

Figure S1. Related to Figure 1A. Characterization of expression and T-DNA insertion mutants of *OsDDM1a*, *OsDDM1b*, and *OsDRM2*.

A. Transcript levels of *OsDDM1a*, *OsDDM1b*, and *OsDRM2* relative to that of Actin 1 in difference organs, detected by qRT-PCR. Bars = means \pm SD from 3 repeats.

B. Diagrams of *OsDDM1a*, *OsDDM1b*, and *OsDRM2* genes. Open triangles indicate the T-DNA insertion sites. Primers used for genotyping are indicated by arrows.

C. Genotyping of T-DNA insertion plants.

D. *osddm1a* and *osddm1b* single mutants displayed no obvious phenotype at seedling and mature stages.

E. Phenotypes of *osddm1a/1b* double mutants in comparison with wild type. The double mutant is severely dwarfed at mature stage. The panicle is much smaller and no viable pollens were produced in the double mutant. Empty and dark colored seeds produced in the double mutant are indicated by arrows. Close-up shows ectopic tillers produced in the double mutant compared to wild type.

F. Phenotype of *osdrm2* mutant in comparison with wild type. The mutant has pleiotropic growth phenotypes at both vegetative and reproductive stages, including growth defects, abnormal panicle and spikelet morphology, and complete sterility.

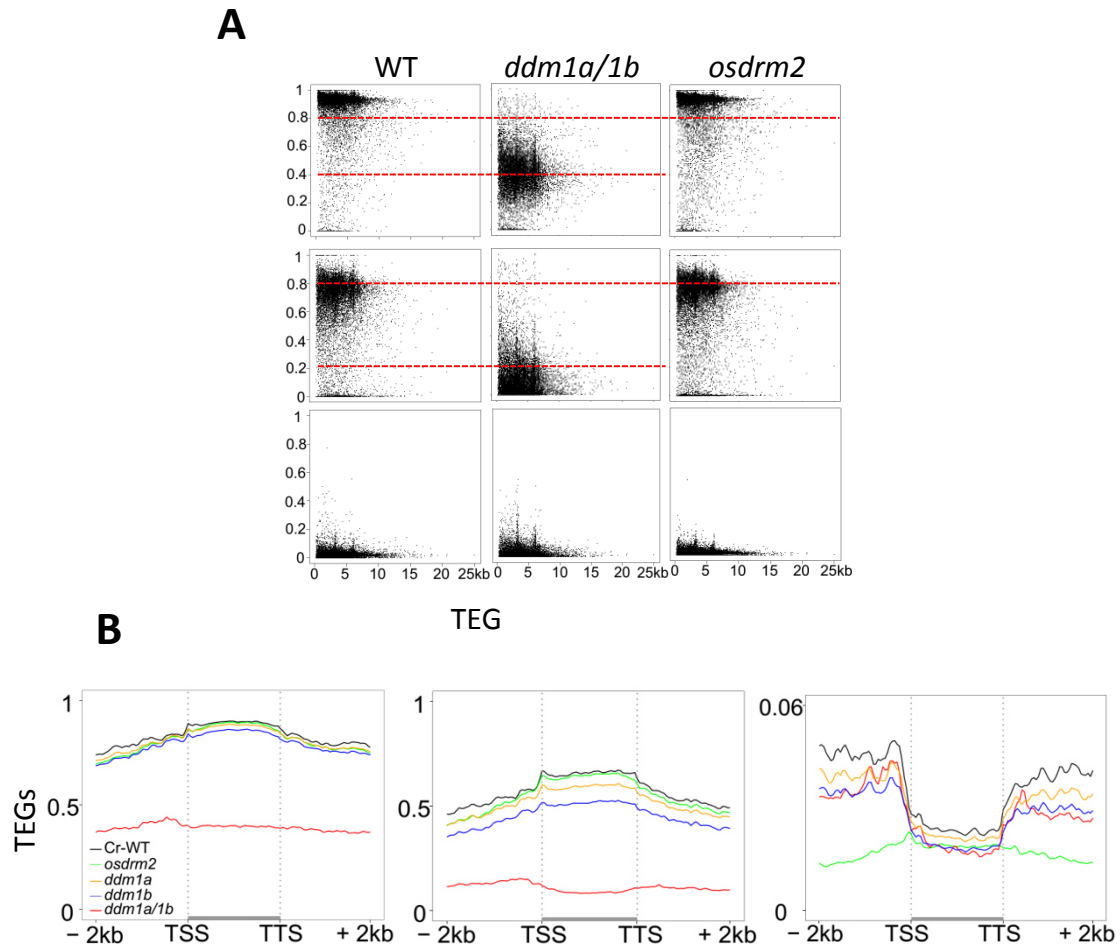


Figure S2. Related to Figure 2. Cytosine methylation average levels at all sequence contexts in TE-related genes (TEG).

A. Cytosine methylation average levels at all sequence contexts plotted against TEG size. Note that high levels of wild type CG methylation (>80) in TEG were reduced to about 40% in average in *osddm1a/1b*, and wild type CHG (80% in average) in TEG were reduced to low levels (below 20%) in *osddm1a/1b*, while remained unchanged in *osdrm2*.

B. Patterns and genome-wide average levels of cytosine methylation (CG, CHG, and CHH) of TEGs with their contiguous 2 kb upstream and downstream flanks in wild type and mutant rice plants.

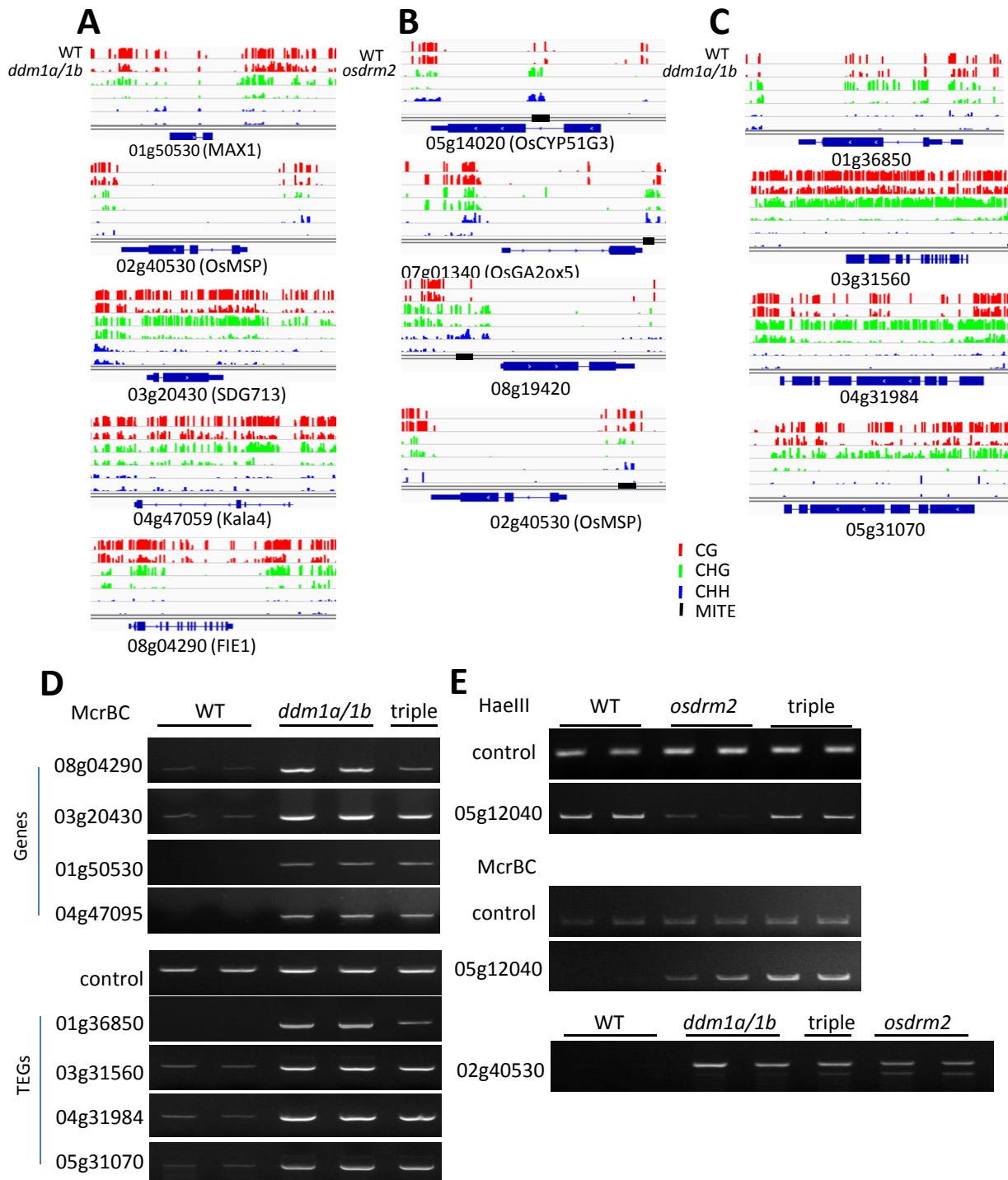


Figure S3. Related to Figure 2. Genome browser and validation of DNA methylation loss in the mutants.

A. Genome browser display of methylation of protein coding genes in *ddm1a/1b*.

B. Genome browser display of methylation of MITE-associated protein-coding genes in *osdrm2*.

C. Genome browser display of methylation of TE-related genes (TEGs) in *osddm1a/1b*.

D. McrBC-digestion and PCR tests of methylation of genes and TEGs (shown in A, C) in *osddm1a/1b* double and *osddm1a/1b osdrm2* triple mutants compared to wild type.

E. McrBC- or Hae III-digestion and PCR tests of methylation of genes (shown in B) in *osdrm2*, *osddm1a/1b*, and the triple mutants compared to wild type.

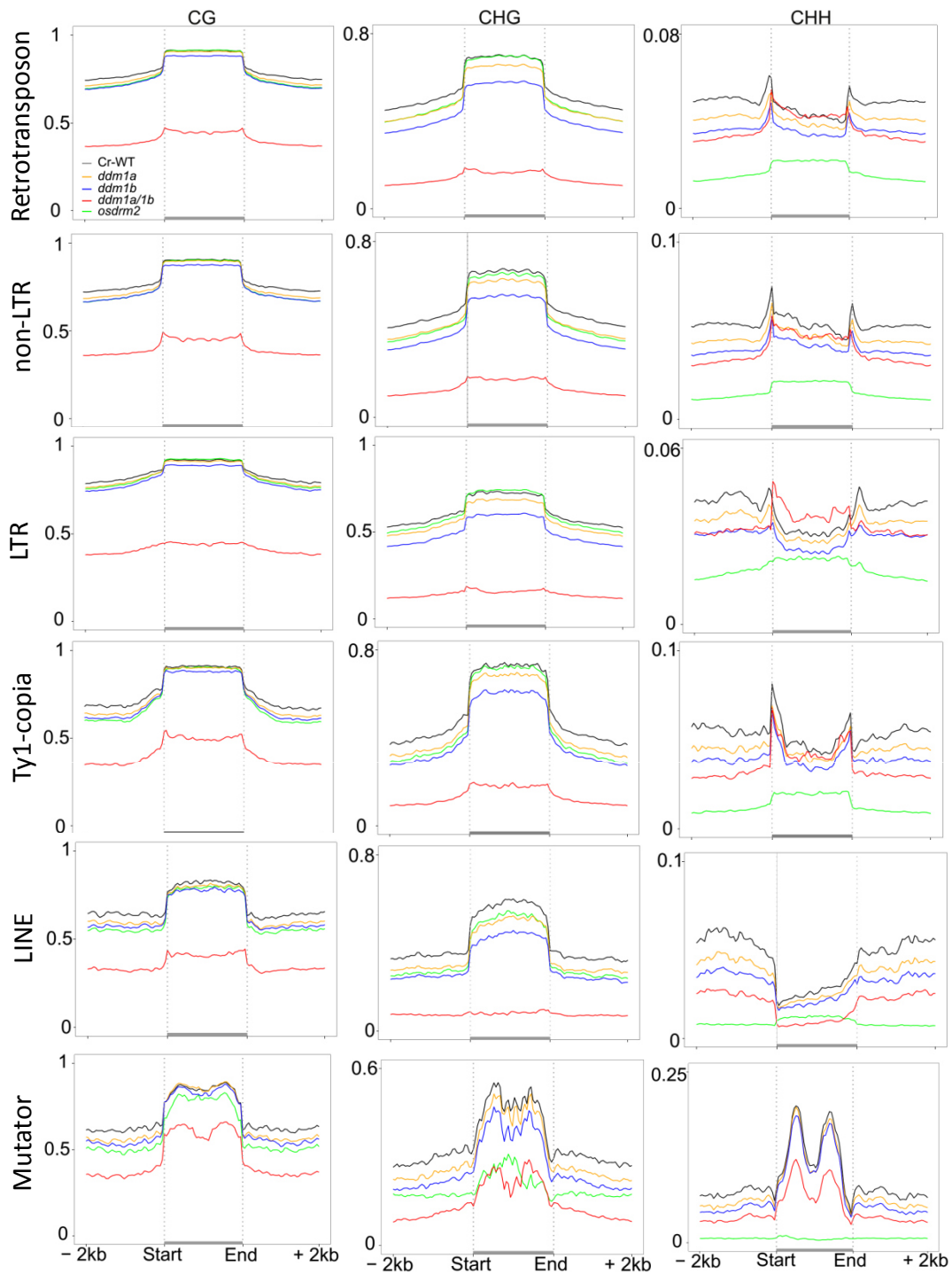


Figure S4. Related to Figure 3. Patterns of cytosine methylation (CG, CHG, and CHH) of additional TE and repeats in wild type and *osddm1a*, *osddm1b*, *osddm1a/1b*, and *osdrm2* mutants. TEs were aligned at the 5' and the 3' ends, and average methylation for all cytosines within each 100 bp interval is plotted.

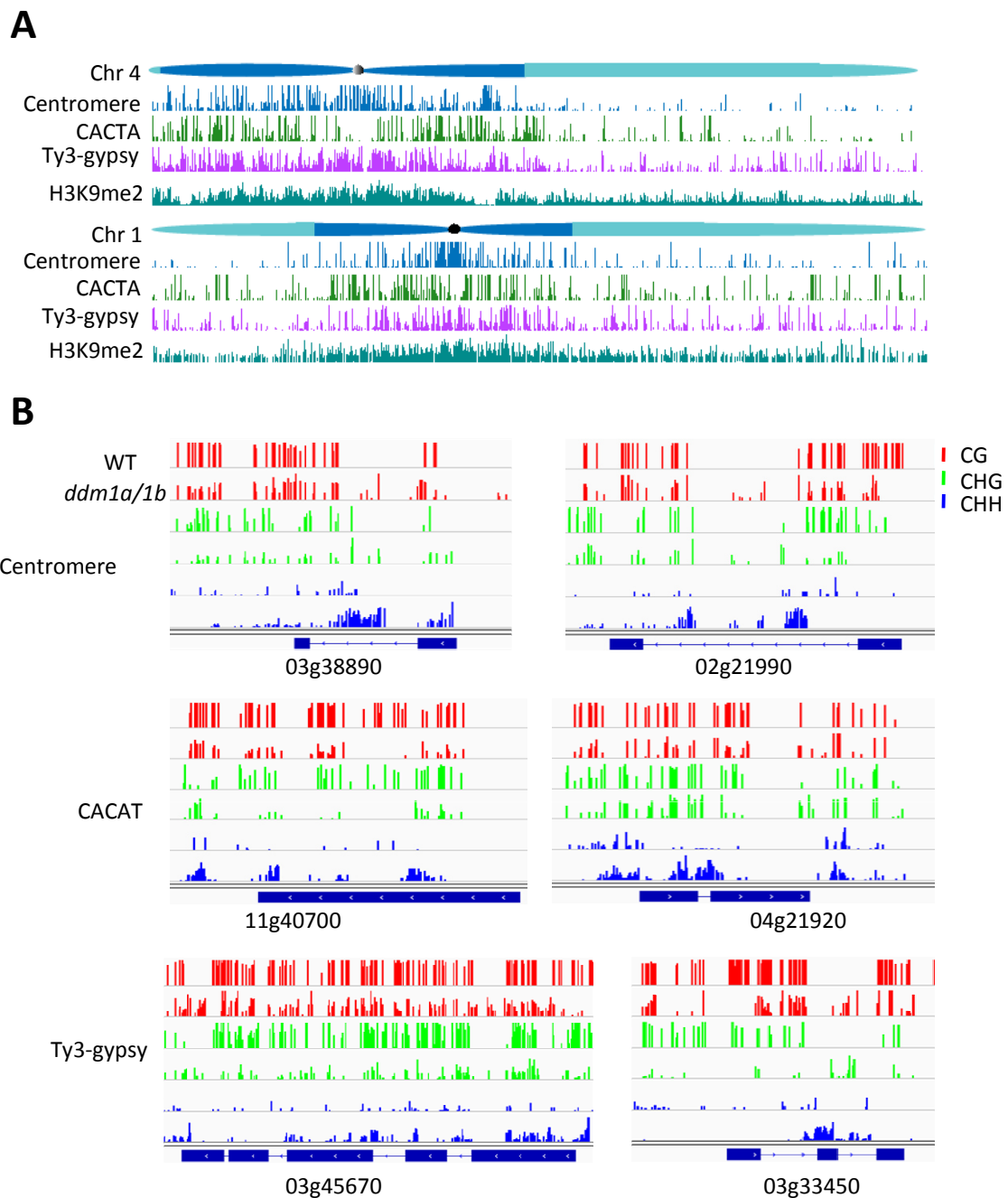


Figure S5. Related to Figure 3. Heterochromatin localization of hyper-CHH methylated TEs in *osddm1a/1b* plants.

A. Distribution of hyper-CHH methylated CACAT, Ty3-gypsy, and centromere repeats in chromosome 1 and chromosome 4.

B. Genome browser of methylation levels at CG, CHG and CHH sites of 2 CACAT, 2 Ty3-gypsy, and 2 centromere repeats in *osddm1a/1b* compared to wild type.

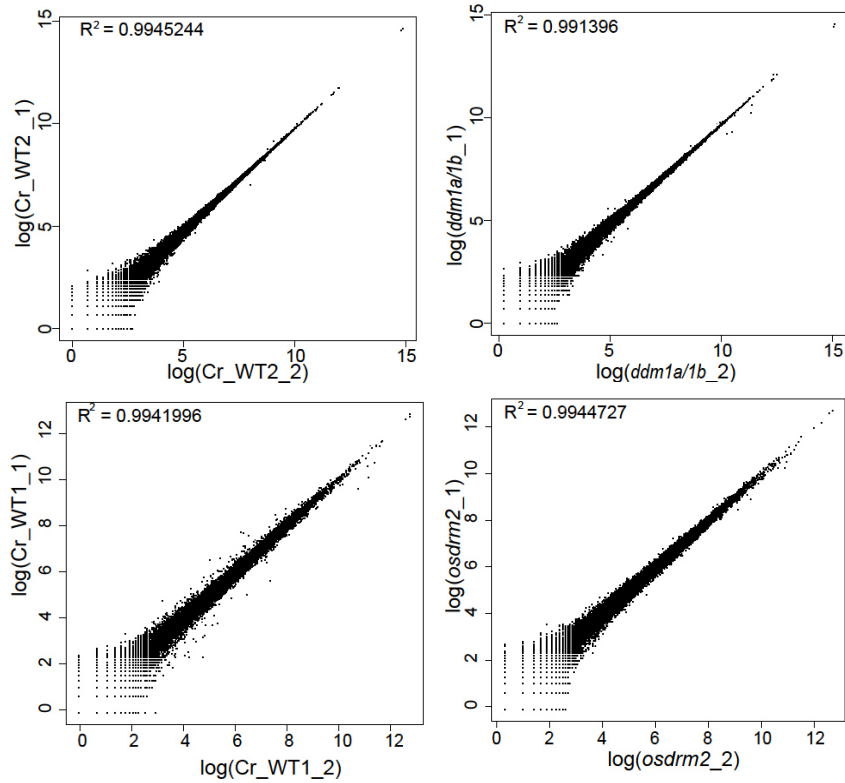
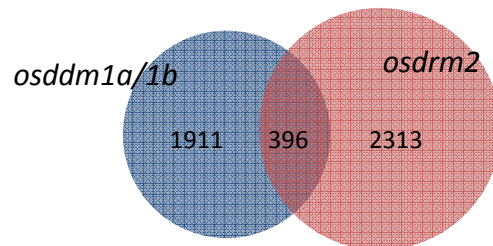
A**B**

Figure S6. Related to Figure 6. A. Transcriptomic analysis.

- A.** Reproducibility of RNA-seq repeats of Cr_WT, *osddm1a/1b*, and *osdrm2* seedling leaves.
- B.** Overlap of differentially expressed genes (> 2 fold changes) in *osddm1a/1b* and *osdrm2* mutants .

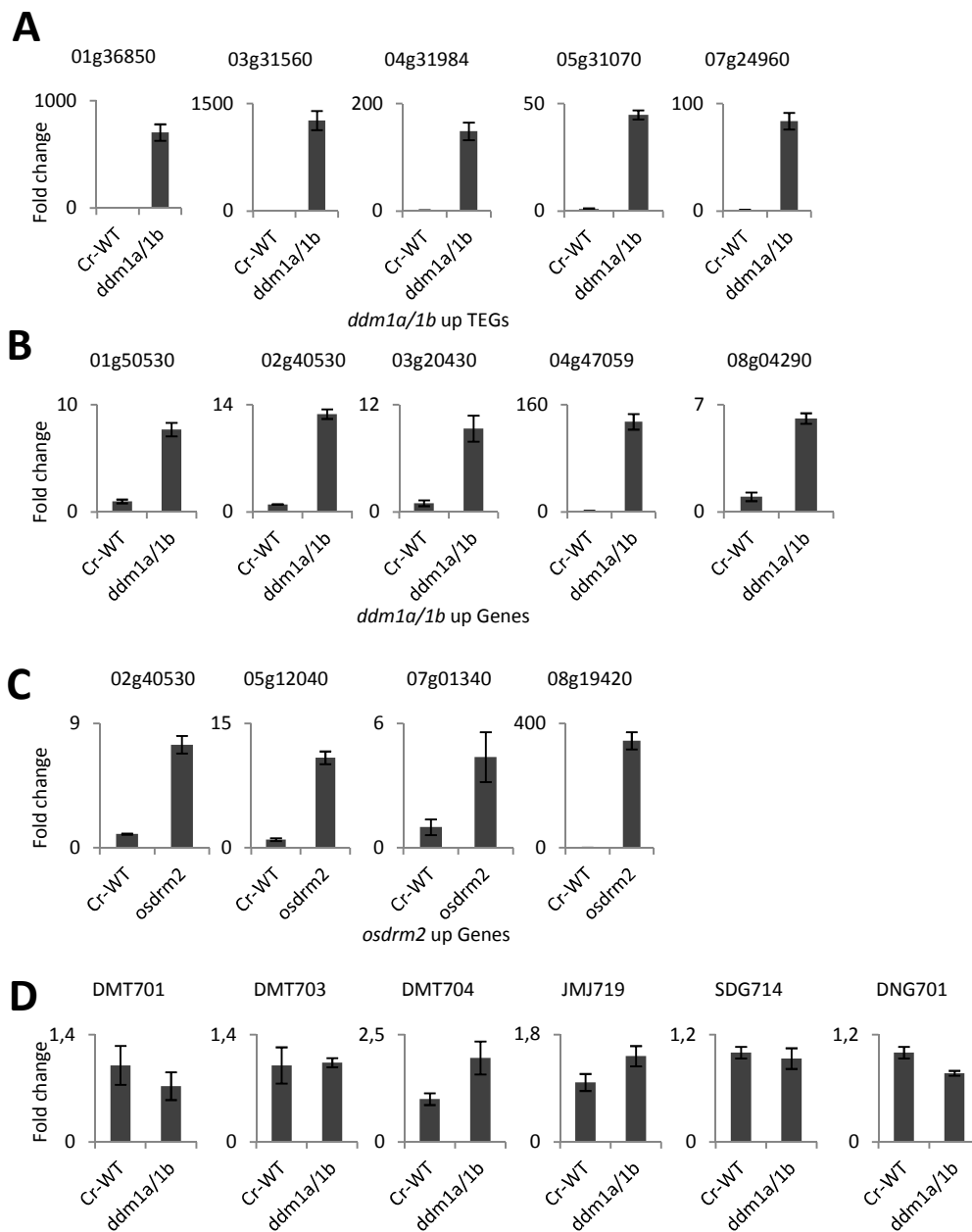


Figure S7. Related to Figure 6. Relative transcripts levels (*to Actin 1*) tested by qRT-PCR. **A.** Relative transcript levels of 5 TE-related genes shown in Fig S3 in *osddm1a/1b* compared Callus-regenerated wild type (Cr-WT). **B.** Relative transcript levels of 5 protein-coding genes shown in Fig S3 in *osddm1a/1b* compared wild type. **C.** Relative transcript levels of 4 protein-coding genes shown in Fig S3 in *osdrm2* compared wild type. **D.** Transcripts levels of H3K9me2 methyltransferase and demethylase genes in *osddm1a/1b* compared wild type. *DMT701* and *DMT704* (*OsCMT3*); *DMT703* (*OsCMT2*); *JMJ719* (*OsIBM1*); *SDG714* (*OsSUVH4*).

Table S1. Cytosine methylation levels in wild type and mutant rice leaves measured by mass spectrometry.

Genotype	n	5-meC content (% of total cytosine)	Percentage of wild-type levels	Percentage decrease
WT	2	18.80±0.51		
<i>ddm1a</i>	3	18.23±0.52	97	3
<i>ddm1b</i>	3	16.65±0.68	88.5	11.5**
<i>ddm1a/1b</i>	3	8.73±0.68	46.4	53.6**

Table S2. Related to Figure 1B; BS-seq data of wild type and mutants.

Sample	Raw Reads	Raw Bases	Clean Reads	Clean Bases	unique mapping	unique reads	Bases	Coverage (X)	all mapping reads	all mapping	Bsmap
Cr_WT_R1	50225388	6.28G	49777516	5.87G	0.606	30148787	3.59G	19.1x	38885978	78.10%	39138323
Cr_WT_R2	50225388	6.28G	49777516	5.87G	0.593	29457713	3.51G		38177954	76.80%	38428905
<i>osdrm2</i> _R1	51984762	6.50G	51598980	6.09G	0.685	35336806	4.20G	22.4x	41945268	81.30%	42353999
<i>osdrm2</i> _R2	51984762	6.50G	51598980	6.09G	0.675	34683659	4.13G		41368623	80.60%	41748375
<i>ddm1a/1b</i> _R1	49576575	6.20G	49086258	5.79G	65.20%	32018224	3.81G	20.1x	38859020	79.20%	39191088
<i>ddm1a/1b</i> _R2	49576575	6.20G	48887400	5.69G	63.30%	30880753	3.67G		37685588	77.20%	38022832
<i>ddm1a</i> _R1	54157402	6.77G	53515752	6.36G	67.90%	36341590	4.37G	22.5x	44846996	83.80%	45231651
<i>ddm1a</i> _R2	54157402	6.77G	53515752	6.36G	67.10%	35850415	4.30G		44460623	83.30%	44818782
<i>ddm1b</i> _R1	52796143	6.60G	52216457	6.18G	67.10%	35042446	4.18G	22.2x	42172955	80.80%	42581410
<i>ddm1b</i> _R2	52796143	6.60G	52216457	6.18G	65.90%	34277335	4.08G		41503003	79.80%	41904424

Table S3. Related to Figure 1B; H3K27me3 and H3K9me2 ChIP-seq data

	Clean paired reads	aligned concordantly exactly 1 time	aligned concordantly >1 times	Fail to align
Anti-H3K27me3	26194106	19755934 (75.42%)	5857015 (22.36%)	581157 (2.22%)
Anti-H3K9me2	31124057	10974036 (35.26%)	19609049 (63.00%)	540972 (1.74%)

Table S4. Related to Figure 6; RNA-seq data (two repeats per sample).

Sample	raw reads	Bases	read count	clean data	pair mapping rate
Cr_WT1_1_R1	37875666	1.93G	34674908	1.62G	96.40%
Cr_WT1_1_R2	37875666	1.93G	34674908	1.61G	
Cr_WT1_2_R1	38232336	1.95G	31678952	1.48G	96.30%
Cr_WT1_2_R2	38232336	1.95G	31678952	1.47G	
<i>osdrm2</i> _1_R1	33093494	1.69G	31219031	1.46G	95.50%
<i>osdrm2</i> _1_R2	33093494	1.69G	31219031	1.45G	
<i>osdrm2</i> _2_R1	33295195	1.70G	21412846	1.00G	95.10%
<i>osdrm2</i> _2_R2	33295195	1.70G	21412846	0.99G	
Cr_WT2_1_R1	26823185	4.05G	26168322	3.52G	92.30%
Cr_WT2_1_R2	26823185	4.05G	26168322	3.30G	
Cr_WT2_2_R1	33143704	5.00G	31678952	4.46G	92.00%
Cr_WT2_2_R2	33143704	5.00G	31678952	4.18G	
<i>ddm1a/1b</i> _1_R1	35700950	5.39G	34976763	4.78G	91.10%
<i>ddm1a/1b</i> _1_R2	35700950	5.39G	34976763	4.49G	
<i>ddm1a/1b</i> _2_R1	27632632	4.17G	27000421	3.70G	91.00%
<i>ddm1a/1b</i> _2_R2	27632632	4.17G	27000421	3.46G	
<i>ddm1a/1b</i> _1_R1	35700950	5.39G	34976763	4.78G	91.10%
<i>ddm1a/1b</i> _1_R2	35700950	5.39G	34976763	4.49G	
<i>ddm1a/1b</i> _2_R1	27632632	4.17G	27000421	3.70G	91.00%
<i>ddm1a/1b</i> _2_R2	27632632	4.17G	27000421	3.46G	

Table S5. Related to Figure 5. Numbers of hypo or hyper DMC, DMR and DMGs in CG, CHG, and CHH contexts in the mutants.

DMCs	CG		CHG		CHH	
	Hypo	Hyper	Hypo	Hyper	Hypo	Hyper
<i>osdrm2</i>	30570	12335	227058	13485	1597161	19924
<i>ddm1a/1b</i>	263798	9161	765968	4436	313235	65719
<i>ddm1a</i>	12734	16710	21892	8006	51391	42193
<i>ddm1b</i>	35929	47668	72650	11042	74172	40674

DMRs	CG		CHG		CHH	
	Hypo	Hyper	Hypo	Hyper	Hypo	Hyper
<i>osdrm2</i>	1169	315	9431	456	90851	492
<i>ddm1a/1b</i>	11314	120	120229	80	48245	8388
<i>ddm1a</i>	110	136	301	101	11490	808
<i>ddm1b</i>	671	567	1177	292	23155	1112

DMGs	<i>osdrm2</i>			<i>ddm1a/1b</i>		
	CG hypo	CHG hypo	CHH hypo	CG hypo	CHG hypo	CHH hypo
non-TE	61	633	11777	229	2569	6034
TE	5	60	1048	525	8674	483
	CG hyper	CHG hyper	CHH hyper	CG hyper	CHG hyper	CHH hyper
non-TE	23	36	48	5	6	154
TE	4	27	22	0	0	414