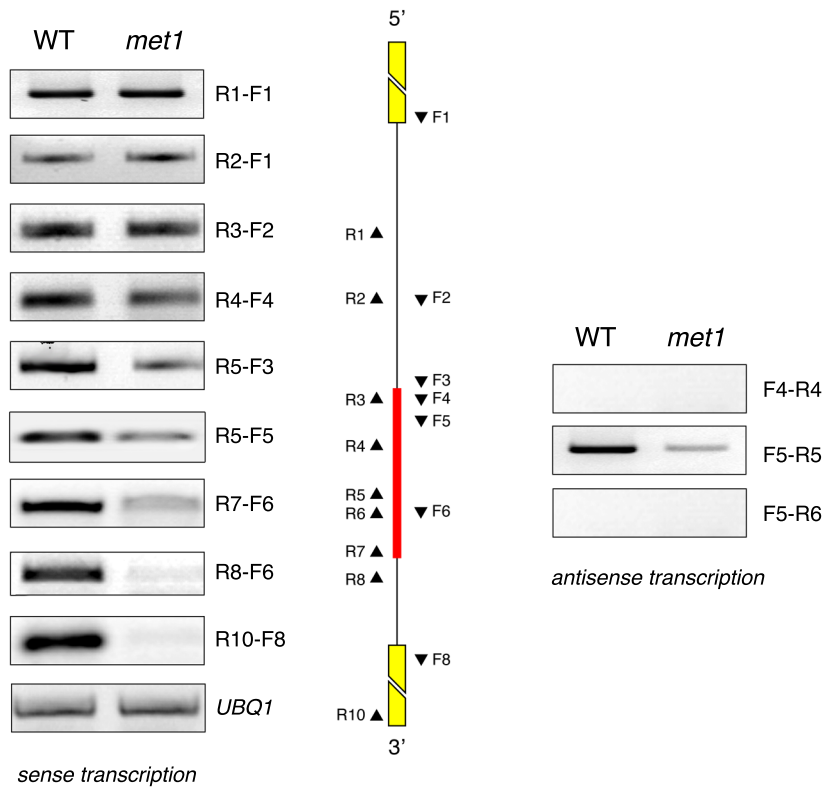
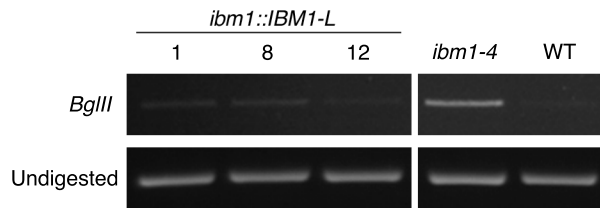


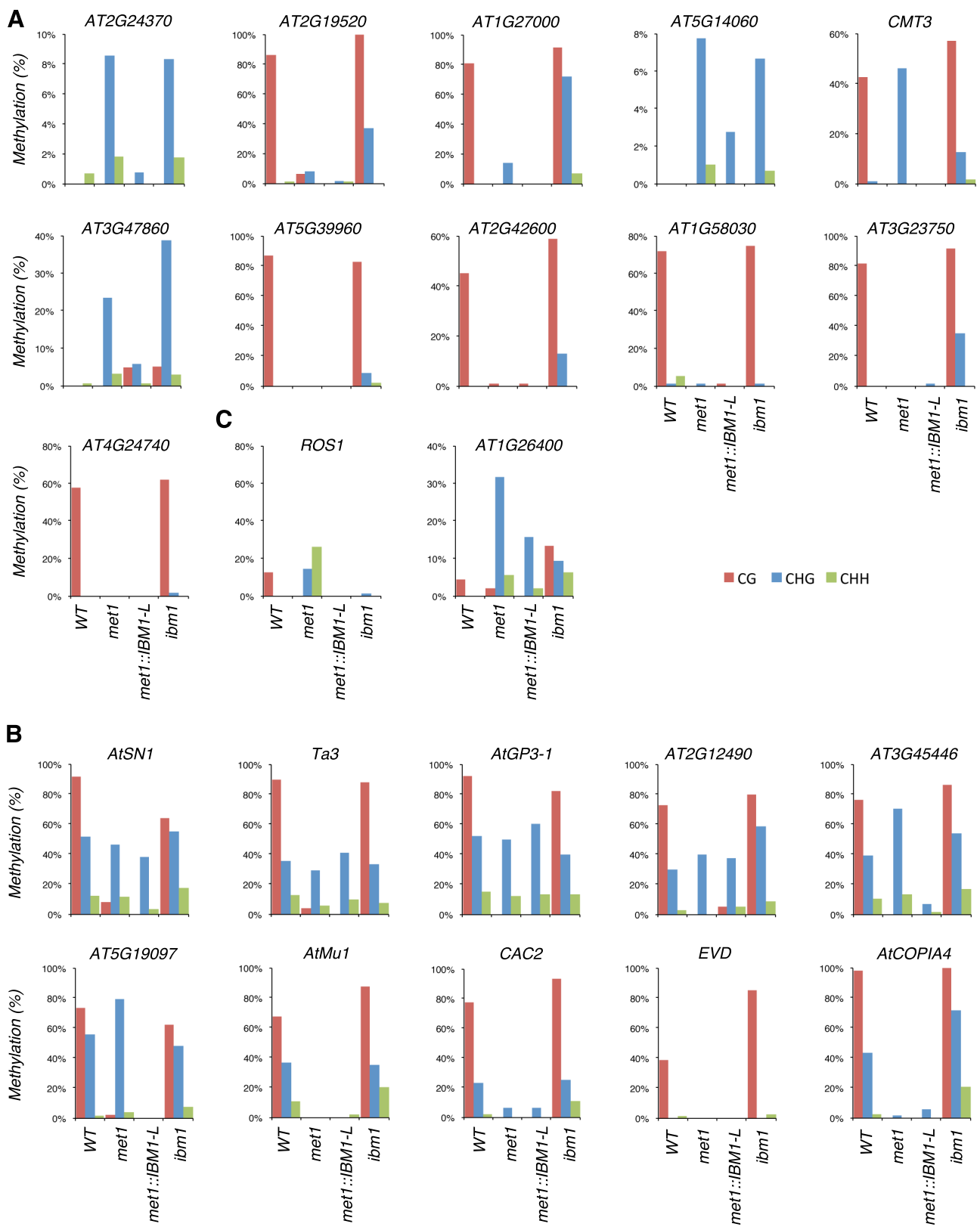
Supplementary Figure S1. Bisulfite sequencing analysis of cytosine methylation at the methylated zone of the large *IBM1* intron in wild-type, *cmt3* and *kyp* plants, and of the *cmt3*-derived and the *kyp*-derived alleles in Ler x *cmt3* and Ler x *kyp* F1 hybrids, respectively. Proportions of methylated cytosines at CG (red), CHG (blue) and CHH (green) sites are indicated as percentages.



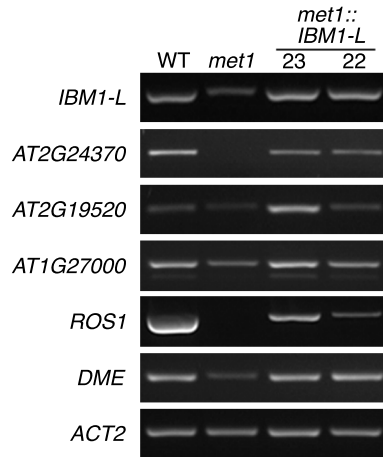
Supplementary Figure S2. RT-PCR analysis of sense (left panel) and antisense (right) *IBM1-L* transcription in wild-type and *met1-3* plants, using primer sets designed along the length of the large intron of the *IBM1* gene (middle). Primer positions, intron, exons and the methylated intronic region are indicated by black triangles, a black line, yellow boxes and a red bar, respectively. The amplification of *UBQ1* was used to normalize the RNA template levels.



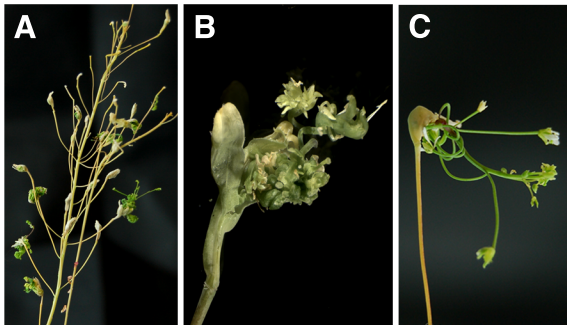
Supplementary Figure S3. CHG methylation at the *BNS* locus in 3 independent *ibm1::IBM1-L* lines and in *ibm1* and wild-type plants. DNA methylation was analyzed by restriction digestion with the methylation-sensitive enzyme *BglIII* followed by PCR with BNS-F2/R3 primers (Supplementary Table 1).



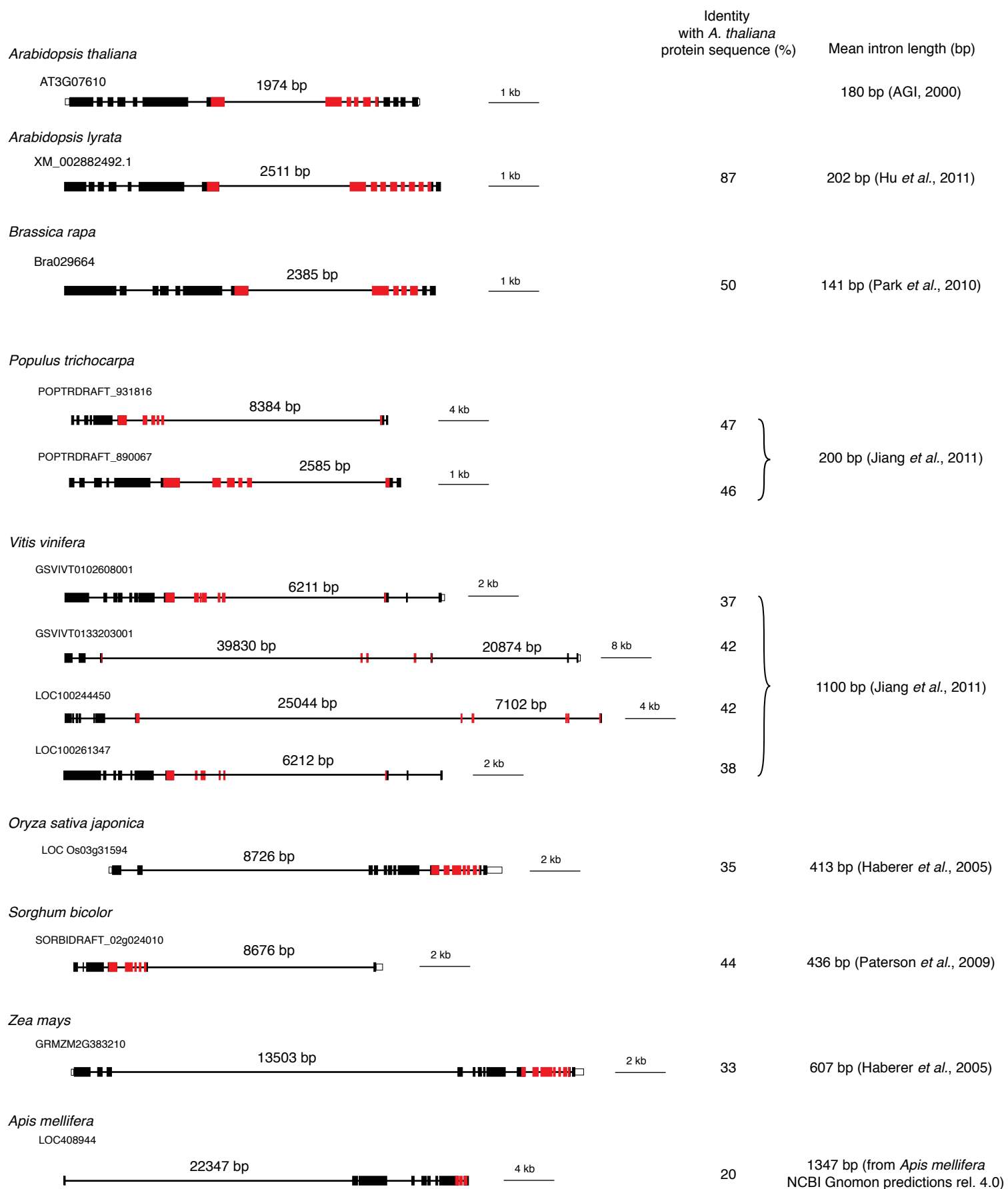
Supplementary Figure S4. Bisulfite sequencing analysis of the DNA methylation patterns at eleven genes (A), ten transposable elements (B) and at the *ROS1* gene and the *AT1G26400* *ROS1* target locus (C), in wild-type, *met1*, *met1::IBM1-L* and *ibm1* plants. Proportions of methylated cytosines at CG (red), CHG (blue) and CHH (green) sites are indicated as percentages.



Supplementary Figure S5. Transcription analysis in 2 independent *met1::IBM1-L* lines. Representative images of RT-PCR analyses at *IBM1-L*, *AT2G24370*, *AT2G19520* and *AT1G27000*, *ROS1* and *DME* genes.



Supplementary Figure S6. Abnormal flower phenotypes observed in some *met1-3* lines. Images of a whole inflorescence shoot (A) and magnification of two abnormal siliques (B & C).

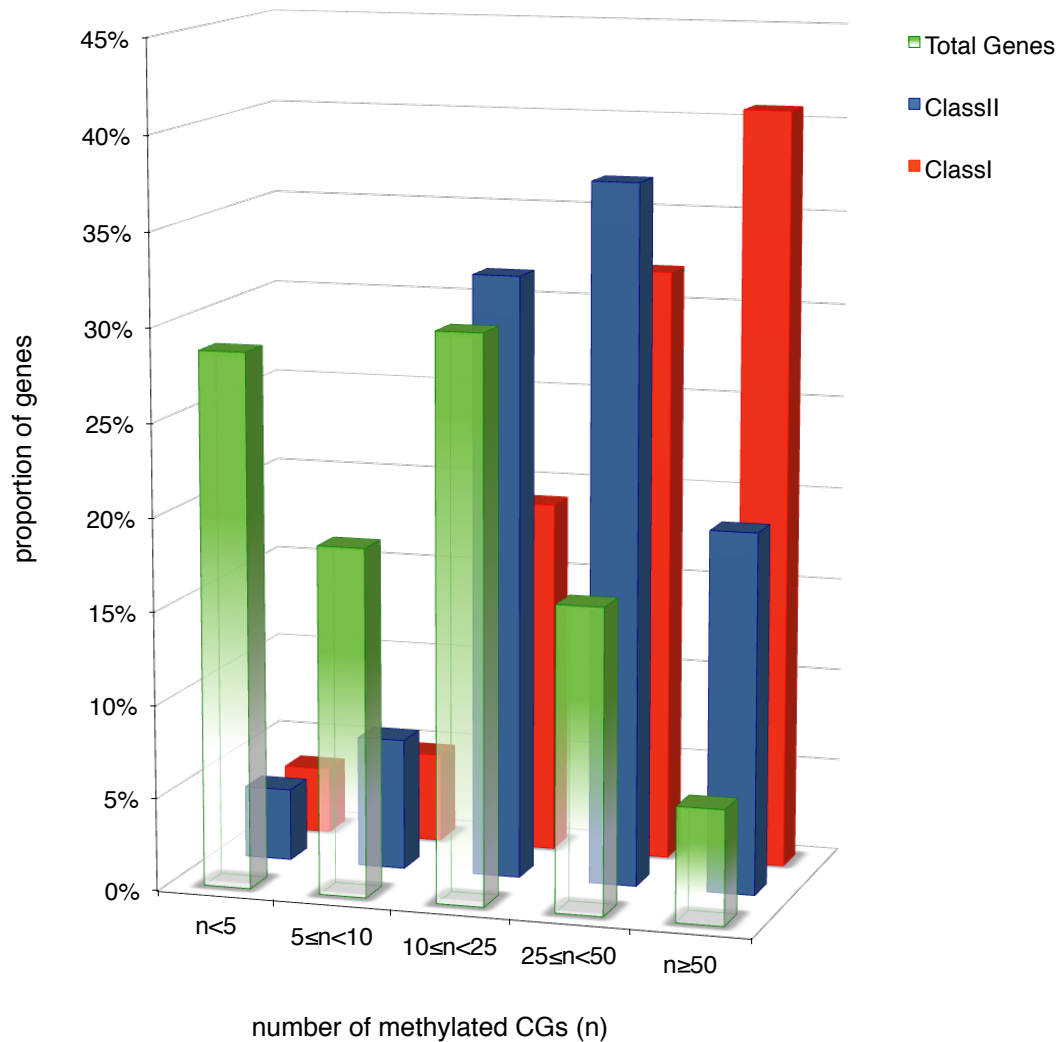


Supplementary Figure S7. Orthologs of the *A. thaliana* *IBM1* gene containing a large intron.

Boxes represent exons, with coding regions in black and untranslated regions in white. The region encoding the jmjC domain is shown in red (Saze *et al.*, 2008). Length of the large introns and scale bars are indicated for each gene. The mean length of introns in each organism and the percentage of identity of each orthologous protein with Arabidopsis *IBM1* protein are indicated.

Orthologous genes were identified by blastp of the Arabidopsis *IBM1* protein sequence against non-redundant protein sequences of the NCBI database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins>). Additional genes annotated as orthologs of the *IBM1* gene in the Gramene database were selected (<http://www.gramene.org/>).

The percentage of identity of each orthologous protein with the Arabidopsis *IBM1* protein sequence were determined by alignment of orthologous protein sequences using the ClustalW2 program (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>).



Supplementary Figure S8. Proportion of genes with the indicated number of methylated CG dinucleotides (n). Shown are Class I and Class II genes displaying DNA hypermethylation in the *ibm1-4* mutant (Miura et al, 2009) and total genes. The number of methylated CG dinucleotides was extracted from previously published datasets (Lister et al, 2008).

Locus	Primer	Sequence (5'-3')	Methods
18S rRNA	18S-F2	TCCTATTGTGTTGGCTTCGG	RT-PCR
	18S-R	CACGTAGCTAGTTAGCAGGCTG	
ACT2 (AT3G18780)	ACT2-F ¹	CTAAGCTCTCAAGATCAAAGGC	RT-PCR
	ACT2-R ¹	AACATTGCAAAGAGTTTCAAGG	
AT1G26400	At1g26400-RT-F1	GATCTTAGCTTCATCCACGTTCAA	RT-PCR, ChIP
	At1g26400-RT-R1	CGTCGACTCCAGGAGAATTT	
	At1g26400-BS-F	CTTAAACTACRCTTTTAACTACTAACTAAT	Bisulfite sequencing
	At1g26400-BS-R	GGGGAATTTAAAGAGATTGATGGATGTTAAAG	
AT1G27000	At1g27000-F	TGTGAATTCTGCTGTTTTGTTG	RT-PCR
	At1g27000-R	ACAACCACATCACGTTCCAA	
	AT1G27000-BS-F	CAAATTATRCAATRATTCCAAATCCATCTCAAA	Bisulfite sequencing, ChIP (with AT1G27000-BS-Fbis/R)
	AT1G27000-BS-Fbis	CATAACCACAACCACATCACRTTCCAAAAC	
	AT1G27000-BS-R	TGAAAAGAGATTTYAGTGTTGTAAGGGAAT	
AT1G58030	AT1G58030-F	ATTACAAAGGCCGTGGACA	RT-PCR, ChIP
	AT1G58030-R	TGTATCGGTGTGGCTGCTTA	
	AT1G58030-BS-F	CCCRITTRITCCCCTCCTACCTATCATCT	Bisulfite sequencing
	AT1G58030-BS-R	GATTTGTGTTAYATATATAAYAAAAGAGATG	
	AT1G58030-BS-Fbis	CCTACCTATCATCTRCATTCTCATCAACAT	
AT2G12490	copia66_F	GTGCCCTGTACCCCTGGAT	RT-PCR
	copia66_R	CTGATTGACTAGAACCGAAGAA	
	AT2G12490-BS-F	CAACACAACRATCAARCTTCAATTC	Bisulfite sequencing
	AT2G12490-BS-Fbis	ATCRATCAARAACATAATCAAAACAAA	
AT2G12490-BS-R	TGAAGGTGATYAAAYGGAAGTAAGT		
AT2G19520	At2g19520-F	AAACAAGGGAACCTAGAAAGCAA	RT-PCR
	At2g19520-R	TCTCTTAAACCACTGACACTATCTTG	
	AT2G19520-BS-F	TATRAARTATATCTCTTAAACCACT	Bisulfite sequencing
	AT2G19520-BS-Fbis	CTCTTAAACCACTRACACTATCTTA	
	AT2G19520-BS-R	GGGAAAYTAGAAGYAAGTAAAATGAGAAAAGT	
AT2G24370	AT2G24370_F1	TGCGGATGTTTGTCTACGAGTTC	RT-PCR, ChIP
	AT2G24370_R1	CATTGGTGGTTTTGCTGTTATCA	
	AT2G24370-F	ATTRTACAATTTTAAACACTTTCTCCACAA	Bisulfite sequencing
	AT2G24370-Fbis	ACACTCTATTRCATCTCCTTATACTAC	
AT2G24370-R	ACTAGAGAAAGGAACCTTGGTTGATTTA		
AT2G42600	AT2G42600-F	AAAACCGAAGAGGTTGCAGA	RT-PCR, ChIP
	AT2G42600-R	ACCCGATCATGACTTCTTGC	
	AT2G42600-BS-F	CCTCRITCCAACRRITCCCACCTCTTCC	Bisulfite sequencing
	AT2G42600-BS-R	GAATGGTYGGAGGATAAAAAGAYAGGAATGG	
AT2G42600-BS-Fbis	CTCCRTATCTTTTTTRCCACCTTCAC		
AT3G23750	AT3G23750-F	TGGATGTGTATGCATTGGTG	RT-PCR, ChIP
	AT3G23750-R	ATGTCGGGTCTTTGTTGAGG	
	AT3G23750-BS-F	TTCACATCRATTCCRAAACTCTCTTC	Bisulfite sequencing
	AT3G23750-BS-R	ATTGGTTGYAYTGATATTGATGTATA	
	AT3G23750-BS-Rbis	GGAAGAGTAAYGAYGAAAGTGGATGTGTATG	
AT3G45446	AT3G45446-F	CCATTGTACTCCCAACACCA	RT-PCR, ChIP
	AT3G45446-R	TGAGAATTATGGCAACAAGGTG	
	AT3G45446-BS-F	TACTCCCAACACCATATCAATACCTT	Bisulfite sequencing
	AT3G45446-BS-R	GTGGATYAGAAAGGTGAGAATTATGG	
	AT3G45446-BS-Fbis	CCATCCACATTAATTTTTCTCCCATC	
AT3G47860	AT3G47860-F	TCAGAAGAGTGTTTTGATTCTGGA	RT-PCR, ChIP
	AT3G47860-R	TTGGCACAATTTGGCTATGA	
	AT3G47860-BS-F	CTCTTRCACTRATCTTATATAAACCTAAT	Bisulfite sequencing
	AT3G47860-BS-R	AYTTAAGATYYGATGTGGGATAAAAT	
	AT3G47860-BS-Rbis	GTAYTTTTGGTTTYTYTGTGGAGTGTATG	
AT4G24740	AT4G24740-F	TGGTCAAGTGCTAGAATGTTGG	RT-PCR, ChIP
	AT4G24740-R	ATGGTTACGATAGTCAAACCAGTTC	
	AT4G24740-BS-F	ACTACACACATRCTACACATCCAA	Bisulfite sequencing
	AT4G24740-BS-R	GTTGGGATAGGGAGAGGAAGGAAATGGTGG	
	AT4G24740-BS-Rbis	GTGAGGYTGYTATGATTGAAATGAAATG	

AT5G14060	AT5G14060-F	GCAATGGAGTGAATGTGCAG	RT-PCR
	AT5G14060-R	CAACAACAACAACAACAATGGA	
	AT5G14060-BS-F	TCTTRACACATTCTTARCAATAACTAC	Bisulfite sequencing, ChIP (with AT5G14060-BS-F/Rbis)
	AT5G14060-BS-R	TTTGATAGATTGTTTTYTTTTGTGAGGGT	
AT5G14060-BS-Rbis	GGGTYATATGTGGTATTGGTTAAAGGG		
AT5G19097	AT5G19097-F	CGAAGCCACAACATTTCCCTT	RT-PCR, ChIP
	AT5G19097-R	CAGCCACTGCGTCTTTTACA	
	AT5G19097-BS-F	CTRAARCATCAATCCACAAATCATTCA	Bisulfite sequencing
	AT5G19097-BS-R	GAAAGAATYTTTTAAAYTGAGAGATTTAGG	
AT5G19097-BS-Fbis	CTTTCRAARCCACAACATTTCCCTT		
AT5G39960	AT5G39960-F	GAGGGAAGAGGACGTATGGA	RT-PCR, ChIP
	AT5G39960-R	TAGGAGTCCCAGCCTAAATCA	
	AT5G39960-BS-F	CTRTTTCRCCTTCCACCACCACCT	Bisulfite sequencing
	AT5G39960-BS-R	GGAAGAGGAYGTATGGAAGTAATGAAAGAAG	
AT5G39960-BS-Fbis	TCCRCCTAAATCAAAATCTTCCTTCAAT		
AtCOPIA4 (AT4G16870)	AtCOPIA4-F ² (JP3067)	CTTGTTGTCTTCCCCGTGT	RT-PCR, ChIP
	AtCOPIA4-R ² (JP3087)	TGACGAAGAGCGTACCTGTG	
	AtCOPIA4-BS-R ³ (JP3100)	GGTTGTYTGTGTTTTTATGGTTYAGATTTATA	Bisulfite sequencing
	AtCOPIA4-BS-F ³ (JP3101)	ATAACTRAACCACARATTCARCCCATTTTCATT	
AtGP3-1 (AT3G11970)	AtGP3-1-F	AAGCATAATAGTACAAAAGTTCCCC	RT-PCR
	AtGP3-1-R	TTCTCTTGGAAAGTTGCAGAC	
	AtGP3-1_BS-F ⁴	ATGYTAGTGTATYTTAAATAGTGTATYTTG	Bisulfite sequencing, ChIP (with AtGP3-1_BS-Fbis/R)
	AtGP3-1_BS-R ⁴	AACRTTACTTTCTTTACCTTTTTATATAA	
AtGP3-1_BS-Fbis	GGTTYAGGATAGATTATTAAYGGGGGAA		
AtMu1 (AT4G08680)	AtMu1-F ⁵	CCGAGAACTGGTTGTGGTTT	RT-PCR
	AtMu1-R ⁵	GCTCTTGCTTTGGTGATGGT	
	AtMu1-BS-F1 ⁶	AATTTTATGGAATGAAGTATATG	Bisulfite sequencing, ChIP (with AtMu1-BS-F1/Rbis)
	AtMu1-BS-R	AAATTTAARCCCTCTTCTCATACA	
AtMu1-BS-Rbis ⁶	TTCTCATACARTRRCTTCAATTT		
AtSN1 (AT3G44000-AT3G44005)	ATS15 ⁷	ACCAACGTGTTGTGGCCAGTGGTAAATC	RT-PCR, ChIP
	ATS15-F4 ⁷	AAAATAAGTGGTGGTTGTACAAGC	
	AtSN1-BS-F	CTCTCAAAAACAAACCCCTTAAAC	Bisulfite sequencing
	AtSN1-BS-R	GTTGAGATGGTGTTRTTGGAAAGG	
AtSN1-BS-R1	GGAAGTTTGTATAGTTTAAARATAAG		
BNS (AT1G73177)	BNS-F2 ⁸	GCTAGAGGTTTTAGTTCCTCTG	<i>BNS</i> methylation analysis
	BNS-R3 ⁸	TTCCATTATGACATTTCAAGGTC	
CAC2 (AT1G35270-AT1G35280)	CAC2-RT-F	TTGGTCCGATAAGAGTTTCGT	RT-PCR
	CAC2-RT-R	CGCAGGTTTCTTTCTTCAGC	
	CAC2-F	GCCTAAGTGTGGCGGACTA	ChIP (with CAC2-bsR2)
	CAC2-bsF2 ⁹	CATATAAACCCCAAAATCAAATC	
CAC2-bsR2 ⁹	ATGGAAAAGGAGAAGGAGGTAT	Bisulfite sequencing	
CAC2-bsR2bis	TYATTGATAGYAGTAGAGGATAG		
CMT3 (AT1G69770)	CMT3-MseI-F	ATGCCAAGGAATCAGTGGTC	RT-PCR
	CMT3-MseI-R	AACTTTTGAATGGCGTTGTG	
	CMT3-R2	TAATCCGCAGTCCCACCT	ChIP (with CMT3-BS-Fbis)
	CMT3-BS-F	CCTAATTTTARTCAAAATTRCTAAAAACATC	
CMT3-BS-Fbis	TCAAAATTTTRCTAAAAACATCAATACTTA	Bisulfite sequencing	
CMT3-BS-R	GGTGTATATGAAAYATTGTAGAATAITTTGAAG		
DME (AT5G04560)	DME-F	TGAGAGGAAGTTTTCCGCTC	RT-PCR
	DME-R	GTGTCGCTTCTGCTCTATGA	
EVD (AT5G17125)	EVD_RT_F ¹⁰	GACCTGACATTTGTTTTGCG	RT-PCR
	EVD_RT_R ¹⁰	GGTGAAGATATCCGCCAAC	
	EVD_TD	ACTCGTGAGTCCCTTCAACGG	ChIP (with EVD-BS-R)
	EVD-BS-F ¹⁰	ACATRATCTTATRCTRATAACCAT	
EVD-BS-R1 ¹⁰	GTAGAGGAYAAATGTTAATTYGTTGTTGG	Bisulfite sequencing	
EVD-BS-R2 ¹⁰	GTGYGAYAAGATYGATGTAGGTTATTG		

IBM1 (<i>AT3G07610</i>)	IBM1-F8	ACAACAAGTCCAAAATGTTG	RT-PCR (<i>IBM1-L</i>)	
	IBM1-R10	TAAACACTCGCTGACATTTTCAGG		
	IBM1-F9	TATGTAGTTGCCTTTGAAG	RT-PCR (<i>IBM1-S</i>)	
	IBM1-R11	GTTCTGAAATCATTATAGATGTGC		
	IBM1-F10	TGAGATCCGCAATGGAAAAG	probe for Northern blot analysis	
	IBM1-R12	CCAAACAATCAACAGCAAC		
	IBM1-F1	TCTGATGCGGTTAGTACCC	RT-PCR along the length of <i>IBM1</i> gene, ChIP (with IBM1- F5/R6)	
	IBM1-R1	CCAAAAACCGAATGCCAGGCC		
	IBM1-R2	TAAGCAGAATGGATGAAGGC		
	IBM1-F2	AGCCTTCATCCATTCTGCTT		
	IBM1-R3	GCGTAATCTTTGCATTCAATGTG		
	IBM1-F3	CAGCATTTTATAAATGTTTGATTG		
	IBM1-F4	ACATTGAATGCAAAGATTACGC		
	IBM1-F5	TAAAGTGTGTTGTGATTGTTTCGA		
	IBM1-F6	ACTTCTTGATATTGTTCCGGAATG		
	IBM1-R4	CATTATTGATACGACAGACG		
	IBM1-R5	GCGAAATTGATGTTGGTCATT		
	IBM1-R6	ATCATTCCGAACAATATCAAGA		
	IBM1-R7	TGGAAAGAGTCATTGAGGTAG		
	IBM1-R8	CACAAAACCAAAATCTACTAAATTAECTATT		
	IBM1_polyR1	TTAGTACTACTCTGTTAACTTAGAGGTA		dCAPS analysis
	IBM1-BS-F	GTTAATYGTYGATATAAYTGGTGTAGTT		Bisulfite sequencing, methylation analysis by restriction digestion
	IBM1-BS-R	CACAAAACCAAAATCTACTAAATTAECTATT		
	IBM1-BS-Fbis	TAGATTTTATATGTTGYTGTTA		
ROS1 (<i>AT2G36490</i>)	ROS1-F	AGAAGAAATCCTACCATCA	RT-PCR	
	ROS1-R	ACCGTCTTCGAGGTAATTC		
	ROS1-R2	AGAGGATGCTCGTCTGGAAG	ChIP (with ROS1-F)	
	ROS1_BS_F ¹¹	TAGAGGAGGYGTTTTTTG	Bisulfite sequencing	
	ROS1_BS_Fbis ¹¹	GAGGYGTTTTTTGAGGAT		
	ROS1_BS_R ¹¹	CATTTTTAACCTAAAAACRAAAAAA		
Ta3 (<i>AT1G37110</i>)	Ta3-middle-F ² (JP1565)	GATTCTTACTGTAAAGAATCGGCATTGAGAGA	RT-PCR	
	Ta3-middle-R ² (JP1566)	TCCAAATTTCTGAGGTGCTTGTAACC		
	Ta3-LTR-F ² (JP1617)	TAGGGTTCTTAGTTGATCTTGTATTGAGCTC	ChIP	
	Ta3-LTR-R ² (JP1618)	TTTGCTCTCAAACCTCTCAATTGAAGTTT		
	Ta3-BS-F (JP1615) ³	GGTTTAATGTTGGTTTAGTGATATTYGGTTTAGT	Bisulfite sequencing	
	Ta3-BS-Rbis (JP1616) ³	AATCAAAAAACRAATAAACCTCRCTCTRATACCACTTATT		
	Ta3-BS-R	CCATTAACACCTTTATCTCAACTCTA		
TUB8 (<i>AT5G23860</i>)	TUB8-F ¹²	ATAACCGTTTTCAAATTCTCTCTCTC	ChIP	
	TUB8-R ¹²	TGCAAATCGTTCTCTCCTTG		
UBQ1 (<i>AT3G52590</i>)	UBQ-FWD (U1) ¹³	ACCGGCAAGACCATCACTCT	RT-PCR	
	UBQ-Rev (U2) ¹³	AGGCCTCAACTGGTTGCTGT		

Supplementary Table 1. List of primers.

¹ Vaillant I, Schubert I, Tourmente S, Mathieu O (2006) MOM1 mediates DNA-methylation-independent silencing of repetitive sequences in Arabidopsis. *EMBO J* 7: 1273-1278

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³ Henderson IR, Zhang X, Lu C, Johnson L, Meyers BC, Green PJ, Jacobsen SE (2006) Dissecting Arabidopsis thaliana DICER function in small RNA processing, gene silencing and DNA methylation patterning. *Nat Genet* 38: 721-725

⁴ Tsukahara S, Kobayashi A, Kawabe A, Mathieu O, Miura A, Kakutani T (2009) Bursts of retrotransposition reproduced in Arabidopsis. *Nature* 461: 423-426

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- ⁸ Saze H, Kakutani T (2007) Heritable epigenetic mutation of a transposon-flanked Arabidopsis gene due to lack of the chromatin-remodeling factor DDM1. *EMBO J* **26**: 3641-3652
- ⁹ Miura A, Nakamura M, Inagaki S, Kobayashi A, Saze H, Kakutani T (2009) An Arabidopsis jmjC domain protein protects transcribed genes from DNA methylation at CHG sites. *EMBO J* **28**: 1078-1086
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- ¹² Mathieu O, Probst A, Paszkowski J (2005) Distinct regulation of histone H3 methylation at lysines 27 and 9 by CpG methylation in Arabidopsis. *EMBO J* **24**: 2783-2791
- ¹³ Zhang W, Sun Y, Timofejeva L, Chen C, Grossniklaus U, Ma H (2006) Regulation of Arabidopsis tapetum development and function by DYSFUNCTIONAL TAPETUM1 (DYT1) encoding a putative bHLH transcription factor. *Development* **133**: 3085-3095