Supporting Information

Shibuya *et al*. 10.1073/pnas.0809294106

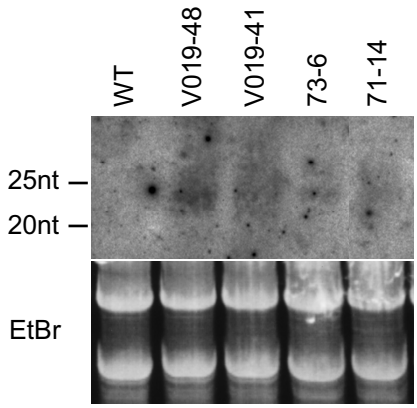

Fig. S1. Detection of small RNAs in transgenic plants. Enriched small RNAs are hybridized with the ^{32}P-labeled sense RNA probe transcribed from the 1701–1800 sequence of *pMADS3* intron 2. EtBr, ethidium bromide stained low-molecular-weight RNAs as a control for equal loading.

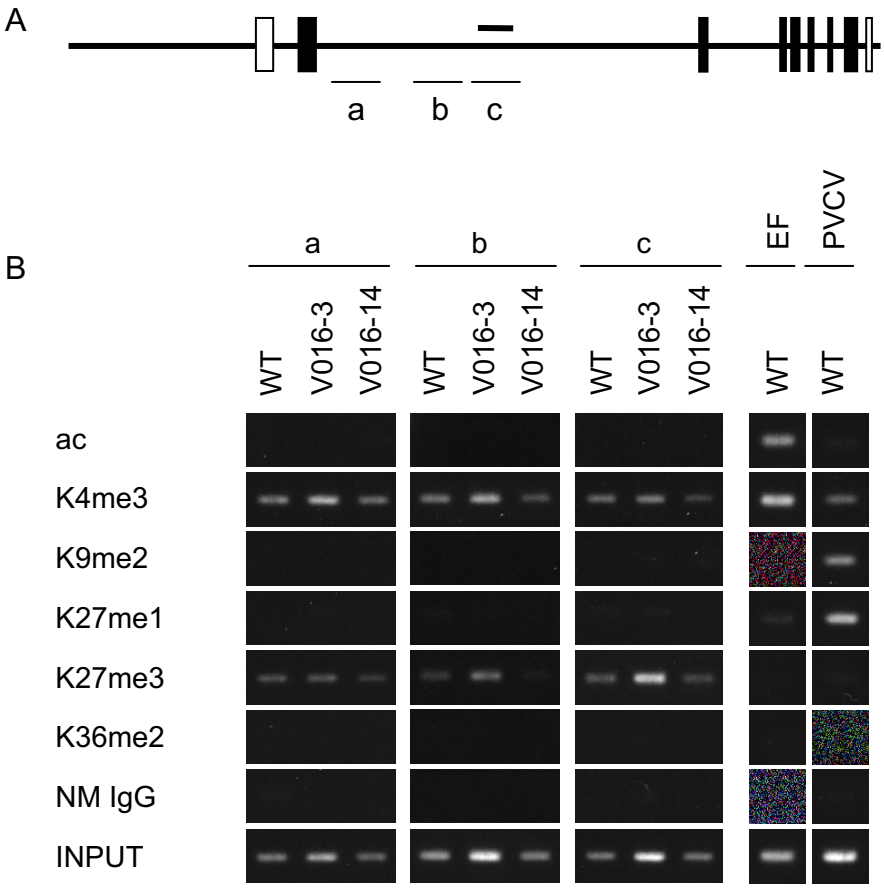

Fig. S2. Histone modifications at *pMADS3* locus of transformant plants. (*A*) Schematic of *pMADS3* locus showing the regions analyzed by ChIP. The *pMADS3* exons are indicated by boxes (filled, coding; open, noncoding). Bars (a–c) represent the regions analyzed by PCR after ChIP. The thick bar above 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 H3ac (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′-AAAGAACCAACACTGAGGAATGA-3′); those for *EF* are EF-77F-ChIP (5′-GCACTGTGATTGATGCTCCT-3′) and EF-258R-ChIP (5′-TTGTTTGACACCAAGGGTGA-3′), and those for Petunia vein-clearing virus (PVCV) are PVCVb-60F (5′-CAACAAGCAACGAGCTTCAA-3′) and PVCVb-333R (5′-TCACATGCCCATTGTGAATC-3′).

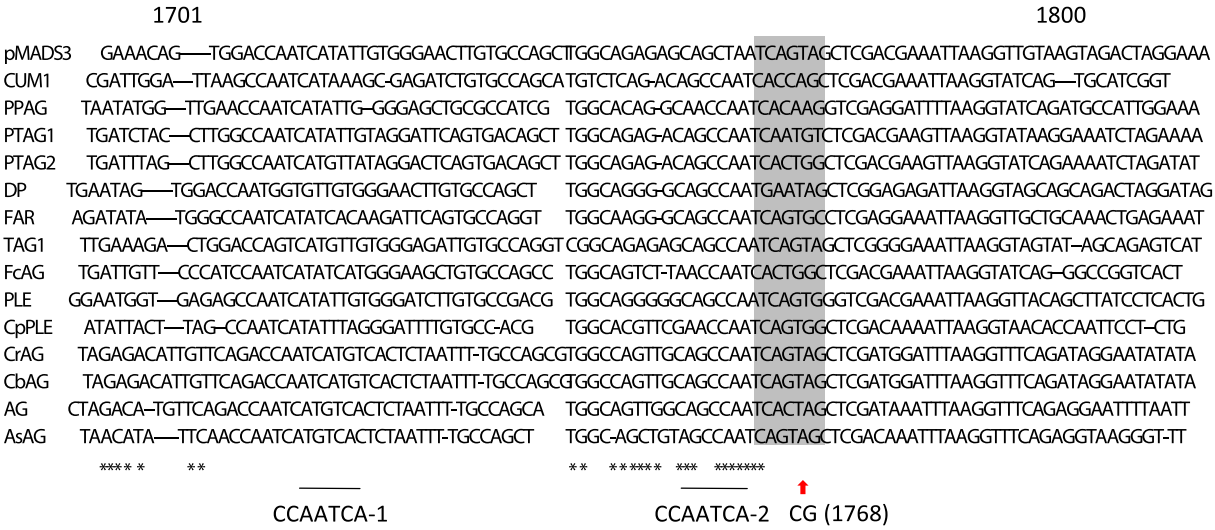

Fig. S3. DNA sequence alignment of intron 2 partial sequences in *pMADS3* homologs. The alignment is generated for the sequences corresponding to the 1701–1800 region of *pMADS3* in 15 *pMADS3* homologs of 13 plant species belonging to 9 different families (see Table S1). Asterisks indicate the bases identical among all of the sequences. The TAGCT*CG*A 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.

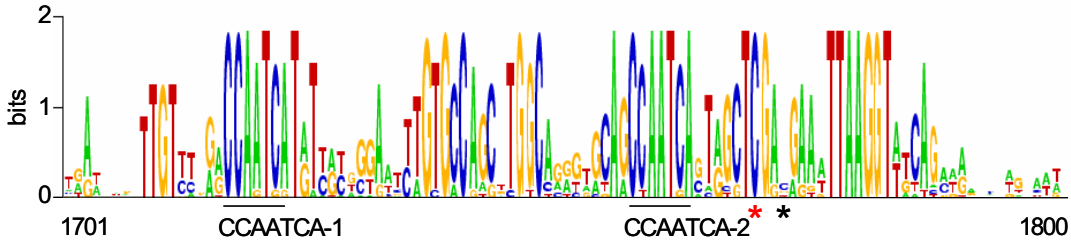

Fig. S4. Sequence logo for the conserved region of *pMADS3* intron 2. The sequence logo is generated for sequences corresponding to the 1701–1800 region of *pMADS3* in 15 *pMADS3* homologs of 13 plant species belonging to 9 different families (see Table S1). Asterisks indicate CG sites at nts 1768 (red) and 1771 (black). Numbers indicate the positions in *pMADS3* intron 2.

Table S1. *AG* orthologs used for sequence alignment

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

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	CGGGATCCATCGATTCTCTTTCTCAACACCCACCCAT
V033	MI2RNAi-1601F	GACTAGTCCATGGTGCCTTTGAAACAAAACCCT
	MI2RNAi-1767R	CGGGATCCATCGATATATATATAGCTACTGATTAGCTGCTCT
V034	MI2RNAi-1768F	GACTAGTCCATGGATATATATCGACGAAATTAAGGTTGTAA
	MI2RNAi-1900R	CGGGATCCATCGATTCAGACTCAATCTGTCTACTC