Supporting Information

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SI Materials and Methods

Manual Large-Scale Genotyping of Soybean Seeds. The tissue (cotyledon) was obtained by drilling a hole in the seed surface without damaging the embryonic axis. The initially drilled material was discarded to eliminate contamination from the seed coat. Collected materials were then transferred into wells in a 384-well plate. The drill and tube were cleaned using compressed air. Subsequent DNA extraction, PCR, and electrophoresis were performed following standard protocols.

Screening and Sequence Data for BAC Clones. Three-dimensional screening, contig development, and BAC sequencing were performed as described previously (1). Sequence data for the related BAC clones (Fig. S2) were deposited in the DNA Data Base in Japan with the accession nos. AP011817 (WBb10K2), AP011820 (WBb220D5), AP011823 (WBb55K17), AP011818 (WBb120K2), AP011819 (WBb20D6), AP011815 (MiB63F7), AP011814 (MiB42G1), AP011816 (MiB93E8), and AP011812 (MiB309A12). All marker positions and physical distances shown in Fig. 2 were based on the contig information obtained in this study.

Southern Hybridization. To confirm the existence of two paralogs of E1 in the soybean genome and deletion of E1 in cultivars with the e1-nl genotype, we selected BanII and BanII+EcoRV restriction enzymes based on sequence analysis of proximate regions where E1 and its two paralogues reside. We also included EcoRV to confirm whether two bands were present, as we predicted that two fragments containing E1 or its paralogue after digestion would be of the same size. To evaluate the effect of transgene copy number, we performed Southern hybridization by using *sGFP* and *E1* probes amplified with the primers sGFP and TI, respectively (Table S4). We used a standard Southern hybridization protocol with the AlkPhos Direct Labeling and Detection System (GE Healthcare Japan).

Screening and Characterization of Ethylmethanesulfonate-Derived Mutations. Dry seeds of OLERICHI50 (PI643976), derived from the cultivar Bay, were soaked in a 0.35% (wt/vol) ethylmethanesulfonate (EMS) solution for 12 h, and then rinsed in tap water for 8 h. M2 seeds were obtained from self-pollinated M1 plants. For Fukuyutaka (JP29688) mutagenesis, some M2 seeds were retreated with EMS and the resultant M2 seeds were referred to as M2-2. The EMS treatments were performed as described earlier. Green leaves were harvested from 3,360 (OLERICHI50), 3,331 (Fukuyutaka M2), and 3,895 (Fukuyutaka M2-2) individual M2 plants for DNA preparation. Genomic DNAs were extracted using the cetyltrimethylammonium bromide method and purified using diatomaceous earth columns. Pooled DNAs from five to eight individuals were used for mutant screening. We performed Targeting Induced Local Lesions IN Genomes screening as described previously (2), except that we used 2% (wt/vol) agarose gel to separate CEL I-digested DNA fragments stained with GelRed (Biotium). Primers used for the Targeting Induced Local Lesions IN Genomes screening were GmE1-5'-F1 (5'-GACAAAACC-TGAACTCAAATT-3') and GmE1-3'-R1 (5'-AAGGATTTCA-TAAGATACAACATT-3'). The PCR using Pfu DNA polymerase consisted of 40 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, without initial denaturation or final extension. CEL I was purified from celery stalks, as described previously (3).

For phenotypic characterization of the three EI mutant lines, we first evaluated phenotypic segregation for flowering time in F₂ populations derived from a cross between homozygous e1-m1 plants and WT OLERICHI50. The F₂ population (96 plants) was grown in a greenhouse with a 15 h light/9 h dark photoperiod extended with metal halide lamps for 30 d after sowing; thereafter, we used a 14 h light/10 h dark photoperiod. The allelic information was acquired by direct sequencing of fragments amplified by using the primer pair 5'-CCGTTTGATTGGTTT-TTGGT-3' (forward) and 5'-AACAAGCGATGAAGGGAAA-A-3' (reverse) and sequenced using the primers 5'-CCCTTC-AGTTTCTGCAGCTC-3' or 5'-AACCGTGCATAGATCTCC-TGCTA-3'. We selected 12 representative plants per genotype for subsequent analysis. For other mutations, we grew offspring of the heterozygous genotypes (E1/e1-m2 or E1/e1-m3) or the homozygous genotypes (e1-m2/e1-m2 or e1-m3/e1-m3) for e1-m2 and e1-m3, along with the original WT cultivar Fukuyutaka as a control, and evaluated their flowering phenotypes under natural day-length conditions. Genotypes were identified by direct sequencing.

Alignment and Phylogenetic Analysis. We searched for expressed sequence tags using the Computational Biology and Functional Genomics Laboratory database (http://compbio.dfci.harvard.edu/ tgi/cgi-bin/tgi/Blast/index.cgi). E1 homologues were also identified using BLASTP searches against Lotus japonicus (www.kazusa.or. jp/lotus) and other species (http://www.phytozome.net) with E < 10^{-5} . Glyma06g23040.1 (corresponding to E1), two paralogues Glyma04g24640.1 and Glyma18g22670.1 (corresponding to Gm18g22670), and other homologous protein sequences were retrieved from the gene family encoding a domain of unknown function (DUF313) at the Angiosperm node (http://www.phytozome.net, version 8.0). First, from this DUF313 family, the sequences with high homology to E1 were selected. Second, some randomly selected angiosperm species were included for analysis as a result of the large size of this gene family. For each selected species, the gene most homologous to E1 was chosen. As some members of a gene family were annotated as having a B3 domain, we added several well characterized B3 domain sequences as well as their homologues in soybean for the alignment and phylogenetic analysis. Sequences were first aligned by using Clustal X2 (4), with default parameter values (protein weight matrix using the Gonnet Series with a gap penalty of 10.00, a gap length penalty of 0.20, and a delay-divergent cutoff of 30%). The central alignments corresponding to the E1 protein sequence were curated, and were realigned by using Clustal X2 with the default parameter values. Thereafter, phylogenetic analysis was performed using MEGA4, with the neighbor-joining method and 1,000 bootstrap iterations (5). The alignment of the E1 protein with other highly homologous sequences from Phaseolus vulgaris, Medicago truncatula, and L. japonicus is shown in Fig. 3A. The condensed tree with a cutoff value of 40% is shown in Fig. 3B.

RNA Preparation and Quantitative PCR. Plants were grown in growth chambers with normal cool white lamps (MLRBOC400FU; Mitsubishi/OSRAM) with long (16 h light/8 h dark), short (12 h light/12 h dark), or intermediate (14.5 h light/9.5 h dark) day length. In the early vegetative growth stage, plants were positioned at the middle of the growth chamber with a light intensity of approximately 200 μ mol·s⁻¹·m⁻²; during the late growth stage, major functional leaves received light with a stronger intensity of approximately 270 μ mol·s⁻¹·m⁻² because they were closer to the top of the growth chamber. All samples for RNA extraction or expression were taken from fully expanded leaves except for our analyses of tissue-specific expression. To minimize damage to the plants, each

sample was collected from the leaf margins of two plants. Total leaf RNAs were extracted using the TRIzol method (Life Technologies) and treated with DNase (Takara Bio). RNA yield and quality were examined with a NanoDrop spectrophotometer (NanoDrop Technologies). We used 1 μ g of total RNA for first-strand cDNA synthesis using ReverTra Ace (Toyobo) and a standard oligo(dT20) primer according to the manufacturer's instructions. The cDNAs were then diluted threefold with PCR-grade water, and 2- μ L aliquots were used as RT-PCR templates.

Semiquantitative RT-PCR. The PCR was performed using Ex-Taq under parameters and conditions described in Table S4. PCR products were resolved in nondenaturing polyacrylamide gel [5% (wt/vol) for stacking and 11–13% (wt/vol) for separating] were fluorescently stained with Vistra Green (GE Healthcare Japan)

- 1. Xia Z, Sato H, Watanabe S, Kawasaki S, Harada K (2005) Construction and characterization of a BAC library of soybean. *Euphytica* 141:129–137.
- McCallum CM, Comai L, Greene EA, Henikoff S (2000) Targeting induced local lesions IN genomes (TILLING) for plant functional genomics. *Plant Physiol* 123:439–442.
- 3. Yang B, et al. (2000) Purification, cloning, and characterization of the CEL I nuclease. Biochemistry 39:3533–3541.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25:4876–4882.

(6). Band intensity on the scanned gel image semiquantitatively represented the gene expression level.

Real-time quantitative RT-PCR. Transcript levels were quantified using real-time quantitative RT-PCR (iCycle iQ; BioRad) using a SYBR Green SuperMix kit (BioRad). We used 2 μ L of diluted cDNA in a 20- μ L reaction volume. Gene expression level was quantified using the nonequal efficiencies method with *GmTubulin* as an internal control (7).

To analyze gene expression in the transgenic plants under longday conditions (16 h light/8 h dark), samples from fully expanded leaves were taken weekly starting 2 wk after emergence. Unless otherwise indicated, leaf samples were taken exactly 4 h after the light was turned on. The growth and flowering status were observed daily.

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- 6. Xia Z, et al. (2007) An integrated high-density linkage map of soybean with RFLP, SSR, STS, and AFLP markers using A single F2 population. *DNA Res* 14:257–269.
- Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:e45.



Fig. S1. Graphical genotypes of soybean recombinants carrying crossovers in the *E1* region in the 2006 to 2009 experiments. *Left*: Recombinants used for finescale mapping of the *E1* locus. *Right*: Phenotypic segregation patterns in the following year in the progenies. Recombination is shown by white bars representing Harosoy (*e1*), black bars representing Harosoy-*E1*, and cross-hatched bars representing the heterozygote. The phenotypic segregation is shown in boxplot format. The interquartile region, median, and range are indicated by the box, the bold vertical line, and the horizontal line, respectively. In 2006 to 2007, with seven recombinants, we were able to delimit the *E1* to a 289-kb region. In 2008 to 2009, with 10 recombinants, we further delimited the *E1* region to a 17.4-kb region (chromosome Gm06 in Glyma1.0; http://www.phytozome.net/soybean). The phenotypic parameter flowering time (R1) and the genotypes at marker TI are summarized in Table S1. The sowing dates (April 2007 and June 2009) appeared to affect the phenotypic segregation ranges, but not the segregation patterns.



Fig. 52. Polymorphisms (gaps or insertion/deletions) between two contigs in the *E1* region built from BAC clones of Misuzudaizu and Williams 82. The deletions and transposed regions are marked in yellow, green, and brown, respectively. The physical positions of the markers (Table S4) used for fine-scale mapping are also shown. Black bars labeled with MIB or WBb represent BAC clones from Misuzudaizu and Williams 82, respectively. Their accession numbers are listed in *SI Materials and Methods*.



Fig. S3. The presence of *E*1 and its two paralogues in the soybean genome were confirmed by Southern hybridization. Three copies of *E*1 were present in cultivars of Williams 82 (*e*1-*as*), Sakamotowase (*e*1-*fs*), Harosoy-*E*1 (*E*1), and Harosoy (*e*1-*as*). The bands marked with stars, which correspond to the *E*1 locus, was absent in the *e*1-*n*1 cultivars Yukihomare and Toyosuzu, indicating that a deletion occurred in the *E*1-anchored regions. Only two bands (one for Toyosuzu, *e*1-*n*1), were present (as predicted) using *Eco*RV, as the two fragments containing *E*1 and *Gm*18g22670 are of similar size. M represents the DNA molecular weight markers (λ .*Hind*III).



Fig. 54. Characterization of *E1* mutants induced by EMS. (A) Three independent mutations occurred at different position in *E1*. (*B*–*D*) Representative sequence electrograms showing the allelic variations at the mutation point (nucleotide) in F2 population derived from a cross between e1-m1 and WT OLERICHI50 (*E1*) (*B*), and in the progenies of from self-pollinated heterozygous genotype of e1-m2 (*C*), and e1-m3 (*D*) with a genetic background of Fukuyutaka (*E1*). The reverse complementary sequences are displayed in *B* and *C*. The mutation positions are marked by black triangles. Phenotypic data for each genotype are shown in Table 1.



Fig. S5. Characterization of the B3-like domains of the E1 protein. (*A*) Comparison of the structural models of the B3-like domains of AtRAV1 and E1. The model was generated by using the Phyre server (http://www.imperial.ac.uk/phyre) based on the NMR solution structure of the B3 domain of *Arabidopsis thaliana* AtRAV1 (Protein Data Bank code 1wid). Both structures are displayed with the same orientation after 3D superimposition. The candidate residues involved in the binding of AtRAV1 to DNA, and their corresponding residues in E1, are shown in the ball-and-stick representation. (*B*) Structure-based sequence alignment of the B3 domains of E1. Sequences of the last six proteins (At, *A. thaliana;* Zm, *Zea mays*) were obtained from the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov) with the accession number shown at the end of each sequence. AlB3, ABSCISIC ACID INSENSITIVE3; ARF, Auxin Response Factor; RAV, Related to ABI3 and VP1; VP1, VIVIPAROUS1. Letters with a gray background are residues conserved in at least two families. The amino acid residues in red and blue, respectively, correspond to secondary structure α -helix and β -strand regions of AtRAV1 derived from its NMR solution structure, whereas those in E1 were obtained by using the Jpred3 secondary structure prediction server (http://www.compbio.dundee.ac.uk/www-jpred). The predicted DNA-contacting residues for AtRAV1 are indicated by asterisks above the sequences.

				Japan)	
Line	E1 genotype	n	Mean	SD	P value [†]
Progeny test in 2007 (NIAS,	Tsukuba, Japan)				
060-1057	e1/e1 ⁺	77	53.7	1.29	_
060–784	E1/e1 ⁺	148	68.8	7.76	_
060–946	E1/e1 ⁺	106	67.5	6.74	_
060–828	E1/E1 ⁺	96	74.5	2.1	_
060–285	e1/e1 [†]	118	54.3	1.69	_
060–250	E1/E1 ⁺	127	76.4	2.08	_
060–52	E1/e1 ⁺	108	64.9	6.05	_
Harosoy-E1	E1/E1 ⁺	5	73.2	0.98	_
Harosoy (e1)	e1/e1 [†]	5	54.2	0.63	_
Association analysis betwee	en phenotypic parameters for flow	ering time (R1)	and E1 genot	ype determine	ed using TI
marker (2009)					
113	e1/e1 [§]	58	37.1	1.31	
144	E1/E1 [§]	102	51.2	0.67	_
177	E1/E1 [§]	60	51.6	0.74	_
241	e1/e1 [§]	26	36.5	1.18	$6.16 imes 10^{-39}$
	E1/e1 [§]	52	46.5	1.13	
	E1/E1 [§]	19	60.4	0.88	
268	E1/E1 [§]	57	50.2	0.61	_
306	e1/e1 [§]	24	36.4	1.58	1.39×10^{-36}
	E1/e1 [§]	47	46.5	1.29	
	E1/E1 [§]	27	50.3	1.08	
337	e1/e1 [§]	59	36.4	1.58	_
378	e1/e1 [§]	25	37.5	0.7	$6.10 imes 10^{-49}$
	E1/e1§	50	47.3	1.04	
	E1/E1 [§]	22	50.8	0.89	
427	E1/E1 [§]	58	51.3	0.75	_
446	e1/e1 [§]	21	36.2	1.41	6.58×10^{-32}
	E1/e1 [§]	56	46.7	0.96	
	E1/E1 [§]	21	50.9	0.87	

Table S1. Progeny test for the recombinant lines used for fine-scale mapping of E1

Flowering time (R1)* in progeny (NIAS Tsukuba,

NIAS, National Institute of Agrobiological Sciences.

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*Differences in R1 among different genotypes were evaluated by means of ANOVA, and a significant difference (P < 0.001) was observed for the whole segregation data set in 2007 or in 2009.

[†]Differences in flowering times among different *E1* genotypes in a progeny derived from the self-pollinated heterozygous parent at the *E1* locus were analyzed by means of ANOVA.

[†]Genotype at the *E1* locus determined based on the segregation pattern in the progeny.

[§]Genotype at the E1 locus determined using the TI marker that was specifically designed for distinguishing between E1 and e1-as.

Table S2.	Flowering	time for	cultivars	carrying	different	E alleles

				Flowering time	(R1, days af	ter emergence)
			Growth	chamber, light	/dark h*	
Cultivar or NIL	Accession no.	Genotype	12/12	14.5/9.5	16/8	Field [†] (NIAS, Japan)
Aokimame	JP 28298	E1,E2,E3,E4	31.0	ND	106.0	89.6
Peking	JP 28432	E1,E2,E3,E4	ND	65.0	98.0	55.2
Bay	PI 553043	E1,E2,E3,E4	29.5	ND	75.0	60.0
Harosoy-E1	PI 547676	E1,e2,E3,E4	31.0	51.0	73.0	46.4
1–136(<i>E1</i>) [‡]	—	E1,E2,e3,E4	ND	66.0	ND	ND
Enrei	JP 28862	E1,e2,e3,E4	ND	51.0	71.0	49.0
9L [§]	—	E1,e2,e3,E4	29.0	ND	58.5	ND
Kariyutaka	JP 86520	E1,E2,e3,e4	ND	32.0	36.0	36.6
130L [§]	_	E1E4	ND	39.0	ND	ND
130E [§]	_	E1e4	ND	33.0	ND	ND
Clark	PI 617268	e1-as,E2,E3,E4	ND	40.0	58.0	42.4
Williams 82	PI 518671	e1-as,E2,E3,E4	29.0	ND	54.0	41.8
Harosoy-E2	PI 547768	e1-as,E2,E3,e4	30.0	ND	57.0	36.9
1–136(<i>e1</i>) [‡]	—	e1-as,E2,e3,E4	ND	40.0	ND	ND
Harosoy	PI 547707	e1-as,e2,E3,E4	29.0	33.5	49.0	35.4
Harosoy-e4	PI 591435	e1-as,e2,E3,e4	29.0	ND	44.0	33.2
Harosoy-e3	PI 547716	e1-as,e2,e3,E4	29.5	ND	43.0	32.3
Sakamotowase	JP 27450	e1-fs,e2,e3,E4	29.0	31.0	30.0	29.7
9E [§]	_	e1-fs,e2,e3,E4	29.0	ND	32.0	ND
Yukihomare	—	e1-nl,e2,E3,e4	ND	34.0	36.0	34.9
Fiskeby V	JP 30465	e1-nl,e2,e3,E4	29.0	ND	30.0	32.0

JP, accessions from the Japanese NIAS GenBank; ND, not done; NIL, near-isogenic line; NIAS, National Institute of Agrobiological Sciences; PI, accessions from the USDA-ARS National Plant Germplasm System.

*Phenotypic data for flowering time were collected from three or four plants in the growth chamber.

[†]Phenotypic data for flowering time were collected from five plants in a field under natural conditions [day length (h:min) ranging from 13:10–14:37] at NIAS.

^{*}NILs were derived from Misuzudaizu \times Mashidougong 503.

 $^{\$}\text{NILs}$ were derived from Miharudaizu \times Sakamotowase.

Table S3. Transcript abundance of *E1* in relation to expression level of *GmFTs*, flowering time, and copy number of *E1* in transgenic plants

			E1 expr	ression*	Gn expre	nFT ssion*	
T ₀ line	No. of T_1 plants	Copy no.	15 d	30 d	15 d	30 d	Mean flowering time \pm SD, d
TG2	2	7–8	_	_	+++	+++	24.5 ± 0.5
TG2	4	1–2	+++	+++	_	_	≥53.0
TG11	2	1–2	+++	+++	_	_	>70.0
TG4	1	3	++	+	±	++	37.0
WT [†]	6	0	_	_	+++	+++	29.1 ± 0.69
VC	6	0	—	_	+++	+++	29.4 ± 0.90

VC, vector control.

*—, \pm , +, ++, and +++ represent no band or very faint, faint, intermediate, and strong bands, respectively, on the PAGE gel in semiguantitative RT-PCR analyses (*SI Materials and Methods*).

[†]Kariyutaka.

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Table S4. List of primers used in this study

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	d	lication	Forward primer (5'-3')	Reverse primer (5'–3')	Forward primer	Reverse primer	Forward primer	Reverse primer	°	cycles	Mq	Origin
mtt mtt mtt mtt mtt mtt mtt 000 Gadivational Gadiva	ppir.	bu D	ATCCCCACTTGAAACACCAA	TCGTGTGGCAATAAACCAGA AAGGATGAGTTTGATAAACAT			19387989 19653961	19387817 19653688	58	30	0.10	Present study http://www.sovbase.org
Description Code/Action/Galactication 2732 956301 956300 951300 9 0 0 Prent num Dist Code/Action/Galactication Arria Arria 966301 967303 957032 95 0 0 Prent num Dist Code/Action/Galactication 3343 959339 957032 95 0 0 Prent num Dist Code/Action/Galactication 3343 959339 957033 95 0 0 Prent num Dist Code/Action/Action 9393 957033 95203 95303 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 95003 9500 9500 9500		ņ	АТТТ	GAATGAAGAA								
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Image Construction disp disp<	niqo	βL	GGAACCTTGTGACCCCTTTT	TCAGACTCGACAAGCCTTCA	42794	43084	19669752	19670042	58	30	0.10	Present study
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	pin	Ъ	CCCTAATTCTGTCCAGGTTCA	CCAAAACGCAACAACAAATG	75944	75261	19702890	19702207	58	30	0.10	Present study
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ping CTCCATGATINC	pin	bu	ATGCTGCCAAGGTAGGAAAA	ACACGACATGAAATCGGTGA	198185	198651	19825139	19825603	58	30	0.10	Present study
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ping TITCACTGARCEARCEARGE ACTGACCAARGIGTACCAATC 322769 32231 1998957 1988677 1988677 1988677 1988677 1988677 1988677 1988677 1989578 20013222 58 30 0.10 Present study ping TGGGGTGGGGGGGGGAGCAT TCGATCGAGGTCATGCAGGTCATCCCTTCC 37835 2007142 20007565 58 30 0.10 Present study quencing TTGGATTGGGGGGGAGCAT - - 2007388 - - - - Present study encing TTGGATTGGGGGGGATCA - - 2007388 - - - Present study encing ATTGAATTGGGGGGATCA - - 2007399 58 30 0.10 Present study encing ATTGAATTGGGATCA - - 20014395 20014305 58 30 0.10 Present study ping ATTGAATTGGAGGATCA - - - - - - Present study ping G	pir.	bu	AAAGGGGATCTCATCCATCA	GCAAAACATGGCTCCAATC	352578	352779	19979415	19979616	58	30	0.10	Present study
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quencing GTCAAAGGAGT - - - - - - - Present study Jancing ATTGGATTIGGGAGGATA - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -	pir.	/gu	TCAGATGAAAGGGAGCAGT	TCCGATCTCATCACCTTTCC	377835	378259	20007142	20007566	58	30	0.10	Present study
Information TriccrAriant George George - 378081 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -	sque	encing	GTCAAAGAAGT									
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	opir.	ng	GCGTGTGCTTGCTTCTCTTAGA	GCGTACTACTTACCCTGTTTGT	Ι	Ι	23874546	23874314	58	30	0.10	http://www.soybase.org
			CTGACT	CTAAAA			50030100		C L	ç	č	

Table S4. Cont.

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				Position ir contig (Willié constructed study*	the ams 82) in this	Positic chromosor (Glymé	n on ne Gm06 a 1.0)	Annealing		Primer	
pair pair	Application	Forward primer (5'-3')	Reverse primer (5'–3')	Forward primer R	everse primer	Forward primer	Reverse primer	temperature, °C	cycles	concentration, pM	Origin
4K	Sequencing/	AACAAGCGATGAAGGGAAAA	ATCATACGGGTTCTACCACTGAC		I	I	I	58	30	0.10	Present study
	transformation										
sGFP	Southern probe	ATGGTGAGCAAGGGCGAGGA	TTACTTGTACAGCTCGTCCATGC	I	I	I	I	58	30	0.10	Present study
GmFT2a	RT experiment	ATCCCGATGCACCTAGCCCA	ACACCAAACGATGAATCCCCA	I	Ι	Ι	Ι	56	25	0.15	Glyma 1 6g 26660. 1 [¶]
GmFT5a ^{ll}	RT experiment	AGCCCGAACCCTTCAGTAGGGA	GGTGATGACAGTGTCTCTGCCCA	I	Ι	Ι	Ι	56	25	0.15	Glyma16g04830.1
E1 (e1-as/	RT experiment	CACTCAAATTAAGCCCTTTCA	TTCATCTCCTCTTCATTTTTGTTG	I	I	I	I	60	28	0.25	AB552962
e1-fs)											
E1-L	RT experiment	AAACACTCAAAGCCCGATCA	CCCTTGTTCATCTCCTCTTCA	Ι	I	I	I	60	28	0.25	Glyma04g24640.11
											Gm18g22670
GmCO-1	RT experiment	GCGTTCCTCTGCAAGGCCGA	CAACGTCAGCAACGTCGTCGT	Ι	Ι	Ι	Ι	60	28	0.25	Glyma18g51320.1
GmCO-2	RT experiment	TGGACCTTGTTGATGACTGCGA	CTCTTGTGAGAAACAACACCGA	Ι	I	I	Ι	60	28	0.25	Glyma08g28370.1
GmCO-3	RT experiment	ACCGCCTCTTTCGGGTTCGTCGT	TGATTTCGGGGGGGGGCGTCCATGAGT	I	I	I	I	60	28	0.25	Glyma13g01290.1
GmCO-4	RT experiment	AAGCCGTGCGATTCCTGCAAGCT	ACGCGAGTTTGTTCGAGCAGT	I	I	I	I	60	28	0.25	Glyma17g07420.1
GmLCL2	RT experiment	TGATGCGAACGTTTTAGATACC	GCAGTCCCTCCTCCTCATT	I	I	I	I	60	28	0.25	EU076434**
GmTubulin	RT experiment	TCTTGGACAACGAAGCCATCT	TGGTGAGGGACGAAATGATCT	ļ	I	ļ	I	60	28	0.25	Glyma05g29000.1
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sequences of relevant clones were deposited in DNA Data Base in Japan with accession numbers AP01181/-AP011820 and AP011823.

These primer pairs tended to be nonspecific, and gave rise to more than a single band. The AFLP-derived primer pairs A21 and A40 were developed when the soybean genome sequence was not available. The S9 primer pair was developed from a specific region and no other good alternative SSR motif was available. For these primers, the presence of one or a few nonpolymorphic bands did not interfere with our mapping as we used only polymorphic bands for our mapping. We did not use these primer pairs during the later stages of fine mapping.

Restriction enzyme treatment with Hifl or Tagl was needed to detect polymorphism between genotypes Harosoy-E1 and e1-fs or e1-as.

Sequencing protocol followed the manufacturer's instructions (Applied Biosystems).

⁵sequence information for each gene is available in the soybean genome database (http://www.phytozome.net/index.php).

⁴This primer pair can amplify both *Glyma1604830.1* and *Glyma19028400.1*, which are similar in size, from genomic DNA. However, restriction analysis with *Bsrl* (to specifically cut *Glyma1928400.1*) and with Mfel and Tap509I (to specifically cut Glyma16904830.1) confirmed that the expression of Glyma19928400.1 could not be detected in the samples used in this study.

**Sequence information for GmLCL2 (Glycine max LHY/CCA1 Like2 gene) was from Liu et al. (1).

1. Liu H, et al. (2009) Analysis of clock gene homologs using unifoliolates as target organs in soybean (Glycine max). J Plant Physiol 166:278–289.