Supplemental Information

Inventory of information:

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- **4 supplemental tables**
- **Supplemental Experimental Procedures**
- **Supplemental References**

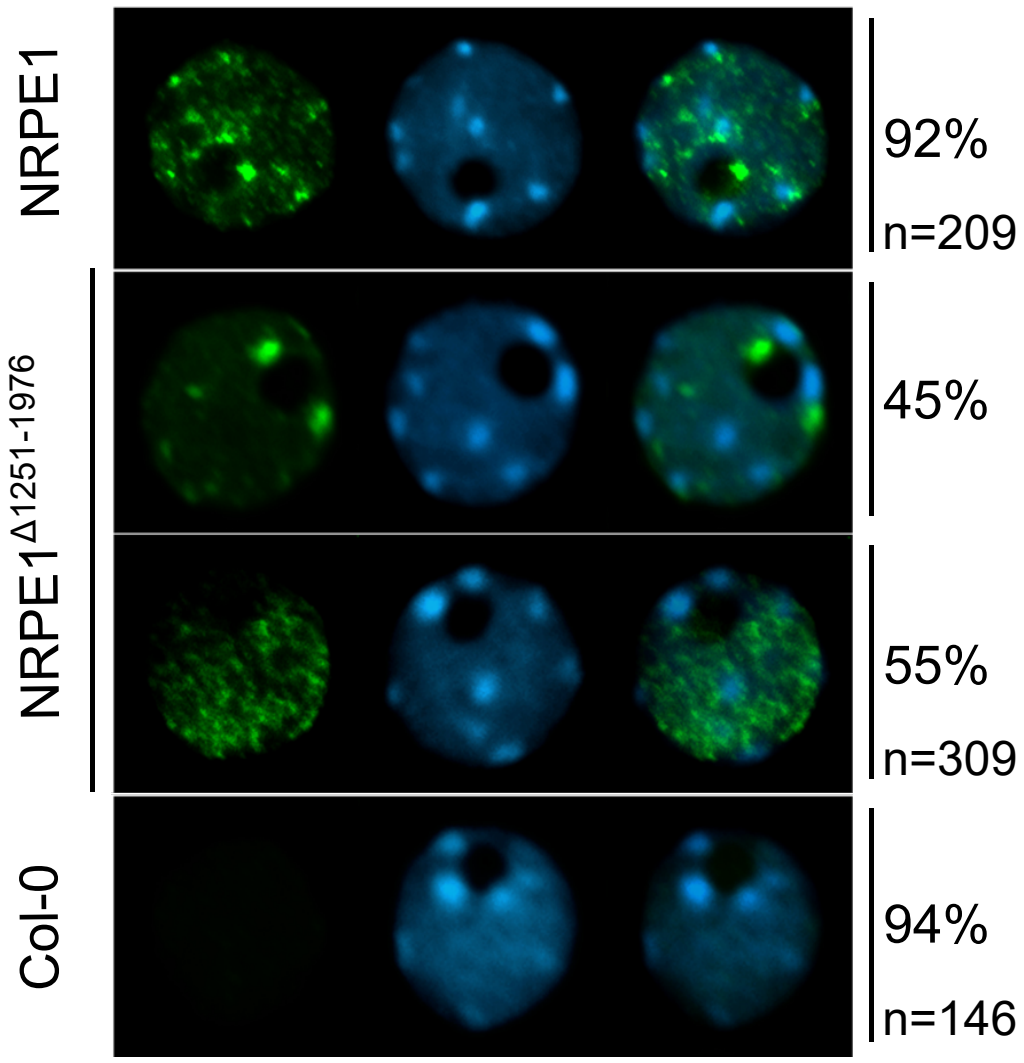
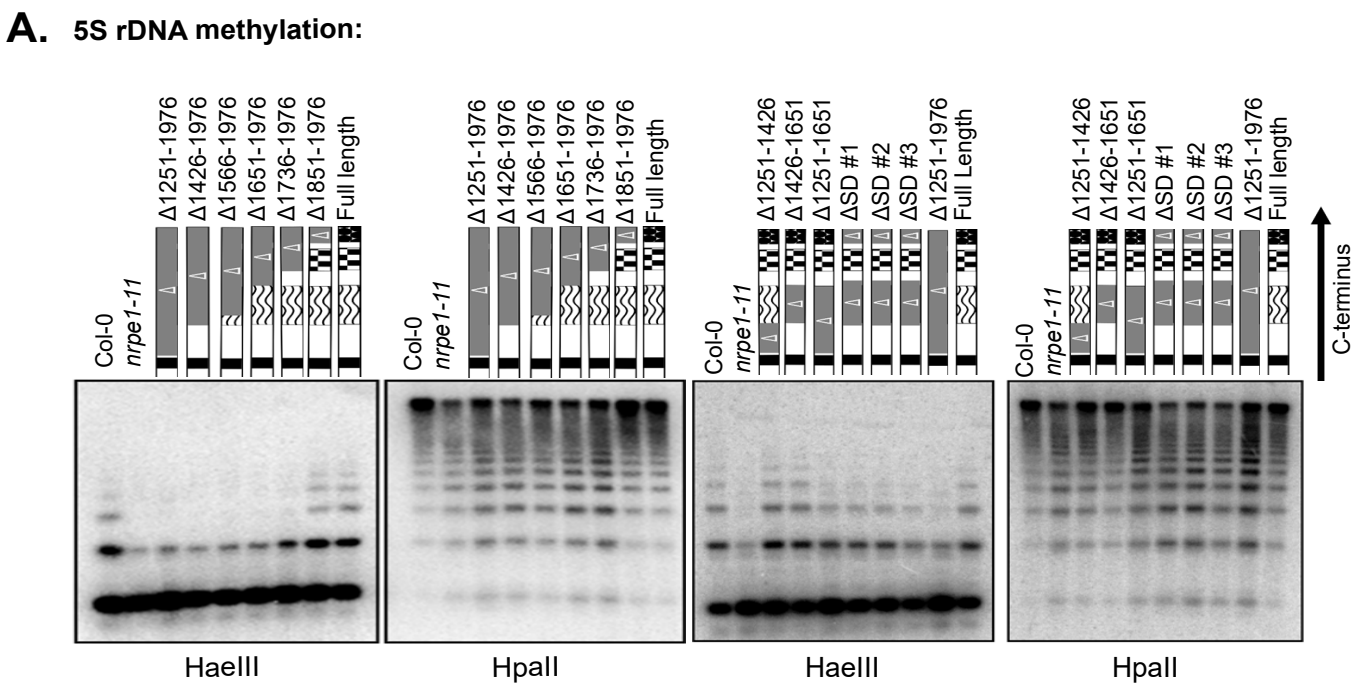
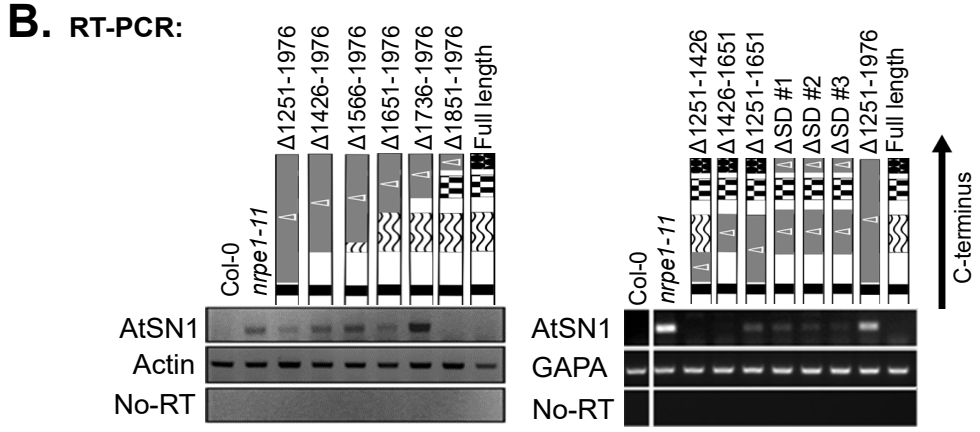

NRPE1
NRPE1Δ1251-1976
Col-0
92%
n=209
45%
55%
n=309
94%
n=146

Figure S1. Nuclear immunolocalization of full-length NRPE1 and NRPE1 missing the CTD (Δ1251-1976). Related to Figure 1. Nuclei were fixed using formaldehyde and incubated with anti-HA antibodies recognizing the HA epitope tag at the C-termini of the recombinant proteins. Nuclei of non-transgenic Col-0 serve as a negative control. Nuclei were counterstained with DAPI. Dark, DAPI-negative regions, appearing as black holes, are nucleoli. Bright Pol V foci frequently observed at the edges of the nucleoli (apparent in the top two rows of images) correspond to Cajal bodies. The number of nucleoli examined, and the frequency of localization patterns that resemble the representative images shown, are provided to the right of the images. Note that Pol V assembled using NRPE1 that is missing the CTD displays a nuclear localization pattern only subtly different from that observed for full-length NRPE1. Whereas full-length NRPE1 is present in a larger number of concentrated foci, CTD-deleted NRPE1 tends to be more diffuse, or present in smaller, punctate foci.


A. 5S rDNA methylation:
Col-0
nrpe1-11
Δ1251-1976
Δ1426-1976
Δ1566-1976
Δ1651-1976
Δ1736-1976
Δ1851-1976
Full length
Δ1251-1426
Δ1426-1651
Δ1251-1651
ΔSD #1
ΔSD #2
ΔSD #3
Δ1251-1976
Full Length
C-terminus
HaeIII
HpaII
HaeIII
HpaII


B. RT-PCR:
Col-0
nrpe1-11
Δ1251-1976
Δ1426-1976
Δ1566-1976
Δ1651-1976
Δ1736-1976
Δ1851-1976
Full length
AtSN1
Actin
No-RT
Δ1251-1426
Δ1426-1651
Δ1251-1651
ΔSD #1
ΔSD #2
ΔSD #3
Δ1251-1976
Full length
C-terminus
AtSN1
GAPA
No-RT

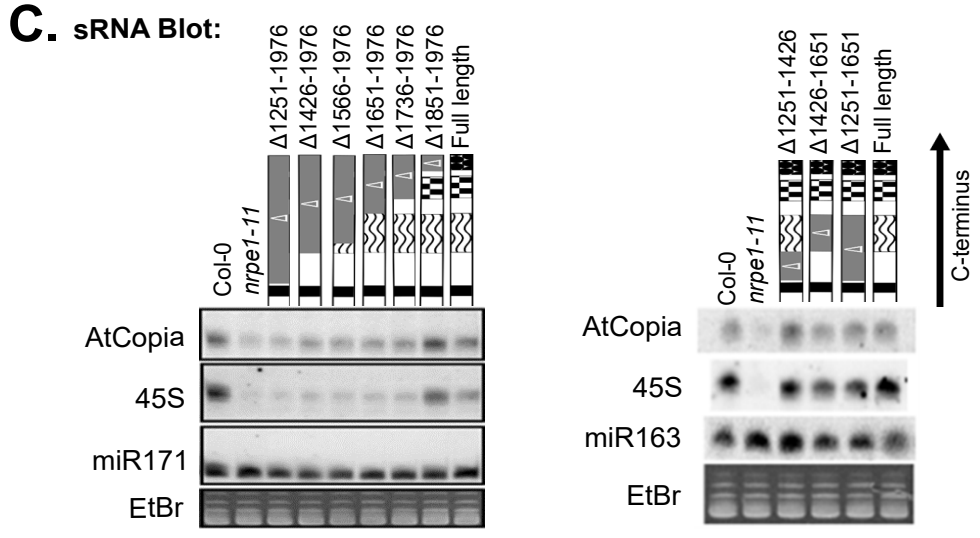

C. sRNA Blot:
Col-0
nrpe1-11
Δ1251-1976
Δ1426-1976
Δ1566-1976
Δ1651-1976
Δ1736-1976
Δ1851-1976
Full length
AtCopia
45S
miR171
EtBr
Δ1251-1426
Δ1426-1651
Δ1251-1651
Full length
C-terminus
AtCopia
45S
miR163
EtBr

Figure S2. Consequences of NRPE1 CTD deletions on 5S rRNA gene silencing, AtSN1 transposon silencing and siRNA abundance. Related to Figure 2.
A. Southern blot analysis of 5S rRNA gene repeats after digestion with the methylation sensitive enzymes, *Hae*III or *Hpa*II. Decreased cytosine methylation results in increased digestion, and smaller products. Note the importance of the DeCL domain, and the combinatorial effects of deletions affecting the linker plus 17 aa repeat subdomains or 17aa repeat plus QS subdomains, which have greater effect than deletions of the individual subdomains.
B. RT-PCR analysis of *AtSN1* retrotransposon transcripts generated when Pol V-dependent silencing is lost. *Actin* and *GAPA* are Pol II transcribed genes included as positive controls. Note that silencing is lost if the DeCL subdomain is deleted.
C. Northern blot analysis of Pol IV and Pol V-dependent 24nt siRNAs corresponding to *AtCopia* transposons or 45S rRNA gene repeats. *miR171* and *miR163* are 21nt micro RNAs unaffected by the RdDM pathway, included as positive controls. Note that siRNA levels are reduced when the DeCL subdomain is deleted.

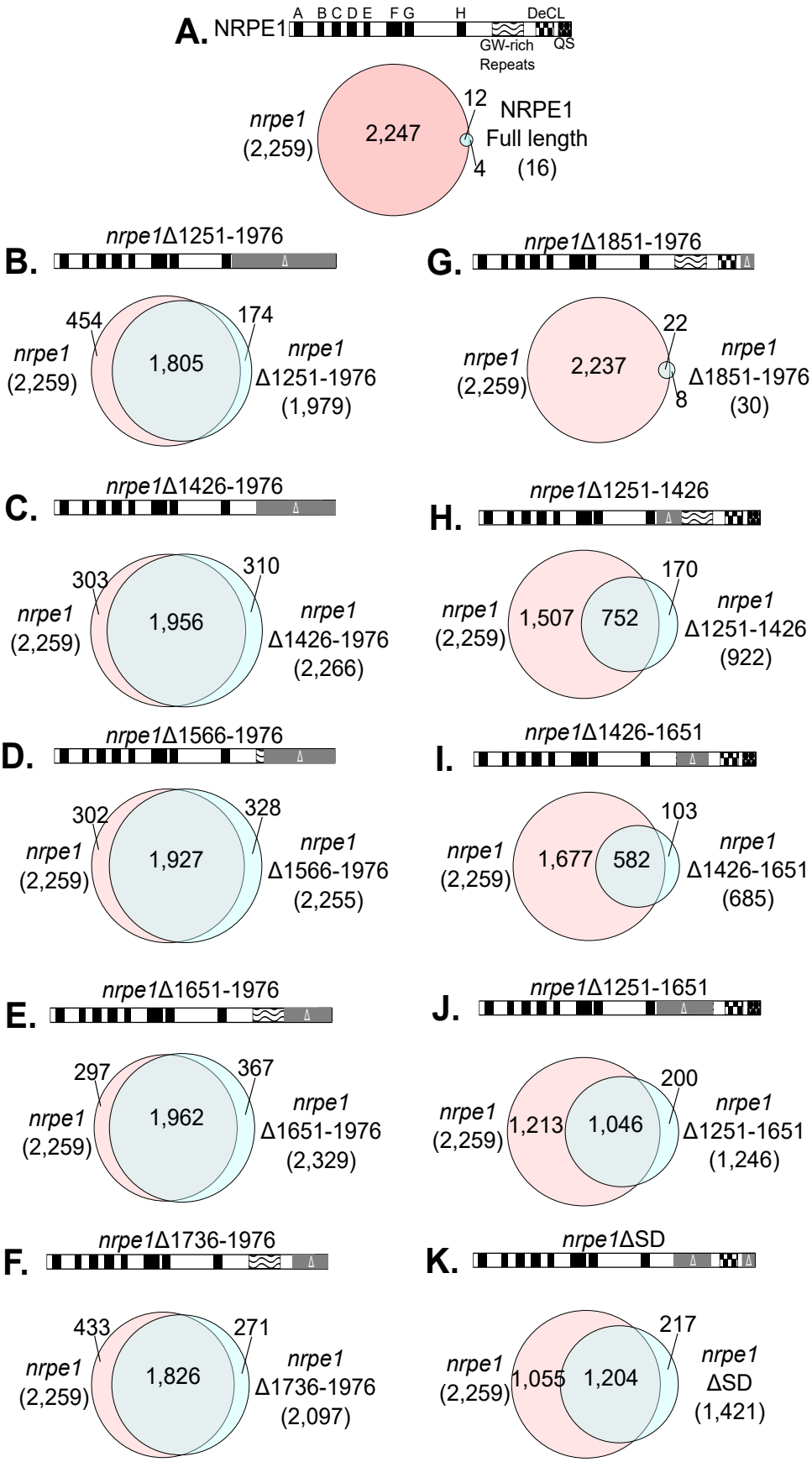

A.
NRPE1
A B C D E F G H DeCL
GW-rich Repeats
QS
nrpe1 (2,259)
2,247
12
4
NRPE1 Full length (16)
B.
nrpe1Δ1251-1976
454
1,805
174
nrpe1 (2,259)
nrpe1 Δ1251-1976 (1,979)
C.
nrpe1Δ1426-1976
303
1,956
310
nrpe1 (2,259)
nrpe1 Δ1426-1976 (2,266)
D.
nrpe1Δ1566-1976
302
1,927
328
nrpe1 (2,259)
nrpe1 Δ1566-1976 (2,255)
E.
nrpe1Δ1651-1976
297
1,962
367
nrpe1 (2,259)
nrpe1 Δ1651-1976 (2,329)
F.
nrpe1Δ1736-1976
433
1,826
271
nrpe1 (2,259)
nrpe1 Δ1736-1976 (2,097)
G.
nrpe1Δ1851-1976
22
2,237
8
nrpe1 (2,259)
nrpe1 Δ1851-1976 (30)
H.
nrpe1Δ1251-1426
1,507
752
170
nrpe1 (2,259)
nrpe1 Δ1251-1426 (922)
I.
nrpe1Δ1426-1651
1,677
582
103
nrpe1 (2,259)
nrpe1 Δ1426-1651 (685)
J.
nrpe1Δ1251-1651
1,213
1,046
200
nrpe1 (2,259)
nrpe1 Δ1251-1651 (1,246)
K.
nrpe1ΔSD
1,055
1,204
217
nrpe1 (2,259)
nrpe1 ΔSD (1,421)

Figure S3. Overlap between CHH DMRs that become hypomethylated in the *nrpe1-11* mutant and DMRs that remain hypomethylated (un-rescued) in each CTD deletion line. Related to Figure 2. All DMRs are defined relative to methylation levels in wild-type Col-0 (see Methods for details).

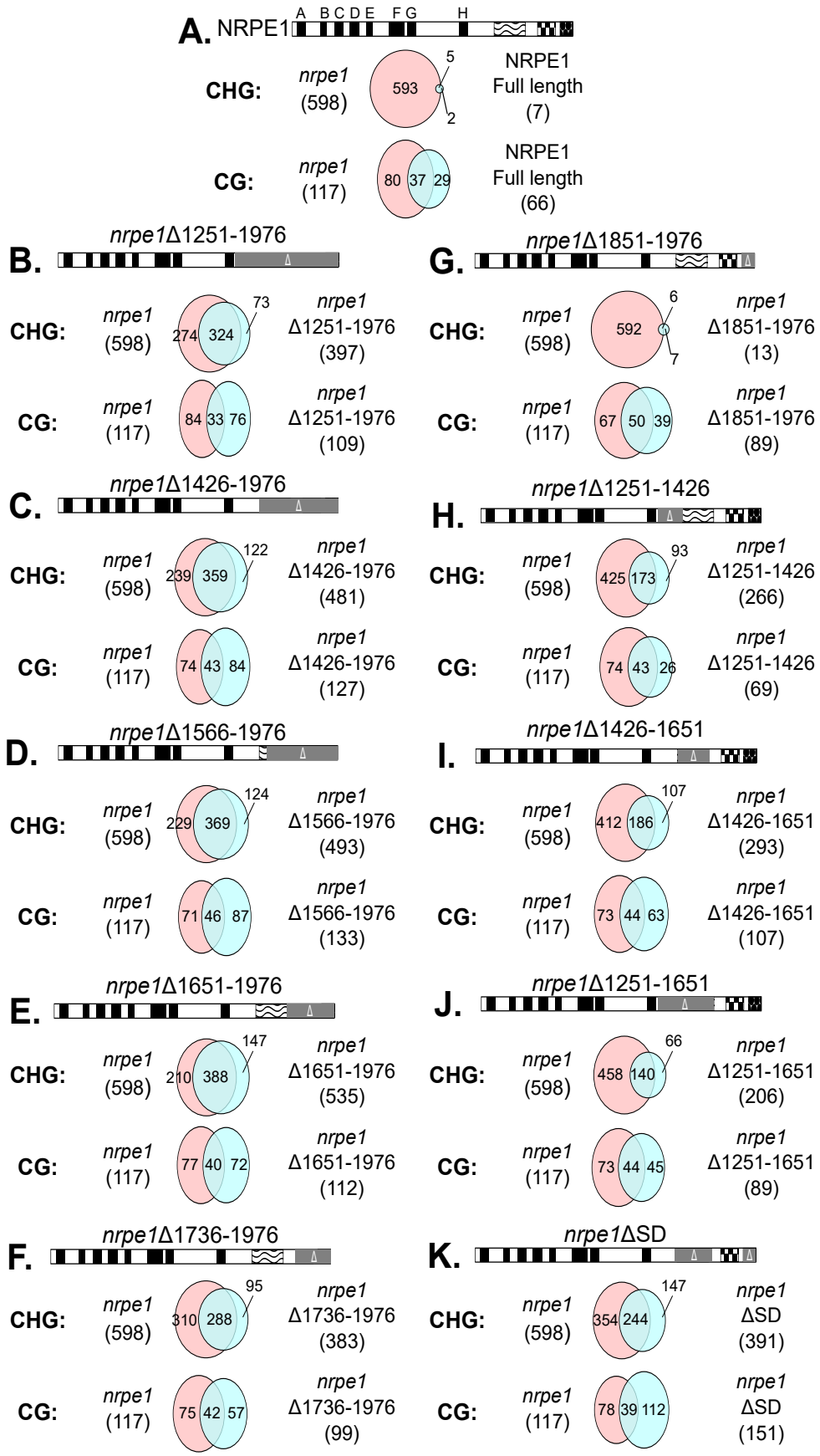
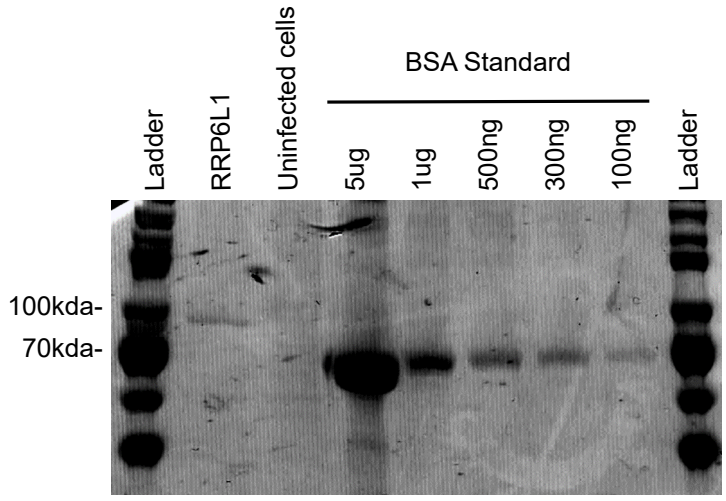
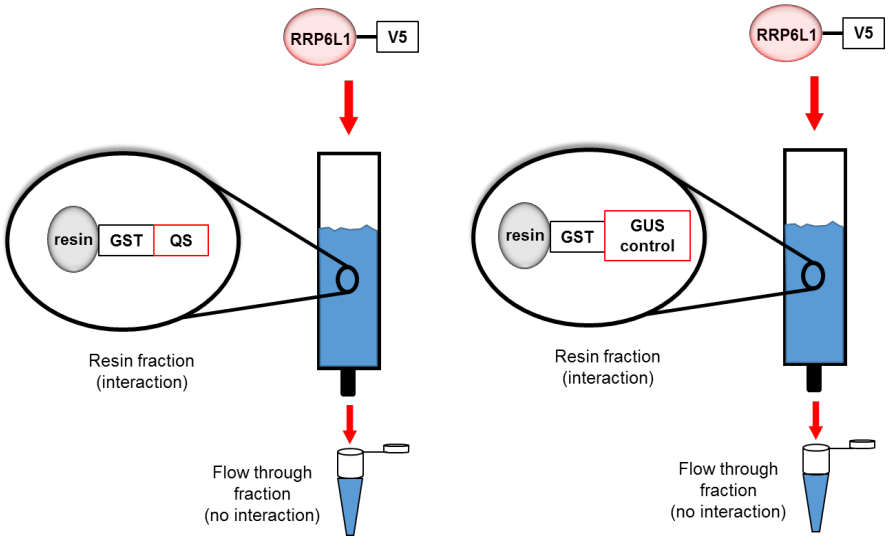

A.
NRPE1
A B C D E F G H
CHG:
nrpe1
(598)
593
5
2
NRPE1
Full length
(7)
CG:
nrpe1
(117)
80 37 29
NRPE1
Full length
(66)
B.
nrpe1Δ1251-1976
CHG:
nrpe1
(598)
274 324
73
nrpe1
Δ1251-1976
(397)
CG:
nrpe1
(117)
84 33 76
nrpe1
Δ1251-1976
(109)
G.
nrpe1Δ1851-1976
CHG:
nrpe1
(598)
592
6
7
nrpe1
Δ1851-1976
(13)
CG:
nrpe1
(117)
67 50 39
nrpe1
Δ1851-1976
(89)
C.
nrpe1Δ1426-1976
CHG:
nrpe1
(598)
239 359
122
nrpe1
Δ1426-1976
(481)
CG:
nrpe1
(117)
74 43 84
nrpe1
Δ1426-1976
(127)
H.
nrpe1Δ1251-1426
CHG:
nrpe1
(598)
425 173
93
nrpe1
Δ1251-1426
(266)
CG:
nrpe1
(117)
74 43 26
nrpe1
Δ1251-1426
(69)
D.
nrpe1Δ1566-1976
CHG:
nrpe1
(598)
229 369
124
nrpe1
Δ1566-1976
(493)
CG:
nrpe1
(117)
71 46 87
nrpe1
Δ1566-1976
(133)
I.
nrpe1Δ1426-1651
CHG:
nrpe1
(598)
412 186
107
nrpe1
Δ1426-1651
(293)
CG:
nrpe1
(117)
73 44 63
nrpe1
Δ1426-1651
(107)
E.
nrpe1Δ1651-1976
CHG:
nrpe1
(598)
210 388
147
nrpe1
Δ1651-1976
(535)
CG:
nrpe1
(117)
77 40 72
nrpe1
Δ1651-1976
(112)
J.
nrpe1Δ1251-1651
CHG:
nrpe1
(598)
458 140
66
nrpe1
Δ1251-1651
(206)
CG:
nrpe1
(117)
73 44 45
nrpe1
Δ1251-1651
(89)
F.
nrpe1Δ1736-1976
CHG:
nrpe1
(598)
310 288
95
nrpe1
Δ1736-1976
(383)
CG:
nrpe1
(117)
75 42 57
nrpe1
Δ1736-1976
(99)
K.
nrpe1ΔSD
CHG:
nrpe1
(598)
354 244
147
nrpe1
ΔSD
(391)
CG:
nrpe1
(117)
78 39 112
nrpe1
ΔSD
(151)

Figure S4. Overlap between CG and CHG DMRs that become hypomethylated in the *nrpe1-11* mutant and DMRs that remain hypomethylated (un-rescued) in each CTD deletion line. Related to Figure 2. All DMRs are defined relative to methylation levels in wild-type Col-0 (see Methods for details).

A.



B. **Interaction assay:**



Immunoblot:

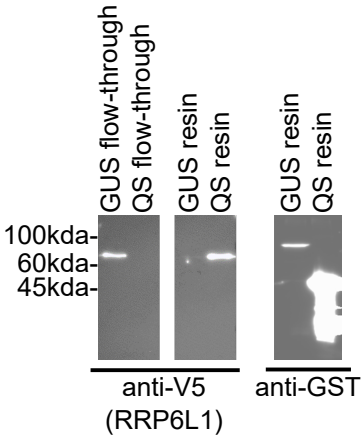


Figure S5. RRP6L1 expressed in insect cells physically interacts with the QS subdomain of NRPE1. Related to Figures 4-5.

A. Coomassie stained gel showing purified RRP6L1, uninfected cell lysate and a Bovine Serum Albumin (BSA) dilution series. Recombinant RRP6L1 was engineered to have C-terminal His and V5 tags and was expressed in insect cells using a baculovirus vector. The His-tag allowed the protein to be purified using nickel affinity resin.

B. *In vitro* assay for RRP6L1 interaction with the QS domain (aa1851-1976) of the Pol V CTD. GST-fused to a QS domain polypeptide or a GUS protein control were expressed in bacterial cells and immobilized on glutathione sepharose resin. The resins were then incubated with RRP6L1, and washed extensively to remove unbound protein. RRP6L1 was retained on the resin with immobilized GST-QS peptide, but not the GST-GUS control resin, as shown by SDS-PAGE and immunoblot analyses of resin and flow-through fractions using anti-V5 (recognizing the epitope tag on RRP6L1) or anti-GST antibodies.

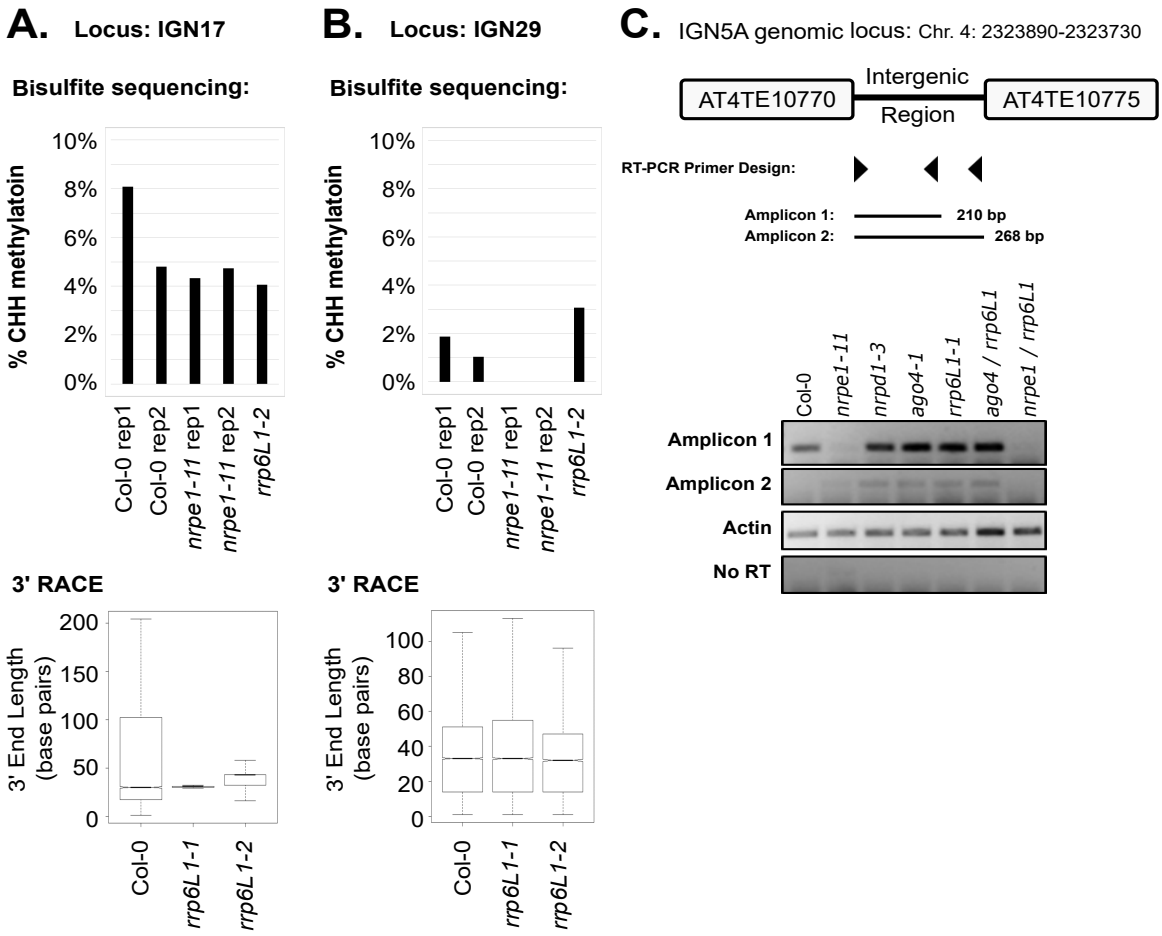

A.
Locus: IGN17
Bisulfite sequencing:
% CHH methylatoin
10%
8%
6%
4%
2%
0%
Col-0 rep1
Col-0 rep2
nrpe1-11 rep1
nrpe1-11 rep2
rrp6L1-2
3' RACE
3' End Length (base pairs)
200
150
100
50
0
Col-0
rrp6L1-1
rrp6L1-2
B.
Locus: IGN29
Bisulfite sequencing:
% CHH methylatoin
10%
8%
6%
4%
2%
0%
Col-0 rep1
Col-0 rep2
nrpe1-11 rep1
nrpe1-11 rep2
rrp6L1-2
3' RACE
3' End Length (base pairs)
100
80
60
40
20
0
Col-0
rrp6L1-1
rrp6L1-2
C.
IGN5A genomic locus: Chr. 4: 2323890-2323730
AT4TE10770
Intergenic Region
AT4TE10775
RT-PCR Primer Design:
Amplicon 1:
210 bp
Amplicon 2:
268 bp
Col-0
nrpe1-11
nrpd1-3
ago4-1
rrp6L1-1
ago4 / rrp6L1
nrpe1 / rrp6L1
Amplicon 1
Amplicon 2
Actin
No RT

Figure S6. Evidence of RRP6L1 trimming of Pol V transcript 3' ends is not detected at loci where methylation is not dependent on RRP6L1. Related to Figure 6 and Table S4. Bisulfite sequencing and 3’ RACE results for Pol V dependent transcripts from: **A.** *IGN17* (Chr1: 13585614-13585914) and **B.** *IGN29* (Chr2: 15314700-15315000). Histograms show %CHH methylation measured by whole genome bisulfite sequencing. Box plots show the measured 3’ end lengths in basepairs relative to the internal gene specific primer used in the 3’ RACE PCR. **C**. RT-PCR of the Pol V-dependent transcript *IGN5*. Strand-specific RT was performed such that Amplicon 2 detects products with extended 3’ ends.

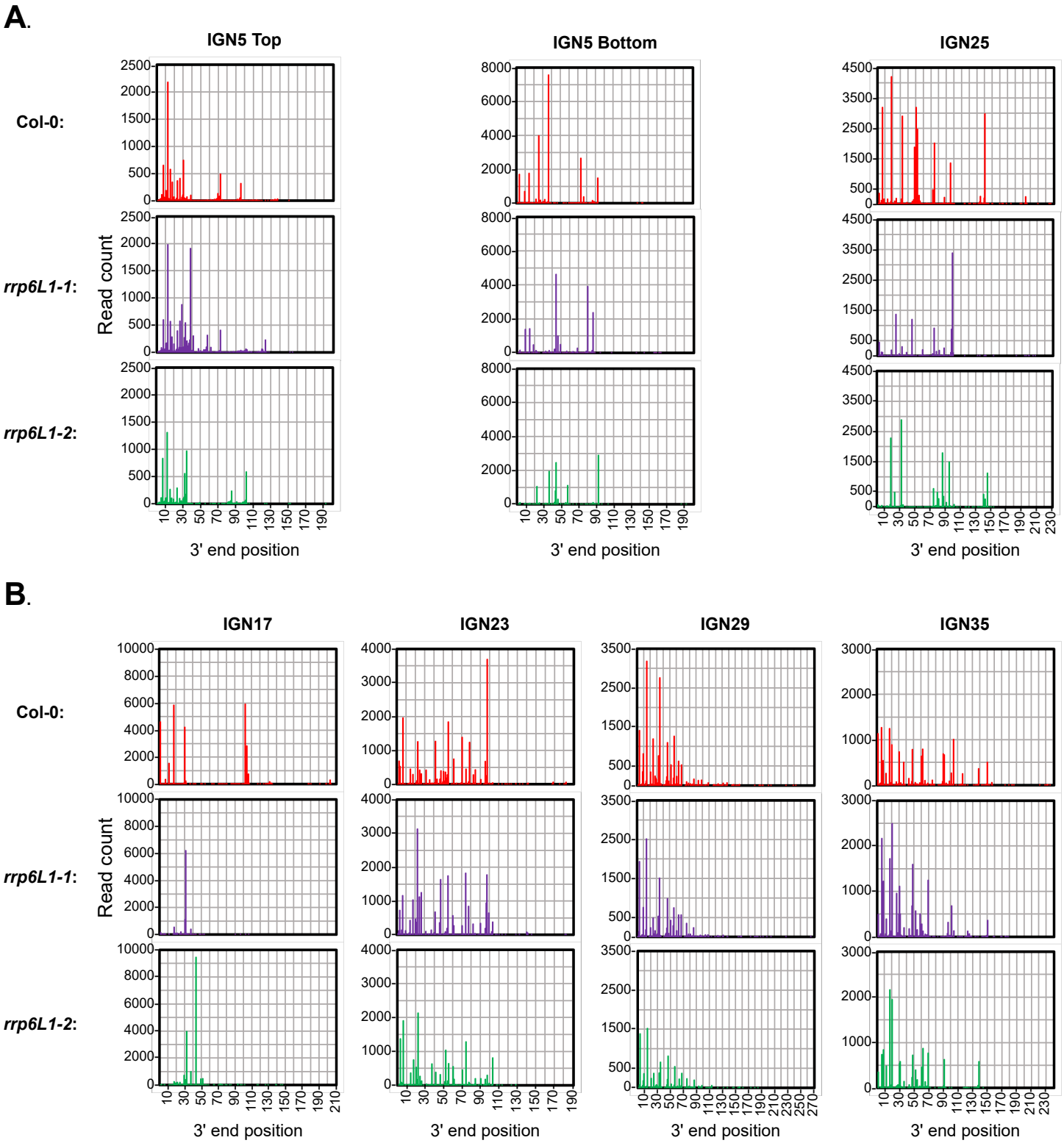

A.
IGN5 Top
IGN5 Bottom
IGN25
Col-0:
rrp6L1-1:
rrp6L1-2:
Read count
3' end position
B.
IGN17
IGN23
IGN29
IGN35
Col-0:
rrp6L1-1:
rrp6L1-2:
Read count
3' end position

Figure S7. Distribution of Pol V transcript 3' ends detected by 3' RACE. Related to Figures 6, S6 and Table S4. Shown are the read counts for each 3' end position (designated in number of nucleotides 3' of the 3' RACE internal gene specific primer) detected in Col-0 and *rrp6L1* mutants at loci that undergo RRP6L1 dependent cytosine methylation (**A**) and loci where methylation is established independent of RRP6L1 (**B**).

Table S1. Genome wide bisulfite sequencing stats. Related to Figures 2 and 4.

Sample	Accession	Source	Reads	Uniquely Mapped reads	% Uniquely Mapping	De-duplicated reads	X Coverage	ChrC mapped cytosines	ChrC methylated cytosines	% Con-version
Col-0 rep1	GSM2451991	This Study	44,460,982	28,174,303	63.37%	18,209,996	21.61	144,836,404	546,268	99.62%
nrpe1-11 rep1	GSM2451993	This Study	40,333,481	26,492,991	65.68%	17,792,319	21.11	133,795,491	473,114	99.65%
NRPE1 full length rep1	GSM2451995	This Study	38,013,343	24,809,228	63.57%	16,762,607	19.89	122,987,872	455,828	99.63%
nrpe1 Δ1251-1651 rep1	GSM2452013	This Study	39,404,933	25,049,535	65.51%	16,831,751	19.97	134,435,407	559,419	99.58%
nrpe1 Δ1736-1976 rep1	GSM2452005	This Study	42,908,764	28,108,382	65.51%	18,273,998	21.68	155,434,322	622,802	99.60%
nrpe1 ΔSD rep1	GSM2452015	This Study	50,595,778	32,648,309	64.53%	20,377,533	24.18	145,903,463	528,060	99.64%
nrpe1 Δ1251-1976 rep1	GSM2451997	This Study	53,439,616	34,979,265	65.46%	22,291,361	26.45	133,919,257	566,206	99.58%
nrpe1 Δ1426-1976 rep1	GSM2451999	This Study	51,368,333	33,002,962	64.25%	21,801,303	25.87	104,634,789	424,011	99.59%
nrpe1 Δ1566-1976 rep1	GSM2452001	This Study	56,843,693	36,706,316	64.57%	23,284,990	27.63	127,791,392	519,814	99.59%
nrpe1 Δ1651-1976 rep1	GSM2452003	This Study	53,562,928	35,115,705	65.56%	22,913,656	27.19	135,499,575	522,918	99.61%
nrpe1 Δ1851-1976 rep1	GSM2452007	This Study	55,574,617	35,954,311	64.70%	23,483,894	27.87	118,852,143	475,319	99.60%
nrpe1 Δ1251-1426 rep1	GSM2452009	This Study	53,129,033	34,028,642	64.05%	21,626,413	25.66	153,671,866	591,426	99.62%
nrpe1 Δ1426-1651 rep1	GSM2452011	This Study	52,653,935	32,656,149	62.02%	20,765,787	24.64	158,511,978	657,066	99.59%
Col-0 rep2	GSM2451992	This Study	38,733,453	24,915,519	64.33%	17,430,896	20.68	107,648,039	507,038	99.53%
nrpe1-11 rep2	GSM2451994	This Study	47,986,860	32,613,597	67.96%	20,687,953	24.55	167,076,843	666,888	99.60%
NRPE1 full length rep2	GSM2451996	This Study	39,119,926	25,903,331	66.22%	17,207,809	20.42	117,907,853	451,212	99.62%
nrpe1 Δ1251-1651 rep2	GSM2452014	This Study	40,469,664	27,130,551	67.04%	17,310,659	20.54	147,108,843	553,722	99.62%
nrpe1 Δ1736-1976 rep2	GSM2452006	This Study	42,943,982	28,722,593	66.88%	18,348,619	21.77	158,837,642	639,988	99.60%
nrpe1 ΔSD rep2	GSM2452016	This Study	53,753,337	35,075,045	65.25%	20,982,367	24.90	181,096,087	612,749	99.66%
nrpe1 Δ1251-1976 rep2	GSM2451998	This Study	37,542,631	24,504,676	65.27%	14,985,591	17.78	96,626,985	409,069	99.58%
nrpe1 Δ1426-1976 rep2	GSM2452000	This Study	44,592,352	28,924,727	64.86%	18,355,489	21.78	108,448,450	440,344	99.59%
nrpe1 Δ1566-1976 rep2	GSM2452002	This Study	47,837,147	31,523,368	65.90%	19,758,216	23.45	105,286,294	439,365	99.58%
nrpe1 Δ1651-1976 rep2	GSM2452004	This Study	40,852,904	27,256,676	66.72%	19,002,414	22.55	84,571,586	328,210	99.61%
nrpe1 Δ1851-1976 rep2	GSM2452008	This Study	51,011,863	34,050,659	66.75%	21,615,480	25.65	125,382,330	482,896	99.61%
nrpe1 Δ1251-1426 rep2	GSM2452010	This Study	41,468,051	28,087,846	67.73%	17,838,446	21.17	136,594,443	642,013	99.53%
nrpe1 Δ1426-1651 rep2	GSM2452012	This Study	46,546,645	30,612,248	65.77%	18,737,480	22.23	164,427,854	609,330	99.63%
ago4	SRR534197	Stroud et. al 2013	275864062	141822231	51.41%	72,649,618	30.35	2,132,733	32,465	98.48%
ago6	SRR534199	Stroud et. al 2013	264475851	155448950	58.78%	79,669,287	33.29	2,109,298	33,817	98.40%
idn2/idnl1/idnl2	SRR534236	Stroud et. al 2013	297740753	191836596	64.43%	98,476,031	41.15	2,159,170	9,851	99.54%
spt5L/ktf1	SRR534238	Stroud et. al 2013	228175457	135524716	59.39%	71,571,920	29.90	2,129,739	20,346	99.04%
rrp6L1	SRR960246	Zhang et. al 2014	22546460	18,235,132	80.88%	17,553,435	26.40	75,322,021	271,250	99.64%
dms3	SRR534216	Stroud et. al 2013	121938777	59,497,931	48.88%	38,079,894	15.91	1,975,813	12,519	99.37%
drd1	SRR534221	Stroud et. al 2013	276695439	164579432	59.48%	78,002,308	32.59	2,148,153	24,427	98.86%
suvh2/suvh9	SRR1023830	Johnson et. al 2014	120048009	73,916,597	61.57%	44,319,269	18.52	2,130,682	27,182	98.72%

Table S2. Differentially methylated regions. Related to Figures 2, 3, and 4.

Table S3. sRNA sequencing data for DMRs. Related to Figure 3F.

Table S4. Read counts for 3' RACE deep sequencing. Related to Figure 6 and S5. *Reads also mapped to a highly similar TE gene, AT3G43686, suggesting a spurious amplification of a derepressed TE in this line. This sequence was filtered from all samples before further analyses

	IGN5 top	**IGN5 btm**	**IGN23**	**IGN25**	**IGN35**	**IGN17**	**IGN29**
Col-0	8,539	22,079	21,930	28,398	14,377	27,611	18,555
nrpe1-11	0	4	248,926*	2	3	2	3
rrp6L1-1	12,888	17,850	24,308	10,636	20,028	9,307	14,880
rrp6L1-2	6,999	11,627	15,845	12,843	13,641	18,209	9,140

Supplemental Experimental Procedures

Nuclear immunolocalization

For immunolocalization experiments, nuclei from 4-week old plants were fixed in 4% formaldehyde and incubated overnight at 4°C with antibodies recognizing the C-terminal HA epitope of NRPE1 transgene products, as described in (Pontes et al., 2006). Chromatin was counterstained with DAPI.

Southern blot methylation assays

For the Southern blot analyses described in Figure S2, 250 ng DNA was digested with *Hpa*II or *Hae*III (NEB) and subject to agarose gel electrophoresis and transfer to uncharged nylon membranes. Membranes were probed with a 5S rDNA gene probe generated by random priming of a full length 5S gene PCR product amplified from clone pTC4.2 (Campell et al., 1992).

RT-PCR

The RT-PCR shown in Figure S2B was conducted using ~1 µg RNA. RNA was treated with RQ1 DNase (Promega) and used to generate random-primed cDNA using degenerate dN6 primers (NEB) and Superscript III Reverse Transcriptase (Invitrogen) according to the manufacturer's instructions. PCR was conducted with GoTaq green (Promega) using primers listed below. PCR products were analyzed by agarose gel electrophoresis.

sRNA blot

RNA was isolated from 350 mg inflorescence tissue using a mirVana miRNA isolation kit (Ambion). 9.5 µg RNA was resolved by denaturing polyacrylamide gel electrophoresis on a 20% (w/v) gel. Gels were electroblotted (20 mA/cm2 for 2 hours) to Magnacharge nylon membranes (0.22 µm; Osmonics) using a semidry transfer apparatus. Riboprobes were generated using the mirVana probe construction kit (Ambion) using oligonucleotides specific for a given small RNA and labeling by T7 polymerase transcription in the presence of α-32P CTP. DNA oligonucleotides are listed in below. Blots were hybridized in 50% formamide, 0.25 M Na2HPO4 (pH 7.2), 0.25 M NaCl, 7% SDS at 42°C (14–16 hours) followed by two 15 minute washes at 37°C in 2× SSC, two 15 minute washes at 37°C in 2× SSC, 0.1% SDS, and a 10 minute wash in 0.5× SSC, 1% SDS.

Heterologous protein expression

Arabidopsis thaliana RRP6L1 cDNA, minus the stop codon, was amplified from total RNA using primers listed below. cDNA clones were recombined into the Baculovirus expression system and expressed in Sf9 cells using the Baculo-Direct kit according to the manufacturer's protocol (Invitrogen). Transformed viruses expressed RRP6L1 modified with a C-terminal 6X His and V5 tag. Protein was purified from P3 virus infected 75 ml Sf9 cultures after incubation for 72 hours. Cells were pelleted by centrifugation at 150 x g for 2 minutes and washed twice with 1X PBS. After the second wash, cells were suspended in binding buffer (0.5 M NaCl, 20 mM Tris-HCl pH 8, 5 mM imidazole) and lysed via sonication with a Biorupter UCD-200 sonicator at 4ºC with the settings at Low for 1 minute, and intervals of 10 seconds on, 10 seconds off. Lysate was cleared by centrifugation at 14,000 x g for 20 minutes. Protein was purified from cell extract using Novagen HisBind slurry according to the manufacturer's protocol. For negative controls, uninfected cells were subject to the same protocol.

In vitro transcription assays

Polymerases were immunoprecipitated from 4 g frozen leaf tissue as described previously, except that 150 mM $NaSO_4$ and 5mM $MgSO_4$ were used in extraction buffer in place of NaCl and $MgCl_2$, 25 µl HA resin was used for the IP, and NP-40 was not added to the wash buffer. Resin-associated polymerases were washed once with CB100 buffer (100 mM potassium acetate, 25 mM HEPES, pH 7.9, 20% glycerol, 0.1 mM EDTA, 0.5 mM DTT, 1 mM PMSF), resuspended in 50 µL CB100 buffer and supplemented with 50 µL 2x transcription reaction buffer (120 mM ammonium sulfate, 40 mM HEPES, pH 7.6, 20 mM magnesium sulfate, 20 µM zinc sulfate, 20% glycerol, 0.16 U/µL RNaseOUT , 20 mM DTT , 2 mM ATP, 2 mM UTP, 2 mM GTP, 0.08 mM CTP, 0.2 mCi/mL alpha ^{32}P-CTP

and 4 pmols of template). Transcription reactions were incubated for 60 minutes at room temperature on a rotating mixer, and stopped by addition of 50 mM EDTA and heating at 75 ˚C for five minutes. Reaction products were enriched using PERFORMA spin columns (EdgeBio) as per manufacturer's protocol and precipitated using 1/10 volume of 3M sodium acetate, pH 5.2, 20 µg glycogen and 2 volumes isopropanol at -20˚C overnight. Precipitated RNA was resuspended in 5 µL 2X RNA loading buffer (NEB), incubated at 70°C for 5 minutes and loaded on to 15% denaturing polyacrylamide gels. Gels were transferred to Whatman 3M filter paper, dried under vacuum for 2 hours at 80 °C, and subjected to phosphorimaging.

Templates for *in vitro* transcription were generated by using equimolar amounts (10 mM each) of DNA template and the RNA primer were mixed in the annealing buffer containing 20 mM HEPES-KOH (pH 7.6) and 100 mM potassium acetate. The mixture was boiled in a water bath and slowly cooled to room temperature. Template sequences are listed below.

In vitro interaction assays

To test for interaction of insect cell expressed RRP6L1 and the QS subdomain of the NRPE1 CTD, the region encoding amino acids 1851-1976 of the NRPE1 cDNA was amplified and cloned into pDEST15 (Invitrogen) (Primers listed below). The *β-Glucuronidase* (GUS) reporter gene, also expressed in pDEST15, was used as a negative control. Vectors were expressed *E. coli* BL21-AI One-Shot chemically competent cells grown in LB broth supplemented with 50ug/ml Carbenicillin. For protein expression, 2.5 ml overnight culture was added to 50 ml LB broth (50 µg/ml Carbenicillin), which was incubated at 37ºC at 225 rpm until an OD600 was reached (~45 minutes). Expression was induced by the addition of L-arabinose to a concentration of 0.2% and cultures were incubated for a further 3 hours. Cells were then pelleted by centrifugation at 10,000 x g for 10 minutes. Pellets were suspended in 10 ml cold binding buffer (140 mM NaCl, 2.7 mM KCl, 10 mM K_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) and lysed via sonication with a Biorupter UDC 200 for 10 minutes on High, 30 seconds on, 30 seconds off. Supernatant was cleared twice by centrifugation for 10 minutes at 10,000 x g and then added to a column containing 300 µl Glutathione Sepharose 4B slurry (Amersham) that had previously been equilibrated with 2 washes of 5 ml binding buffer. Supernatant was incubated with glutathione slurry for 2 hours with end over end rotation at 4ºC. Sepharose was washed 2X with 10 ml binding buffer, followed by the addition of 450 ng insect cell expressed RRP6L1 suspended in 10 ml binding buffer. Columns were incubated overnight with end over end rotation at 4ºC. Sepharose was then washed 3 times with 10 ml binding buffer. Sepharose or flow-through fractions were suspended in SDS page buffer and western blots were performed as described in the section, *Immunoprecipitation and western blot analysis*, using commercially available anti-GST or anti-V5 antibodies.

Bisulfite sequencing analyses

Base calling, adapter trimming, and read size selection (>/= 35bp) was performed using bcl2fastq v2.16.0.10. Reads were further quality processed to remove end methylation bias and low quality reads using Cutadapt version 1.9.1 (Martin, 2011) with the following commands:

cutadapt -U 7 -u 2 -q 25

Cleaned reads were mapped to the *Arabidopsis thaliana* TAIR10 genome, followed by the removal of PCR duplicates, and extraction of methylation information for cytosines with a minimum of 5 read coverage using Bismark version 0.16.1 default settings (Krueger and Andrews, 2011). The bisulfite conversion rate was calculated based on the number of methylated cytosines divided by total mapped cytosines (converted and un-converted) to the chloroplast genome.

Differently methylated regions (DMRs) were defined using information for cytosines in the CG, CHG, or CHH context for two replicates of each genotype relative to Col-0 using the R package methylKit version 0.9.5 (Akalin et al., 2012). Default methylKit commands were used with the following parameters: The genome was split into 300 base pair sliding windows with a step size of 200 base pairs and a minimum coverage requirement for each window of 10 informative cytosines with at least 5 read coverage each. Significant CHH and CHG hypo-DMRs were defined as those regions with a minimum 25% decrease in methylation relative to Col-0 and a q-value of less than or equal to 0.01. For CG methylation a minimum 40% decrease cutoff relative to Col-0 was used. To quantify the number of DMRs in each line, overlapping DMRs were merged into a single region. To quantify percent

methylation across regions of interest, the methylKit regionCounts function was utilized with the genomic coordinates of interest input as a bed file. In box plots, outliers 1.5 times the interquartile range beyond the upper or lower quartile were omitted. Box plots were generated using the boxplot command in R and heatmaps and clustering analyses were generated using the heatmap2 R package with the default clustering method. Differentially methylated regions defined for each genotype are available in Table S2. Accession numbers for previously published bisulfite sequencing data analyzed are in Table S1.

sRNA sequencing analyses

Raw reads were adapter and quality trimmed and size selected using Cutadapt version 1.9.1 using the following commands:

cutadapt -a TGGAATTCTCGGGTGCCAAG -q 20 -m 15 -M60

Reads were first filtered of all structural RNAs (tRNAs, rRNAs, snRNAs, and snoRNAs) by mapping to a genome consisting of sequences corresponding to all genomic coordinates identified as structural RNAs on TAIR (www.arabidopsis.org) using bowtie2 default commands. All unmapped reads were saved for further analysis.

Filtered and cleaned reads were mapped to the *Arabidopsis* TAIR10 genome using ShortStack version 3.4 default settings (Johnson et al., 2016). sRNA counts for regions of interest were extracted from bam files using the ShortStack --locifile file function. Counts were normalized as reads per million based on total mapped reads. Accession numbers for previously published sRNA data for Col-0 and *nrpd1-3* are SRR2075819 and SRR2505369, respectively.

3'RACE amplicon sequencing

RNA was extracted from ~2.5 week old above ground tissues. Since Pol V transcripts lack a poly-A tail (Wendte and Pikaard, 2017; Wierzbicki et al., 2008), a 3' poly-A tail was added to 10 µg RNA prior to reverse transcription using commercially available Poly-A polymerase according to the manufacturer's protocol (NEB). Poly-A tailed RNA was cleaned using the Zymogen RNA clean and concentrate kit. RNA was reverse transcribed using SuperScript III (Invitrogen) with a oligo-d(T) primer that added nested PCR primer binding sequences. Primary PCR was performed on 1 µl cDNA using Platinum Taq (Invitrogen) according to the manufacturer's protocol with 3' RACE Primer 1 and the appropriate gene specific primer. Nested PCR was also performed with Platinum Taq on 0.6 µl primary PCR product using 3' RACE Primer 2 and the appropriate internal gene specific primer. PCR conditions for both PCR reactions were: 94ºC 2 minutes, 35 cycles of (94ºC 30 seconds, 60ºC 30 seconds, 72ºC 30 seconds). PCR product sizes were verified to be 500 basepairs or less on ethidium bromide-stained agarose gels, pooled, and cleaned using a Qiagen PCR clean-up kit according to the manufacturer's protocol. Primer sequences are listed below

Pooled and cleaned PCR products for each genotype (up to 1.2 µg) were prepared for Illumina sequencing with NEBnext end repair enzyme and Klenow fragment (3'-5' exo -) from NEB according to the manufacturer's protocols. After each enzyme treatment, products were cleaned with the Zymogen Clean and Concentrator-5 kit. Adapters (NextFlex PCR free DNA barcodes from BIOO Scientific) were ligated to end repaired, A-tailed PCR products using T4 DNA ligase (NEB) according to the manufacturer's protocol at 16ºC for 2 hours. Libraries were cleaned using 1 volume AMPure XP beads (Beckman Coulter) according to the manufacturer's instructions and quality checked and quantified with an Agilent Tapestation. 250 basepair single end reads were generated on an Illumina MiSeq by the Indiana University Center for Genomics and Bioinformatics (IU CGB).

Base calling and adapter trimming was performed using bcl2fastq v2.16.0.10 by the IU CGB. Reads were further processed with the following steps to ensure that only reads containing true 3' end sequences were included in further analyses. Reads were first selected for and trimmed of the 3' RACE Primer 2 sequence using Cutadapt version 1.9.1 with the following commands:

cutadapt -g ctactactaggccacgcgtcgactagtac -q 20,20 -m 15 --discard-untrimmed

Reads were then selected for and trimmed of the artificially added poly-A tail with the following:

cutadapt -g "t(210)" -m 15 --discard-untrimmed

Trimmed reads were then mapped to sequences extracted from the *A. thaliana* TAIR10 genome corresponding to the PCR target regions (consisting of genomic sequences from the following coordinates: Chr4: 2318164-2323164 (*IGN5* top strand); Chr4: 2323282-2328282 (*IGN5* bottom strand); Chr4: 2577970-2582970 (*IGN23*); Chr4: 5459147-5464147 (*IGN25*); Chr2: 15314824-15319824 (*IGN29*); Chr4: 6549057-6554057 (IGN35); Chr1: 13585635-13590635 (*IGN17*) using bowtie2 in local alignment mode (Langmead and Salzberg, 2012). SAM output files were converted to bam files, sorted, indexed, and split into individual bam files for each PCR target region using samtools (Li et al., 2009). Bam files were converted to bed files using bedtools (Quinlan and Hall, 2010). Bed files were further filtered for high quality mapping scores (MapQ >= 40) and reads that mapped to the appropriate strand, selected for by the GSP. Read coordinates in the bed files were used to calculate the 3' end lengths relative to the coordinate of the 3' end of the internal gene-specific primer. Boxplots of relative 3' end lengths reported in Figures 6 and S6 were generated using the R boxplot function.

Oligonucleotides used in this study

NRPE1 construct cloning

nrpe1 Δ1251-1976	nrpe1-F	CAC CGC GTA CTA CAA ACG GAA ACG GTC A
	nrpe1-d1251-R	GAT AAA GAA GAA ACA GAT GTG TAC AGC TTC CTT
nrpe1 Δ1426-1976	nrpe1-F	CAC CGC GTA CTA CAA ACG GAA ACG GTC A
	nrpe1-d1426-R	CCA CGA TTT GTC TGA AAC AGA TTT GTG TCC
nrpe1 Δ1566-1976	nrpe1-F	CAC CGC GTA CTA CAA ACG GAA ACG GTC A
	nrpe1-d1566-R	CCC CAT ACC CCA ACC AGC AGG
nrpe1 Δ1651-1976	nrpe1-F	CAC CGC GTA CTA CAA ACG GAA ACG GTC A
	nrpe1-d1651-R	GTC TTC TGC AGT GGG ACT TGG C
nrpe1 Δ1736-1976	nrpe1-F	CAC CGC GTA CTA CAA ACG GAA ACG GTC A
	nrpe1-d1736-R	CTC AGA GGT GAA TGA GTC CAA GCG
nrpe1 Δ1851-1976	nrpe1-F	CAC CGC GTA CTA CAA ACG GAA ACG GTC A
	nrpe1-d1851-R	GAA TTC ATT GAC AAG TAC TTT ACG AAA CCT
nrpe1 Δ1251-1426	d1251-1426 mut-F	GTG TAC AGC TTC CTT GAC AAA AAG AAC TGG GGA ACT GAA TCA GC
	d1251-1426-F	GAC AAA AAG AAC TGG GGA ACT GAA TCA GC
	d1251-1426 mut-R	AAG GAA GCT GTA CAC ATC TGT TTC TTC TTT ATC ATC TAG ACC AGT CTG C
	d1251-1426-R	ATC TGT TTC TTC TTT ATC ATC TAG ACC AGT CTG C
nrpe1 Δ1251-1651	d1251-1651 mut-F	GTG TAC AGC TTC CTT AAG GAT ACC AAT GAG GAT GAT AGA AAT CCG TG
	d1251-1651-F	AAG GAT ACC AAT GAG GAT GAT AGA AAT CCG TG
	d1251-1651 mut-R	AAG GAA GCT GTA CAC ATC TGT TTC TTC TTT ATC ATC TAG ACC AGT CTG C
	d1251-1651-R	ATC TGT TTC TTC TTT ATC ATC TAG ACC AGT CTG C
nrpe1 Δ1426-1651	d1426-1651-F	GTT TCA GAC AAA TCG TGG AAG GAT ACC AAT GAG
	d1426-1651-R	CTC ATT GGT ATC CTT CCA CGA TTT GTC TGA AAC
NRPE1 1426-1851 (Y2H)	NRPE1 1426-F	CAC CAT GTG GGA CAA AAA GAA CTG GGG AAC TG
	NRPE1 1851-R	TCA AGG TTT CGT AAA GTA CTT GTC AAT GAA TTC

NRPE1 1426-1651 (Y2H)	NRPE1 1426-F	CAC CAT GTG GGA CAA AAA GAA CTG GGG AAC TG
	NRPE1 1651-R	TCA GTC TTC TGC AGT GGG ACT TGG C
NRPE1 1851-1976 (Y2H)	NRPE1 1851-F	CAC CAT GCC TCG GCC TAG CGG AAA CAG
	NRPE1 1976-R	TTA TGT CTG CGT CTG GGA CGG
Chop PCR Figure 2A, 6		
IGN5B	Forward	CGCAGCGGAATTGACATCCTATC
	Reverse	TCGGAAAGAGACTCTCCGCTAGAAA
IGN5A	Forward	CCCGAGAAGAGTAGAACAAATGCTAAAA
	Reverse	CTGAGGTATTCCATAGCCCCTGATCC
IGN23	Forward	ACTGAAAATTGTAAACAAAGAAACGGCACTACA
	Reverse	GATCGGTCCATAAACTTGTTGGGTTT
IGN25	Forward	TCAAACCAAACCCCGAACTT
	Reverse	ATTCGTGTGGGCTTGGCCTCTT
IGN26	Forward	CTCTTTCAGTGCGACAGCCTCAT
	Reverse	CGGCCAGGAAACCCTAACTTCC
IGN35	Forward	GCCGGGCTTAGAGGATAGGTAC
	Reverse	TTCCTCTTTGAGCTTGACCA
P6	Forward	GGCTTCGATAGGAAGAATGCCC
	Reverse	GTGAAACTGCCAGATCCAAATTC
P9	Forward	CCGTTTCTGGGTAGGTCGGC
	Reverse	CCAATTCTTGACTGGAGTGGAC
AtSN1	Forward	ACCAACGTGCTGTTGGCCCAGTGGTAAATC
	Reverse	AAAATAAGTGGTGGTTGTACAAGC
soloLTR	Forward	ATAAAACTCGAAACAAGAGTTTTCTTATTGCTTTC
	Reverse	TAATGGTATTATTTTGATCAGTGTTATAAACCGGA
RT PCR Figure 2C and 5C		
IGN5A amplicon 1	RT	CGCAGCGGAATTGACATCCTATC
	PCR	TCGGAAAGAGACTCTCCGCTAGAAA
IGN5A amplicon 2	RT	CGCAGCGGAATTGACATCCTATC
	PCR	ACAAGGACCCAACCATGTCCGC
IGN22	RT	CGGGTCCTTGGACTCCTGAT
	PCR	TCGTGACCGGAATAATTAAATGG
IGN23	RT	ACTGAAAATTGTAAACAAAGAAACGGCACTACA
	PCR	GATCGGTCCATAAACTTGTTGGGTTT
IGN24	RT	CGCATACGATGGTCGGAGAGTT
	PCR	GCTTATCATTATCCAAACTTGATCCTATCCTAAA
IGN25	RT	CTTCTTATCGTGTTACATTGAGAACTCTTTCC
	PCR	ATTCGTGTGGGCTTGGCCTCTT

IGN26	RT	CGGCCAGGAAACCCTAACTTCC
	PCR	CTCTTTCAGTGCGACAGCCTCAT
IGN29	RT	CATGTGTTGTTGTGTGTTTCACTAT
	PCR	TAAAACTTTTCCCGCCAACCA
IGN34	RT	CCCTTTCATCGACACTGACA
	PCR	ATGAATAACAAATTTGGAGTCGTC
IGN35	RT	GACGGACCAAACGATTTCAT
	PCR	TTCCTCTTTGAGCTTGACCA
IGN36	RT	CAGTTTTGGGTGCGGTTTAT
	PCR	GACAAAAATTGCTTTAGACCATGA
AtSN1b	RT	CCTTTCCAAGACACCATCTCAACAAC
	PCR	TCCTCAACAAAAATAATTCCGAACGAC
TUB8	RT	TCGAATCGATCCCGTGCT
	PCR	TCACATACAAGGTGGCCAATG
qRT PCR Figure 5A		
IGN5A	GSP RT primer	CGCAGCGGAATTGACATCCTATC
	PCR P1	TACATGAAGAAAGCCCAAACC
	PCR P2	ATTGGGCCGAATAACAGC
IGN22	GSP RT primer	CGGGTCCTTGGACTCCTGAT
	PCR P1	GCCCCACAAATGGAAGAGGTCT
	PCR P2	TCGTGACCGGAATAATTAAATGG
IGN29	GSP RT primer	CATGTGTTGTTGTGTGTTTCACTAT
	PCR P1	CGTTTGTTTATGTAGGGCGAAAG
	PCR P2	TAAAACTTTTCCCGCCAACCA
ACT2	GSP RT primer	AAGAATGGAACCACCGATCCAGACA
	PCR P1	GCTGACCGTATGAGCAAAGA
	PCR P2	CTGTACTTCCTTTCAGGTGGT
RT PCR Figure S1B		
AtSN1	P1	ACC AAC GTG CTG TTG GCC CAG TGG TAA ATC
	P2	AAA ATA AGT GGT GGT TGT ACA AGC
Actin	P1	TCA TAC TAG TCT CGA GAG ATG ACT CAG ATC ATG TTT GAG
	P2	TCA TTC TAG AGG CGC GCC ACA ATT TCC CGT TCT GCG GTA G
GAPA	P1	GGT AGG ATC GGG AGG AAC
	P2	GAT AAC CTT CTT GGC ACC AG
sRNA blot Figure S1C		
miR171		TGATTGAGCCGCGCCAATATCcctgtctc
miR163		TTGAAGAGGACTTGGAACTTCGATcctgtctc

45S		CAATGTCTGTTGGTGCCAAGAGGGAAAAGGGCcctgtctc
AtCopia		TTATTGGAACCCGGTTAGGAcctgctc
In vitro transcription templates Figure 2D		
CTAATTCGCAACGAAAGCTTCGATCTGACTGTGTACGCCTGGTCCGACTCG		
UGCAUAAAGACCAGGC		
Cloning for heterologous expression		
RRP6L1 full cDNA	Forward	CACCATGAGATTTGATGATCCCATGGATGAGTTC
	Reverse	AATGTTAAGAAAGCCACGTTTCATATTCATG
NRPE1 aa 1851-1976	Forward	CAC CAT GCC TCG GCC TAG CGG AAA CAG
	Reverse	TTA TGT CTG CGT CTG GGA CGG
3' RACE		
IGN5 top	GSP1 external	TGTTGGTTATTGGGCCGCATGATACA
	GSP2 internal	AGCATTTGTTCTACTCTTCTCGGGAACT
IGN5 bottom	GSP1 external	TGAAGAAAGCCCAAACCATACACT
	GSP2 internal	GCTGTTATTCGGCCCAATAGCC
IGN17	GSP1 external	AACCCTAGCCTTTCATTAAAACCCTCTC
	GSP2 internal	CCCTCTCTGAATTCATCTCTAGTATTT
IGN23	GSP1 external	ACTGAAAATTGTAAACAAAGAAACGGCACTACA
	GSP2 internal	GCCATTAGTTTTAGATGGACTGCAA
IGN25	GSP1 external	CTGGCATTCCTTATTTGCAAGG
	GSP2 internal	CTTCTTATCGTGTTACATTGAGAACTCTTTCC
IGN29	GSP1 external	CGTTTGTTTATGTAGGGCGAAAG
	GSP2 internal	TGGTTGGCGGGAAAAGTTTTA
IGN35	GSP1 external	TGGTCAAGCTCAAAGAGGAA
	GSP2 internal	CAAGCGGAAATTAACGGTCAAATAAC
3'RACE RT primer		GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTTT
3'RACE PCR primer 1		CUACUACUACUAGGCCACGCGTCGACTAGTAC
3'RACE PCR primer 2		GGCCACGCGTCGACTAGTAC

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