Table S1 Potential off-target analysis at the three target sites of *GmFT2a* in the T1 generation

Target site	Putative off-target site				No. of plants sequenced[c]	No. of plants with mutations
	Gene locus	Region	Sequence[a]	MMs[b]		
GmFT2a-SP1	Glyma19g24640 19:-30300772	intron	GTgGtGAaCCTCTCGTTGTT GGG	3	18	0
	Glyma16g26690 16:+30780526	exon	GTAGGGAcCCTCTaGTTGT TGGG	2	18	0
GmFT2a-SP2	Glyma12g35200 12:-38349846	utr	CACAcGTgGgaAACCAACC AAGG	4	35	0
	Glyma15g10400 15:+7538434	intron	CtCAtGTTtTCAACCAAgCA AGG	4	35	0
GmFT2a-SP3	Glyma09g11720 9:-11946423	utr	gtCtGATATTaACCCTTGGTT GG	4	11	0
	Glyma16g26690 16:-30780651	exon	CACCGATATTTAttCTTGGT TGG	2	11	0

[a] Mismatched bases are shown in lowercase letters. [b] No. of mismatched bases. [c] T1 plants identified as biallelic mutants of *GmFT2a*.

Text S1 Frameshift mutations at three target sites of *GmFT2a* generated premature translation termination codons (PTCs)

CDS of *GmFT2a* (WT, wild-type)

ATGCCTAGTGGAAGTAGGGATCCTCTCGTTGTTGGGGGAGTAATTGGGGATGTATTGGATCCTTTTGAATATTCTATTCC
TATGAGGGTTACCTACAATAACAGAGATGTCAGCAATGGATGTGAATTCAAACCCTCACAAGTTGTCAACCAACCAAG
GGTAAATATCGGTGGTGATGACCTCAGGAACTTCTATACTTTGATTGCGGTTGATCCCGATGCACCTAGCCCAAGTGAC
CCCAATTTGAGAGAATACCTCCATTGGTTGGTGACTGATATCCCAGCAACAACAGGGGCTAGTTTCGGCCATGAGGTT
GTAACATATGAAAGTCCAAGACCAATGATGGGGATTCATCGTTTGGTGTTTGTGTTATTTCGTCAACTGGGTAGGGAGA
CCGTGTATGCACCAGGATGGCGCCAGAATTTCAACACTAAAGAATTTGCTGAACTTTACAACCTTGGATTGCCAGTTG
CTGCTGTCTATTTCAACATTCAGAGGGAATCTGGTTCTGGTGGAAGGAGGTTATACTAA

Protein sequence of *GmFT2a* (WT, wild-type)

MPSGSRDPLVVGGVIGDVLDPFEYSIPMRVTYNNRDVSNGCEFKPSQVVNQPRVNIGGDDLRNFYTLIAVDPDAPSPSDPN
LREYLHWLVTDIPATTGASFGHEVVTYESPRPMMGIHRLVFVLFRQLGRETVYAPGWRQNFNTKEFAELYNLGLPVAAVYF
NIQRESGSGGRRLY

***GmFT2a*-SP1 (1-bp insertion)**

CDS

ATGCCTAGTGGAAGTAGGGATCCTCTCGTTTGTTGGGGGAGTAATTGGGGATGTATTGGATCCTTTTGAATATTCTATTC
CTATGAGGGTTACCTACAATAACAGAGATGTCAGCAATGGATGTGAATTCAAACCCTCACAAGTTGTCAACCAACCAA
GGGTAAATATCGGTGGTGATGACCTCAGGAACTTCTATACTTTGATTGCGGTTGATCCCGATGCACCTAGCCCAAGTGA
CCCCAATTTGAGAGAATACCTCCATTGGTTGGTGACTGATATCCCAGCAACAACAGGGGCTAGTTTCGGCCATGAGGT
TGTAACATATGAAAGTCCAAGACCAATGATGGGGATTCATCGTTTGGTGTTTGTGTTATTTCGTCAACTGGGTAGGGAG
ACCGTGTATGCACCAGGATGGCGCCAGAATTTCAACACTAAAGAATTTGCTGAACTTTACAACCTTGGATTGCCAGTT
GCTGCTGTCTATTTCAACATTCAGAGGGAATCTGGTTCTGGTGGAAGGAGGTTATACTAA

Protein sequence

MPSGSRDPLVCWGSNWGCIGSF **Stop**

***GmFT2a*-SP1 (8-bp deletion)**

CDS

ATGCCTAGTGGAAGTAGGGATC--------GTTGGGGGAGTAATTGGGGATGTATTGGATCCTTTTGAATATTCTATTCCTATG
AGGGTTACCTACAATAACAGAGATGTCAGCAATGGATGTGAATTCAAACCCTCACAAGTTGTCAACCAACCAAGGGTA
AATATCGGTGGTGATGACCTCAGGAACTTCTATACTTTGATTGCGGTTGATCCCGATGCACCTAGCCCAAGTGACCCCA
ATTTGAGAGAATACCTCCATTGGTTGGTGACTGATATCCCAGCAACAACAGGGGCTAGTTTCGGCCATGAGGTTGTAA
CATATGAAAGTCCAAGACCAATGATGGGGATTCATCGTTTGGTGTTTGTGTTATTTCGTCAACTGGGTAGGGAGACCGT
GTATGCACCAGGATGGCGCCAGAATTTCAACACTAAAGAATTTGCTGAACTTTACAACCTTGGATTGCCAGTTGCTGC
TGTCTATTTCAACATTCAGAGGGAATCTGGTTCTGGTGGAAGGAGGTTATACTAA

Protein sequence

MPSGSRDRWGSNWGCIGSF **Stop**

***GmFT2a*-SP2 (1-bp insertion)**

CDS

ATGCCTAGTGGAAGTAGGGATCCTCTCGTTGTTGGGGGAGTAATTGGGGATGTATTGGATCCTTTTGAATATTCTATTCC
TATGAGGGTTACCTACAATAACAGAGATGTCAGCAATGGATGTGAATTCAAACCCTCACAAGTTGTCAACCAAACCAA
GGGTAAATATCGGTGGTGATGACCTCAGGAACTTCTATACTTTGATTGCGGTTGATCCCGATGCACCTAGCCCAAGTGA
CCCCAATTTGAGAGAATACCTCCATTGGTTGGTGACTGATATCCCAGCAACAACAGGGGCTAGTTTCGGCCATGAGGT
TGTAACATATGAAAGTCCAAGACCAATGATGGGGATTCATCGTTTGGTGTTTGTGTTATTTCGTCAACTGGGTAGGGAG

ACCGTGTATGCACCAGGATGGCGCCAGAATTTCAACACTAAAGAATTTGCTGAACTTTACAACCTTGGATTGCCAGTT

GCTGCTGTCTATTTCAACATTCAGAGGGAATCTGGTTCTGGTGGAAGGAGGTTATACTAA

Protein sequence

MPSGSRDPLVVGGVIGDVLDPFEYSIPMRVTYNNRDVSNGCEFKPSQVVNQTKGKYRW **Stop**

***GmFT2a*-SP2 (4-bp deletion)**

CDS

ATGCCTAGTGGAAGTAGGGATCCTCTCGTTGTTGGGGGAGTAATTGGGGATGTATTGGATCCTTTTGAATATTCTATTCC

TATGAGGGTTACCTACAATAACAGAGATGTCAGCAATGGATGTGAATTCAAACCCTCACAAGTTGTCAA----CCAAGGG

TAAATATCGGTGGTGATGACCTCAGGAACTTCTATACTTTGATTGCGGTTGATCCCGATGCACCTAGCCCAAGTGACCC

CAATTTGAGAGAATACCTCCATTGGTTGGTGACTGATATCCCAGCAACAACAGGGGCTAGTTTCGGCCATGAGGTTGT

AACATATGAAAGTCCAAGACCAATGATGGGGATTCATCGTTTGGTGTTTGTGTTATTTCGTCAACTGGGTAGGGAGACC

GTGTATGCACCAGGATGGCGCCAGAATTTCAACACTAAAGAATTTGCTGAACTTTACAACCTTGGATTGCCAGTTGCT

GCTGTCTATTTCAACATTCAGAGGGAATCTGGTTCTGGTGGAAGGAGGTTATACTAA

Protein sequence

MPSGSRDPLVVGGVIGDVLDPFEYSIPMRVTYNNRDVSNGCEFKPSQVVNQG **Stop**

***GmFT2a*-SP3 (14-bp deletion)**

CDS

ATGCCTAGTGGAAGTAGGGATCCTCTCGTTGTTGGGGGAGTAATTGGGGATGTATTGGATCCTTTTGAATATTCTATTCC

TATGAGGGTTACCTACAATAACAGAGATGTCAGCAATGGATGTGAATTCAAACCCTCACAAGTTGTCAACCAACC-------

-------GTGGTGATGACCTCAGGAACTTCTATACTTTGATTGCGGTTGATCCCGATGCACCTAGCCCAAGTGACCCCAATTT

GAGAGAATACCTCCATTGGTTGGTGACTGATATCCCAGCAACAACAGGGGCTAGTTTCGGCCATGAGGTTGTAACATAT

GAAAGTCCAAGACCAATGATGGGGATTCATCGTTTGGTGTTTGTGTTATTTCGTCAACTGGGTAGGGAGACCGTGTATG

CACCAGGATGGCGCCAGAATTTCAACACTAAAGAATTTGCTGAACTTTACAACCTTGGATTGCCAGTTGCTGCTGTCTA

TTTCAACATTCAGAGGGAATCTGGTTCTGGTGGAAGGAGGTTATACTAA

Protein sequence

MPSGSRDPLVVGGVIGDVLDPFEYSIPMRVTYNNRDVSNGCEFKPSQVVNQPW **Stop**

***GmFT2a*-SP3 (1-bp insertion)**

CDS

ATGCCTAGTGGAAGTAGGGATCCTCTCGTTGTTGGGGGAGTAATTGGGGATGTATTGGATCCTTTTGAATATTCTATTCC

TATGAGGGTTACCTACAATAACAGAGATGTCAGCAATGGATGTGAATTCAAACCCTCACAAGTTGTCAACCAACCAAA

GGGTAAATATCGGTGGTGATGACCTCAGGAACTTCTATACTTTGATTGCGGTTGATCCCGATGCACCTAGCCCAAGTGA

CCCCAATTTGAGAGAATACCTCCATTGGTTGGTGACTGATATCCCAGCAACAACAGGGGCTAGTTTCGGCCATGAGGT

TGTAACATATGAAAGTCCAAGACCAATGATGGGGATTCATCGTTTGGTGTTTGTGTTATTTCGTCAACTGGGTAGGGAG

ACCGTGTATGCACCAGGATGGCGCCAGAATTTCAACACTAAAGAATTTGCTGAACTTTACAACCTTGGATTGCCAGTT

GCTGCTGTCTATTTCAACATTCAGAGGGAATCTGGTTCTGGTGGAAGGAGGTTATACTAA

Protein sequence

MPSGSRDPLVVGGVIGDVLDPFEYSIPMRVTYNNRDVSNGCEFKPSQVVNQPKGKYRW **Stop**

Text S1 Frameshift mutations at three target sites of *GmFT2a* generated premature translation termination codons (PTCs). CDS, coding sequence. Blue capital letter, target sequence. Red capital letter, protospacer adjacent motif. Underline, insertions. Dashes, deletions. Yellow rectangle, termination codon.

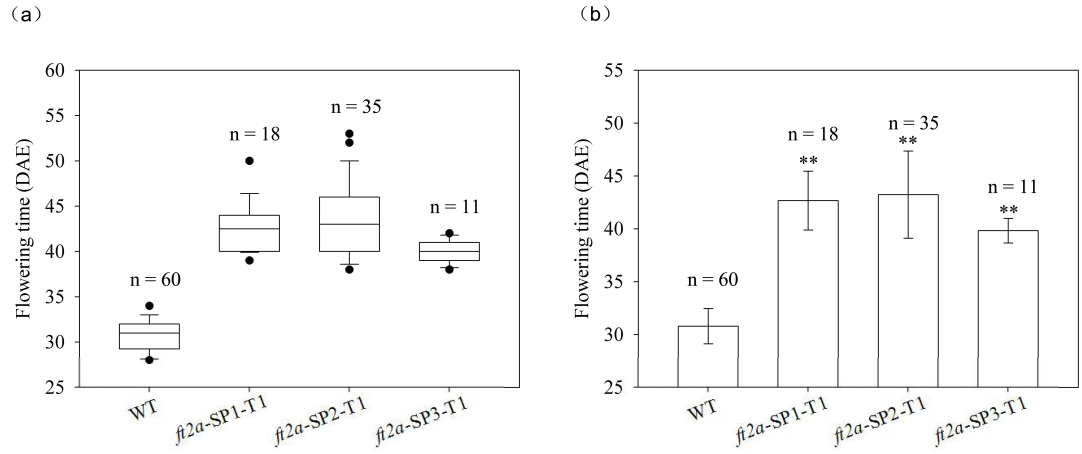

Figure S1 Homozygous *ft2a* mutants at three target sites in the T1 generation delayed flowering time under natural conditions (summer) in Beijing, China (N39°58', E116°20'). WT, wild-type plant (Jack). *ft2a*-SP1-T1, *ft2a*-SP2-T1 and *ft2a*-SP3-T1 were homozygous mutants of *GmFT2a* at the three target sites in the T1 generation, respectively. n, exact numbers of individual plants identified. The flowering time are shown as the mean values ± standard deviation. **, homozygous T1 *ft2a* mutants exhibit highly significant late flowering ($P < 0.01$). DAE, days after emergence.

Table S2 T2 *ft2a* mutants under LD and SD conditions

T1 homozygous *ft2a* mutants	The types of homozygous mutations	No. of the progeny plants under LD conditions	No. of the progeny plants under SD conditions	Total
ft2a-SP1-T1#11.14	1-bp insertion at *GmFT2a*-SP1	4	3	7
ft2a-SP1-T1#11.15	1-bp insertion at *GmFT2a*-SP1	8	6	14
ft2a-SP1-T1#11.16	1-bp insertion at *GmFT2a*-SP1	7	6	13
ft2a-SP2-T1#16.04	1-bp insertion at *GmFT2a*-SP2	7	8	15
ft2a-SP2-T1#16.11	1-bp insertion at *GmFT2a*-SP2	7	8	15
ft2a-SP2-T1#16.12	1-bp insertion at *GmFT2a*-SP2	8	7	15
ft2a-SP3-T1#30.02	14-bp deletion at *GmFT2a*-SP3	3	3	6
ft2a-SP3-T1#30.08	14-bp deletion at *GmFT2a*-SP3	6	6	12
ft2a-SP3-T1#30.12	14-bp deletion at *GmFT2a*-SP3	6	6	12
Total		56	53	109

Text S2 The sequences of the Cas9 and *Arabidopsis U6* promoter used in the present study.

Cas9 DNA sequence

atggcccctaagaagaagagaaaggtcggtattcacggcgttcctgcggcgatggacaagaagtatagtattggtctggacattgggacgaattccgttggctgggccgtgatcaccgatgagt
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tctacgagacgcggatcgacttgagtcagctcgggggggataagagaccagcggcaaccaagaaggcaggacaagcgaagaagaagaagtag

***Arabidopsis U6* promoter**

ttcgttgaacaacggaaactcgacttgcctttccgcacaatacatcatttcttcttagctttttttcttcttcttcgttcatacagttttttttgtttatcagcttacatttttcttgaaccgtagctttcgttttcttctt
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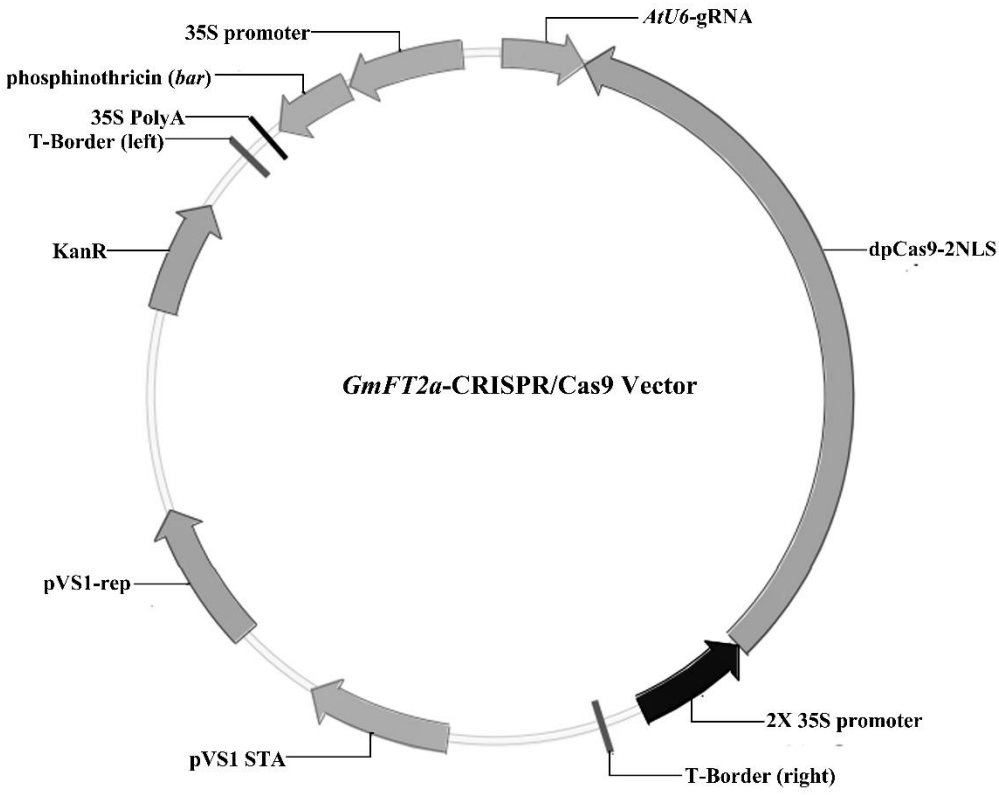

Figure S2 Schematic illustrating the basic architecture of the constructs used for CRISPR/Cas9-mediated genome editing. *AtU6*, *Arabidopsis U6* promoter. gRNA, guide-RNA. dpCas9, Cas9 codon-optimized for dicotyledons. NLS, nuclear localization sequence. The *bar* gene driven by a CaMV 35S promoter is used as a screening marker. KanR, kanamycin resistance gene. pVS1-rep, pVS1 replication origin. pVS1 STA, pVS1 stability function.

Table S3 Primer sequences used in the present study

Primer name	Primer sequence	Size (bp)	Purpose
GmFT2a-F	ATTCATAACAAAGCAAACGAG	653	To amplify the regions which span the target sites
GmFT2a-R	ACTTGACCTTCCCTTAAACAC		
OFF1-F	CGGAGACAAAATGCAGCAGG	684	To examine potential off-target site 1
OFF1-R	GGCCTACAACTATGCTCCCC		
OFF2-F	GGGTCATGCAGAAACTCACCT	533	To examine potential off-target site 2
OFF2-R	TGTGACGTTTGCATGTCTTTTT		
OFF3-F	GGGAAGAGTTTCGGGTCGTT	608	To examine potential off-target site 3
OFF3-R	AAGGTTTCTCATGGTCGGTGT		
OFF4-F	GGCATCATTGCCCCATTTGTT	426	To examine potential off-target site 4
OFF4-R	AACCAAGGAATCAAGTGAAAGCAT		
OFF5-F	AAATTTTATGGCACCACTACCTGC	701	To examine potential off-target site 5
OFF5-R	CTCATCACCATCGCCATCTCT		
OFF6-F	GGGTCATGCAGAAACTCACCT	533	To examine potential off-target site 6
OFF6-R	TGTGACGTTTGCATGTCTTTTT		
Cas9-F	TTGGGGCTCACACCAAACTT	910	To amplify part of the Cas9 coding sequence
Cas9-R	CGATCGCCTTCTTTTGCTCG		
sgRNA-F	AAAAGGCCCCTGGGAATCTG	593	To amplify the region from the *AtU6* promoter to downstream vector sequence spanning the sgRNA
sgRNA-R	GATGAAGTGGACGGAAGGAAGGAG		
GmActin-F	CGGTGGTTCTATCTTGGCATC	249	To amplify *GmActin* as a normalization control.
GmActin-R	GTCTTTCGCTTCAATAACCCTA		
q*GmFT2a*-F	GGATTGCCAGTTGCTGCTGT	160	qRT-PCR analysis (*GmFT2a*)
q*GmFT2a*-R	GAGTGTGGGAGATTGCCAAT		
q*GmActin*-F	CGGTGGTTCTATCTTGGCATC	249	qRT-PCR analysis (*GmActin*)
q*GmActin*-R	GTCTTTCGCTTCAATAACCCTA		