Supporting Information

Deng et al. 10.1073/pnas.1103175108

SI Materials and Methods

Analysis of the CArG-Box Motif. The fuzznuc program (1) was used to search for motifs against the four parts of the FLC binding data. Then, 800 randomly selected promoter and genomic sequences were used as the negative control. The TOMTOM program (2) was used to compare the CArG-box motif of the FLC protein with other proteins with default parameters.

Gene Expression Analysis and Quantitative RT-PCR. RNA was extracted from ~100 mg of seedlings by using the RNeasy Plant Mini Kit according to the manufacturer's instructions (Qiagen). For quantitative RT-PCR, DNase-treated RNA was reverse transcribed with an oligo(dT) primer and SuperScript III reverse transcriptase (Invitrogen), and at least triplicate reactions were amplified by using either a Rotor-Gene 2000 Real-Time Cycler (Corbett Research) or a 7900HT Fast Real-Time PCR System (Applied Biosystems) with SYBR green. The primers used are listed in Table S4.

1. Rice P, Longden I, Bleasby A (2000) EMBOSS: The European Molecular Biology Open Software Suite. *Trends Genet* 16:276–277.

2. Gupta S, Stamatoyannopoulos JA, Bailey TL, Noble WS (2007) Quantifying similarity between motifs. *Genome Biol* 8:R24.

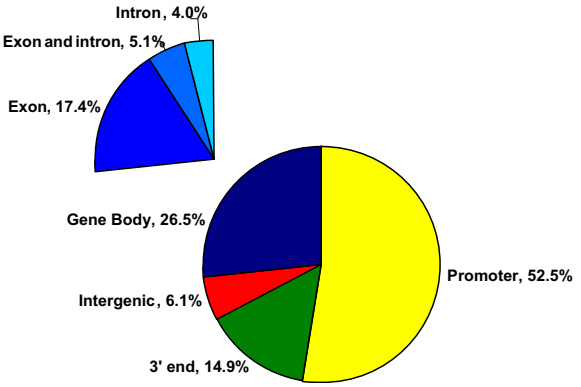

Fig. S1. The genomic locations of FLC binding sites were determined relative to the nearest genes and characterized into four classes: promoter, 3′ end, gene body, and intergenic. The gene body class was divided into exon, intron, and exon and intron.

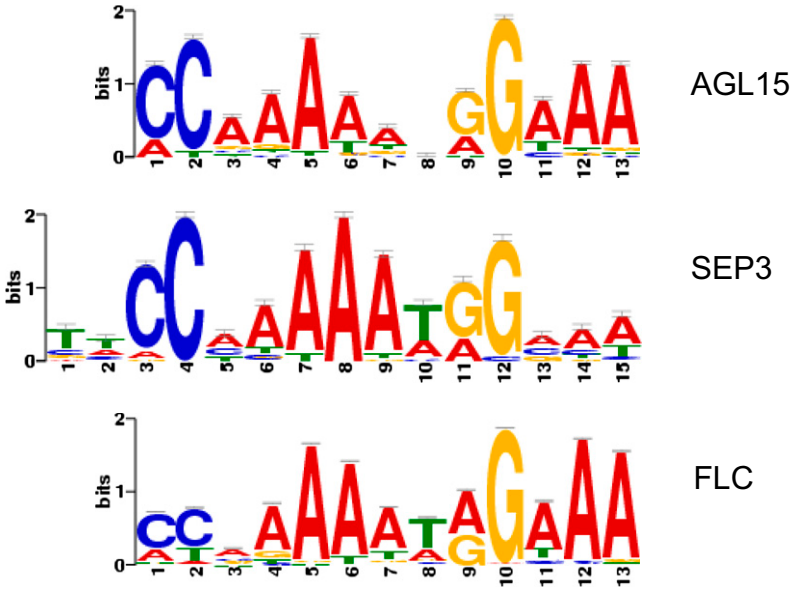

Fig. S2. Comparison of the FLC CArG-box motif with other DNA motifs. A comparison of the FLC CArG-box motif against the DNA motif database was performed by using the TOMTOM program. The DNA motifs with high similarity to the FLC CArG-box motif were included here.

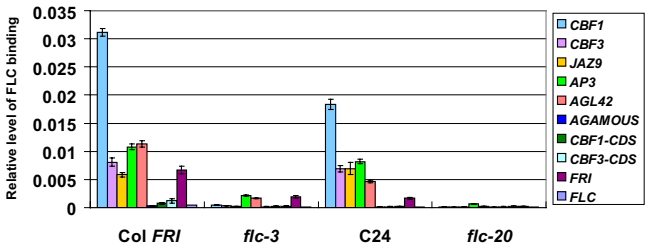

Fig. S3. ChIP-PCR validation for selected FLC target genes. ChIP was done with an FLC antibody in Col *FRI* and C24 and compared with the corresponding *flc* mutant: *flc-3* and *flc-20*. Input DNA was used as the reference in the PCR. *AGAMOUS*, *CBF1-CDS*, *CBF3-CDS*, and *FLC* were used as the negative controls with no FLC binding sites. Error bars represent SEs.

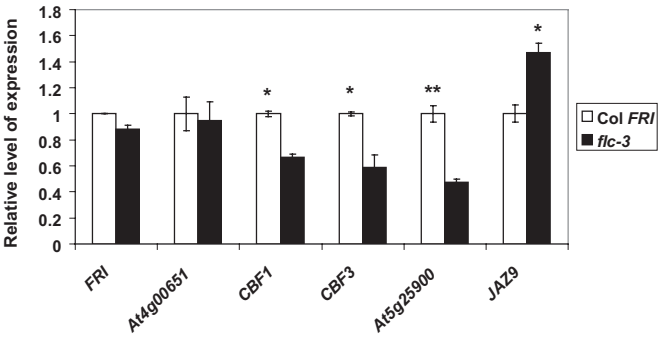

Fig. S4. Expression levels of candidate FLC target genes were determined in Col *FRI* and *flc-3* by using quantitative RT-PCR. *Actin* was used as the internal reference. The expression level of each gene in *flc-3* was normalized to the level in Col *FRI*. Error bars represent SEs. Asterisks indicate a significant change (*$P < 0.05$; **$P < 0.01$; Student's *t* test).

Table S1. QuEST parameters for the determination of FLC binding sites

Peak category	Parameter	*n*
Stringent peak	ChIP enrichment	50
	ChIP to background enrichment	3
	ChIP extension enrichment	3
Recommended peak	ChIP enrichment	30
	ChIP to background enrichment	3
	ChIP extension enrichment	3
Relaxed peak	ChIP enrichment	10
	ChIP to background enrichment	3
	ChIP extension enrichment	3

Table S2. Motif analysis with the fuzznuc program

	Sequence category					
Peaks/ motifs	Stringent	Recommended	Relaxed1	Relaxed2	Promoter-random	Genomic-random
			CCWWWWWWRG			
Total peaks	41	52	200	205	800	800
Motif present	38	45	168	124	477	374
Percentage, %	92.68	86.54	84.00	60.49	59.63	46.75
			CCWWWWWWRGWAA			
Total peaks	41	52	200	205	800	800
Motif present	20	31	93	68	125	71
Percentage, %	48.78	59.62	46.50	33.17	15.63	8.88

Table S3. Transcription factor families enriched in the FLC target genes

Transcription factor family	No. in FLC targets	Total FLC targets	No. in genome	Total annotated genomic genes	Fisher *P* value
MADS box	11	505	109	27,416	<0.001
AP2-EREBP	10	505	138	27,416	<0.01
NAC	7	505	96	27,416	<0.05

Table S4. Primers used in this study

Name	Sequence (5′ to 3′)
ChIP primers	
SEP3 ChIP For	AGATGAGAATCGGACGGCT
SEP3 ChIP Rev	TCTATTTTGGGTAACGAGGTCC
SPL15 ChIP For	GGCCTGACCCGAACACATT
SPL15 ChIP Rev	CCTCTATTCTCCTCCGTCCTTA
SPL3 ChIP For	ACTGTTCAGTCTTGCTTTTCCA
SPL3 ChIP Rev	CGGGTTCAAATATGCATGTTGT
JAZ6 ChIP For	AGGACACGTGAAGTATTGTTATGTG
JAZ6 ChIP Rev	GGCCTATGAAGTATGAACGCTATAA
SVP ChIP For	CACGATTTACTTTCCATTTCAGTCG
SVP ChIP Rev	GAGAGTAAAGAGAAGAGATGGAGGA
DIN10 ChIP For	TGCAACAGGAAAGTTTTACAAAT
DIN10 ChIP Rev	GACGACCTTATCACCAACTAAATTG
AGL16 ChIP For	TGCCATGTGTCAAAACATAACAAGC
AGL16 ChIP Rev	GAGATGTGGTTTTGTTGATCGAAAG
SOC1 ChIP For	TGTGTCGCAAATATGATGGAC
SOC1 ChIP Rev	CCATCCAACTAGATATTTATGGGAG
FT ChIP For	GGTGGAGAAGACCTCAGGAA
FT ChIP Rev	GTGGGGCATTTTTAACCAAG
FD ChIP For	CAATCCCACCCATCTTGACT
FD ChIP Rev	TCGGTGGACAAGTGATTTGA
CBF1 ChIP For	AGAGTGGTAAGGTGAAGAAAACAAC
CBF1 ChIP Rev	AATCCTCTAAACCCGTGGTTC
CBF3 ChIP For	AGAGTGAAGTGACGCGACACG
CBF3 ChIP Rev	CACAAGATAACCCAGTGCCA
JAZ9 ChIP For	TTTGTGGCGTTTGGTGTTTGGC
JAZ9 ChIP Rev	ATGCACCCGGAAACGCCTTAGC
AP3 ChIP For	TTGTAGGCAGAGGCTAGGTGAG
AP3 ChIP Rev	CGCGAACGAGTTTGAAAGTG
AGL42 ChIP For	AATATTGCTCAAAGCCTCAAAGTCG
AGL42 ChIP Rev	CCCTATTTTGGCAAACTTAACG
AGAMOUS ChIP For	CCCAAAGATTTTAGTGCCTCA
AGAMOUS ChIP Rev	GGTTCAAGTTGGGCAATCAC
CBF1-CDS ChIP For	CCTTATCCAGTTTCTTGAAACAGAG
CBF1-CDS ChIP Rev	GCGAAGTTGAGACATGCTGA
CBF3-CDS ChIP For	CCTTATCCAGTTTCTTGAAACAGAG
CBF3-CDS ChIP Rev	GGTTTGAAATGTTCCGAGCC
FRI ChIP For	AGAGAGAGATTTTTTGAGGAGGGAG
FRI ChIP Rev	CGATGTCGTTTCAAATCTTTCC
FLC ChIP For	CCCAGGTAAGGAAAAGGCG
FLC ChIP Rev	TCTCTGTGACGCATCCGTCG
RT-PCR primers	
SEP3 RT-PCR For	CAAGAGAGGCCTTAGCAGAACTTA
SEP3 RT-PCR Rev	TTTGTCTCAGTCAGCATGCG
SPL15 RT-PCR For	CGCTCCATCTCTTTACGGAAAC
SPL15 RT-PCR Rev	GGCTGAGCCATTGTAACCTT
SPL3 RT-PCR For	CAACAATGCAGCAGGTTTCA
SPL3 RT-PCR Rev	AAACAGACAGAGACACAGAGGATT
JAZ6 RT-PCR For	AAAATTCGATCTCAAAGGACAAC
JAZ6 RT-PCR Rev	GCTTTTGTCAAGATTCATGTGACTC
SVP RT-PCR For	GAGTTCTGTAGCTCCAGCATGA
SVP RT-PCR Rev	CCGCTTGTTCTCATCCATC
DIN10 RT-PCR For	CTTCTTCGCTTTCTGGCATTG
DIN10 RT-PCR Rev	CGAACCGCCGGTTTAATCGT
AGL16 RT-PCR For	GATTTCTCCAGCTCCAGCATGA
AGL16 RT-PCR Rev	GGTGAACGAGATTCCCCTCTC
TEM1 RT-PCR For	GGCGGAAGCAGCGTCGTTTT
TEM1 RT-PCR Rev	TCTGCTCAAACTCATCGGCG
TOE3 RT-PCR For	GGGAGTCACATATTTGGGACTG
TOE3 RT-PCR Rev	AAACCCAGTGCTTTGCCTT
SMZ RT-PCR For	GGGAATCTCATATTTGGGATTG
SMZ RT-PCR Rev	TGAGTTTTGAATTGGGTGCA
SOC1 RT-PCR For	ATTCGCCAGCTCCAATATGC

Table S4. Cont.

Name	Sequence (5′ to 3′)
SOC1 RT-PCR Rev	TGAGCTGCTCAATTTGTTCC
FT RT-PCR For	ACTATATAGGCATCATCACCGTTCGTTACTCG
FT RT-PCR Rev	ACAACTGGAACAACCTTTGGCAATG
FRI RT-PCR For	CACCGCTGGCATTTAAAGAA
FRI RT-PCR Rev	AGGAAAGCTTCTATCACGGTAGA
At4g00651 RT-PCR For	CAGTTTAAAGCCTGCAACTCA
At4g00651 RT-PCR Rev	AAATAGACCTCTGGCTCTTGGT
CBF1 RT-PCR For	CCTTATCCAGTTTCTTGAAACAGAG
CBF1 RT-PCR Rev	GCGAAGTTGAGACATGCTGA
CBF3 RT-PCR For	CCTTATCCAGTTTCTTGAAACAGAG
CBF3 RT-PCR Rev	GGTTTGAAATGTTCCGAGCC
At5g25900 RT-PCR For	GGTGCTAATGCACAGAAACG
At5g25900 RT-PCR Rev	CAACATCAATTGCACCTTCC
JAZ9 RT-PCR For	CTGTCGAAGAACGAGGGTTAA
JAZ9 RT-PCR Rev	GGGAGATATGTCATTAAAGACGCT

For, forward; Rev, reverse.

Other Supporting Information Files

Dataset S1. List of FLC binding sites and the associated target genes

Dataset S1 (XLS)