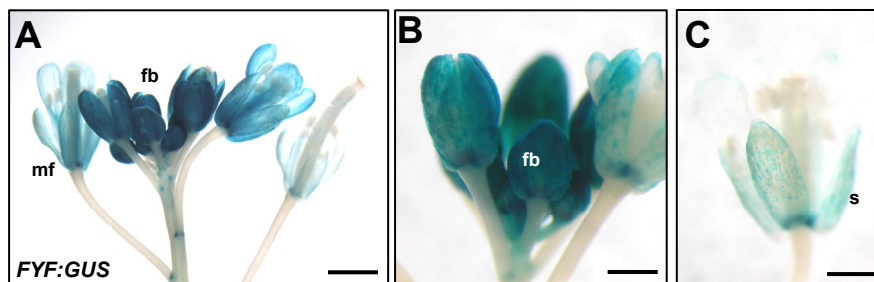
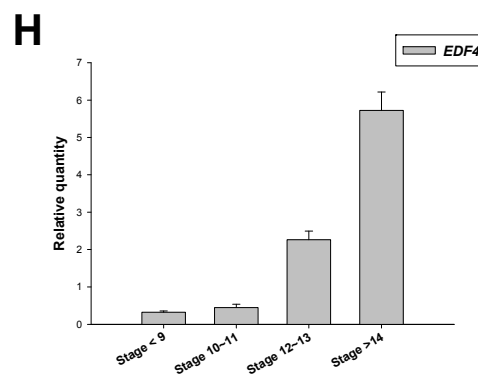
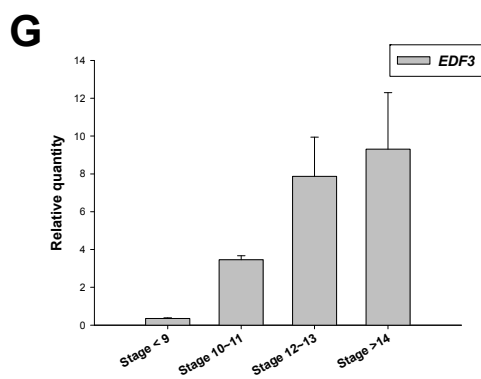
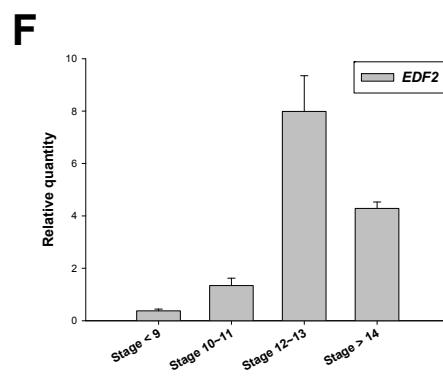
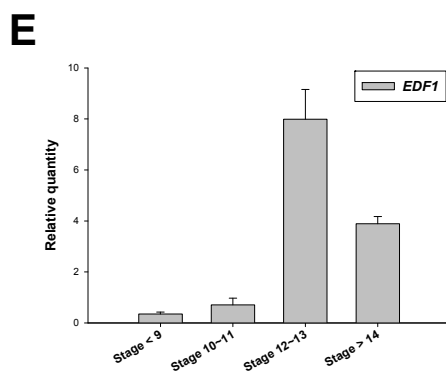
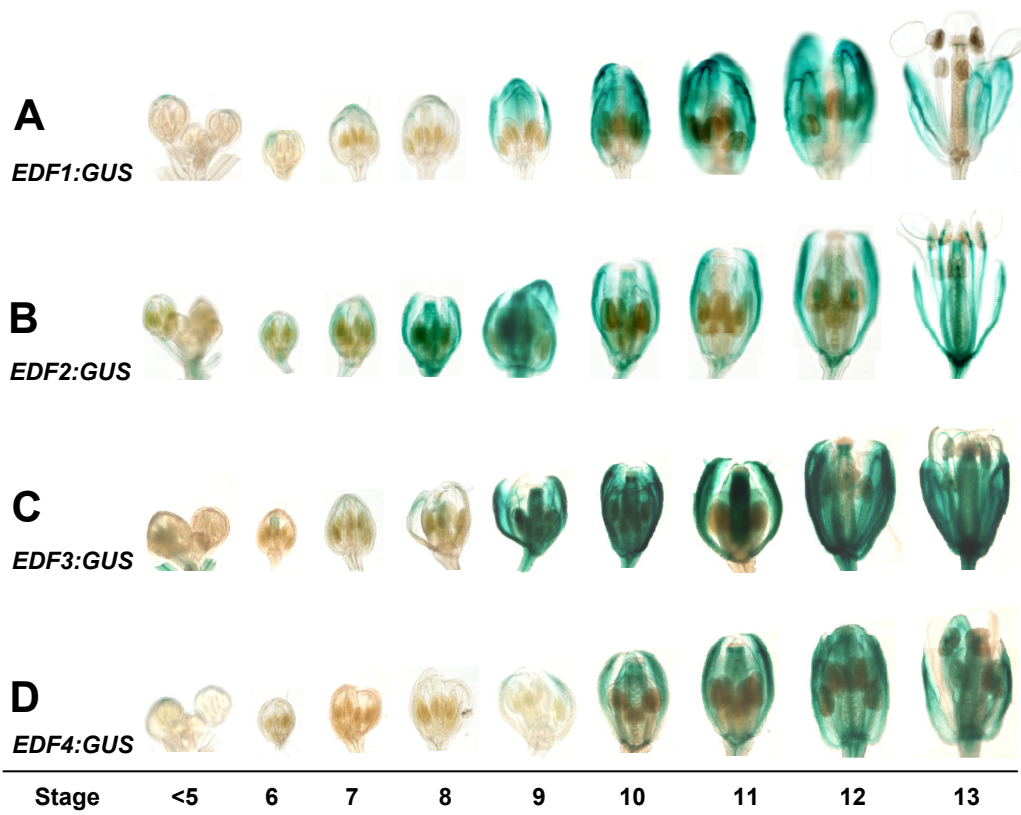


Supplemental Figure S1. A protein sequence comparison of EDF1/2/3/4, which are in the RAV family of the AP2/ERF-related proteins.

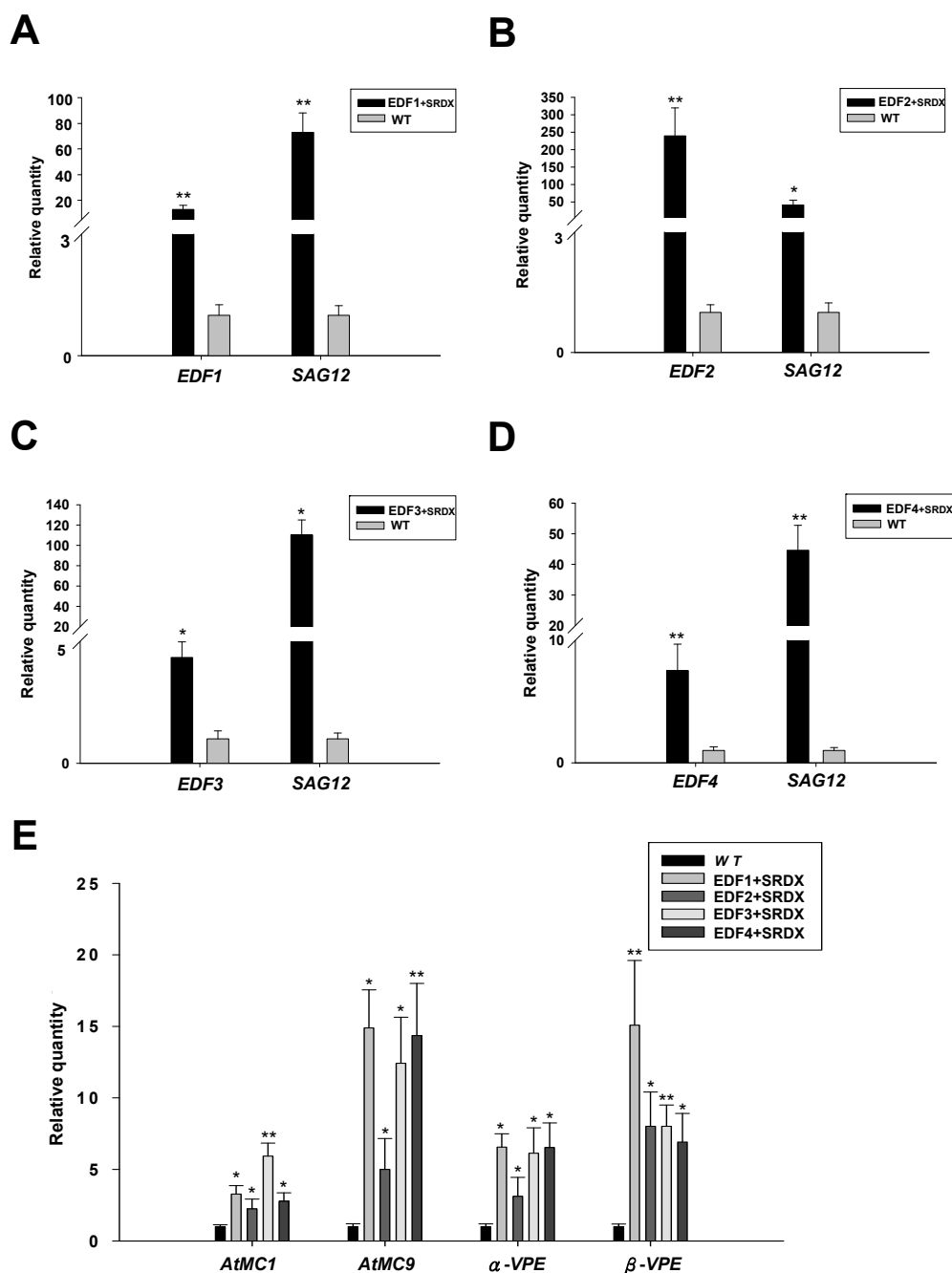
The three lines represent the three motifs [the AP2/ERF domain (blue), the NLS (red) and the B3 domain (green)] that are conserved among the AP2/ERF proteins in the RAV family. The amino acid residues identical to EDF1 are highlighted in black; the amino acid residues similar to EDF1 are highlighted in gray. To improve the alignment, dashes were introduced into the sequence. The five conserved RLFgV residues shared with the AP2/ERF repressors are indicated by a red box. This sequence alignment was generated by the ClustalW Multiple Sequence Alignment Program at the DNA Data Bank of Japan (<http://clustalw.ddbj.nig.ac.jp/top-e.html>).



Supplemental Figure S2. GUS staining patterns in *FYF:GUS Arabidopsis* plants. A, Strong GUS staining was observed in the flower buds (fb) of *FYF:GUS Arabidopsis*. The GUS staining was reduced in the young and mature flowers (mf). B to C, Close-up images of the flower buds (fb) (B) and mature flowers (C) from (A). s, sepal. Scale bars: 2 mm in (A); 1 mm in (B) and (C).



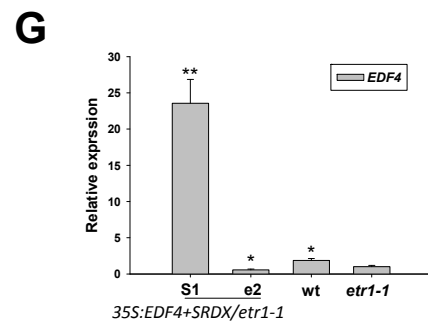
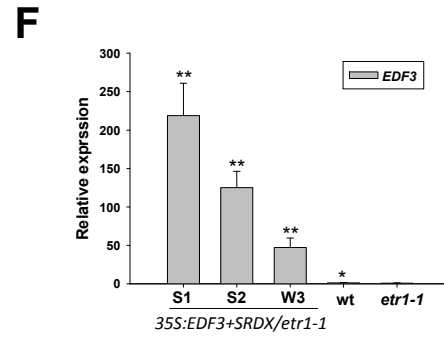
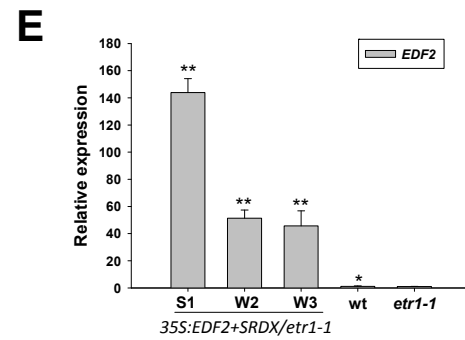
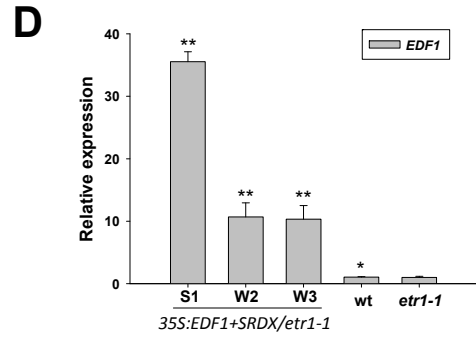
Supplemental Figure S3. GUS staining patterns in *EDFs:GUS Arabidopsis* plants and the detection of *EDFs* expression in flowers at different developmental stages. A to D, GUS staining images for different developmental stages (<5, 6, 7, 8, 9, 10, 11, 12, and 13) of *EDF1/2/3/4:GUS* flowers. GUS staining was absent in the flower buds before stage 8, and stronger staining increased in the young and mature flowers (after stage 9) of *EDF1:GUS Arabidopsis* (A). GUS staining was absent or barely detected in the flower buds before stage 7, and stronger staining increased in the young and mature flowers after stages 8, 9 and 10 of *EDF2:GUS* (B), *EDF3:GUS* (C) and *EDF4:GUS* (D) *Arabidopsis*, respectively. E to H, The detection of *EDF1* (E), *EDF2* (F), *EDF3* (G) and *EDF4* (H) expression in wild-type flowers at four different developmental stages (<9, 10-11, 12-13 and >14). The sample for mRNA isolation was collected at 8 hours after turn on the light under long-day conditions (16-h light/8-h dark). The mRNA levels were determined by real-time quantitative PCR. The transcript levels of *EDFs* were determined using two to three replicates and were normalized using *UBQ10*. The error bars represent the standard deviation. Each experiment was repeated twice with similar results.



Supplemental Figure S4. The detection of senescence associated gene expression in *35S:EDFs+SRDX* plants.

A to D, The expression of the *SAG12* gene in *35S:EDF1+SRDX* (A), *35S:EDF2+SRDX* (B), *35S:EDF3+SRDX* (C) and *35S:EDF4+SRDX* (D) plants.

E, The expression of *AtMC1*, *AtMC9*, *α-VPE* and *β-VPE* genes in *35S:EDFs+SRDX* plants. The mRNA levels were determined by real-time quantitative PCR. Total RNA was isolated from the floral buds and the open flowers at position 1-3 from one wild-type Columbia plant (WT) and from one *35S:EDFs+SRDX* plant. The transcript levels of these genes were determined using two to three replicates and were normalized using *UBQ10*. The expression of each gene is given relative to that of the wild-type plant, which was set at 1. The asterisks indicate a significant difference from the wild type (WT) value (*means $P \leq 0.05$, **means $P \leq 0.01$). Expression statistic analysis was measured by student's T-test, each $n=3$.



Supplemental Figure S5. A phenotypic analysis of flower senescence and abscission in *etr1-1* mutants ectopically expressing the *EDFs*+*SRDX* constructs.

A, The flowers along the inflorescence of *etr1-1* (first row), *35S:EDF1+SRDX/etr1-1* (second row), *35S:EDF2+SRDX/etr1-1* (third row), *35S:EDF3+SRDX/etr1-1* (fourth row) and *35S:EDF4+SRDX/etr1-1* (fifth row) plants. The numbers indicate the positions of the flowers.

B, Inflorescence stem of a *35S:EDF2+SRDX/etr1-1* plant. The perianth organs of the flowers were completely senescent and abscised from the base of the flower after position 1, these organs remained hanging on the siliques even after these organs had been abscised from the flowers for two weeks.

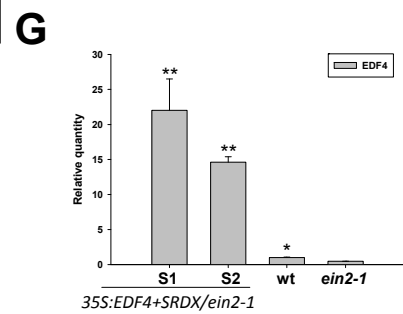
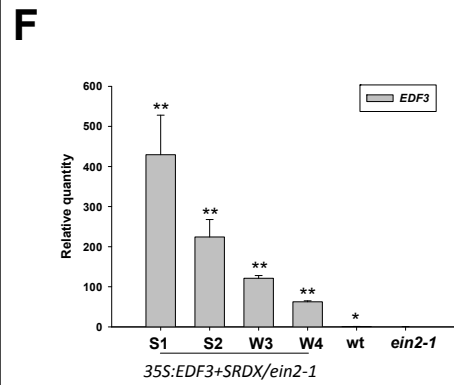
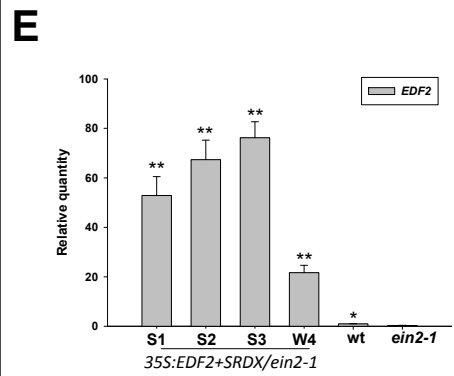
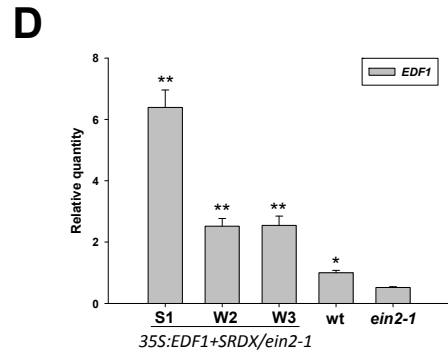
C, Close-up image of the *etr1-1* (left) and *35S:EDF2+SRDX/etr1-1* (right) flowers at position 1. The perianth organs were completely senescent and abscised in the *35S:EDF2+SRDX/etr1-1* flowers, whereas the floral organs were fresh in the *etr1-1* flower. s, sepal; p, petal; st, stamen; c, carpel.

D, The expression of *EDF1+SRDX/EDF1* in *35S:EDF1+SRDX/etr1-1* plants. The mRNA accumulation was determined by real-time quantitative PCR. Total RNA was isolated from one wild-type Columbia plant (wt), one *etr1-1* plant, one *35S:EDF1+SRDX/etr1-1* plant (#S1) with a severe mutant phenotype and two *35S:EDF1+SRDX/etr1-1* plants (#W2, W3) with a wild-type-like phenotype.

E, The expression of *EDF2+SRDX/EDF2* in *35S:EDF2+SRDX/etr1-1* plants. The mRNA accumulation was determined by real-time quantitative PCR. Total RNA was isolated from one wild-type Columbia plant (wt), one *etr1-1* plant, one *35S:EDF2+SRDX/etr1-1* plant (#S1) with a severe mutant phenotype and two *35S:EDF2+SRDX/etr1-1* plants (#W2, W3) with a wild-type-like phenotype.

F, The expression of *EDF3+SRDX/EDF3* in *35S:EDF3+SRDX/etr1-1* plants. The mRNA accumulation was determined by real-time quantitative PCR. Total RNA was isolated from one wild-type Columbia plant (wt), one *etr1-1* plant, two *35S:EDF3+SRDX/etr1-1* plants (#S1, S2) with a severe mutant phenotype and one *35S:EDF3+SRDX/etr1-1* plant (#W3) with a wild-type-like phenotype.

G, The expression of *EDF4+SRDX/EDF4* in *35S:EDF4+SRDX/etr1-1* plants. The mRNA accumulation was determined by real-time quantitative PCR. Total RNA was isolated from one wild-type Columbia plant (wt), one *etr1-1* plant, one *35S:EDF4+SRDX/etr1-1* plant (#S1) with a severe mutant phenotype and one *35S:EDF4+SRDX/etr1-1* plant (#e2) with a *etr1-1*-like phenotype. The transcript levels of these genes in (D) to (G) were determined using two to three replicates and were normalized using *UBQ10*. The expression of each gene is given relative to that of the wild-type plant, which was set at 1. The error bars represent the standard deviation. Each experiment was repeated twice with similar results. The asterisks in (D) to (G) indicate a significant difference from the *etr1-1* value (*means $P \leq 0.05$, **means $P \leq 0.01$). Expression statistic analysis was measured by student's T-test, each $n=3$.



Supplemental Figure S6. A phenotypic analysis of flower senescence and abscission in *ein2-1* mutants ectopically expressing the *EDFs*+*SRDX* constructs.

A, The flowers along the inflorescences of *ein2-1* (first row), *35S:EDF1+SRDX/ein2-1* (second row), *35S:EDF2+SRDX/ein2-1* (third row), *35S:EDF3+SRDX/ein2-1* (fourth row) and *35S:EDF4+SRDX/ein2-1* (fifth row) plants. The numbers indicate the positions of the flowers.

B, An inflorescence of a *35S:EDF3+SRDX/ein2-1* plant. The perianth organs of the flower were completely senescent and abscised at position 1.

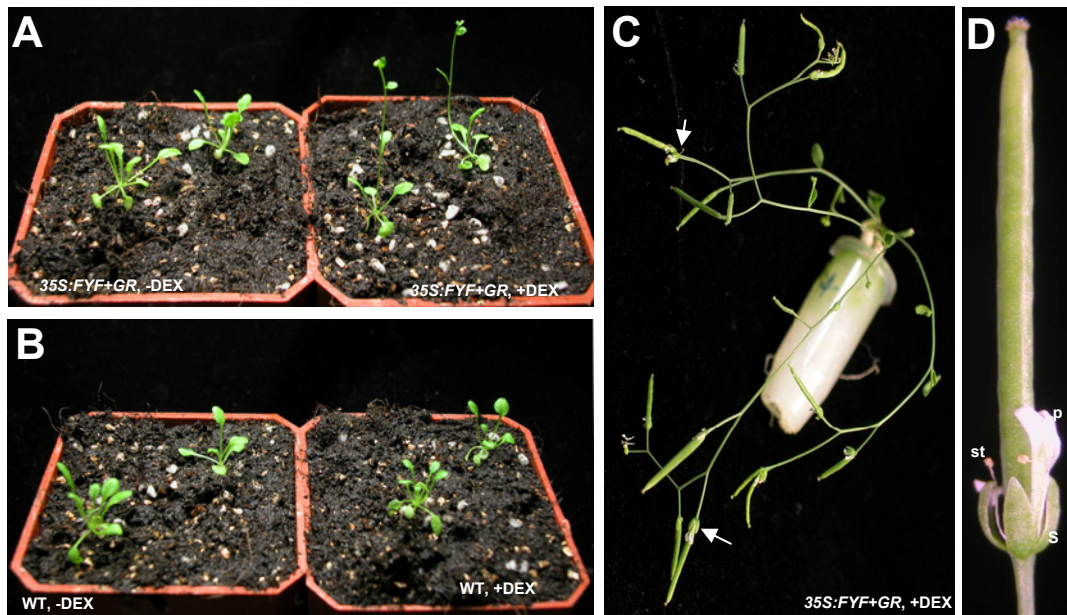
C, Close-up images of *ein2-1* (left) and *35S:EDF3+SRDX/ein2-1* (right) flowers at position 0. The perianth organs were senescent and chlorotic in *35S:EDF3+SRDX/ein2-1* flowers, whereas the floral organs were fresh in *ein2-1* flowers. s, sepal; p, petal; st, stamen.

D, The expression of *EDF1+SRDX/EDF1* in *35S:EDF1+SRDX/ein2-1* plants. The mRNA accumulation was determined by real-time quantitative PCR. Total RNA was isolated from one wild-type Columbia plant (wt), one *ein2-1* plant, one *35S:EDF1+SRDX/ein2-1* plant (#S1) with a severe mutant phenotype and two *35S:EDF1+SRDX/ein2-1* plants (#W2, W3) with wild-type-like phenotype.

E, The expression of *EDF2+SRDX/EDF2* in *35S:EDF2+SRDX/ein2-1* plants. The mRNA accumulation was determined by real-time quantitative PCR. Total RNA was isolated from one wild-type Columbia plant (wt), one *ein2-1* plant, three *35S:EDF2+SRDX/ein2-1* plants (#S1, S2, S3) with a severe mutant phenotype and one *35S:EDF2+SRDX/ein2-1* plant (#W4) with wild-type-like phenotype.

F, The expression of *EDF3+SRDX/EDF3* in *35S:EDF3+SRDX/ein2-1* plants. The mRNA accumulation was determined by real-time quantitative PCR. Total RNA was isolated from one wild-type Columbia plant (wt), one *ein2-1* plant, two *35S:EDF3+SRDX/ein2-1* plants (#S1, S2) with a severe mutant phenotype and two *35S:EDF3+SRDX/ein2-1* plants (#W3, W4) with wild-type-like phenotype.

G, The expression of *EDF4+SRDX/EDF4* in *35S:EDF4+SRDX/ein2-1* plants. The mRNA accumulation was determined by real-time quantitative PCR. Total RNA was isolated from one wild-type Columbia plant (wt), one *ein2-1* plant and two *35S:EDF4+SRDX/ein2-1* plants (#S1, S2) with a severe mutant phenotype. The transcript levels of these genes were determined using two to three replicates and were normalized using *UBQ10*. The expression of each gene was set relative to that of the wild-type plant, which was set to 1. The error bars represent the standard deviation. Each experiment was repeated twice with similar results. The asterisks in (D) to (G) indicate a significant difference from the *ein2-1* value (*means $P \leq 0.05$, **means $P \leq 0.01$). Expression statistic analysis was measured by student's T-test, each $n=3$.



Supplemental Figure S7. The ectopic expression of *FYF+GR* delayed flower senescence and abscission after dexamethasone (DEX) treatment.

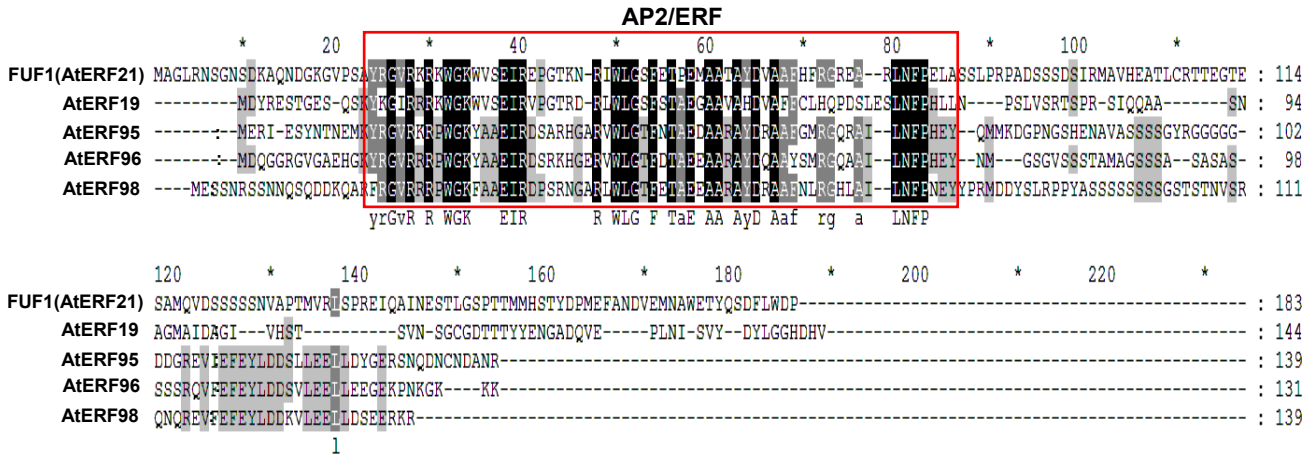
A, The *35S:FYF+GR* plants grew normally and were indistinguishable from wild-type plants without dexamethasone (DEX) treatment (left). Upon the treatment with DEX, the *35S:FYF+GR* plants flowered early (right).

B, The DEX-treated (right) and untreated (left) wild-type plants were indistinguishable.

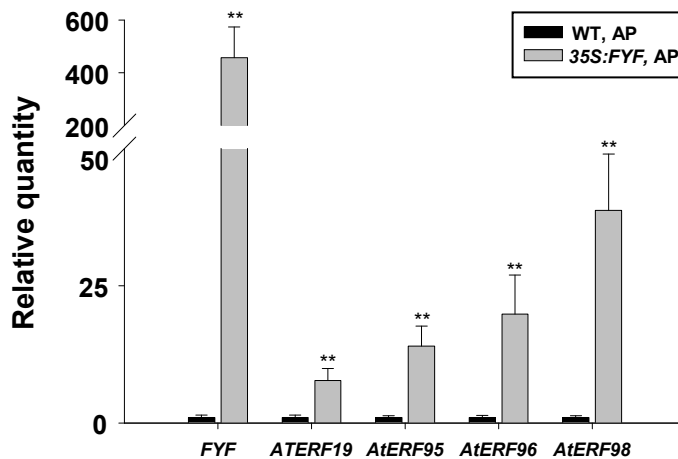
C, Upon DEX treatment, the *35S:FYF+GR* plants showed delayed flower senescence and abscission and continued to grow when transferred to a tube containing cotton and water. The perianth organs did not senesce and remained attached to the bases of the mature and elongated siliques (arrow).

D, Sepals (s), petals (p) and stamens (st) did not senesce and remained associated with the developing siliques in the *35S:FYF+GR* plants from (C).

A



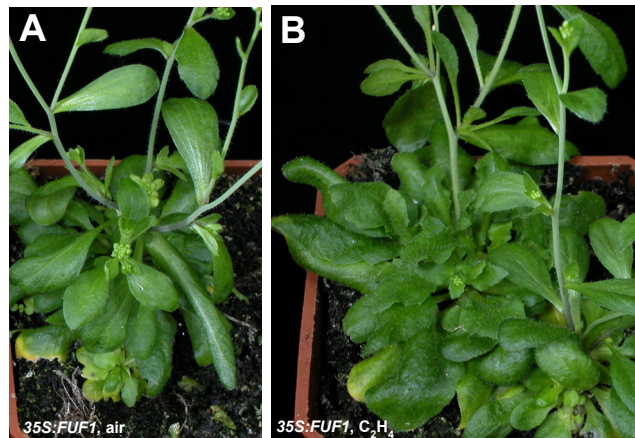
B



Supplemental Figure S8. A protein sequence comparison of FUF1 (AtERF21) and four other ERF proteins (AtERF19, 95, 96 and 98) whose transcripts are up-regulated by *FYF*.

A, The red box indicates the AP2/ERF domain that is conserved among the AP2/ERF family members. The amino acid residues identical to FUF1 are highlighted in black; amino acid residues similar to FUF1 are highlighted in gray. To improve the alignment, dashes were introduced into the sequence. This sequence alignment was generated by the ClustalW Multiple Sequence Alignment Program at the DNA Data Bank of Japan (<http://clustalw.ddbj.nig.ac.jp/top-e.html>).

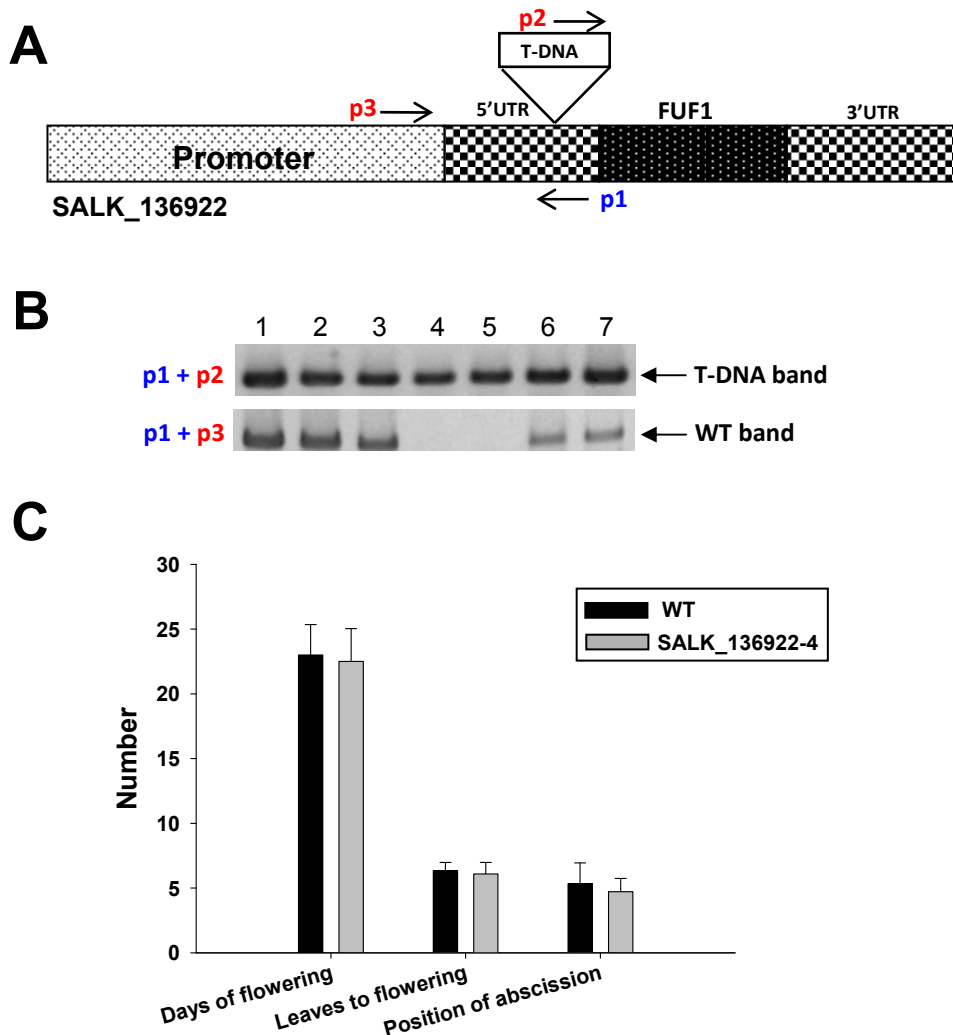
B, The expression levels of *FYF* and four ERF genes (*AtERF19*, *95*, *96* and *98*) in flowers after stage 12 and pollination (AP) in wild-type (WT) and *35S:FYF* plants, as determined by real-time quantitative RT-PCR. The expression of each gene is given relative to that of the wild-type plant, which was set at 1. The asterisks indicate a significant difference from the wild type (WT) value (**means $P \leq 0.01$). Expression statistic analysis was measured by student's T-test, each $n=3$.



Supplemental Figure S9. An analysis of the effect of ethylene on leaves of *35S:FUF1 Arabidopsis*.

A, The rosette leaves of a *35S:FUF1* transgenic plant were green in the presence of air only.

B, The rosette leaves from *35S:FUF1* transgenic plants remained green after ethylene treatment.



Supplemental Figure S10. Analysis of *FUF1* T-DNA insertion line SALK_136922.

A, A schematic representation of the *FUF1* genomic structure with the T-DNA inserted into the 5'-UTR in line SALK_136922. The position of the primers used was indicated by the arrows. The forward primers, p2 (in 3' end of the T-DNA) and p3 (in the 3' end of the promoter), are in red color. The reverse primer p1 (in the 3' end of the 5'-UTR) is in blue color.

B, Test the homozygosity for the progenies of line SALK_136922. Total DNA isolated from 7 progenies of line SALK_136922 (#1-7) was used to detect the presence of the T-DNA fragment by PCR. The primers 1 and 2 (p1 + p2) were used to detect the presence of the T-DNA fragment whereas primers 1 and 3 (p1 + p3) were used to detect the presence of the wild-type genomic DNA sequence of *FUF1*. The detection of both PCR products indicated the heterozygosity of the T-DNA insertion (plant #1, 2, 3, 6, 7) whereas the detection of the PCR product from p1+p2 only indicated the homozygosity of the T-DNA insertion (plant #4, 5).

C, The detection of the flowering time (days of flowering), the number of leaves produced upon the flowering (leaves to flowering) and the position of the flower start to show abscission of the flower organs (position of abscission) for wild-type plant (WT) and homozygous T-DNA insertion line of SALK_136922-4 (plant #4 in B). The result indicated the SALK_136922-4 plant is phenotypically indistinguishable from wild-type plants.