Supplemental Data

The AT-hook motif-containing protein AHL22 regulates flowering initiation by modifying *FLOWERING LOCUS T* chromatin in *Arabidopsis*

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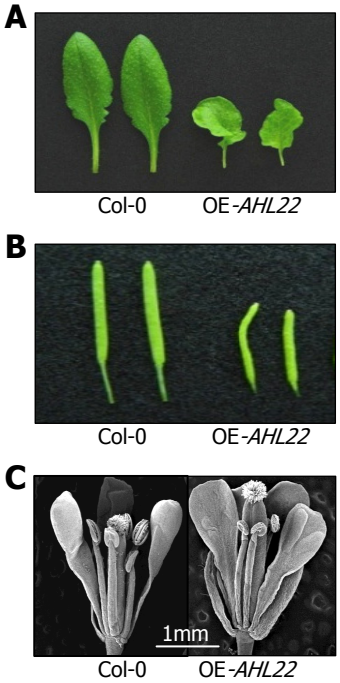
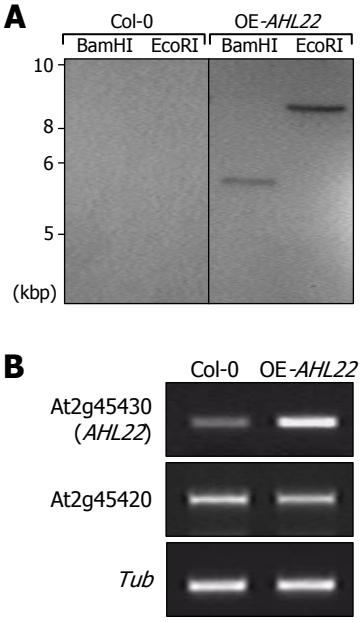
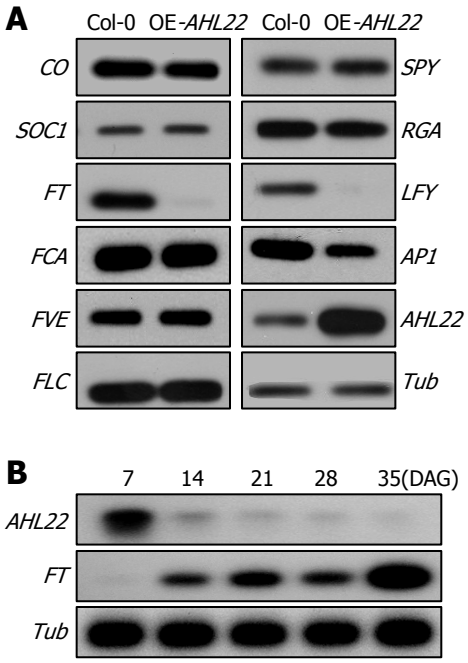


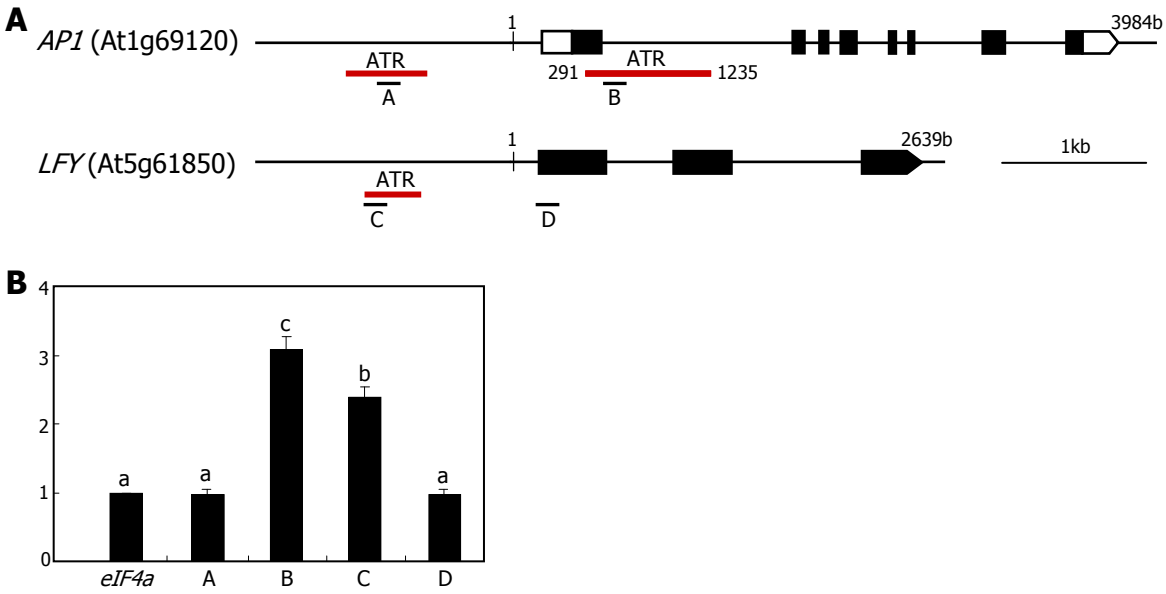
Supplemental Figure 1. Pleiotropic phenotypes of OE-*AHL22* mutant.
A, Altered leaf morphology. Two representative leaves were photographed for each plant genotype. *B*, Short, curled siliques. Two representative siliques were photographed for each plant genotype. *C*, Altered floral structure. Floral structures were compared by scanning electron microscopy. Parts of sepals and petals were removed to visualize the internal structures of flowers.



Supplemental Figure 2. Activation of *AHL22* gene in OE-*AHL22* mutant.
A, Genomic Southern blot hybridization. Genomic DNA was digested with either BamHI or EcoRI. The gel blot was probed with ^{32}P-labeled 35S enhancer sequence. kbp, kilobase pair. The result showed that there was a single T-DNA insertion event in OE-*AHL22* mutant.
B, Activation of *AHL22* gene in OE-*AHL22* mutant. The At2g45420 gene adjacent to the *AHL22* gene locus was included in the assays. A tubulin gene (*Tub*) was used as control for constitutive expression. Transcript levels were compared by semi-quantitative RT-PCR.



Supplemental Figure 3. Molecular characterization of OE-*AHL22* mutant.
Transcript levels were compared by RT-PCR-based Southern blot hybridization. A tubulin gene (*Tub*) was included as control for constitutive expression.
A, Expression of flowering time genes in OE-*AHL22* mutant. Whole plants grown on ½ X Murashige & Skoog (MS)-agar plates (hereafter, referred to as MS-agar plates) for 2 weeks under long days (16-h light/8-h dark) were used for extraction of total RNA.
B, Temporal expression patterns of *AHL22* and *FT* genes. Plants were grown in soil for up to 35 days after germination (DAG). Temporal expression patterns of the *AHL22* and *FT* genes were compared.

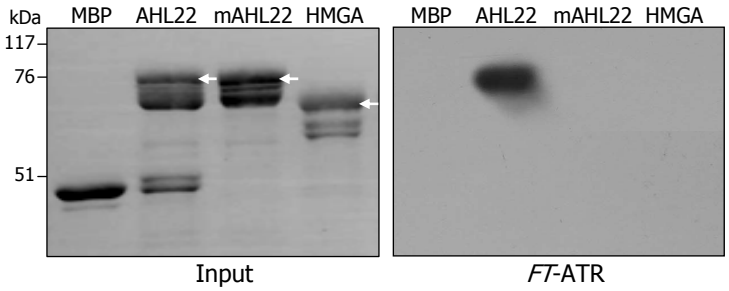


Supplemental Figure 4. Chromatin immunoprecipitation (ChIP) assays on AHL22 binding to intergenic and intragenic ATRs of *AP1* and *LFY* loci.

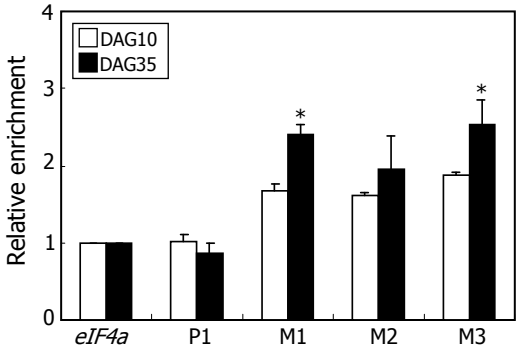
A, Prediction of ATRs in *AP1* and *LFY* loci.

Black bars indicate exons, and white bars indicate untranslated regions. Potential matrix attachment regions (MARs), which are characterized by stretches of AT-rich sequences (ATRs) [Rudd, S., Frisch, M., Grote, K., Meyers, B. C., Mayer, K., and Werner, T. (2004) *Plant Physiol*. **135**, 715-722], were predicted using the SMARTest software (http://www.genomatrix.de). One intragenic ATR was predicted in *AP1* locus as in *FT* locus. The intragenic *AP1*-ATR includes parts of exon 1 and intron 1 and consists of 945 nucleotides. Note that there is no intragenic ATR in *LFY* locus. b, bases. kb, kilobases. One intergenic ATR, which is closest to the transcriptional start site, was indicated for each of *AP1* and *LFY* loci. Sequence regions, marked by A – D, were chosen for ChIP assays.

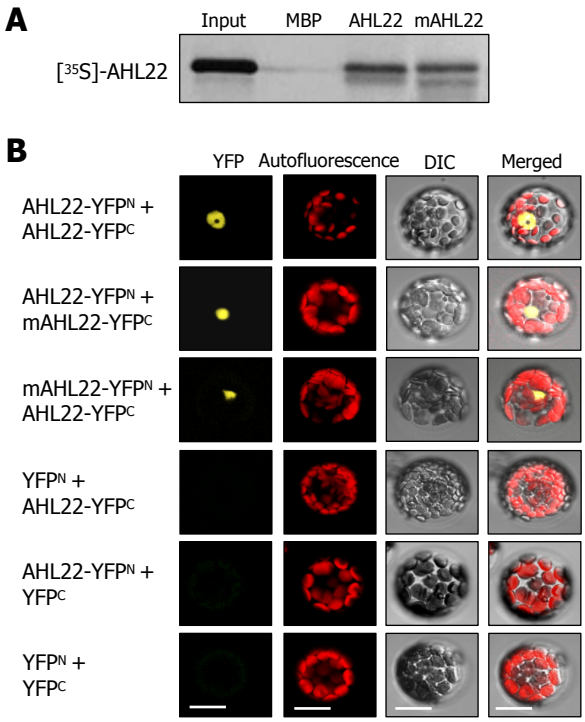
B, ChIP assays on AHL22 binding to *AP1 and LFY* ATRs. The 35S:*MYC-AHL22* transgenic plants grown on MS-agar plates for 35 days were used. Primer pairs specific to the sequences (A – D) were used. Three measurements were averaged and statistically treated. Different letters represent significant difference at $P<0.05$ (one-way ANOVA with Fisher's post hoc test). Bars indicate standard error of the mean.



Supplemental Figure 5. *in vitro* binding assays on HMGA binding to *FT*-ATR.
Recombinant high mobility group A (HMGA) protein (At1g14900) was prepared as maltose binding protein (MBP) fusion in *Escherichia coli* cells in a similar manner with recombinant MBP-AHL22 fusion (*left panel*). White arrows indicate recombinant full-size MBP or MBP fusion proteins. The same amounts of proteins shown on the protein gel and ^{32}P-labelled DNA fragments were used in the *in vitro* binding assays (*right panel*). The mutated AHL22 protein (mAHL22) was also included in the assays. kDa, kilodalton. Note that recombinant HMGA protein does not bind to *FT*-ATR.



Supplemental Figure 6. ChIP assays on kinetic binding of AHL22 to *FT*-ATR.
The 35S:*MYC-AHL22* transgenic plants that overexpress the *MYC-AHL22* gene fusion under the control of the Cauliflower Mosaic Virus (CaMV) 35S promoter, in which a MYC-coding sequence was fused in-frame to the 5' end of the *AHL22* gene, were grown on MS-agar plates under long days, and aerial plant parts were harvested 10 and 35 days after germination (DAG10 and DAG35, respectively). Primer pairs specific to M1, M2, and M3 sequences were used. Three measurements were averaged for each assay and statistically treated (*t*-test, *P<0.01). Bars indicate standard error of the mean. Note that relative enrichments of the *FT*-ATR sequence are higher 35 days after germination (DAG35) than at DAG10 in the M1 and M3 sequences.



Supplemental Figure 7. AHL22-AHL22 interactions *in vitro* and in *Arabidopsis* protoplasts.
A, *in vitro* pull-down assays. *in vitro* translated, [^{35}S]methionine-labeled AHL22 polypeptides were pulled down with recombinant MBP-AHL22 fusion protein prepared in *E. coli* cells. mAHL22, in which the core sequence of the AT-hook motif (RGRP) was mutated to RGAA, was similarly prepared as MBP-mAHL22 fusion in *E. coli* cells. Input represents 5% of the protein sample used in each assay.
B, Bimolecular fluorescence complementation (BiFC) assays in *Arabidopsis* protoplasts. The cYFP and nYFP fusions were cotransformed into *Arabidopsis* protoplasts and visualized by differential interference contrast microscopy (DIC) and fluorescence microscopy. *Scale bar*, 10 μm.

Supplemental Table 1. Primers used in this study.

Primers for RT-PCR

Primers	Sequences
TUB-F	5′-TCACCTTCTTCATCGCAGTT
TUB-R	5′-ATTTGCACCGTACTTTGACT
eIF4a-F	5′-TACTGGGAAAACAGCAACTT
eIF4a-R	5′-TGATCTCAAGAGCTTCTGGT
CO-F	5′-ATCAGCGAGTTCCAATTCTA
CO-R	5′-GGAACCATTGTCGTTGTAGT
SOC1-F	5′-CTGAGGCATACTAAGGTCG
SOC1-R	5′-GAACAAGGTAACCCAATGAA
FT-F	5′-AGACGTTCTTGATCCGTTTA
FT-R	5′-GTAGATCTCAGCAAACTCGC
FCA-F	5′-GTTTCATCTTCTGCCACATT
FCA-R	5′-TAAATTTTGGTTTGGTTGCT
FVE-F	5′-GTTGTTTGATCGTAGGAAGC
FVE-R	5′-AACATGCGACTTGAACTTCT
FLC-F	5′-AGCGAATTGAGAACAAAAG
FLC-R	5′-GCTCCCACATGATGATTATT
SPY-F	5′-AACTACCGCTGAATAAACCA
SPY-R	5′-GGTGAACTCTGTTTTTGAGC
RGA-F	5′-TCCATTACTCTCCTCCACAC
RGA-R	5′-CAAGAAGGGTTATCTGAGGAA
LFY-F	5′-TTGAAGCTTCTTCGTCTAGG
LFY-R	5′-ATGAGCCCTAAAGAGCTTCAGAATCTG
AP1-F	5′-GCGGCGAAGCAGCCAAGGTTGCAGTTG
AP1-R	5′-CTAAGAACAAACCAAAACCG
AHL22-F	5′-CTAAGAACAAACCAAAACCG
AHL22-R	5′-GCAACCTTGTATAACGAC
At2g45420-F	5′-CTCAAGAGGTTCTCATCAGCAGTA
At2g45420-R	5′-CTTGGAGGTCATGACTGTTT

Primers for qRT-PCR and ChIP

Primers	Sequences
eIF4a-real-F	5′-TGACCACACAGTCTCTGCAA
eIF4a-real-R	5′-ACCAGGGAGACTTGTTGGAC
FT-M1-F	5′-GACATGTAGCTACTACCTTTTTTCTATTCA
FT-M1-R	5′-CAATGGAGATATTCTCGGAGGTG
FT-M2-F	5′-GTTTGTGCACTAACTCAACTCTTTAATTA
FT-M2-R	5′-ATTTTATATGTCTCCTTCTATTAAATGTAAAATG
FT-M3-F	5′-CAACTTCGAGAGTGCGATGC
FT-M3-R	5′-GACCACTTTAAAGTGAAAAAAACAAAT
FT-P1-F	5′-CCGAGTTAATGCAAATCCGA
FT-P1-R	5′-GAACGTCTCCAACAACTCTGCT
AHL22-real-F	5′-TCCCAACGAGCACTCTTCAG
AHL22-real-R	5′-GTGATTATCTCCTCCTCCTCCG
FT-real-F	5′-AGACGTTCTTGATCCGTTTA
FT-real-R	5′-GGTTGCTAGGACTTGGAACATC
AP1-A-F	5′-TGATGAAACAATAATACCGTAAGCA
AP1-A-R	5′-TGGTGTTTTCCACGTGTCTTC
AP1-B-F	5′-CCATTTTTGGATTTTTTGATTAGC
AP1-B-R	5′-GATTAAACATACACCCTTCTATATGCTC
LFY-C-F	5′-CAGTCTCTCAGAACTTCGATTTGAC
LFY-C-R	5′-GAACTAAAAATACAAATTAAAGTGTGGG
LFY-D-F	5′-GATTATGGATCCTGAAGGTTTCACG
LFY-D-R	5′-AAGCAGCCGTCTGCGGTGT

F, forward primer; R, reverse primer.