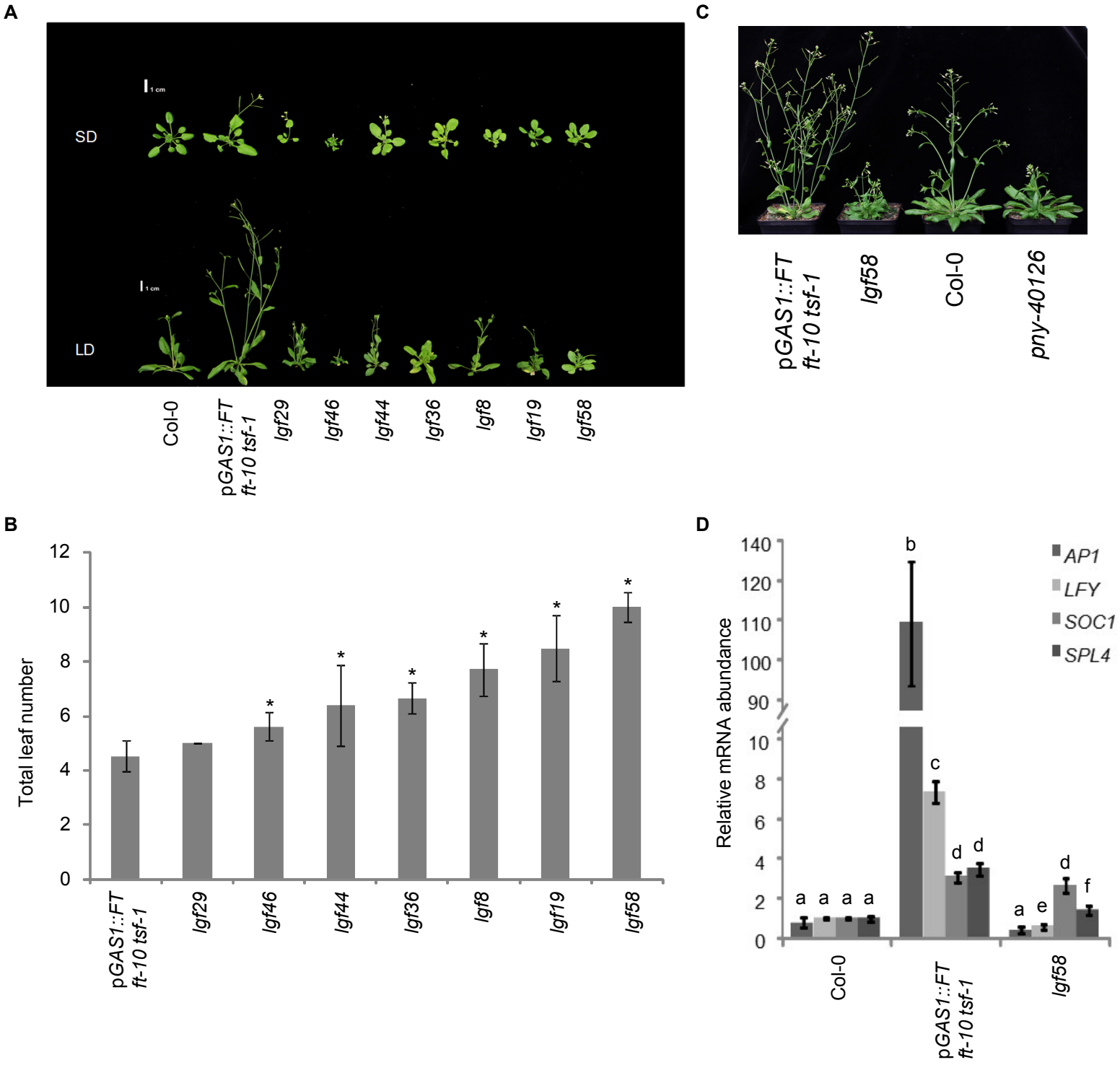



**Figure S1. *late flowering in pGAS1::FT ft-10 tsf-1* (*lgf*) mutants and characterization of *lgf58*.** (A) *lgf* mutants identified in a sensitized genetic screen. Bar: 1 cm. (B) Comparison of flowering time of identified *lgf* mutants. (C) Phenotype of *lgf58* compared to p*GAS1::FT ft-10 tsf-1*, Col-0 and *pny-40126* mutant. lgf58 showed short stature, lanceolated leaves, phyllotactic abnormalities and late flowering. (D) Quantification by RT-qPCR of expression levels of FT-transcriptionally regulated genes in *lgf58* mutant. Error bars in (B) and (C) indicate s.d. Letters shared in common between the genotypes indicate no significant difference (t-test, $P < 0.05$). Asterisks indicate statistical differences between Col-0 and other genotypes (t-test; $P < 0.05$)

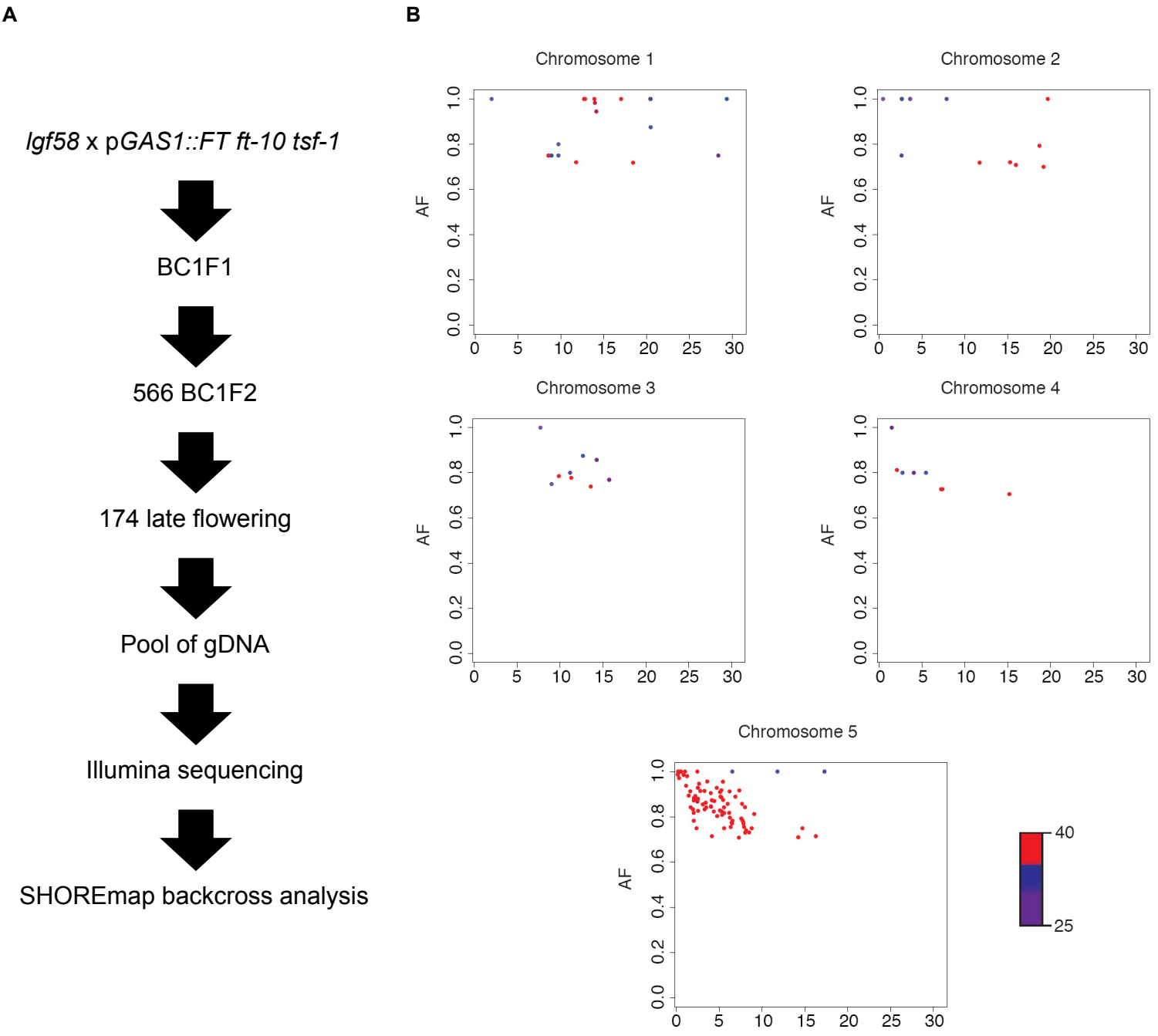



**Figure S2. Cloning-by-sequencing of *lgf58* mutation.** (A) Workflow employed to identify the causal mutation in the *lgf58* mutant. (B) Graphics showing the allelic frequency estimations at EMS-induced mutations (AF, *y* axis) across the five chromosomes (Mb, *x* axis) of *lgf58*. AFs were calculated dividing the number of reads supporting the mutant allele by the number of all reads aligning to a given marker. The color code indicates the resequencing consensus (SHORE) score. EMS-mutations showing a SHORE score higher than 25 were selected. AFs in chromosome 5 were higher as compared with other regions in the genome.

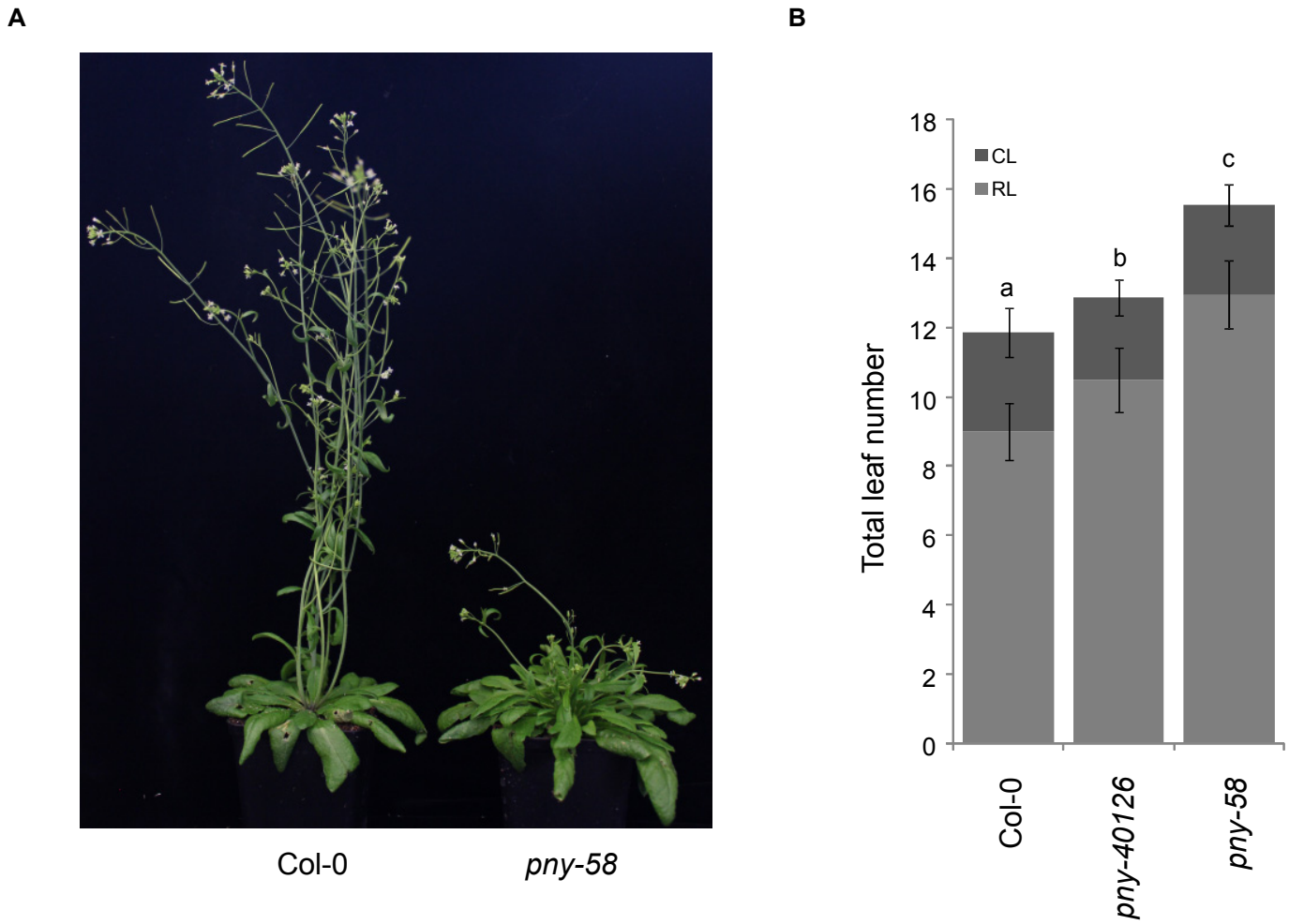
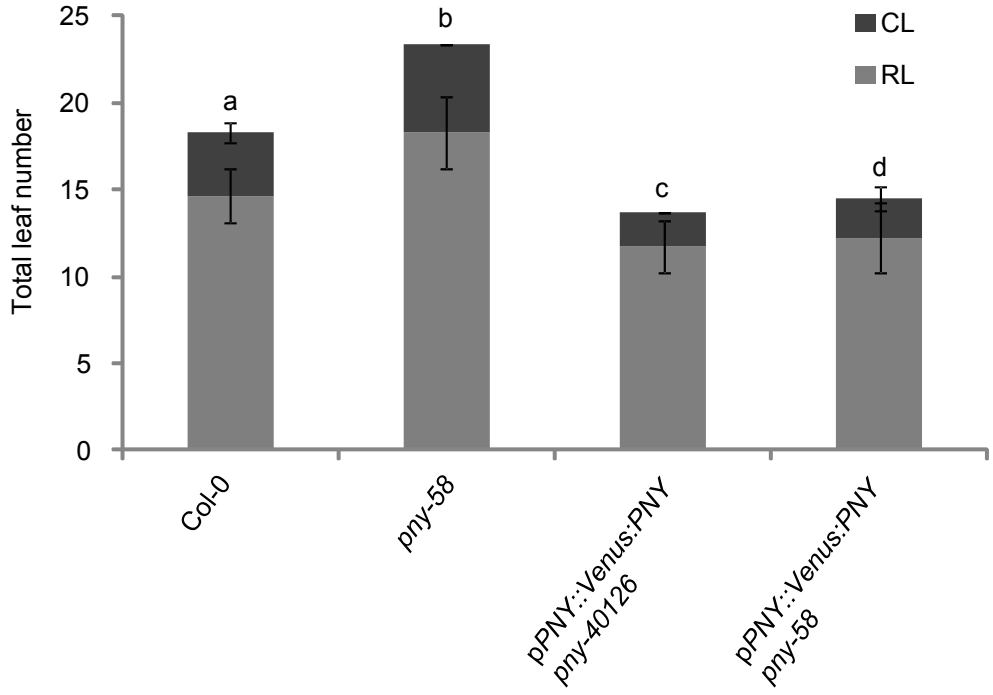



**Figure S3. Characterization of mutant plants carrying the *pny-58* allele.** (A) Phenotypic comparison between Col-0 and *pny-58*. *pny-58* displayed late flowering, short stature and phyllotactic abnormalities (B) Comparison of flowering time between *pny* mutant plants. *pny-40126* (Smith et al., 2003) and *pny-58* mutant plants. Error bars indicate s.d. Letters shared in common between the genotypes indicate no significant difference (t-test, $P < 0.05$).

A




B


**Figure S4. *PNY* mutations caused suppression of FT.** (A) Complementation assay of *pny-58* mutant. p*PNY::Venus:PNY* was crossed to *pny-58*. F2 *pny-58* plants carrying the transgene recapitulated early flowering. (B) The *PNY* mutant allele *pny-40126 in pGAS1::FT ft-10 tsf-1* caused late flowering. Error bars indicate s.d. Letters shared in common between the genotypes indicate no significant difference (t-test, $P < 0.05$).

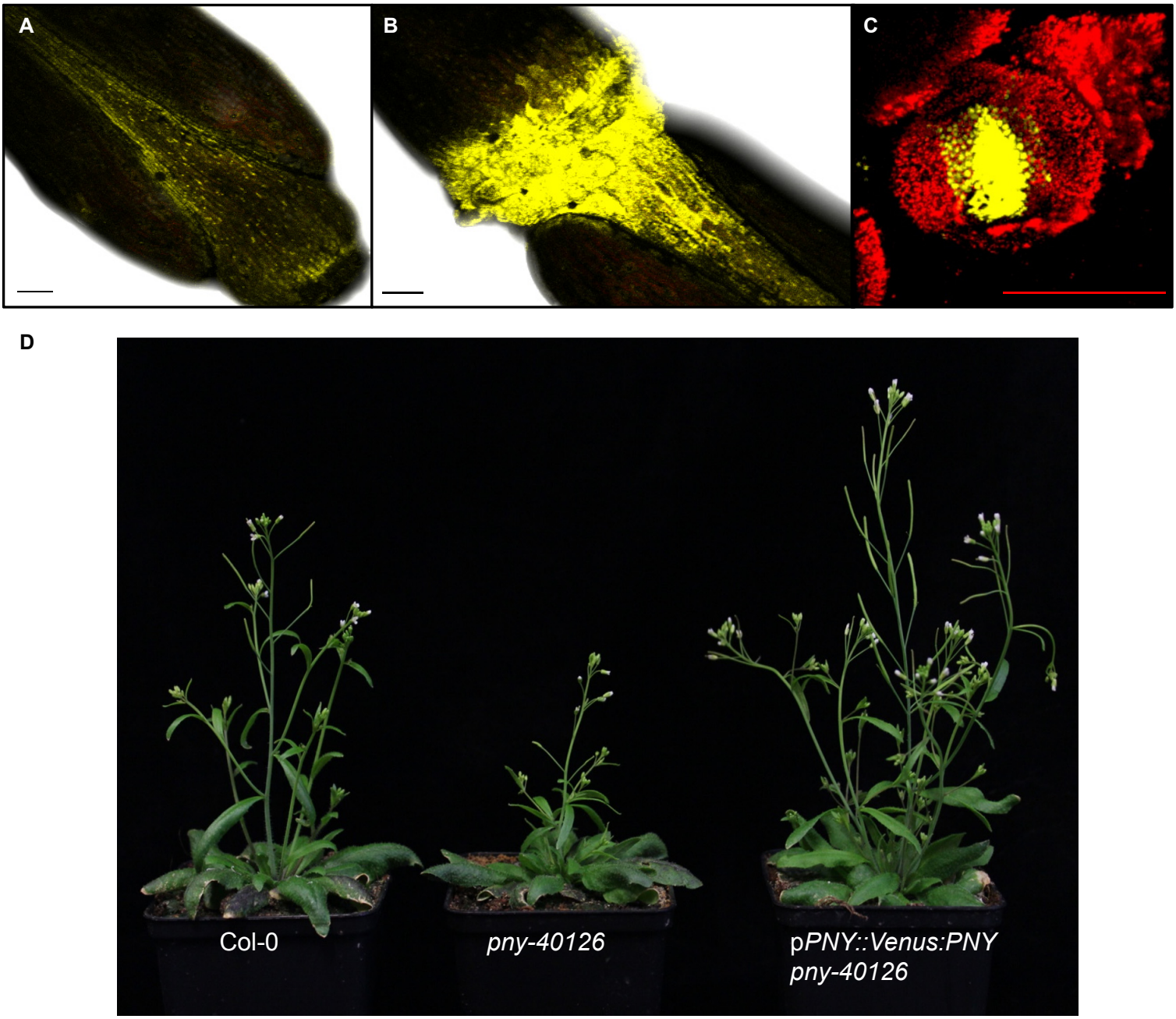



**Figure S5. Functional characterization of p*PNY::Venus:PNY*.** Expression of p*PNY::Venus:PNY* in the distal (A) and proximal (B) fruit regions and the central region of a young floral bud (C). Complementation assay of *pny-40126* mutant with p*PNY::Venus:PNY*. p*PNY::Venus:PNY* restored the wild type phenotype in the *pny-40126* mutant. Scale bars: 100 µM.

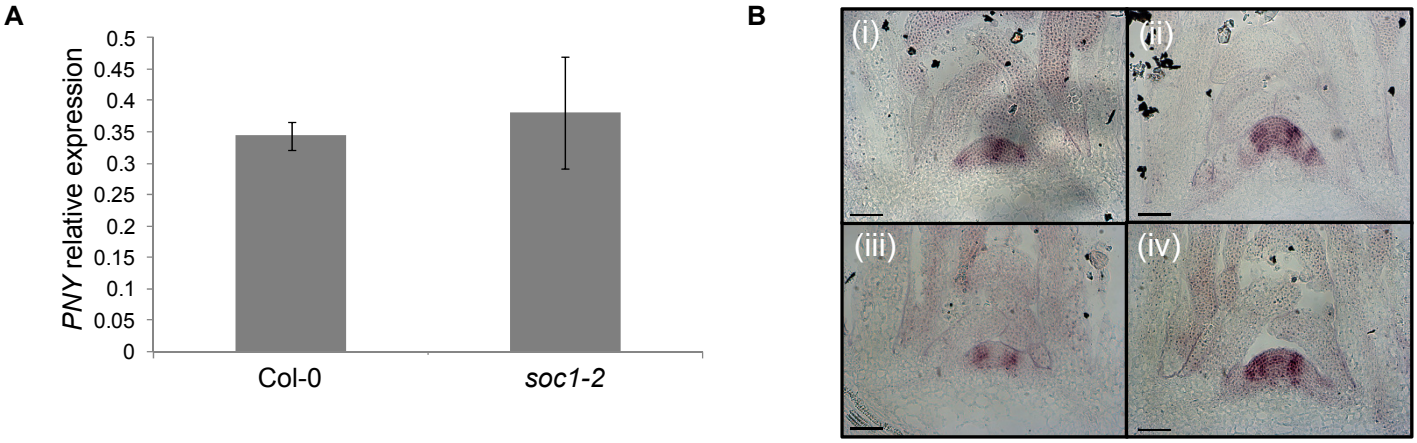



**Figure S6. Photoperiod control of *PNY* pattern of expression.** (A) Expression levels of *PNY* in *soc1-2* mutant compared to Col-0. Plants were grown under SDs for two weeks. Aerial parts were used for RNA extraction. Error bars indicate s.d. (B) Pattern of expression of *PNY* in Col-0 (i and ii) and *ft-10 tsf-1* mutants (iii and iv) at vegetative (i and iii) and reproductive stages (ii and iv). Scale bars: 50 µM.

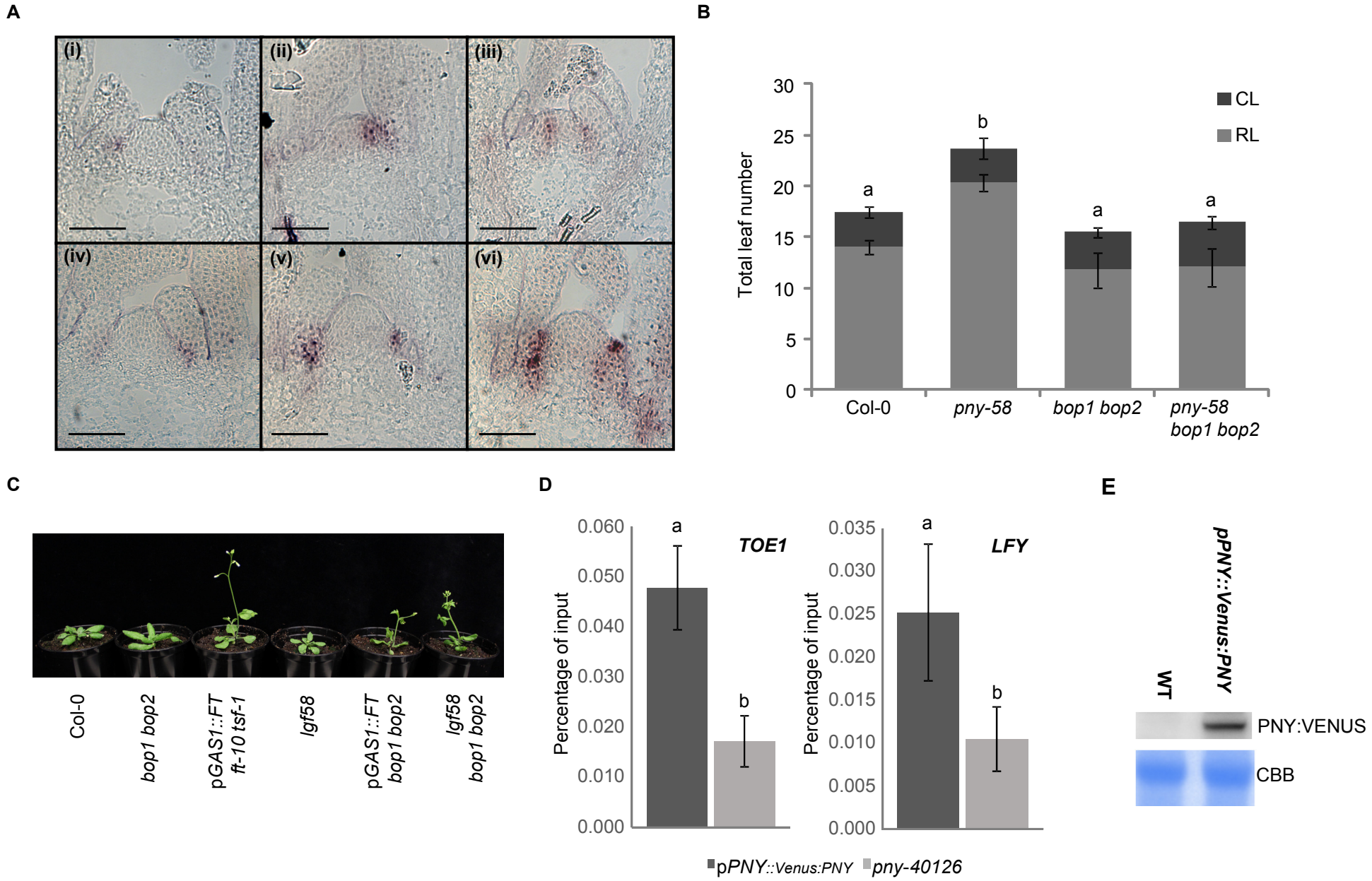



**Figure S7. *PNY* controls expression of *BOP1/2* genes.** (A) Expression pattern of *BOP2* in shoot meristems of Col-0 (i and iv), *pny-58* (ii and v) and *pny-40126* (iii and vi) during vegetative (I, ii and iii) and floral transition (iv, v and vi) stages. Scale bars: 50 µM. (B) Flowering time of the triple mutants *pny-58 bop1-3 bop2-1*. Letters shared in common between the genotypes indicate no significant difference in flowering time (ANOVA test, Holm-Sidak method, P = 0.05). (C) Picture of plants carrying various mutant combinations for *PNY, BOP1/2* and *FT*. (D) ChIP-qPCR to test PNY binding on *TOE1* and *LFY*. Error bars in (B) and (D) indicate s.d. Letters shared in common between the genotypes indicate no significant difference (t-test, $P < 0.05$). (E) Venus:PNY protein levels were detected by immunoblotting assay using an anti-GFP antibody (upper panel) in WT and pPNY:: Venus:PNY inflorescences. CBB, Coomassie Brilliant Blue was used as a loading control (lower panel).

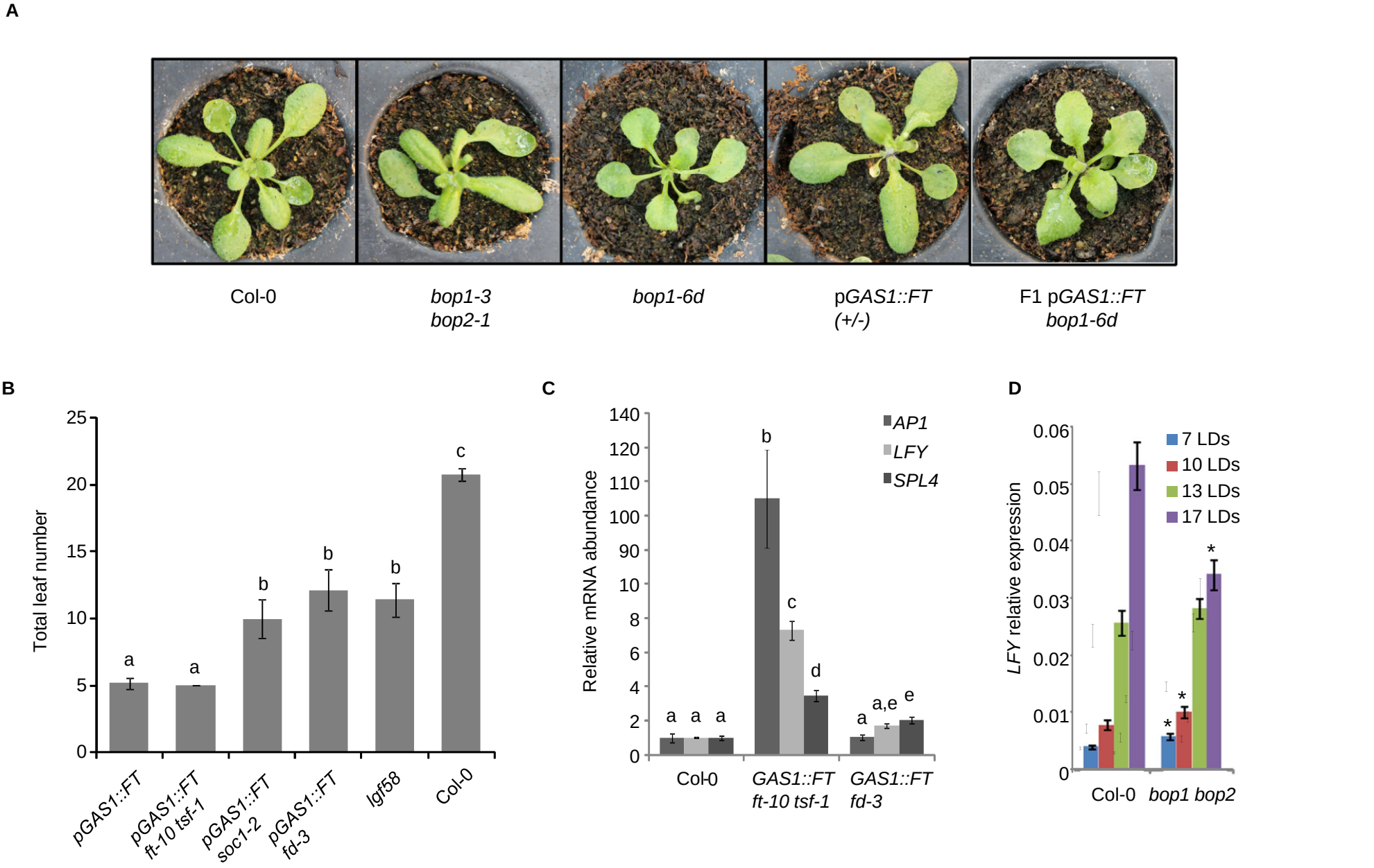



**Figure S8. *BOP1/2* genes interfere *FT* signaling pathway by affecting *FD* expression.** (A) Phenotypes of plants misexpressing *BOP* genes. (B) Flowering time of different suppressors *GAS1::FT* (C) Expression levels of FT-transcriptional regulated genes in *GAS1::FT fd-3* mutant. Letters shared in common between the genotypes indicate no significant difference (t-test, $P < 0.05$). Expression levels of *LFY* in wild type and *bop1 bop2* plants at different shoot meristem developmental stages. RNA was extracted from shoot apices of plants grown during 7 LDs (vegetative stage), 10-13 LDs (floral transition) and 17 LDs (reproductive stage). Asterisks indicate statistical differences between Col-0 and *bop1 bop2* (t-test, $P < 0.05$). Error bars in (B), (C) and (D) indicate s.d.

**Supplemental Table I. Candidate loci identified by SHOREmap**

| Chr[1] | Pos[2] | R[3] | M[4] | N[5] | AF[6] | Sh[7] | Region[8] | Gene ID[9] | Type[10] | R[11] | M[12] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 149076 | C | T | 74 | 0.99 | 40 | intronic/noncoding | AT5G01360 | | | |
| 5 | 185516 | C | T | 39 | 1 | 40 | intronic/noncoding | AT5G01450 | | | |
| 5 | 318400 | C | T | 34 | 0.97 | 40 | intergenic | | | | |
| 5 | 355398 | C | T | 5 | 1 | 32 | intergenic | | | | |
| 5 | 369679 | C | T | 73 | 1 | 40 | intronic/noncoding | AT5G01950 | | | |
| 5 | 397204 | C | T | 61 | 1 | 40 | CDS | AT5G02030 | Nonsyn | Q | STOP |
| 5 | 407433 | C | T | 78 | 1 | 40 | CDS | AT5G02070 | Nonsyn | G | R |
| 5 | 458448 | C | T | 91 | 1 | 40 | CDS | AT5G02250 | Nonsyn | L | F |
| 5 | 543204 | C | T | 51 | 1 | 40 | intronic/noncoding | AT5G02470 | | | |
| 5 | 832812 | C | T | 69 | 0.99 | 40 | CDS | AT5G03380 | Nonsyn | G | E |
| 5 | 1003048 | C | T | 23 | 1 | 40 | intergenic | | | | |
| 5 | 1130080 | C | T | 60 | 0.94 | 40 | CDS | AT5G04140 | Nonsyn | P | L |
| 5 | 1228282 | C | T | 49 | 0.98 | 40 | intronic/noncoding | AT5G04360 | | | |
| 5 | 1432099 | C | T | 42 | 0.89 | 40 | intronic/noncoding | AT5G04895 | | | |
| 5 | 1610524 | C | T | 21 | 0.91 | 40 | intergenic | | | | |
| 5 | 1656838 | C | T | 32 | 0.84 | 40 | CDS | AT5G05570 | Nonsyn | R | W |
| 5 | 1934078 | C | T | 25 | 0.83 | 40 | intergenic | | | | |
| 5 | 2016343 | C | T | 36 | 0.78 | 40 | CDS | AT5G06580 | Nonsyn | R | K |
| 5 | 2017876 | C | T | 45 | 0.82 | 40 | CDS | AT5G06590 | Nonsyn | P | S |
| 5 | 2027212 | C | T | 48 | 0.87 | 40 | CDS | AT5G06600 | Nonsyn | A | T |
| 5 | 2051145 | C | T | 53 | 0.88 | 40 | CDS | AT5G06670 | Syn | L | L |
| 5 | 2197116 | C | T | 58 | 0.89 | 40 | CDS | AT5G07070 | Nonsyn | G | E |
| 5 | 2355749 | C | T | 9 | 0.75 | 40 | intergenic | | | | |
| 5 | 2444272 | C | T | 17 | 1 | 40 | intergenic | | | | |
| 5 | 2450732 | C | T | 59 | 0.87 | 38 | intronic/noncoding | AT5G07700 | | | |
| 5 | 2454751 | C | T | 64 | 0.88 | 40 | CDS | AT5G07710 | Syn | L | L |
| 5 | 2486468 | C | T | 26 | 0.93 | 40 | intergenic | | | | |
| 5 | 2508220 | C | T | 44 | 0.88 | 40 | intronic/noncoding | AT5G07842 | | | |
| 5 | 2531039 | C | T | 62 | 0.83 | 40 | CDS | AT5G07930 | Nonsyn | P | S |
| 5 | 2646127 | C | T | 53 | 0.95 | 40 | intronic/noncoding | AT5G08230 | | | |
| 5 | 2785739 | C | T | 64 | 0.91 | 40 | CDS | AT5G08590 | Nonsyn | S | L |
| 5 | 3077886 | C | T | 59 | 0.86 | 40 | CDS | AT5G09870 | Nonsyn | S | F |
| 5 | 3269438 | C | T | 64 | 0.91 | 40 | CDS | AT5G10390 | Nonsyn | G | E |
| 5 | 3297090 | C | T | 45 | 0.83 | 40 | CDS | AT5G10470 | Syn | P | P |
| 5 | 3422067 | C | T | 44 | 0.86 | 40 | CDS | AT5G10820 | Nonsyn | V | M |
| 5 | 3477286 | C | T | 48 | 0.84 | 40 | CDS | AT5G10990 | Nonsyn | R | W |
| 5 | 3606362 | C | T | 22 | 0.96 | 40 | intergenic | | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 4017212 | C | T | 57 | 0.9 | 40 | CDS | AT5G12400 | Nonsyn | T | I |
| 5 | 4025076 | C | T | 44 | 0.85 | 40 | CDS | AT5G12420 | Nonsyn | A | T |
| 5 | 4056217 | C | T | 38 | 0.84 | 40 | intronic/noncoding | AT5G12850 | | | |
| 5 | 4136159 | C | T | 55 | 0.87 | 40 | intronic/noncoding | AT5G13030 | | | |
| 5 | 4141967 | C | T | 10 | 0.71 | 40 | intergenic | | | | |
| 5 | 4358564 | C | T | 42 | 0.82 | 40 | CDS | AT5G13550 | Nonsyn | G | E |
| 5 | 4422944 | C | T | 47 | 0.87 | 40 | CDS | AT5G13700 | Nonsyn | A | T |
| 5 | 4701808 | C | T | 39 | 0.93 | 40 | intronic/noncoding | AT5G14580 | | | |
| 5 | 4751336 | C | T | 45 | 0.8 | 40 | CDS | AT5G14720 | Nonsyn | G | E |
| 5 | 5069593 | C | T | 48 | 0.83 | 40 | CDS | AT5G15580 | Nonsyn | G | R |
| 5 | 5092346 | C | T | 60 | 0.91 | 40 | CDS | AT5G15650 | Syn | I | I |
| 5 | 5144009 | C | T | 32 | 0.89 | 40 | 3prime_UTR | AT5G15760 | | | |
| 5 | 5243092 | C | T | 67 | 0.92 | 40 | CDS | AT5G16040 | Nonsyn | V | I |

(1) Chr: chromosome. (2) Position: position of the mutated nucleotide. (3) R: nucleotide in the reference genome (p*GAS1::FT ft-10 tsf-1*). (4) M: nucleotide in *lgf58*. (5) N: number of reads supporting the mutation. (6) AF: allele frequency. (7) Sh: SHORE Score (max. 40). (8) Region: region of the locus where the mutation was identified. (9) Gen ID: gene identifier. (10) Type: type of mutation (nonsynonymous or synonymous). (11) AR: amino acid in the reference genome (p*GAS1::FT ft-10 tsf-1*). (12) AM: amino acid in *lgf58*.

## Supplemental Table II. List of primers used in this work

***RT-qPCR***

| Target[1] | Fw/Rv[2] | Sequence[3] |
|---|---|---|
| AP1 | Y28 | ATGAGAGGTACTCTTACGCCGA |
| | Y29 | CAAGTCTTCCCCAAGATAATGC |
| LFY | K228 | TGAACATCGCTTGTCGTCAT |
| | K229 | CGACGATCCGGTACAGCTA |
| SOC1 | K288 | GTGATCTCCACTCAACAAAAA |
| | K289 | CAACAAGAGAGAAGCAGCTTTA |
| PNY | K481 | CAACAACCCATCTTCGTCCT |
| | K482 | CCTCCGTTTGTGCTGCTATT |
| SPL4 | K221 | CATCATTCAAGCGACCACAG |
| | K222 | TTGGCAAGGAAAAGCTAGGA |
| BOP1 | K574 | CGACATCCTTCGAACCCTAA |
| | K575 | GCTCGTGTGTTCGTCTTTCA |
| BOP2 | K576 | GGAAGGTATGAGTCGGCATC |
| | K577 | TGCATGCCCCTCTTCTTAAT |
| FD | MR13 | CATCAACCTTGCTTCCATCC |
| | MR14 | GGTTTTGGTTGTGGTGGTTT |
| PEX4 | K007 | TTACGAAGGCGGTGTTTTTC |
| | K008 | GGCGAGGCGTGTATACATTT |

***pny-58* genotyping**

| Target[1] | Fw/Rv[2] | Sequence[3] |
|---|---|---|
| PNY | K617 | TCCATGTGACGTTTTGAAGG |
| | K618 | TTCGAATGATCCCATCACAG |

***ChIP-qPCR***

| Target[1] | Fw/Rv[2] | Sequence[3] |
|---|---|---|
| SOC1 (+) | K523 | TGATTGGCACGATTCTGAAA |
| | K524 | GAGGTGAGGATTAAAATGATGTTG |
| SOC1 (-) | K288 | GTGATCTCCACTCAACAAAAA |
| | K289 | CAACAAGAGAGAAGCAGCTTTA |
| BOP1 (1) | X272 | AAGCACTTCTTCTGTCTCAT |
| | X273 | AGAAAAGCTGGAGTTTCCAG |
| BOP1 (2) | X274 | TCATGTCGGTAAGACGTGT |
| | X275 | TCCTAGGGTTTTGCTTTCTG |
| BOP1 (3) | X264 | GAGAGAGAGTGAAAAGACAA |
| | X265 | CCCAAATCCATCAATTTTTG |
| BOP1 (4) | X268 | GCGCCACTAATAACTTTATGG |
| | X269 | CGTTAATTAAGTTCAGGAGC |

| | | |
|---|---|---|
| BOP1 (5) | X270 | GTCATTTCTCTCTAAACTCT |
| | X271 | AGATCTGAATGGGCGGACCG |
| BOP2 (6) | X282 | GAGAAAACTAGTCTCCAAACTCTCG |
| | X283 | ATCGTCGTGATTGGCCTAGT |
| BOP2 (7) | X284 | ACGACTGTCAGTGCCCTTCT |
| | X285 | TTATCTACGTCGTGCGTTCG |
| BOP2 (8) | X290 | GTGTTGCTCAGGCTTTCACA |
| | X291 | GAATACAAAGGTGGGCCAAA |
| BOP2 (9) | X292 | CGAAACGCTTTTGATTCGAT |
| | X293 | TCGTCTGCTTTCGGAAACTT |
| LFY | X294 | TGCATGCATTCACACATAGTACACAT |
| | X295 | TATTATCCGCCGAGCAATAGACTGTA |
| TOE1 | X296 | CCATCATGGTAAGTGGTAACCAAGTC |
| | X297 | GAGACCCATTATTGGGAGTAACCAAA |

***In situ* probes**

| **Target[(1)]** | **Fw/Rv[(2)]** | **Sequence[(3)]** |
|---|---|---|
| PNY | K481 | CAACAACCCATCTTCGTCCT |
| | K483 | TAATACGACTCACTATAGGGCCTCCGTTTGTGCTGCTATT |
| BOP2 | K576 | GGAAGGTATGAGTCGGCATC |
| | K640 | TAATACGACTCACTATAGGGTGCATGCCCCTCTTCTTAAT |
| FD | FDT3-2F | ATTAACCCTCACTAAAGGGATTTCATCCTCATCACCATCG |
| | FDT7-2R | TAATACGACTCACTATAGGG ACCAGAGCCTCGAAAGAGGT |

***Molecular cloning of pPNY::Venus:PNY***

| **Target[(1)]** | **Fw/Rv[(2)]** | **Sequence[(3)]** |
|---|---|---|
| PNY-cds | A1-F | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCTTTGATATACATATTGATC GTGCTTCAAAAAGAC |
| | A2-R | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATTGAATCCAATTTCATT TCTTAAAAAGATAACATTTG |
| PNY-prom | A3-F | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGGCCCTAAATATTTTG TTTTTAAAAAAAATGA |
| | A4-R | GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAGAGGAGGAGGGTGAAA GTGGAAGT |
| I-PIPE-1 | A5-F | CATCTCATCGACATCATCCTTTCCCATGGTGAGCAAGGGCGAGG |
| | A6-R | CGCTGCCGCAGCGGCAGCAGCCGCAGCGCCCTTGTACAGCTCGTCCATGC CG |
| I-PIPE-2 | A7-F | TGCCGCTGCGGCAGCGGCTGATGCATACGAGCCTTATCATGTT |
| | A8-R | TCTTATAATGCCCACTTTGTACAAGAAAGCTGGGTCCTATTGAATCCAATT TCATTTCTTAAAAAGATAA |
| V-PIPE | A9-F | TTATCTTTTTAAGAAATGAAATTGGATTCAATAGGACCCAGCTTTCTTGTA CAAAGTGGGCATTATAAGA |
| | A10-R | CCTCGCCCTTGCTCACCATGGGAAAGGATGATGTCGATGAGATG |

(1) Target: gene, genomic region or DNA fragment flanked by the given primers. (2) Fw/Rv: forward and reverse primers. (3) Sequence: DNA sequence 5' > 3' direction.