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 *Ban*II and *Ban*II+*Eco*RV restriction enzymes based on sequence analysis of proximate regions where *E1* and its two paralogues reside. We also included *Eco*RV 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′-GACAAAACCTGAACTCAAATT-3′) and GmE1-3′-R1 (5′-AAGGATTTCATAAGATACAACATT-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 *E1* mutant lines, we first evaluated phenotypic segregation for flowering time in F_2 populations derived from a cross between homozygous *e1-m1* plants and WT OLERICHI50. The F_2 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′-CCGTTTGATTGGTTTTTGGT-3′ (forward) and 5′-AACAAGCGATGAAGGGAAAA-3′ (reverse) and sequenced using the primers 5′-CCCTTCAGTTTCTGCAGCTC-3′ or 5′-AACCGTGCATAGATCTCCTGCTA-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. 3*A*. The condensed tree with a cutoff value of 40% is shown in Fig. 3*B*.

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 $\mu mol \cdot s^{-1} \cdot m^{-2}$; during the late growth stage, major functional leaves received light with a stronger intensity of approximately 270 $\mu mol \cdot s^{-1} \cdot m^{-2}$ 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 μg 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-μL 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) (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 μL of diluted cDNA in a 20-μL 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 long-day 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.

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.
2. 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.
4. 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.
5. Tamura K, Dudley J, Nei M, Kumar S (2007) *MEGA4*: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599.
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.
7. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29:e45.

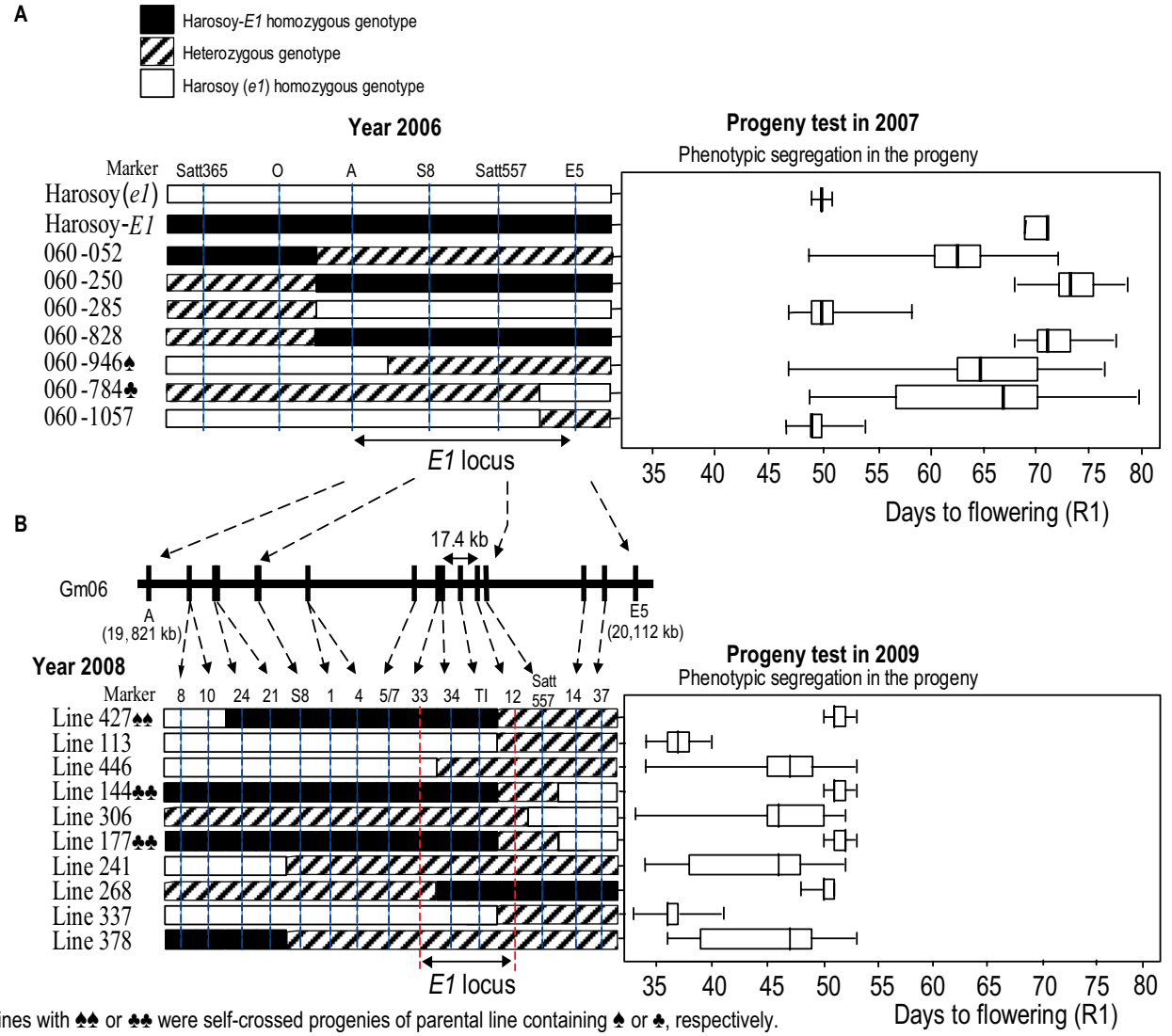

Fig. S1. Graphical genotypes of soybean recombinants carrying crossovers in the *E1* region in the 2006 to 2009 experiments. *Left*: Recombinants used for fine-scale mapping of the *E1* locus. *Right*: Phenotypic segregation patterns in the following year in the progenies. Recombination is shown by white 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.

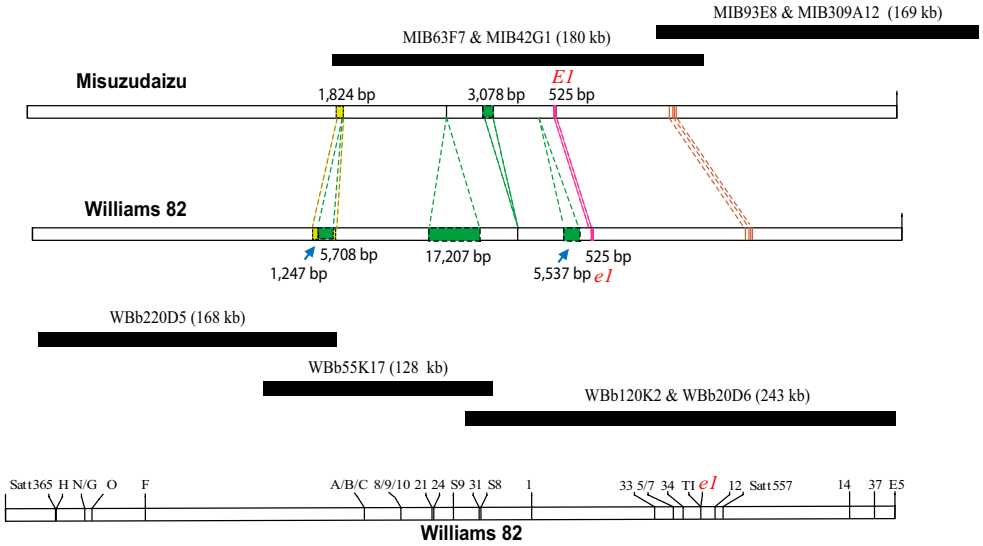

Fig. S2. Polymorphisms (gaps or insertion/deletions) between two contigs in the *E1* region built from BAC clones of Misuzudaizu and Williams 82. The deletions and transposed regions are marked in yellow, green, and brown, respectively. The physical positions of the markers (Table S4) used for fine-scale 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*.

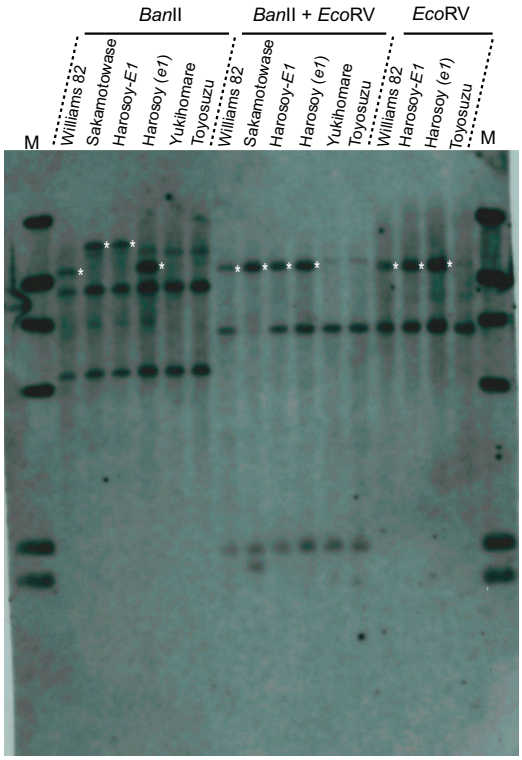

Fig. S3. The presence of *E1* and its two paralogues in the soybean genome were confirmed by Southern hybridization. Three copies of *E1* were present in cultivars of Williams 82 (*e1-as*), Sakamotowase (*e1-fs*), Harosoy-*E1* (*E1*), and Harosoy (*e1-as*). The bands marked with stars, which correspond to the *E1* locus, was absent in the *e1-nl* cultivars Yukihomare and Toyosuzu, indicating that a deletion occurred in the *E1*-anchored regions. Only two bands (one for Toyosuzu, *e1-nl*), were present (as predicted) using *Eco*RV, as the two fragments containing *E1* and *Gm18g22670* are of similar size. M represents the DNA molecular weight markers (λ*Hind*III).

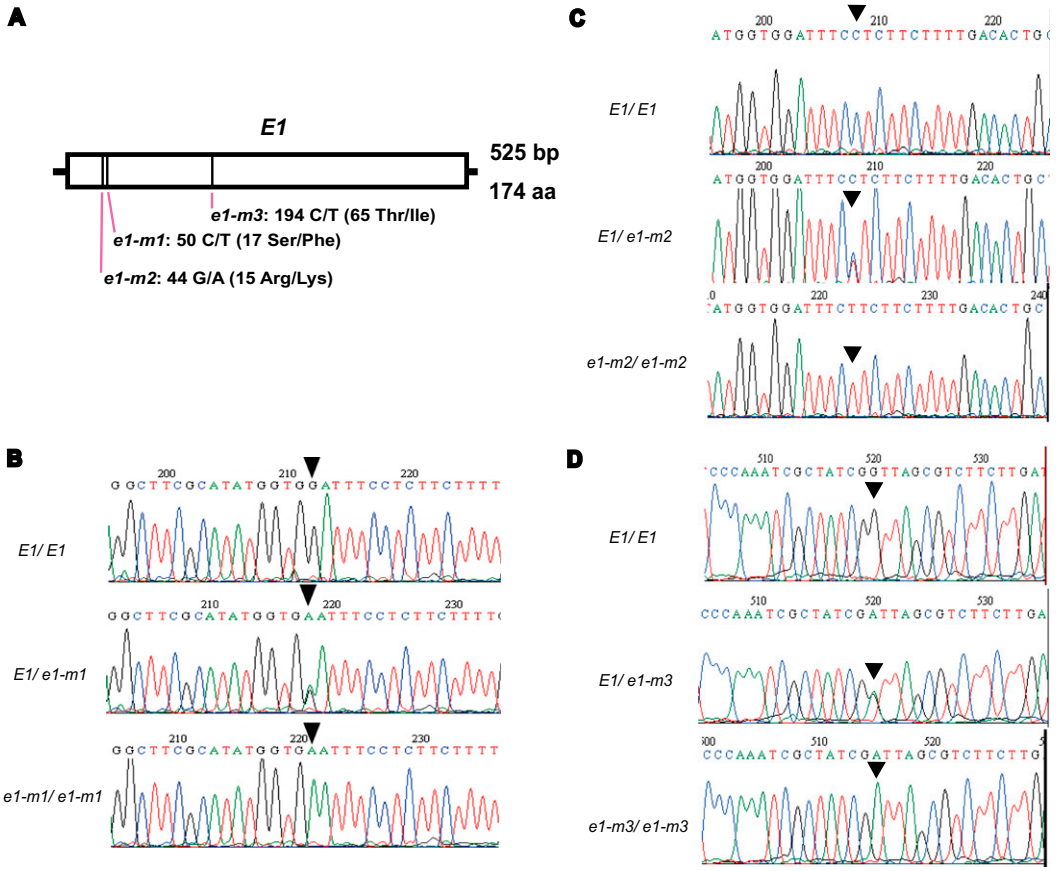

Fig. S4. Characterization of *E1* mutants induced by EMS. (*A*) Three independent mutations occurred at different position in *E1*. (*B–D*) Representative sequence electrograms showing the allelic variations at the mutation point (nucleotide) in F2 population derived from a cross between *e1-m1* and 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.

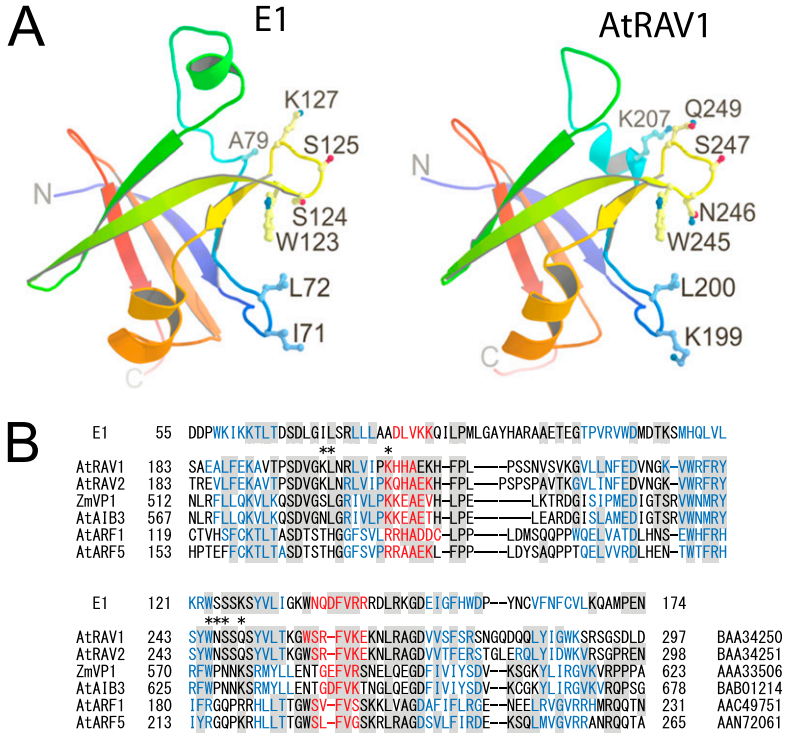

Fig. S5. Characterization of the B3-like domains of the E1 protein. (*A*) Comparison of the structural models of the B3-like domains of AtRAV1 and E1. The model was generated by using the Phyre server (http://www.imperial.ac.uk/phyre) based on the NMR solution structure of the B3 domain of *Arabidopsis 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. AIB3, 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 structural 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.

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

Line	E1 genotype	Flowering time (R1)* in progeny (NIAS Tsukuba, Japan)			
		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 between phenotypic parameters for flowering time (R1) and *E1* genotype determined 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	6.16×10^{-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	6.10×10^{-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	

NIAS, National Institute of Agrobiological Sciences.

*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 after 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(*E1*)[‡]	—	*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(*e1*)[‡]	—	*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 × Mashidougong 503.
[§]NILs were derived from Miharudaizu × 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 expression*		*GmFT* expression*		
T_0 line	No. of T_1 plants	Copy no.	15 d	30 d	15 d	30 d	Mean flowering time ± 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.
*—, ±, +, ++, and +++ represent no band or very faint, faint, intermediate, and strong bands, respectively, on the PAGE gel in semiquantitative RT-PCR analyses (*SI Materials and Methods*).
[†]Kariyutaka.

Table S4. List of primers used in this study

Primer pair	Application	Forward primer (5′-3′)	Reverse primer (5′–3′)	Position in the contig (Williams 82) constructed in this study*		Position on chromosome Gm06 (Glyma 1.0)		Annealing temperature, °C	PCR cycles	Primer concentration, pM	Origin
				Forward primer	Reverse primer	Forward primer	Reverse primer				
A40†	Mapping	ATCCCCACTTGAAACACCAA	TCGTGTGGCAATAAACCAGA	—	—	19387989	19387817	58	30	0.10	Present study
Satt365	Mapping	TGCTCCCCTCTGCCTTTTTTTCTATTTT	AAGGATGAGTTTGATAAACATGAATGAAGAA	27003	26730	19653961	19653688	58	30	0.10	http://www.soybase.org
H	Mapping	CACGATGCTGAGTGGACAAG	GGATGAGTTTGATAAACATGAATGA	27352	26732	19654310	19653690	58	30	0.10	Present study
N	Mapping	GGAACCTTGTGACCCCTTTT	TCAGACTCGACAAGCCTTCA	42794	43084	19669752	19670042	58	30	0.10	Present study
G	Mapping	ATCTTGCGAGAAAACGTTGG	TGAGCTTGCCTTGTAATCCA	43325	43826	19670283	19670772	58	30	0.10	Present study
O	Mapping	GGGGATTGTTGTTTTTGAAGG	TCCTCAAGCCTCAATGGACT	46763	47080	19673709	19674026	58	30	0.10	Present study
F	Mapping	CCCTAATTCTGTCCAGGTTCA	CCAAAACGCAACAACAAATG	75944	75261	19702890	19702207	58	30	0.10	Present study
A	Mapping	TCCTGTAAGAATGGCGACAA	GGCTATGCTGCTTCTCTGCT	194844	195474	19821798	19822428	58	30	0.10	Present study
B	Mapping	TGGCCCTTGAGGAATCATAG	TGCAGTGATGAAACACATGG	196923	197520	19823877	19824474	58	30	0.10	Present study
C	Mapping	ATGCTGCCAAGGTAGGAAAA	ACACGACATGAAATCGGTGA	198185	198651	19825139	19825603	58	30	0.10	Present study
8	Mapping	CCTCCATGAGTACCTGATTTCC	CTGTTGTTGAGGTGACAATACACA	214531	214961	19841483	19841913	58	30	0.10	Present study
9	Mapping	TCAGCAAAATTGTGTTATGATTGA	CGATCCAACATCTCCTCCAT	214732	214518	19841684	19841470	58	30	0.10	Present study
10	Mapping	CTTGTAGCGTAAGTGGAAGGAA	GAATATATCCACTGGGGATTGC	217555	218730	19844507	19845680	58	30	0.10	Present study
10	Mapping	CTTGTAGCGTAAGTGGAAGGAA	GAATATATCCACTGGGGATTGC	217555	218730	19844507	19845680	58	30	0.10	Present study
24	Mapping	TGCTCTGTAGGACCACATGC	GGCAAAAAGCCTCCCATAAT	231776	232008	19858725	19858957	58	30	0.10	Present study
21	Mapping	AATTGCTCTTCCCATTACGC	CCATTGTTAGAGGATGCCAAC	232530	232679	19859479	19859628	58	30	0.10	Present study
S9†	Mapping	GCGCTACAGTTCAACCCTTC	TGTTGGCCTTTCAATCTTGT	243091	243471	19870040	19870420	58	30	0.10	Present study
S8	Mapping	AATTGCAAGCTGTCACATGG	GGTCCACTGTTCAAGGGATG	257416	258035	19884366	19884985	58	30	0.10	Present study
1	Mapping	ATGTTGAGACATTGACCACTGC	CTAAAATGCAACATGAGGGTGA	285813	286966	19912763	19913916	58	30	0.10	Present study
4	Mapping	CTACAAACTCACGAACCCACAA	CTTCCACCTAGCGTCTTCAAGT	288811	289882	19915761	19916832	58	30	0.10	Present study
5	Mapping	AAAGGTCAAAGGGGATCTCATC	CTATGGGAGCTTCTAACGAGGA	352571	352848	19979408	19979685	58	30	0.10	Present study
7	Mapping	AAAGGGGATCTCATCCATCA	GCAAAAACATGGCTCCAATC	352578	352779	19979415	19979616	58	30	0.10	Present study
33	Mapping	TTTTCACTGACACACCAAGGTC	ACCTGCCAAAAGTGTACCAATC	362769	362931	19989677	19989839	58	30	0.10	Present study
34	Mapping	ACTTGGCATCAGGATAATCGAC	GTCCCACTCGAAGTCATTTGAA	368170	373985	19995078	20003292	58	30	0.10	Present study
TI‡	Mapping/ sequencing	TCAGATGAAAGGGAGCAGTGTCAAAAGAAGT	TCCGATCTCATCACCTTTCC	377835	378259	20007142	20007566	58	30	0.10	Present study
U	Sequencing§	TTGCCTATGTTGGGTGCATA	—	378081	—	20007388	—	—	—	—	Present study
3	Sequencing	ACTTGAACTTTGGGAGGATTCA	—	379402	—	20008709	—	—	—	—	Present study
12	Mapping	ATTCGACGTCTTCTCTCGTATT	TTGTACAGAGACCAGGGGAAAG	385412	385623	20014395	20014606	58	30	0.10	Present study
Satt557	Mapping	GCGGGATCCACCATGTAATATGTG	GCGCACTAACCCTTTATTGAA	389763	389946	20018746	20018929	58	30	0.10	http://www.soybase.org
14	Mapping	GGCTGAGTGAGTTTGGGTAAA	GGGACATTACATAGTCAAACAAAAA	458434	458618	20087417	20087601	58	30	0.10	Present study
37	Mapping	TGAGACACATTTAGCAGCGAAT	CTGCTGCCATTAATCAATCA	471975	472194	20100955	20101174	58	30	0.10	Present study
E5	Mapping	GAA TAG CTG AGG CCA TCC AA	GCC AAC AGA GAG ATC AGC AA	483403	483021	20112383	20112001	58	30	0.10	Present study
A32	Mapping	ATCCGGTGGAGGAGCATTAT	AACGGCTCTGTCACAATCAA	—	—	20381118	20380964	58	30	0.10	Present study
A29	Mapping	GGGATGGGAGCATAATCAAA	GAGTCCTGAGTAAACGGCAAA	—	—	20639370	—	58	30	0.10	Present study
A21†	Mapping	AACCGAATGCAAAGTTGGAG	GGATTGACCAACATGGAACC	—	—	20642878	20642651	58	30	0.10	Present study
A28	Mapping	CTCGTTGCAAAGTCTCGTTG	CCGCCACTAATTGCAAAAAT	—	—	20902344	20902601	58	30	0.10	Present study
Satt489	Mapping	GCGTGTGCTTGCTTCTCTTAGACTGACT	GCGTACTACTTACCCTGTTTGTCTAAAA	—	—	23874546	23874314	58	30	0.10	http://www.soybase.org
Satt289	Mapping	GCGCCCAGGTTTAAAAGT	CTGCCCCATCACTAGCCCTTCTT	—	—	27585996	27545782	58	30	0.10	Present study

Table S4. Cont.

Primer pair	Application	Forward primer (5′-3′)	Reverse primer (5′–3′)	Position in the contig (Williams 82) constructed in this study*		Position on chromosome Gm06 (Glyma 1.0)		Annealing temperature, °C	PCR cycles	Primer concentration, pM	Origin
				Forward primer	Reverse primer	Forward primer	Reverse primer				
4K	Sequencing/ transformation	AACAAGCGATGAAGGGAAAA	ATCATACGGGTTCTACCACTGAC	—	—	—	—	58	30	0.10	Present study
sGFP	Southern probe	ATGGTGAGCAAGGGCGAGGA	TTACTTGTACAGCTCGTCCATGC	—	—	—	—	58	30	0.10	Present study
GmFT2a	RT experiment	ATCCCGATGCACCTAGCCCA	ACACCAAACGATGAATCCCCA	—	—	—	—	56	25	0.15	*Glyma16g26660.1*¶
GmFT5a‖	RT experiment	AGCCCGAACCCTTCAGTAGGGA	GGTGATGACAGTGTCTCTGCCCA	—	—	—	—	56	25	0.15	*Glyma16g04830.1*
E1 (e1-as/ e1-fs)	RT experiment	CACTCAAATTAAGCCCTTTCA	TTCATCTCCTCTTCATTTTTGTTG	—	—	—	—	60	28	0.25	AB552962
E1-L	RT experiment	AAACACTCAAAGCCCGATCA	CCCTTGTTCATCTCCTCTTCA	—	—	—	—	60	28	0.25	*Glyma04g24640.1*/ *Gm18g22670*
GmCO-1	RT experiment	GCGTTCCTCTGCAAGGCCGA	CAACGTCAGCAACGTCGTCGT	—	—	—	—	60	28	0.25	*Glyma18g51320.1*
GmCO-2	RT experiment	TGGACCTTGTTGATGACTGCGA	CTCTTGTGAGAAACAACACCGA	—	—	—	—	60	28	0.25	*Glyma08g28370.1*
GmCO-3	RT experiment	ACCGCCTCTTTCGGGTTCGTCGT	TGATTTCGGGGGCGTCCATGAGT	—	—	—	—	60	28	0.25	*Glyma13g01290.1*
GmCO-4	RT experiment	AAGCCGTGCGATTCCTGCAAGCT	ACGCGAGTTTGTTCGAGCAGT	—	—	—	—	60	28	0.25	*Glyma17g07420.1*
GmLCL2	RT experiment	TGATGCGAACGTTTTAGATACC	GCAGTCCCTCCTCCTCATT	—	—	—	—	60	28	0.25	EU076434**
GmTubulin	RT experiment	TCTTGGACAACGAAGCCATCT	TGGTGAGGGACGAAATGATCT	—	—	—	—	60	28	0.25	*Glyma05g29000.1*

*Sequences of relevant clones were deposited in DNA Data Base in Japan with accession numbers AP011817–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 *Hin*fI or *Taq*I was needed to detect polymorphism between genotypes Harosoy-*E1* and *e1-fs* or *e1-as*.

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

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

¶This primer pair can amplify both *Glyma16g04830.1* and *Glyma19g28400.1*, which are similar in size,from genomic DNA. However, restriction analysis with *Bsr*I (to specifically cut *Glyma19g28400.1*) and with *Mfe*I and *Tap509*I (to specifically cut *Glyma16g04830.1*) confirmed that the expression of Glyma*19g28400.1* 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.