Supplemental Information Appendix

Supplemental Text

To characterize TCM and TCdM DMRs, we calculated the DNA methylation levels of high parent (Col and C24 for Col_DMRs and C24_DMRs, respectively), low parent (C24 and Col for Col_DMRs and C24_DMRs, respectively) and reciprocal F1 hybrids and PAV of F1 hybrids for each DMR. As expected, at NI DMRs, methylation levels of F1 hybrids fall between those of high and low parents, and are not significantly different from PAV values (**Figure 2A**). However, we found that the F1 methylation levels are significantly higher and lower than PAVs at TCM and TCdM DMRs, respectively (**Figure 2A**).

To investigate how high and low parent alleles of F1 contribute to the deviation of F1 methylation levels from PAVs, we evaluated allele-specific methylation patterns of DMRs containing at least one single nucleotide polymorphism (SNP) between Col and C24. At NI DMRs, F1 high-parent alleles and low-parent alleles maintained similar methylation levels as their high- and low- parents, respectively (**Figure 2B**), suggesting that parental methylation patterns were maintained at NI DMRs in F1 hybrids. We uncovered At3g44800 as an NI DMR (**SI Appendix, Figure S1C**) and employed Chop PCR (methylation-sensitive restriction digestion followed by PCR) to validate this classification (**SI Appendix, Figure S1D and E**). The Col and C24 alleles in F1 were distinguished by CAPS-PCR (Cleaved Amplified Polymorphic Sequences PCR), which was designed based on a SNP within this region (**SI Appendix, Figure S1D and E**). We examined the allele-specific DNA methylation status of At3g44800 for 41 and 42 individual F2 plants, derived from crossing reciprocal F1 hybrid plants, by methylation-sensitive PCR followed by CAPS-PCR (**Table 1**). The results show that the ratio of methylated plants to unmethylated plants is approximately 3:1, and that two thirds of the methylated plants are heterozygous, coinciding with Mendelian segregation (**Table 1**). These results confirmed that the DNA methylation of NI DMRs is faithfully inherited without methylation interactions during hybridization and subsequent meiosis.

Supplemental Figures

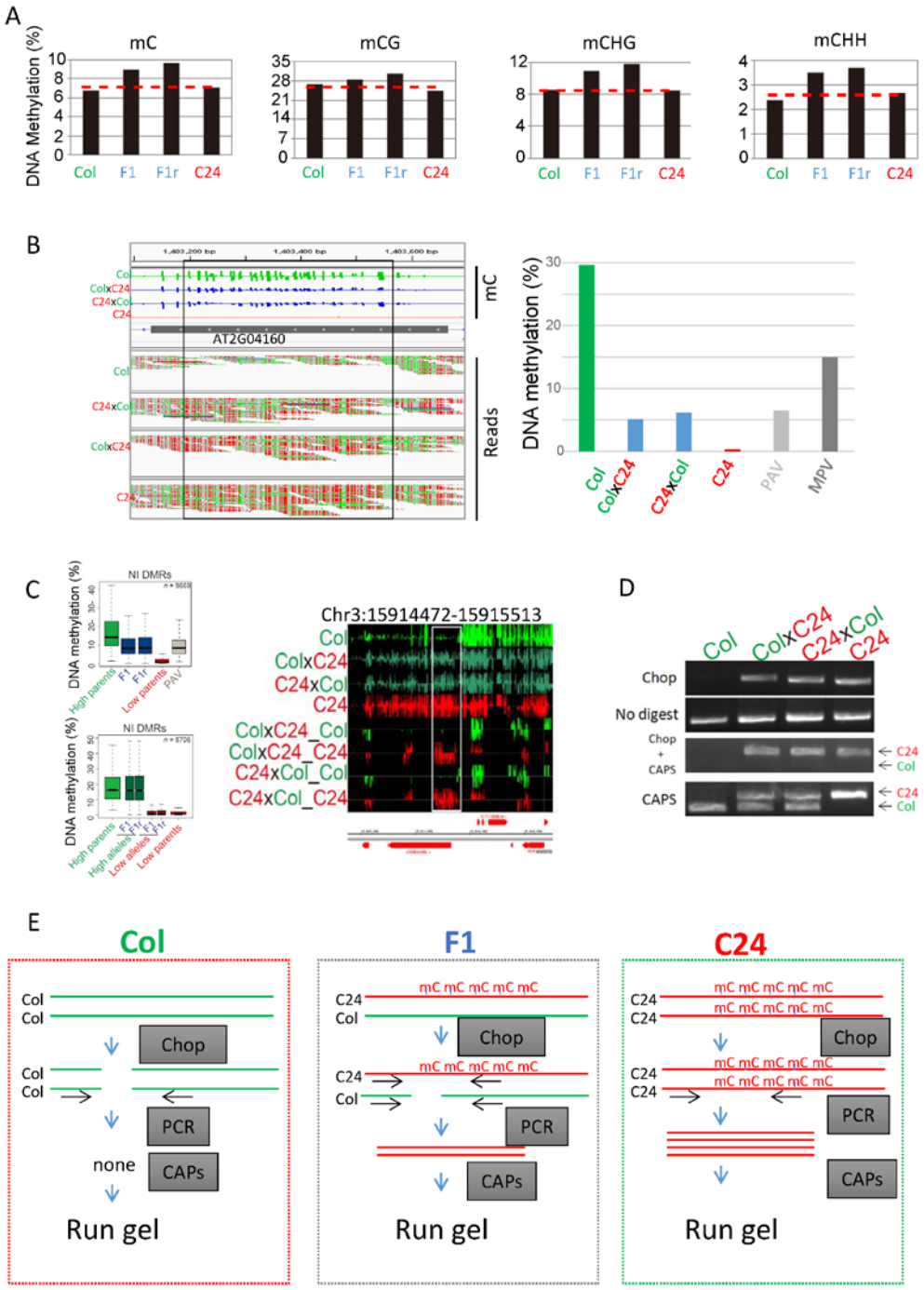

Figure S1. Sequencing and validation of DNA methylation status in Col, C24 and their hybrid progeny.

(A) F1 hybrids have higher methylation levels than parents. The mC, mCG, mCHG and mCHH levels of Col, C24, F1 (Col X C24) and F1r (C24 X Col) are shown. (B) An example region showing that PAV (predicated additive value) is a better estimate of F1 methylation level than MPV (mid-parent value). (C) NI DMRs. Left panel: whole (top) and allelic (bottom) methylation levels of NI DMRs. High parent, the parent with higher methylation level at the DMR; Low parent, the parent with lower methylation

level at the DMR; High allele, allele from the high parent. Low allele, allele from the low parent. Right panel， an example of a region showing allele-specific methylation status in Col, C24, and F1 hybrids by Integrated Genome Browser (IGB) screenshot. (D) Examination of allele-specific methylation status based on CAPS-PCR (Cleaved Amplified Polymorphic Sequences PCR) and methylation-sensitive PCR. (E) A schematic of the CAPS-PCR and methylation sensitive PCR approach.

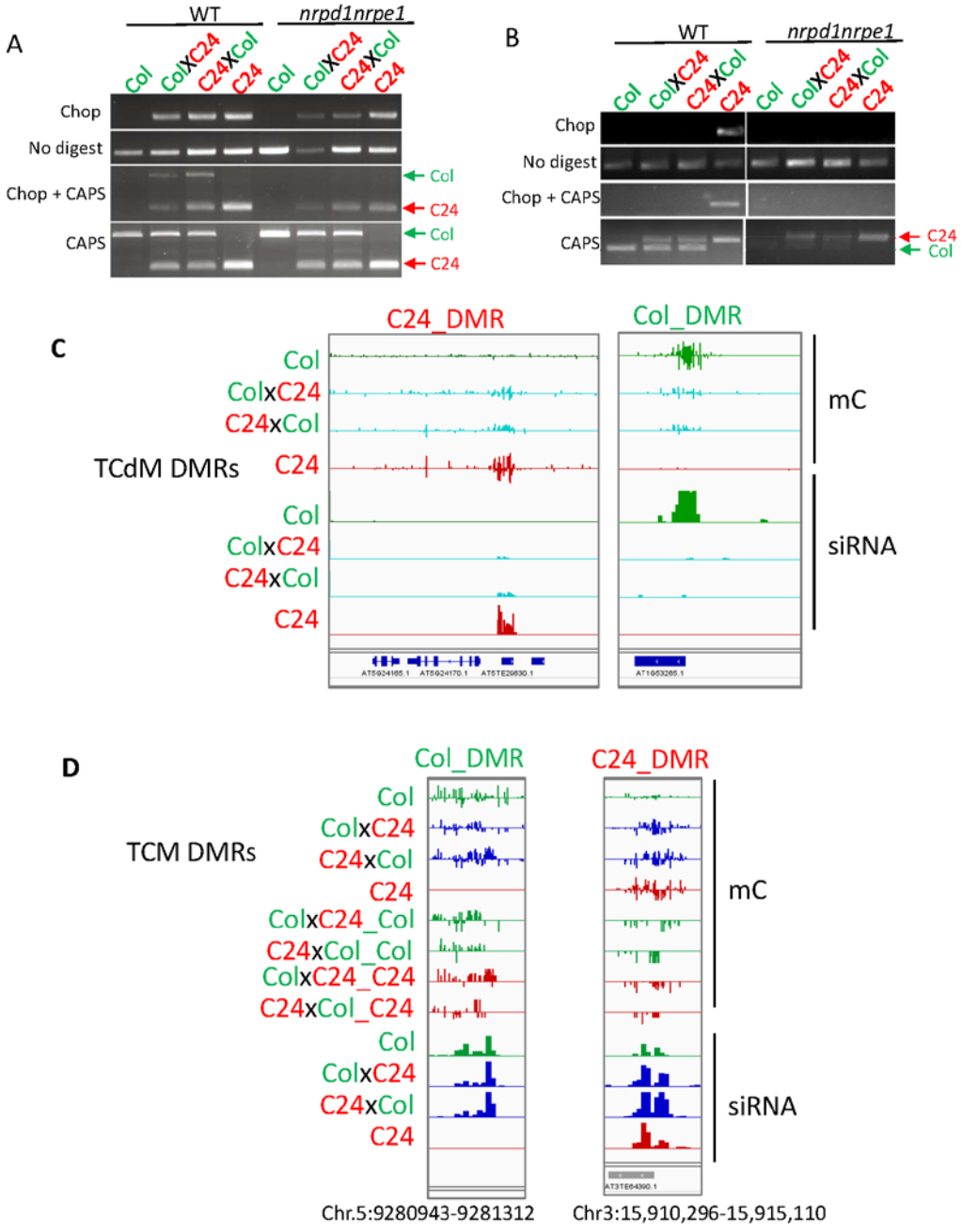

Figure S2. Methylation status of TCM DMRs and TCdM DMRs.
(A) and (B) show validation of methylation status of a TCM DMR and a TCdM DMR, respectively, which are shown in figure 2C and D, by using CAPS-PCR and methylation sensitive PCR. Methylation status was examined in parents and F1 hybrids in both wild type and *nrpd1nrpe1*. (C) and (D) Examples of TCdM DMRs and TCM DMRs. DNA methylation and siRNA accumulation patterns were compared in Col, C24 and their F1 hybrids. IGV screenshots from whole genome bisulfite sequencing and siRNA sequencing data are shown. Vertical bars on each track indicate DNA methylation levels or siRNA abundance. Col_DMR and C24_DMR indicate DMR with higher methylation level in Col and C24 parents, respectively.

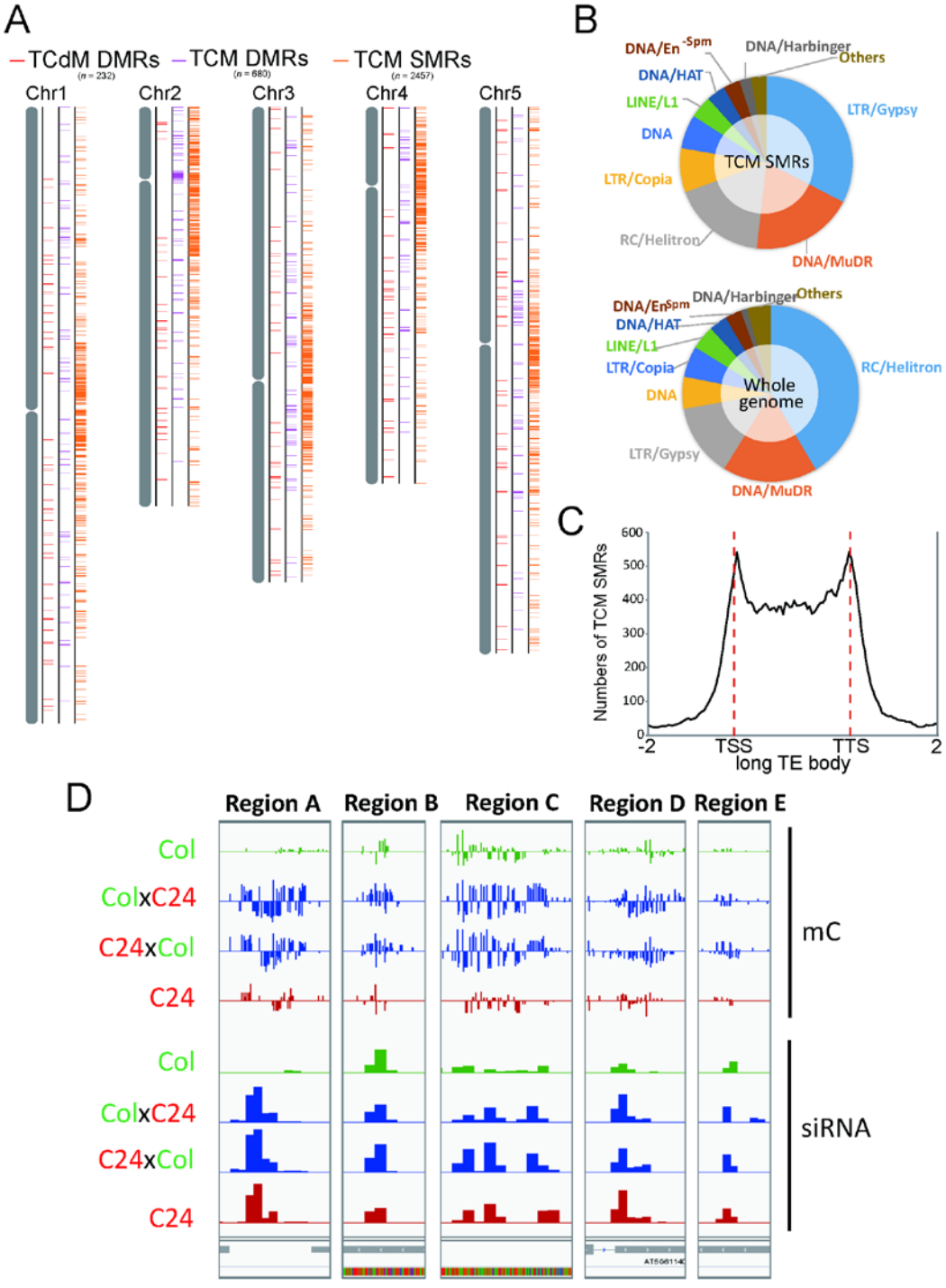

Figure S3. Characterization of TCM SMRs.

(A) Chromosomal distribution of TCM DMRs, TCdM DMRs and TCM SMRs. (B) Distribution of different types of TEs (transposable elements) in TCM SMRs. Genome-wide distribution of TEs is shown as a control. (C) Distribution of TCM SMRs in long TE and nearby 2kb regions. (D) Examples of TCM SMRs. Levels of DNA methylation and siRNA in Col, C24 and their F1 hybrids are displayed.

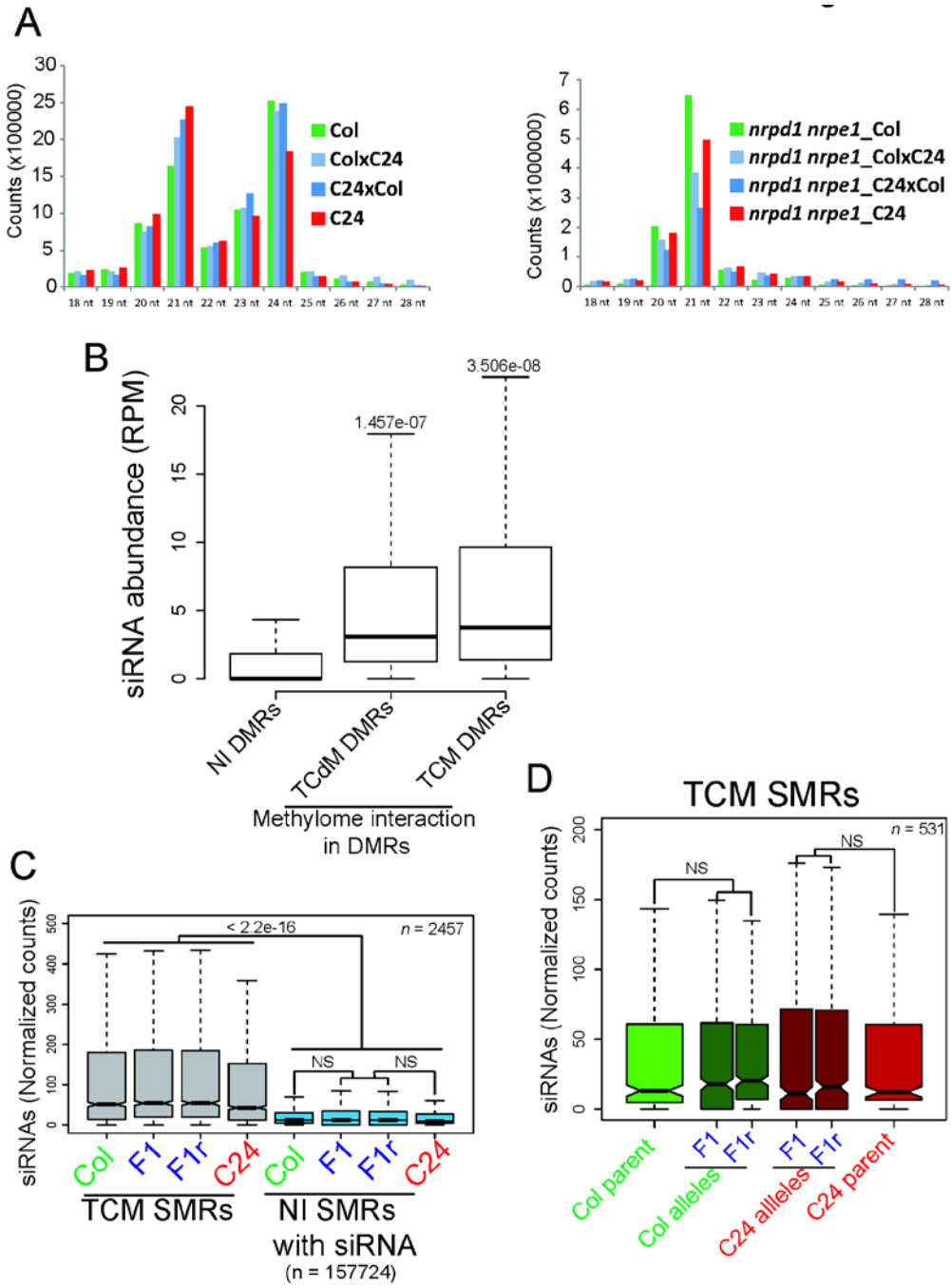

Figure S4. Small RNA sequencing data analysis.

(A) Length distribution of siRNA sequencing reads in the parents and hybrids of wild type (left panel) and *nrpd1nrpe1* (right panel). (B) Comparison of siRNA levels among NI DMRs, TCdM DMRs and TCM DMRs. DMRs with TCM and TCdM have significantly higher siRNA accumulation than NI DMRs. *P*-values < 0.001 indicate statistical significance (two-tailed *t*-test). (C) Comparison of siRNA levels between TCM SMRs and NI SMRs. NI DMRs that have siRNAs in either Col or C24 were used. *P*-values < 0.001 indicate statistical significance (two-tailed *t*-test). NS, not significant. (D) Allele-specific analysis of siRNAs in TCM SMRs. NS, not significant (*P*-values > 0.05).

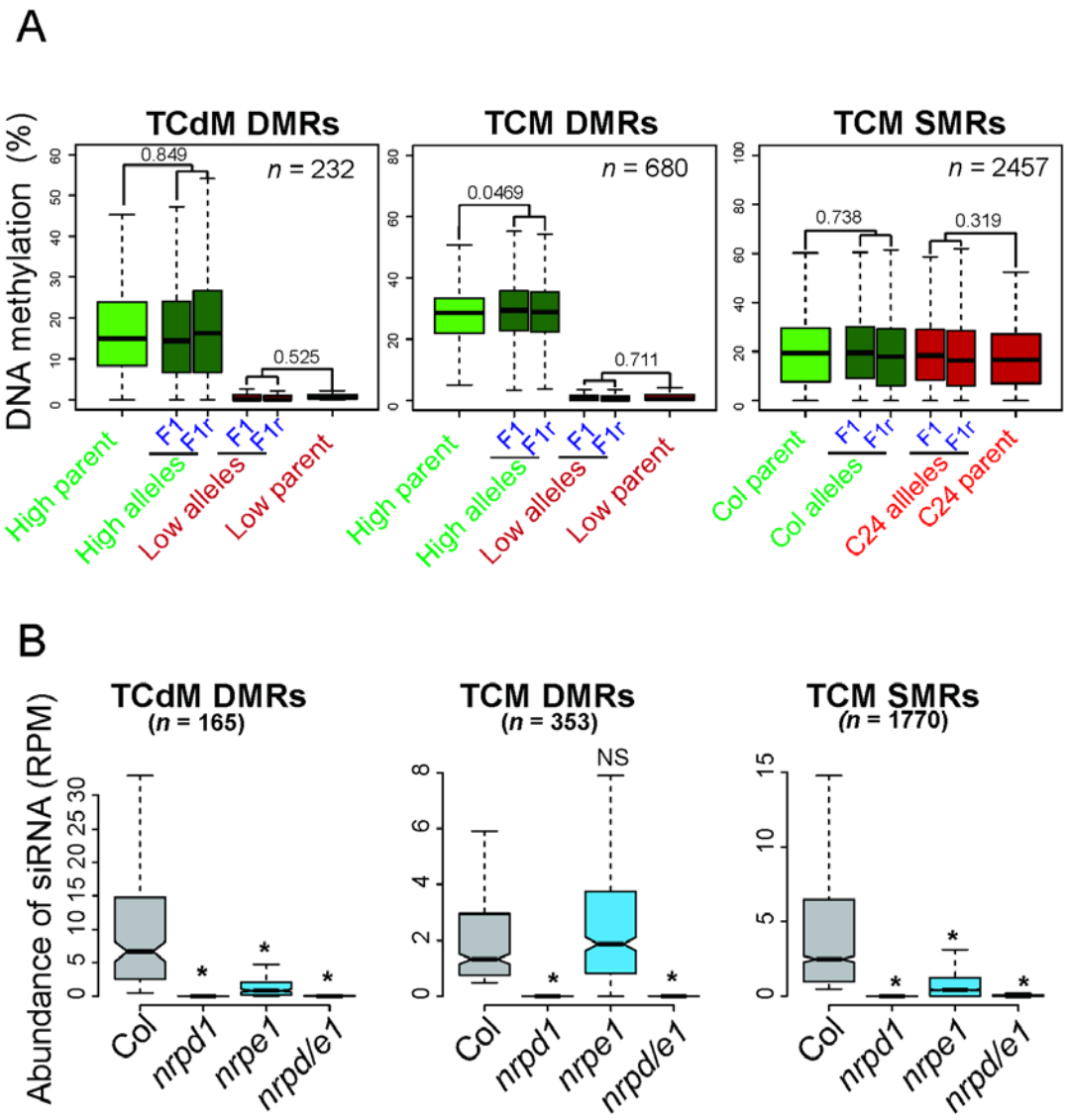

Figure S5. Role of Pol IV/Pol V in methylation interactions.

(A) Allele-specific methylation levels of TCdM DMRs, TCM DMRs and TCM SMRs in *nrpd1nrpe1* (Col), *nrpd1nrpe1* (C24) and *nrpd1nrpe1* F1 hybrids. (B) Published siRNA-sequencing data (Law et al., 2013) was analyzed similarly as in Figure 5C . NS, not significant (two-tailed *t*-test); **P*-values < 0.01 indicate statistical significance.

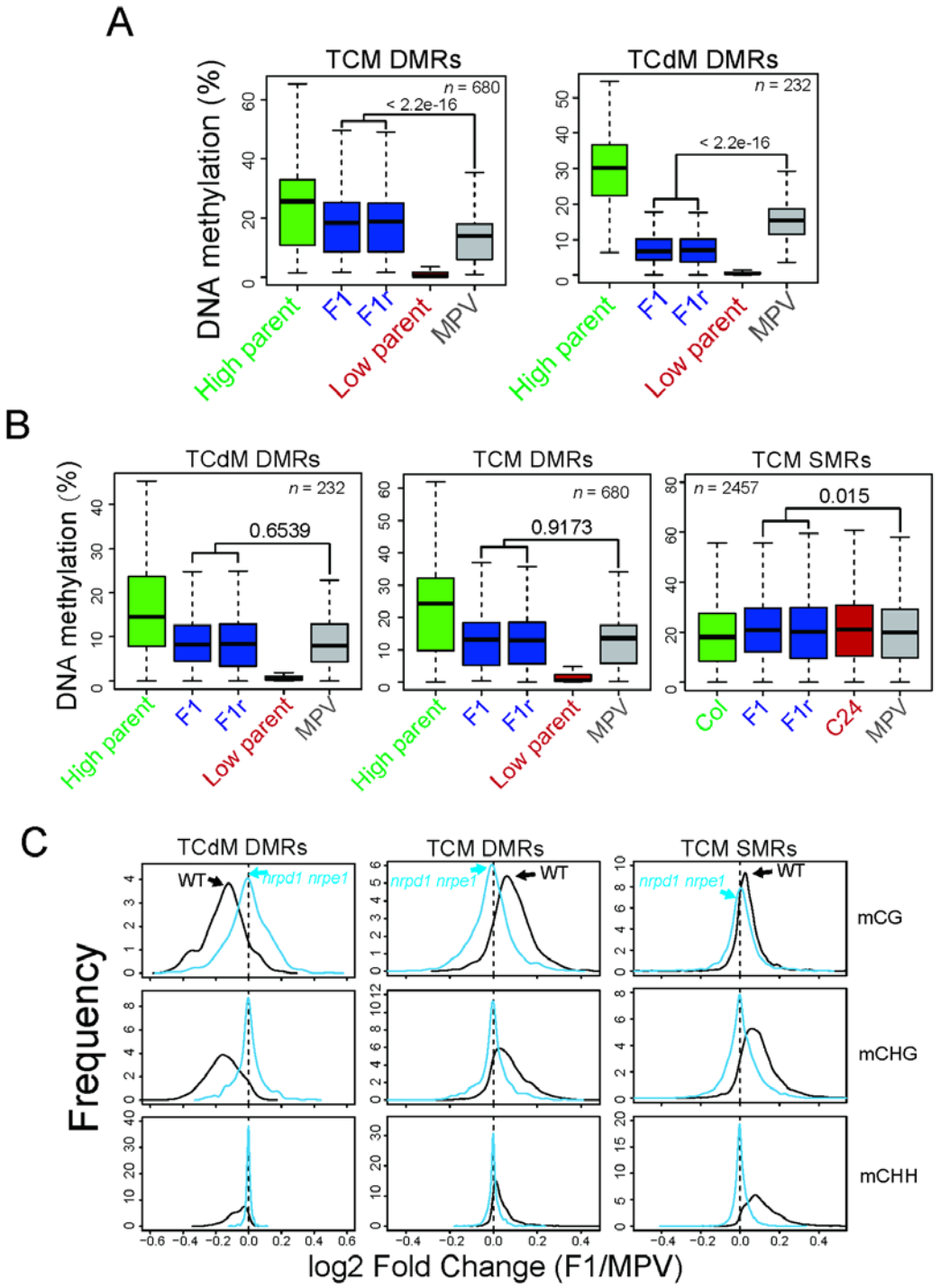

Figure S6. Comparisons between F1 methylation levels and MPV.
(A) Methylation levels of TCM DMRs and TCdM DMRs are shown. High parent, the parent with higher methylation level at the DMR. Low parent, the parent with lower methylation level at the DMR (related to Figure 2A). (B) Methylation levels of TCdM DMRs, TCM DMRs and TCM SMRs in *nrpd1nrpe1* (Col), *nrpd1nrpe1* (C24) and *nrpd1nrpe1* F1 hybrids (related to Figure 5A). (C) For TCdM DMRs, TCM DMRs and TCM SMRs, the differences of mCG, mCHG, and mCHH levels between F1 and MPVs are shown for wild type and *nrpd1nrpe1* (related to Figure 5B).

Table S1 Segregation of DNA methylation in F2 for NI DMRs

Loci	F2 types	Methylated	unMethylated	P-value, (χ2 test)
Chr3:15914472-15915513	ColXC24	33	8	3:1, 0.42
	C24XCol	30	11	3:1, 0.79
Chr5:10611968-1061258	ColXC24	38	9	3:1, 0.35
	C24XCol	27	7	3:1, 0.55

Table S2 Information for *nprd1* and *npre1* in Col and C24

Gene Name	Gene ID	Col Alleles	C24 Alleles
NRPD1	AT1G63020	SALK_128428	T-DNA insertion in the 8th exon
NRPE1	AT2G40030	SALK_029919C	gDNA_5189(from ATG), 31bp deletion

Table S3 Primers and enzymes for gneotyping *nprd1* and *npre1* in Col and C24

Gene ID	Forward (5' > 3')	Reverse (5' > 3')	Enzymes	Applied Alleles	Products Size
LBb1.3 (T-DNA left boarder)	ATTTTGCCGATTTCGGAAC				
nrpd1 _Col	GATCTGTTCAGCTTGCTCGTC	TTAATGTTCTTCATGCGGGAC	N/A	N/A	N/A
nrpe1 _Col	ATTTCTTCTTTGATGGGGGAG	TGTCGTGGATATGACCATTTG	N/A	N/A	N/A
C24_LB (T-DNA left boarder)	TTGACCATCATACTCATTGCTG		N/A	N/A	N/A
nrpd1 _C24	TGCTGTTTGCCGTTCCGTGGT	AGCGCGCTGTAAGCTGCCTC			
nrpe1 _C24	TCCTACAATGCCACCGATCCCGA	ACTGCTCCCCCATCAAAGAAGAAATCG	Deletion	Mutant	110/75

Table S4 Information for Bisulfite Sequencing

Samples	Raw reads	Mapped Reads	PE rate	Depth	Conversion Rates	C	CG	CHG	CHH
Col	49,025,828	45,083,334	91.96%	35.208	99.60%	6.75%	26.74%	8.56%	2.38%
ColxC24	51,362,438	43,716,788	85.11%	29.847	99.63%	8.91%	28.56%	10.91%	3.52%
C24xCol	44,700,556	38,811,690	86.83%	27.732	99.66%	9.60%	30.75%	11.79%	3.71%
C24	48,760,236	39,186,398	80.37%	31.338	99.61%	7.05%	24.37%	8.46%	2.68%
*nrpd1 nrpe1*_Col	53,251,678	48,276,346	90.66%	37.56	99.63%	6.33%	22.98%	7.27%	2.02%
*nrpd1 nrpe1*_ColxC24	47,470,162	39,989,978	84.24%	29.958	99.56%	6.73%	24.63%	8.00%	2.28%
*nrpd1 nrpe*1_C24xCol	44,579,028	37,602,472	84.35%	27.9	99.60%	7.11%	25.98%	8.35%	2.36%
*nrpd1 nrpe1*_C24	45,013,286	35,386,974	78.61%	27.792	99.59%	7.95%	27.40%	9.44%	2.92%

Table S5 Information for small RNA sequenceing

Samples	**Clean Reads**	**Mapped Reads**	**Filtered Reads(Remove tRNA and rRNA)**
Col	10552277	10379121	9180692
ColxC24	10607709	10174506	9422817
C24xCol	10367039	9866192	9398099
C24	10486948	9788878	8870041
*nrpd1 nrpe1*_Col	10783693	10628963	10288240
*nrpd1 nrpe1*_ColxC24	10581498	10339475	9205247
*nrpd1 nrpe*1_C24xCol	10519046	10277826	9050497
nrpd1 nrpe1 _C24	10649792	10462314	9908733

Table S6 Primers for Chop-PCR and CAPS.

Sites	Forward (5’ > 3’)	Reverse (5’ > 3’)	Enzymes for CAPs	Applied allele of CAPS
Chr.3:7286624-7286946	ACACCTTGACTATACGGCATCCACC	TCCACTGAAACTTGCAGCGTGT	DraI	C24
Chr.5:9280943-9281312	TGGCTTACGGTGGTTTGGACCCT	TAGACCATTTTGCCCTCAATTTTCC	PspGI	Col
Chr3:15914472-15915513	GCTCTCCGACACTGATGCACAA	AGGCTGCGGGAAGATGACGA	AseI	Col
Chr5:10611968-1061258	TCGGCTTCATACGCATGACAAGTT	CCGCGTGTCAATGTGCAGCC	PspGI	Col