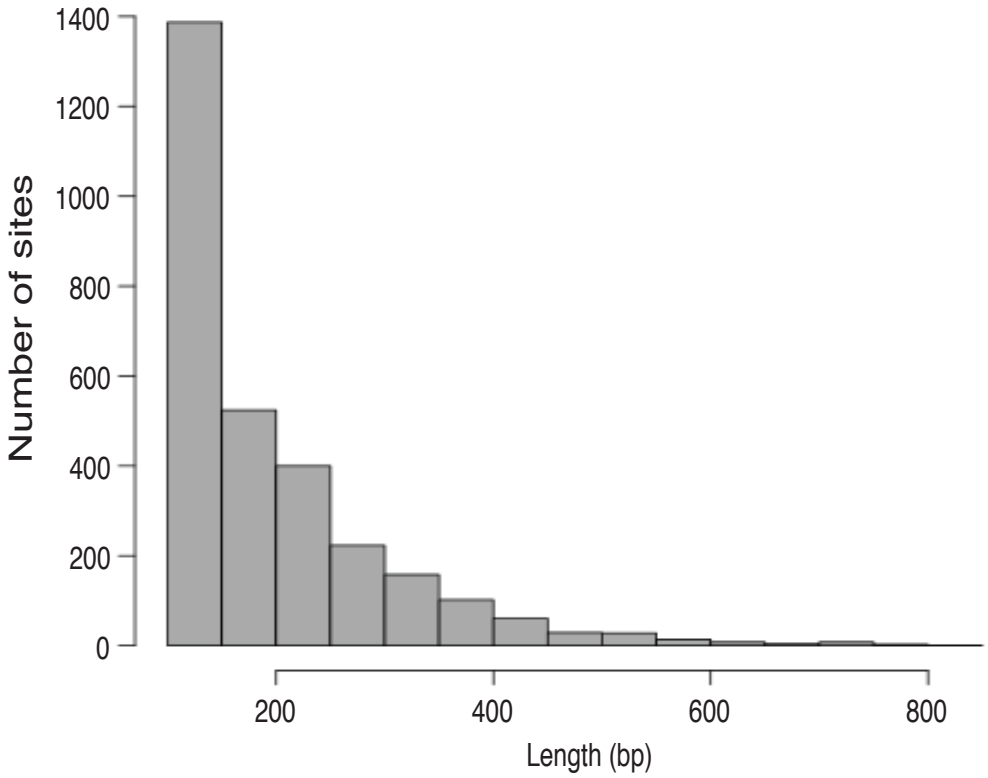
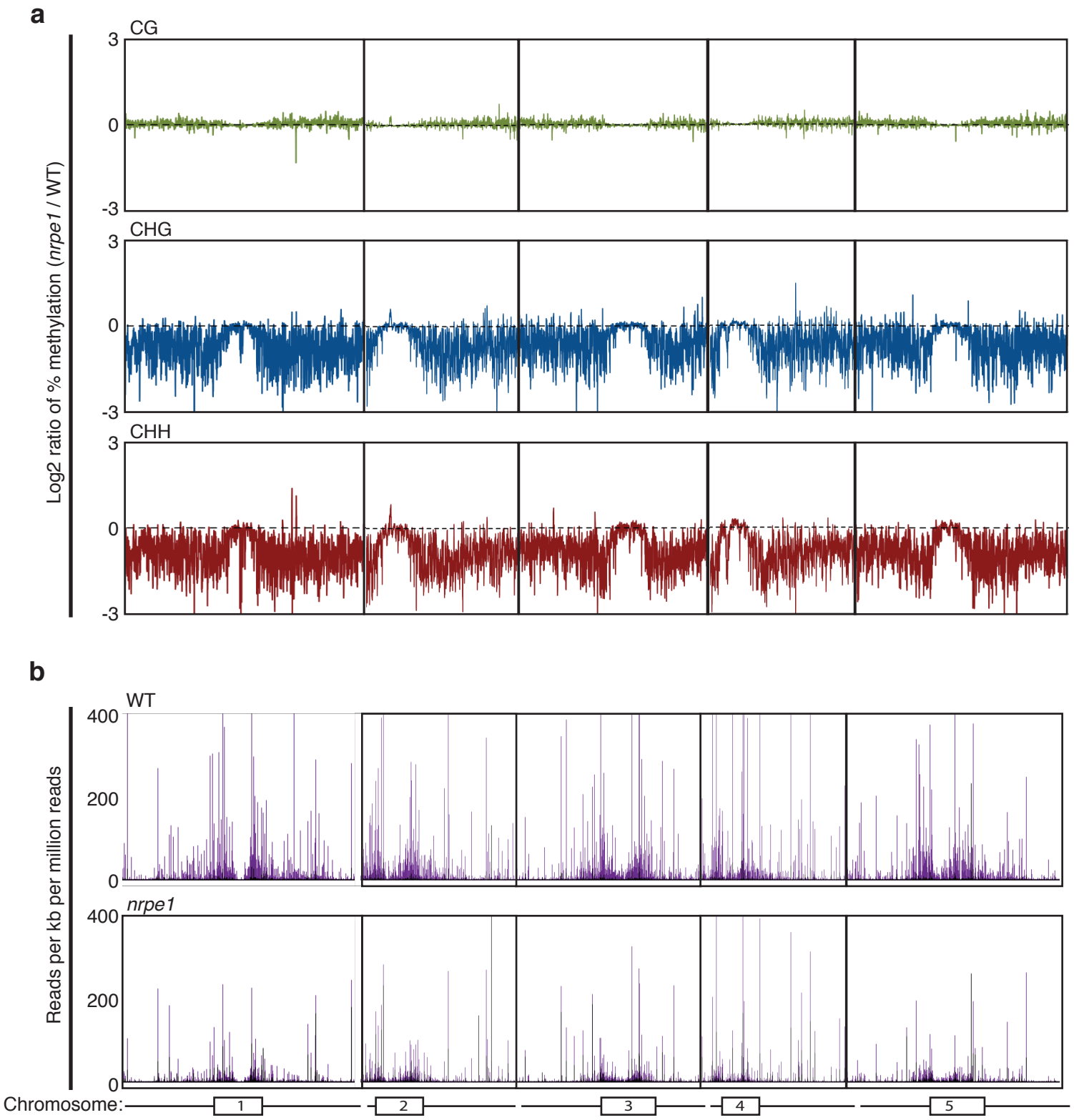
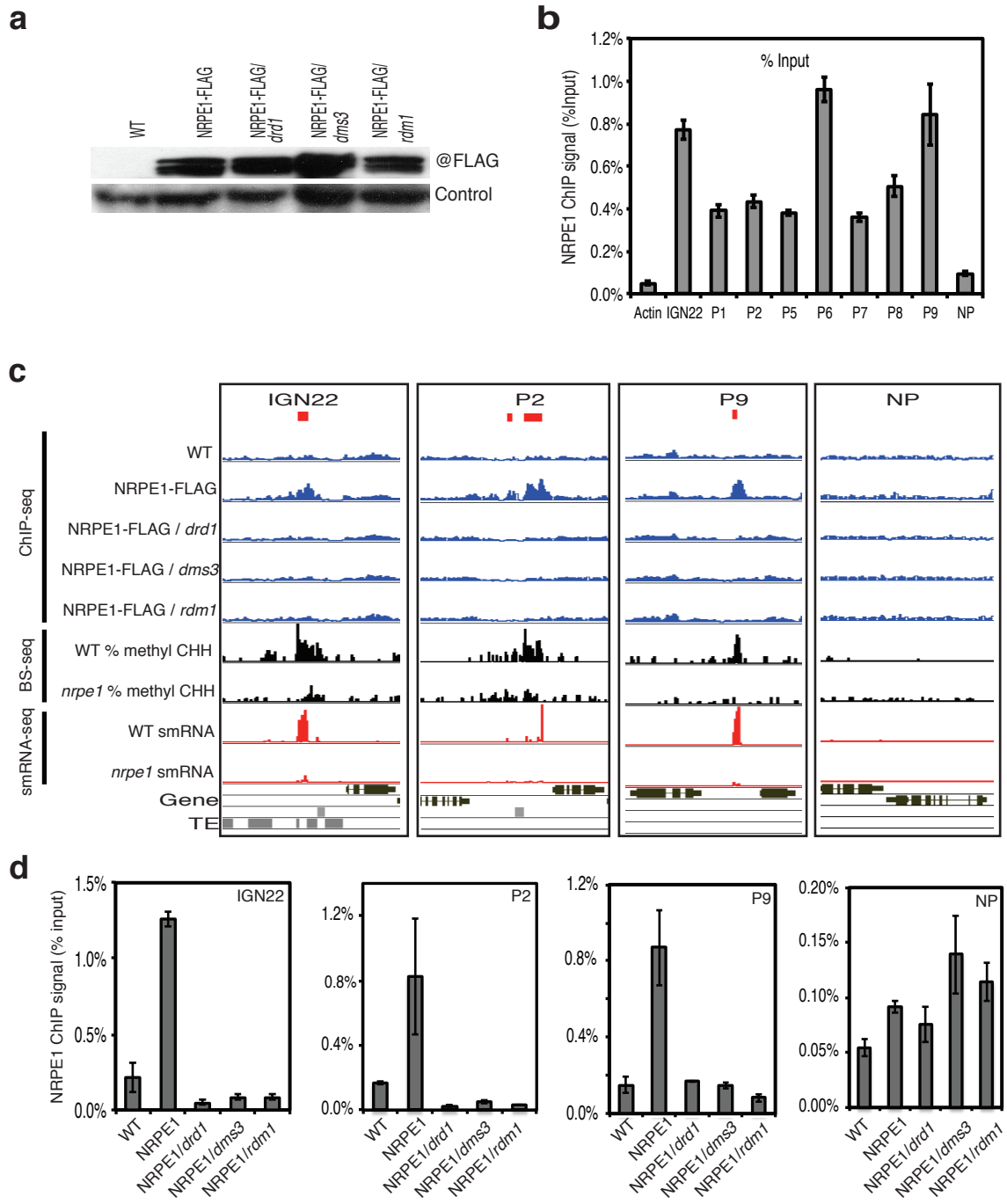
Supplementary Results



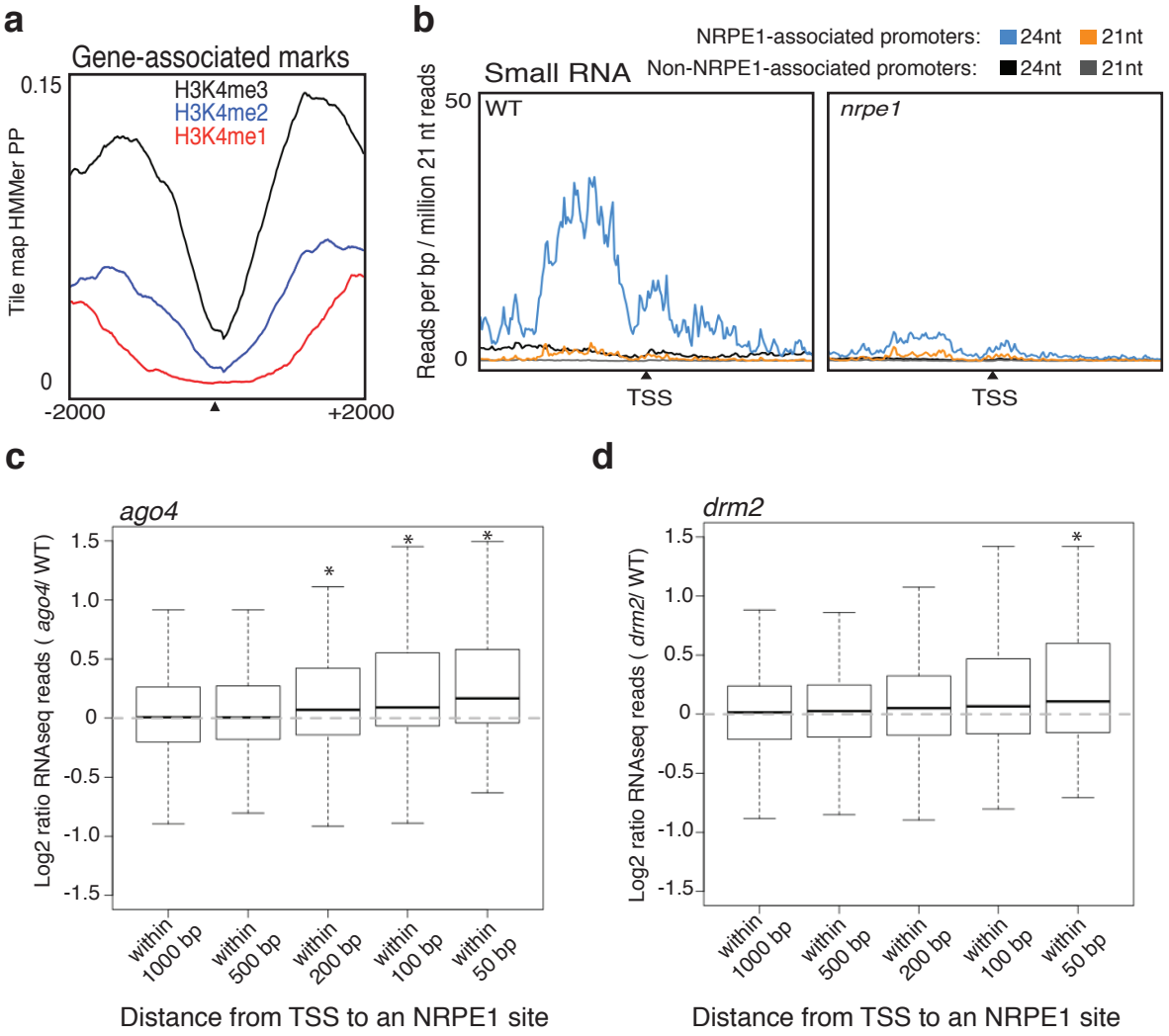
Supplementary Figure 1 The size distribution of NRPE1 sites.



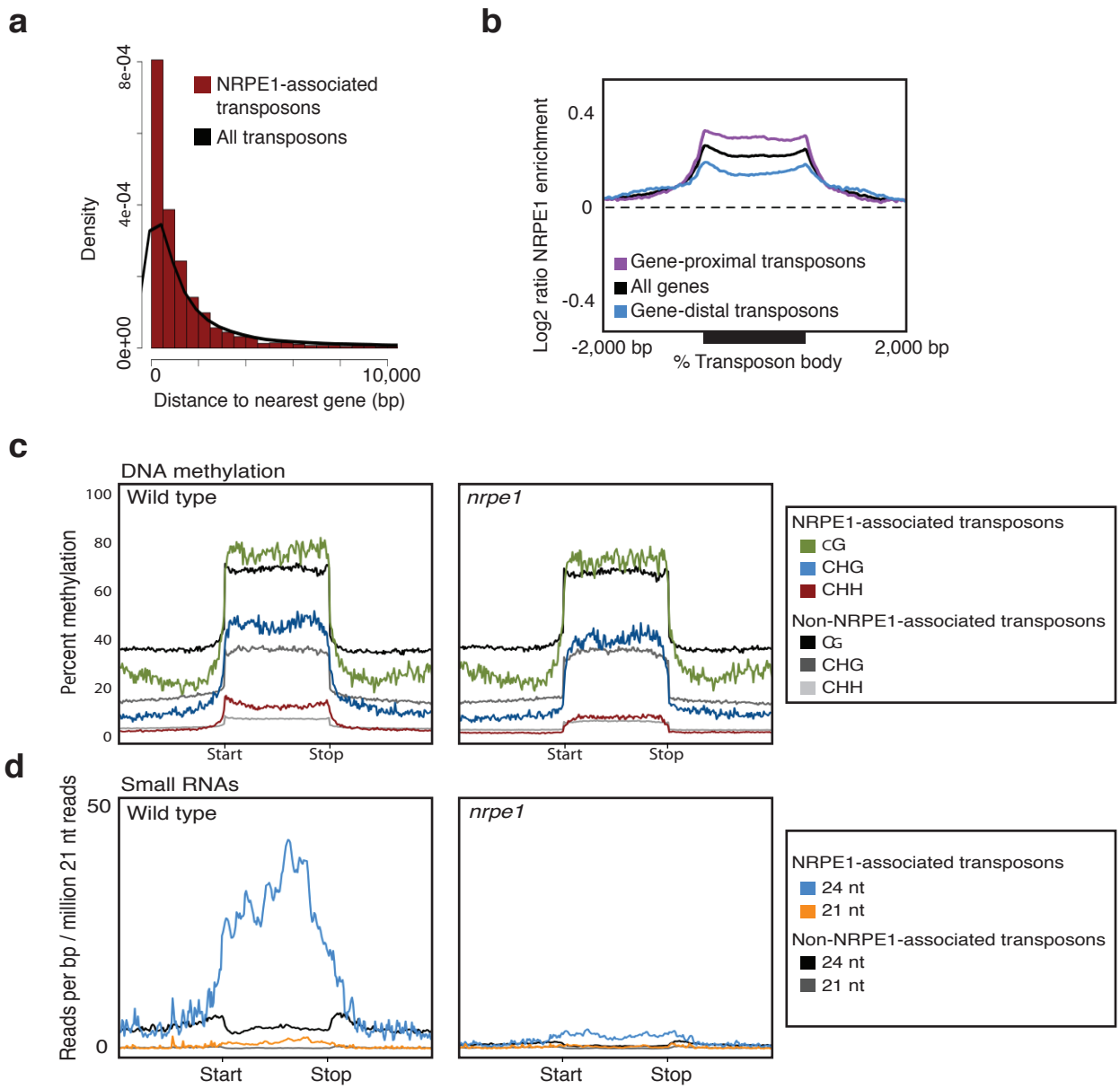
Supplementary Figure 2 Epigenetic marks in *nrpe1* mutant. (**a**) Chromosomal views of log2 ratio of methylation levels in all three cytosine contexts for *nrpe1* mutants relative to WT as assayed by whole-genome bisulfite sequencing. (**b**) 24nt (purple) and 21nt (black) small RNA levels in WT and *nrpe1* plants as assayed by small RNA Illumina sequencing. For small RNA abundance, read counts for each library were normalized to number of mapping reads for that library. Schematic representations of chromosomes are shown as in Figure 1A.



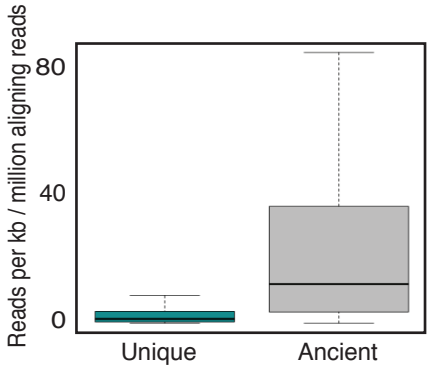
Supplementary Figure 3 Identification of new Pol V-dependent transcripts. (**a**) Western blot of NRPE1 in DDR mutants with WT as a negative control. (**b**) Validation of ChIP-seq peaks (P#) by qPCR at single loci. IGN22 is a previously described Pol V transcript (ref 13), NP is a region not showing NRPE1 enrichment. (**c**) Genome-browser views of NRPE1 peaks. (**d**) Validation of ChIP-seq data for NRPE1 binding to chromatin in NRPE1, *drd1*, *dms3*, and *rdm1* mutants at IGN22, P2, P9 and NP loci. Error bars represent the standard deviation of three biological replicates.



Supplementary Figure 4 Characterization of NRPE1-associated promoters. (**a**) Metaplots of gene-associated H3K4 chromatin marks at NRPE1-enrichment sites (the triangle denotes the midpoint of the NRPE1 binding site with plots extending +/-2000bp) using previously published datasets (reference 18). (**b**) small RNAs at NRPE1 associated promoters. Triangles denote the transcriptional start site (TSS) with all plots/heatmaps extending 2000 bp upstream or downstream. (**c-d**) Boxplot of log2 ratios of normalized RNAseq read counts for *ago4* (**c**) and *drm2* (**d**) mutants over WT for protein coding genes with an NRPE1 site in their promoter (1 kb upstream) with subgroups within those genes classified based on the distance between the TSS and NRPE1 site. * indicates P<0.05 (Mann-Whitney Test).



Supplementary Figure 5 NRPE1 is enriched at gene-proximal transposons that are targets of RdDM. (**a**) Histogram showing the distance between transposons and nearest protein coding gene. (**b**) Metaplot showing NRPE1-enrichment at gene-proximal (within 1 kb) and –distal (>1 kb) transposons. (**c**) Metaplots showing the DNA methylation as assayed by BS-seq at transposons. (**d**) Metaplots of small RNA abundance as assayed by smRNA-seq at transposons. All metaplots extend +/-2000 bp from the body of the transposons.



Supplementary Figure 6 Unique transposons are less transcribed than ancient transposons in wild type plant. Boxplot of RNA-seq reads in wild type showing that unique transposons are generally more lowly expressed than the ancient transposons.

Supplemental Table 3. List of primers used for NRPE1 ChIP validation and new IGN transcripts detection by real-time PCR.

	Primer numbers	Primer sequences from 5' to 3'
Actin	JP2699 JP2700	AGCACGGATCGAATCACATA CTCGCTGCTTCTCGAATCTT
IGN22	JP9978 JP9979	CGGGTCCTTGGACTCCTGAT TCGTGACCGGAATAATTAAATGG
P1	JP10069 JP10070	GGATGTATATACGACTTTTAG GCTGAAGTGTGGAATCTATATG
P2	JP10051 JP10052	CTAAAGCCCATCAGAGAAACC GCTTTGATTGTTTTAACCGGTG
P5	JP10079 JP10080	CCCCAAATCAAATCTCACCC CTCTATATTTTGTATATTAATTCC
P6	JP10059 JP10060	GGCTTCGATAGGAAGAATGCCC GTGAAACTGCCAGATCCAAATTC
P7	JP10053 JP10054	GTCCGTTGGAGATTCTATTGCC GATGGATGATATATTCTATATTTG
P8	JP10073 JP10074	GAAAACAAAAGTTATACTTTG GGTGTTTCATTCACTATCGTCC
P9	JP10075 JP10076	CCGTTTCTGGGTAGGTCGGC CCAATTCTTGACTGGAGTGGAC
NP	JP10081 JP10082	GTTCAATGAATAAGAATCACTGAG CCATGTCTTGTGCATTGTCAGAATCAG

Supplemental Table 4. Illumina sequencing library statistics.

ChIP-seq	Library	Mapping	Mapping Uniquely	Mapping Non-unique
	NRPE1-FLAG	61748706	53323310	8425396
	WT(Columbia)	90457956	79443656	11014300
	NRPE1-FLAG replicate	19056215	15975032	3081183
	NRPE1-FLAG ; *drd1*	28602282	25137147	3465135
	NRPE1-FLAG ; *rdm1*	24907986	22080393	2827593
	NRPE1-FLAG ; *idn1*	26664363	23412699	3251664
	WT(Columbia) replicate	25696190	21832253	3863937
BS-seq*	**Library**	**Mapping**	**Mapping Uniquely**	**Mapping Non-unique**
	WT(Columbia)	-	34759527	-
	nrpe1	-	50979638	-
small RNA-seq	**Library**	**Mapping**	**Mapping Uniquely**	**Mapping Non-unique**
	WT(Columbia)	4464770	1897762	2567008
	nrpe1	8248924	2680443	5568481

*For BS-seq libraries, only uniquely mapping read counts were recorded from the BSseeker wrapper.