

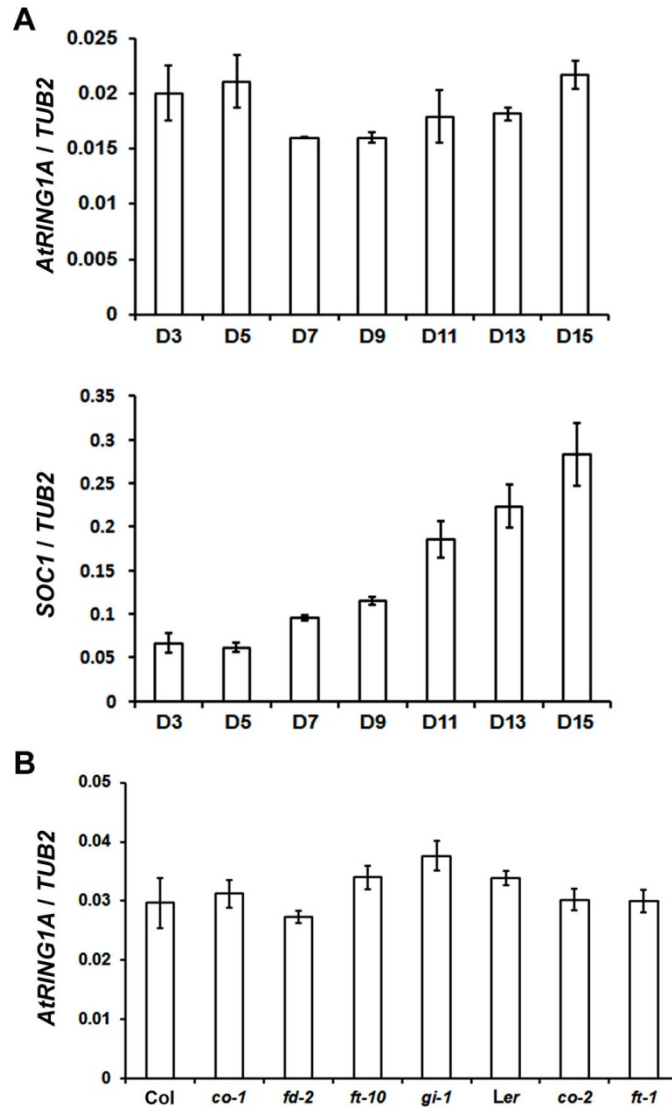
**Fig. S1.** *Atring1b-2*, *Atbmi1a-1* and *Atbmi1b* exhibit similar flowering time to wild-type plants under long days and short days. Values were scored from at least 15 plants of each genotype. Error bars indicate s.d.

***AtRING1A:GUS***

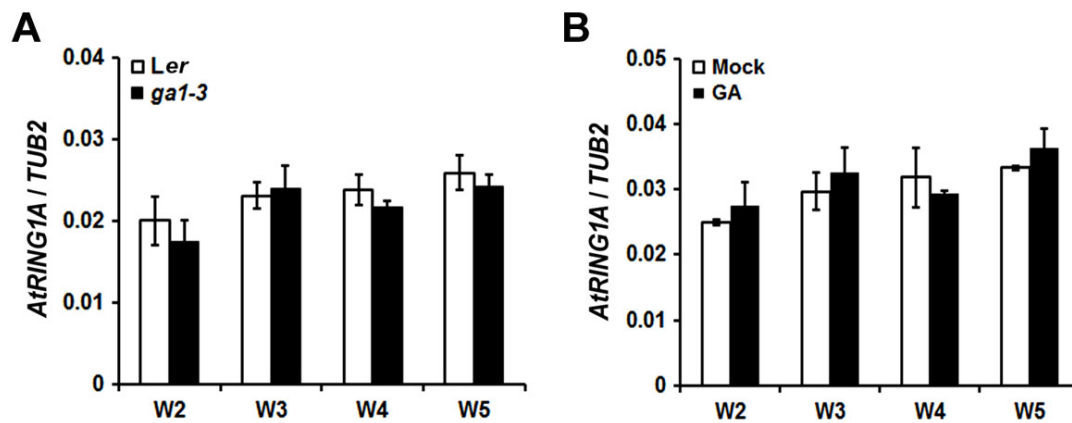


**15-day-old**

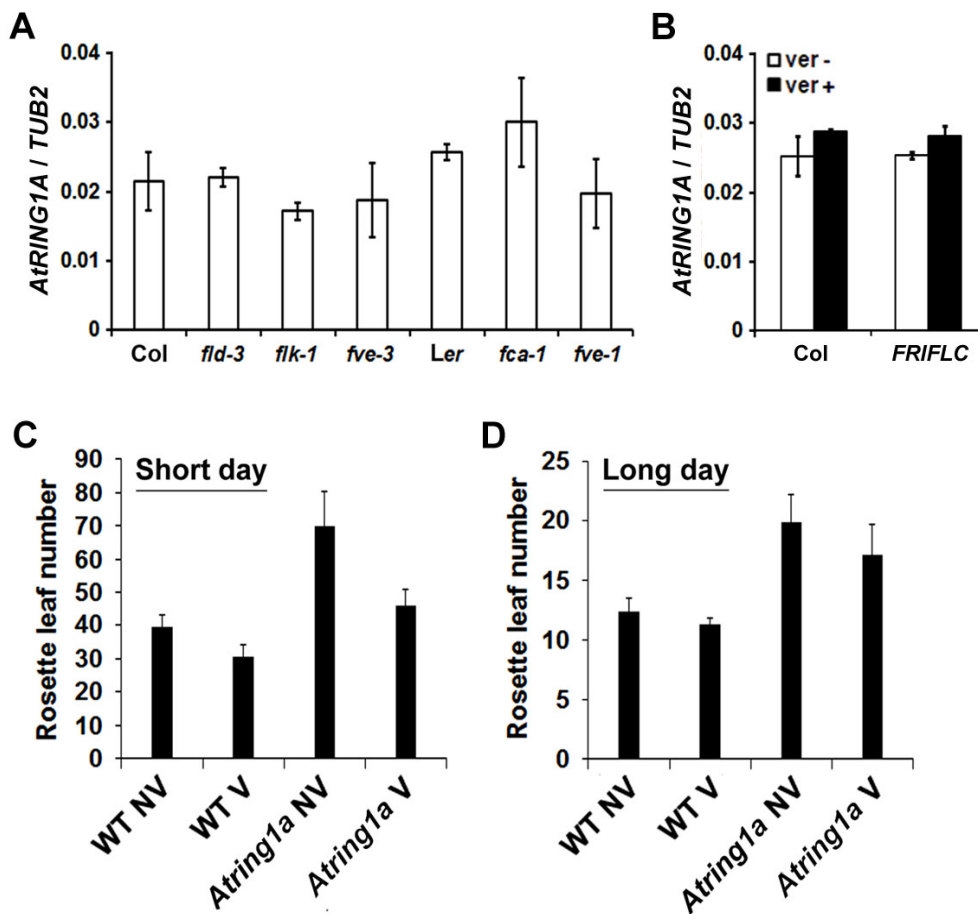
**Fig. S2. GUS staining of a 15-day-old *AtRING1A:GUS* seedling. Bar = 1 mm.**



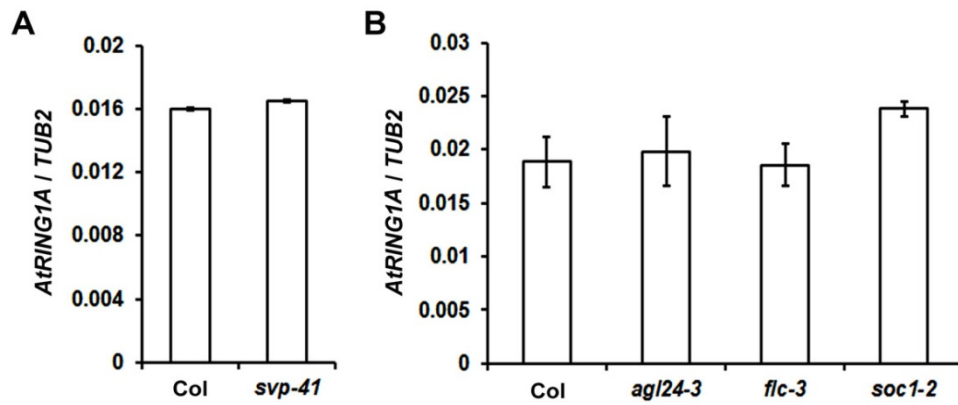
**Fig. S3. *AtRING1A* expression is not affected by the photoperiod pathway. (A)** Temporal expression of *AtRING1A* determined by quantitative real-time PCR in wild-type seedlings grown under LDs (upper panel). The expression of *SOC1*, which is regulated by the photoperiod pathway, was examined as a positive control (lower panel). Error bars indicate s.d. **(B)** *AtRING1A* expression determined by quantitative real-time PCR in 9-day-old mutants of the photoperiod pathway. *AtRING1A* expression was normalized to *TUB2* expression. Error bars indicate s.d.



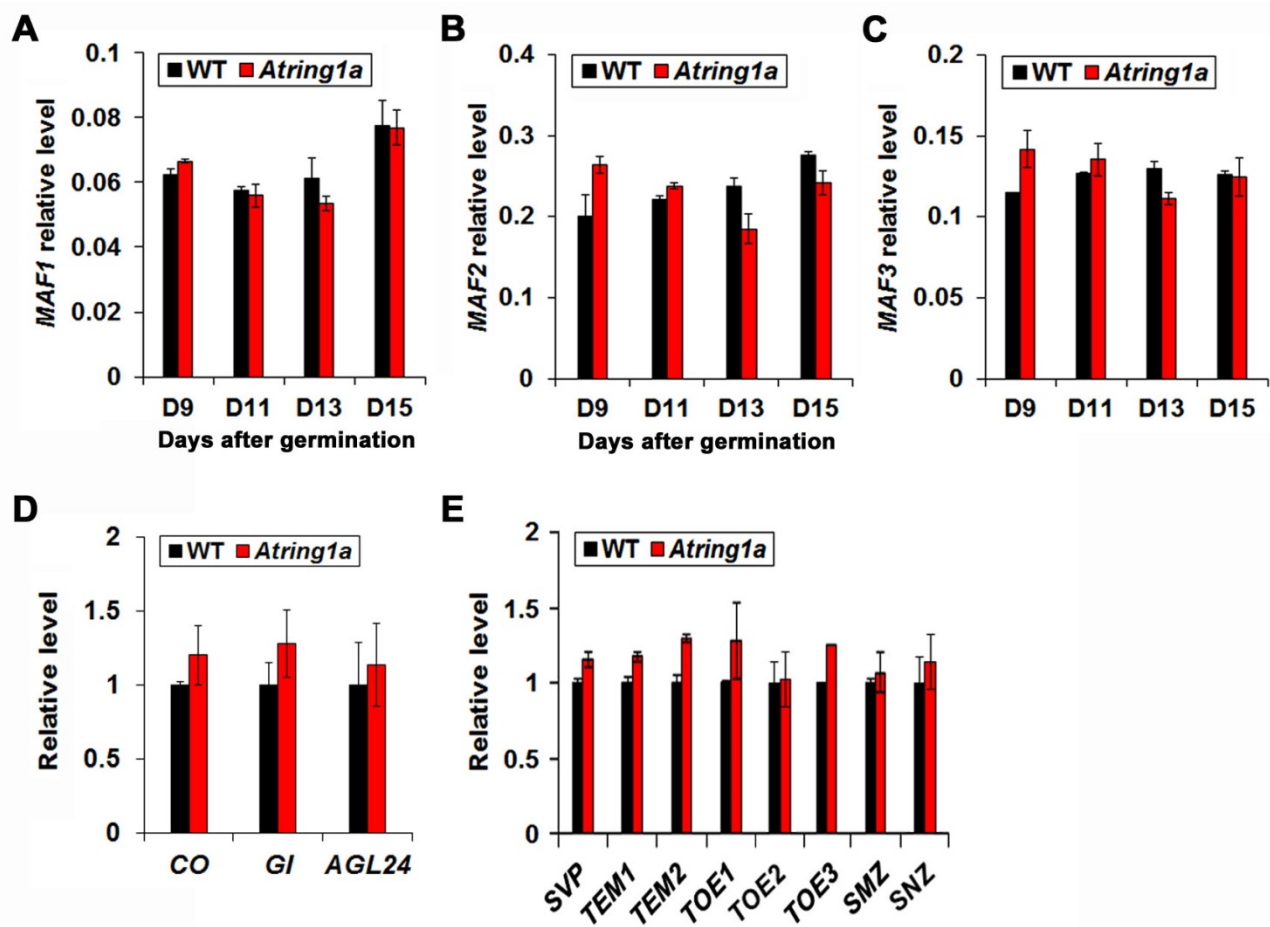
**Fig. S4. *AtRING1A* expression is not affected by the GA pathway.** (A) Comparison of *AtRING1A* expression in GA-deficient mutant *gal-3* and wild-type plants. Seedlings grown under SDs from week 2 (W2) to week 5 (W5) were collected for expression analysis. Error bars indicate s.d. (B) Effect of GA treatment on *AtRING1A* expression in wild-type plants grown under SDs. Exogenous GA (100  $\mu$ M) or 0.1% ethanol (mock) was applied weekly onto wild-type Col plants grown under SDs. Seedlings treated from week 2 (W2) to week 5 (W5) were collected for expression analysis. *AtRING1A* expression was normalized to *TUB2* expression. Error bars indicate s.d.



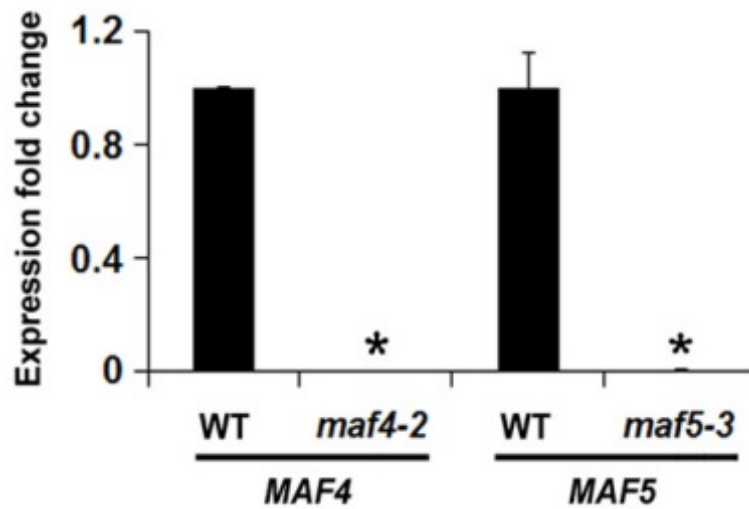
**Fig. S5. *AtRING1A* expression is not regulated by the autonomous and vernalization pathways.** (A) *AtRING1A* expression in 9-day-old mutants of the autonomous pathway grown under LDs. (B) Effect of vernalization treatment on *AtRING1A* expression. For vernalization treatment, seeds were sown on MS medium and vernalized at 4°C under low light condition for 8 weeks. The 9-day-old seedlings grown under LDs were harvested for expression analysis. *AtRING1A* expression in (A) and (B) was examined by quantitative real-time PCR, and normalized to *TUB2* expression. Error bars indicate s.d. (C,D) Flowering time of *Atring1a* and wild-type plants with (V) and without (NV) vernalization treatment grown under SDs (C) and LDs (D). After vernalization treatment, the seedlings were transferred to soil and grown under SDs or LDs. Values were scored from at least 15 plants of each genotype. Error bars indicate s.d.



**Fig. S6. *SVP*, *AGL24*, *SOC1* and *FLC* do not affect *AtRING1A* expression. (A,B) *AtRING1A* expression in several flowering time mutants. *AtRING1A* expression was examined by quantitative real-time PCR in 7-day-old wild-type and *svp-41* seedlings (A), and 9-day-old wild-type and several other mutant seedlings (B) grown under LDs. *AtRING1A* expression was normalized to *TUB2* expression. Error bars indicate s.d.**

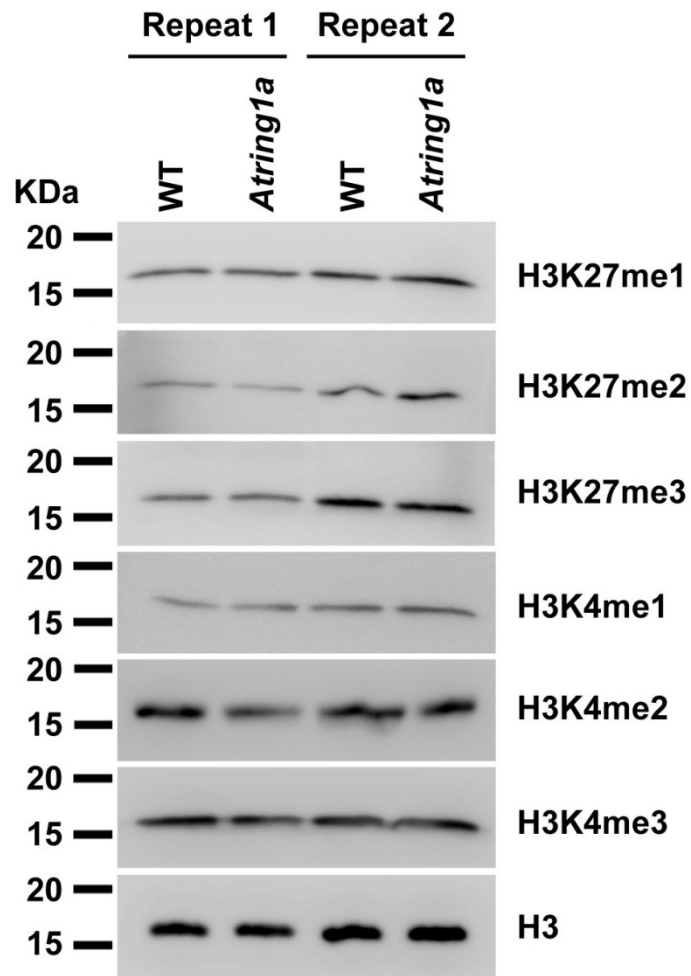


**Fig. S7. Expression of *MAF1*, *MAF2*, *MAF3*, and other important floral repressors is not regulated by *AtRING1A*.** (A-C) Temporal expression of *MAF1* (A), *MAF2* (B) and *MAF3* (C) determined by quantitative real-time PCR in developing *Atring1a* and wild-type seedlings grown under LDs. Gene expression was normalized to *TUB2* expression. (D,E) Expression of *CO*, *GI* and *AGL24* (D), and *SVP*, *TEM1*, *TEM2*, *TOE1*, *TOE2*, *TOE3*, *SMZ* and *SNZ* (E) determined by real-time PCR in 9-day-old *Atring1a* and wild-type seedlings grown under LDs. Gene expression was normalized to *TUB2* expression, and expression levels in wild-type seedlings are all set as 1. Error bars indicate s.d.

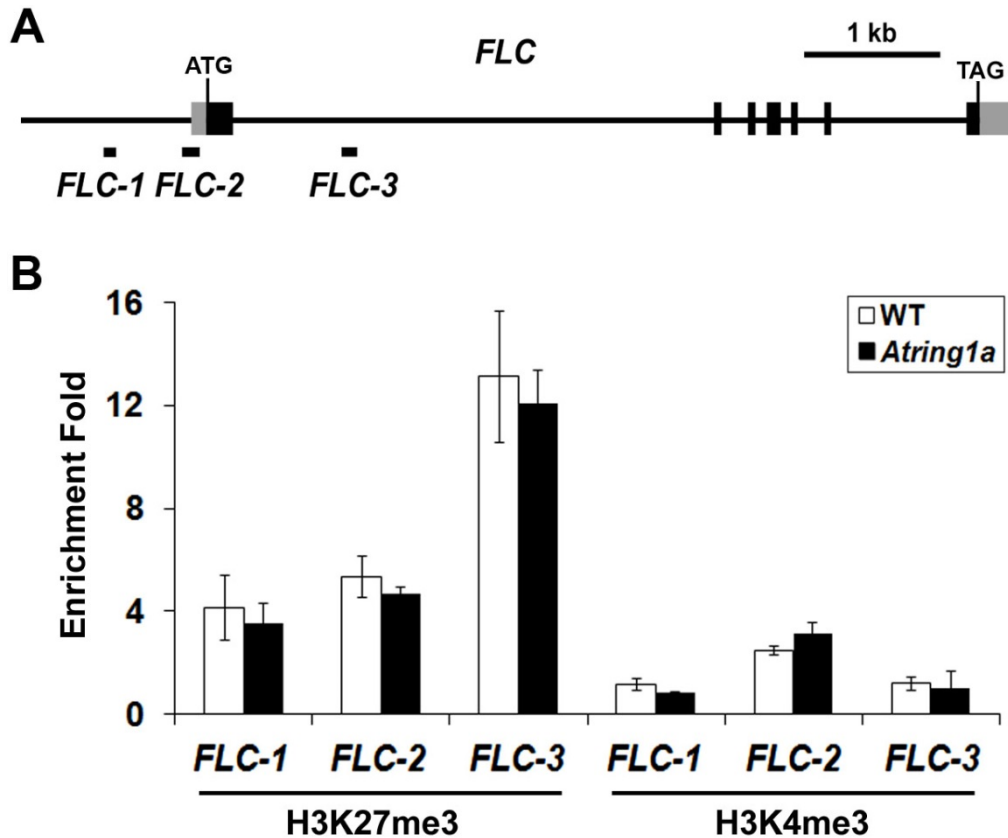


**Fig. S8. Expression of *MAF4* and *MAF5* is undetectable in *maf4-2* and *maf5-3*, respectively.** Gene expression was determined by quantitative real-time PCR in 9-day-old wild-type and mutant plants. Results were normalized against the expression levels of *TUB2*. Asterisks indicate that quantitative real-time PCR analysis of *MAF4* and *MAF5* in *maf4-2* and *maf5-3* obtains very high Ct values, respectively, because of their barely detectable levels.

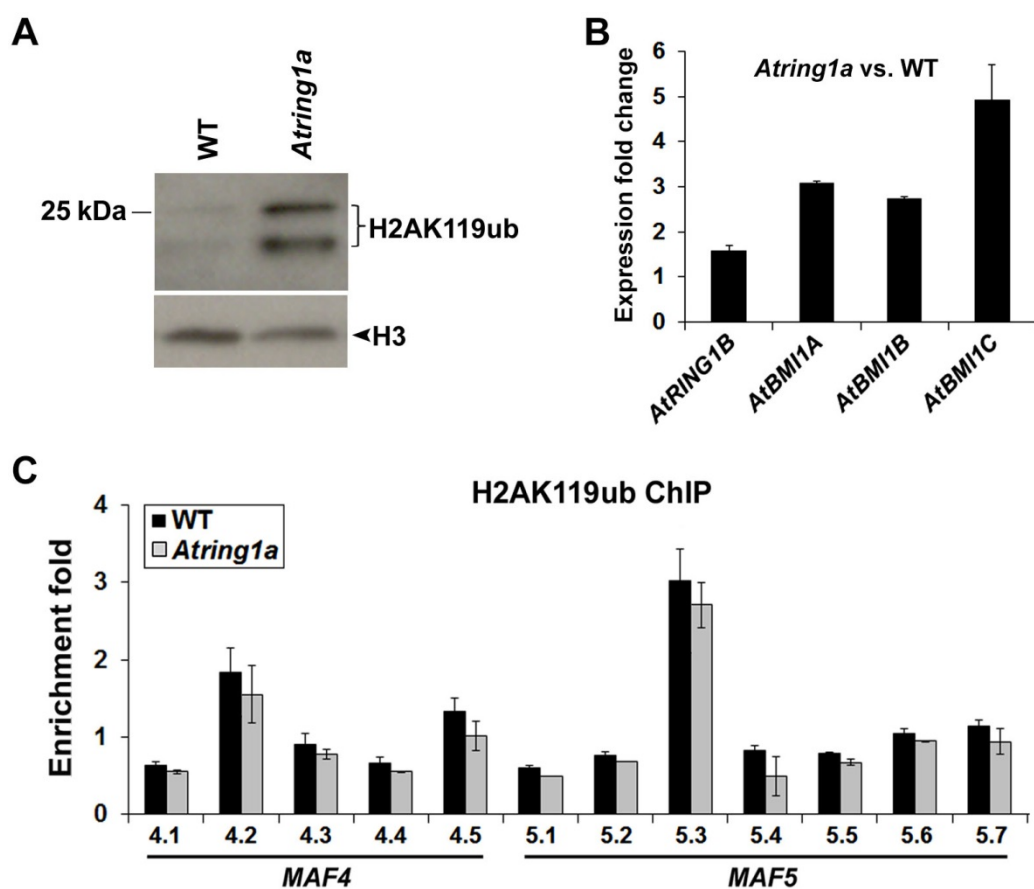




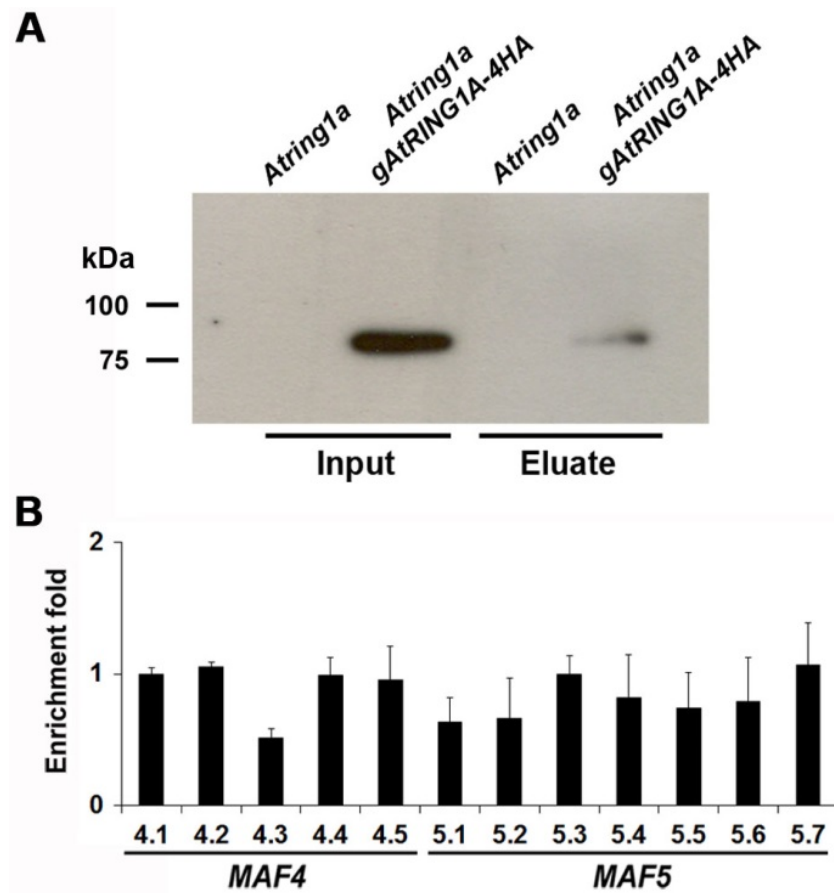
**Fig. S9. Analysis of global H3K27 and H3K4 methylation levels in wild-type and *Atring1a* plants by immunoblotting.** Nuclear extracts of 9-day-old *Atring1a* and wild-type seedlings were subjected to Western blot analysis using various antibodies.



**Fig. S10. *AtRING1A* does not obviously affect H3K27me3 and H3K4me3 enrichment at *FLC*.** (A) Schematic diagram of the *FLC* genomic region. Exons and untranslated regions are represented by black and grey boxes, respectively, while introns and other genomic regions are represented by black lines. The translation start site (ATG) and stop codon (TAG) are indicated. DNA fragments amplified in ChIP assays are indicated below the *FLC* genomic region that carries both H3K27me3 and H3K4me3 marks. (B) ChIP analysis of H3K27me3 and H3K4me3 levels at *FLC* in 9-day-old wild-type and *Atring1a* seedlings. Error bars indicate s.d. of three biological replicates.



**Fig. S11. *AtRING1A* does not significantly affect H2AK119ub levels at *MAF4* and *MAF5*.** (A) Analysis of global H2AK119ub levels in wild-type and *Atring1a* plants by immunoblotting. Nuclear extracts of 9-day-old *Atring1a* and wild-type seedlings were subjected to Western blot analysis using anti-H2AK119ub and anti-H3 antibodies. The different bands shown in the blot indicate different H2Aub isoforms. (B) Quantitative real-time PCR analysis of expression of *AtRING1B*, *AtBM11A*, *AtBM11B* and *AtBM11C* in 9-day-old *Atring1a* and wild-type seedlings grown under LDs. Results were normalized against the expression levels of *TUB2*. Gene expression levels in wild-type seedlings are all set as 1. (C) ChIP analysis of H2AK119ub levels at *MAF4* and *MAF5* in 9-day-old wild-type and *Atring1a* seedlings. A genomic fragment of *ACTIN7* (*At5g09810*) was amplified as an internal control for measurement of H2AK119ub enrichment. Error bars indicate s.d of three biological replicates. There is no statistically significant difference in ChIP enrichment fold between wild-type and *Atring1a*.



**Fig. S12. AtRING1A is not associated directly with *MAF4* and *MAF5* genomic regions.** (A) Western blot analysis using anti-HA antibody shows the expression of AtRING1A-4HA in nuclear extracts (Input) or immunoprecipitated fractions (Eluate) of 9-day-old *Atring1a gAtRING1A-4HA* seedlings. (B) ChIP analysis shows no significant binding of AtRING1A-4HA to *MAF4* and *MAF5* genomic regions. Enrichment fold was calculated first by normalizing the amount of a target DNA fragment against a genomic fragment of *ACTIN7*, and then by normalizing the value for *Atring1a gAtRING1A-4HA* against that for *Atring1a*. Error bars indicate s.d. of three biological replicates.

**Table S1. Primers used in this study****Primers pairs used for plasmid construction**

<b>Primer name</b>	<b>Primers</b>
gAtRING1A-F	5'-CACCTAACTCAGCAGGACAAGGAGG-3'
gAtRING1A-R	5'-CTGTAAAAAAGTAAAAAAGACTAAGC-3'
gAtRING1A-4HA-F	5'-ACCGGAAGAAGCAAACCTGAGTATCCATATGACGTTCCAGA-3'
gAtRING1A-4HA-R	5'-TTAGGCTCCAAGTTTCTTCATCTAGTAGCGTAATCTGGAA-3'
gAtRING1A-F (EcoRI)	5'-CGGAATTCTAACTCAGCAGGACAAGGAGG-3'
gAtRING1A-R (BamHI)	5'-CGGGATCCCTCAGTTTGCTTCTTCCGGTA-3'

**Primers pairs used for gene expression analysis (quantitative real-time PCR)**

<b>Gene name</b>	<b>Primers</b>
<i>SOC1</i>	5'-AGCTGCAGAAAACGAGAAGCTCTCTG-3' 5'-GGGCTACTCTCTTCATCACCTCTTCC-3'
<i>FT</i>	5'-CTTGGCAGGCAAACAGTGTATGCAC-3' 5'-GCCACTCTCCCTCTGACAATTGTAGA-3'
<i>FLC</i>	5'-CTAGCCAGATGGAGAATAATCATCATG-3' 5'-TTAAGGTGGCTAATTAAGTAGTGGGAG-3'
<i>SVP</i>	5'-CAAGGACTTGACATTGAAGAGCTTCA-3' 5'-CTGATCTCACTCATAATCTTGTCAC-3'
<i>AGL24</i>	5'-GAGGCTTTGGAGACAGAGTCGGTGA-3' 5'-AGATGGAAGCCCAAGCTTCAGGGAA-3'
<i>CO</i>	5'-TCAGGGACTCACTACAACGACAATGG-3' 5'-TTGGGTGTGAAGCTGTTGTGACACAT-3'
<i>GI</i>	5'-GGGTAAATATGCTGCTGGAGA-3' 5'-CAGTATGACACCAGCTCCATT-3'
<i>MAF1</i>	5'-GGCATAACCCTTATCGGAGATTTGAAGCCA-3' 5'-CTTTGTGCGATGAGACCATTGCGTCGTTTG-3'
<i>MAF2</i>	5'-AACTCGGAATTATCTGCCACTCAAAG-3' 5'-CTTCCCCATCATTAGTTCTGTCTTC-3'

<i>MAF3</i>	5'-GAAAGGGAGAAGTTGCTGATAGAAGAG-3' 5'-AGCACAAGAACTCTGATATTTGTCTAC-3'
<i>MAF4</i>	5'-TGGCCAAGATCCTCAGTCGTTATGA-3' 5'-GCTGCTCTTCCAGGGACTTTAGACA-3'
<i>MAF5</i>	5'-GATGGAGCTTGTGAAGAACCTTCAGG-3' 5'-CAGCCGTTGATGATTGGTGGTTACTTG-3'
<i>TEM1</i>	5'-ATCCACTGGAAAGTCCGGTCTA-3' 5'-GAATAGCCTAACCACAGTCTGAACC-3'
<i>TEM2</i>	5'-TGGTCCGAGAGAAAACCCG-3' 5'-TCAACTCCGAAAAGCCGAAC-3'
<i>TOE1</i>	5'-CAGCGTGGAGTTAGCTTGAGG-3' 5'-CGTTCCAGTAAAGGCGATGATCC-3'
<i>TOE2</i>	5'-ATGGAGAACCACATGGCTGC-3' 5'-GGTGCTGTAGCTGCTACGGC-3'
<i>TOE3</i>	5'-GATCTTAGCTCAGAGACGACGAG-3' 5'-CATTGCTAGCGATAGATCGCTC-3'
<i>SMZ</i>	5'-AGGGAGAAGGAGCCATGAAGTTTGGTG-3' 5'-GTCTTCAGAGGTTTCATGGTTGCCATG-3'
<i>SNZ</i>	5'-CAGCAGATTATTACATGGGTTTG-3' 5'-GGTTTAATTTCTGTGATCGGTAGA-3'
<i>AtRING1A</i>	5'-ATCTCTGTTGCCGACCCACT-3' 5'-GCCGCATCTTCTCCTACTCT-3'
<i>TUB2</i>	5'-ATCCGTGAAGAGTACCCAGAT-3' 5'-AAGAACCATGCACTCATCAGC-3'

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**Primers pairs used for gene expression analysis (semi-quantitative PCR)**

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Gene name	Primers
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<i>AtRING1A</i>	5'-CCATCTTCTATATCTGGAGACC-3' 5'-GTGTTGAACGACTTGTAGACCG-3'
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<i>TUB2</i>	5'-ATCCGTGAAGAGTACCCAGAT-3' 5'-TCACCTTCTTCATCCGCAGTT-3'
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**Primers pairs used for ChIP assays (quantitative real-time PCR)**

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Product name	Primers
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<i>FLC-1</i>	5'-CAAGCTGATACAAGCATTTACCAA-3' 5'-TTGAGCTATTGCCATATGTGTGGACA-3'
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*FLC-2* 5'-CCGACGAAGAAAAAGTAGATAGGCAC-3'  
 5'-CCCAAACCTGAGGATCAAATTAGG-3'

*FLC-3* 5'-CTTTGAATCACAATCGTCGTGTG-3'  
 5'-ACGTGCATATACAAATCCAAGAGAAC-3'

*MAF3 (3.1)* 5'-GTCTAGCCCAAAGAAGAAGATAGAAACG-3'  
 5'-GGAGGCAGAGTCGTAGAGTTTTCC-3'

*MAF4 (4.1)* 5'-CCATAATTTAAATATGGTGGCCCA-3'  
 5'-AGCCGAACCAAATTTCAAACC-3'

*MAF4 (4.2)* 5'-CGGCGAGTTATGCAGACATCACA-3'  
 5'-GTGGCAGAGATGATGATAAGAGCGA-3'

*MAF4 (4.3)* 5'-AGGGTCTATAGACTGGAACAGATGC-3'  
 5'-GCTAGCTAGAACCCTTTTCCTTAAGC-3'

*MAF4 (4.4)* 5'-GCTAGTTTCTTGGTAGCTCGGCTG-3'  
 5'-CATTCTTACTTCGTGTCGTCTGTGATC-3'

*MAF4 (4.5)* 5'-ATTCTTGAATCCTCTGAAACTCCG-3'  
 5'-TGGACACCATCACAACCTTTATTCAG-3'

*MAF5 (5.1)* 5'-TACTGTTAAGCCCAGATTCGGC-3'  
 5'-ATTGATGTCAATCGCGTACCCT-3'

*MAF5 (5.2)* 5'-GTTTCTCATACAGCCCAATACATGC-3'  
 5'-GATTGGATTTAGTTCATTCCACCG-3'

*MAF5 (5.3)* 5'-CAGGATCTCCGACCAGTTTATACAGAC-3'  
 5'-GAGGAGTTGTAGAGTTTGCCGGT-3'

*MAF5 (5.4)* 5'-CGTGGTGGTAATCCGTAATTCATGT-3'  
 5'-CAAATGGCACTCGTTTCCACTAGA-3'

*MAF5 (5.5)* 5'-GTGTTTTTCGCTTGAGATTGTGGT-3'  
 5'-CGTGATGTCCGTGATCTATTGC-3'

*MAF5 (5.6)* 5'-GAAAGAGAAAATTGTGTCCTGGAAA-3'  
 5'-CTCTATTGAATTGTTAGTTGTTCCGC-3'

*MAF5 (5.7)* 5'-CTACACACTTTCTGGTGAAACCC-3'  
 5'-CAGTTCTTAAAATGATCTTTTCATGTG-3'

*TUB2* 5'-ATCCGTGAAGAGTACCCAGAT-3'  
 5'-AAGAACCATGCACTCATCAGC-3'

*ACT7* 5'-CGTTTCGCTTTCCTTAGTGTTAGCT-3'  
 5'-AGCGAACGGATCTAGAGACTCACCTTG-3'

*MU*

5'-TTACAAGGAATCTGTTGGTGGT-3'  
5'-AACATAGGTTTAGAGCATCTGC-3'

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