

# Supporting Information

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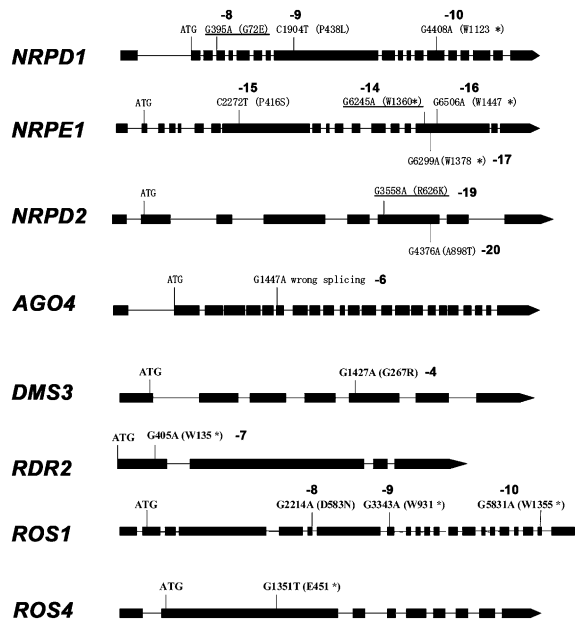


Fig. S1. Diagram of mutations identified in this study. The positions of mutations are counted from the first ATG in the genomic sequence. The changed amino acid is indicated in parentheses.

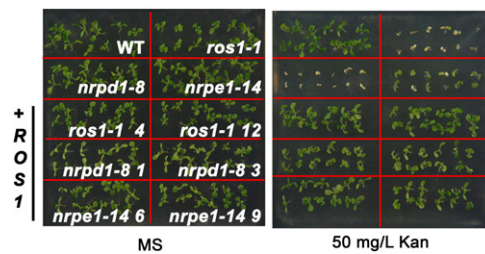
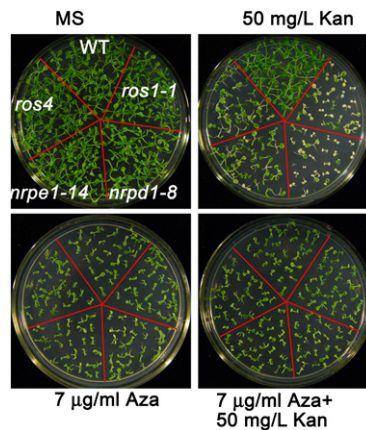
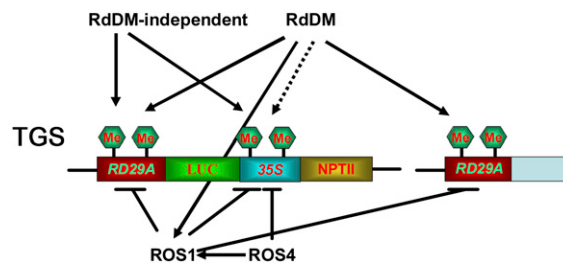


Fig. S2. The 35S promoter-driven *ROS1* rescues the kanamycin (Kan) sensitivity of *ros1*, *nrpd1-8*, and *nrpe1-14*. Two independent transgenic lines were used for the Kan-sensitivity assay.



**Fig. 53.** Transcriptional gene silencing (TGS) of *35S-NPTII* in different mutants as affected by 5-aza-2'-deoxycytidine (Aza) treatment. The mutant seeds were germinated on Murashige and Skoog (MS) medium or MS medium supplemented with 50 mg/L Kan, 7 µg/mL Aza, or 7 µg/mL Aza plus 50 mg/L Kan, and compared for Kan sensitivity.



**Fig. 54.** A working model for regulation of *RD29A* and *35S* promoters by different epigenetic factors. The TGS of *RD29A* is regulated by both the RNA-directed DNA methylation (RdDM) (polymerase IV and polymerase V) and RdDM-independent (DDM1, MET1, HDA6) pathways (1), and the silencing of the *35S* promoter is mainly regulated by the RdDM-independent pathway. The dotted line indicates a possible role of the RdDM pathway in regulation of the *35S* promoter. *ROS1* expression is positively regulated by the RdDM pathway. *ROS1* can suppress the silencing of transgene *RD29A*, endogenous *RD29A*, as well as the *35S* promoter through active DNA demethylation, whereas *ROS4* suppresses the silencing of the *35S* promoter by affecting *ROS1* function or directly through histone acetylation.

1. Chinnusamy V, Zhu JK (2009) RNA-directed DNA methylation and demethylation in plants. *Sci China C Life Sci* 52:331–343.

**Table S1. Primers used in this study**

Primers for complementation assay (5'-3')	
CCGGAATTCCTCGACGATACGTGTCGAACATTC	ROS4-promoter-EcoR I-F
CCGGAGCTCTCCACAACTAACTGCATATCCAC	ROS4-promoter-SacI-R
CCGGAGCTCATGCTTCCCGGAGCTGAGATTG	ROS4-cDNA-SacI-F
CCCCCGGGATTCCGAATCTGAGAGCTATCAGAGTC	ROS4-cDNA-XmaI-R
CGCGGATCCATGGAGCAAAAGCTCATTTCTG	MYC-BamHI-F
CCGGTCGACTTATACCGAGTTCAAGTCCTTTCAG	MYC-Sall-R
Primers used in gene expression-related real-time PCR (5'-3')	
GTCAAGACCGACCTGTCCGGG	NPTIIRT-F
GTCCTGATAGCGGTCCGCCACA	NPT IIRT-R
CACAGGGAATGACACGGTCTTG	4G02960RT-F
CGGAGTGCAATGTCAACTTTGGTG	4G02960RT-R
CCTTTCATCGGACTCATCGCTG	3G47330RT-F
GGCAGATCAGCTTGAGAAAGAGGC	3G47330RT-R
CATGTTGATGATGCTGTACTGAACC	4G28960RT-F
CTGCCTTTTCTCAAGCTGATCTG	4G28960RT-R
GAACATTGGCTCTCGACGACTG	5G03090RT-F
CAAGCTCGAAAACCGATCC	5G03090RT-R
CGGGGAATTGGCAGCTTTGTC	3G02550RT-F
TCAAATCGGTTGAGCCAGTAGCC	3G02550RT-R
GGTGGTGGGAACTCCAATCAC	3G20810RT-F
GCCTTGGGAACTCGTTTCGTC	3G20810RT-R
CCTGGATTGCCACACC	EF1a-F
AGTCTGCCTCATGTCC	EF1a-R
Primers for Northern blotting (5'-3')	
GTCAAGACCGACCTGTCCGGG	NPTIIRT-F
GTCCTGATAGCGGTCCGCCACA	NPTIIRT-R
CGCATGATTTGATGGAGGAGCCATAG	RD29ANor-F
GGATCAAACAGAGGAAC CACCACTC	RD29ANor-R
GAAGCTCCCAGGACACCACGAC	COR47Nor-F
CAGCGAATGTCCACTCCCAC	COR47Nor-R
Primers used in ChIP-related quantitative PCR (5'-3')	
TTCCCGATCCTATCTGTCACTT	35SCHIP-F
GATCTTGTAGAGAGAGACTGGT	35SCHIP-R
CGACGGCTATACTCACTTCGG	3G47330CHIP-F
AGTCAACAACCCCATAGG	3G47330CHIP-R
AGTAATGGAGAACGATGAGCCAG	4G02960CHIP-F
CACGAAATTAGCAAACCTCTCGTACC	4G02960CHIP-R
GCCCAAAGAAAAATGTAAATACTCGGG	4G28960CHIP-F
CGGCTTCCTTCTGTGATTCA	4G28960CHIP-R
CGGAAGAGAGGAACTACTTTCTGCG	5G03090CHIP-F
AGCAAGGACGACGATTCTCCC	5G03090CHIP-R
GAGTTTAGTGGATTCTGTATGTATCCTC	3G02550CHIP-F
GGGCAATCGGTTTATTTGGTTTTTATAC	3G02550CHIP-R
GACGGACCAAAAACGTAAGAACTCG	3G20810CHIP-F
TCGTACACTTGGAAAACCGC	3G20810CHIP-R
GATGATGCGCAAGAGCTG	actin2CHIP-F
GCCTCATCACCTACGTAGGCAT	actin2CHIP-R
Primers used in bisulfite sequencing (5'-3')	
ATGATTATATGATGGGTTAATAGATATGGAT	Rd29ABi-F
CACAATAAATTTAAATAAATTCAAAAAATTA	Rd29AEnBi-R
AAAAAATTAATAAATTTTCACTACATAC	Rd29ATransBi-R
Primers for vector coding <i>ROS4-GFP</i> fusion protein (5'-3')	
CGGCCTAGGATGCTTCCCGGAGCTGAGATTG	ROS4-cDNA-AvrII-F
CGGGGTACCTTCCGAATCCTGAGAGCTATCAGAGTC	ROS4-cDNA-KpnI-R
GGTACCATGGTGAGCAAGGGCGAGGAGC	GFP-KpnI-F
GAGCTTACTTGTACAGCTCGTCCATGCCGAGAG	GFP-SacI-R
Primers for vector coding <i>ROS4::GUS</i> (5'-3')	
CAGGTCGACCTCGACGATACGTGTCGAACATTC	ROS4-promoter-Sall-F
CCGGAATTCCTCCACAACTAACTGCATATCCAC	ROS4-promoter-EcoR I-R

F, forward; R, reverse.