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Supplemental Information

Intragenic DNA methylation regulates insect gene expression and reproduction through the MBD/Tip60 complex

Guanfeng Xu, Hao Lyu, Yangqin Yi, Yuling Peng, Qili Feng, Qisheng Song, Chengcheng Gong, Xuezhen Peng, Subba Reddy Palli, and Sichun Zheng

Transparent Methods

Insects and inhibitor treatments

The *B. mori* strain P50 was obtained from the Research and Development Center of the Sericulture Research Institute of the Academy of Agricultural Sciences of Guangdong Province, China. Larvae were reared on fresh mulberry leaves at 25°C and a photoperiod of 12 h light:12 h darkness. *S. litura* indivudals were obtained from the Institute of Entomology, Sun Yat-sen University (Guangzhou, China). Larvae were reared on artificial medium at 25°C and 70% humidity under a photoperiod of 12 h light:12 h darkness. Specimens of *L. migratoria* were obtained from the School of Life Sciences, Henan University (Kaifeng, China). Larvae were reared under a 14-h:10-h light:dark cycle and at 30 ± 2°C; larvae and adults were supplied with fresh leaves of *Setaria viridis (L.) Beauv* and wheat bran.

The DNA methylation inhibitor 5-aza-2'-deoxycytidine (5-aza-dC) (Sigma, California, USA) was dissolved in ddH_2O. Two microliters of 5-aza-dC at a concentration of 10 μg/μL was injected into the abdomen of *B. mori* and *S. litura* on pupa day 1 and *L. migratoria* on adult day 1. The same volume of ddH_2O was injected as a control. Three replicates of approximately 30-40 individuals per replicate were carried out.

Library construction and mapping

Genomic DNA was extracted from the ovaries of seven-day-old pupae using the phenol extraction method. After genomic DNA was extracted, DNA concentration and integrity were detected by a NanoDrop spectrophotometer and agarose gel

electrophoresis, respectively. DNA libraries for bisulfite sequencing were prepared by Gene Denovo Biotechnology Co (Guangzhou, China). Briefly, genomic DNA was fragmented into 100-300-bp fragments by sonication (Covaris, Woburn, USA) and purified with a MiniElute PCR Purification Kit (QIAGEN, Dusseldorf, Germany). The fragmented DNAs were end-repaired, and a single "A" nucleotide was added to the 3' end of the blunt fragments. The genomic fragments were ligated to methylated sequencing adapters and fragments with adapters were bisulfite converted using a Methylation-Gold kit (ZYMO, Irvine, USA). Finally, the converted DNA fragments were PCR amplified and sequenced using an Illumina HiSeqTM 2500. Short reads generated by Illumina sequencing were aligned to the *B. mori* reference genome (Wang et al., 2005; Xia et al., 2004).

Methylation level analysis

To obtain high-quality clean reads, raw reads were filtered by removing reads containing more than 10% unknown nucleotides and low-quality reads containing more than 40% low-quality (Q-value $\leqslant$ 20) bases. The obtained clean reads were mapped to the species reference genome using BSMAP software (Xi and Li, 2009) (version: 2.90) by default. Then, a custom Perl script was used to call methylated cytosines, and the methylated cytosines were tested with the correction algorithm described in Lister et al. (Lister et al., 2009). The methylation level was calculated based on the methylated cytosine percentage of each chromosome and in different regions of the genome for each sequence context (CG, CHG, and CHH). To assess

different methylation patterns in different genomic regions, the methylation profile at flanking 2-kb regions and gene bodies (or transposable elements) was plotted based on the average methylation levels for each window.

RNA-seq analyses

Total RNA was extracted from the ovaries of 7-day-old pupae using TRIzol reagent (TaKaRa, Dalian, China) according to the manufacturer's instructions. RNA-seq was performed using the Illumina HiSeq^{TM} 4000 platform by Gene Denovo Biotechnology, and paired reads with an average length of 150 bp were generated. The clean reads that were filtered from the raw reads were used for mapping to reference *B. mori* genomes (Wang et al., 2005; Xia et al., 2004). Using the assembled transcripts generated after mapping, the gene expression level of each gene was normalized using FPKM values (fragments per kilobase of exon per million fragments mapped) with Cufflinks software (Trapnell et al., 2012).

ChIP-seq analyses

H3K27ac ChIP-seq data were obtained from NCBI with the BioProject accession number PRJNA450142 (Cheng et al., 2018). We used Bowtie2 to map paired-end clean reads to the silkworm reference genome. Highly enriched peaks were obtained by MACS2 using standard settings.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using TRIzol (TaKaRa, Dalian, China), and cDNAs were synthesized using First-Strand cDNA Synthesis Kit (TaKaRa, Dalian, China) following the manufacturer's protocol. qPCR was performed using 2×SYBR Premix

EXTaq^{TM} Kit (TaKaRa, Dalian, China). The relative mRNA level of gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Data were normalized to the housekeeping gene ribosomal protein 49 (Rp49) and analyzed by the $2^{-\Delta\Delta Ct}$ method. All data consisted of three biological replicates. The sequences of the qRT-PCR primers used are shown in Table S9.

Construction of the reporter luciferase vector

Genomic DNA was extracted from *Bm12* cells. The promoter of the actin gene, including 91 bp of the core promoter and 63 bp of the 5' UTR, was cloned into the pMD-18T vector (TaKaRa, Dalian, China) according to the sequence of the *B. mori* genome SilkDB (http://silkworm.genomics.org.cn). The methyl-CpG fragment was amplified by PCR. The actin promoter and methyl-CpG fragment were cloned into the luciferase reporter pGL3-basic vector (Promega, Madison, USA). The primers used for constructing the plasmids are listed in Table S9.

Cell transfection and transcriptional activity determination

The *B. mori* cell line DZNU-Bm-12 (*Bm12*) originally developed from ovarian tissues (Khurad et al., 2009) was maintained at 28°C on Grace medium (Invitrogen, California, USA) supplemented with 10% fetal bovine serum (FBS) (HyClone, Utah, USA).

Bm12 cells at the logarithmic growth phase were inoculated into Grace Insect medium in 12- or 24-well culture plates (Corning, New York, USA) and cultured for 12 h. Cell transfection was conducted when the cells were at 80% density. The Renilla luciferase vector pRL-SV40 was cotransfected for normalization of firefly luciferase

activity.

For transfection, a mixture of 30 μL containing 1 μg of the pGL3-derived reporter plasmid DNA, 100 ng internal control plasmid (pRL-SV40), and 3 μL Fugene HD transfection reagent (Promega, Wisconsin, USA) in Opti-MEM Reduced Serum Medium (Life Technologies, Massachusetts, USA) was added to the cells, which were cultured for 48 h at 28°C, harvested and used for the transcriptional activity assays or isolation of protein and RNA.

For cotransfection, a mixture of 30 μL containing 0.5 ng of the wild-type or mutated pGL3-derived reporter plasmid DNA, 0.5 μg overexpression plasmid or 1 μg dsRNA, 100 ng internal control plasmid (pRL-SV40 vector) and 4 μL Fugene HD transfection reagent in Opti-MEM Reduced Serum Medium was added to the cells in Grace medium.

Luciferase activity was assessed as follows. Briefly, after 48 h of transfection, the cells were washed twice with filtered PBS and then lysed in 100 μL Passive Lysis Buffer (Promega, Wisconsin, USA). The samples were centrifuged at 800 × g for 5 min at room temperature. The supernatant was used to analyze the luciferase activity with Dual-Luciferase Assay System according to the manufacturer's protocol and a luminometer (IBA7300, Veritas, Turner Biosystems, California, USA). Luciferase activity was normalized to that of Renilla luciferase.

Expression and purification of recombinant proteins

Open reading frames of select genes were amplified using cDNA or synthesized by TSINGKE (Beijing, China). The cDNAs were subcloned into the pPET-28a, pGEX-6P-1 or EGFP vector with a 6×His, GST tag or GFP tag to generate

recombinant expression vectors, respectively. The recombinant proteins were expressed in *Escherichia coli* (BL21) or *Bm12* cells. The specific primers and restriction sites used for each protein expression are shown in Table S9.

For purification of His-tagged protein, the transformed *E. coli* cells were collected by centrifugation and resuspended in binding buffer (0.5 M NaCl, 20 mM Tris-HCl, 5 mM imidazole, pH 7.9 and 1 mM PMSF). The suspension was centrifuged after being lysed by sonication and then purified with Ni-chelating affinity chromatography using His-Bind® 12 Kit according to the manufacturer's protocol (Novagen, Wisconsin, USA).

For purification of GST-tagged protein, the transformed *E. coli* cells were collected by centrifugation and resuspended in PBS. The suspension was centrifuged after being lysed by sonication and then purified using a GST Protein Purification Kit according to the manufacturer's protocol (Beyotime, Shanghai, China).

Nuclear protein preparation and electrophoretic mobility shift assay (EMSA)

Nuclear proteins were extracted from tissues or cells according to the instructions of NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Scientific, Massachusetts, USA). EMSA was conducted using LightShift Chemiluminescent EMSA Kit (Thermo Scientific, Massachusetts, USA). Wild-type oligonucleotides labeled with biotin at the 5' end or methylated at cytosine were synthesized by Invitrogen (Shanghai, China). Oligonucleotide probes were heated at 95°C for 10 min in 50 mM Tris-acetate buffer at pH 4.1 and then slowly cooled to room temperature. Binding assays were performed according to the manufacturer's protocol. Briefly,

nuclear protein extracts or purified proteins were incubated with 20 μL of binding buffer containing 50 ng of poly (dI-dC), 2.5% glycerol, 0.05% NP-40, 50 mM potassium chloride, 5 mM magnesium chloride, 4 mM EDTA and 0.1 pmol of a methylated and biotinylated end-labeled double-stranded probe. Cold probes (unlabeled) at different concentrations were added to the binding mixture as competitor. For a mutant probe, 20 fmol of a nonmethylated but biotinylated end-labeled double-stranded probe was used. Two micrograms of MBD2/3 antiserum or 2 μg of normal rabbit IgG (control) was added to detect supershifted bands. After electrophoresis and transfer to nylon membranes, the protein bands were visualized by using LightShift Chemiluminescent EMSA Kit according to the manufacturer's protocol.

GST pull-down assays

Expression of soluble BmMBD2/3-GST protein was induced in DH5α cells. Nuclear proteins of *Bm12* cells overexpressing BmTip60-EGFP proteins were extracted according to the instructions of NE-PER Nuclear and Cytoplasmic Extraction Reagents. BmMBD2/3-GST proteins were mixed with 40 μL of Pierce™ Glutathione Agarose (Thermo Scientific, Massachusetts, USA) and incubated at 4°C with gentle agitation for 1 h. After washing three times with PBS, 100 μg nuclear extract containing BmTip60-EGFP proteins was incubated with Glutathione Agarose-BmMBD2/3 overnight at 4°C in 500 μL PBS with gentle agitation. After incubation, the protein-protein complexes were washed three times with 600 μL PBS. The bound proteins were eluted in 30 μL SDS-PAGE sample buffer (50 mM Tris, 100

mM DTT, 2% SDS, 0.1% bromophenol blue, 10% glycerol) and subjected to 12% SDS-PAGE. Target proteins were identified by Western blot with an anti-GFP antibody (ab290, Abcam, Cambridge, UK).

Chromatin immunoprecipitation (ChIP) assay

ChIP was performed in *Bm12* cells following the instructions of Pierce™ Magnetic ChIP Kit (Thermo Scientific, Massachusetts, USA). Approximately 4 x10^6 cells were crosslinked with 1% formaldehyde for 10 min at room temperature after transfection with overexpression vectors for BmMBD2/3-3×FLAG, BmTip60-3×FLAG or EGFP (as a control) for 48 h and then decrosslinked with glycine. The cells were washed twice with PBS and collected by centrifugation at 3000 × g for 5 min. The cell pellets were broken with extraction buffer containing protease/phosphatase inhibitors. The nuclei were collected and treated with MNase diluted in MNase Digestion Buffer for 15 min at 37°C, and the nuclei were released from the cells by ultrasonic breaking with several pulses and 20 s ice-cold intervals, followed by centrifugation at 9000 × g for 5 min. The protein-DNA complexes were immunoprecipitated by using rabbit anti-FLAG antibodies (#14793, Cell Signaling Technology, MA, USA) or normal rabbit IgG (as a control) (Thermo Fisher Scientific, Massachusetts, USA). The IP reactions were incubated overnight at 4°C with constant mixing. The DNA/protein/antibody complexes were purified by incubation with ChIP Grade Protein A/G Magnetic Beads for 2 h at 4°C with mixing. DNA was crosslinked and purified using the column method according to the manufacturer's instructions

(Thermo Scientific, Massachusetts, USA) and detected by qRT-PCR (Table S9). The enriched sequence in the immunoprecipitated DNA samples was normalized to the DNA present in the 10% input. The PCR products of the enriched promoters were confirmed by sequencing. The antibodies used in this study are listed in Table S10.

RNA interference (RNAi)

For RNAi in the *Bm12* cell line, a 400~600-bp unique fragment of the ORF of target genes was chosen as the template for synthesizing gene-specific dsRNA using T7 RiboMAXTM Express RNAi System (Promega, Wisconsin, USA). dsRNA (4 μg) was used to transfect *Bm12* cells with 6 μL Fugene HD transfection reagent in Opti-MEM Reduced Serum Medium. The cells were collected at 48 h after transfection. For embryo RNAi, dsRNA (20-50 ng) was injected into embryos within 2 h of fertilization, and three replicates of 300~400 embryos per replicate were carried out. To detect the knockdown efficiency, qRT-PCR was performed using the specific primers listed in Table S9.

Western blot and far-western blot analyses

Tissues or *Bm12* cells were homogenized in Cell Lysis Buffer (Beyotime, Shanghai, China). For western blot, proteins (30~100 μg) were mixed with 5× loading buffer and then separated by 12% SDS-PAGE, followed by transfer to a nitrocellulose blot membrane. The membrane was blocked with Tris-buffered saline (pH 9.0) with 0.5% Tween-20 (TBST) containing 3% (w/v) BSA, followed by hybridization

overnight in TBST with 1% BSA and the primary antibody. After the membranes were washed in TBST three times, a horseradish peroxidase (HRP)-conjugated antibody (Dingguo Biotechnology, Beijing, China) was applied. An anti-tubulin antibody (Dingguo Biotechnology, Beijing, China) was used to verify equal loading of the protein samples on the gel. The antibodies used in this study are listed in Table S10.

For far-western blot, the purified recombinant BmTip60-His or BmMBD2/3-GST protein (3 mg) was separated by 12% SDS-PAGE and transferred to nitrocellulose membranes. The membranes were washed in TBST to remove SDS, blocked with 3% (wt/vol) BSA in TBST, and then probed with another purified protein (2 mg/mL), BmMBD2/3-GST or BmTip60-His, in TBST containing 0.1% BSA. The blots were incubated overnight; after washing, the membranes were probed with antibodies against the GST or His tag, followed by a horseradish peroxidase (HRP)-conjugated antibody.

Immunofluorescence

Bm12 cells at the logarithmic growth phase were subcultured on glass coverslips (WHB, Shanghai, China) and grown to a proper density. A mixture of 30 μL containing 0.5 μg of BmMBD2/3-EGFP, 0.5 μg of BmTip60-flag-EGFP plasmid DNA, and 3 μL Fugene HD transfection reagent in Opti-MEM Reduced Serum Medium was added, and the cells were cultured for 48 h at 28°C.

The *Bm12* cells on glass coverslips were fixed with 4% paraformaldehyde, blocked in PBS containing 5% BSA and 0.5% Triton-X (PBT) for 1-2 h, and then

incubated with an anti-flag primary antibody (Cell Signaling Technology, D6W5B) at 4°C for 3 h. The cells were washed three times with 0.2% PBT (0.2% Triton-X in PBS) and incubated with Alexa Fluor^{TM}594 goat anti-rabbit IgG (Invitrogen, California, USA) for 2 h. DAPI (Beyotime, Shanghai, China) was added to stain the nucleus. Primary and secondary antibodies were diluted 1:200 and 1:400 in PBS containing 5% BSA and 0.2% Triton-X (PBT), respectively. The cells were observed and imaged using an FV3000 confocal microscope (Olympus, Japan).

For 5mC staining, fixed tissues or cells were first incubated with 2 M HCl solution for 20 min and then neutralized with 100 mM Tris-HCl (pH 8.5) for 10 min at room temperature. After blocking with 3% BSA in 0.5% PBT buffer, the tissues or cells were incubated with an anti-5mC antibody (diluted 1:500 in 0.2% PBT buffer, Abcam, Cambridge, UK); Alexa FluorTM 594 goat anti-rabbit IgG (diluted 1:400 in 0.2% PBT buffer, Invitrogen, California, USA) was used as the secondary antibody. The nuclei were stained with DAPI (Beyotime, Shanghai, China) for 10 min. Wing tissues stained with anti-5mC and DAPI were observed and imaged using an FV3000 confocal microscope (Olympus). The antibodies used in this study are listed in Table S10.

Bioinformatics analysis of protein sequences

Phylogenetic tree analysis was conducted using MEGA7.0. The protein sequences were aligned with ESPript 3.0 (http://espript.ibcp.fr/ESPript/ESPript/), and the protein domains were analyzed with SMART (http://www.smart.embl-heidelberg.de/). The phylogenetic tree was edited with Evolview (http://www.evolgenius.info/).

Egg number and hatch rate statistics

To record egg number, *B. mori* and *S. litura* were injected with 5-aza-dC on the first day of pupation, and *L. migratoria* was injected with 5-aza-dC on the first day of adulthood. Ovaries of *B. mori*, *S. litura* or *L. migratoria* were dissected from adult females on the first, third or tenth day. Eggs were collected from females treated with 5-aza-dC or ddH_2O (control) and counted.

To calculate the hatch rate, dsRNA (20-50 ng) was injected into *B. mori, S. litura* and *L. migratoria* embryos within 2 h after fertilization. The larvae hatched from the resulting eggs of *B. mori* and *L. migratoria* were recorded at 9-13 days after dsRNA injection; the hatched larvae of *S. litura* were recorded at 3-5 days after dsRNA injection. The hatch rate was calculated as follows: hatch rate=larval number/egg number.

Statistical analysis

Data are presented as the mean ± SEM. “n” represents the number of biological replicates. P values for the purpose of group comparisons were calculated using ANOVA (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Supplemental Information

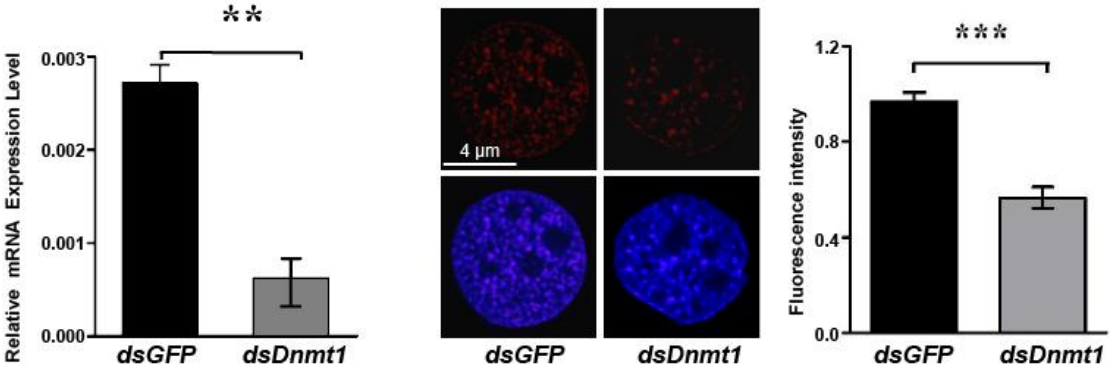

Figure S1. 5mC was detected by immunofluorescent staining in the nuclei of *B. mori* ovarian cell line (Bm12) after *BmDnmt1* RNAi. Related to Figure 1. Relative mRNA levels of *BmDnmt1* after *dsDnmt1* or *dsGFP* (control) was transfected into Bm12 cells. The mRNA levels of *BmDnmt1* was normalized to the expression level of the house-keeping gene, ribosomal protein 49 (*RP49*) (n=3) (left). The immunofluorescent staining (middle) (Scale bar, 4 μm); the fluorescence intensity performed using ImageJ software (right). Data are presented as the mean ± SEM. **P < 0.01, ***P < 0.001 by ANOVA.

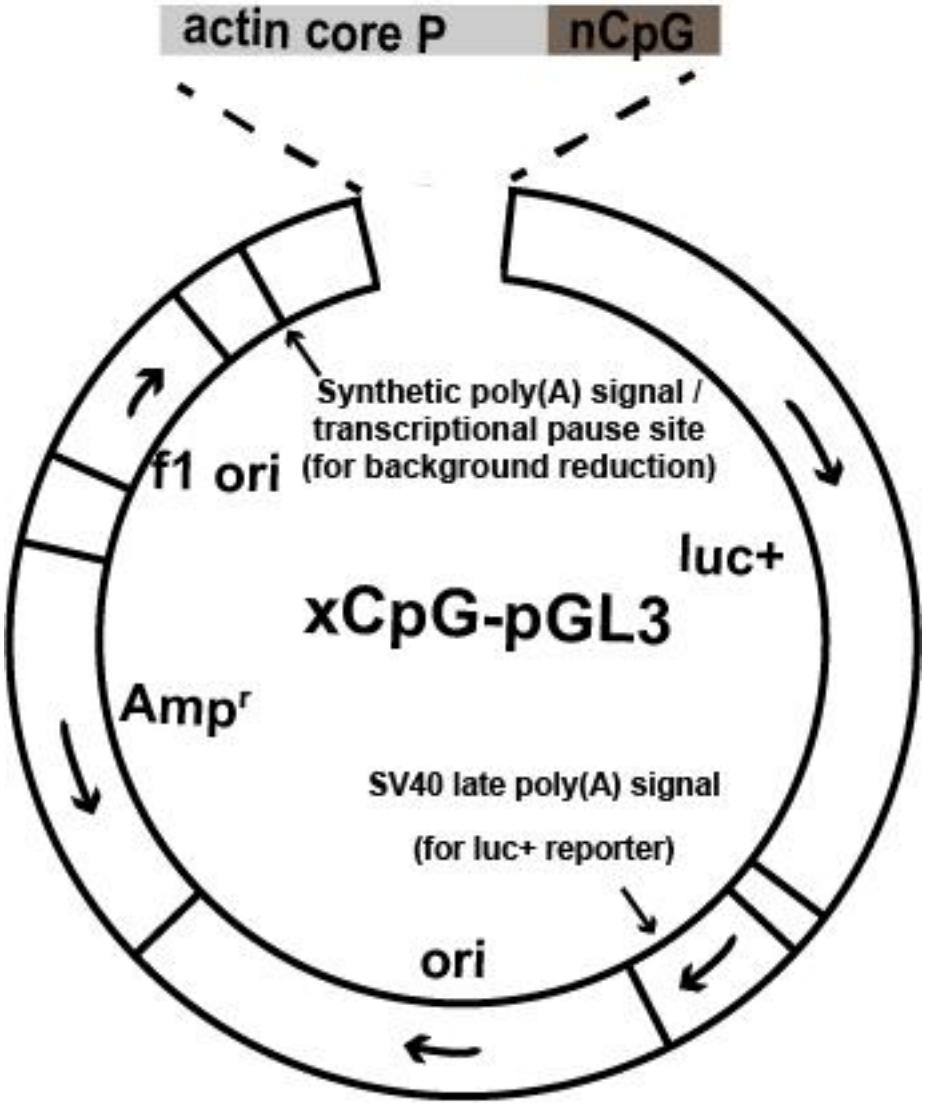

Figure S2. The vector for luciferase activity determination was constructed with 0, 2, 4 and 6 repeats of methyl-CpG fragment (ATCGAT) between the luciferase gene body and actin core promoter. Related to Figure 2. The sequences of the actin core promoter and 5'UTR are shown in Table S2.

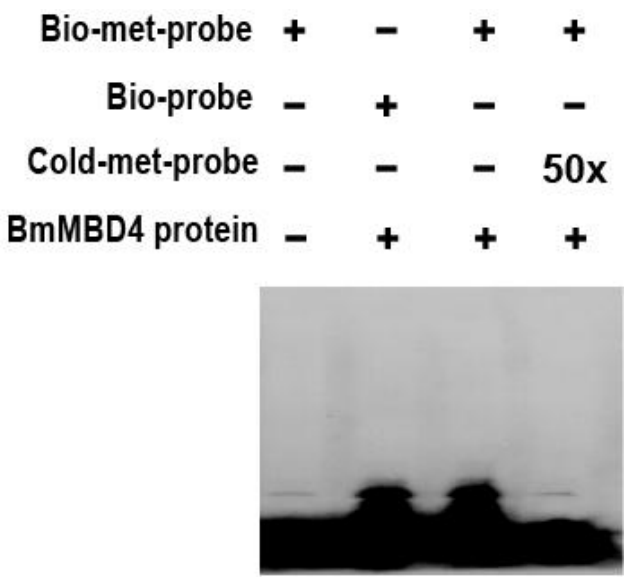

Figure S3. EMSA shows that the purified BmMBD4 did not bind with the methyl-CpG probes. Related to Figure 3. The sequences of the probes in the study are shown in Table S9.

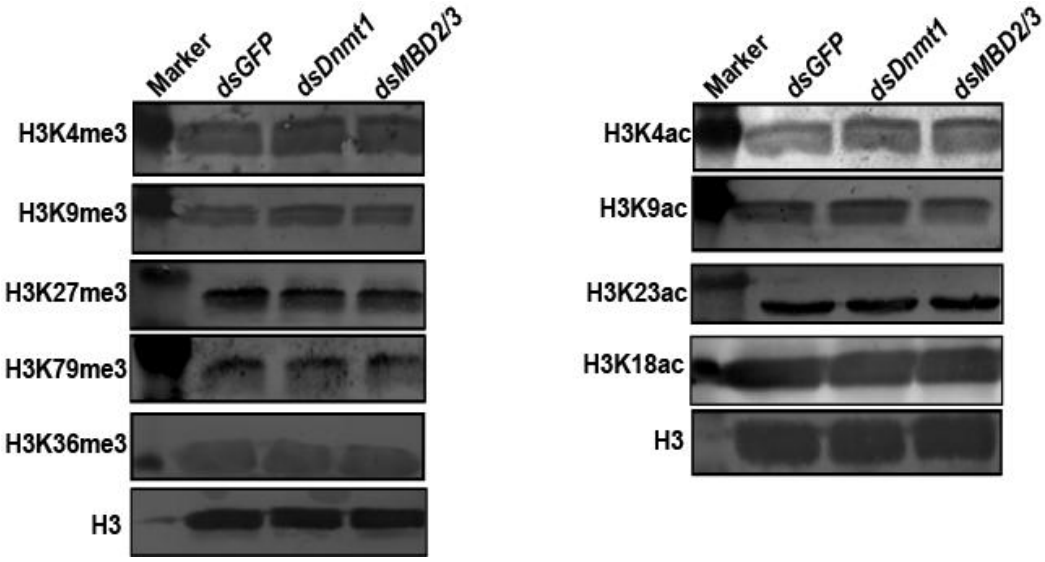

Figure S4. Western blot analyses of histone modification levels after the knockdown of *BmDnmt1* or *BmMBD2/3* in *B. mori* Bm12 cells. Related to Figure 3. Left: H3K4me3, H3k9me3, H3K27me3, H3K79me3, H3K36me3; Right: H3K4ac, H3K9ac, H3K23ac, H3K18ac. Marker: 15 kDa.

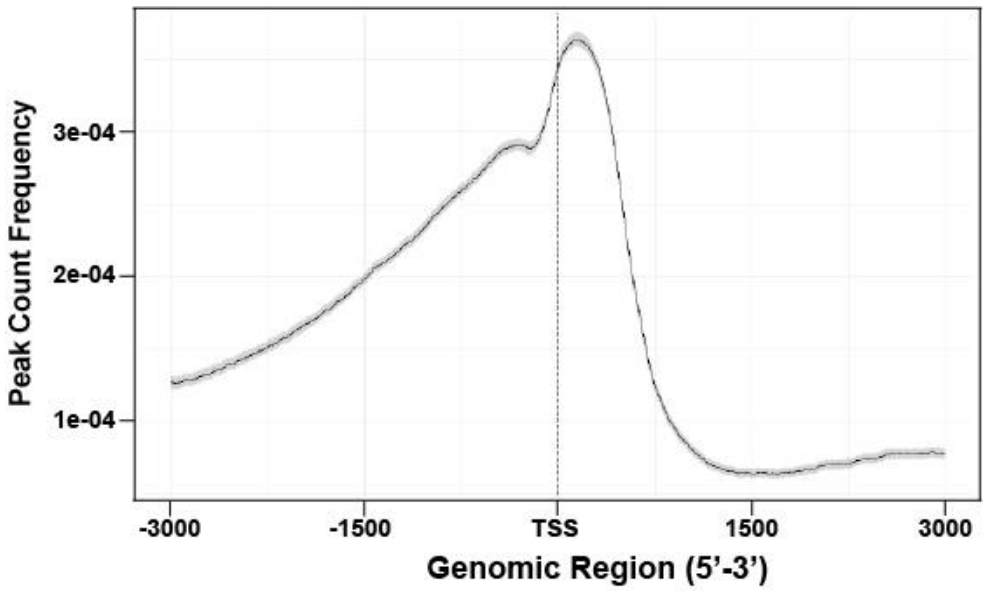

Figure S5. Distribution of H3K27ac in the genome by ChIP-seq using H3K27ac antibody. Related to Figure 3.

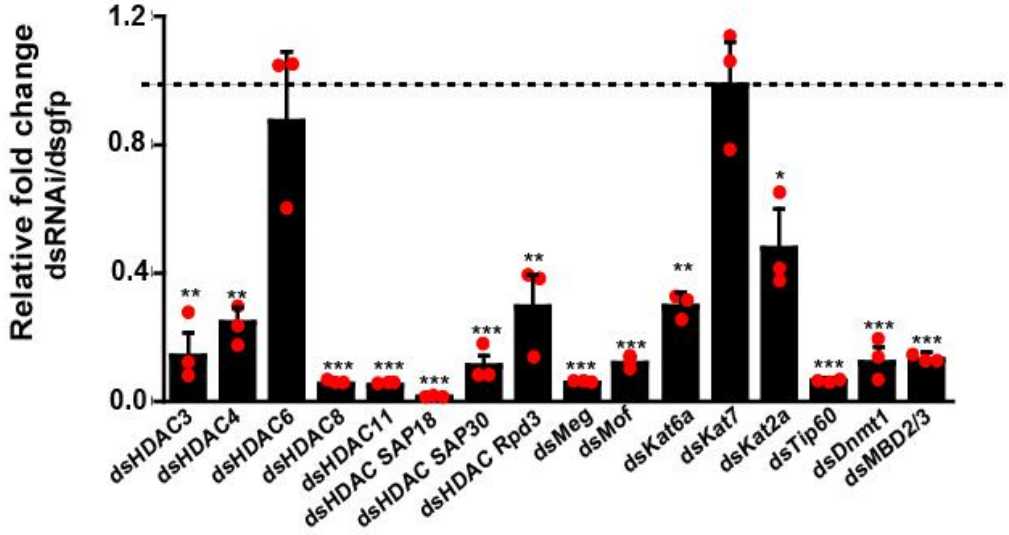

Figure S6. qPCR analyses of RNAi effectiveness in the Bm12 cells. Related to Figure 3. Relative mRNA levels are presented as the mRNA level in dsRNA-transfected samples divided by the control (*dsgfp*), which was normalized to the expression level of the house-keeping gene, ribosomal protein 49 (*RP49*). Genes

include *Dnmt1*, *MBD2/3*, *acetyltransferases (HATs)* and *deacetylases* (*HDACs*) (n=3). "n" represents the number of biological replicates. Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 by ANOVA analysis.

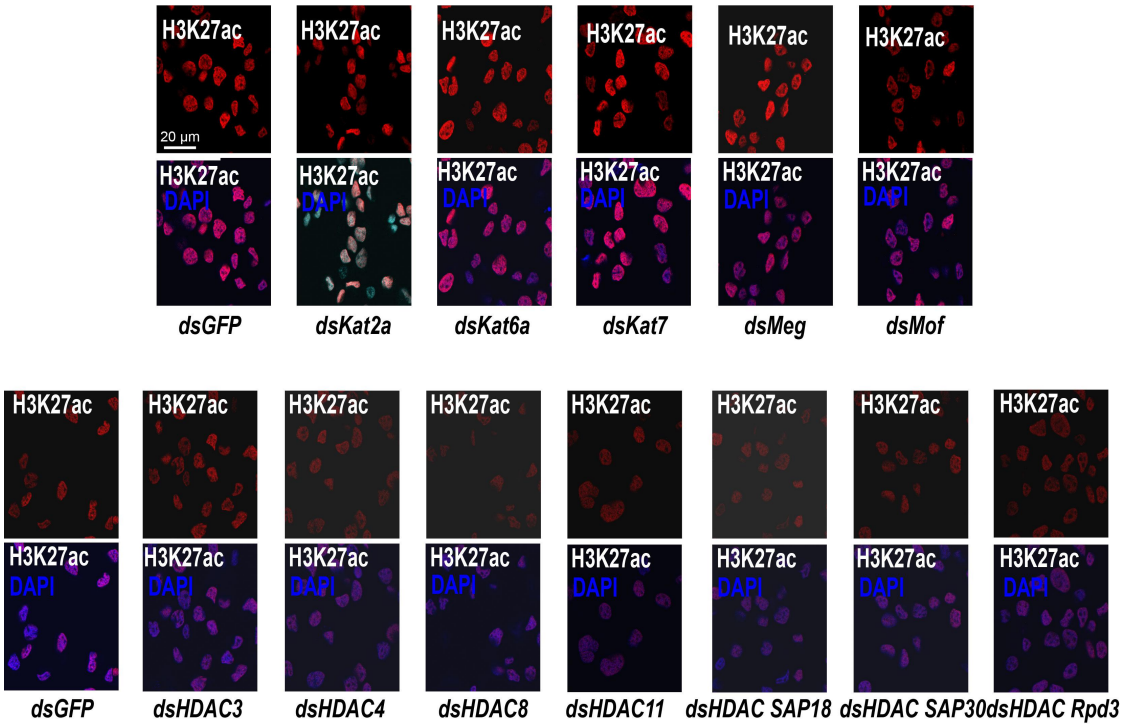

Figure S7. Immunofluorescence analyses of the H3K27ac levels after the knockdown of HATs (a-f) or HDACs (g-n) in the Bm12 cells. Related to Figure 3. Blue: DAPI, red: H3K27ac (Scale bar, 20 μm).

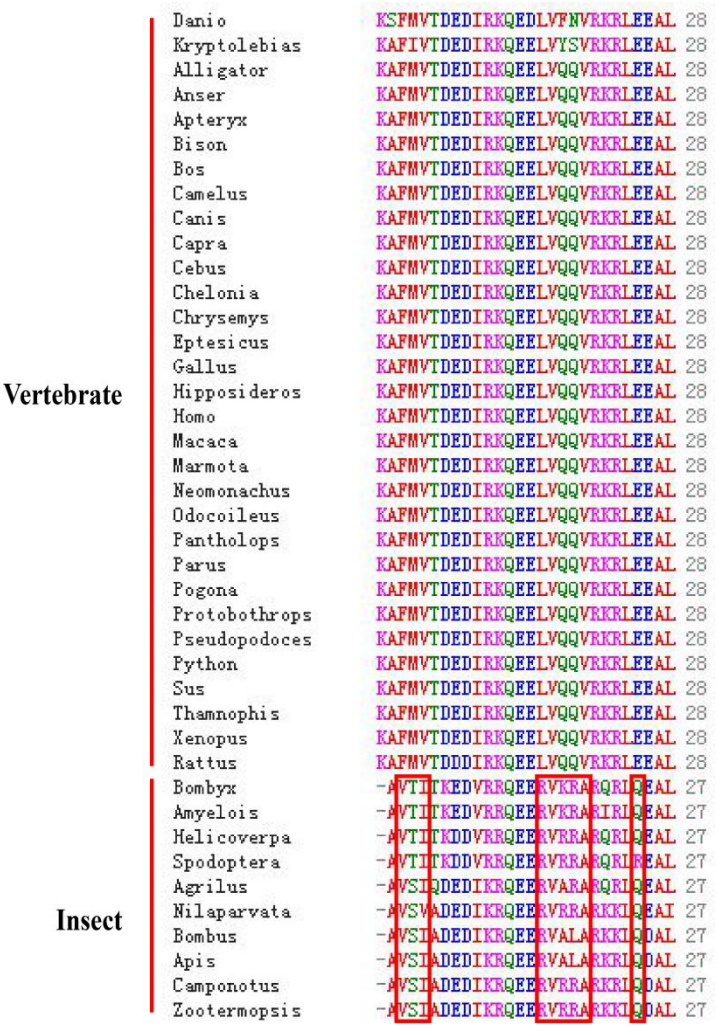

Figure S8. Conservative analysis of coiled-coil domain of MBD2/3 or MBD2/3 in different species. Related to Figure 4. The MBD2/3 protein contains two domains, methyl-CpG binding domain (MBD, specially binding to mCpGs) and a coiled-coil domain (interacting with other proteins).

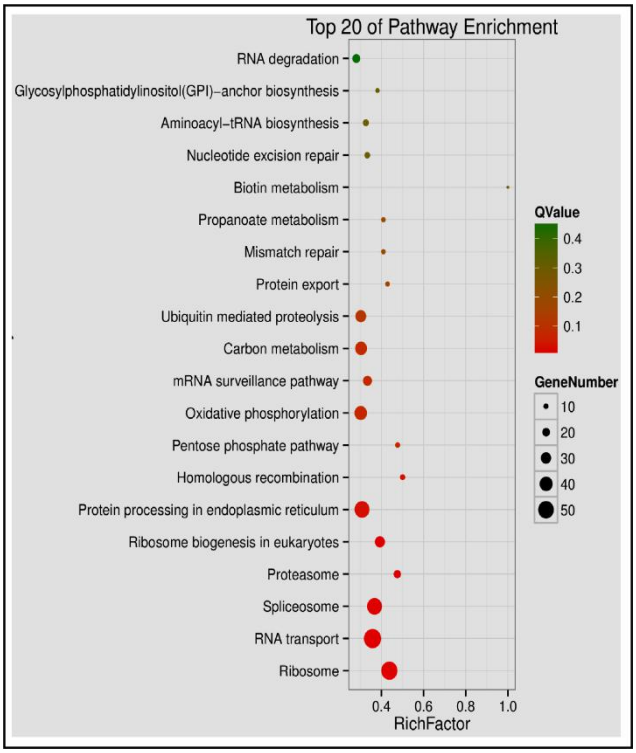

Figure S9. Top 20 pathway enrichment of genes with high DNA methylation rate in the genome of *B. mori* ovary. Related to Figure 6.

Table S1. DNA methylation levels in different regions of the genome. Related to Figure 1. mCs are divided into three categories according to their sequence characteristics: mCG, mCHG and mCHH (H represents A, C or T). The methylation level was calculated as the total number of mCs divided by the total number of Cs, CGs, CHGs and CHHs in the whole genome.

Sample	Region	C(%)	CG(%)	CHG(%)	CHH(%)
Ovary-1	Genome	0.52	0.81	0.42	0.45
	Genebody	0.78	2.24	0.43	0.46
	Exon	1.02	3.24	0.43	0.45
	Intron	0.71	1.9	0.43	0.46
	CDS	1.02	3.24	0.43	0.45
Ovary-1	Genome	0.63	0.96	0.51	0.54
	Genebody	0.93	2.72	0.52	0.55
	Exon	1.23	4	0.52	0.54
	Intron	0.85	2.32	0.52	0.56
	CDS	1.23	4	0.52	0.54

Table S5. Reads and methylated sites number statistics in the whole genome by BS-seq analysis. Related to Figure 1 and 2. mCs are divided into three categories according to their sequence characteristics: mCG, mCHG and mCHH (H represents A, C or T). The methylation proportion was calculated as the number of mCGs, mCHGs and mCHHs divided by the total number of mCs in whole genome.

Sample	Total Reads	Mapped Reads	Mapped Ratio(%)	Sequence Depth
Ovary Bisulfite Sequence-1	227,885,236	196,463,231	86.21	51.08
Ovary Bisulfite Sequence-2	201,184,054	171,451,740	85.22	44.58

Pattern	mCG	mCHG	mCHH
Ovary-1 Number	303,813	10,636	48,939
Ovary-1 Proportion	83.61%	2.93%	13.47%
Ovary-2 Number	333,953	17,738	82,309
Ovary-2 Proportion	76.95%	4.09%	18.97%

Table S6. Identification and description of the hypermethylated genes studied in Figure 2. Related to Figure 2.

Gene	Chromosome	Methylation level(%)	Description
BGIBMGA013120	nscaf3062	11.95888957	Arp2/3 complex subunit [Bombyx mori]
BGIBMGA013896	nscaf3099	12.1352089	PREDICTED: exosome complex component RRP41 [Bombhyx mori]
BGIBMGA005486	nsca2828	10.07141628	PREDICTED: histone RNA haipinbiding protein [Bombyx mori]
BGIBMGA006154	nscaf2847	10.84927891	mannose-P-dolichol uiliztion defect 1 protein [Bombyx mori]
BGIBMGA001054	nscaf1898	11.84594721	ubiquitin carboxyl-teminal hydrolase 46 [Bombyx mori]
BGIBMGA009845	nsca2970	10.2865735	NADH dehydrogenase isoform 2 [Bombyx mori]
BGIBMGA009698	nscaf2964	10.20651311	nascent polypeptide associated complex protein alpha subunit [Bombyx mori]
BGIBMGA001105	nscaf1898	12.64991839	PREDICTED: nitrie oxide synthase-interacting protein homolog [Bombyx mori]
BGIBMGA007945	nsca2888	10.61053109	PREDICTED: pre-mRNA-splicing factor 38B [Bombyx mori]

BGIBMGA010975	nscaf3013	12.16418691	PREDICTED: protein FRGI homolog [Bombyx mori]
BGIBMGA009840	nscaf2970	11.90055899	PREDICTED: protein lin-37 homolog [Bombyx mori]
BGIBMGA001106	nscaf1898	11.6113326	ribosomal pronein L21 [Bombyx mori]
BGIBMGA002610	nscaf2529	10.09892159	PREDICTED: 39s ribosomal protein L42, mitochondrial [Bombyx mori]
BGIBMGA006330	nscaf2852	11.74673515	PREDICTED: serine/threonine-protein kinase Warts [Bombyx mori]

Table S7. The sequences of the core promoter and 5'UTR of actin gene. The red represents the TATA box and transcription start site (TSS); The blue represents the 5'UTR. Related to Figure 2.

actin core promoter + 5'UTR	aataatacgcgaatgatgataacgtgttacgttacataatcgttgcataactagt gaagtgaaatttttataaaaaaaaacagtttcggaattagtgtaatgccgttgct actaaataagaaataagtttattgaacgcacatttcaaaatg

Table S8. List of histone deacetylases (HDACs) (left) and histone acetyltransferases (HATs) (right) in the silkworm genome. Related to Figure 3.

Histone deacetyase(HDAC)	
Protein introduction	**Accession number**
HDAC3	XM_012697024
HDAC4	XM_021346265
HDAC6	XM_012688445
HDAC8	XM_021349792
HDAC11	XM_004925365
HDAC SAP18	XM_004921692
HDAC SAP30	XM_004927722
HDAC Rpd3	XM_004931383.3
Histone acetyltransferase(HAT)	
Protein introduction	**Accession number**
HAT Tip60	XP_004928297
HAT MOF protein	NP_001093305
CREB-binding protein	XP_021204790
HAT KAT2A	XP_004922629
HAT KAT7	XP_021205742
HAT KAT6A	XP_021202172
Meg protein 3	XP_004928644

Table S10. The list of antibodies used in this study. Related to Figure 1, 3, 4 and 5.

Antibody name	Product code
Anti-5-methylcytosine (5-mC)	ab10805
Anti-histone H3 (tri methyl K27)	ab6002
Anti-histone H3 (tri methyl K36)	ab9050
Anti-histone H3 (tri methyl K79)	ab2621
Anti-histone H3 (acetyl K27)	ab4729
Anti-histone H3 (acetyl K23)	ab177275
Anti-histone H3 (acetyl K4)	ab176799
Anti-histone H3 (acetyl K9)	ab4441
Anti-histone H3 (acetyl K18)	ab1191
Anti-histone H3	ab1791
Anti-EGFP	ab290
Anti-FLAG Antibody	D6W5B