## **Supporting Information**

## Shibuya et al. 10.1073/pnas.0809294106

DN A C

<



Fig. S1. Detection of small RNAs in transgenic plants. Enriched small RNAs are hybridized with the <sup>32</sup>P-labeled sense RNA probe transcribed from the 1701–1800 sequence of *pMADS3* intron 2. EtBr, ethidium bromide stained low-molecular-weight RNAs as a control for equal loading.



**Fig. 52.** Histone modifications at *pMADS3* locus of transformant plants. (*A*) Schematic of *pMADS3* locus showing the regions analyzed by ChIP. The *pMADS3* exons are indicated by boxes (filled, coding; open, noncoding). Bars (a– c) represent the regions analyzed by PCR after ChIP. The thick bar above intron 2 shows the region targeted by IR in V016 plants. (*B*) ChIP analysis with antibodies to acetylated H3, H3K4me3, H3K9me2, H3K27me1, H3K27me3, H3K36me2, and normal mouse IgG (NM IgG) as a negative control in wild-type (WT) and V016 plants. A euchromatin control (EF: elongation factor) reacted with antibodies to acetylated H3 and H3K4me3, and weakly with that to H3K27me1. A heterochromatin control (PVCV: petunia vein-clearing virus) reacted with antibodies to H3K9me2 and H3K27me1, and weakly with that to H3K4me3. Petals from floral buds (1–3 cm in length) were crosslinked with 0.1% formaldehyde and the chromatin fraction was extracted with EpiQuik plant ChIP kit (Epigentek). Immunoprecipitation was performed with antibodies to H3K9me2 (Upstate no. 06-599), H3K4me3 (Abcam no. ab8580), H3K9me2 (Upstate no. 07-441), H3K27me1 (Upstate no. 07-448), and H3K36me2 (Upstate no. 07-369) by using EpiQuik plant ChIP kit (Epigentek) according to the manufacturer's instruction. The primers used in PCR for *pMADS3* intron2 are MI-1479F-ChIP (5'-ATTGGCTGCTGAAAATGGAC-3') and MI-1829R-ChIP (5'-AAAGAACCAAACACAGGAATGA-3'); those for *EF* are EF-77F-ChIP (5'-CAACAAGCAACGAACGAACGAACTGAAC-3') and PVCVb-333R (5'-TCACATGCCCATTGTGAATC-3').

1701	1800
pMADS3 GAAACAG-TGGACCAATCATATTGTGGGAACTTGTGCCAG	CTIGGCAGAGAGCAGCTAATCAGTAGCTCGACGAAATTAAGGTTGTAAGTAGACTAGGAAA
CUM1 CGATTGGA-TTAAGCCAATCATAAAGC-GAGATCTGTGCCAGC	ATGTCTCAG-ACAGCCAATCACCAGCTCGACGAAATTAAGGTATCAG—TGCATCGGT
PPAG TAATATGG-TTGAACCAATCATATTG-GGGAGCTGCGCCATCG	TGGCACAG-GCAACCAATCACAAGGTCGAGGATTTTAAGGTATCAGATGCCATTGGAAA
PTAG1 TGATCTAC—CTTGGCCAATCATATTGTAGGATTCAGTGACAGC	T TGGCAGAG-ACAGCCAATCAATGTCTCGACGAAGTTAAGGTATAAGGAAATCTAGAAAA
PTAG2 TGATTTAG—CTTGGCCAATCATGTTATAGGACTCAGTGACAGC	T TGGCAGAG-ACAGCCAATCACTGGCTCGACGAAGTTAAGGTATCAGAAAATCTAGATAT
DP TGAATAG—TGGACCAATGGTGTTGTGGGAACTTGTGCCAGCT	TGGCAGGG-GCAGCCAATGAATAGCTCGGAGAGATTAAGGTAGCAGCAGACTAGGATAG
FAR AGATATA—TGGGCCAATCATATCACAAGATTCAGTGCCAGGT	TGGCAAGG-GCAGCCAATCAGTGCCTCGAGGAAATTAAGGTTGCTGCAAACTGAGAAAT
TAG1 TTGAAAGA—CTGGACCAGTCATGTTGTGGGAGATTGTGCCAGG	T CGGCAGAGAGCAGCCAATCAGTAGCTCGGGGAAATTAAGGTAGTAT-AGCAGAGTCAT
FcAG TGATTGTT—CCCATCCAATCATATCATGGGAAGCTGTGCCAGCC	TGGCAGTCT-TAACCAATCACTGGCTCGACGAAATTAAGGTATCAG-GGCCGGTCACT
PLE GGAATGGT—GAGAGCCAATCATATTGTGGGATCTTGTGCCGACG	TGGCAGGGGGCAGCCAATCAGTGGGTCGACGAAATTAAGGTTACAGCTTATCCTCACTG
CpPLE ATATTACT—TAG-CCAATCATATTTAGGGATTTTGTGCC-ACG	TGGCACGTTCGAACCAATCAGTGGCTCGACAAAATTAAGGTAACACCAATTCCT-CTG
CrAG TAGAGACATTGTTCAGACCAATCATGTCACTCTAATTT-TGCCAGC	CGTGGCCAGTTGCAGCCAATCAGTAGCTCGATGGATTTAAGGTTTCAGATAGGAATATATA
CbAG TAGAGACATTGTTCAGACCAATCATGTCACTCTAATTT-TGCCAG	CETGGCCAGTTGCAGCCAATCAGTAGCTCGATGGATTTAAGGTTTCAGATAGGAATATATA
AG CTAGACA-TGTTCAGACCAATCATGTCACTCTAATTT-TGCCAGCA	TGGCAGTTGGCAGCCAATCACTAGCTCGATAAATTTAAGGTTTCAGAGGAATTTTAATT
Asag TAACATA—TTCAACCAATCATGTCACTCTAATTT-TGCCAGCT	TGGC-AGCTGTAGCCAAT <mark>CAGTAG</mark> CTCGACAAATTTAAGGTTTCAGAGGTAAGGGT-TT
*** * **	** ***** *** ****
CCAATCA-1	CCAATCA-2 CG (1768)

**Fig. S3.** DNA sequence alignment of intron 2 partial sequences in *pMADS3* homologs. The alignment is generated for the sequences corresponding to the 1701–1800 region of *pMADS3* in 15 *pMADS3* homologs of 13 plant species belonging to 9 different families (see Table S1). Asterisks indicate the bases identical among all of the sequences. The TAGCTCGA motif including the CG at nt 1768 is shaded. CCAATCA-1 and CCAATCA-2 are the CCAATCA boxes reported by Hong *et al.* (23). Numbers indicate the positions in the *pMADS3* intron 2.

PNAS PNAS





DN AS

## Table S1. AG orthologs used for sequence alignment

PNAS PNAS

Gene	Species	Family	GenBank accession no.
pMADS3	Petunia x hybrida	Solanaceae	AB076051
CUM1	Cucumis sativus	Cucurbitaceae	AY254704
PPAG	Prunus persica	Rosaceae	EU072354
PTAG1	Populus balsamifera	Salicaceae	AF052570
PTAG2	Populus balsamifera	Salicaceae	AF052571
DP	Ipomoea nil	Convolvulaceae	AB281192
FAR	Antirrhinum majus	Veronicaceae	AY935268
TAG1	Lycopersicon esculentum	Solanaceae	AY254705
FcAG	Fagopyrum cymosum	Polygonaceae	AB089696
PLE	Antirrhinum majus	Veronicaceae	AY935269
CpPLE	Carica papaya	Caricaceae	EF645801
CrAG1	Capsella rubella	Brassicaceae	AY253263
CbAG1	Capsella bursa-pastoris	Brassicaceae	AY253261
AG	Arabidopsis thaliana	Brassicaceae	AL021711
AsAG	Aurinia saxatilis	Brassicaceae	AY253249

## Table S2. Primers used for generation of inverted-repeat constructs

PNAS PNAS

Constructs	Name	Sequence
V001	MADSi2RNAi-1F	CATGCCATGGGTAATCTTTTAACAAAAAAAATT
	MADSi2RNAi-1R	CGGGATCCATCGATCCCCAACAAATTTCTGGTTCT
V002	MADSi2RNAi-2F	CATGCCATGGTGGGTGGTTGGTGTAAGTTT
	MADSi2RNAi-2R	CGGGATCCATCGATCCAGATACTTTCTTACTCCATA
V003	MADSi2RNAi-3F	CATGCCATGGATTTTCATTACCTTTTGAAC
	MADSi2RNAi-3R	CGGGATCCATCGATCTATGTGTTCTTAACACCTGCTA
V004	MADSi2RNAi-4F	CATGCCATGGCTAACTACAACTCTACTGTG
	MADSi2RNAi-4R	CGGGATCCATCGATCTAGACCATAAAAATGTTAG
V005	MI2RNAi-951F	GACTAGTCCATGGTATACACTCTACTCTAGCTT
	MI2RNAi-1250R	CGGGATCCATCGATTTCTTTGCTGTTTAAGTTCC
V006	MI2RNAi-951F	GACTAGTCCATGGTATACACTCTACTCTAGCTT
	MI2RNAi-1500R	CGGGATCCATCGATCCGTCCATTTTCAGCAGCCA
V007	MI2RNAi-1251F	GACTAGTCCATGGACAGAAGTGGAAGAAAGAGA
	MI2RNAi-1700R	CGGGATCCATCGATAACGGTTCAGATCTATGCCA
V008	MI2RNAi-1501F	GACTAGTCCATGGTTGTGATGTGATCAGATGAG
	MI2RNAi-1800R	CGGGATCCATCGATTTTCCTAGTCTACTTACAAC
V009	MI2RNAi-1501F	GACTAGTCCATGGTTGTGATGTGATCAGATGAG
	MI2RNAi-2050R	CGGGATCCATCGATTAGAAAGAGTGCCTTAACCA
V010	MI2RNAi-1801F	GACTAGTCCATGGTGAGAGTCATTCCTCAGTGT
	MI2RNAi-2050R	CGGGATCCATCGATTAGAAAGAGTGCCTTAACCA
V015	MI2RNAi-1501F	GACTAGTCCATGGTTGTGATGTGATCAGATGAG
	MI2RNAi-1700R	CGGGATCCATCGATAACGGTTCAGATCTATGCCA
V016	MI2RNAi-1601F	GACTAGTCCATGGTGCCTTTGAAACAAAACCCT
	MI2RNAi-1800R	CGGGATCCATCGATTTTCCTAGTCTACTTACAAC
V017	MI2RNAi-1501F	GACTAGTCCATGGTTGTGATGTGATCAGATGAG
	MI2RNAi-1600R	CGGGATCCATCGATTTTTATCAATAATCTATAAT
V018	MI2RNAi-1601F	GACTAGTCCATGGTGCCTTTGAAACAAAACCCT
	MI2RNAi-1700R	CGGGATCCATCGATAACGGTTCAGATCTATGCCA
V019	MI2RNAi-1701F	GACTAGTCCATGGGAAACAGTGGACCAATCATA
	MI2RNAi-1800R	CGGGATCCATCGATTTTCCTAGTCTACTTACAAC
V032	MI2RNAi-1251F	GACTAGTCCATGGACAGAAGTGGAAGAAAGAGA
	MI2RNAi-1400R	CGGGATCCATCGATTCTCTTTCTCAACACCCCACCCAT
V033	MI2RNAi-1601F	GACTAGTCCATGGTGCCTTTGAAACAAAACCCT
	MI2RNAi-1767R	CGGGATCCATCGATATATATATAGCTACTGATTAGCTGCTCT
V034	MI2RNAi-1768F	GACTAGTCCATGGATATATATCGACGAAATTAAGGTTGTAA
	MI2RNAI-1900R	CGGGATCCATCGATTCAGACTCAATCTGTCTACTC