# Supporting Information

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### SI Materials and Methods

**Plant Material and Generation of Transgenic Plants.** The *Arabidopsis thaliana* accession Landsberg *erecta* (L*er*) was used as the wild type unless otherwise specified. The *ap1-1* and *ap1-4* alleles are in the L*er* background (1), and *ahk2-5*, *ahk3-7*, *cre1-2*, and *pTCS::GFP-ER*, *pLOG1::GUS* and *p35S::AGL24* lines are in Columbia (Col-0) background (2–4). The *ap1-1* mutation was introduced into *ahk* mutants and the *pTCS::GFP-ER* and *pLOG1::GUS* lines by crossing, and $F_2$ or $F_3$ generations were used for phenotype analysis. Sibling plants obtained from the same crossing were used as controls. Genotyping primers are listed in Table S1.

The stem-loop precursor *AmiR-LOG1* was constructed by integrating an amiRNA sequence targeting *LOG1* into the *miR159a* stem-loop precursor following the protocol of Niu et al. (5). Oligonucleotide-directed mutagenesis was used to replace the mature *miR159a* sequence with a synthetic sequence targeting *LOG1* (see Table S2 for primers). The *AmiR-LOG1* precursor was ligated into a BJ36 vector containing the 5.0 kb upstream regulatory sequence from the *AP1* gene (6) and was subcloned into the pMOA34 binary transformation vector. Full-length cDNAs for *IPT8* and *CKX3* were introduced similarly into the BJ36 vector under control of the *AP1* upstream regulatory sequence and were subcloned into pART27 (see Table S2 for primers). The *pAP1::IPT8* construct was transformed into L*er*, and *pAP1::amR-LOG1* and *pAP1::CKX3* constructs were transformed into *ap1-4* using the *Agrobacterium*-mediated floral dip method (7).

**Plant Growth and Cytokinin Treatment Conditions.** Plants were grown in the greenhouse on soil at 22 °C under long-day conditions (16 h light/8 h dark). For cytokinin treatment, inflorescences were sprayed with a solution containing N6-benzylaminopurine (Sigma-Aldrich) and 0.05% Tween 20. Plants were treated three times at 1-wk intervals, and phenotype analysis was performed 15 d after treatment. A solution containing 0.05% Tween 20 alone was used as a mock control.

**RT-PCR and Quantitative Real-Time PCR.** Total RNA was extracted from inflorescences of 12 plants 6 d after bolting using the AxyPrep Multisource RNA Miniprep kit (Corning). First-strand cDNA synthesis was performed with 2 μg total RNA using TransScript One-Step gDNA Removal and cDNA synthesis SuperMix (TransGen) and 22-mer oligo dT primers according to the manufacturer's instructions. RT-PCR analysis was performed in a 20-μL reaction volume using Taq DNA polymerase (TianGen) and gene-specific primers (Table S3). Quantitative real-time PCR (qRT-PCR) was performed on a Bio-Rad CFX96 real-time PCR detection system with KAPA SYBR FAST qPCR kit (KAPA Biosystems). Relative expression by qRT-PCR was normalized to *TUB6* (*At5g12250*), which has been shown to be a superior reference gene for qRT-PCR analysis, being constant in various treatments (8). Gene-specific primers (Table S3) were used to amplify and detect each gene.

**ChIP.** ChIP experiments were performed according to published protocols (8). Inflorescences of ~4-wk-old *p35S::AP1-GR ap1-1 cal-1* plants were induced with dexamethasone (Dex) as described (9). Inflorescence material from treated and untreated plants (800 mg) was harvested 2 h later and was fixed with 1% formaldehyde under vacuum for 10 min. Nuclei were isolated and lysed, and chromatin was sheared to an average size of 1,000 bp by sonication. The sonicated chromatin served as input or positive control. Immunoprecipitations were performed with an antibody against glucocorticoid receptor (GR) (PA1-516; Affinity Bioreagents). The precipitated DNA was isolated, purified, and used as a template for PCR. RT-PCR was performed as described above (see Table S4 for primers). The data are presented as degree of enrichment of *CKX3* or *LOG1* promoter fragments. The amount of precipitated DNA used in each assay was determined empirically so that an equal amount of *ACT2* (*At3g18780*) was amplified. Two independent sets of biological samples were used.

**Protoplast Transient Expression Assay.** To produce the effector constructs, full-length *AP1* was amplified from *Arabidopsis* cDNA and inserted into the pBI221 vector to generate pBI221-AP1. PCR was used to fuse the EDLL domain (10) to AP1 to generate pBI221-AP1-EDLL. To generate the *LOG1* and *CKX3* promoter-driven *LUC* reporter gene, the *LOG1* and *CKX3* promoter was amplified from *Arabidopsis* genomic DNA. PCR fragments were inserted into the corresponding sites of the YY96 vector (11) to produce *pLOG1::LUC and pCKX3::LUC* (Fig. 3*H*; see Table S2 for primers).

Isolation of *Arabidopsis* protoplasts and PEG-mediated transfection were performed as described previously (12). The reporter construct, effector plasmid, and a *p35S::GUS* construct (internal control) were cotransformed into protoplasts. After transformation, the protoplasts were incubated at 23 °C for 12–15 h. The protoplasts were pelleted and resuspended in 100 μL of 1× CCLR buffer (Promega). For the GUS enzymatic assay, 5 μL of the extract was incubated with 50 μL of 4-methylumbelliferyl-β-D-glucuronide assay buffer [50 mM sodium phosphate (pH 7.0), 1 mM β-D-glucuronide, 10 mM EDTA, 10 mM β-mercaptoethanol, 0.1% sarkosyl, 0.1% Triton X-100] at 37 °C for 15 min, and the reaction was stopped by adding 945 μL of 0.2 M $Na_2CO_3$. For luciferase activity assay, 5 μL of the extract was mixed with 50 μL of luciferase assay substrate (Promega), and the activity was detected with the Modulus Luminometer/Fluometer with a luminescence kit. The reporter gene expression levels were expressed as relative LUC/GUS ratios.

**Microscopy.** Morphological characterization of flowers was based on light microscopy of at least 20 flowers using a Nikon SMZ1000 dissecting microscope. Fixed tissue sections of flowers from 24-d-old plants were stained with 0.02% toluidine blue O and were observed under an Olympus BX60 microscope. GUS staining was performed with 0.1 M ferricyanide and 0.1 M ferrocyanide, as described (13). Images were taken with a Nikon DS-Ri1 camera.

Confocal microscopy images were taken with a Nikon C2 Si confocal microscope. Excitation and detection windows for GFP were as previously described (14). To detect the signal of propidium iodide staining, a 488-nm laser line was used for excitation, and a 585- to 615-nm band-pass filter was used for detection. Autofluorescence was excited at 488 nm and was detected in the 660- to 700-nm range.


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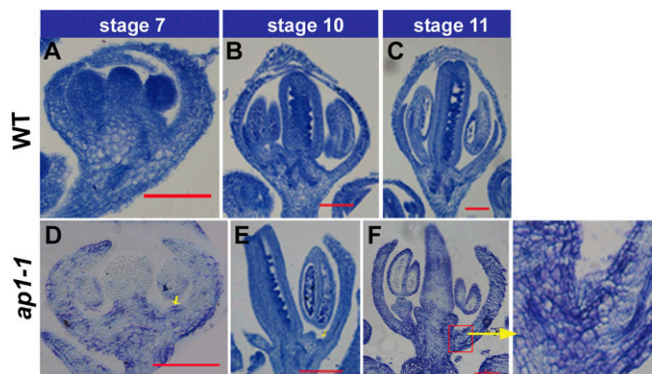


**Fig. S1.** Initiation of sepal axil secondary flowers in *ap1-1*. Longitudinal sections of developing flowers at stage 7 (*A* and *D*), stage 10 (*B* and *E*), and stage 11 (*C* and *F*) in wild-type (*A*–*C*) and *ap1-1* (*D*–*F*) plants. Bulging floral meristems for secondary flowers (arrows) are observed in *ap1-1* mutants. (Scale bars: 1 mm.)

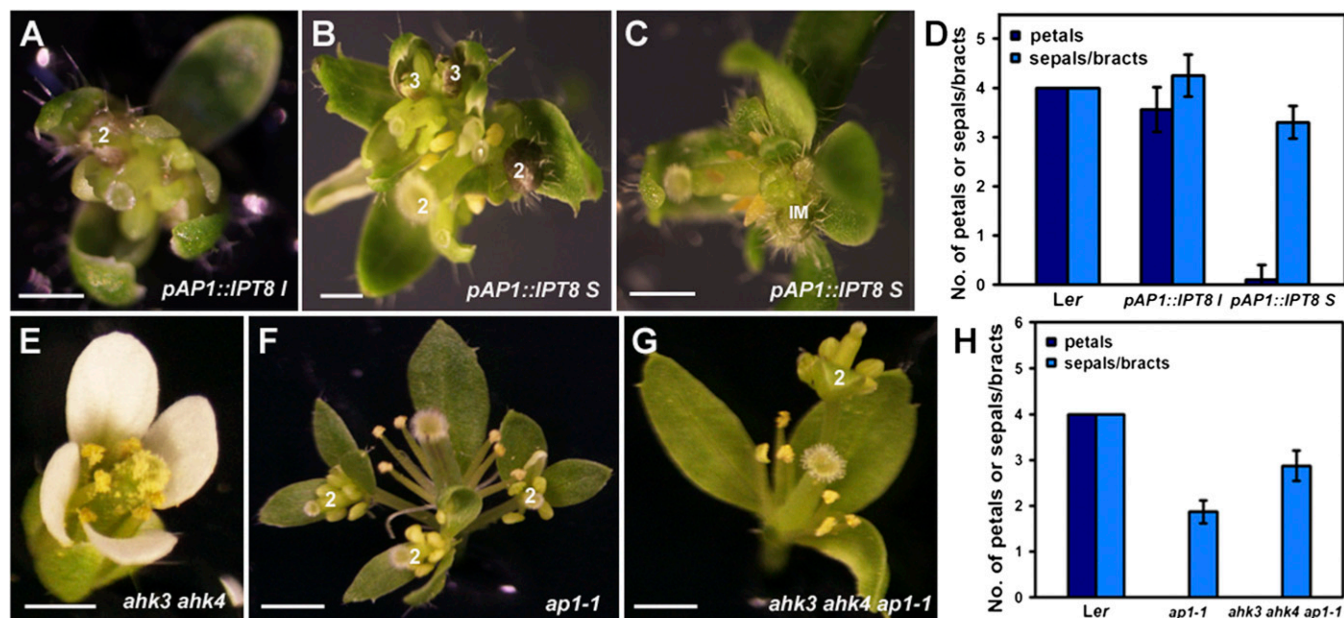


**Fig. S2.** Effects of cytokinin signaling on floral organ patterning. (*A*–*D*) Flower phenotype of an intermediate *pAP1::IPT8* transgenic line (*A*), which is indicated as *pAP1::IPT8 I* and was used for Figs. 2 and 5,, and a strong *pAP1::IPT8* transgenic line (*B* and *C*), which is indicated as *pAP1::IPT8 S*. The basal flower for each line is shown in *A*–*C*, and the mean number of petals and sepals/bracts per flower are shown in *D*. Error bars indicate SD. (*E*–*H*) Flower phenotype of *ahk3 ahk4* (*E*), *ap1-1* (*F*), and *ahk3 ahk4 ap1-1* (*G*) mutants. The basal flower is shown for each genotype in *E*–*G*, and the mean number of petals and sepals/bracts per flower are shown in *H*. Error bars indicate SD. IM, sepal/bract axil inflorescence meristem; 2, secondary flower; 3, tertiary flower. (Scale bars: 500 μm.)

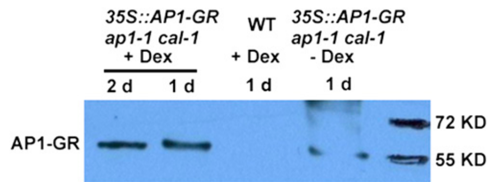


**Fig. S3.** Nuclear accumulation of the AP1-GR fusion protein after Dex treatment. Protein gel blot detection of the AP1-GR fusion protein using crude nuclear extracts isolated from *p35S::AP1-GR ap1-1 cal-1* and wild-type L*er* plants treated with 10 μM Dex. Samples were harvested 1 and 2 d after treatment.

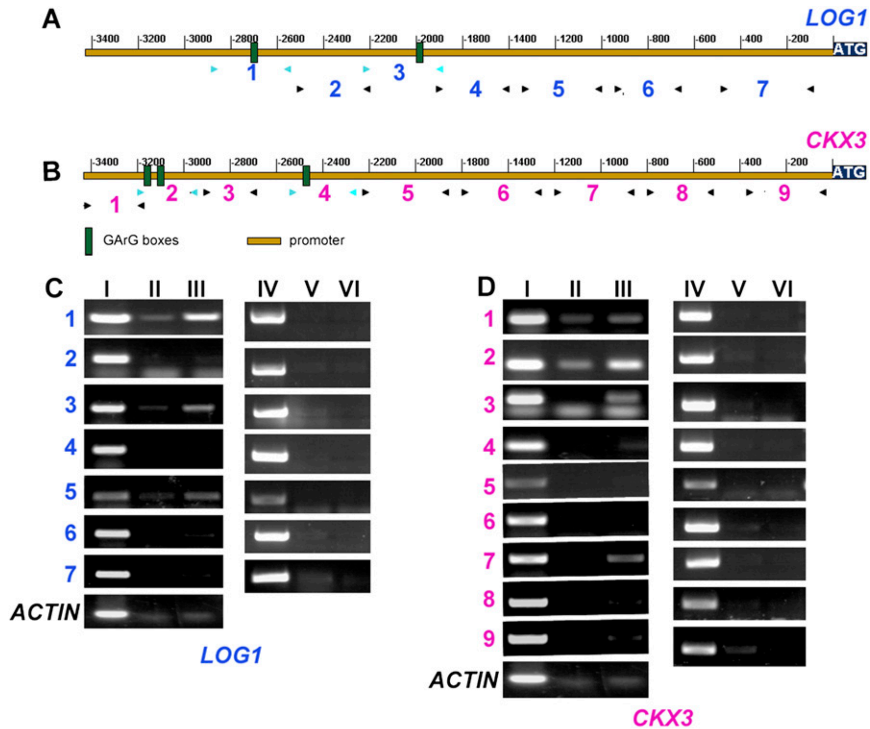


**Fig. S4.** ChIP enrichment test by PCR. Genomic fragments near *LOG1* (*A* and *C*), *CKX3* (*B* and *D*), and *ACT2* as a negative control are shown. ChIP was carried out with an anti-GR antibody using *p35S::AP1-GR ap1-1 cal-1* inflorescence 2 h after mock (−Dex) or Dex (+Dex) treatment, together with total DNA input (input) and no-antibody (−anti-GR) controls.

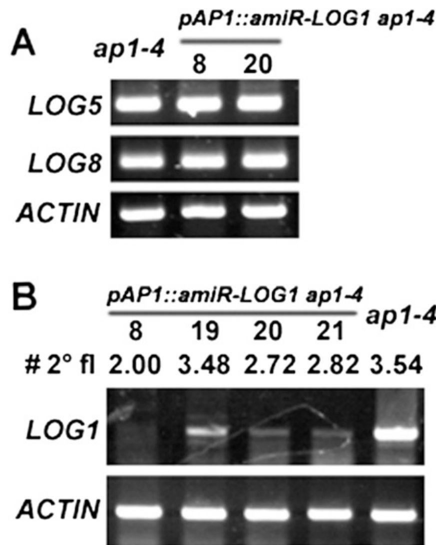


**Fig. S5.** RT-PCR analysis of *LOG* gene expression in independent *pAP1::amiR-LOG1 ap1-4* lines. (*A*) Expression of *LOG5* and *LOG8* in two *pAP1::amiR-LOG1 ap1-4* transgenic lines and in *ap1-4*. (*B*) Expression of *LOG1* and mean number of sepal axil secondary flowers per pedicel at the fourth flower in four independent *pAP1::amiR-LOG1 ap1-4* transgenic lines and in *ap1-4*. Lane 8 of *pAP1::amiR-LOG1 ap1-4* was used in Fig. 5.

**Table S1. Primers used for genotyping**

| Primer | Sequence (5′–3′) |
|---|---|
| AHK2-5-F | GCAAGAGGCTTTAGCTCCAA |
| AHK2-5-R | TTGCCCGTAAGATGTTTTCA |
| AHK3-7-F | CCTTGTTGCCTCTCGAACTC |
| AHK3-7-R | CGCAAGCTATGGAGAAGAGG |
| CRE1-2-F | CTCCATGTGCTTGCTTATTAGTCGT |
| CRE1-2-R | CACACACTGACCTGATCAATTGCA |
| GABI-T | ATATTGACCATCATACTCATTGC |
| SK-LS2 | TGGACGTGAATGAATGTAGACACGTCGA |
| SAIL-LB3 | GAATTTCATAACCAATCTCGATACAC |
| AP1-1 | GGTACACAGACAAACTGGTCGATG |
| AP1-8 | GCTTGCAAGTCTTCCCCAAGATAAGGC |
| AP1-9 | TCATTGCATGAGTGCAACTGTG |
| AP1-10 | ATATTGTGGCCTTGGTTCTGCGGATC |

**Table S2. Primers used to make constructs**

| Primer | Sequence (5′–3′) |
|---|---|
| IPT8-F | *atcggtacc*ATGCAAAATCTTACGTCCACATTCGTCTC |
| IPT8-R | *agctctaga*TCACACTTTGTCTTTCACCAAGAAGCGTT |
| LOG1-F | *agatct*TGATCTGACGATGGAAGTACAAGTGGGACTTAGCGCTACATGAGTTGAGCAGGGTA |
| LOG1-R | GTACAAGTGGGACTTAGCGCTAGAAGAGTAAAAGCCATTA |
| CKX3-F | *cgggatccC*TAACTCGAGTTTATTTTTTGA |
| CKX3-R | *cccccggg*ATGGCGAGTTATAATCTTCGT |
| AP1-F | *gctctagagc*ATGGGAAGGGGTAGGGTTC |
| AP1-R | *ggggtacccc*TGCGGCGAAGCAGCCAA |
| LOG1-1-F | *cccaagcttggg*CCTAACCATGTGACGGTAAT |
| LOG1-1-R | *cgggatcccg*GTGATTAGTGATGGTTTG |
| LOG1-3-F | *cccaagcttggg* ATGATGGATAGTCGGTACT |
| LOG1-3-R | *cgggatcccg* TCCCAAGAGATCAGACTAT |
| CKX3-2-F | *cccaagcttggg*CTGAAGTAAAGGTCGCTGA |
| CKX3-2-R | *cgggatcccg* TGCTAAACGCACGTGTAG |
| CKX3-4-F | *cccaagcttggg* TGGCTGCCAATGGATTTAA |
| CKX3-4-R | *cgggatcccg* TCCGGGATCGTAAATGTATT |

Lowercase italic indicates added restriction enzyme sites and protective bases for cloning, and underlining indicates the *amiR-LOG1* sequence.

**Table S3. Primers used for RT-PCR and qRT-PCR**

| Primer | Sequence (5′–3′) |
| --- | --- |
| TUB6-F | GAAACCTTGAAGACAGTCGCAAT |
| TUB6-R | GCAATCTGGTGCTGGAAACAA |
| ACT2-FP | GTCGTACAACCGGTATTGTGC |
| ACT2-RP | CACAAACGAGGGCTGGAACAAG |
| GFP-Q-F: | GTGAAGGTGATGCAACATACGG |
| GFP-Q-R: | AAGTCGTGCCGCTTCATATGA |
| ARR5-Q-F | TCTGAAGATTAATTTGATAATGACGG |
| ARR5-Q-R | TCACAGGCTTCAATAAGAAATCTTCA |
| LOG1-RT-F | ATGGAGATAGAATCAAAGTTCA |
| LOG1-RT-R | AGTCCCACTGGTTTATCAT |
| LOG1-Q-F | TTTTGTGGAAGTAGTGCTGGTAATA |
| LOG1-Q-R | AAATCAAACCCATTAAACCAAT |
| CKX3-RT-F | CACAGTCAACGAGGAAAT |
| CKX3-RT-R | CCAAGCCTAACTCGAGTTTATTTTTTGA |
| CKX3-Q-F | CCCACCGGATAACTGGAGAT |
| CKX3-Q-R | CACGATGTCGTTTGACCATT |
| AGL24-Q-F | GAGGCTTTGGAGACAGAGTCGGTGA |
| AGL24-Q-R | AGATGGAAGCCCAAGCTTCAGGGAA |
| SOC1-Q-F | AGCTGCAGAAAACGAGAAGCTCTCTG |
| SOC1-Q-R | GGGCTACTCTCTTCATCACCTCTTCC |
| SVP1-Q-F | CAAGGACTTGACATTGAAGAGCTTCA |
| SVP1-Q-R | CTGATCTCACTCATAATCTTGTCAC |

**Table S4. Primers used for ChIP-PCR**

| Primer | Sequence (5′–3′) |
| --- | --- |
| LOG1-1-F | ATGATGGATAGTCGGTACT |
| LOG1-1-R | TCCCAAGAGATCAGACTAT |
| LOG1-2-F | CTACAATAAGTGTGTGCTCT |
| LOG1-2-R | AGTACCGACTATCCATCAT |
| LOG1-3-F | CCTAACCATGTGACGGTAA |
| LOG1-3-R | GTGATTAGTGATGGTTTGCC |
| LOG1-4-F | ATAGTCTGATCTCTTGGGAT |
| LOG1-4-R | ACTCGATGACAAACCATATG |
| LOG1-5-F | ACGTACCCATTAGGCTATTA |
| LOG1-5-R | GGTTGATCCAAACCAATCA |
| LOG1-6-F | ATATGCGGTTGTCGTAGT |
| LOG1-6-R | CTATGCACTTGATATAAACACG |
| LOG1-7-F | TCCTCAAACTCGAGACATT |
| LOG1-7-R | CTAGCTCATACGCTTCCT |
| CKX3-1-F | AATCATTACCCTAATTCCT |
| CKX3-1-R | GCGACCTTTACTTCAG |
| CKX3-2-F | CTGAAGTAAAGGTCGCTG |
| CKX3-2-R | TGCTAAACGCACGTGTA |
| CKX3-3-F | CGGGTACAACGTCTTATTG |
| CKX3-3-R | CAGGCCTGATCATAGGTAT |
| CKX3-4-F | TGGCTGCCAATGGATTTAA |
| CKX3-4-R | TCCGGGATCGTAAATGTATT |
| CKX3-5-F | GTGCACCTAAAAACATTCTTTA |
| CKX3-5-R | AACTCGTGGTATACCACAG |
| CKX3-6-F | GCAAATGGAATTGGAGTGT |
| CKX3-6-R | AGATGATGTTTCCTTGTCAC |
| CKX3-7-F | AGGTCCGATTGTTTACAAC |
| CKX3-7-R | TGTACCATAATCATCATGGG |
| CKX3-8-F | GTGATGATTATGGTGGTGG |
| CKX3-8-R | GGTGTCGCTCACTAGTTAA |
| CKX3-9-F | AGATTCTCACGTGACTTTGA |
| CKX3-9-R | GCATGCAGACACATGTATT |