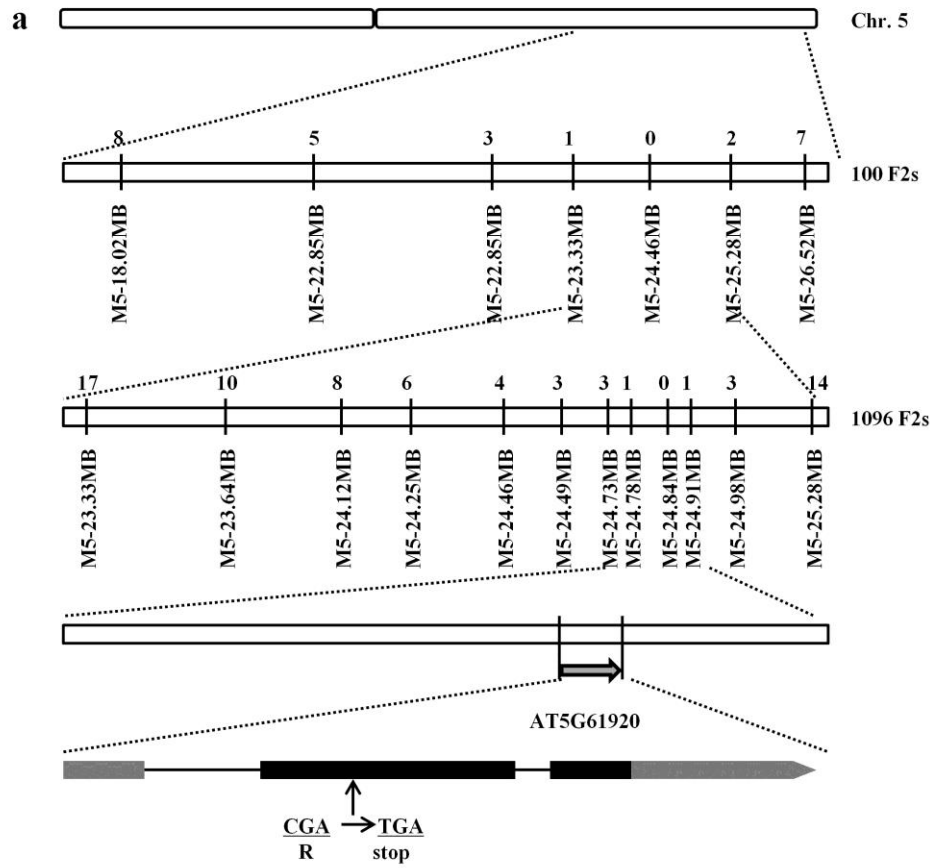


## **Supplementary Information**

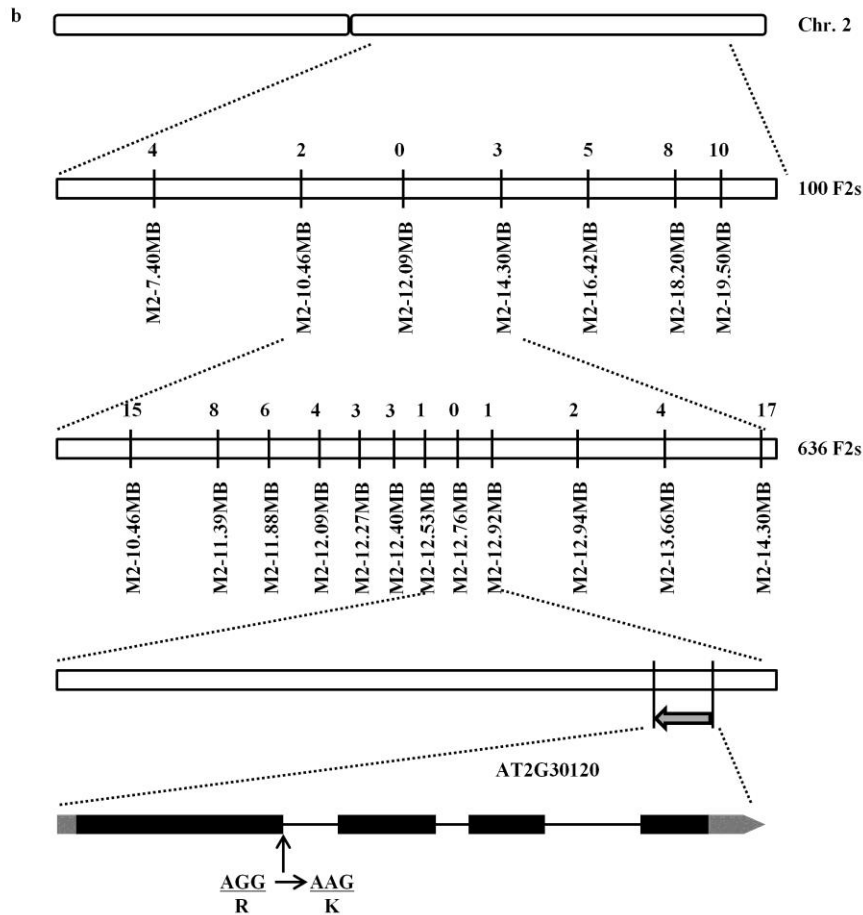
**Two *FLX* family members are non-redundantly required to establish the vernalization requirement in Arabidopsis**

Joohyun Lee & Richard M. Amasino

Supplementary Figure S1a



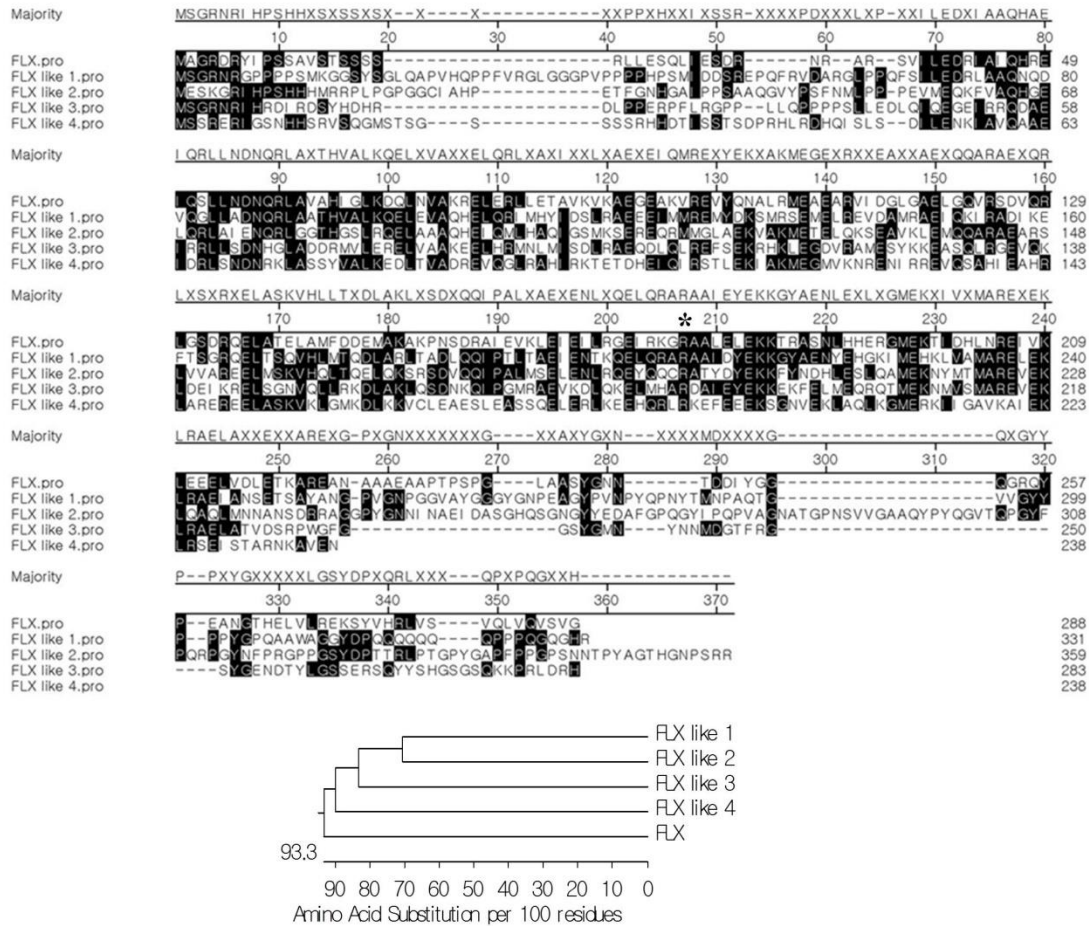
## Supplementary Figure S1b



### Supplementary Figure S1 | Positional cloning of *fl4-1* and *flx-3*

(a, b) The physical location of *fl4-1* and *flx-3* was determined in a segregating population derived from crossing mutants to Ler *FRI FLC* and selecting early-flowering F2 segregants. DNA sequencing identified mutations between the makers, M5-24.78 MB and M5-24.91 MB for *fl4-1*, and M2-12.53 MB and M2-12.92 for *flx-3*. The number of recombinant individuals for each marker is indicated. The gray arrow indicates the direction of gene transcription. Black and gray boxes indicate coding regions and open reading frames, respectively.

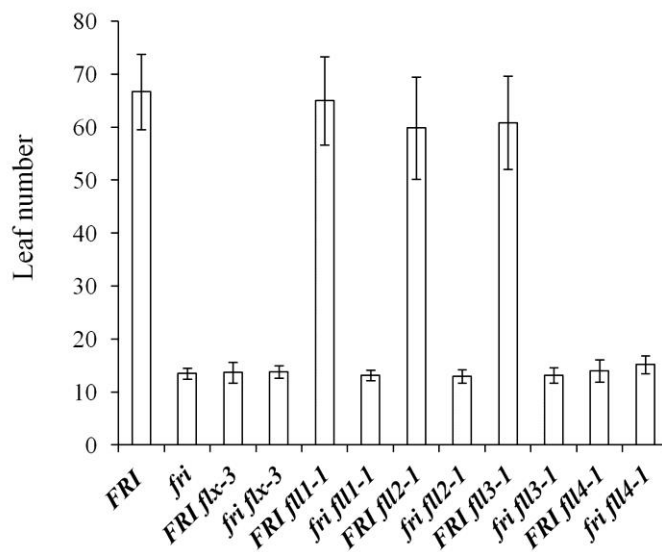
## Supplementary Figure S2



## Supplementary Figure S2 | *FLL4* encodes a protein with homology to *FLX*

Protein sequence alignments were performed using ClustalW. The tree and bootstrap analyses were performed using the neighbor-joining algorithm in MEGA version 4.0. Values indicate the number of times (in percent) that each branch topology was found during bootstrap analysis. The scale bar indicates an evolutionary distance of 0.2 amino acid substitutions per position in the sequence.

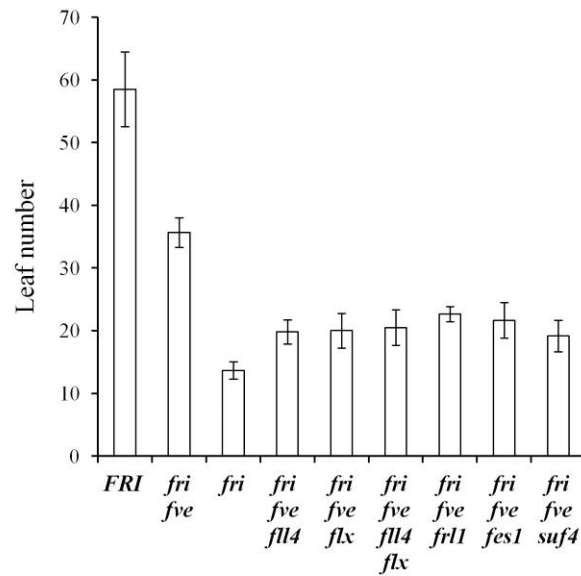
### Supplementary Figure S3



### Supplementary Figure S3 | *FLA* and *FLX* are the only *FLX* family members for which single-gene mutations affect flowering behavior

Flowering of loss of function mutations in *FLX* family members. Flowering was monitored as number of primary leaves formed prior to flowering in long days without vernalization. All of the flowering time data are presented as mean values of 12 individual plants. Error bars indicate standard deviation.

## Supplementary Figure S4



### Supplementary Figure S4 | Loss of all known *FRI* components suppresses the delayed flowering time of *fve*

Flowering behavior of all known *FRI* components in a *fri fve* background in long days without vernalization. All of the flowering time data are presented as mean values of 12 individual plants. Error bars indicate standard deviation.

## Supplementary Table S1

genes	forward primers (5' to 3')	reverse primers (5' to 3')
1. RT-PCR		
<i>FLC</i>	TCCGGCAAGCTCTACAGCTTC	AGCATGCTGTTTCCCATATCGATC
<i>VIN3</i>	CTGCTTCGCTCTCAAAGATCTG	CTCAATGCATTCAATATCTTCAAACG
<i>FLX</i>	AGATTCGTAAAGGAAGGGCTGCTC	GCGCTCATGGTGAAGGTTACTAGC
<i>FLL1</i>	AGTTGCAATGGCTCGGGAAGCTG	ACCACCATAAGCAACTCCACCAG
<i>FLL2</i>	AGCAAATACCTGCTCTGATGTCTG	TCATAGTCATATGTTGCCCTGCAC
<i>FLL3</i>	GGGTGAAATCAGAAGGCAGGATGC	ACCATTCGGTCGTCAGCTAGTC
<i>FLL4</i>	AGTTTAGAGGCGTCGAGTCAAGAG	CTTCTCTTCCTCGAACTCTTTCCG
<i>UBQ10</i>	CTACCGTGATCAAGATGCAGATC	TTGTCGATGGTGTCGGAGCTTTC
2. complementation		
<i>FLX</i>	CACCATGGCCGGACGAGATCGTTA TA	GTA AAA CCG CAA TCT TAT CAT AAG CGT TG
<i>FLL4</i>	CACCATGTCTTCAAGGGAGAGGATA G	AGC CAG ATT CGT AGA TTA ACC C

## Supplementary Table S1 | Primers used in the manuscript