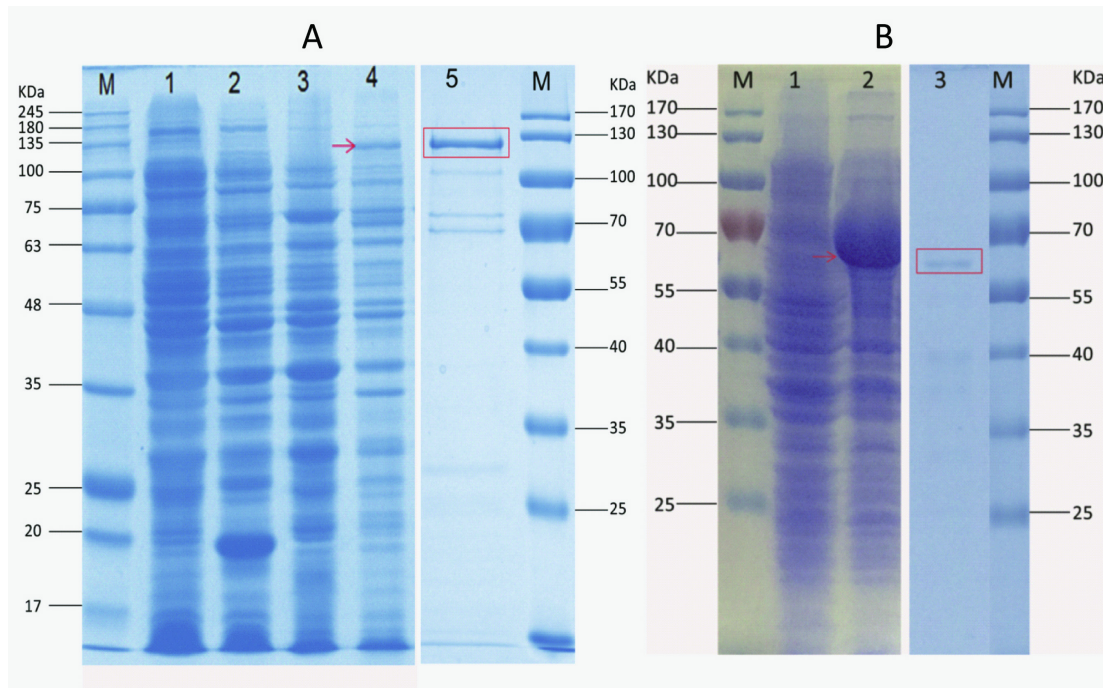
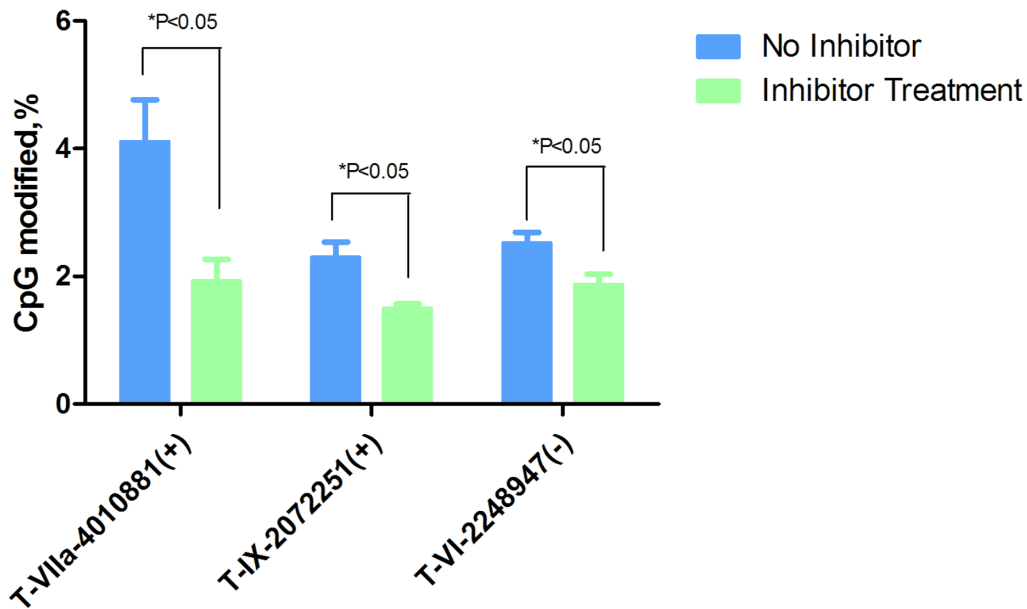


## Supporting Information



**Figure S1. The expression and purification of TgDNMTa and TgDNMTb-conserved domain.** A, the Coomassie staining gel including expression and purification of TgDNMTa. Lane 1 and 2, the empty vector of PET32a(+) without and with IPTG initiated; Lane 3 and 4, the recombinant PET32a(+)-TgDNMTa vector without and with IPTG initiated; Lane 5, the purification of TgDNMTa. B, the Coomassie staining gel including expression and purification of TgDNMTb-conserved domain, Lane 1 and 2, the recombinant PET32a(+)-TgDNMTb- conserved domain vector without and with IPTG initiated; Lane 3, the purification of TgDNMTb-conserved domain. The red arrow represented the expression of the proteins; the red box represented the purification of the proteins.



**Figure S2. 5-AzaC treatment results in DNA demethylation or hemi-demethylation in *T. gondii*.**

Tachyzoites were treated with 5-AzaC for 30 min and washed with PBS to eliminate the inhibitor, and then DNA from no inhibitor group and inhibitor treatment group were extracted and used to detect the methylation level using methylation-sensitive or insensitive restriction enzymes digestion coupled with qPCR. The methylation level of the selected three sites in the Inhibitor Treatment group is significantly lower than that in the No Inhibitor group. Error bars: SEM.

**Table S1. qPCR primer sequences for validation of methylated sites.**

**Table S2. TgDNMTa's Similarities to Protein Data Bank (PDB) Chains.**

**Table S3. TgDNMTb's Similarities to Protein Data Bank (PDB) Chains.**

**Table S4. Information for 2,660 methylated cytosines in tachyzoites.**

**Table S5. Information for 4,402 methylated cytosines in bradyzoites.**

**Table S6. The information for the 624 genes with different CpG methylation levels in the upstream region and the 1550 genes with different cytosine methylation level in the CDS between tachyzoites and bradyzoites.**

**Table S7. The Gene Ontology (GO) and KEGG pathways enrichment terms for 624 genes with different CpG methylation levels in the upstream region between tachyzoites and bradyzoites.**

**Table S8. The Gene Ontology (GO) and KEGG pathways enrichment terms for 1550 genes with different cytosine methylation levels in the CDS of tachyzoites and bradyzoites.**

**Table S9. All 190 genes with different CpG methylation levels in the upstream region and with a transcription level of " | log<sub>2</sub> expression ration | > 1", 576 genes with different cytosine methylation level in the CDS region and with a transcription level of " | log<sub>2</sub> expression ration | >1".**

**Table S1**

<b>Methylated location</b>	<b>Primer</b>	<b>Primer sequence</b>
T-IV-1336110+	Forward	AAATGTCCACGACGGTTT
	Reverse	ACGGGAGTCTACACTCTTTC
T-VIIa-4010881(+)	Forward	CCCGCCTTTGGTCATGG
	Reverse	AAACCCAGAAGAGGCATTGAG
T-VI-2248947(-)	Forward	CACGTCGGTTACGCTAGAAA
	Reverse	TGATGGAACATCACATCCAGATAA
T-IX-2072251(+)	Forward	TCACATTCTGTAGTGGGCTTG
	Reverse	GCTTGTGTGGGCCCTATT
B-III-521081(-)	Forward	GAAGGATTCACCTGCCTGTC
	Reverse	TTCACAAAGACAGCTGAGAGAC
B-III-571920(+)	Forward	GTCACCTTCGTCGTC AAGAA
	Reverse	CAGGAGTAGAAGGCAGGATTG
B-IX-1630654(-)	Forward	GAGTCCGTTGATGGGACAAG
	Reverse	CCGCTGGCGTACTCATTTA
B-V-2608103(-)	Forward	GAAACTGGCCGATGTCTATCT
	Reverse	TGTTCTCCAGCTTCGTTTTAG
B-V-1971557(-)	Forward	GTCGTGCCAGCACAATTC
	Reverse	CTTTGCCGCAACTCTGTATT
B-II-702983(+)	Forward	CATTTGCAAGATTGTCGGTAGG
	Reverse	GTCACCTCTAGAACCGGAAA
B-V-1331914(-)	Forward	ATTTGTCTCTCGAATCCGTCTG
	Reverse	CGACGAAGACCTCTCGATTC

qPCR primer sequences for methylated sites validated.

**Table S2**

PDB Structure	PDB Molecular Description	Taxon	% Identity	P-value
1g55_A	DNA cytxsine METHYLTRANSFERASE DNMT2	Homo sapiens	48	$3.4 \times 10^{-43}$
4h0n_A	DNMT2	Spodoptera frugiperda	42	$3.8 \times 10^{-33}$
4h0n_B	DNMT2	Spodoptera frugiperda	42	$3.8 \times 10^{-33}$
4h0n_C	DNMT2	Spodoptera frugiperda	42	$3.8 \times 10^{-33}$
4h0n_D	DNMT2	Spodoptera frugiperda	42	$3.8 \times 10^{-33}$
3qv2_A	5-cytosine DNA methyltransferase	Entamoeba histolytica	38	$1.9 \times 10^{-28}$

TgDNMTa's Similarities to Protein Data Bank (PDB) Chains

**Table S3**

PDB Structure	PDB Molecular Description	Taxon	% Identity	P-value
1g55_A	DNA CYTXSINE METHYLTRANSFERASE DNMT2	Homo sapiens	49	$2.3 \times 10^{-28}$
4h0n_A	DNMT2	Spodoptera frugiperda	45	$2 \times 10^{-14}$
4h0n_B	DNMT2	Spodoptera frugiperda	45	$2 \times 10^{-14}$
4h0n_C	DNMT2	Spodoptera frugiperda	45	$2 \times 10^{-14}$
4h0n_D	DNMT2	Spodoptera frugiperda	45	$2 \times 10^{-14}$
3qv2_A	5-cytosine DNA methyltransferase	Entamoeba histolytica	33	$1.2 \times 10^{-6}$

TgDNMTb's Similarities to Protein Data Bank (PDB) Chains