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Supplementary Information for

Anaphase Promoting Complex/Cyclosome regulates RdDM activity by degrading DMS3 in Arabidopsis

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Supplemental Materials and Methods

Plant Materials and Regents

T-DNA insertion mutants of *nrpe1-12* (SALK_033852) and *pol iv* (SALK_128428) were obtained from ABRC (www.arabidopsis.org). Seeds of *dms3-5* (SALK_068723), *ros1-1 rdm1-1*(1), and 35S::DRD1-Flag transgenic plants were provided by Dr. Xin-jian He. The *apc8-1* mutant and the pCYCB1;1::CYCB1;1-GFP plants were described previously (2). We constructed co-suppression (cs) lines of *APC10*. MG132 (Sigma, Cat#M7449), Protease inhibitor cocktail (Roche, Cat#11873580001), CHX (Sigma, Cat#C7698), GFP-Trap beads (Chromotek, Cat#gta-20), E1 (Boston Biochem, E-300), E2 (Boston Biochem, E2-625), anti-H3K9me2 (upstate, 17-648), Hydroxyurea (HU) (Sigma, H8627), anti-Hsc70 (Stressgen, #SPA-818), anti-AGO4 (Agrisera, AS09617), anti-UBQ11 (Agrisera, AS08307), anti-GFP (Cavans, MMS-118R), anti-GST (abmart, M20007), anti-His (abmart, M30111), and anti-FLAG (Sigma, #F7425) were purchased. We generated anti-NRPE1 (the peptide "CDKKNSETESDAAAWG" as the antigen) (3), anti-RDM1 (recombinant protein as the antigen), and anti-DMS3 (recombinant His-DMS3 protein as the antigen) antibodies.

Plasmid Construction

DMS3 coding sequences were obtained using the primer pair DMS3F1/R1, then cloned into the pENTR1A vector to obtain pEN-DMS3. Then pEN-DMS3 was transferred into pGBTK7-GW to obtain DMS3-BD, and into pB7FWG2 to obtain 35S::DMS3-YFP. The 35S promoter was replaced by the endogenous promoter of *DMS3* using primers DMS3F2/R2 to obtain proDMS3::DMS3-YFP. To construct His-DMS3 and GST-DMS3, the coding sequences of *DMS3* were obtained using the primer pair DMS3F3/R3, then cloned into pET28b and pGEX2TK, respectively. To construct cLUC-DMS3 and DMS3-nLUC, the coding sequence of *DMS3* was obtained using the primer pair DMS3F4/R4 and DMS3 F5/R5, then cloned into 35S::cLUC and 35S::nLUC, respectively. To construct $DMS3^{R1}$, $DMS3^{R2}$, and $DMS3^{R3}$, pEN-DMS3 was site-mutagenized using the primer pairs DMS3F6/R6, DMS3F7/R7, DMS3F8/R8, respectively. *APC10* coding sequence was obtained using the primer pair APC10F1/R1, then cloned into pENTR1A vector to obtain pEN-APC10. pEN-APC10 was transferred into pGADT7-GW to obtain

APC10-AD, then into pEarleyGate101 and pEarleyGate::3xFlag to obtain 35S::APC10-YFP and 35S::APC10-Flag. For co-suppression (cs) lines of *APC10*, *bonsai*-like plants with reduced expression of *APC10* from 35S::APC10-YFP transgenic plants were identified as the cs lines. *APC10* coding sequences were obtained using the primer pair APC10F2/R2 and APC10F3/R3, cloned into pGEX2TK and 35S::nLUC, to obtain GST-APC10 and APC10-nLUC, respectively. To construct other APC-AD, CDS sequences were obtained using the primer pairs APC2F1/R1, APC4F1/R1, APC6F1/R1, APC7F1/R1, APC8F1/R1, APC11F1/R1, APC13F1/R1, respectively, then cloned into pGADT7. To construct 35S::APC8-YFP, *APC8* CDS was obtained using the primer pair APC8F2/R2, then cloned into the plant expression vector pCambia2302. To construct GST-RDM1 and His-RDM1, *RDM1* CDS was obtained using the primer pair RDMF1/R1, then cloned into pGEX2TK and pET28b, respectively. To construct RDM1-BD, the *RDM1* CDS was obtained using the primer pair RDMF2/R2, then cloned into pGBTK7. To construct RDM1-nLUC and RDM1-cLUC, the *RDM1* CDS was obtained using the primer pair RDMF3/R3 and RDMF4/R4, then cloned into 35S::nLUC and 35S::cLUC, respectively. To construct $RDM1^{R}$, pEN-RDM1 was site-mutagenized using the primer pairs RDM1F5/R5. To construct DRD1-BD and cLUC-DRD1, the *DRD1* CDS was obtained using the primer pair DRD1F1/R1 and F2/R2, then cloned into pGBTK7 and 35S::cLUC, respectively. To construct 35S::DRD1-YFP, the *DRD1* CDS was obtained using the primer pair DRD1F3/R3, then cloned into pCambia2302. To construct other RdDM components into BD, including NRPE1, NRPE5, NRPE7, NRPD1, NRPD2, AGO4, DRM2, KTF1, SUVH2, SUVH9, MORC1, MORC2, MORC6, and DRB4, the coding sequences of corresponding genes were amplified using their respective primers and cloned into pGBTK7. Primers are listed in Appendix Dataset S5.

RNA Sequencing and Small RNA Sequencing

Total RNA isolated from 14-day-old seedlings of Col-0, *apc8-1*, *nrpe1-12*, or *pol iv* was extracted by Trizol (Ambion). For RNA sequencing, 1 μg Dnase I-digested total RNA was treated with a Ribo Zero kit (Epicenter) to obtain a ribosomal RNA-depleted RNA ($ribo^{-}$ RNA), then used for library preparation with the Illumina TruSeq Stranded Total RNA HT Sample Prep Kit (P/N15031048), and then subjected to PE150 deep sequencing

with Illumina HiSeq 2500 at Genergy (Shanghai, China). There were two biological replicates for each genotype. The sequencing reads were filtered to remove reads with more than 5 nucleotides and with sequencing scores smaller than 20. Adaptor sequences were removed to extract RNAs. The obtained RNA sequences were mapped back to the *A. thaliana* reference genome (TAIR 10) using Tophat/Cufflinks 2 (4). To visualize the abundance of TEs, the "bedtools genomecoverage" command of bedtools (5) was used to generate the bedgraph files of the individual RNA libraries, then normalized by the total number of reads in each RNA library. The normalized bedgraph files were then loaded into Integrative Genomics Viewer (6) to visualize the abundance of selected TEs. FeatureCounts (7) was then used to calculate the normalized abundance of TE-derived RNAs using the TAIR10 Transposon Element annotation. The counts of TEs were used to find deregulated TEs in *apc8-1* and *nrpe1-12* by comparing count values in Col-0 using edgeR (8). The count values of TEs commonly de-repressed in *apc8-1* and *nrpe1-12* were used to perform biclustering analysis with the clustergram command in MatLab (MathWorks, Natick, MA). The expected number of overlapped TEs was obtained by dividing the number of up-regulated TEs in *apc8-1* by the total number of TEs (118 / 31189), then multiplied by the number of up-regulated TEs in *nrpe1-12* (211). The number of overlapping TEs between those up-regulated in *apc8-1* and *nrpe1-12*, respectively, was evaluated by a $\chi 2$-test against the expected number of overlapping TEs. The classes of TEs deregulated in *apc8-1* and *nrpe1-12* were assigned based on the Transposon Families in the TAIR database. The percentages of different classes of deregulated TEs in *apc8-1* and *nrpe1-12* were evaluated using the Kolmogorov-Smirnov test. For small RNA sequencing, 2 μg total RNA was used for library preparation with the TruSeq® Small RNA Sample kit (Part # 15004197), and then subjected to SE50 deep sequencing with Illumina HiSeq 2500 at Genergy (Shanghai, China). There were two biological replicates for each genotype. The sequencing reads were filtered to remove reads with more than 5 nucleotides and with sequencing scores smaller than 20. Adaptor sequences were removed to extract small RNAs. Redundant sequences were eliminated, and unique small RNAs were counted. Unique small RNAs were mapped back to the *A. thaliana* reference genome using SOAP2 (9). Custom programs were then used to calculate the normalized abundance (RPTM, Reads Per Ten Million sequencing tags) of

siRNAs. The normalized abundances of siRNAs in *apc8-1* and *pol iv* were compared to those in Col-0 using edgeR by setting a bcv value of 0.1. Unique 24-nt siRNAs with RPTM>=50, negative log2 fold change values, and a multiple test corrected *P*-values smaller than 0.05 were defined as down-regulated siRNAs. The numbers of siRNAs for *pol v* and *dms3* in 500 bp windows across the whole genome were extracted from Table S3 of (10). The regions that have log2 fold change values of mutant vs Col-0 smaller than -1 were defined as Pol V- and DMS3-dependent siRNA generation regions. The down-regulated 24-nt siRNAs in *apc8-1* that were aligned to the Pol V- and DMS3- dependent siRNA generation regions were regarded as Pol V- and DMS3-dependent siRNAs, respectively. The raw and processed data for both RNA seq and small RNA seq have been deposited into the NCBI GEO database under the series accession number GSE106972. The original data can be reached by the link: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE106972 and the secure token: afyhqmwgxnqnlep.

Cell-free Decay Assay

Ten-day-old seedlings of Col-0 and *apc8* were ground in liquid nitrogen, and the powder was dissolved with two volumes of lysis buffer (25 mM Tris_HCl (pH 7.5), 10 mM NaCl, 10 mM $MgCl_2$, 2 mM DTT, 10% glycerol), and then the supernatant was collected by spinning at 13200 rpm for 15 min three times. Total protein extracts from each were adjusted to equal concentrations with the degradation buffer. The supernatant with the addition of 500 μM Cycloheximide (CHX) and 5mM ATP was incubated at 28 ℃ as indicated. For the in vitro cell-free decay assay, purified recombinant proteins (1 μg) of His-DMS3 or His- $DMS3^{R3}$ were incubated at 28 ℃ with the supernatants (2 mg) of the Col-0 or *apc8* lysates. For the in vivo cell-free decay assay, the supernatants of Col-0 or *apc8* lysates were directly incubated at 22 ℃. For MG132 treatment, 50 μM MG132 was added as indicated. The reaction was stopped by adding 2 × SDS-PAGE loading buffer and the extracts were resolved by 8% SDS–PAGE after boiling. Proteins were detected with His antibody. Relative amounts of His-DMS3 were calculated with a chemiluminescent imaging system (Tanon5200, Tanon).

In vitro Ubiquitination Assay

In vitro APC/C-mediated ubiquitination was carried out as described (11), using purified His-DMS3 or His-DMS3^{R3} (1 μg per reaction) as substrate. APC/C was immunopurified as the E3 from 10-day-old seedlings from transgenic plants of 35S::APC8-YFP using GFP-Trap agarose beads (Chromotek). The other ingredients, including E1, E2 (UbcH5c and UbcH10), and Ub, were from Boston Biochem (Cambridge, MA). The ubiquitination reaction mix was incubated for 10 min at 37℃ and stopped by the addition of SDS-PAGE loading buffer with 100 mM DTT. Western blotting analysis was performed to determine the ubiquitination of DMS3 using anti-DMS3 antibody.

Gel Filtration

Gel filtration was carried out as described (12) with modifications. 0.5 g of two-week-old seedling tissue of Col-0, *apc8-1*, and DMS3-OE were ground in liquid nitrogen with 1.8 ml of lysis buffer (50 mM Tris_HCl (pH 8.0), 150 mM NaCl, 5 mM $MgCl_2$, 0.5% Triton X-100, 2 mM DTT, 10% glycerol, protease inhibitors) and incubated at 4 ℃ for 1 hr. The lysates were filtered with two layers of miracloth, and total protein extracts were centrifuged at 4 ℃ for 15 min at 13200 rpm, the supernatant was transferred into the new tube and repeated spin for four more times until there was no visible pellet. The supernatant was then filtered through a 0.22 micron filter prewashed with lysis buffer, and the flow through was concentrated to 50 μl with Amicon Ultra centrifugal filter units (Millipore, UFC501096, MWCO 10 KDa) prewashed with equilibration buffer (50 mM Tris_HCl (pH 8.0), 150 mM NaCl, 5 mM $MgCl_2$, 0.5 mM DTT, 10% glycerol). Lysates was gradually equilibrated with equilibration buffer to reduce the effects of Triton X-100 from the lysis buffer and high salt concentrations from plant tissue, in which 1 ml equilibration buffer was added three times when 100 μl lysates were left. Then, ~50 μl sample for each genotype was loaded onto a Superose 6 increase 3.2/300 GL column (GE Healthcare, 17-5172-01) prewashed with equilibration buffer, and fractions collected per 50 μl. Fractions were separated with 6-12% SDS-PAGE and probed by western blotting using antibodies for NRPE1, RDM1 and DMS3. Standard proteins were used to determine the protein sizes in the eluted fractions.

Northern Blotting of Small RNAs

Total RNA was extracted using Trizol reagent (Invitrogen) from inflorescences. 15 µg of the enriched small RNA was separated by denaturing 15% (w/v) PAGE and transferred to a nylon membrane. The ^{32}P-labeled oligo nucleotides complementary to siRNA were synthesized as probes. Hybridization was performed using hybridization buffer (Ambion), and signals were detected using a Phosphor imager. U6 was used as a loading control. The probes used are listed in Appendix Dataset S5.

Yeast Two-Hybrid Assay

The yeast strain AH109 was co-transformed with pGADT7 and pGBKT7 constructs. Yeast cells were grown on the synthetic dropout medium minus Leu and Trp (-LT). Positive yeast colonies were streaked on both -LT and -LTHA (synthetic dropout medium minus Trp, Leu, His, and Ade) for growth assays. Growth of transformed positive yeast strains on -LT indicates interaction between GAL-AD fusion protein and GAL4-BD fusion protein in the corresponding yeast strains.

ChIP

ChIP was performed according to (13). Briefly, chromatin from seedlings of Col-0, *apc8*, and *nrpe1-12* plants was immunoprecipitated with anti-NRPE1 and anti-H3K9me2 antibodies. Co-precipitated DNAs were identified via qPCR. There were three biological replicates. The primer sets used for PCR are listed in Appendix Dataset S5.

Recombinant Protein Expression and Pull Down Assays

His-DMS3, His-RDM1, GST-DMS3, and GST10-APC10 were expressed in *E. coli* BL21. Expression was induced for 15 h with 0.2 mM IPTG at 18 ℃. Cells were disrupted by sonication and proteins purified by Ni-NTA agarose and Glutathione Sepharose 4B agarose, respectively. For the semi *in vivo* pull down assays, 2 µg of recombinant proteins (His-DMS3 conjugated on Ni-NTA agarose; GST-APC10 or GST-DMS3 conjugated on Glutathione Sepharose 4B agarose were incubated with 800 µl of cell lysates from inflorescences from 35S::APC8-YFP, 35S:: DMS3-YFP, or 35S::DRD1-Flag transgenic plants for 2 h at 4℃ on a rotating wheel. For the *in vitro* pull down

assays, 2 μg of His-DMS3 conjugated on Ni-NTA agarose or GST-APC10 conjugated on Glutathione Sepharose 4B agarose were incubated with the indicated amount of purified recombinant protein for 2 h at 4 ℃ on a rotating wheel. Beads were washed 8 times with lysis buffer. The bound (pellet) proteins were detected by immunoblotting with corresponding antibodies. 1/5 of the pellet fractions, and 1% of the purified recombinant proteins used in the pull down assay were loaded.

DNA Methylation Assay

14-day-old seedlings from at least ten T1 individuals per each transgene construct/genotype were pooled for each experiment. DNA was extracted using a Plant Genomic DNA Kit (TIANGEN, cat. DP305). DNA methylation was tested by bisulfite sequencing and chop-PCR. For bisulphite sequencing, 100 ng of genomic DNA was sodium bisulfite-converted and purified using the BisulFlash DNA modification kit (Epigentek, Cat. P-1026-050). Purified DNA was amplified and PCR products were cloned into pMD19-T (Takara, Cat. 6013). At least 12 clones were sequenced per sample. For chop-PCR, 100 ng of genomic DNA was digested with 2 units of the methylation-sensitive restriction enzyme AluI (New England Biolabs) for 30 min at 37 ℃, followed by 20 min at 80 ℃ to inactivate the enzyme. A 20 μl PCR reaction was then assembled using 2 μl of the digestion with 0.4μM primer pairs, 0.2 mM dNTPs, and 0.4 units Taq (Takara) in 10x PCR Buffer. The samples were subjected to 32 cycles for soloLTR and 30 cycles for IGN26, at 94 ℃ for 30 seconds, 55 ℃ for 30 seconds, and 72 ℃ for 30 seconds, then the products analyzed on a 3% TBE agarose gel. The abundance of PCR products indicates the DNA methylation levels of the amplified loci. Equal amounts of genomic DNA were amplified as an internal control. There were three biological replicated or each chop-PCR. The primer pairs used for Chop-PCR are listed in Appendix Dataset S5.

Split Luciferase Complementation Imaging Assay

The BiFC assay was performed as described (14). Plasmids were introduced into *Agrobacterium* strain GV3101, and the OD values were monitored using an ultraviolet-visible spectrophotometer, then co-infiltrated with indicated ratios of different

combinations into young leaves of *N. benthamiana*. Plants were grown in the dark for 1 d and then transferred to long-day conditions (16 h light/8 h dark) for 2 d. Luciferin (1 mM) was sprayed evenly onto infiltrated leaves and LUC activity was monitored with LB985 NightShade with indiGo software (Berthhold Tech).

Co-immunoprecipitation (Co-IP)

Inflorescences of 35S::APC8-YFP or pAPC8::APC8-YFP transgenic plants were ground in liquid nitrogen and homogenized in two volumes of protein lysis buffer (50 mM Tris_HCl (pH 8.0), 150 mM NaCl, 50 mM $MgCl_2$, 0.2% Nonidet P-40, 2 mM DTT, 5% glycerol, 20 μM MG132, 1 mM PMSF, proteinase inhibitor) and centrifuged for 15 min at 13200 rpm. After preclearing with protein A agarose, the lysate was incubated with GFP-Trap agarose beads (Chromotek), anti-NRPE1-conjugated Protein A agarose, or anti-DMS3-conjugated protein A agarose for 2 h. The immune complexes were then washed eight times with 1ml of lysis buffer. Proteins retained on the beads were resolved on SDS-PAGE. Endogenous anti-NRPE1, endogenous anti-DMS3, and anti-GFP antibodies were used to detect NRPE1 DMS3, and APC8 proteins, respectively, with western blot analyses.

Synchronization by Hydroxyurea (HU) Treatment

The synchronization was performed as described (15). Seeds of pCYCB1;1-CYCB1-GFP and the 35S::DMS3-YFP plants were plated on sterilized nylon membrane on the Murashige and Skoog (MS) medium and grown vertically in 16 h/8 h growth chamber at 22 ℃. For synchronization, after 2 days of germination and 5 days of growth, the nylon membrane was transferred into the liquid MS medium containing 2 mM hydroxyurea (HU) for indicated time points. Two biological replicates, each containing at least ~500 plants, were analyzed. Roots tips were immediately cut with a scalpel and used for subsequent western blotting analysis.

Western Blotting

For extraction of total proteins, 7-10 day-old seedlings were ground to a fine powder in liquid nitrogen, and homogenized in two volumes of lysis buffer (50 mM Tris_HCl (pH

8.0), 150 mM NaCl, 0.2% Nonidet P-40, 2 mM DTT, 10% glycerol, protease inhibitor) then centrifuged for 15 min at 13200 rpm. The supernatant was mixed with 5× SDS loading buffer and boiled at 95℃ for 5 min. After centrifugation at 12,000 rpm for 10 min, the supernatant containing total proteins was separated on SDS-PAGE gels, and then proteins detected on western blots using corresponding antibodies. Band intensities were measured with a Tanon-5200 Gel Image System (Version 4.2.5) software.

Gene Expression Analysis by Quantitative RT-PCR (qRT-PCR)

Total RNAs were extracted from inflorescences or seedlings using Trizol (Invitrogen) and 2 μg of Dnase I-treated total RNA was used for reverse transcription (PrimeScript™ II reverse transcriptase, TaKaRa). For RdDM loci, random hexmer-priming was used, while other coding genes were oligo dT-primed. For qRT-PCR, cDNAs were combined with SYBR master mix (Bio-Rad). qRT-PCR was performed in triplicate with a Bio-Rad C1000 Thermocycler. The data were collected and analyzed with Bio-Rad real-time PCR detection systems and software. Primers are listed in Appendix Dataset S5.

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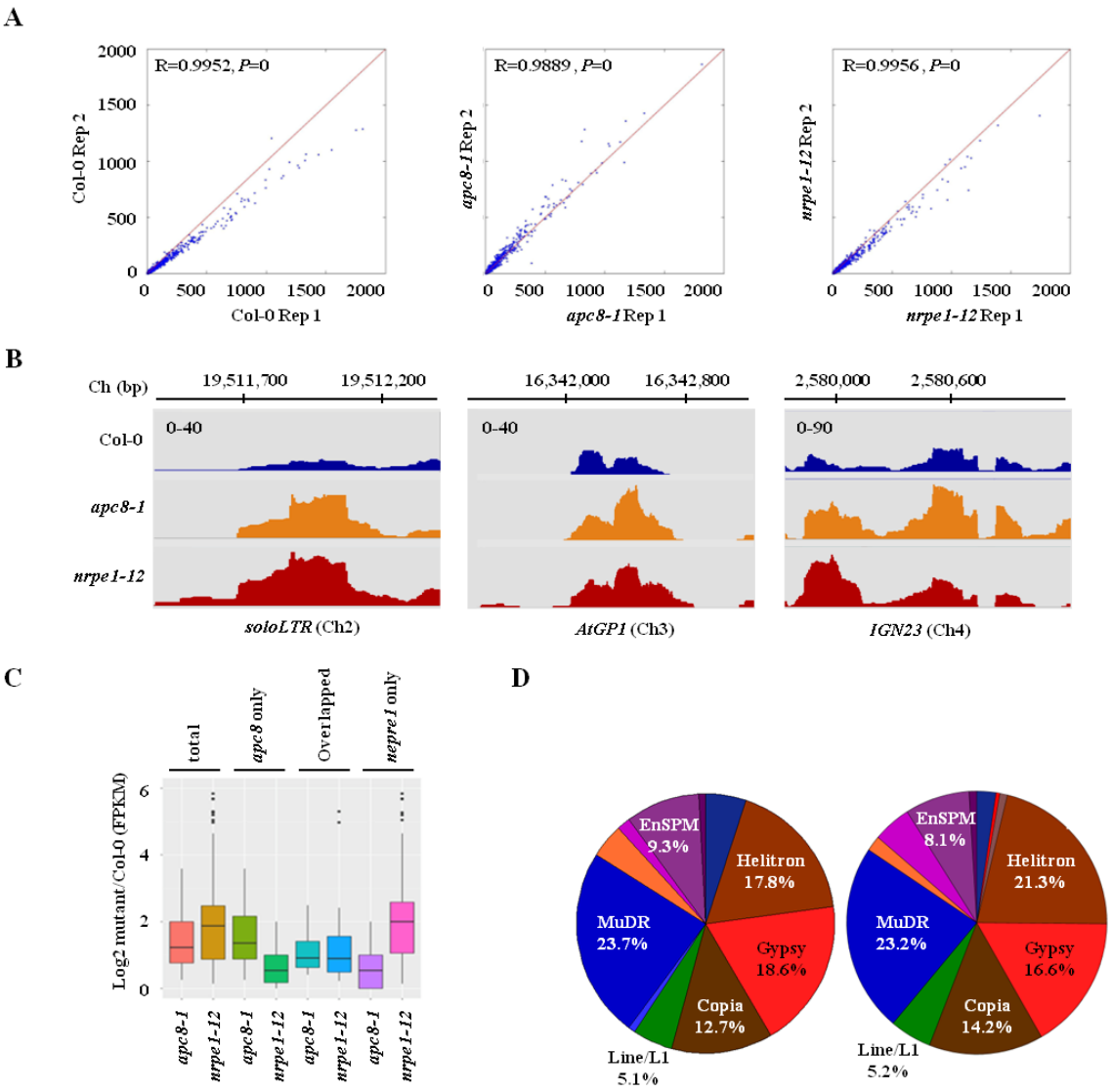

Fig. S1. APC/C is required for a subset of TE silencing.

(A) Reproducibility of expression levels between biological replicates in Col-0, *nrpe1-12*, and *apc8-1*. The Pearson's correlation coefficients and their *P*-values are indicated for each graph. Rep 1 and Rep 2 indicate two biological replicates.

(B) Normalized expression levels of transcripts from flanking region of typical RdDM loci in Col-0, *apc8-1*, and *nrpe1-12*. Sequencing reads in each genotype are indicated by different colors. The X-axis indicates the chromosomal location of RdDM loci flanking regions (~3 kb upstream of *soloLTR*, ~6 kb downstream of *AtGP1*, and ~2 kb downstream of *IGN23*), and the Y-axis indicates normalized peaks. Same scales ("0-100"

indicates numbers of normalized reads coverage) are identical for each genotype.

(C) Boxplot showing expression of de-repressed TEs in Col-0, *apc8-1*, and *nrpe1-12*.

(D) Piecharts showing the categories of up-regulated TEs in *apc8-1* and *nrpe1-12*. The numbers indicate the percentage of indicated subfamilies relative to total up-regulated TEs in each mutant. The same color indicates same family of TE in *apc8-1* and *nrpe1-12*. The classes of TEs deregulated in these two mutants did not show significant difference (P = 1, Kolmogorov-Smirnov test).

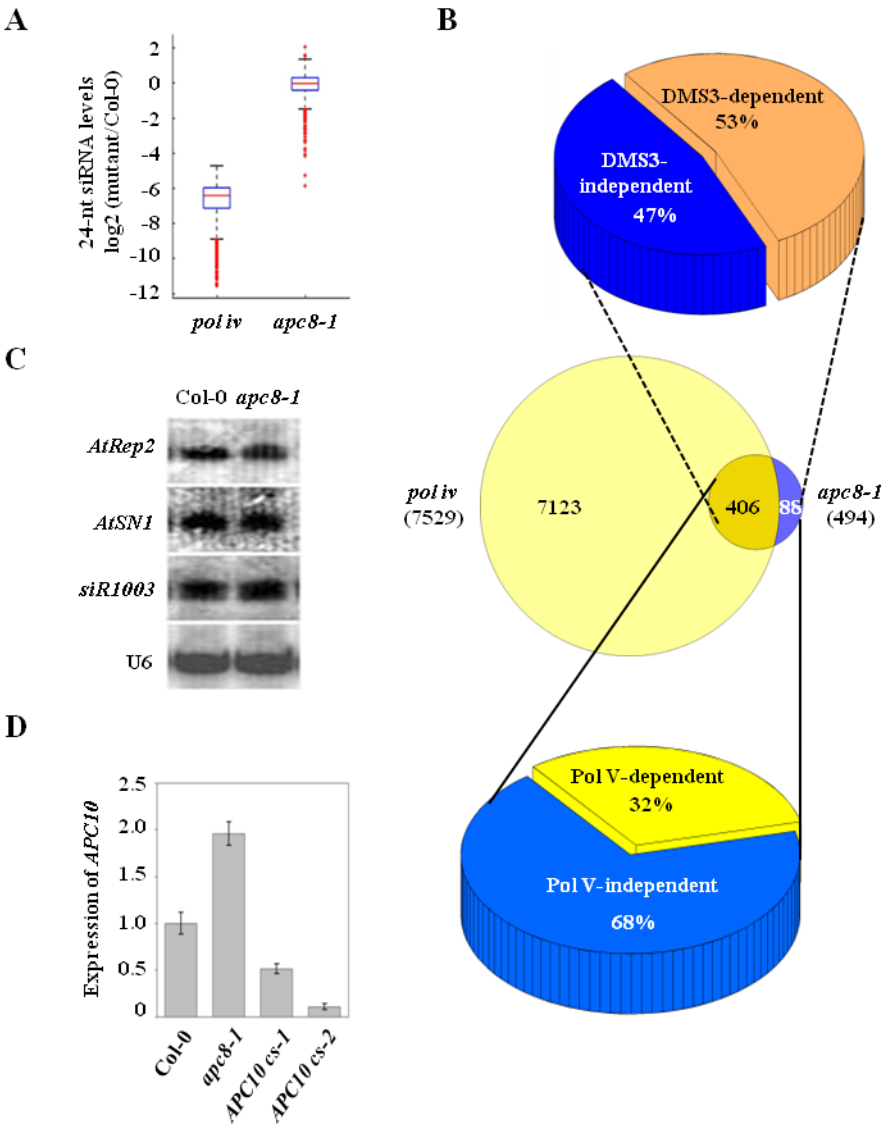

Fig. S2. APC/C is required for a subset of TE silencing.

(A) Small RNA sequencing analysis of 24-nt siRNAs in Col-0, *apc8-1*, and *pol iv*. Small RNA libraries were generated from inflorescences. The log2-transformed ratios of *apc8-1*/Col-0 and *pol iv*/Col-0 were plotted. Each dot represents one unique siRNA. Thick lines indicate mean values.

(B) Overlap of down-regulated 24-nt siRNAs between *apc8-1* and *pol iv* mutants. The Venn diagram shows the numbers of 24-nt siRNAs decreased between *apc8-1* and *pol iv*. The piecharts show the dependency of down-regulated siRNAs in *apc8-1* on Pol V and DMS3. See Materials and Methods for details.

(C) Northern blotting of *AtSN1*, *AtPEP2*, and *siR1003* in Col-0 and *apc8-1*. The experiments were repeated three times. U6 was used as a loading control.

(D) qRT-PCR analysis of *APC10* in Col-0, *apc8-1*, and two independent cs lines of *APC10*. Total RNA from inflorescences of each genotype was analyzed. *UBQ5* was used as an internal control. Data are presented as mean ± standard errors (SE), n = 3.

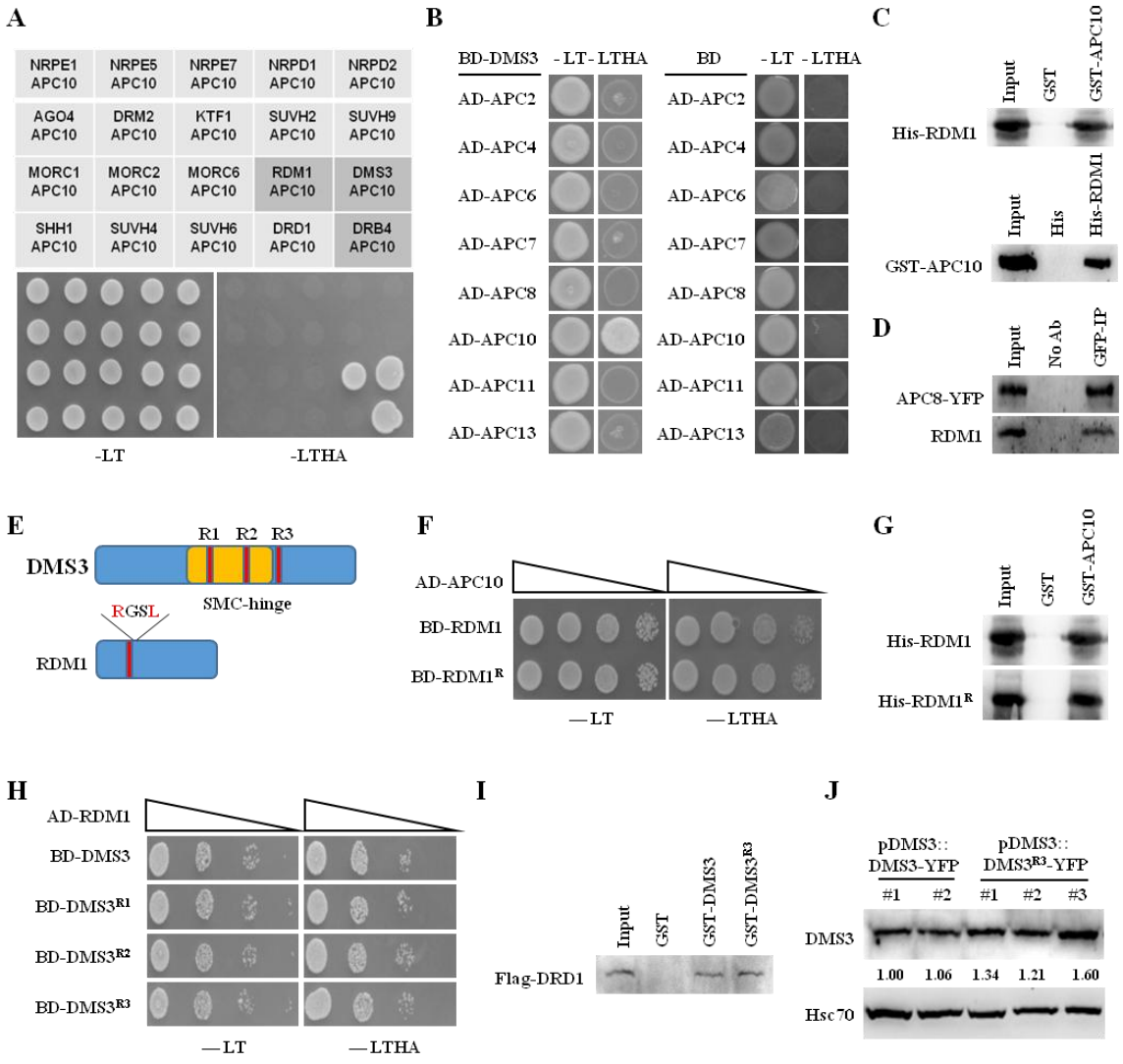

Fig. S3. Effects of mutated D-box on RDM1 and DMS3 function.

(*A*) Interaction between APC10 and RdDM regulators determined by Y2H assays. Yeast cells co-transformed by indicated combination of plasmids were grown on non-selective (-LT) and selective (-LTHA) medium.

(*B*) Y2H assays to test the interaction between DMS3 and different APC/C subunits. Empty BD vector was the negative control.

(C) Reciprocal *in vitro* pull down assays to examine the RDM1-APC10 interaction. Precipitates were analyzed by western blotting using anti-GST and anti-His antibodies.

(D) Co-IP between APC8 and RDM1. GFP-Trap beads were used to immunoprecipitate RDM1 in inflorescences of 35S::APC8-YFP transgenic plants. Anti-

RDM1 and anti-GFP antibodies were used to detect corresponding proteins by western blotting.

(E) Schematic showing the putative D boxes (R1, R2, and R3) in DMS3 and RDM1.

(F) Y2H assays to test the interaction between APC10 and RDM1 or mutated RDM1 ($RDM1^{R}$). Yeast cells co-transformed by indicated combination of plasmids were diluted and spotted onto non-selective (-LT) and selective (-LTHA) media.

(G) *In vitro* pull down assays to examine the interaction between APC10 and RDM1 or mutated RDM1 ($RDM1^{R}$). Protein precipitates were analyzed by western blotting using an anti-His antibody.

(H) Y2H assays to test the interaction between RDM1 and mutated DMS3. Yeast cells co-transformed by indicated combination of plasmids were diluted and spotted onto non-selective (-LT) and selective (-LTHA) media.

(I) Semi *in vivo* pull down to detect the interaction between DMS3 and DRD1. GST-DMS3 or DMS3-$DMS3^{R3}$ were incubated with cell lysates from 35S::DRD1-Flag plants, and anti-YFP antibody was used to detect DRD1 retained on the beads.

(J) Western blotting showing the protein amounts in individual transgenic plants of DMS3 and $DMS3^{R3}$. Total proteins from seedlings were subjected to SDS-PAGE and followed by western blots using anti-DMS3 antibody. Hsc70 was used as the loading control. Quantification was from three biological replicates.

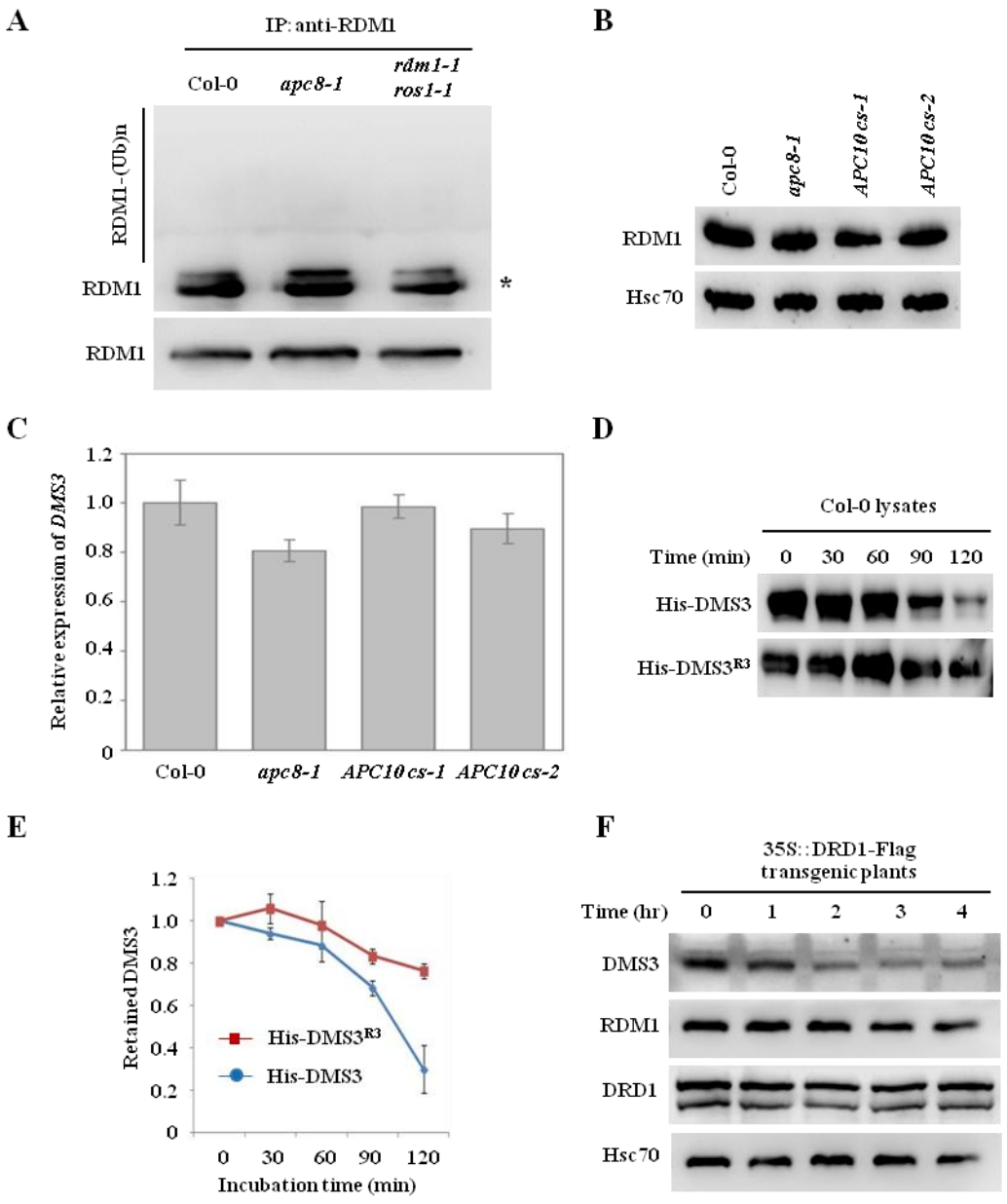

Fig. S4. RDM1 and DRD1 are not regulated by APC/C *in vivo*.

(A) Detection of RDM1 ubiquitination in Col-0, *ros1-1 rdm1-1*, and *apc8-1*. Anti-RDM1 immunoprecipitates were immunoblotted with anti-RDM1 for Ub-RDM1 conjugates (upper), RDM1 levels, and the IP efficiency (lower). The bands at the bottom of the upper panel indicate the expected size of RDM1 in plants. The bottom panel indicates the IP efficiency of anti-RDM1.

(B) Western blotting to determine RDM1 levels in seedlings of Col-0, *apc8-1*, and

two independent cs lines of *APC10*. Hsc70 was the loading control.

(C) qRT-PCR analysis to assess expression of *DMS3* in Col-0, *apc8-1*, and two independent cs lines of *APC10*. The *UBQ5* gene was the internal control. Data are presented as mean ± standard errors (SE), n = 3.

(D) Decay rate of DMS3 and DMS3^{R3} examined by the cell-free protein decay assay. Fixed amounts of His-DMS3 or His-DMS3^{R3} were incubated with equal amounts of cell lysates of Col-0 for the indicated times. Levels of DMS3 were determined with anti-His antibody.

(E) Statistical analysis of the decay rate of His-DMS3 and His-DMS3^{R3} in **(***D***)**. Data are presented as mean ± standard errors (SE), n = 3.

(F) Decay rate of the DMS3, RDM1, and DRD1 in 35S::DRD1-Flag transgenic plants. Total proteins were extracted and incubated for the indicated times. The levels of DMS3, RDM1, and DRD1 were determined with anti-DMS3, anti-RDM1, or anti-Flag antibodies. Hsc70 was the loading control.

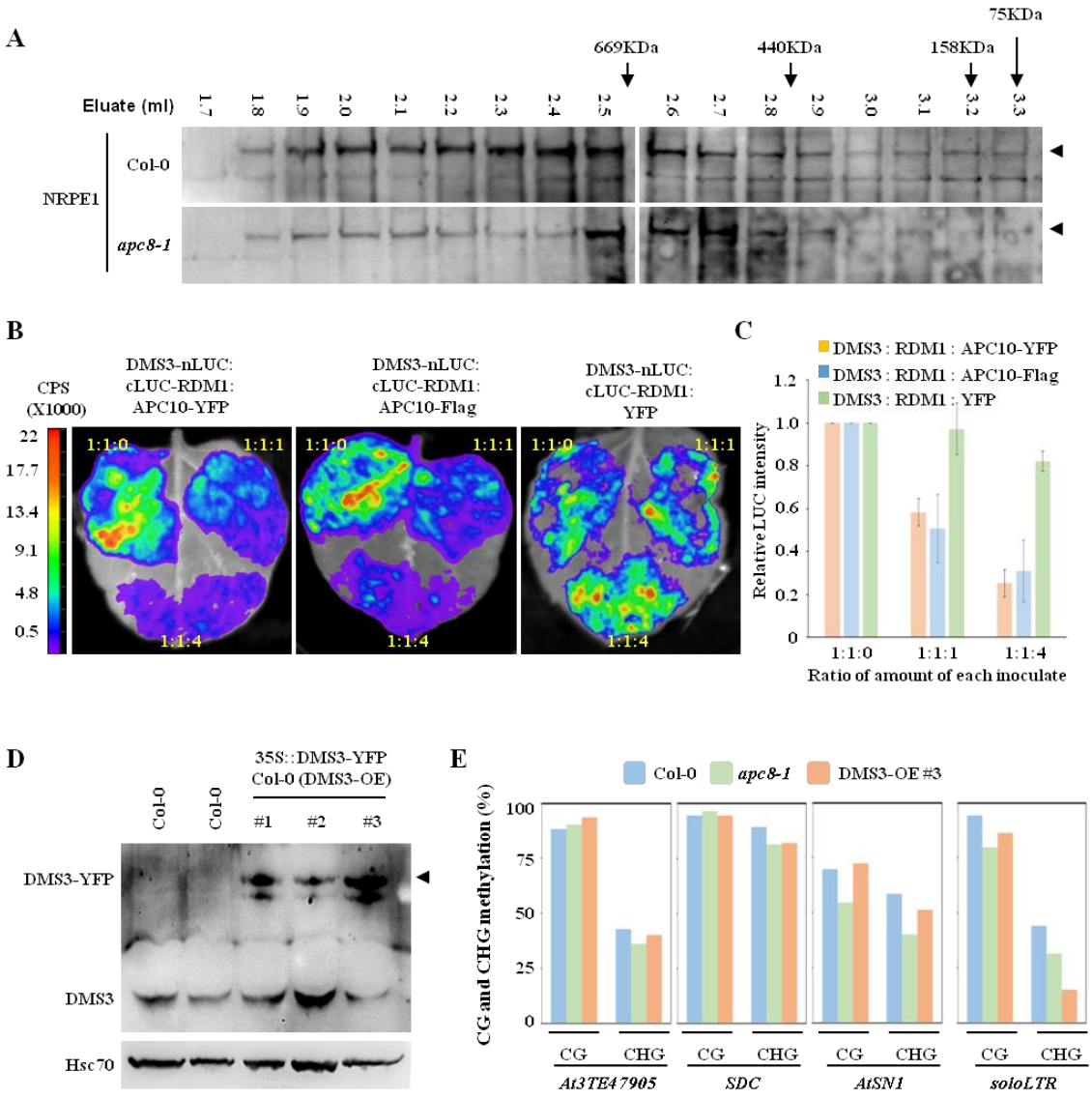

Fig. S5. Gel filtration of NRPE1 and DNA methylation in DMS3-OE plants.

(A) Gel filtration of NRPE1 in Col-0 and *apc8-1*. Elution profiles of NRPE1 on a Superose 6 column were detected by western blotting with an anti-NRPE1 antibody. Eluate fractions and sizing standards are indicated. Specific bands are marked by arrowheads.

(B) Split luciferase complementation assays were performed to examine the interaction between RDM1 and DMS3 after the addition of APC10. Three plasmids were inoculated into tobacco leaves at the indicated ratios, and the YFP alone (pCambia2302) was used as the negative control.

(C) Statistical analysis of LUC activity produced from the interaction between RDM1 and DMS3 after the addition of APC10 or YFP alone in (*B*). Relative intensities of LUC were normalized with indiGo software (Berthhold Tech). Data are presented as mean ± SE; n = 3.

(D) Western blotting to determine DMS3-YFP and DMS3 levels in seedlings of Col-0, and the over-expression lines of *35S::DMS3-YFP* in the Col-0 background (DMS3-OE). #1, 2, and 3 indicate individual transgenic lines. Hsc70 was used as the loading control.

(E) CHG/CG methylation of *AtSN1, SDC, soloLTR* and *At3TE47905* was determined by bisulfite sequencing in Col-0, *apc8-1*, and DMS3-OE plants.

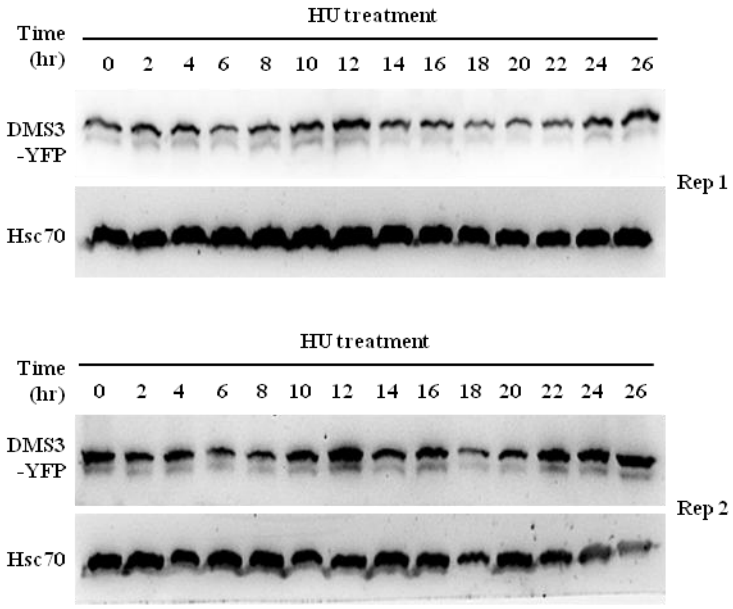

Fig. S6. The protein level of DMS3 fluctuates during cell cycle progression.

Western blotting analysis detected the transgene DMS3-YFP levels of root tips at indicated time points after hydroxyurea (HU) treatments. Hsc70 was used as the loading control. Two biological replicates were shown.

Supplemental Datasets

Dataset S1. De-repressed TEs in Col-0, *apc8-1*, *nrpe1-12*.

TE loci with average count values of at least 1 were identified in RNA sequencing libraries, and are listed with information of their annotation and normalized reads.

Dataset S2. Categories of up-regulated TEs in *apc8-1* and *nrpe1-12*.
Up-regulated TE loci (Table S1) were assigned based on the Transposon Families in the TAIR database. The percentages of different classes of deregulated TEs in *apc8-1* and *nrpe1-12* were evaluated using the Kolmogorov-Smirnov test.

Dataset S3. List of 24-nt siRNA in *apc8-1*and *pol iv*.
24-nt siRNAs with RPTM>=50 in Col-0 and fold-changes smaller than -1 in *pol iv* were identified in small RNA sequencing libraries, and are listed with information of their annotation and read numbers.

Dataset S4. Primary data for locus-specific bisulfite sequencing.

Dataset S5. Primer sequences used in this study.