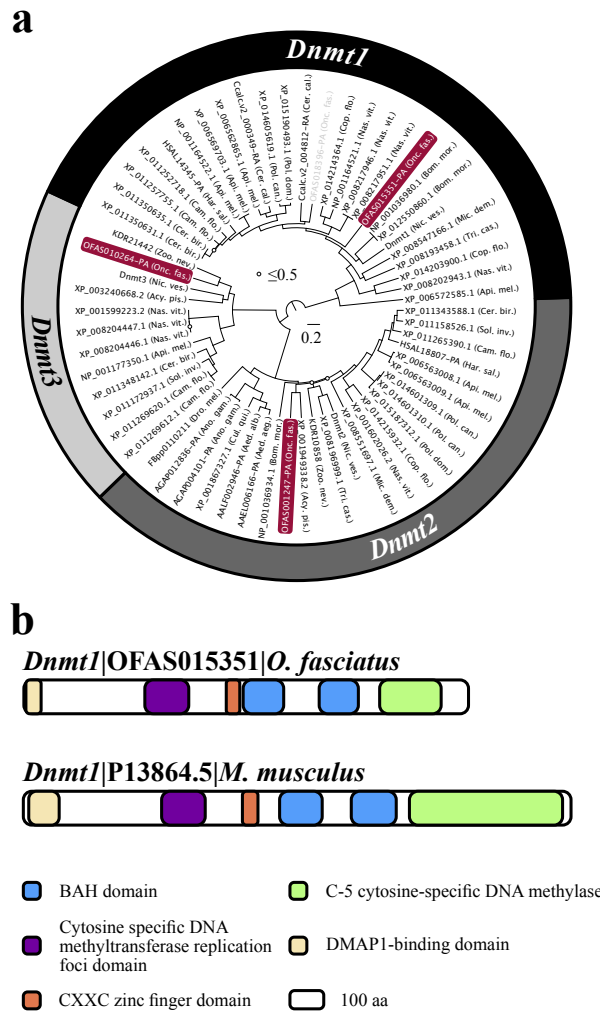
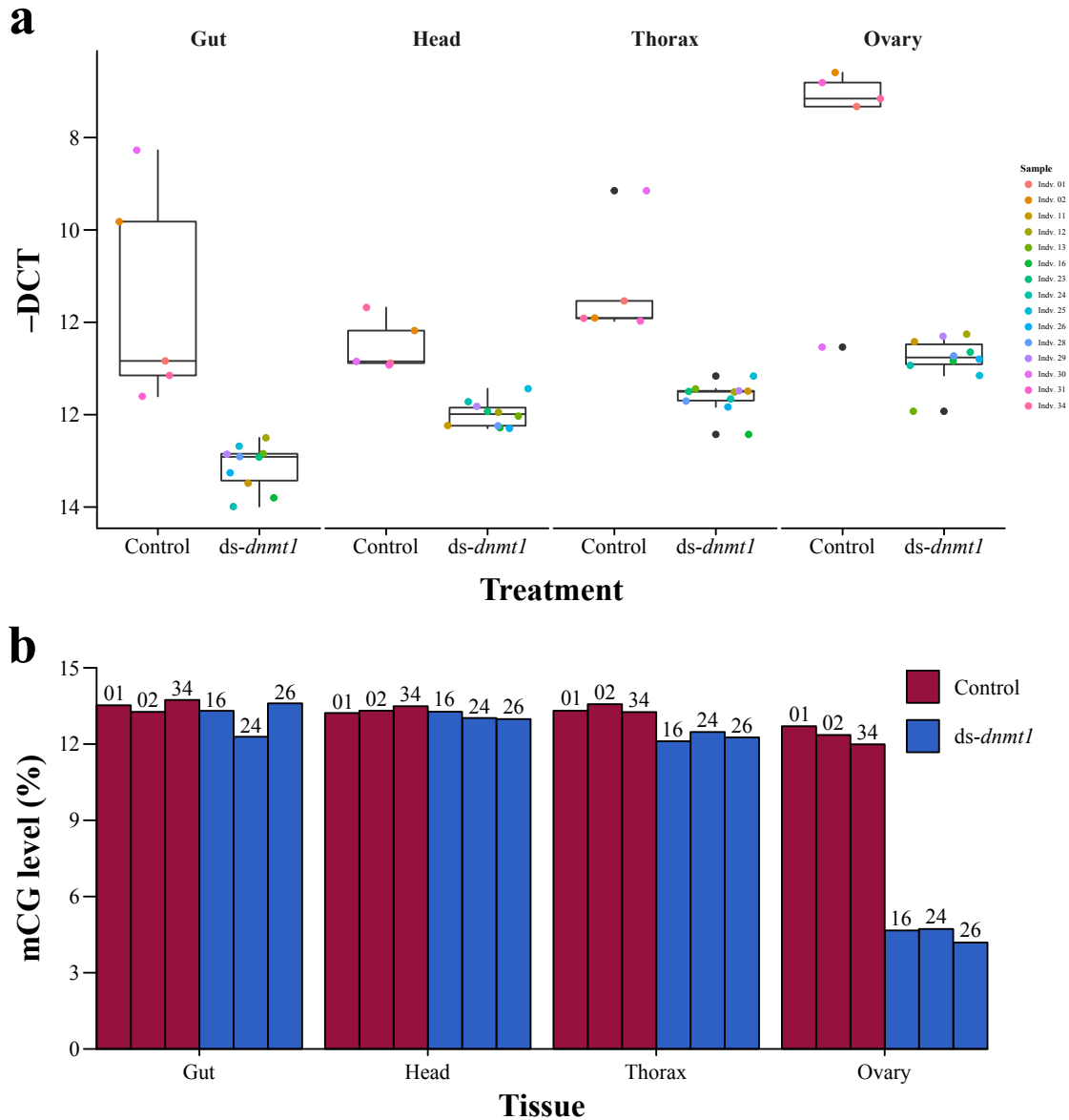


1 Extended data figures and tables

Extended Data Fig. 1



2 **Extended Data Fig. 1 Identification of *O. fasciatus* DNA methyltransferases.** **a**,
3 Phylogenetic relationship of DNA methyltransferases identified the *de novo* (*Dnmt3*) and
4 maintenance (*Dnmt1*) DNA methyltransferase in *O. fasciatus*. Node support with ≤ 0.5
5 posterior probability is indicated – other nodes are ≥ 0.95 . Branch lengths are in amino
6 acid substitutions per site. Species names are represented as abbreviations: Acy. pis.:
7 *Acyrtosiphon pisum*, Aed. aeg.: *Aedes aegypti*, Aed. alb.: *Aedes albopictus*, Ano. gam.:
8 *Anopheles gambiae*, Api. mel.: *A. mellifera*, Bom. mor.: *Bo. mori*, Cam. flo.:
9 *Camponotus floridanus*, Cop. flo.: *Copidosoma floridanum*, Cul. qui.: *Culex pipiens*
10 *quinquefasciatus*, Dro. mel.: *Drosophila melanogaster*, Har. sal.: *Harpegnathos saltator*,
11 Mic. dem.: *Microplitis demolitor*, Nas. vit.: *Nasonia vitripennis*, Nic. ves.: *Nicrophorus*
12 *vespilloides*, Onc. fas.: *O. fasciatus*, Cer. bir.: *Ooceraea (Cerapachys) biroi*, Pol. can.:
13 *Polistes canadensis*, Pol. dom.: *Polistes dominula*, Sol. inv.: *Solenopsis invicta*, Tri. cas.:
14 *Tribolium castaneum*, and Zoo. nev.: *Z. nevadensis*. **b**, A to scale representation of
15 *Dnmt1* and protein domains identified in *O. fasciatus* and *M. musculus*.



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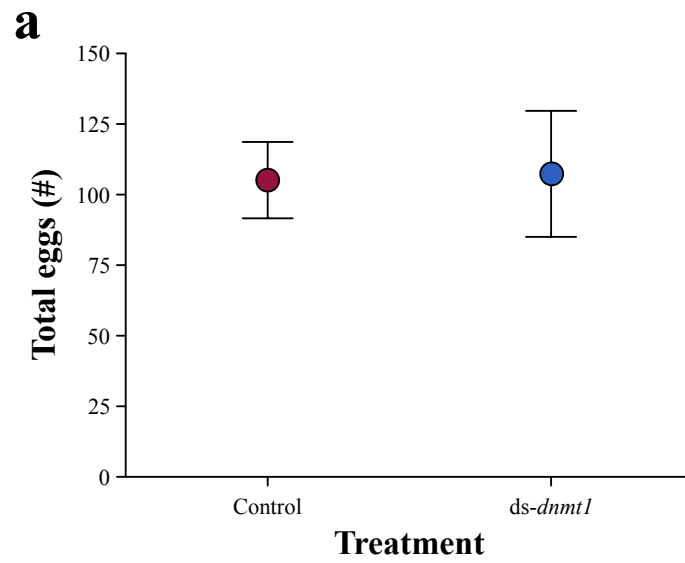
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Extended Data Fig. 2 DNA methylation consequences following post-transcriptional knockdown of *Dnmt1* are restricted to ovaries. **a**, Assessment of RNAi treatment targeting *Dnmt1* using qRT-PCR demonstrates successful reduction in transcription compared to control across all tissues sampled. Colored dots indicate independent biological replicates. **b**, Genome-wide CG methylation level across tissues sampled. Numbers at the top of each bar corresponds to independent biological replicates.

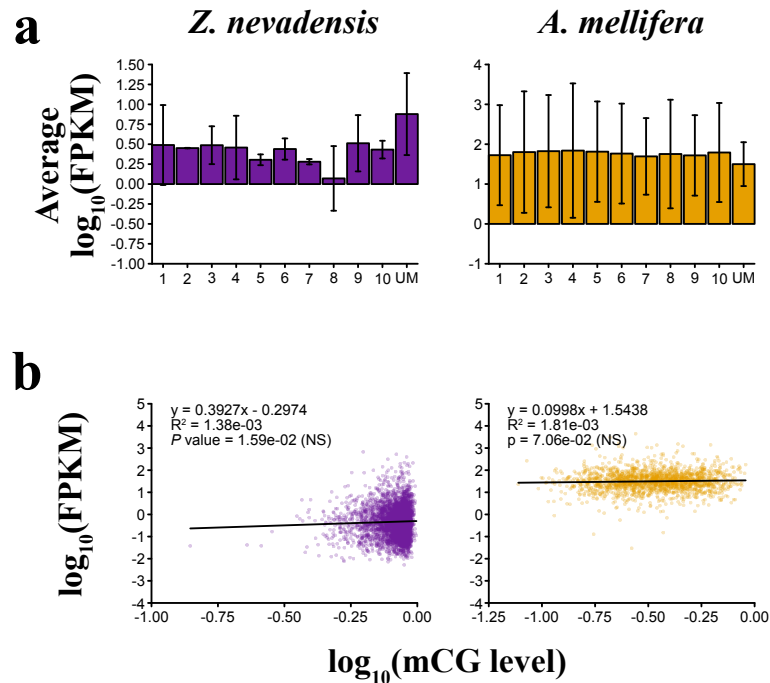


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24 **Extended Data Fig. 3 Eggs laid in *O. fasciatus* ds-dnmt1 and control females. a,**

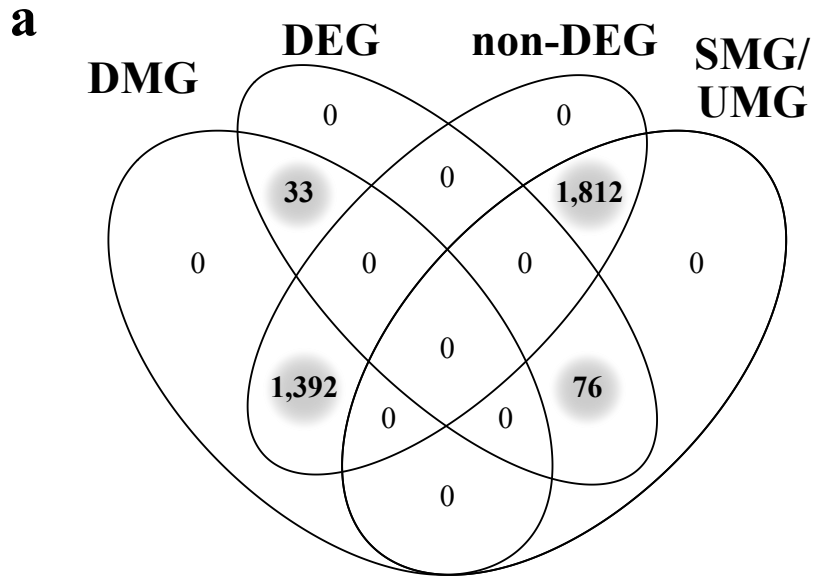
25 Number of eggs laid by ds-dnmt1 and control females 8-days post-injection. Dots

26 indicate mean expression level, and error bars indicate standard error of the mean.

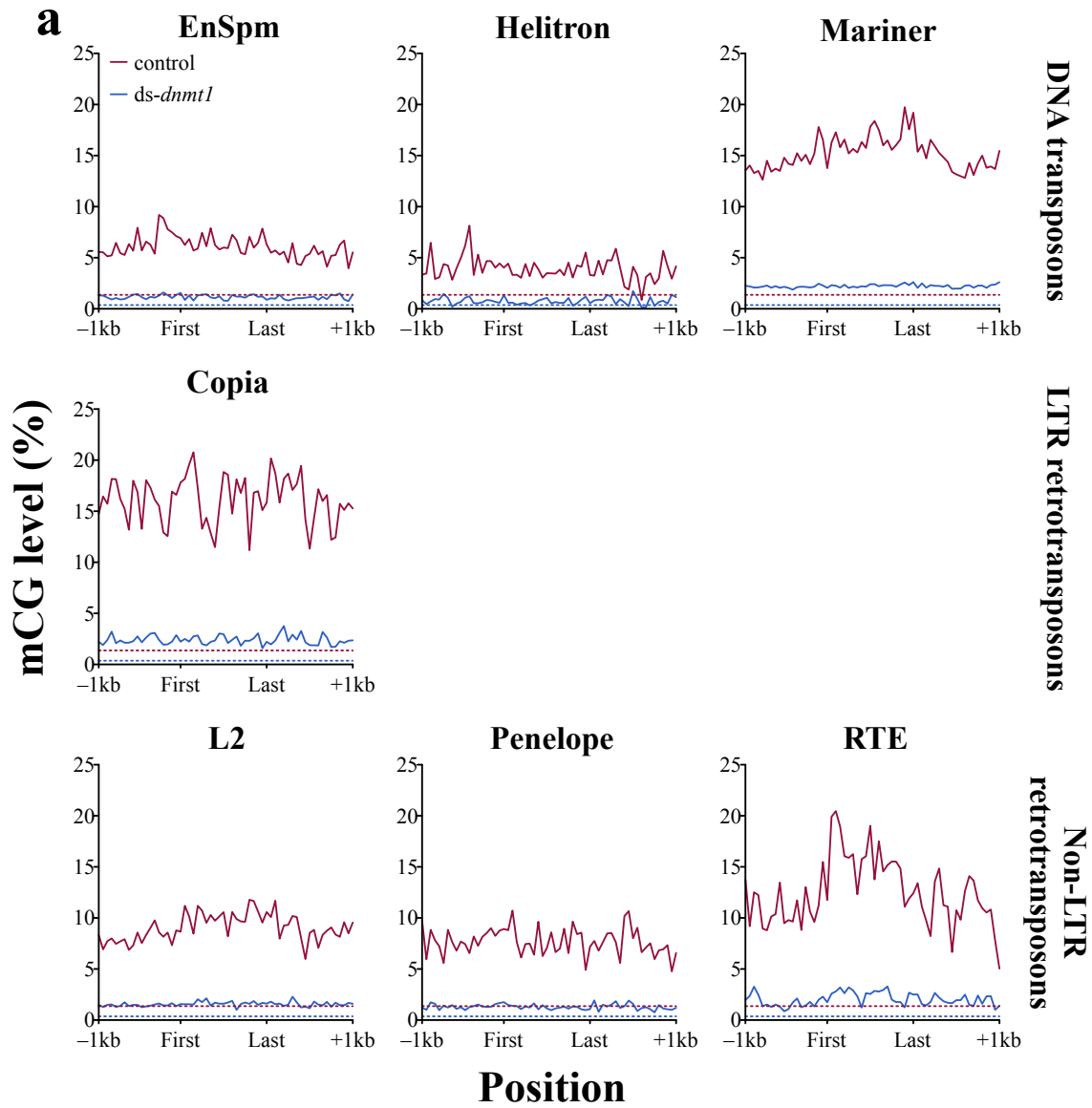


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28 **Extended Data Fig. 4 mCG in *A. mellifera* and *Z. nevadensis* is not associated with**
 29 **transcription. a**, Gene expression level for deciles of increasing mCG (1–10) and un-
 30 methylated genes (UM). Error bars represent 95% confidence interval of the mean. **b**,
 31 Regression of gene expression against a continuous measure of mCG among all genes
 32 with >0 FPKM and weighted mCG. Raw *P* values are provided for each regression, and
 33 significance (S) or non-significance (NS) is indicated in brackets following Bonferroni
 34 correction.



35
 36 **Extended Data Fig. 5 Loss of mCG in *O. fasciatus* ovaries has a limited effect on**
 37 **transcription. a**, Combinational overlap of genes that are differential CG-methylated and
 38 expressed, and similarly CG-methylated and expressed between *O. fasciatus* ds-*dnmt1*
 39 and control. Gene groups: Differentially Methylated Gene (DMG), Differentially
 40 Expressed Gene (DEG), Similarly Methylated Gene (SMG)/UnMethylated Genes
 41 (UMG), and non-Differentially Expressed Gene (non-DEG). More stringent thresholds
 42 were used to group genes as CG-methylated or unmethylated (see **Methods**).
 43



44

45 **Extended Data Fig. 6 DNA methylation of TEs in *O. fasciatus*.** a, Levels of mCG

46

across the bodies and 1kb flanking sequence of different annotated TEs in *O. fasciatus*.

47 **Extended Data Table 1 PCR primers used to validate the presence of a single *Dnmt1***
 48 **ortholog in the *O. fasciatus* genome.**

Primer name	Primer sequence (5' to 3')
Of_DMNT1-1_3603S	TGGTATTTTACAAGCTGGAAATTAT
Of_DNMT1-2_424A	TAGCAACACACTTGCGAATTCT
Of_DNMT1-2_465A	ATCAGTTATCATTTTTTCTTCA
Of_DNMT1-2_1S	ATGTCAGATCTTCCTGAAATCAGGA

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51 **Extended Data Table 2 Primer sequences for use in producing template DNA for**
 52 **use in the MegaScript transcription kit to generate double stranded RNAs for**
 53 **injection and for quantitative real-time PCR to assess expression levels of *Dnmt1*.**

Primer	Sequence (5' to 3')
Parental RNAi primer sequences	
PCR primers to produce <i>Dnmt1</i> templates for transcription of sense RNA strand	
SENSE + T7	taatacgaactactataggagaTGATGCTCGGCCTCAAAACAAGAT
ANTIENSENSE	ACTCCAGGAGGTGGAACAGTAGTCT
PCR primers to produce <i>Dnmt1</i> templates for transcription of antisense RNA strand	
SENSE	TGATGCTCGGCCTCAAAACAAGAT
ANTIENSENSE + T7	taatacgaactactataggagaACTCCAGGAGGTGGAACAGTAGTCT
PCR primers to produce <i>Red</i> templates for transcription of sense RNA strand	
SENSE + T7	taatacgaactactataggagaCGAATTGGGCCCGACGTCGCA
ANTIENSENSE	TTCAGCTTCAGGCCTTGTGGAT
PCR primers to produce <i>Red</i> templates for transcription of antisense RNA strand	
SENSE	CGAATTGGGCCCGACGTCGCA
ANTIENSENSE + T7	taatacgaactactataggagaTTCAGCTTCAGGCCTTGTGGAT
Quantitative Real-Time PCR primers	
<i>Dnmt1</i> forward	TGTGGTGTGTTGTAAGCCTGT
<i>Dnmt1</i> reverse	GGTGTGTAGGTGTTGCTGG
Actin forward	CTGTCTCCCGAAAAGAGAATATG
Actin reverse	TCTGTATGGATTGGAGGATCTA
GAPDH forward	ACGGTTCAAGGAGAAGTTAG
GAPDH reverse	ACGGTTCAAGGAGAAGTTAG

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56 **Extended Data Table 3 Overlap between *O. fasciatus* (control) CG-methylated and**
 57 **unmethylated genes in none and ≥ 1 other insect species used in this study.**

Number of species (N)	CG methylated in <i>O. fasciatus</i> (control) and N species	Unmethylated in <i>O. fasciatus</i> (control) and N species
0	913	516
1	1515	1677
2	2029	1848
3	1581	1796
4	479	1755
5	1	563

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60 **Extended Data Table 4 Significance (*P* value) of TE expression between ds-*dnmt1***
 61 **and control tissues.**

Transposable element	Ovary 1	Ovary 2	Gut	Head	Thorax
Chapeav	5.00E-01	1.00E+00	1.00E+00	1.00E+00	2.49E-01
Copia	5.00E-01	6.92E-01	3.55E-01	3.15E-01	8.23E-01
EnSpm	5.00E-01	5.88E-01	6.52E-01	1.47E-01	8.17E-01
Gypsy	5.00E-01	7.00E-01	5.00E-01	3.92E-01	7.29E-01
Helitron	5.00E-01	9.46E-01	7.84E-01	9.95E-01	9.86E-01
Jockey	5.00E-01	8.64E-01	7.84E-01	7.53E-01	1.00E+00
L2	5.00E-01	7.08E-01	6.16E-01	6.41E-01	3.53E-01
Mariner	5.00E-01	4.83E-01	1.00E+00	1.00E+00	1.00E+00
Penelope	5.00E-01	4.41E-01	2.99E-01	8.48E-01	7.18E-01
RTE	5.00E-01	4.27E-01	5.78E-01	4.58E-01	5.06E-01

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