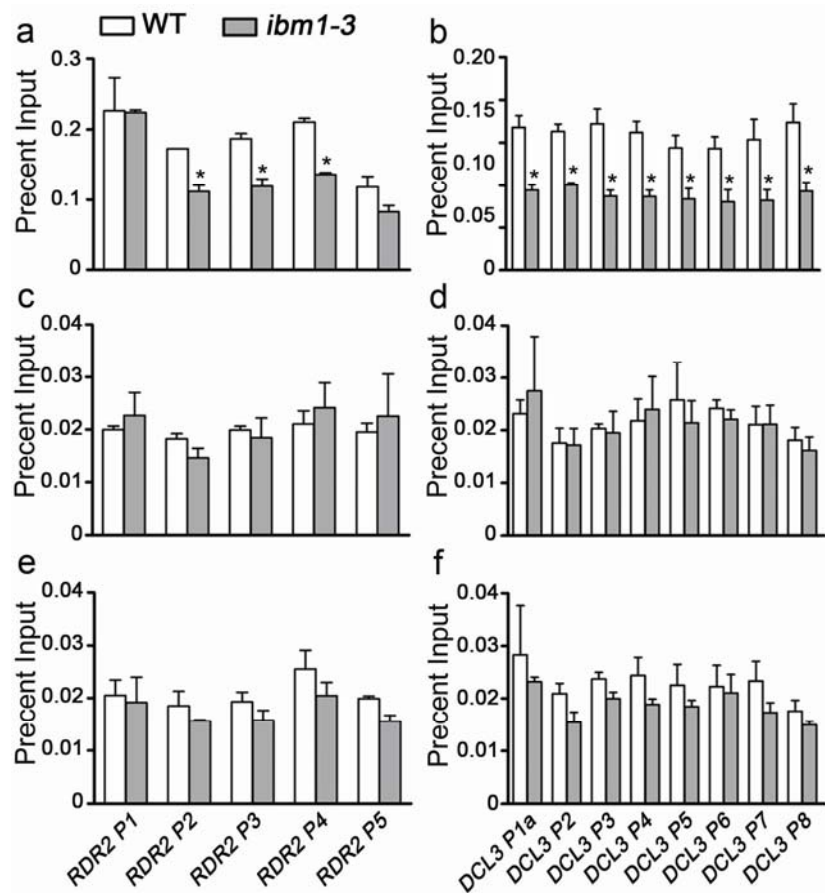
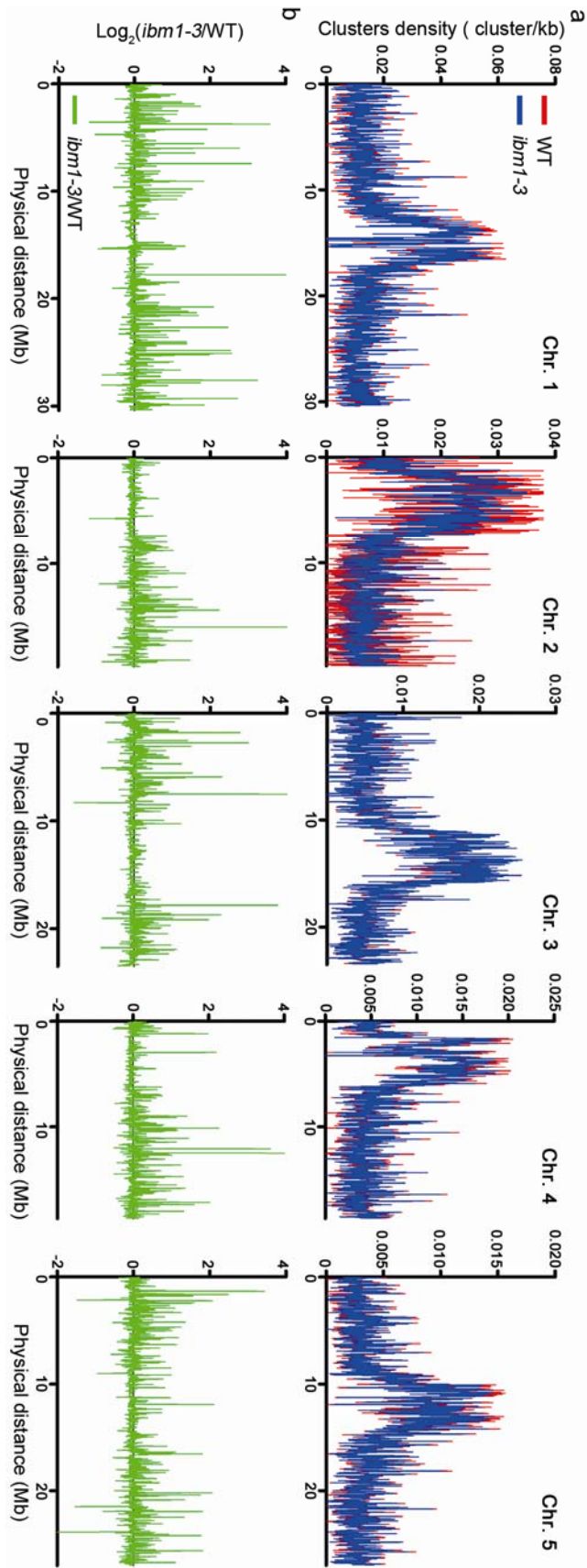


**Supplementary Materials:**

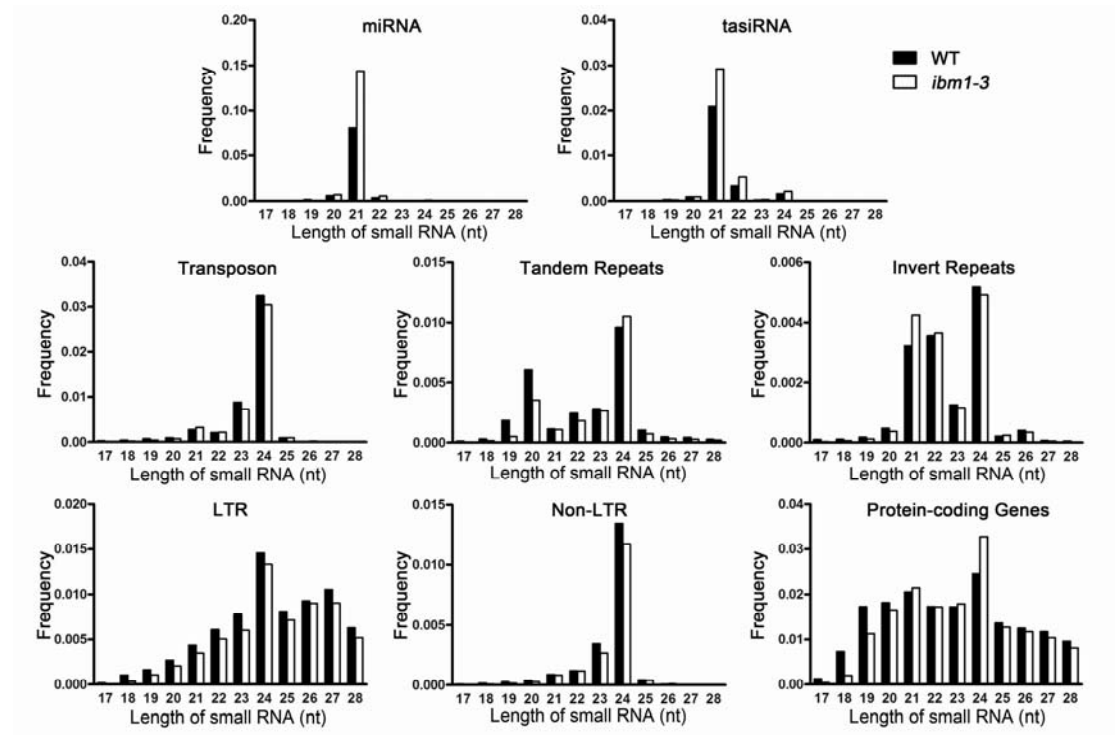


**Supplementary Figure 1.** IBM1 affects the association of Pol II with *RDR2* and *DCL3* chromatin. The association of the total Pol II ([a] and [b]), the CTD serine 2 phosphorylated Pol II ([c] and [d]), and the CTD serine 5 phosphorylated Pol II ([e] and [f]), with the chromatin of *RDR2* and *DCL3* were analyzed by ChIP. Ten-day-old plants were used for ChIP, and the enrichments were normalized to the input. All results are shown as the mean value  $\pm$  S.E. of three biological replicates. WT: wild-type. Asterisks indicate the significant difference using Student's *t* test ( $p < 0.05$ ).

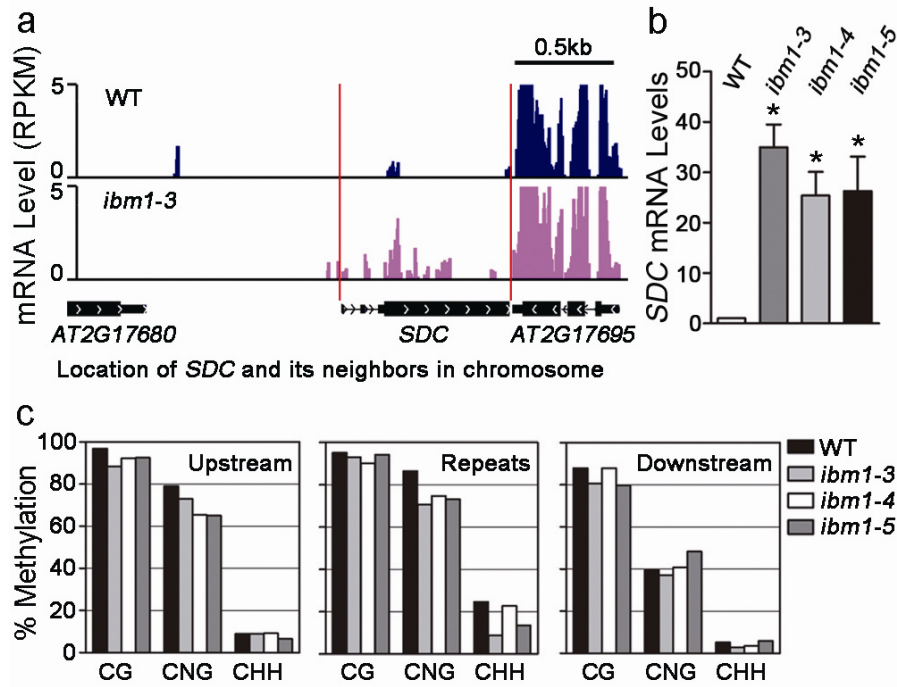


**Supplementary Figure 2.** Global view of the small RNA clusters in *ibm1* and wild-type. Clustered small RNA loci were used to compare the diversity and

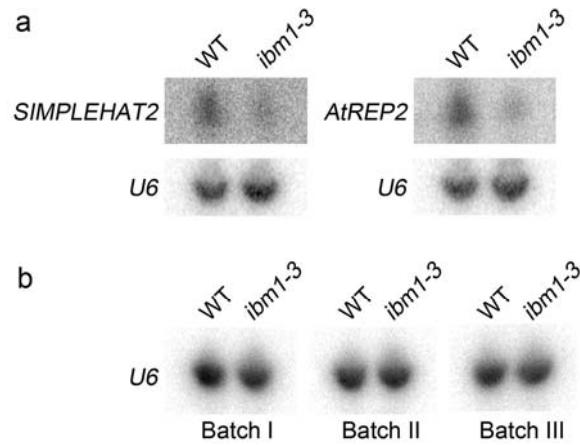
conservation of small RNAs between wild type and *ibm1-3*. The densities of small RNA clusters within 50-kb sliding windows with a 25-kb slide were determined along the chromosomes and normalized using the total number of clusters. The number of clusters per kb in the genome is presented. Most of the small RNA clusters accumulated near centromeres, while the variations between wild-type and *ibm1-3* corresponded more closely to euchromosomes.



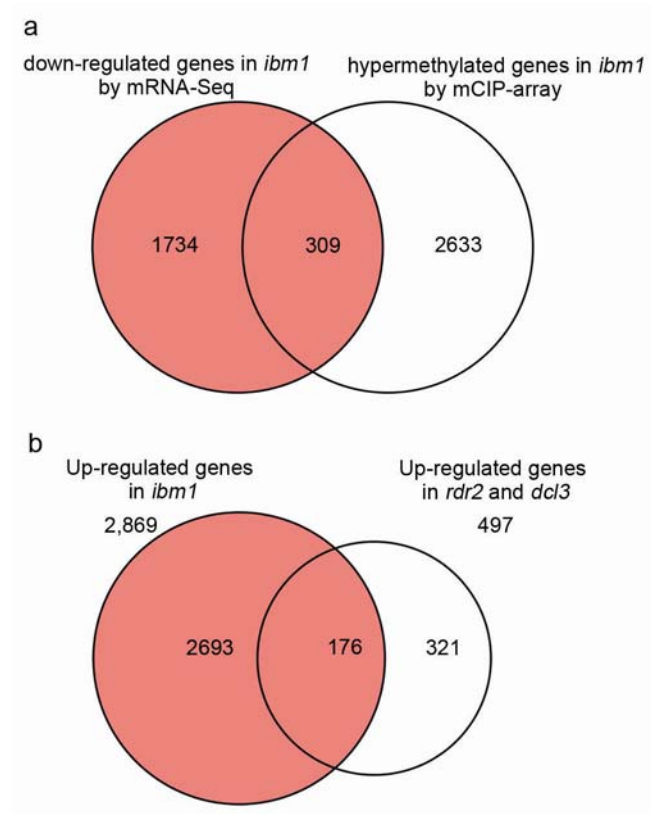
**Supplementary Figure 3.** Size distribution of the miRNAs, tasiRNAs, and small RNAs generated from different locations. The size distribution of different species of small RNAs was compared between wild type and *ibm1-3*. The percentage of reads of a given length from all small RNA reads was calculated. WT: wild-type.



**Supplementary Figure 4.** Transcription and DNA methylation analysis of *SDC* in *ibm1* alleles. **(a)** The mRNA-Seq reads that mapped to *SDC* (*AT2G17690*) were distinctly enriched in *ibm1-3*. The IGV viewer shows the read accumulation in *SDC*, but not in its flanking genes, *AT2G17680* and *AT2G178695*. The X-axis represents the location of *SDC* and its neighbor genes on chromosome II; while the Y-axis represents the RPKM value of mRNA reads by IGV. **(b)** *SDC* expression in the *ibm1* alleles was validated by qPCR. **(c)** Analysis of DNA methylation status in the tandem repeats of *SDC* promoter and in the flanking regions by bisulphate sequencing. WT: wild-type. Asterisks indicate the significant difference using Student's *t* test ( $p < 0.05$ ).



**Supplementary Figure 5.** Northern blot analysis of *SIMPLEHAT2*, *AtREP2*, and *U6* in *ibm1* and wild-type. (a) The siRNA biogenesis for *SIMPLEHAT2* and *AtREP2* are decreased in *ibm1*. Northern blot analysis of endogenous siRNAs at the *SIMPLEHAT2* and *AtREP2* locus in *ibm1-3* and wild type, the *U6* were used as a loading control. The probes for siRNA northern blots for *AtREP2*, *SIMPLEHAT2* and *U6* are listed in Supplementary Table 10. (b) Northern blot analysis of *U6* in three replicates of wild-type and *ibm1-3* plants as the equal loading for quantitative analysis of small RNAs in Figure 6c. WT: wild-type.



**Supplementary Figure 6.** Comparison of the overlap of genes from different approaches. (a) Diagram of the overlap between the genes significantly decreased in transcription and DNA hypermethylated genes in *ibm1*. The transcriptional profile for IBM1-mediated genes was from Illumina mRNA-Seq in the present work; the DNA methylation data was from Miura et al. *EMBO J.*, 2009, **28**, 1078-1086. (b) Diagram of the overlap between the genes with significantly up-regulated expression in *ibm1*, *rdr2* and *dcl3*. The transcriptional profile in for IBM1-mediated genes was from Illumina mRNA-Seq in the present work; the transcriptional profile for RDR2- and DCL3-mediated genes was from Kasschau et al. *PLoS Biol.*, **5**, e57, 2007.

**Supplementary Table 1.** Summary of the wild-type and *ibm1-3* mRNA-Seq libraries

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mRNA-Seq libraries

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	Total Reads	Genome Matches	Unique Mapped	Exon	Intron	Intregenic
Wild-type	17,348,542	14,241,086	12,388,179	12,131,311	226,000	30,868
<i>ibm1-3</i>	26,709,084	20,105,480	16,655,823	16,342,263	274,136	39,424
Total	44,057,626	34,346,566	29,044,002	28,473,574	500,136	70,292

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**Supplementary Table 2.** Summary of the differentially expressed genes in *ibm1-3* and wild-type as determined by mRNA-Seq

	log <sub>2</sub> -fold change > 0.5 or < -0.5	log <sub>2</sub> -fold change > 1 or < -1
Number of up-regulated gene	2869	1335
Number of down-regulated gene	2043	672
Total	4912	2007

**Supplementary Table 3.** Gene expression level as determined by mRNA-Seq

Name	TAIR Code	Normalized mRNA of WT <sup>a</sup>	Normalized mRNA of <i>ibm1-3</i> <sup>a</sup>	Chisquare. Test	Q-value	<i>ibm1-3</i> /WT
<i>DCL3</i>	AT3G43920	1.69	0.80	3.48E-06	2.52E-05	0.47
<i>RDR2</i>	AT4G11130	1.53	0.83	0.00129	0.00569	0.54
<i>DCL1</i>	AT1G01040	4.98	3.65	1.62E-05	0.000105	0.73
<i>DCL2</i>	AT3G03300	3.98	3.35	0.0510	0.139	0.84
<i>DCL4</i>	AT5G20320	2.27	2.70	0.0923	0.226	1.19
<i>RDR6</i>	AT3G49500	4.14	4.86	0.0838	0.211	1.17
<i>SDC</i>	AT2G17690	0.23	1.79	4.93E-06	3.47E-05	7.78

WT: wild-type

a. RPKM value

**Supplementary Table 4.** Bisulfite sequencing of the *RDR2* locus

	CG		CNG		Asymmetric		Clone No.
Sites in the region	9		6		22		
Wild-type	225	85.6 %	1	0.5 %	5	0.7 %	31
<i>ibm1-3</i>	262	80.9 %	112	51.9 %	46	5.8 %	36
<i>ibm1-4</i>	76	76.8 %	22	33.3 %	3	1.2 %	11
<i>ibm1-5</i>	244	90.4 %	118	65.6 %	44	6.7 %	30

DNA methylation in intragenic region (+2509 to +2702) of *RDR2*

**Supplementary Table 5.** Bisulfite sequencing of the *DCL3* locus

	CG		CNG		Asymmetric		Clone No.
Sites in the region	8		7		16		
Wild-type	166	74.1 %	2	1.0 %	0	0.0 %	28
<i>ibm1-3</i>	145	69.7 %	112	62.2 %	55	13.2 %	26
<i>ibm1-4</i>	172	74.1 %	109	53.7 %	52	11.2 %	29
<i>ibm1-5</i>	164	75.9 %	123	65.1 %	63	14.6 %	27

DNA methylation in intragenic region (+4204 to +4396) of *DCL3*

**Supplementary Table 6.** Summary of the small RNA libraries of wild-type and *ibm1-3*

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Small RNA libraries

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	Total Reads	Genome Matches	Small RNAs <sup>a</sup>	Unique Mapped	Multiple Mapped
Wild-type	24,911,693	21,461,549	5,088,056	2,310,925	2,777,131
<i>ibm1-3</i>	24,845,025	22,014,858	6,562,528	3,195,421	3,367,107
Total	49,756,718	43,476,407	11,650,584	5,506,346	6,144,238

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a. After eliminating tRNA, rRNA, snRNA and snoRNA matched reads from all genome matched ones.

**Supplementary Table 7.** Bisulfite sequencing of the tandem repeats in *SDC*

Upstream							
	CG		CNG		Asymmetric		Clone No.
Sites in the region	4		1		20		
Wild-type	93	96.9 %	19	79.2 %	43	9.0 %	24
<i>ibm1-3</i>	88	88.0 %	18	72.0 %	44	8.8 %	25
<i>ibm1-5</i>	74	92.5 %	13	65.0 %	26	6.5 %	20
Repeats							
Sites in the region	7		9		15		
Wild-type	160	95.2%	187	86.6 %	89	24.7 %	24
<i>ibm1-3</i>	163	93.1 %	159	70.7 %	33	8.8 %	25
<i>ibm1-5</i>	132	94.3 %	133	73.9 %	41	13.7 %	20
Downstream							
Sites in the region	4		6		39		
Wild-type	95	88.0 %	64	39.5 %	55	5.2 %	27
<i>ibm1-3</i>	58	80.6 %	40	37.0 %	20	2.9 %	18
<i>ibm1-5</i>	67	79.8 %	61	48.4 %	49	6.0 %	21

DNA methylation in *SDC* tandem repeats (-954 to -731), upstream (-1101 to -955) and downstream (-657 to -443) regions of repeats

**Supplementary Table 8.** Bisulfite sequencing of indirect targets of IBM1

<b><i>AT1G28135</i></b>							
	CG		CNG		Asymmetric		Clone No.
Sites in the region	13		7		41		
Wild-type	183	82.8 %	73	61.3 %	236	33.9 %	17
<i>ibm1-3</i>	202	77.7 %	46	32.9 %	100	12.2 %	20
<b><i>AT1G31580</i></b>							
Sites in the region	12		11		16		
Wild-type	236	98.3 %	139	63.2 %	64	20.0 %	20
<i>ibm1-3</i>	158	87.8 %	84	50.9 %	17	7.1 %	15
<b><i>AT1G33370</i></b>							
Sites in the region	3		2		21		
Wild-type	38	84.4 %	16	53.3 %	114	36.2 %	15
<i>ibm1-3</i>	40	74.1 %	18	50.0 %	134	32.8 %	18
<b><i>AT2G01422</i></b>							
Sites in the region	6		13		42		
Wild-type	70	97.2 %	110	70.5 %	92	18.3 %	12
<i>ibm1-3</i>	42	87.5 %	53	51.0 %	6	1.8 %	8
<b><i>AT2G13431</i></b>							
Sites in the region	5		7		33		
Wild-type	72	72.0 %	49	35.0 %	51	7.7 %	20
<i>ibm1-3</i>	42	64.6 %	15	16.5 %	13	3.0 %	13
<b><i>AT3G29639</i></b>							
Sites in the region	2		1		26		
Wild-type	37	97.4 %	10	52.6 %	155	31.4 %	19
<i>ibm1-3</i>	16	88.9 %	2	22.2 %	48	20.5 %	9
<b><i>AT3G50480</i></b>							
Sites in the region	7		3		59		
Wild-type	133	100.0 %	49	86.0 %	877	78.2 %	19
<i>ibm1-3</i>	116	97.5 %	42	82.3 %	726	72.4 %	17
<b><i>AT4G04293</i></b>							
Sites in the region	1		5		28		
Wild-type	16	84.2 %	40	42.1 %	65	12.2 %	19
<i>ibm1-3</i>	12	92.3 %	19	29.2 %	22	6.0 %	13

**Supplementary Table 9.** Overlap of the increased siRNAs and DNA hypermethylation in *ibm1*

Group	Region	24-nt siRNA of WT <sup>a</sup>	24-nt siRNA of <i>ibm1-3</i> <sup>a</sup>	<i>ibm1-3</i> /WT
All protein-coding genes	Promoter	8.73	8.03	0.92
	Gene Body	3.42	3.63	1.06
	Downstream	6.16	6.13	0.99
Hypermethylated genes	Promoter	10.39	10.00	0.96
	Gene Body	0.88	3.03	3.46*
	Downstream	4.22	4.99	1.18

a. RPKM

Asterisks indicate  $p < 0.01$

WT: wild-type

The siRNAs data is from the present work, and DNA methylation data is from Miura *et al.* *EMBO J.*, 2009, **28**, 1078-1086.



**Supplementary Table 10. Primers used in this work**

<b>Name</b>	<b>Sequence</b>	<b>Employ</b>
IBM1-3TF	GAGAAGGAAGTTGAGGCCAAAATATACG	T-DNA identification in <i>ibm1-3</i> , pair to LBb1
IBM1-3F	CGTTATTATTGGATATACTGCATTAAT	Identification of <i>ibm1-3</i>
IBM1-3R	CAAGCCATTAGATAAATACGAGTATAAG	
IBM1-4F	CTTGTTGATTTCTACCTCAATGACTCT	Identification of <i>ibm1-4</i>
IBM1-4R	ATCAAAACAGCATCACCAAGCTTCTGATT	
IBM1-5F	GTGGACTCTTTCGTTGAACTCCTCTTTTTC	Identification of <i>ibm1-5</i>
IBM1-5R	GGAAGCTCAACTGGCAGCAACT	
NRPD1A-ReF	GACTTGTGAAGATGGTCTGCAGTTG	qPCR
NRPD1A-ReR	GTCTTCGAATGTCCCGTCTATTCTTAC	
RDR2-ReF	TGGCGAGAGATAACCGGAGGTATG	qPCR
RDR2-ReR	CTTCTCATCGCGATGGTTTGGATTG	
DCL3-ReF	GCCTACTTTCGATACCTCGGAAGA	qPCR
DCL3-ReR	GCATACATCACAGCCTCACGATTG	
AGO4-ReF	CACTCGCTCTCCTATGTGTACCAAAG	qPCR
AGO4-ReR	CATGGCTTGATGATGTCTCAGACTGATC	
AtSDC-ReF	CATCGTGTCTAGTGATCGGCATC	qPCR
AtSDC-ReR	CAGCGTAAGTAAGACCTTCGTCAAG	
IBM1-ReF	GTTAAGAAGATGATAGTCCATGCAGTTG	qPCR and RT-RCP
IBM1-ReR	TGATATCTCCTCTTCAGCTGCTTC	qPCR and RT-RCP
IBM1-NS	CGGGATCCATGGATTCTGTGGAGGAAGAAGGTG	for 35S::YFP::IBM1
IBM1-CAS	GCGTCGACCTACATCTTCTCCATTTCTAATCTGTCAATG	construct
RDR2-P1-F	TGGCGATAGAGAAGATTGTGGAAG	ChIP
RDR2-P1-R	GCAGAGGTAATAAAACGGTAAACTAG	
RDR2-P2-F	CAGAGCTACTTTCGCAAGATTGATGA	ChIP
RDR2-P2-R	CAAGAACTCTAATATCTCCAGGGTGAA	
RDR2-P3-F	AGCGAGGACACGGTAGCTTATG	ChIP
RDR2-P3-R	CGTTAGCAGCTCCATAGTATATCATC	
RDR2-P4-F	AGACGAACTCTGTCCGGAACTAAG	ChIP
RDR2-P4-R	CCAAGTGTACTTTGGGATTGGTTG	
RDR2-P5-F	GTAGGGCATGTGACAGAATAACCA	ChIP
RDR2-P5-R	GCATTTTCATCTTCATTTGACACC	
DCL3-P1a-F	CGTGTGTCAGAATGTGTTAAATTG	ChIP
DCL3-P1a-R	CTCTCTATTCACCAAGAAAACAGTGT	
DCL3-P1-F	CATCCAAACTCGGAACATACATTG	ChIP
DCL3-P1-R	TATCCGGGTAATCTGGTAGAAATGTAC	
DCL3-P2-F	GAGAGAATTATAACTGCGAAAGTGATC	ChIP
DCL3-P2-R	AAGAACAGGTAACCTTGCCATG	
DCL3-P3-F	TATGAGTGATCCTCCTAGCAGAAATG	ChIP
DCL3-P3-R	CGACACCAGCTAATTGACGAATTA	
DCL3-P4-F	CGTAAGCGATTCTTCTGCTATGA	ChIP

DCL3-P4-R	CAATCCCATACCTGATTA AAAAGGGAA	
DCL3-P5-F	ACCGAATATCCATGCGCATTG	ChIP
DCL3-P5-R	CTAAGCTGGCTGGCCAACATTA	
DCL3-P6-F	TGGATTGTCTGCTTCTCTCCATATG	ChIP
DCL3-P6-R	TTCTCTCCA ACTCTATGAGCTCATC	
DCL3-P7-F	TGAGTTTTGCAAGAAGCATCTGTG	ChIP
DCL3-P7-R	GACTCGGGATACATGAGAGCAGA	
DCL3-P8-F	GCTCATAGACATTACTACAGTTGAAG	ChIP
DCL3-P8-R	TCGAGGCACAGTGAAAAGCTC	
DCL3-Bisu-F	GGATAGTGATGGGAATTAGTGTAATATTT	Bisulfite analysis
DCL3-Bisu-R	TAAAACATCCACCTCAAAAAAACTC	
RDR2-Bisu-F	TTTGGAGTATGGTTAAGTGATGTT	Bisulfite analysis
RDR2-Bisu-R	TACTTTTCTCCCTTCTAAAAAAA	
SDC-Bisu-F1	TAAGTTTTATTATTTGGATTTAAAGYGGATAATAT	Bisulfite analysis of <i>SDC</i>
SDC-Bisu-R1	AATCTCTAAAATTTTTTTTATTTTACTCATTCTAC	upstream and repeats regions
SDC-Bisu-F2	GTATAGAGGTTTTAAAGTAGAATGAGTAAAAT	Bisulfite analysis of <i>SDC</i>
SDC-Bisu-R2	ACATTTATATCRATAAATTTTAATCRAAAAATATAT	downstream region
AT1G28135-BS1F	TGTTAAATATATTTTTGAAYYAGATTGG	Bisulfite analysis
AT1G28135-BS1R	CCACATTACCRTTTTTRATTCRATCAA ACTT	
AT1G31580-BS1F	AAGAAAGAYAAGGTTGAAAGAGAGTAA	Bisulfite analysis
AT1G31580-BS1R	CAATCTAAAATACTTTATTCCRCCAACACTTA	
AT1G33370-BS1F	ATGATATGTTTATGAATTATTGTYTAAATATTTTG	Bisulfite analysis
AT1G33370-BS1R	AACAATCTACATATACATTTTTTRCARCCATTTTAT	
AT2G01422-BS3F	GGYTTTcTYAGYAYCGGAAYCGAAGT	Bisulfite analysis
AT2G01422-BS3R	ATAAAACCATCATCCARARACCCAAA	
AT2G13431-BS1F	TGTTTGGTGATTATAGYTTYTTTTG	Bisulfite analysis
AT2G13431-BS1R	AAAACCAATCAARCTCTTAACRCAAATTCAA	
AT3G29639-BS2F	TAAGGGTGATGTATGTTTGGAAGT	Bisulfite analysis
AT3G29639-BS2R	TATTATTGCATAAAAATGACAAACAACAATTAT	
AT3G50480-BS1F	GAATAAAGATGGATGYYYATTATAAGG	Bisulfite analysis
AT3G50480-BS1R	TTCTCTTCTTTAACRATTTTCTTTRTARAATCATT	
AT4G04293-BS1F	YAGGTAAGGGGAAATATTGGGT	Bisulfite analysis
AT4G04293-BS1R	CTCCRATCATCTARATRCTCATTTTCA	
AT1G28135F	AGTTGATTACGTTGCTCTCGC	qPCR
AT1G28135R	TTTCTTGCCCTGAGCCCTG	
AT1G31580F	ATTGTCCCATTTCATGTTCC	qPCR
AT1G31580R	TCAGTGACTTGGTGAGTTTTTTTG	
AT1G33370F	GCACCAATGGTTCAGTGGTAG	qPCR
AT1G33370R	TACACCGTCTGGGATCGAA	
AT2G01422F	ATCCATCCAAGGCAGAAGC	qPCR
AT2G01422R	GAGTGAGAGCCTCATGAAGGA	
AT2G13431F	CTTTTACCCAGGTGAGCAAAC	qPCR
AT2G13431R	CAAGCTCTTAACGCAAATTCAAG	

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AT3G29639F	TGGAAACCAGAAAGCAAAGAG	qPCR
AT3G29639R	CCACAGACACAGAAACTCATATACAG	
AT3G50480F	CGATAGAACTTGAATTAGTAAGGGTAGC	qPCR
AT3G50480R	CGACTTGTATCGCCTTCATTTTC	
AT4G04293F	GACGACTACTTCACCCAACGTG	qPCR
AT4G04293R	CAGAATCTACGCAAGCACTCTAATG	
AT1G33370	AACGTACTGCCAAACCCGTCCCGC	siRNA qPCR
AT2G01422	AGGCGGCGGCTGAAGTTTTAGAGA	siRNA qPCR
AT4G04293	AATGAGCATCTAGATGATCGGAGC	siRNA qPCR

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