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Supporting Online Material for

A Role for RNAi in the Selective Correction of DNA Methylation Defects

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Correction: In Fig. S3 on page 7, the author has removed one “remethylatable sequence,” leaving five (instead of six in the original figure) to match the results indicated in Tables S1 and S2. Also, Figure S9C from the original SOM is now Figure S10B, which is reflected in the main article’s SOM citations.

A role for RNAi in the selective correction of epigenetic defects

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Legends of Tables S1 to S4

Supplemental References

Material and Methods

Plant Material

Plants were from the *A. thaliana* Col-0 accession. The *ddm1-2*, *met1-1*, *dcl2-1*, *dcl3-1*, *rdr2-1*, *nRPD1a-1*, and *nRPD1b-1* mutants have been previously described (1-5). The *ddm1-2* mutant plants used in this study were derived from a *ddm1/DDM1* plant stock that had been maintained in the heterozygous state by repeated backcrossing to a wild type Columbia line over six generations to remove EMS-induced mutations unlinked to *ddm1* (A kind gift from Eric Richards, Washington University, Saint Louis, MO, USA). Homozygous *ddm1/ddm1* progeny was subsequently selfed for four generations. Plants were grown under long-day conditions, either *in vitro* in liquid for 10-day-old seedlings (6), or in soil otherwise.

Analysis of DNA methylation and transcription

DNA and RNA were extracted from seedlings, rosette leaves, stems and inflorescences using DNeasy and RNeasy Qiagen kits, respectively.

Digestion with the McrBC enzyme (New England Biolabs), which cuts methylated DNA, was followed by quantitative PCR (McrBC-qPCR) with specific primer pairs. Digestion was carried out using 500 ng of genomic DNA. Quantitative PCR was performed on equal amounts (2.5 ng) of digested and undigested DNA samples, using an ABI 7900 machine and Eurogentec SYBR green I MasterMix Plus. Primers are listed in Table S1. Results were expressed as percentage of molecules lost through McrBC digestion (Table S2). Most *ddm1*-hypomethylated sequences (47 out of 55) were chosen within the ~500 kb, repeat-rich interval that forms the heterochromatic knob on the short arm of chromosome 4, and were thus expected to be inherited as a single block. As controls, sequences

from 14 known genes or single copy unannotated regions that are unmethylated in wt and *ddm1* were included in the analysis, together with five sequences that are equally methylated in the two genetic backgrounds (Table S1).

Sodium bisulfite sequencing was performed as previously described (7). Primer sequences for bisulfite sequencing analysis were designed using Methyl Primer Express® software v1.0 and are listed in Table S3.

Reverse transcription (RT) was performed on 1 µg of total RNA using an oligodT primer and Superscript II (Invitrogen). Quantitative PCR was performed as described above on 1/40 of the RT reaction. Results were expressed as percentage of expression relative to the mean value obtained for three genes (*At2g36060*; *At4g29130*; *At5g13440*) with invariant expression over hundreds of publicly available microarray experiments. All primers used are listed in Tables S1 and S4.

Small RNA Isolation, Cloning, and 454 Sequencing

Small RNA isolation, gel purification and cloning were performed as previously described (8), using 200 µg RNA from wt and *ddm1* seedlings. Libraries were sequenced using 454 Life Sciences pyrosequencing technology (71 623 reads for wt, and 176 257 reads for *ddm1*). Sequence reads were matched against the Arabidopsis genome using MUMmer v3.0 software (9). Only small RNA sequences with perfect matches over their entire length (15-30 nt) were analyzed further (31 878 reads in wt and 76 753 reads in *ddm1*; GEO accession number: GSE13419). Sequences were characterized using the TAIR7 release of the Arabidopsis genome annotation (www.arabidopsis.org), together with a novel annotation of transposable elements (<http://urgi.versailles.inra.fr/gbrowse/cgi-bin/gbrowse/atha/>). miRNAs were identified using miRBase (April 11th, 2008 <http://microrna.sanger.ac.uk/>) and the ASRP database (May, 2008; <http://asrp.cgrb.oregonstate.edu/db/download.html>). Sequences obtained from wt were only used for quality control. Publically available small RNA deep sequencing data obtained from wt seedlings (178 646 reads matching the genome; (10) were used for comparison with *ddm1* (Fig. 3A), to take advantage of their deeper coverage.

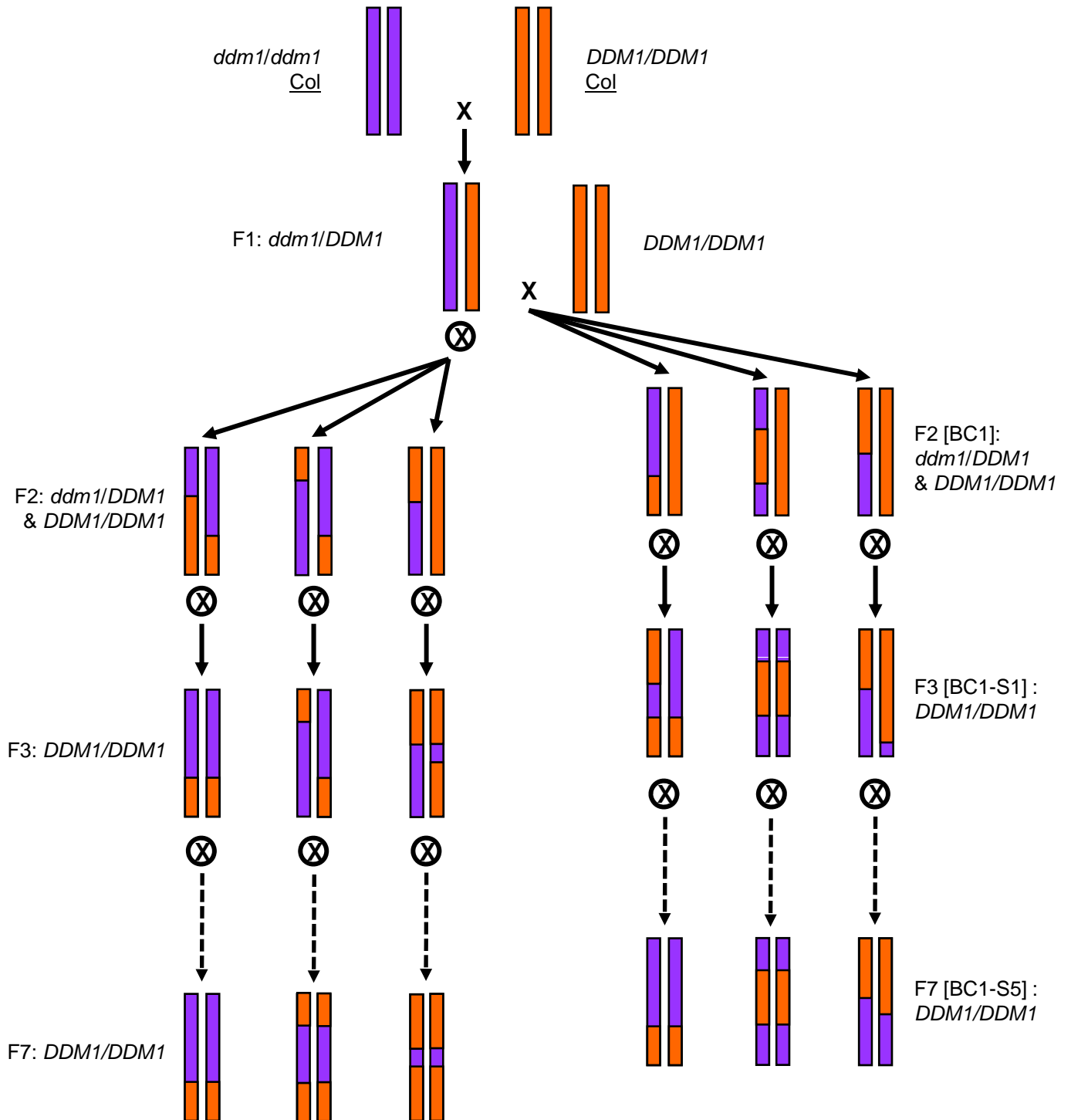
Densities (Fig. 3B, Table S1) were calculated by first considering each sequence probed by McrBC-PCR together with 300 bp on either side. Thus, densities were calculated over a 700-800 bp region in each case, using the following formula:

$$ND = \frac{\sum \left(\frac{NR_i}{NM_i} \right)}{TNR \times \text{Region Length}} \times 10^8$$

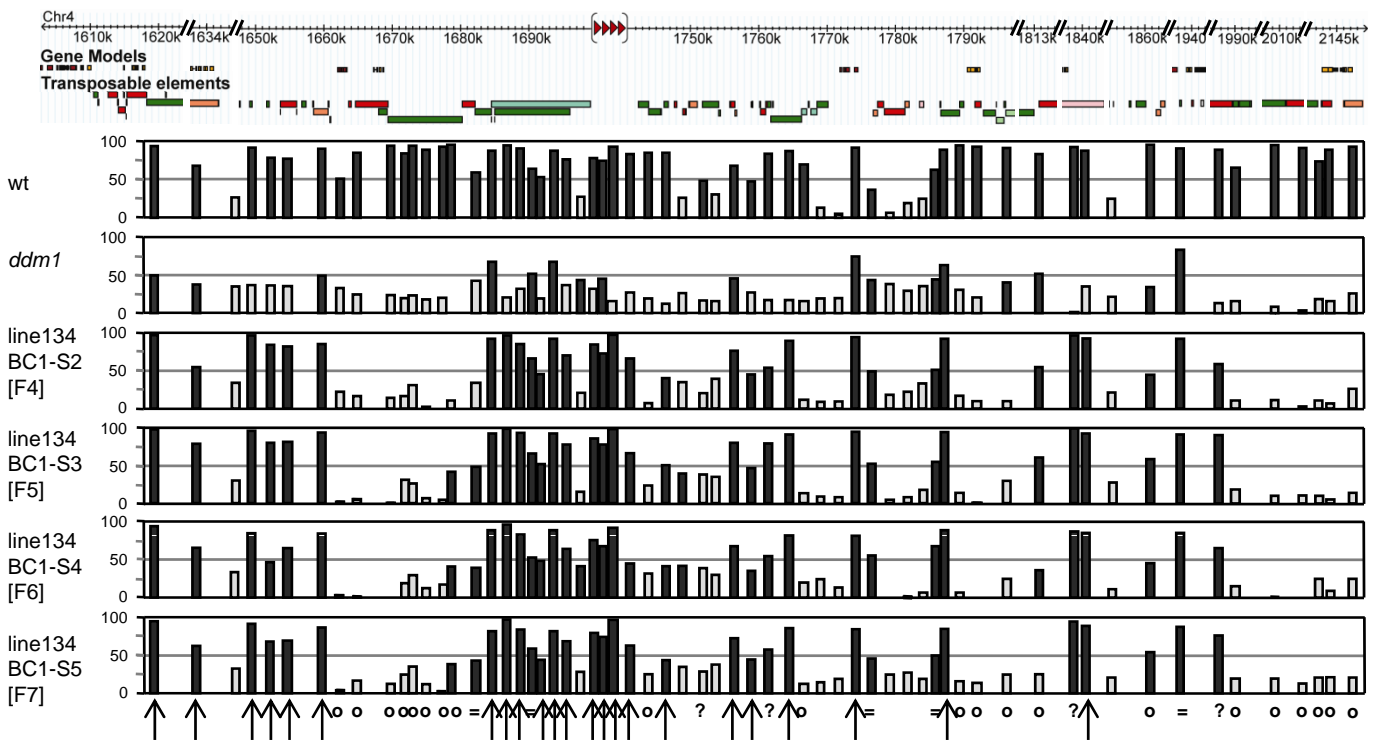
where NR is the number of reads corresponding to match M_i , NM_i is the number of total matches for that sequence across the genome, TNR is the total number of matching reads in the library, Region length is in bp. Densities are expressed as the number of reads per unique match per kb per 10^5 reads. This formula thus corrects for the fact that many siRNAs match multiple positions within the genome.

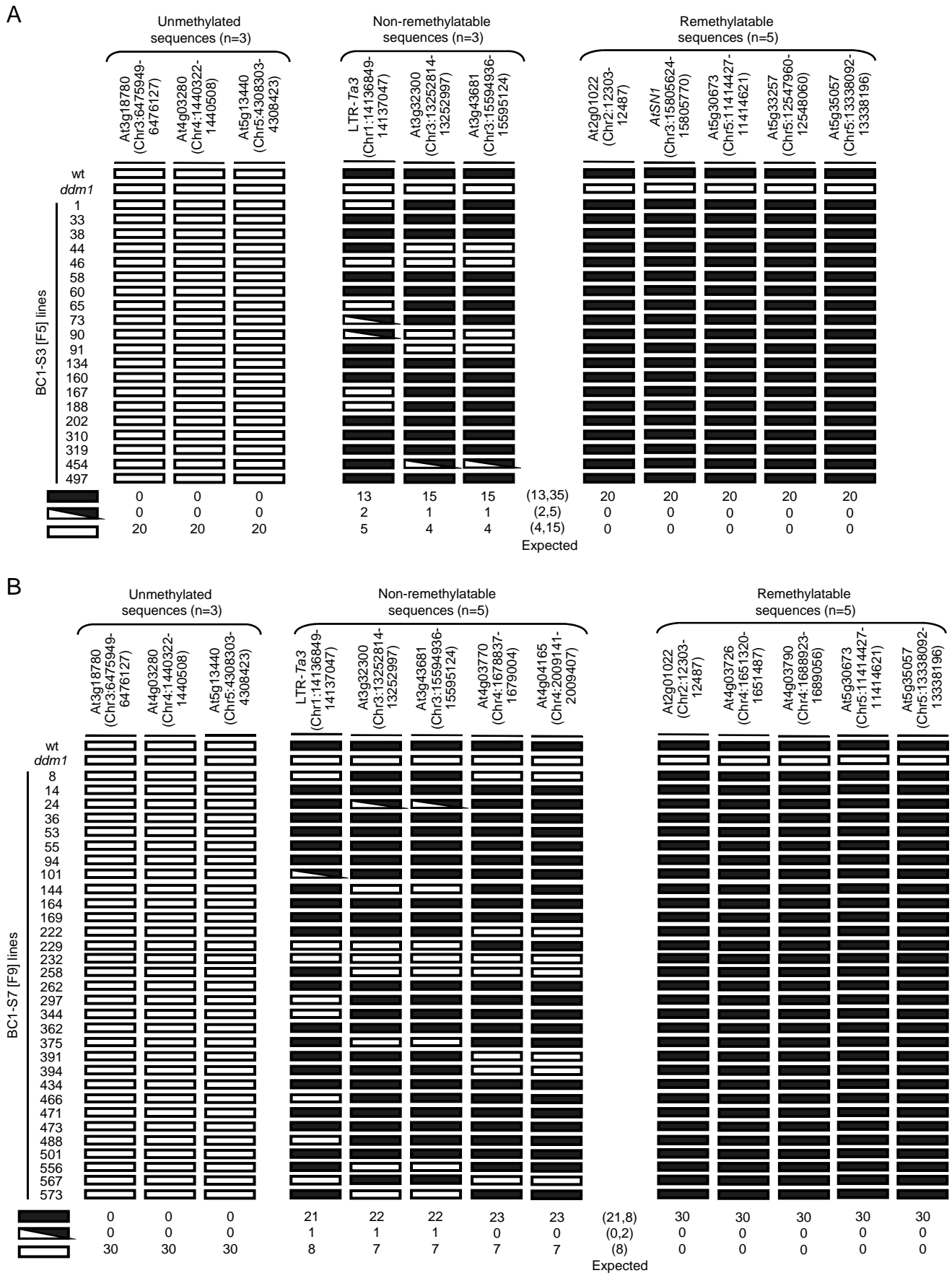
Frequency distribution of siRNA sizes (Fig. 3C) was calculated by first removing reads (approximately 25-28%) corresponding to known miRNA genes and tasiRNAs as well as reads smaller than 16 nt or larger than 28 nt.

Teixeira et al., Figure S1

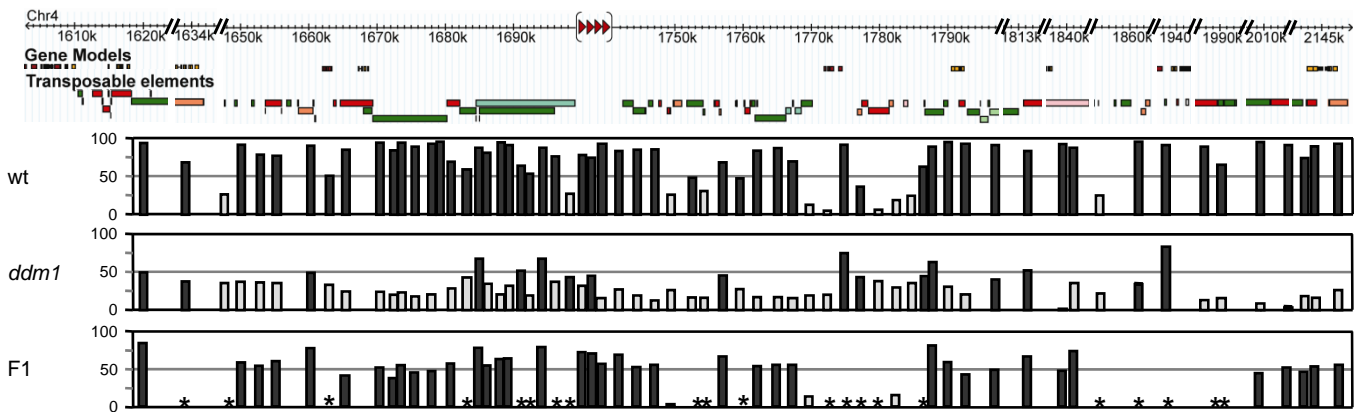


Teixeira et al., Figure S2

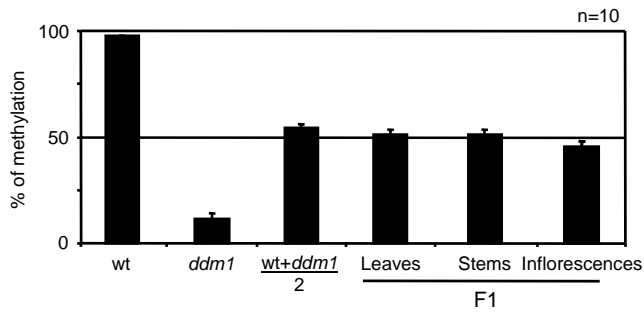




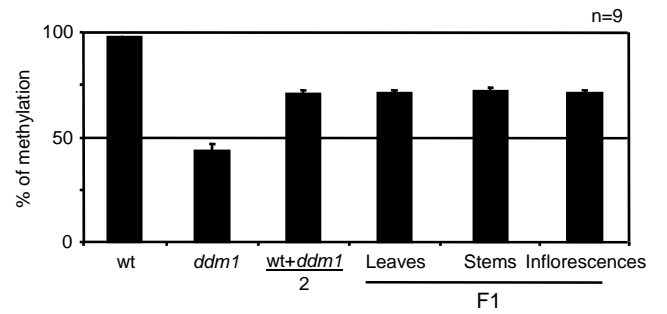
A



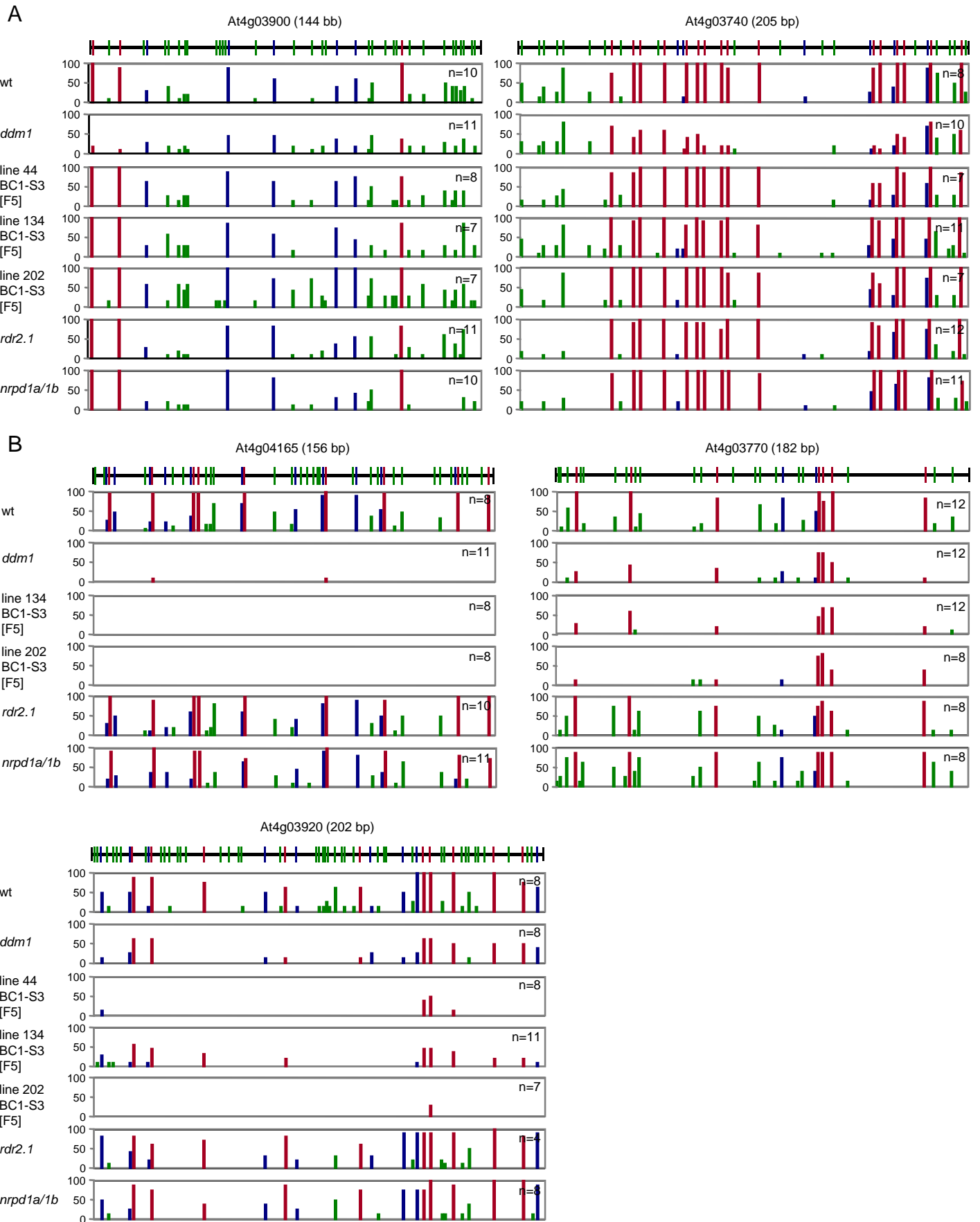
B



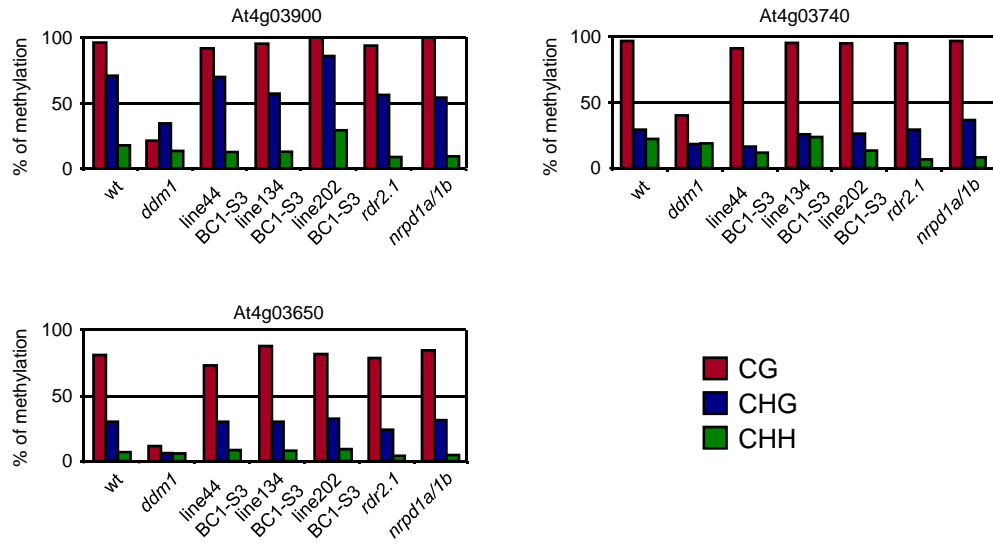
C



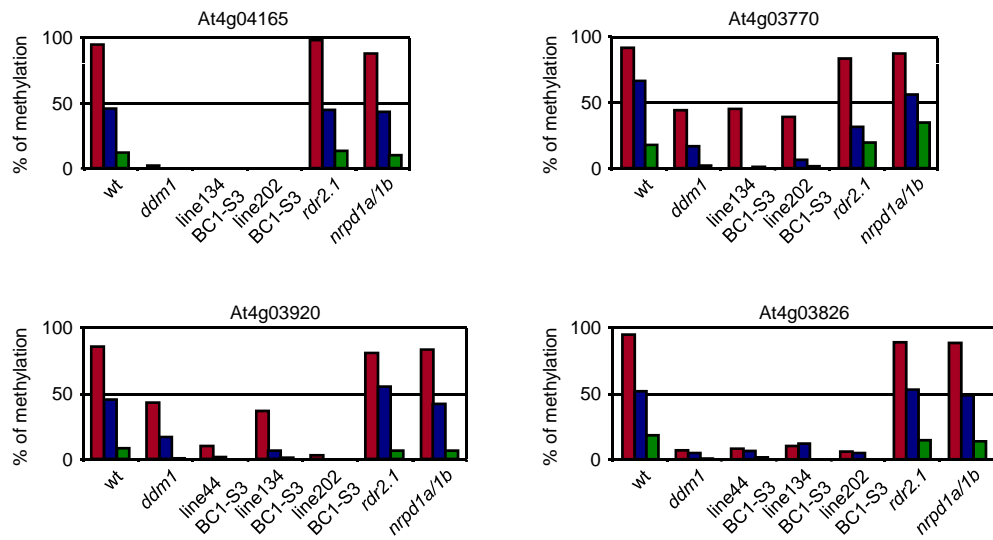
Teixeira et al., Figure S5

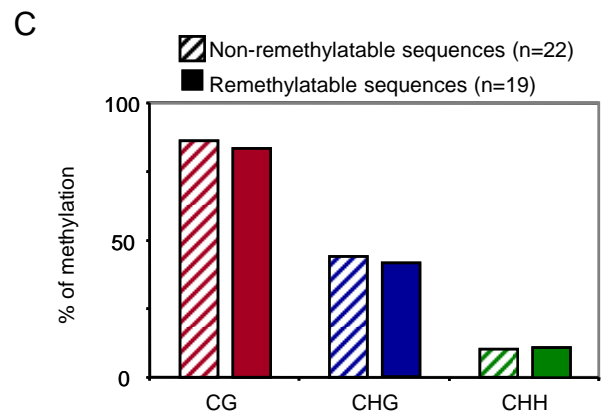
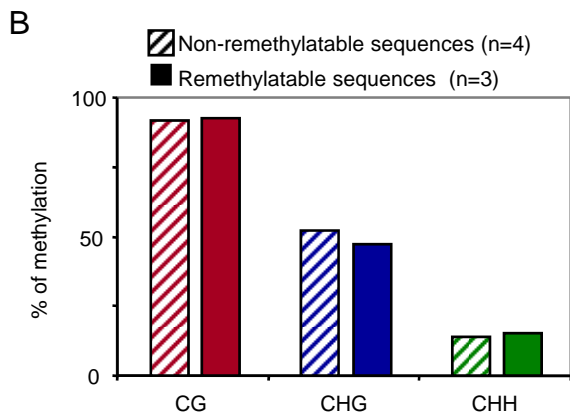
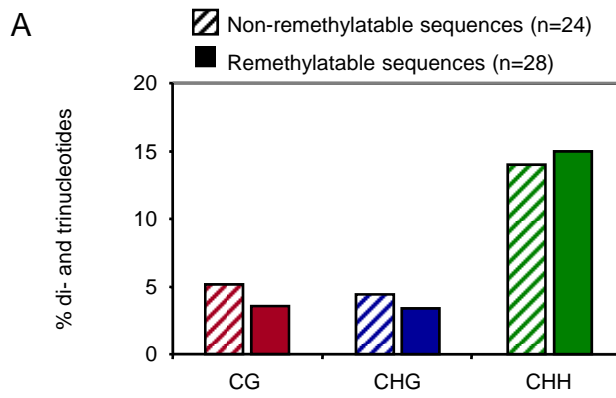


A

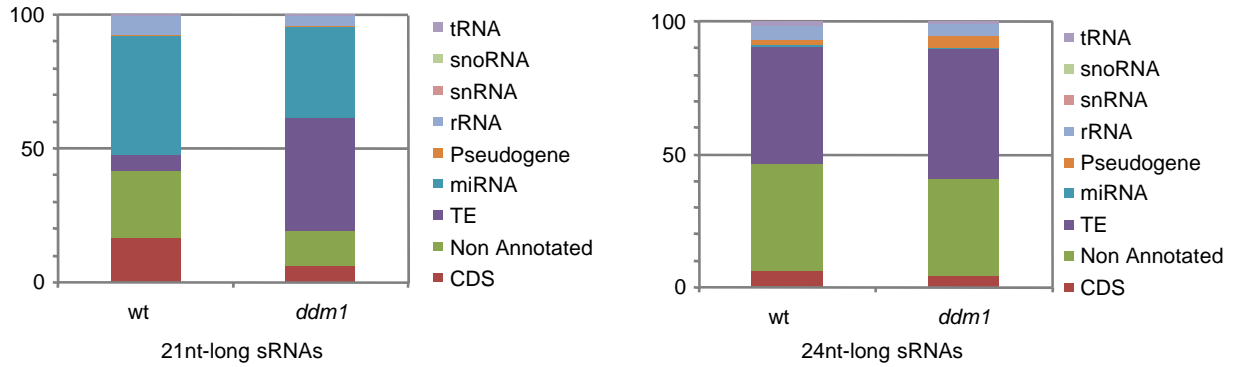


B

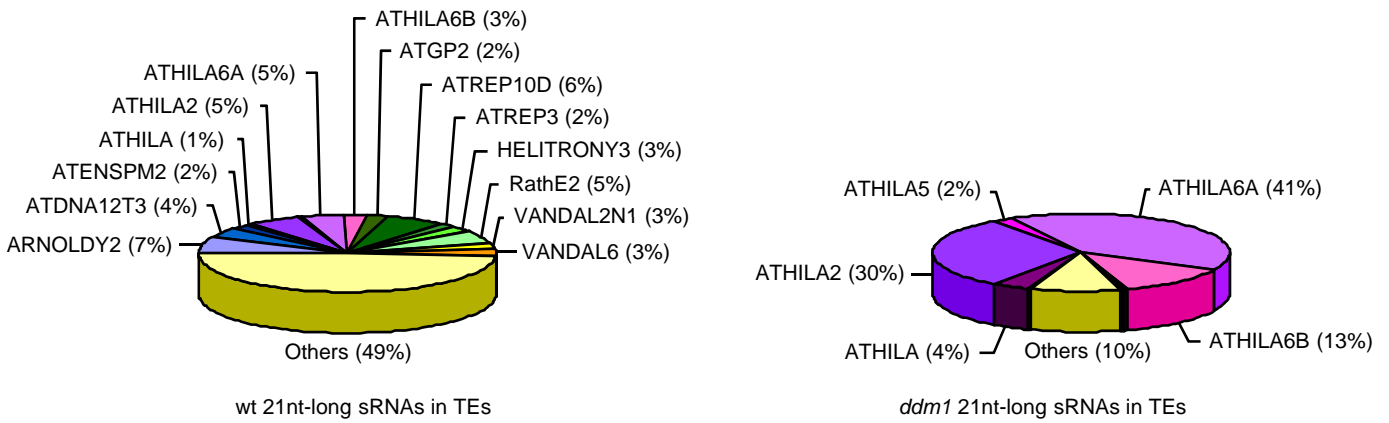




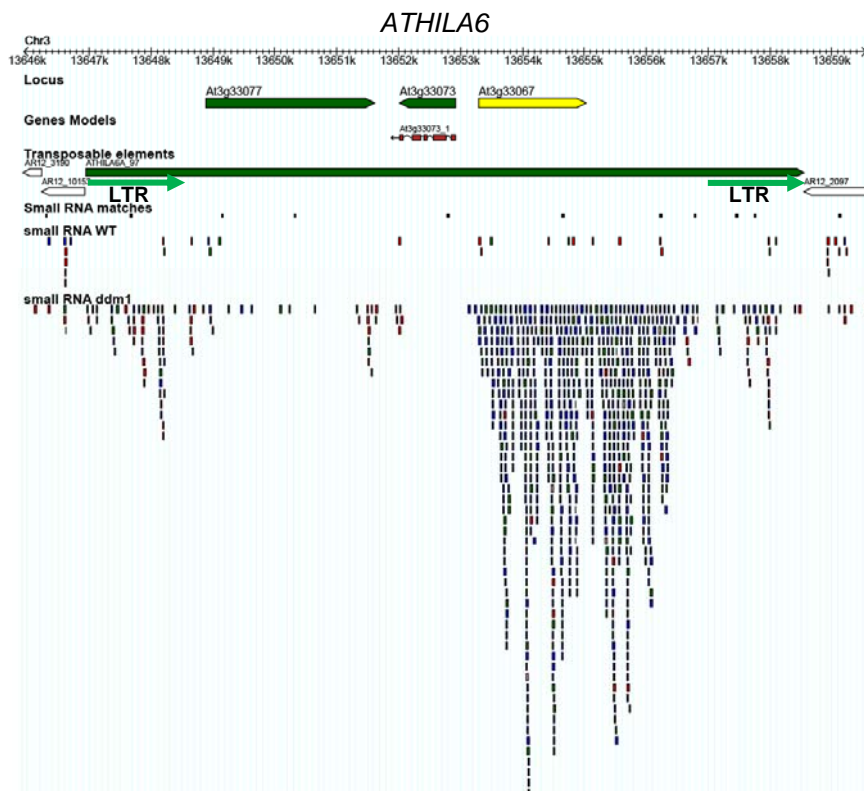
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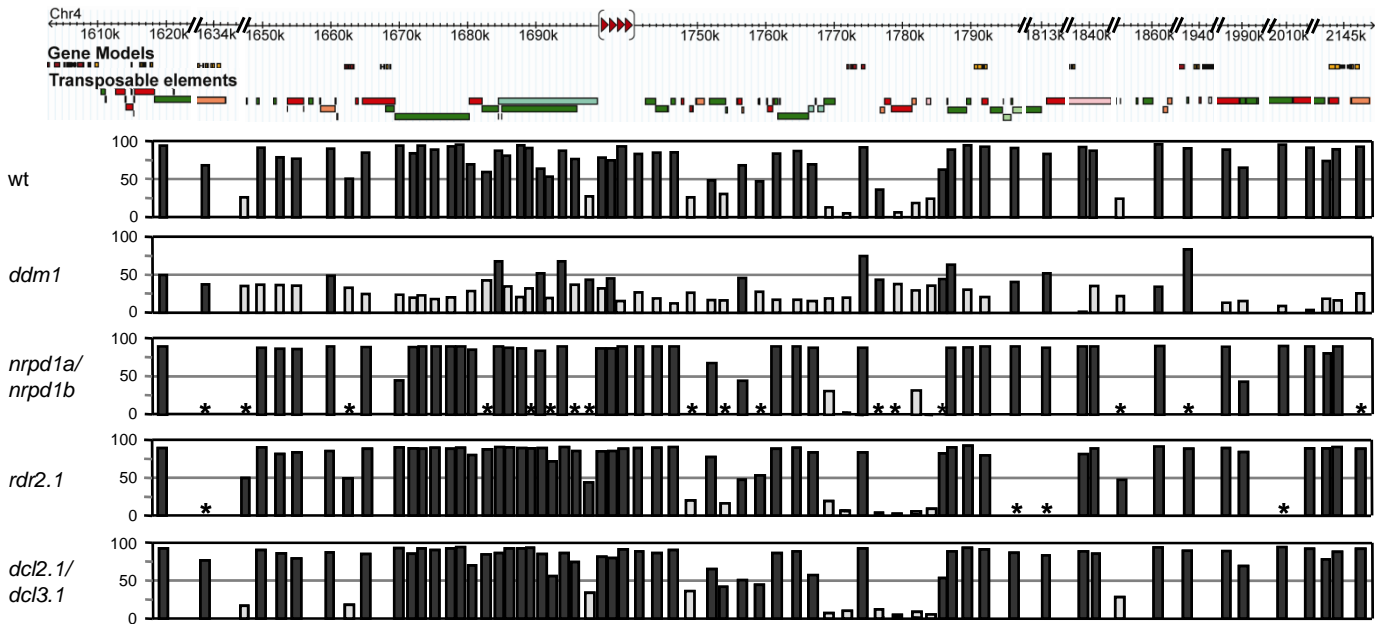
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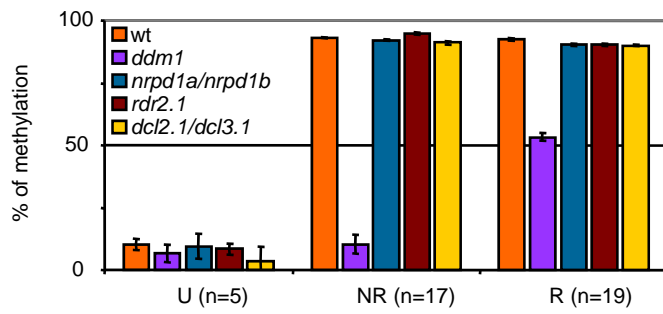
C



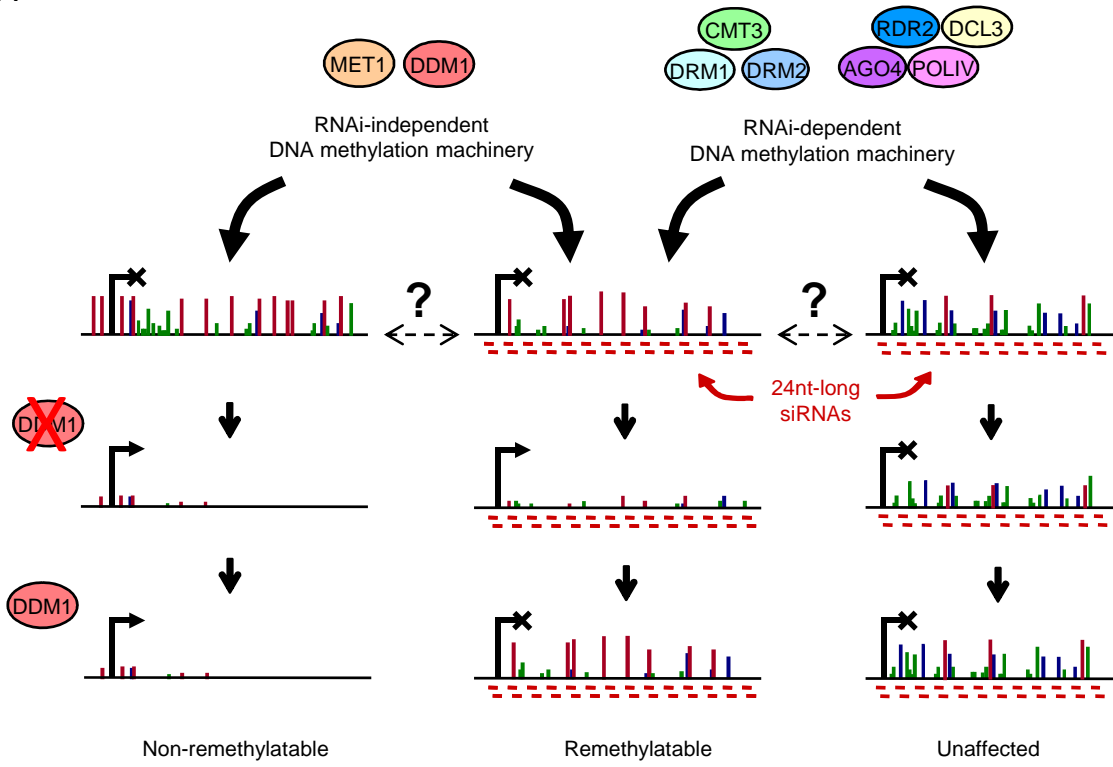
A



B



A



B

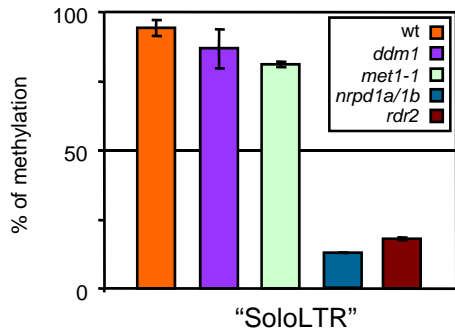


Fig S1. Schematic representation of the different genetic crosses performed to investigate remethylation in the progeny of *ddm1* plants with restored DDM1 function. The *ddm1* and wt parents were from the Col accession. Reciprocal crosses were performed between *ddm1* and wt as well as between F1 progeny and wt. F2 plants of *DDM1/DDM1* and *ddm1/DDM1* genotype and obtained through backcrossing (BC1) as well as selfing of F1 parents were selected for DNA methylation analysis. F2 plants of *DDM1/DDM1* genotype were also propagated by selfing for another five generations for DNA methylation analysis. Purple and orange color lines indicate chromosomal segments inherited from *ddm1* and wt, respectively.

Fig. S2. MrcBC-qPCR analysis of DNA methylation in a single progeny line over successive generations (BC1-S2 [F4] to BC1-S5 [F7]). Representation is as in Fig. 1A of the main text.

Fig S3. Segregation analysis of DNA methylation in *DDM1/DDM1* BC1-S3 [F5] and BC1-S7 [F9] lines. **(A)** Results obtained using 20 independent F5 lines and 11 sequences located outside the knob region of chromosome 4. **(B)** Results obtained using an additional 30 independent F9 lines and 13 sequences located within as well as outside the knob. Name and position of probed sequences are indicated at the top. DNA methylation levels were measured by MrcBC-qPCR. Black rectangles indicate, high, wt methylation, white rectangles indicate either absence of methylation or *ddm1*-induced hypomethylation. Sectorized rectangles indicate levels of methylation that are intermediate between that of wt and *ddm1*, suggestive of heterozygosity. Observed values for segregation of methylation states are indicated below each graph, as well as values expected in the case of Mendelian segregation.

Fig S4. MrcBC-qPCR analysis of DNA methylation in F1 progeny. **(A)** Representation as in Fig. 1A. Stars indicate sequences not analyzed in the F1 progeny. **(B, C)** Average DNA methylation level of 10 non-remethylatable **(B)** and 9 remethylatable **(C)** sequences in wt and *ddm1* seedlings, as well as in leaves, stems and inflorescences of F1 progeny. Averages between wt and *ddm1* are also indicated.

Fig S5. Results of bisulfite sequencing for **(A)** two remethylatable (At4g03900 and At4g03740) and **(B)** three non-remethylatable (At4g04165, At4g03770, and At4g03920) sequences located within the heterochromatic knob. Sequence size and position of cytosine sites are indicated at the top of each panel. The percentage of methylation at each cytosine in wt, *ddm1*, three independent *DDM1/DDM1* BC1-S3 [F5] lines, *rdr2.1*, and *npr1a/1b* is indicated by vertical bars (red, CG; blue, CHG; green, CHH). The number of clones sequenced is shown in each case.

Fig. S6. Summary of bisulfite sequencing results obtained for **(A)** 3 remethylatable sequences (At4g03900, At4g03740 and At4g03650) and **(B)** 4 non-remethylatable sequences (At4g04165, At4g03770, At4g03920, and At4g03826). Red, CG; blue, CHG; green, CHH.

Fig. S7. Composition and wt DNA methylation of non-remethylatable and remethylatable sequences. **(A)** CG, CHG and CHH composition of 24 non-remethylatable (stippled bars) and 27 remethylatable (solid bars) sequences. **(B)** Percentage of methylation of CG, CHG and CHH sites in wt plants as measured by bisulfite sequencing of 4 non-remethylatable (stippled bars) and 3 remethylatable (solid bars) sequences in wt (for individual results, see fig. S5 and S6). **(C)** Percentage of methylation of CG, CHG and CHH sites in wt plants as measured at 22 non-remethylatable (stippled bars) and 18 remethylatable (solid bars) sequences by mining single-base resolution methylome data (11).

Fig. S8. Annotation of small RNA populations and expression analysis of genes involved in RNAi-dependent DNA methylation. **(A)** Annotation of 21-nt and 24-nt RNAs matching the Arabidopsis genome in wt and *ddm1*. **(B)** Annotation of 21-nt RNAs matching transposable elements in wt and *ddm1*. **(C)** Genome browser view of a representative ATHILA6 retrotransposon located on chromosome 3 (positions 13646944 to 13658552), with matching small RNAs (blue: 20-21 nt; green: 22-23 nt; red: 24-25 nt). Note that most 21-nt RNAs that accumulate in *ddm1* match the 3' sub-terminal part of the element, which includes the predicted precursor for miR854 (12).

Fig. S9. DNA methylation analysis in RNAi mutants. **(A)** McrBC-qPCR analysis. Representation as in Fig. 1A. Stars indicate sequences not analyzed in the different RNAi mutants. **(B)** Summary of McrBC-qPCR data.

Fig. S10. **(A)** Model of DNA methylation control over repeat elements in Arabidopsis. Percentage of methylation of individual cytosines is indicated by vertical bars of varying height (red: CG; blue: CHG; green: CHH). Sequences are shown as being potentially transcribed upon loss of DNA methylation. For simplicity, progressivity of remethylation over successive generations is not illustrated. This model is an oversimplification, as there is likely a continuum of situations (question marks over double arrows) between the two extremes represented here. **(B)** McrBC-qPCR analysis of “SoloLTR” (13) methylation in wt, *ddm1*, *met1-1*, *nrd1a/1b*, and *rdr2*.

Teixeira et al, Table S1

Name of primer pair	Chr.	Position	Forward Primer	Reverse Primer	Annotation	Sequence type	wt 21nt sRNA* (number of matching reads)	wt 24nt sRNA* (number of matching reads)	dΔml 21nt sRNA (number of matching reads)	dΔml 24nt sRNA (number of matching reads)	Density of wt 24-nt sRNA (per kb per 10 ⁵ 24-nt sRNA reads)	Density of dΔml 24-nt sRNA (per kb per 10 ⁵ 24-nt sRNA reads)
LRTx3	1	14136849..14137047	TTTGCTCTCAAACTCAATTGAAGTT	TAGGTTCTGATGATCTTGTATGAGCTC	(ATOP1A66)	NR	0	0	0	0		
At2g01022	2	12303..12487	CGAATGAATCCCTTACCCAAC	AGCCAGCATCGGGAGGAT	AT2g01022 (ATP1)	R	1	17	3	21	2,670772447	14,33404346
Act1n2	3	6475949..6476127	GCCATCCAGTGTCTCTCTC	CCCTCGTAGATTGACACAGT	AT3g18780	U	0	0	0	0	0	0
SRF	3	8247700..8248287	GCCGACGAAATGATCGGAG	CTTCGGACGAAATGATCGGAG	AT3g23130	U	0	0	0	0	0	0
At3g32300	3	13252814..13252997	ACGCTCCGATGTTCCTCTA	TTCTGAGATCCGCGAAGTAT	AT3g32300 (ATLANTYS2)	NR	0	0	0	0	0	0
At3g43681	3	15594936..15595124	TCCGAACTTCAATGCTCTGA	CACGCTCCGCAACTACAA	AT3g43681 (ATLANTYS2)	NR	0	1	0	0	0,47290559	0
AtSN1	3	15805624..15805770	AACGTGCTTGTGCCCAAGT	CTGGAATTTCAAGCCCAAGT	(RatH3_cons)	R	0	0	0	0	2,504966221	0
At4g03280 (ORF)	4	1440322..1440508	CTCATCTCTTCCCTCCCTAC	TGAACTCTCTTCCCTCCCTAC	At4g03280	U	0	0	0	0	0	0
At4g03650 (ORF)	4	1621496..1621645	CGGTTCATCCAGTTCATAT	TASCCTTCGCCACTTCGCTG	AT4g03650 (ATP1)	R	1	19	1	21	2,432817826	10,61640969
LB (65)	4	1633554..1633696	ATTAGAAGGGTGGCGAAGT	AGSAGCTTCAATCCAAATGA	AT4g03690 (HELITRON2)	R	2	1	0	0	1,259235041	0
LB (50)	4	1648850..1648985	TGTGATTTTGTCTTCGCTTGT	GCGACTCCCAATTTCTCAAG	non-annotated	U	0	0	0	0	0	0
LB (47,5)	4	1651320..1651487	GGGTGAACGTGTCCAAATAC	GGCAATGGCTTACTGCTCAT	AT4g03726	R	0	8	0	0	13,40009961	0
LB (45)	4	1654018..1654138	TCTTAAAGATTTGCTCCCTCA	GCTTTTGAAGTTTTPGAAATGG	AT4g03730 (ATENSIPM2)	R	0	0	6	0	2,595423334	60,78731733
LB (42,5)	4	1656372..1656505	AKTCAGGGGCTTCAAGT	AKTCAGGGGCTTCAAGT	non-annotated	R	0	0	0	0	4,713007058	0
LB (37,5)	4	1661412..1661566	TCATTGTGCCCAATATTCG	CGATGTTTTCCGACGATT	non-annotated	R	1	5	0	1	12,39194165	9,674374112
LB (35)	4	1663903..1664062	TGCAGAAACAGACTAACGA	TGCAGAAACAGACTAACGA	(HELI TRON1C)	NR	0	0	0	0	4,924123222	0
LB (32,5)	4	1666416..1666551	GGCACGAGCTTGTAAAGAG	GGGTCTTGTGACTTTTGGA	AT4g03745 (ATENSIPM2)	NR	0	1	0	0	0,508491102	0
LB (27,5)	4	1671382..1671533	AATTAAGCTCCCTATCTCTG	CTCCGAGAGCCAGTAGAG	AT4g03760 (ATLANTYS2)	NR	0	0	0	0	0	0
At4g03770 (ORF)	4	1673729..1673971	CGATGACGAGAGTTGACCC	CGCTTTTCCGCTTTATGCA	AT4g03770 (ATLANTYS2)	NR	0	1	0	0	0,158826027	0
LB (25)	4	1673860..1674024	GAGGACCCGATCTCCTCAT	CGAGAACCCTCCGTAAGC	AT4g03770 (ATLANTYS2)	NR	0	1	0	0	0,174710621	0
LB (22,5)	4	1676428..1676568	GGTGTAAACGAGAAATCGT	TGGAGGCTGCTGCTAGATT	AT4g03770 (ATLANTYS2)	NR	0	6	1	0	4,37714638	0
LB (20)	4	1678837..1679004	GGCCTTATCTCCTGTCTCTG	ATTTTGGGAAATCGGGAAC	AT4g03770 (ATLANTYS2)	NR	0	0	1	0	0	0
At4g03770 (PRO)	4	1679370..1679513	GAGATCTCAGAAAGATTCTGTC	TGAATGGGTTGTTGGTTTT	(ATLANTYS2)	NR	0	0	0	0	0	0
LB (15)	4	1683804..1683944	GCTCCAGGAGTTTTTCAGCA	GAGGCTGAGAGCGAATCGT	AT4g03780 (ATHILA3)	M	0	1	0	3	2,525276758	7,275702075
At4g03790 (LTR)	4	1685514..1685735	AATCTAAGTCCCAACGCAAT	TGGAATTTGACCCAGACAT	(ATHILA2)	R	9	31	8	48	1,618305793	5,527556641
At4g03790 (ORF)	4	1688437..1688644	AATCTGGGAGGAGGAGAGA	CATGACGACACCCTGTTT	AT4g03790 (ATHILA2)	R	2	10	315	18	0,668129825	6,483779019
LB (10)	4	1688923..1689056	TCCTCCGCTGAGATATATG	AGTCGCCCACTGATGATGT	AT4g03790 (ATHILA2)	R	5	12	274	5	4,405156431	2,261713407
LB (7,5)	4	1691371..1691492	TCCCTGACCTGATGCTCA	AGCTCACTGATGCTGCTCA	AT4g03790 (ATHILA2)	R	0	0	0	0	0,838849512	2,330516092
At4g03790 (ORF)	4	1692632..1692733	ATCCGAATGCAACATTAAGC	GATGCTGATGCTTCCAAA	AT4g03790 (ATHILA2)	R	0	2	0	0	0,837841792	0
LB (2,5)	4	1696529..1696673	TGTGGAGCTGAGATCTGTT	TGTGGAGCTGAGATCTGTT	AT4g03795 (ATHILA3)	R	0	0	1	0	5,210478347	0,653627068
LB (0)	4	1698790..1698964	TCCTAATCCCTAAGTGTGCA	TTCCCTCAACCTCAGATCTC	(ATHILA3)	U	0	0	2	0	1,718989046	2,642687217
AtenAT1	4	1718375..1718813	CGTTTCCAAAGGTAAGACTT	CGTGTGTTAAACCCGAGTCC	(ATENSAT1)	R	1	35	0	5	4,881136864	1,711466356
AtenAT2	4	1718819..1719123	CGATTCCTCTCAGACTATG	CGCTTCCTCTCAGACTATG	(ATENSAT1)	R	0	0	0	0	3,488485676	2,162176657
AtenAT3	4	1719334..1719448	AGAAAGTTTGTACCCGCTAAG	TGGCAAAGCCGGAAGATTG	(ATENSAT1)	R	0	0	0	0	1,390251821	6,202044535
RB (0)	4	1742330..1742467	CGACGAGAGGCTCACTCTAA	CGACGAGAGGCTCACTCTAA	(ATHILA4)	R	0	0	0	0	0,197984802	0
RB (2,5)	4	1744850..1745011	CTGCTGACCATGCAACAACT	TTCCGATGGGACGAGAGTGT	AT4g03800 (ATLANTYS1)	NR	0	0	0	0	2,455591065	0
RB (5)	4	1747375..1747513	GACAGCTTGTATCCGCTCAT	CGATGACCAACAAGAGTGT	AT4g03800	R	0	5	0	0	3,165150408	0
RB (7,5)	4	1749837..1750010	CGTACGAAATTTTTCAGTG	GATGACGAAATTTTTCAGTG	(ATREF10)	U	0	0	0	0	0	0
RB (10)	4	1752876..1753037	CGCGAAATCTGAATGGAAAT	TAACTTTCCGACCACTCT	AT4g03810 (ATCOPIA95)	I	0	0	0	0	0	0
RB (12,5)	4	1754561..1754691	CGTCACTGATCAATGAACCAT	TGAACCTGATCACTGACCTCA	non-annotated	U	0	0	0	0	2,239885891	0
RB (15)	4	1757308..1757437	TCTGACCCAGTCCCAATA	CCGACCCGACCAACTCAG	non-annotated	R	1	16	0	1	21,07465438	10,00614227
RB (17,5)	4	1759668..1759805	TTGSCCTGTGATGATGTA	TGACGGGACCACTCCCTTA	non-annotated	M	1	0	0	0	9,378694106	0
RB (20)	4	1762377..1762505	ACGSCAGGAGATCTCTCAT	ATTCGCGGCTTTGTTTCC	AT4g03813	I	0	0	0	0	2,566902199	0
RB (22,5)	4	1765040..1765190	ACCCAGGTGTGAGTGTATA	CAAGCCGAGTCAACCACT	AT4g03816 (ATHILA2)	R	0	0	0	0	0,725577688	0
RB (25)	4	1767385..1767517	GGCGGCTCAAAATGGAAT	GATCGGCTCAAAATGGAAT	AT4g03816	NR	0	0	0	0	0	0
RB (27,5)	4	1769744..1769853	GGTTTGAAGAGCCCAATTT	CGAAGTTGCAAAAGTTGAT	AT4g03816 (ATCOPIA28)	U	0	0	0	0	0	0
RB (30)	4	1772313..1772471	CGGATATCTCTCTCTGTTG	CGGCACTCTCTCTCTGTTG	AT4g03820	U	0	0	0	0	0	0
RB (32,5)	4	1774813..1774954	CGAACAGCAGAGATATGTTGC	TGGATATGATCTGCTTCTGA	non-annotated	U	0	0	0	0	2,521868827	0
RB (35)	4	1777125..1777324	CACATAGATCAATGAGATTGAG	CACATAGATCAATGAGATTGAG	(ATREF10A)	R	0	3	30	1	5,799972189	1,232185431
RB (37,5)	4	1779784..1779915	GCTTAAAGCCCATATTTGA	CGTAAAGCTTGTTCACCA	non-annotated	U	0	0	0	0	0	0
RB (40)	4	1782212..1782344	CAATCATTTGAGTGCACAG	TTCCCAAGCTATGCGCGAGT	non-annotated	U	0	0	0	0	0	0
RB (42,5)	4	1784473..1784608	TGATCTTCCACAGCAATGC	GATCTCTTCCACAGCAATGC	AT4g03827	U	0	6	0	0	0	0
RB (45)	4	1786490..1786688	CGGAAACCAATATAGADTAG	CGTCAACAGCTCCAAATGC	AT4g03825 (ATHILA2)	M	0	0	0	4	0	1,129922983
At4g03826 (PRO)	4	1787486..1787683	ACGATATTTCCGCGTCA	ACCCCAACAGAGTCCCTCA	(ATHILA2)	R	7	11	1	22	1,527170913	2,782524867
RB (47,5)	4	1789783..1789958	CCCAAGCTTCCGAAGTGA	ACCAAGCTCCAGACTTTCC	(TAT1_ATH)	NR	0	0	0	0	0	0
RB (50)	4	1792310..1792462	CGTGGAGAGGTTCAAGCTG	CGAGCCCTAAGCTTCTCTG	AT4g03830	NR	0	0	0	0	0	0
RB (54)	4	1798611..1798805	GTCCGCCCAATAAGAGAG	CTCAGCCCACTCCCTCATG	(ATOP2)	NR	3	8	1	0	3,428334846	1,4221797635
RB (71)	4	1813024..1813155	AGAGAGGGGAGTGTAACT	TCACTCCCTCCATCGTGTG	AT4g03870	NR	0	3	0	8	0,123724603	0,811834536
At4g03900 (PRO)	4	1838222..1838363	ACGTTGAGAGAAATCCGAGAA	TGTTTGTGATATGCTGCTG	(VANDAL2)/ATENSIPM5	I	0	2	0	0	1,681245885	0
At4g03900 (ORF)	4	1839865..1840024	CGGTTGTATCACTAGTGGTTC	AGCTCGAGGATGATTTGTC	AT4g03900 (ATENSIPM5)	R	0	29	8	7	1,377361077	6,845676304
At4g03920 (PRO)	4	1852522..1852710	TATTCGATGTCCGGAAGCTC	CGAGTTTGGAAAGCCCAAT	non-annotated	U	0	0	0	0	0	0
At4g03920 (ORF)	4	1860690..1860868	CTCGAGCCCAAGCTGCTCTG	CTCGAGCCCAAGCTGCTCTG	AT031920	NR	0	0	0	4	8,533461507	0
At4g04040 (PRO)	4	1938485..1938636	CCCTCTTCCGATATGTTGCA	CGTCTTTTACTCCGATGTTGC	non-annotated	M	0	1	0	3	2,488288683	29,13906024
At4g04140 (PRO)	4	1986726..1986871	TTACATTAAGGATCGGGTGT	ATTCAGCATAGCAACAAC	AT4g04140 (VANDAL20)	I	0	0	0	0	0	0
At4g04140 (ORF)	4	1989072..1989241	CGGAATTAGTAAACAGTCCGTA	GAAACACACCGAAACTGCA	(VANDAL20)	NR	0	0	0	0	0	0
At4g04140 (ORF)	4	2009141..2009407	CTGAGGCTCATGAGTCGAT	GGGACAGCTTTTTCAGTATC	AT4g04165 (ATLANTYS2)	NR	0	0	0	0	0,539464434	0
At4g04170 (PRO)	4	2013208..2013379	CGATCTTCTGATGCTGCTG	TGGACACTTCTGATGCTGCTG	AT4g04170 (ATENSIPM2)	U	0	0	0	0	0	0
At4g04380 (ORF)	4	2141753..2141930	TGGTGTTTTGTGCTGTCTC	AGCCTTGGATGAGGAGCTG	AT4g04380 (ATCOPIA328)	NR	0	0	0	0	0	0
At4g04390 (PRO)	4	2142644..2142828	TCCCTTCTCCGTTATCTCCA	CCGCACTCAAAACCAGCA	AT4g04390 (VANDAL9)/VANDAL12)	NR	0	0	0	0	0	0
At4g04390 (ORF)	4	2146697..2146868	CGCTTTACCAGAGTTTCAT	AGAGCCCAACCAAGCTTCT	AT4g04390 (VANDAL11)	NR	0	1	0	0	2,423741635	0
At5g13440	5	4308903..4308423	ACAGCCAAATTTTTCGTAGC	ACAGAGTCCAGATGTCATGTT	AT5g13440	U	0	0	0	0	0	0
At5g30673	5	1141447..11414621	CGATATCTCCGACAGT	CGATATCTCCGACAGT	(ATHILA2)	R	1	29	1	0	1,616191585	12,44279111
At5g32527	5	12547958..12548058	ACCAGCCGAGTCAACCATAT	CATTTGCTCGAGTCTCTG	AT5g32527 (ATHILA2)	R	2	11	0	2	3,171160014	0,806762553
At5g35057	5	13338092..13338196	TGCTAGATCGAGTGTGCTGT	CGAGCCCTGAGAGCAGAG	AT5g35057 (ATHILA2)	R	11	10	273	6	1,306598741	3,094895903

* From R. Rajagopalan, H. Vaucheret, J. Trejo, D. P. Bartel, C. G. Davis, 2007 (2006).

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Name of primer pair	Sense	Antisense	Product Size (bp)	Sequence type
Bs-ara-At4g03650 (ORF)	AGTAAAGATGGTAGAGTTAAAGAAGTAG	ACAACATCATCAATCCTTAACAA	199	R
Bs-ara-At4g03760 (ORF)	GGGAGTAAGAGAAATGTTAAGTTTTTT	ACTTTAAAAACAAAAACACTTATTTTCA	237	NR
Bs-ara-LB (47, 5)	ATGYTGGATTTATTATTGAGGTTTTGA	TCACACTTTTCCCAAAAAAATCT	262	R
Bs-ara-At4g03900 (ORF)	TATGATGGAATTATATTGAAATTYAGGA	CRACAAACRTTTTTCTTCTATAATATAA	200	R
Bs-ara-At4g03920 (ORF)	GTTGGGGTATGYAGATTGATATAATATG	CCTTTACTTCCAATAACATAAATAACA	254	NR
Bs-ara-LB (37, 5)	GTTTGTGTGTGATTATAATTTGTTTGTA	TTCCAACCAATATTATTATCTRAAAA	260	NR
Bs-ara-ta26b10	GATGAAAAATGAAAAAGTTGAATAGTA	AAAAAAAAAAAAACAAAAAACCTC	210	NR

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	Chr.	Position and size	Primer sequences
NRPD1a	1	23363737-23363954 218 bp	5' CATTGCCTCCTGACTATTGGA 3' 5' TCCTTCTTGATGAGCCTCTGA 3'
AGO4	2	11547777-11547977 201 bp	5' TTTCTGTGGTGCTTGAGGAA 3' ; 5' AGTTTTTGGACCGGTTAGGC 3'
At2g36060	2	15149917-15150067 150 bp	5' TGAAGTCGTGAGACAGCGTTG 3' ; 5' GGGCTTCTCCATTGTTGGTC 3'
NRPD1b	2	16723780-16723989 210 bp	5' TGATGGTTTCAGCACCATGT 3' 5' GCTTCAGCCTTATGCGACTC 3'
DCL2	3	771620-771849 230 bp	5' CTGCAAAATCAAACCTCGGAAG 3' ; 5' GTTGGATGCAGGGTCAAATC 3'
DCL3	3	15766880-15767064 185 bp	5' GCAAAACCATCTGTGAGCAG 3' 5' AAGGCACTGCTTTTGCTTGT 3'
RDR2	4	6783466-6783656 191 bp	5' GAAGCAGGCCTCGTCTAATG 3' 5' GCAGTTGAGATCACCCCAAG 3'
At4g29130	4	14352280-14352408 128 bp	5' GGCGTTTTCTGATAGCGAAAA 3' ; 5' ATGGATCAGGCATTGGAGCT 3'
At5g13440	5	4308303-4308423 120 bp	5' ACAAGCCAATTTTGTGAGC 3' ; 5' ACAACAGTCCGAGTGTGATGTT 3'
SoloLTR	5	9872531-9872826 296 bp	5' TGCTTTCTTTCTTTCTTTCTTTTC 3' ; 5' AAACCGGATAAGTATGGATGTCA 3'

Table S1. List of primer pairs used for McrBC-qPCR analysis, sequence annotation and summary of small RNA data. Note that for each of the three primer pairs corresponding to the satellite repeat *ATENSAT1*, (*AtenSAT1-3*), only one out of 22 matching positions is indicated.

Table S2. Raw McrBC-qPCR data. **(A)** Average percentage (a minimum of two measurements) of molecules lost through McrBC digestion. **(B)** Standard deviation.

Table S3. List of primer pairs used for sequencing of bisulfite-treated DNA.

Table S4. Additional primer pairs used for RT-PCR and McrBC-qPCR analysis.

Supplemental References

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