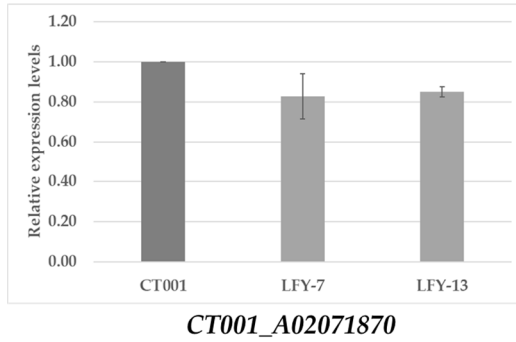
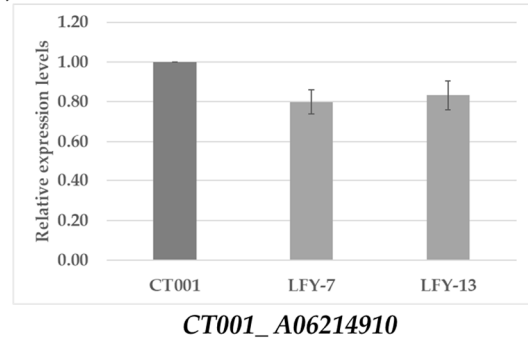


|     |        |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |   |          |          |          |          |          |          |
|-----|--------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|---|----------|----------|----------|----------|----------|----------|
| (A) |        | <u>G</u> | <u>A</u> | <u>A</u> | <u>C</u> | <u>G</u> | <u>T</u> | <u>T</u> | <u>G</u> | <u>G</u> | <u>G</u> | <u>T</u> | <u>C</u> | <u>G</u> | <u>T</u> | <u>G</u> | <u>G</u> | <u>C</u> |   | <u>G</u> | <u>T</u> | <u>C</u> | <u>A</u> | <u>G</u> | <u>G</u> |
|     | LFY-1  | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        |   | G        | T        | C        | A        | G        | G        |
|     | LFY-2  | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        | A | G        | T        | C        | A        | G        | G        |
|     | LFY-4  | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        | A | G        | T        | C        | A        | G        | G        |
|     | LFY-5  | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        |   | G        | T        | C        | A        | G        | G        |
|     | LFY-6  | C        | A        | A        | C        | G        | T        | T        | T        | G        | T        | T        | C        | G        | T        | G        | G        | C        |   | G        | T        | C        | A        | G        | G        |
|     | LFY-7  | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        | A | G        | T        | C        | A        | G        | G        |
|     | LFY-11 | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        |   | G        | T        | C        | A        | G        | G        |
|     | LFY-13 | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        | A | G        | T        | C        | A        | G        | G        |
|     | LFY-14 | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        |   | G        | T        | C        | A        | G        | G        |

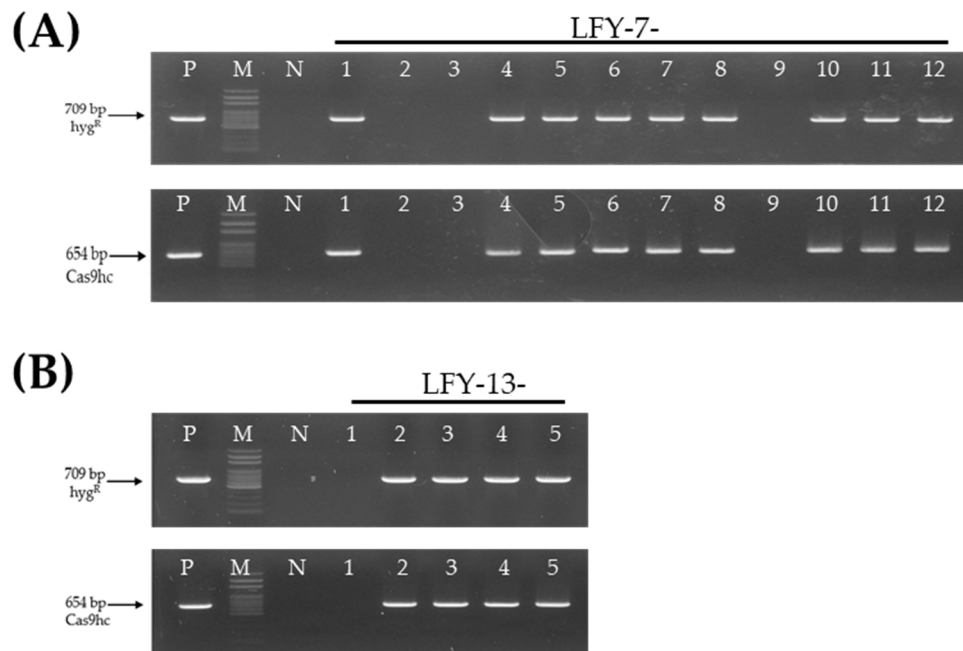
  

|     |        |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |   |          |          |          |          |          |          |
|-----|--------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|---|----------|----------|----------|----------|----------|----------|
| (B) |        | <u>G</u> | <u>A</u> | <u>A</u> | <u>C</u> | <u>G</u> | <u>T</u> | <u>T</u> | <u>G</u> | <u>G</u> | <u>G</u> | <u>T</u> | <u>C</u> | <u>G</u> | <u>T</u> | <u>G</u> | <u>G</u> | <u>C</u> |   | <u>G</u> | <u>T</u> | <u>C</u> | <u>A</u> | <u>G</u> | <u>G</u> |
|     | LFY-1  | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        | T | G        | T        | C        | A        | G        | G        |
|     | LFY-2  | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        |   | G        | T        | C        | A        | G        | G        |
|     | LFY-4  | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        |   | G        | T        | C        | A        | G        | G        |
|     | LFY-5  | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        |   | G        | T        | C        | A        | G        | G        |
|     | LFY-6  | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        |   | G        | T        | C        | A        | G        | G        |
|     | LFY-7  | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        | A | G        | T        | C        | A        | G        | G        |
|     | LFY-11 | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        |   | G        | T        | C        | C        | G        | G        |
|     | LFY-13 | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        | C | G        | T        | C        | A        | G        | G        |
|     | LFY-14 | G        | A        | A        | C        | G        | T        | T        | G        | G        | G        | T        | C        | G        | T        | G        | G        | C        |   | G        | T        | C        | A        | G        | G        |

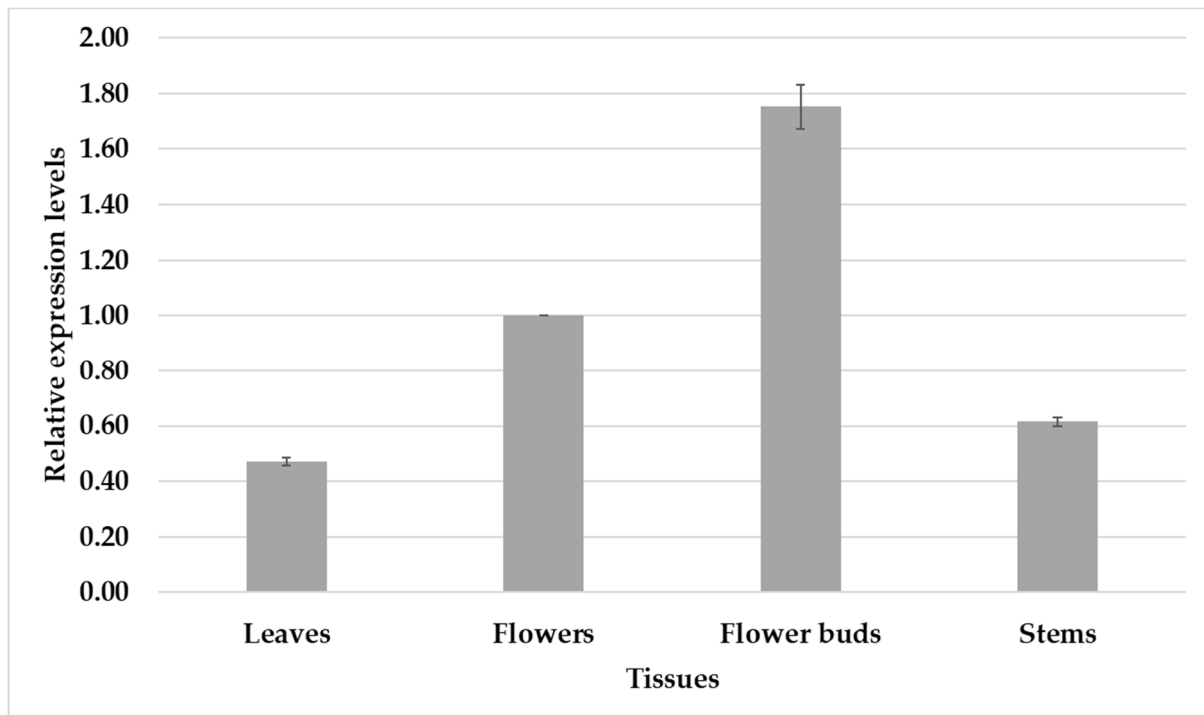
**Figure S1.** Sequence-based detection of mutations induced by CRISPR/Cas9 system in the *BrLFY* paralogs in the E<sub>0</sub> *LFY*-edited lines. (A) Mutagenesis of *CT001\_A02071870* at the genomic DNA level in the E<sub>0</sub> *LFY*-edited lines. (B) Mutagenesis of *CT001\_A06214910* at the genomic DNA level in the E<sub>0</sub> *LFY*-edited lines. sgRNA sequence is underlined in black and blue rectangle indicates PAM sequence. Nucleotide mutations are indicated using red font.

**(A)****(B)**

**Figure S2.** Comparison of the *BrLFY* paralogs expression levels between the inbred line 'CT001' and E<sub>0</sub> *LFY*-edited lines. **(A)** *CT001\_A02071870* expression levels in the LFY-7 and LFY-13 edited lines. **(B)** *CT001\_A06214910* expression levels in the LFY-7 and LFY-13 edited lines. Values are averages from three independent experiments; the bars on the graph indicate the standard error of the means (n = 3). *BrActin7* gene was used as the endogenous housekeeping gene for normalization.



**Figure S3.** Identification of the E<sub>1</sub> *LFY*-edited lines using polymerase chain reaction (PCR) analysis. **(A)** PCR analysis with Hyg<sup>R</sup> and Cas9hc primer sets of E<sub>1</sub> *LFY*-7-edited lines. **(B)** PCR analysis with Hyg<sup>R</sup> and Cas9hc primer sets of E<sub>1</sub> *LFY*-13-edited lines. The 709 bp and 654 bp expected PCR products are indicated with an arrow, respectively. P, positive control; M, 100 bp DNA ladder; N, negative control; Numbering lane, *LFY*-edited lines.



**Figure S4.** Expression levels of *CT001\_A06214910* gene in different tissues of the inbred line 'CT001.' Based on the expression level of *CT001\_A06214910* quantified in flowers, the relative expression levels of *CT001\_A06214910* gene in leaves, flower buds, and stems were shown. Values are averages from three independent experiments; the bars on the graph indicate the standard error of the means (n = 3). *BrActin7* gene was used as the endogenous housekeeping gene for normalization.

**Table S1.** List of primer sets for polymerase chain reaction (PCR) analysis.

| Name                     | Primer         | Sequence (5'→3')            | Expected product size (bp) |
|--------------------------|----------------|-----------------------------|----------------------------|
| <b>Hyg<sup>R</sup></b>   | F <sup>z</sup> | CGT CTG CTG CTC CAT ACA AG  | 709                        |
|                          | R              | TGT CGA GAA GTT TCT GAT CGA |                            |
| <b>Cas9<sup>hc</sup></b> | F              | CCG CCA GGA GGA CTT CTA CC  | 654                        |
|                          | R              | ATG TTC TCG GGC TTG TGG CG  |                            |
| <b>CT001_A06214910</b>   | F              | CCA TAG TAC AGT ATC CAT GA  | 488                        |
|                          | R              | GCG AGG ATG AGC GTT AAA     |                            |
| <b>CT001_A020718710</b>  | F              | TGG TAC TTT CTG TTT GGA G   | 412                        |
|                          | R              | TAC CGT TCC CAA CCA AAG     |                            |

<sup>z</sup>: F, forward primer; R, reverse primer.

**Table S2.** List of degenerate primers and T-DNA-border specific primers for variable argument-thermal asymmetric interlaced polymerase chain reaction (VA-TAIL PCR) analysis.

| Target Region                   | Name                     | Sequence (5'→3')                                    |
|---------------------------------|--------------------------|---|
| <b>Zinc finger protein LSD1</b> | <b>BrAD1<sup>z</sup></b> | GAM RTG NCT VAM WTT G <sup>y</sup>                  |
|                                 | <b>BrAD2</b>             | DTA ASA TGN HNT TGC T                               |
| <b>Left border of T-DNA</b>     | <b>LSP1</b>              | ATA GTG GAA ACC GAC GCC <u>CCA GCA</u> <sup>x</sup> |
|                                 | <b>LSP2</b>              | <u>CCA GCA</u> CTC GTC CGA GGG CAA AG               |
| <b>Right border of T-DNA</b>    | <b>RSP1</b>              | AAA GTA TAC CCC TAC GAC GTG <u>CCC G</u>            |
|                                 | <b>RSP2</b>              | <u>GCC CGA</u> CTA CGC CTA ACA CCC AG               |

<sup>z</sup>: BrAD primers are degenerate primers.

<sup>y</sup>: Mixed bases. M = A/C; R = A/G; V : A/C/G; W : A/T; D = A/G/T; S : G/C; H = A/C/T; N = any base.

<sup>x</sup>: Underlined nucleotide sequences indicate the overlapping sequence.