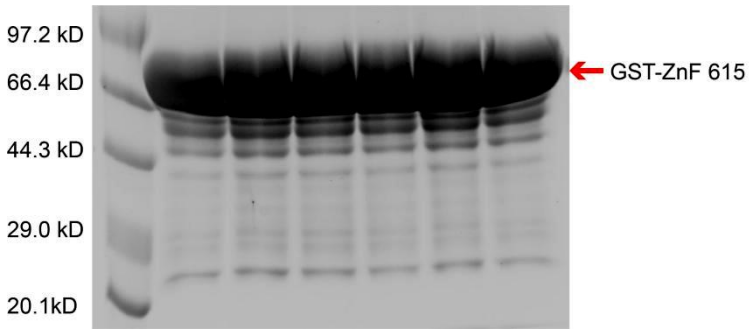
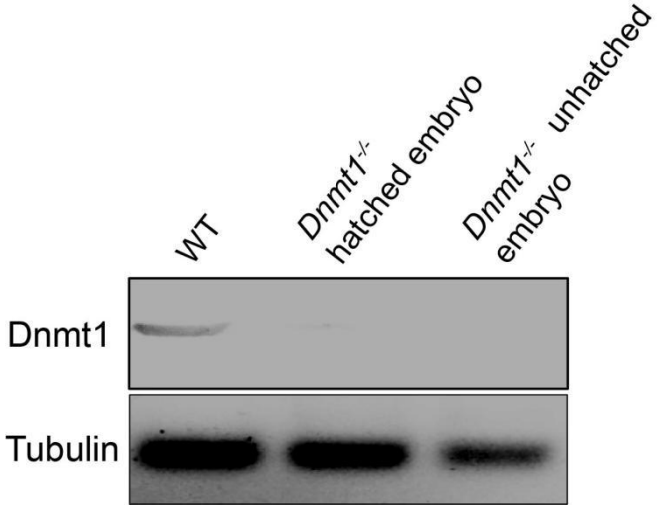
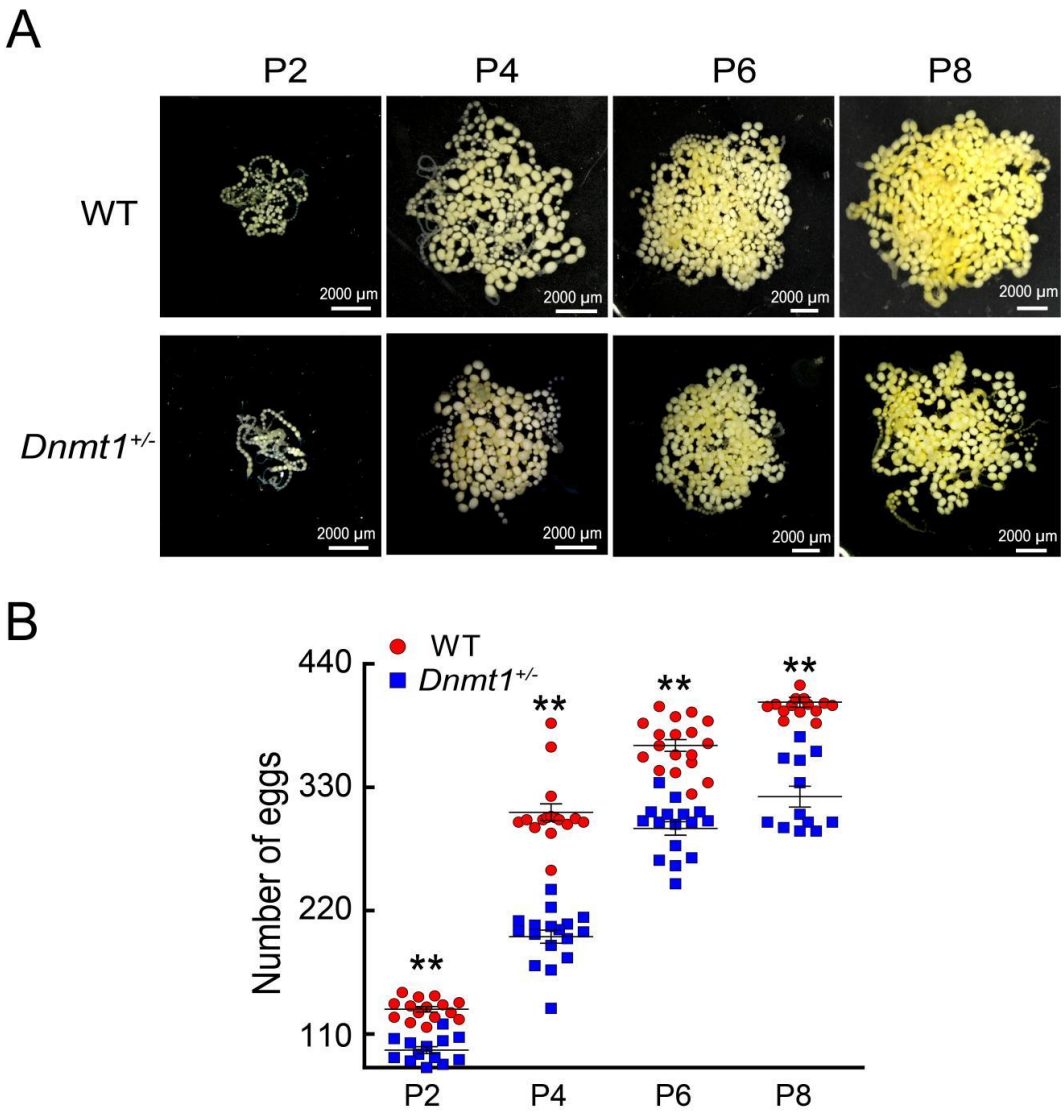
Supplementary Materials



Supplementary Figure S1. SDS-PAGE analysis of purified recombinant GST-ZnF615 proteins.

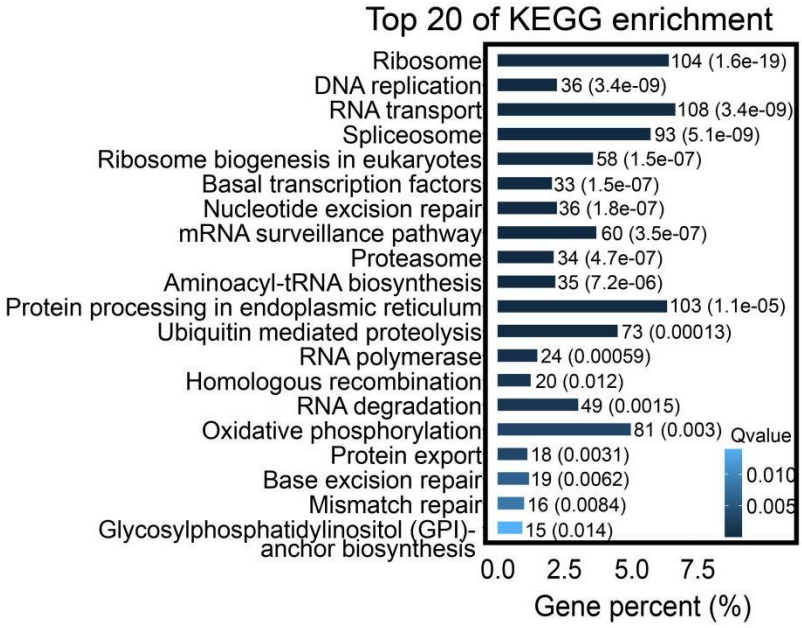


Supplementary Figure S2. Western blot analysis of Dnmt1 proteins in wild-type (WT), *Dnmt1*$^{-/-}$ hatched, and unhatched embryos.

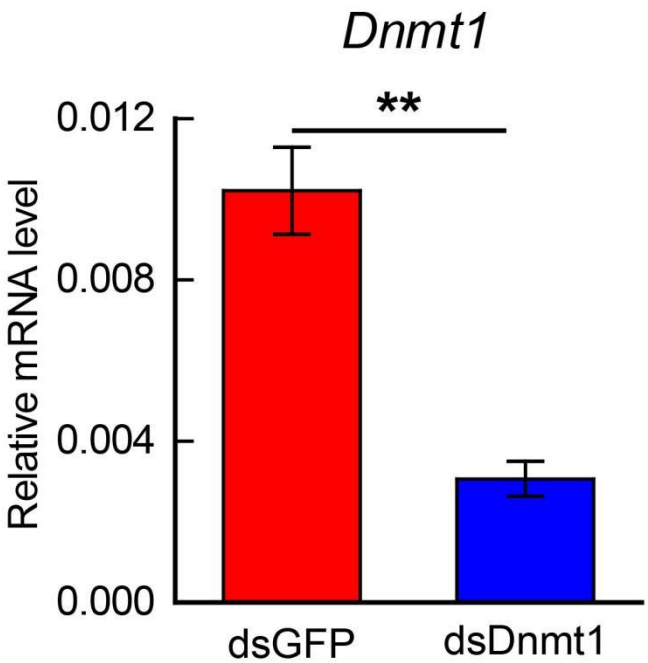


Supplementary Figure S3. Morphology of *Dnmt1*$^{+/-}$ *B. mori* ovaries.

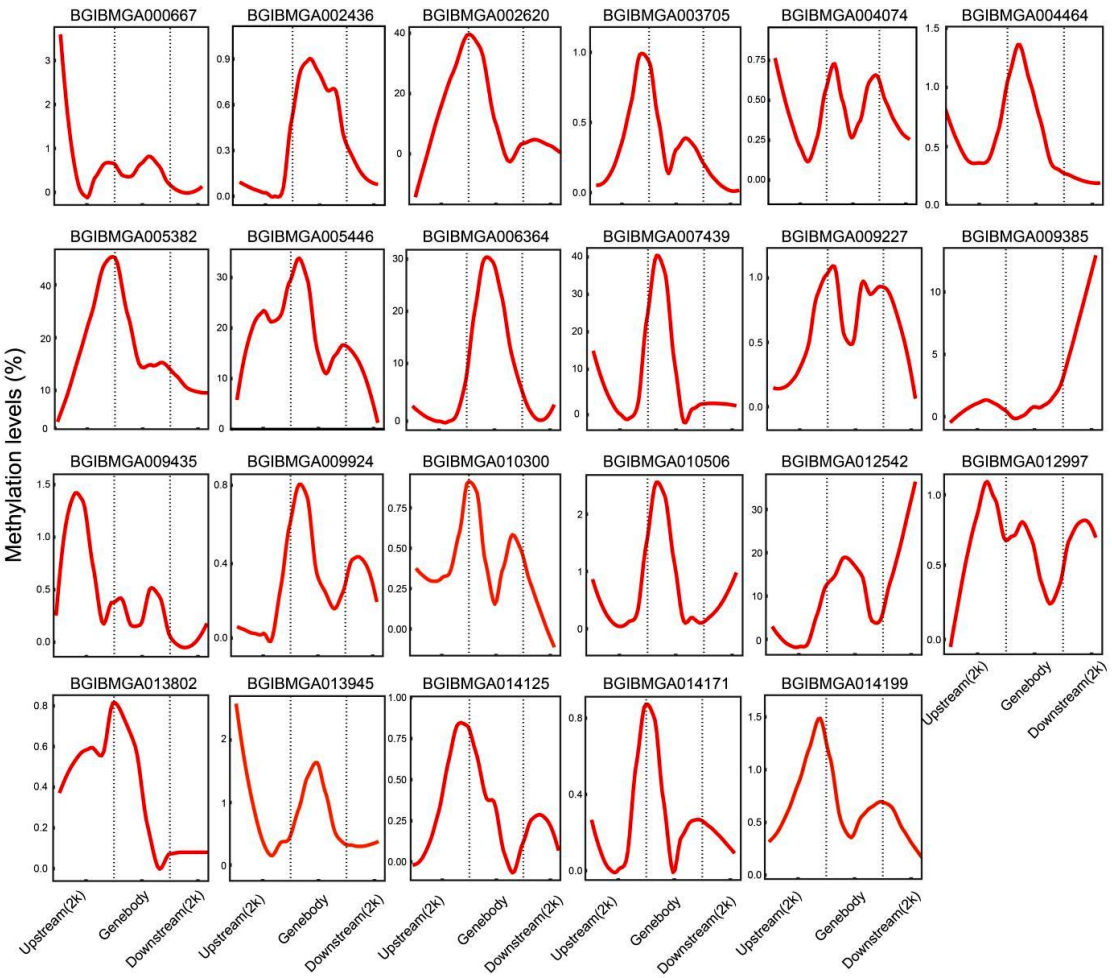
A: Morphology of ovaries between *Dnmt1*$^{+/-}$ and WT females from two-day-old pupae (P2) to eight-day-old pupae (P8). B: Changes in egg numbers of *Dnmt1*$^{+/-}$ and WT females from P2 to P8. Significance of results was determined by *t*-test (**P*<0.05, ***P*<0.01).



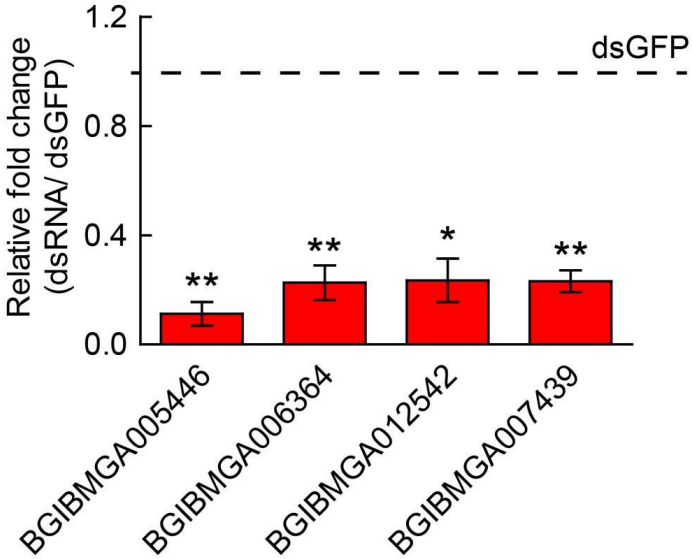
Supplementary Figure S4. Top 20 pathway-enriched genes with high DNA methylation rates in embryonic genome of *B. mori*.



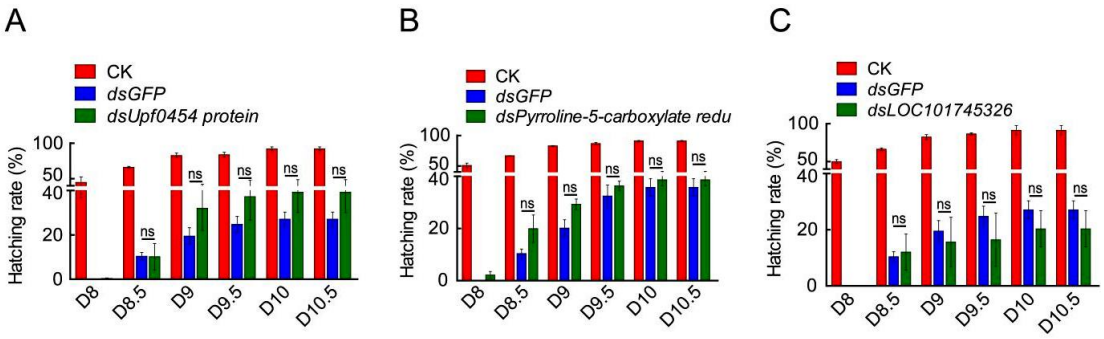
Supplementary Figure S5. Analysis of *Dnmt1* RNAi efficiency for Figure 2D in *B. mori* embryos by qRT-PCR. Significant differences were determined by *t*-test (**P*<0.05, ***P*<0.01).



Supplementary Figure S6. mCpG level analysis in selected genes from Figure 2F.



Supplementary Figure S7. Analysis of RNAi efficiency of four genes from Figure 3B and Supplementary Figure 8 in *B. mori* embryos by qRT-PCR. Significant differences were determined by *t*-test (**P*<0.05, ***P*<0.01).

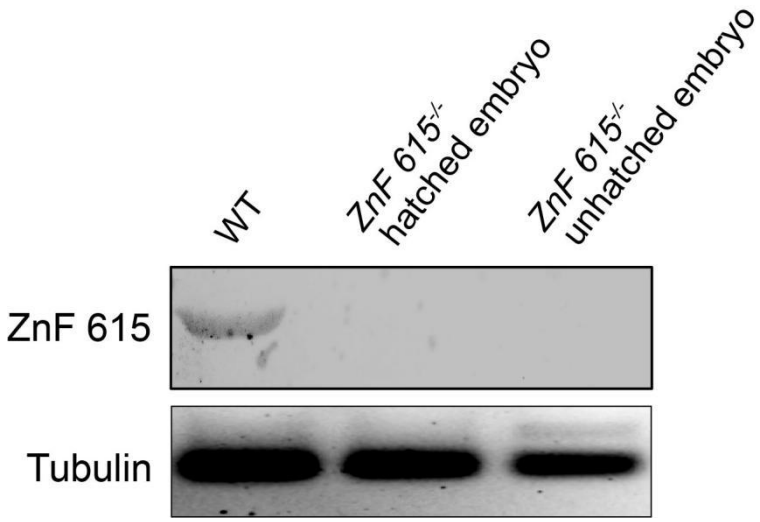


Supplementary Figure S8. Changes in hatching rate after DNA-methylated gene RNAi.

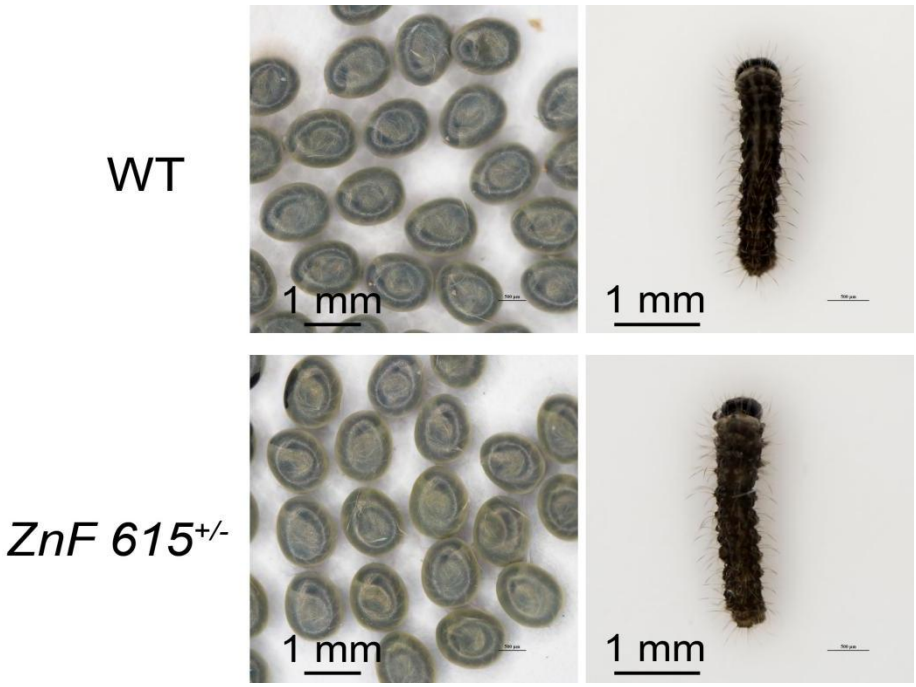
A-C: Changes in hatching rate after *BGIBMGA005446* (*UPF0454 protein*) (A), *BGIBMGA006364* (*pyrroline-5-carboxylate reductase*) (B), and *BGIBMGA012542* (*uncharacterized protein LOC101745326*) (C) RNAi. *dsRNA* was injected into *B. mori* embryos within 2 h after oviposition. Dn: n-Day of embryonic development.

Region 1 AAATGAATACTCCTGATCCAACCACTTCTACAAATGAATCAACCACCTCTACAAACGAACGTCGTTATTCAAAGAGGATTAGACGTCCCAAAATACAAGA
Region 2 AGATATGATCTATAATTTTTTGATTACCGAGGGATCAGATCCTAAGTCAGTTGAGGACACATTGGAACGTTCGGATCGAGAGTTATGGCACACAGCCATG
Region 3 TTAGATGGATACAATTCGCTGATGCAAAACAAAAACAGTGAACAGAATTAAACTTAATAAATGTATAAAACTTATGTGGTTAAGTGGGGGTGTTAGAACT
Region 4 GGCCTTCTTAGTATATGTAACCATCATAAGTTTATATTAAGAAGAATAGTTGTTGCCTAGCAACAATTTGTTAGTCGTATAATTTTATGTAAAGAATAAA
Region 5 TAATAAAGCCGAGTGTCCCATATGTAAGCTTCGTTTCAAGAGTGAAGCTTCCGTAGACTTGCACAGGCGACACACGCACAAGAAGAAGTTCCAGTATAAA
Region 6 AGCTGCCCTAAAACACTTAGTGATGCGTGAGTGTCACGAATGATTTTTAGTAGTCAGTATTTATTTACGTTTTAACGTGTCAATAGTTTTAACTAAGTAA
Region 7 AAATGAAAGTATTGTATTTTTTTTTTTGTCGTGTAACATTTATAAAACTGGCTAATTATTGGGAGCAGCTGTGCTACGCACGGACAATCCACCCGTCCTGT
Region 8 TGGCTTAGGGCGAGTTTTTCTAACTTGTATGTATGGAGTTGGAACAGCTTCCCATCGTTTGCCGCTAGGGGCGTTGTACTAACTCCATACCAAATTTGAG
Region 5 mut TAATAAAGCatAGTGTCCCATATGTAAGCTTatTTTCAAGAGTGAAGCTTCatTAGACTTGCACAGGatACACAatCACAAGAAGAAGTTCCAGTATAAA
Region 7 mut AAATGAAAGTATTGTATTTTTTTTTTTGTatTGTAACATTTATAAAACTGGCTAATTATTGGGAGCAGCTGTGCTAatCAatGACAATCCACCatTCCTGT

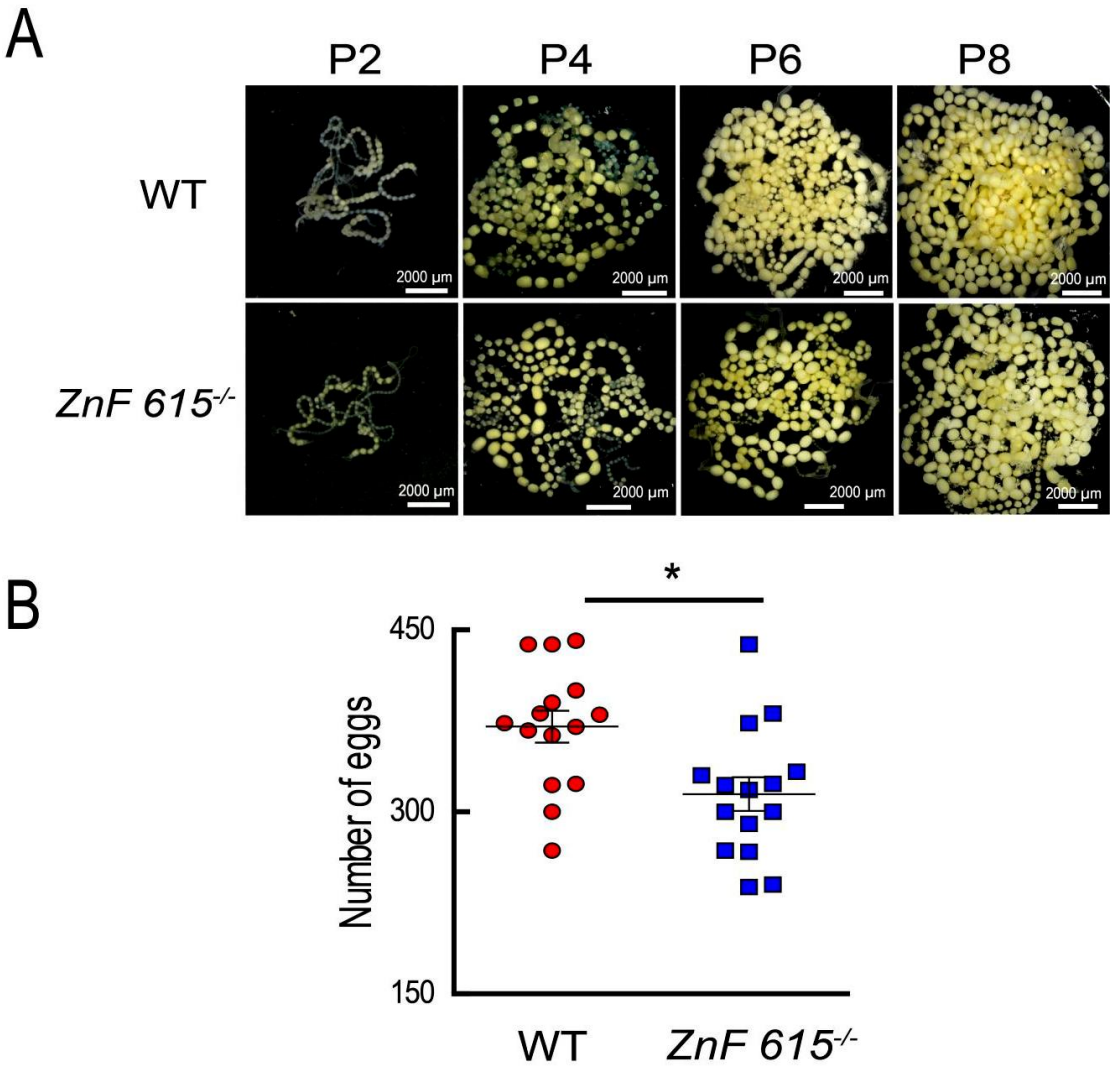
Supplementary Figure S9. Sequences of regions 1–8 and mutant regions 5 and 7 of *ZnF615* gene.



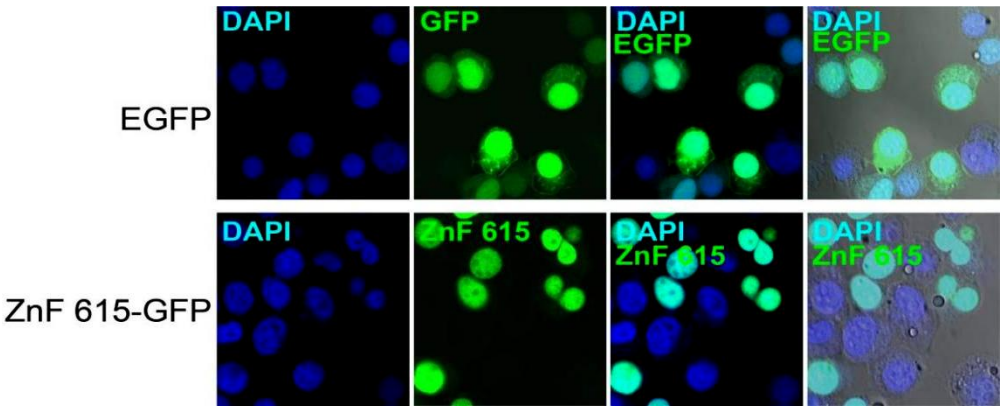
Supplementary Figure S10. Western blot analysis of ZnF615 proteins in WT, *ZnF615*$^{-/-}$ hatched, and unhatched embryo mutants.



Supplementary Figure S11. Phenotypes of WT and *ZnF615*$^{+/-}$ *B. mori*.

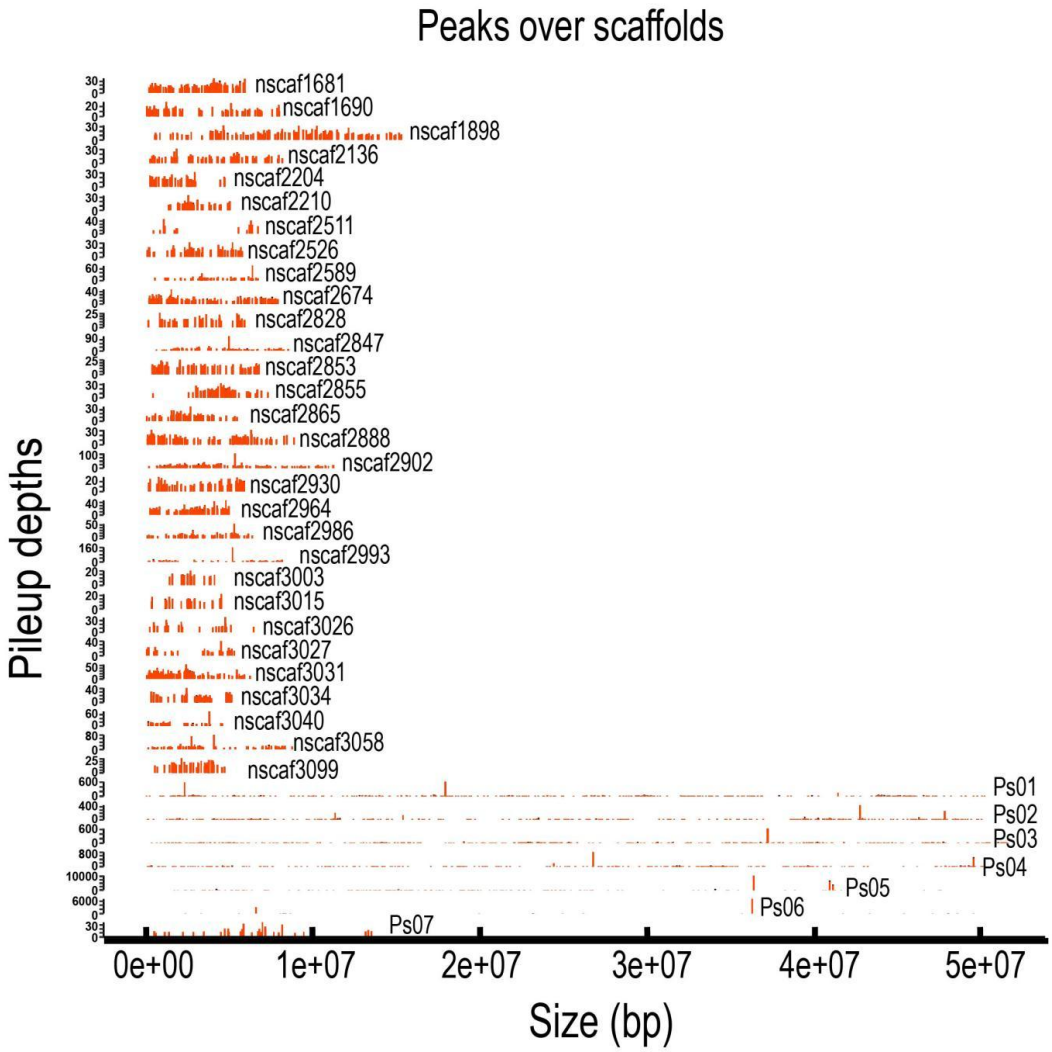


Supplementary Figure S12. Morphology of ovary in *ZnF615*$^{-/-}$ *B. mori*.
A: Morphology of *ZnF615*$^{-/-}$ ovaries from two-day-old pupae (P2) to eight-day-old pupae (P8). B: Changes in egg numbers of *ZnF615*$^{-/-}$ adult females (right). Significance of results was determined by *t*-test (**P*<0.05).

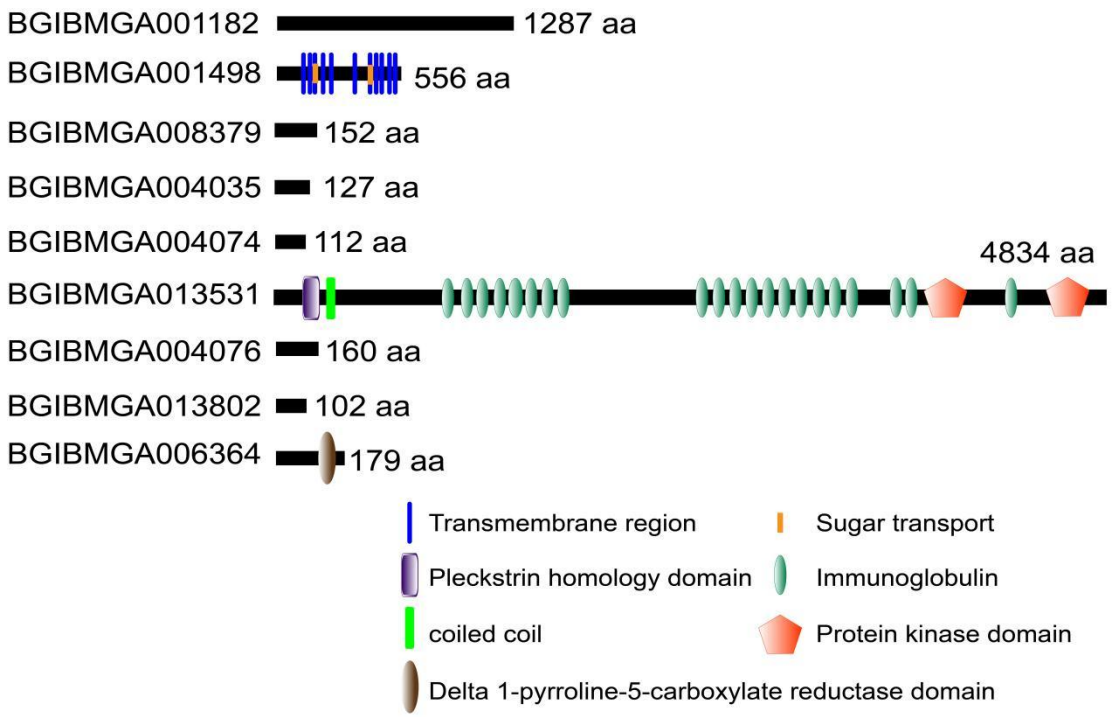


Supplementary Figure S13. Immunofluorescence localization of ZnF615 protein in *Bm*12 cells. *Bm*12 cells were transfected with EGFP-N1 and ZnF615-GFP. Blue:

DAPI.



Supplementary Figure S14. Distribution of ZnF615 DAP-seq peaks in different chromosomes of silkworm genome.



Supplementary Figure S15. Functional domain analysis of genes differentially expressed after *Dnmt1* RNAi and *ZnF615* binding in Figure 7D.

Supplementary Tables

Supplementary Table S1. DNA methylation levels of all genes in genome of *B. mori* embryos (blastoderm stage) by bisulfite sequencing (BS-seq) analysis.

Supplementary Table S2. DEGs after *Dnmt1* RNAi by RNA-seq analysis.

Supplementary Table S3. Description and methylation level analysis of DEGs **after *Dnmt1* RNAi** in Figure 2F.

Supplementary Table S4. FPKM of all genes in six different embryonic development stages by RNA-seq analysis.

Supplementary Table S5. Sequences of promoter and 5'UTR of *actin* gene. Red represents TATA box and transcription start site (TSS); blue represents 5'UTR.

actin promoter + 5'UTR	aaaacaaactggagccgatactgtgtaaattgtgataacggctcttttatatagtt tatcctcacgagtcggttctcatttactaaggtgtgctcgaacagtgcgcattcg catctacgtacctacttgtcacttatttaataatactatgtaagttttaattttaaaatt gcgaaagaaaaaaaaacatatttatttatttgtaaaatttgaatttcgaaggttctc cgtccctttacctttaagtattacatatgtttgagtgtttttttttaataatacgcgaa tgatgataacgtgttacgttacataatcgttgcataactagtgaagtgaaatttttt ataaaaaaaaaacagtttcggaattagtgtaatgccgttgctactaaataagaaat aagtttattgaacgcacatttcaaaatg

Supplementary Table S6. Annotation and description of peak-related genes by ZnF615 DAP-seq.

Supplementary Table S7. Identification and description of genes differentially expressed after *Dnmt1* RNAi and ZnF615 binding.

Gene ID	Gene name
BGIBMGA001182	Adhesive plaque matrix protein
BGIBMGA001498	Synaptic vesicle glycoprotein 2B
BGIBMGA008379	Uncharacterized protein LOC101738308
BGIBMGA004035	Chemosensory protein isoform X1
BGIBMGA004074	Cuticular protein glycine-rich 19 precursor
BGIBMGA013531	Obscurin
BGIBMGA004076	Uncharacterized protein LOC110385826
BGIBMGA013802	AGAP005312-PA-like protein
BGIBMGA006364	Pyrroline-5-carboxylate reductase

Supplementary Table S8. Identification and description of nutrient metabolism-related DEGs **enriched by** *Dnmt1* RNAi.

Gene ID	Gene name
BGIBMGA007324	Homogentisate 1,2-dioxygenase
BGIBMGA011646	beta-N-acetylglucosaminidase 1

BGIBMGA002450	Myrosinase 1
BGIBMGA012226	Succinate dehydrogenase flavoprotein
BGIBMGA010537	Lactase-phlorizin hydrolase
BGIBMGA007197	Uncharacterized family 31 glucosidase KIAA1161
BGIBMGA009924	Glucose dehydrogenase-like
BGIBMGA002827	Carbonyl reductase [NADPH] 1
BGIBMGA010676	Stearoyl-CoA desaturase 5
BGIBMGA011072	Lipase 3
BGIBMGA011777	Lipase 3-like
BGIBMGA010611	Stearoyl-CoA desaturase 5
BGIBMGA003517	bile salt-activated lipase-like

Supplementary Table S9. Sequences of primers used in this study.

Supplementary Tables S1-4, 6, 9 are listed as separate excel files due to their large size.