Supporting Information

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SI Materials and Methods

Plant Materials. *nrpe5a-1* (FLAG 607D12), *nrpe5b-1* (SALK_134107) *and nrpe5c-1* (GABI_454F05) homozygous plants were genotyped by PCR (1, 2, 3). The endogenous copy of the gene was detected using primers 149/152, 513/514 and 515/516 respectively. The T-DNA insertions were followed using primers Tag6/149, LBA1/513 and 8409/515.

Semiquantitative and Real-Time PCR Amplifications. Primers 786/787 for EF1–4α were used as calibration control and primers 603/604, 149/152, 513/514 and 515/516 were used for *NRPB5a*, *NRPE5a*, *NRPE5b* and *NRPE5c* respectively. Primers LTR625-F and LTR625-R for *IG/LINE* and GAPA-F, GAPA-R for *GAPA* (At3g26650) a constitutively expressed gene as control, were reported in Huettel *et al.* (4). RT-PCR conditions were as follows: 94°C for 3 min, followed by 22 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, for GAPA or 28 for IG/LINE. Real-time PCR amplification was performed using the same primers and LightCycler 480 SYBR Green I Master (ROCHE Diagnostics) to analyze expression of IG/LINE. Data were analyzed using Light cycler software (Roche). Relative transcript levels were calculated using Δ CT method (5). GAPA was used as internal reference.

Immunoprecipitation. For PolII immunoprecipitation, plant extracts were prepared as described in Li *et al.* (6). Dynabeads M-280 Sheep anti-Mouse IgG (Invitrogen) were used to bind the anti-NRPB1 8WG16 antibody (Abcam) using 3 μl for 100 μl of beads resuspended in PBS/BSA (5 mg/ml BSA in isotonic (PBS) pH 7.2–7.6 as recommended by the manufacturer) and rotated O/N at 4°C. After 3 washes in isotonic PBS, the coated Dynabeads were resuspended into the plant extracts and rotated for 2h. After 3 washes in IP buffer, the bound material was recovered by a direct addition of SDS/PAGE loading buffer. The same experiment was carried out without any antibody as a negative control.

1. Alonso JM, *et al.* (2003) Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* 301:653–657.
2. Samson F, *et al.* (2002) FLAGdb/FST: a database of mapped flanking insertion sites (FSTs) of Arabidopsis T-DNA transformants. *Nucleic Acids Res* 30:94–97.
3. Rosso MG, *et al.* (2003) An Arabidopsis thaliana T-DNA mutagenized population (GABI-Kat) for flanking sequence tag-based reverse genetics. *Plant Mol Biol* 53:247–259.
4. Huettel B, *et al.* (2006) Endogenous targets of RNA-directed DNA methylation and PolIV in *Arabidopsis*. *EMBO J* 25:2828–2836.
5. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25:402–408.
6. Li CF, *et al.* (2006) An ARGONAUTE4-containing nuclear processing center colocalized with Cajal bodies in *Arabidopsis thaliana*. *Cell* 126:93–106.

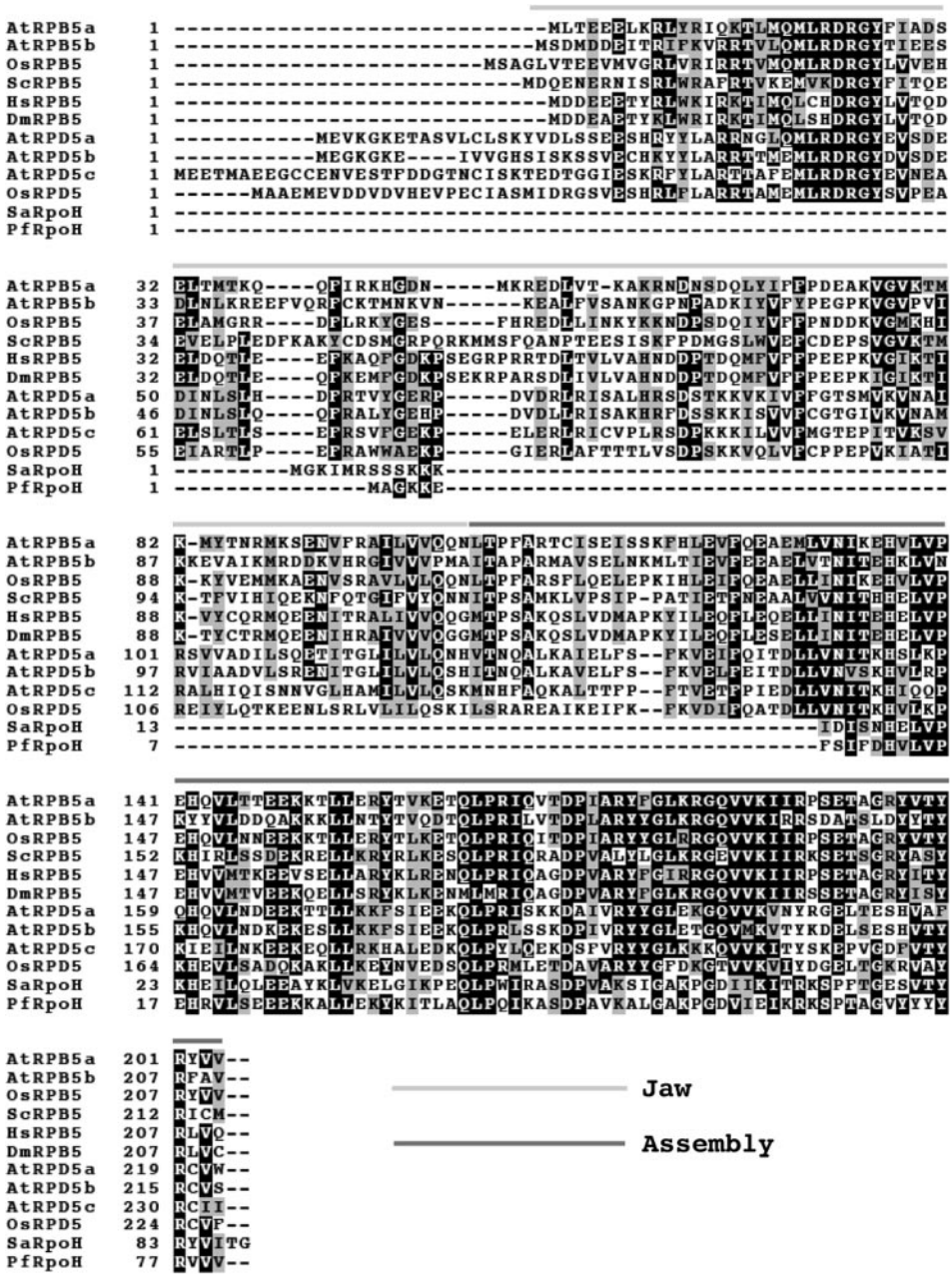

Fig. S1. Amino acid sequence alignments of *Arabidopsis thaliana* RPB5-type proteins with their counterparts in animal, fungi and archaeal RNA polymerases. Boundaries of both the eukaryote-specific N-terminal Jaw domain and the conserved C-terminal Assembly domain are indicated gray/dark lines. Abbreviations: Sc, *Saccharomyces cerevisiae*; At, *Arabidopsis thaliana*; Os, *Oryza sativa*; Hs, *Homo sapiens*; Dm, *Drosophila melanogaster*; Pf, *Pyrococcus furiosus*; Sa, *Sulfolobus acidocaldarius*

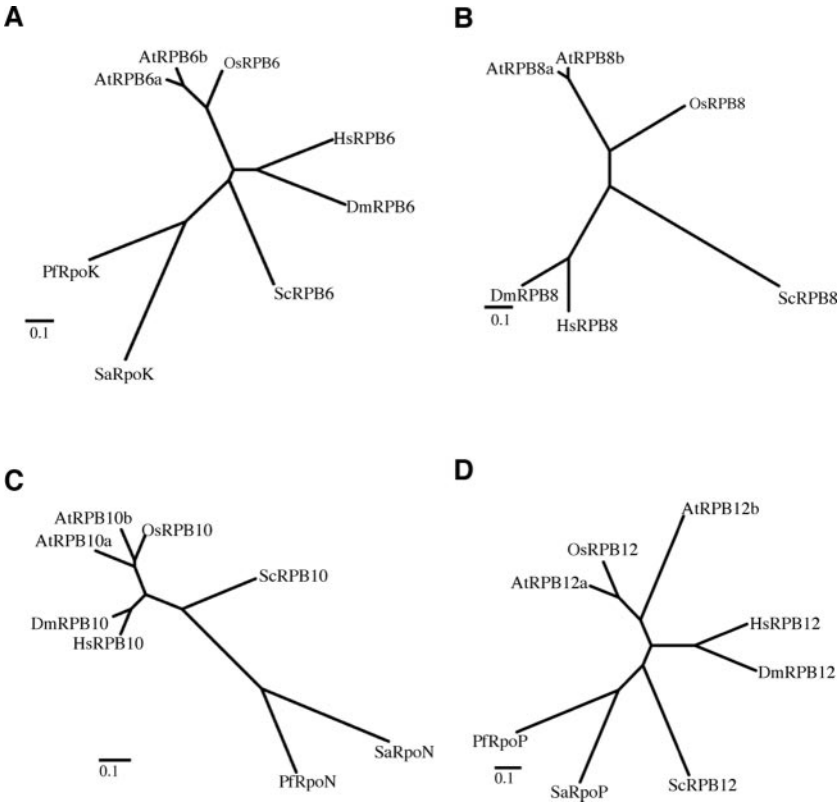

Fig. S2. Evolutionary relationships between RPB6-, 8-, 10- and 12-type proteins and related factors (*A*, *B*, *C*, and *D* respectively). The unrooted phylogenetic trees were inferred from the full-length protein alignment. Abbreviations: Sc, *Saccharomyces cerevisiae*; At, *Arabidopsis thaliana*; Os, *Oryza sativa*; Hs, *Homo sapiens*; Dm, *Drosophila melanogaster*; Pf, *Pyrococcus furiosus*; Sa, *Sulfolobus acidocaldarius*.

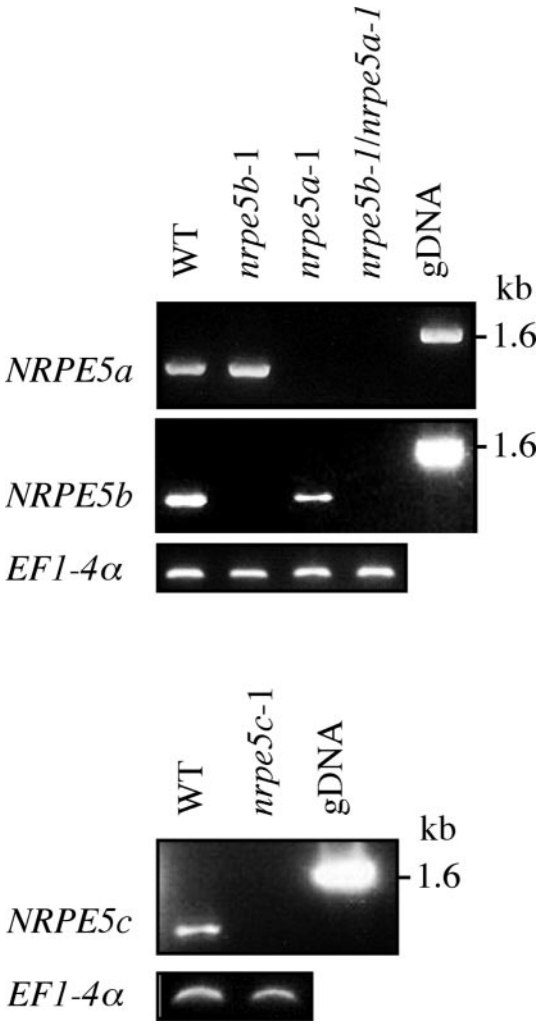

Fig. S3. *NRPE5* genes expression in wild-type plants and homozygous mutants shown by RT-PCR on flower total RNA. gDNA represents the genomic DNA control. *EF1–4α* is used as a control to standardize the RT-PCR reactions.

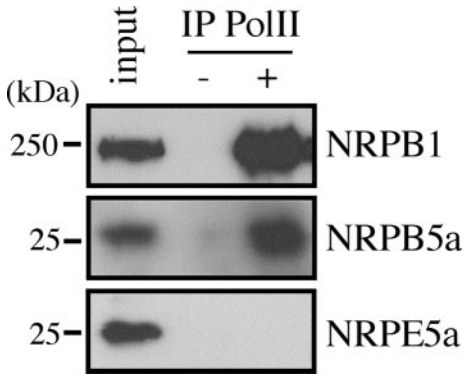

Fig. S4. Immunoprecipitation of PolII. PolII complex was immunoprecipitated from Columbia inflorescences using the anti-NRPB1 8WG16 antibody (+). As a control, the same experiment was performed without antibody (-). The ''input'' lane corresponds to the plant extracts prior immunoprecipitation. Theses samples were used run onto SDS/PAGE gels and subjected to Western blots using anti-NRPB1, anti-NRPB5a and anti-NRPE5a antibodies.

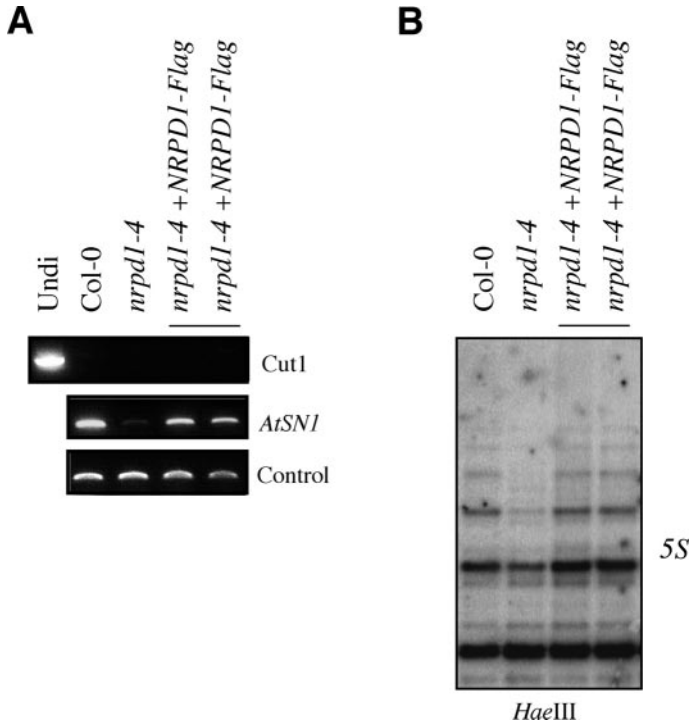

Fig. S5. Complementation of the *nrpd1a-4* mutant. (*A*) *AtSN1* DNA methylation in *nrpd1a-4* is rescued by transgenes expressing a Flag-tagged version of NRPD1a. Two independent T1 transgenic lines are shown. Undi represents the Col-O undigested DNA control. (*B*) 5S DNA methylation in *nrpd1a-4* is restored by a transgene expressing a Flag-tagged version of NRPD1(NRPD1a). Genomic DNA from wild-type, *nrpd1–4* and two independent T1 transgenic lines were digested by HaeIII and subjected to Southern Blot analysis using 5S probe.

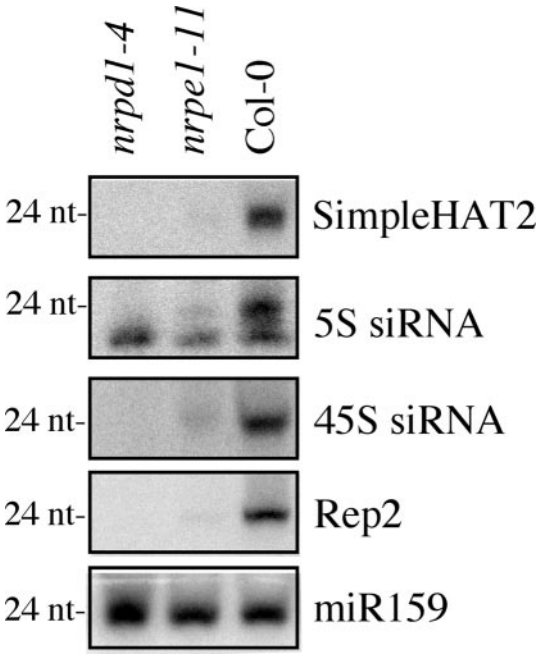

Fig. S6. sRNA accumulation in *nrpd1/nrpe1* mutants at PolIV(PolIVa)/PolV(PolIVb)-dependent loci. Analysis of sRNA accumulation by Northern blot done with 30 μg of total RNA from inflorescences from wild-type (Col-0), *nrpd1–4* and *nrpe1–11*.

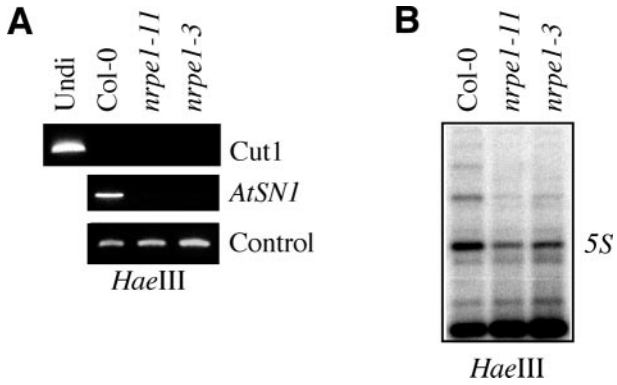

Fig. S7. Loss of CNN methylation at 5S and *AtSN1* loci in wild-type and *nrpe1* mutants. (A) HaeIII digested DNA were used as templates for *AtSN1* PCR reactions. Undi represents the Col-0 undigested genomic DNA. (B) Southern blot analysis of methylation at the 5S locus. Genomic DNA from wild-type, *nrpe1–11* and *nrpe1–3* mutants were digested by HaeIII.

Table S1. Primers and peptides used in this study

Name	Sequence
Primers used for genotyping and RT-PCR	
149	5′′-gtgaaagggaaagagacagct-3′′
152	5′′-tcaccacacacatcggaaggc-3′′
352	5′′-aagcttctttcgtttagagatcatcttaaggcggcg-3′′
462	5′′-gtcgacatggaagacgattgtgaggagcttcag-3′′
463	5′′-ccatgggcgggttttcggagaaaccaccgga-3′′
465	5′′-ggatccgtcgacccatcagtaaggaaaatttagatacg-3′′
513	5′′-gggaaagagatagtggttgg-3′′
514	5′′-agaaacgcatcggtagtaac-3′′
515	5′′-acaatggccgaagaagggtgc-3′′
516	5′′-acaattgtaagcacacaccaaggacac-3′′
603	5′′-gatgttgacggaagaggaggtc-3′′
604	5′′-cgataggtaacataacgaccagccg-3′′
786	5′′-ctaaggatggtcagacccg-3′′
787	5′′-cttcaggtatgaagacacc-3′′
Primers used for sRNA probes	
5S	5′′-atgccaagtttggcctcacggtct-3′′
45S	5′′-gtctgttggtgccaagagggaaaagggctaat-3′′
si02	5′′-gttgaccagtccgccagccgat-3′′
Rep2	5′′-gcgggacgggtttggcaggacgttacttaat-3′′
SimpleHat	5′′-tgggttacccattttgacaccccta-3′′
TR2558	5′′-ttcatcagcatgaccgatagctta-3′′
TR2558	5′′-tttgttcaatcattcattggccat-3′′
miR159	5′′-tagagctcccttcaatccaaa-3′′
U6	5-aggggccatgctaatcttctc-3
Peptide used for antibody production	
NRPE5a_ pep: TKHSLKPQHQVLNDE	
NRPE5c_ pep: KEPVGDFVTYRCII	