAATAGGAGCAAGA	
Actin-55-F	CGTTTCGCTTTCCTTAGTGTTAGCT	ChIP primers measure the region at the beginning of Actin7, as high expression reference for ChIP data normalization.
Actin-188-R	AGCGAACGGATCTAGAGACTCACCTTG	
IGN5-set3-F	CCCTTAAGCGGACATGGTT	ChIP primers for IGN5 as reference gene which is not a Pol II target
IGN5-set3-R	AATGTCGGCCAATCTTCTTG	
STM-F	GCCCATCATGACATCACATC	ChIP primers for STM as low expression reference gene.
STM-R	GGGAACTACTTTGTTGGTGGTG	