

Table S1. Quality control of WGBS by FastQC report.

NCBI name	Analysis name		Raw reads	Clean read	Clean Total bases (Gbp)	Sequence length	%GC
SCSP_M1	MN12	Forward	212222274	210360748	29.7	20-150	26
		Reverse	212154342	210360748	29.6	20-150	27
CHIP_F2	MN23	Forward	227536909	224804503	31.7	20-150	25
		Reverse	227422091	224804503	31.7	20-150	25
WIOBA_M3	MN31	Forward	236345033	234393749	33.3	20-150	26
		Reverse	236270708	234393749	33.2	20-150	27
ITAS_F4	MN46	Forward	243635591	241390700	34.2	20-150	25
		Reverse	243547146	241390700	34.1	20-150	27
ITAS_F4-7	MN47	Forward	182312811	180625448	25.5	20-150	25
		Reverse	182252852	180625448	25.5	20-150	27
TX1	TX1	Forward	316537539	313646302	44.5	20-150	25
		Reverse	316473939	313646302	44.2	20-150	27
TX2	TX2	Forward	348266018	345089411	48.9	20-150	25
		Reverse	348184624	345089411	48.6	20-150	27
TX3	TX3	Forward	334586555	331084894	47.1	20-150	25
		Reverse	334495331	331084894	46.7	20-150	27
TX5	TX5	Forward	346119734	341618924	48.4	20-150	25
		Reverse	346169082	342618924	48.1	20-150	27
TX7	TX7	Forward	333116469	329540571	46.7	20-150	26
		Reverse	333032874	329540571	46.2	20-150	27
TX8	TX8	Forward	315427820	312574478	44.3	20-150	25
		Reverse	315358874	312574478	44	20-150	27

Table S2. Summary of mapping efficiency and methylation percentages according to Bismark from WGBS data.

Sample	Sex	Mapping efficiency (bt2_PE_report.txt) (%)	C methylated in CpG context (%)	C methylated in CHG context (%)	C methylated in CHH context (%)
MN12	male	13	11	3.5	4.5
MN23	female	13.2	11.5	3.5	4.5
MN31	male	13.4	11.3	3.5	4.4
MN46	female	13.2	10.9	3.4	4.3
MN47	female	13.2	10.9	3.5	4.4

TX1	female	16.8	15.3	3.5	4.3
TX2	female	16.9	15.6	3.4	4.2
TX3	female	16.9	14.5	3.4	4.2
TX5	male	15.9	12.9	3.4	4.2
TX7	male	16.5	12.2	3.5	4.3
TX8	Male	16.7	13.6	3.9	4.7

Table S3. Read coverage per cytosine for MN and TX *I. scapularis* using Methylkit from WGBS.

Sample	Sex	Min.	1st Qu	Median	Mean	3rd Qu.	Max.
MN12	male	10	11	14	25.02	22	3948
MN23	female	10	11	13	24.27	21	4355
MN31	male	10	11	13	24.95	21	4285
MN46	female	10	11	13	23.23	19	4976
MN47	female	10	11	14	25.65	23	3285
Mean					24.624		
TX1	female	10	11	13	18.11	17	2525
TX2	female	10	11	13	16.9	17	2421
TX3	female	10	11	12	16.81	16	2446
TX5	male	10	11	13	24.56	20	2709
TX7	male	10	11	13	28.28	23	3019
TX8	Male	10	11	13	23.66	20	2774
Mean					21.3866667		

Table S4. General summary statistics for nanopore sequencing of *I. scapularis*.

	Seq stats							Methylation stats		Mapped stats		
Location	# of reads	# of bases	Avg* length	Max length	N50	AvgQual _α	GC (%)	5mC (%)	5hmC (%)	Reads mapped	Bases mapped	Mean CpG cov _¥
Minnesota	17,259,423	29,584,813,871	1,714	173,450	2,784	14.96	45.11	8.8	0.47	15,894,116	28,680,865,979	11.53
Texas	9,431,723	25,878,880,925	2,744	137,572	5,840	15.51	45.93	6.2	0.34			10.83

* Avg = average

_α AvgQual = Average Quality

_¥ Cov = coverage

Table S9. Whole Genome sequencing information by FastQC and SAMtools.

NCBI name		Raw reads	Total bases (Gbp*)	Total de read after trimming	Total de bases (Gbp) after trimming	Sequence length	%GC	% mapped reads	% reads properly paired
MNFem3	Forward	318402016	32	318402012	31	31-101	46	96.87	85.02
	Reverse	318402016	32	318402012	31	25-101	46		
MNMale4	Forward	319809486	32.1	319809485	31.5	30-100	46	97	85.44
	Reverse	319809486	32.1	319809485	31.5	40-100	47		
TXFem1	Forward	350823886	35.2	350823882	34.2	32-100	46	98.08	88.95
	Reverse	350823886	35.2	350823882	34.2	28-100	46		
TXMale2	Forward	344827946	34.6	344827944	33.4	31-100	46	98.12	89.3
	Reverse	344827946	34.6	344827944	33.4	35-100	46		
	Mean	333465834	33.475	333465831	32.525		46.125	97.5175	87.1775

*Gbp = Gigabasepairs

Table S10. Summary of variable sites filtered out using VCFtools.

Process	Total of SNPs*	Remaining SNPs (%)
Variable sites identified by FreeBayes	143,654,820	100
Remove indels and non-biallelic SNPs	102,196,460	71.1402931
Retain sites with $\leq 10\%$ missing data	73,779,049	51.35856145
Filter by quality ($Q \geq 20$)	46,994,192	32.71327199
Filter by minor allele count ($MAC \geq 2$)	20,162,323	14.03525687
Remove SNPs with excessive coverage	19,799,421	13.78263604

* SNPs = Single Nucleotide Polymorphisms

Table S11. Candidate differentially methylated promoter region in MN ticks compared to TX ticks.

Type	Putative promoter	Upstream of gene	# meth. sites	Meth. location	qvalue	Meth. diff
Hypomethylation	Promoter 1	Transmembrane protein 50A	12	249461842	1.46171E-05	-38.699957
				249461850	7.51694E-05	-35.07085
				249461862	6.54663E-06	-36.564681
				249461883	3.26788E-06	-36.411658
				249461885	2.27E-08	-40.936639
				249461890	3.94441E-08	-39.730965
				249461895	1.00475E-07	-40.034364
				249461906	2.16651E-07	-38.297521
				249461924	2.42781E-05	-33.676471
				249461944	0.000649297	-28.713398
				249461981	5.18055E-05	-33.84911
				249461990	0.002743926	-26.877166
	Promoter 2	Protein-cysteine N-palmitoyltransferase Rasp isoform X1	17	7843518	1.14371E-08	-36.111871
				7843521	5.67424E-10	-38.895558
				7843535	5.45E-12	-41.253088
				7843538	4.26069E-10	-38.295554
				7843550	1.06507E-11	-40.674603
				7843565	3.66545E-09	-36.783099
				7843568	1.19902E-10	-37.057431
				7843580	3.73868E-08	-33.716649
				7843583	1.56997E-08	-33.731824
				7843586	1.80072E-08	-34.920635
				7843592	3.6161E-08	-34.467763
				7843612	1.81E-07	-32.138643
				7843624	3.86117E-08	-35.763999

				7843630	2.00414E-08	-38.166164
				7843639	1.29339E-08	-39.497324
				7843643	1.07736E-10	-43.729097
				7843648	1.81328E-10	-43.570451
	Promoter 3	Uncharacterized gene	3	68310865	0.000342469	-30.414439
				68310868	0.002030451	-26.570048
				68310880	0.004740045	-25.501801
	Promoter 4	Tissue factor pathway inhibitor 2-like	7	80414213	8.82946E-05	-31.902166
				80414232	3.37768E-06	-34.744063
				80414274	2.43215E-06	-38.457636
				80414299	3.96E-07	-41.830574
				80414305	4.51589E-05	-35.814592
				80414314	2.33926E-05	-37.47528
				80414324	9.79257E-05	-35.399543
	Promoter 5	Uncharacterized LOC115332125, transcript variant X1	1	23928215	0.000268202	-30.649569
Hypermethylation	Promoter 6	Histone H3	2	99962	5.50E-51	44.8236178
	Promoter 7	Histone H1-III-like	1	99964	5.50E-51	43.0059303
	Promoter 8	Histone H3	1	382331	8.81E-10	28.9148716
	Promoter 9	Uncharacterized gene (LOC115318757)	2	382331	8.81E-10	28.9148716
	Promoter 10	Uncharacterized LOC121834116	2	166422007	1.85E-05	27.3872679
				166422014	9.62E-05	26.8047709
	Promoter 11	Small subunit ribosomal RNA	1	396069	1.20E-30	30.4785091
	Promoter 12	Small subunit ribosomal RNA	2	396097	4.15E-26	33.2956273
				576242	3.27E-29	32.6821789
				602534	1.04E-06	28.3660299
				603460	9.46E-29	42.1125903

	Promoter 13	5.8S ribosomal RNA	1	604220	4.49E-12	32.3264441
				692371	1.03E-19	37.8276854
	Promoter 14	5.8S ribosomal RNA	2	693783	3.23E-27	37.809597

meth. sites = Number of methylated sites

Meth. location = Location of the methylated sites

Meth. diff = Methylation differences

Table S12. Enriched MN hypomethylated genes ontology terms associated with biological processes using PANTHER.

Analysis Type:	PANTHER Overrepresentation Test (Released 20240807)						
Annotation Version and Release Date:	PANTHER version 19.0 Released 2024-06-20						
Analyzed List:	Client Text Box Input (<i>Ixodes scapularis</i>)						
Reference List:	<i>Ixodes scapularis</i> (all genes in database)						
Test Type:	FISHER						
Correction:	FDR						
PANTHER GO-Slim Biological Process	<i>Ixodes scapularis</i> - REFLIST (20494)	Input (82)	Expected	over/under	Fold Enrichment	Raw P-value	FDR
Purine nucleotide catabolic process (GO:0006195)	7	2	0.03	+	71.41	3.28E-04	2.33E-02
Nucleotide catabolic process (GO:0009166)	8	2	0.03	+	62.48	4.36E-04	2.10E-02
Rho protein signal transduction (GO:0007266)	8	2	0.03	+	62.48	4.36E-04	2.04E-02
Purine-containing compound catabolic process (GO:0072523)	8	2	0.03	+	62.48	4.36E-04	1.98E-02
Nucleoside phosphate catabolic process (GO:1901292)	10	2	0.04	+	49.99	6.97E-04	2.72E-02
Homophilic cell adhesion via plasma membrane adhesion molecules (GO:0007156)	30	3	0.12	+	24.99	2.32E-04	3.80E-02
Axon guidance (GO:0007411)	30	3	0.12	+	24.99	2.32E-04	3.45E-02

Neuron projection guidance (GO:0097485)	30	3	0.12	+	24.99	2.32E-04	3.16E-02
Cell morphogenesis involved in neuron differentiation (GO:0048667)	31	3	0.12	+	24.19	2.56E-04	2.79E-02
Axonogenesis (GO:0007409)	31	3	0.12	+	24.19	2.56E-04	2.62E-02
Cell-cell adhesion via plasma-membrane adhesion molecules (GO:0098742)	32	3	0.13	+	23.43	2.82E-04	2.56E-02
Cellular anatomical entity morphogenesis (GO:0032989)	33	3	0.13	+	22.72	3.09E-04	2.66E-02
Cell projection morphogenesis (GO:0048858)	33	3	0.13	+	22.72	3.09E-04	2.53E-02
Neuron projection morphogenesis (GO:0048812)	33	3	0.13	+	22.72	3.09E-04	2.41E-02
Plasma membrane bounded cell projection morphogenesis (GO:0120039)	33	3	0.13	+	22.72	3.09E-04	2.30E-02
Cell morphogenesis (GO:0000902)	37	3	0.15	+	20.26	4.35E-04	2.16E-02
Axon development (GO:0061564)	39	3	0.16	+	19.23	5.09E-04	2.25E-02
Neuron projection development (GO:0031175)	43	3	0.17	+	17.44	6.79E-04	2.71E-02
Neuron development (GO:0048666)	44	3	0.18	+	17.04	7.26E-04	2.77E-02
Cell-cell adhesion (GO:0098609)	49	3	0.2	+	15.3	9.96E-04	3.47E-02
Cell development (GO:0048468)	53	3	0.21	+	14.15	1.25E-03	4.27E-02
Import into cell (GO:0098657)	55	3	0.22	+	13.63	1.39E-03	4.48E-02
Cellular nitrogen compound catabolic process (GO:0044270)	75	4	0.3	+	13.33	2.33E-04	2.94E-02
Heterocycle catabolic process (GO:0046700)	75	4	0.3	+	13.33	2.33E-04	2.73E-02
Aromatic compound catabolic process (GO:0019439)	85	4	0.34	+	11.76	3.77E-04	2.28E-02
Organic cyclic compound catabolic process (GO:1901361)	86	4	0.34	+	11.62	3.94E-04	2.22E-02
Intracellular signal transduction (GO:0035556)	123	5	0.49	+	10.16	1.35E-04	3.17E-02
Signal transduction (GO:0007165)	337	7	1.35	+	5.19	4.04E-04	2.13E-02

Phosphorus metabolic process (GO:0006793)	302	6	1.21	+	4.97	1.34E-03	4.37E-02
Signaling (GO:0023052)	363	7	1.45	+	4.82	6.28E-04	2.64E-02
Cell communication (GO:0007154)	378	7	1.51	+	4.63	7.97E-04	2.97E-02
Cellular response to stimulus (GO:0051716)	553	9	2.21	+	4.07	3.62E-04	2.28E-02
Cellular component organization or biogenesis (GO:0071840)	726	11	2.9	+	3.79	1.45E-04	2.97E-02
Response to stimulus (GO:0050896)	599	9	2.4	+	3.76	6.44E-04	2.63E-02
Cellular aromatic compound metabolic process (GO:0006725)	673	10	2.69	+	3.71	3.47E-04	2.37E-02
Heterocycle metabolic process (GO:0046483)	688	10	2.75	+	3.63	4.12E-04	2.11E-02
Nucleobase-containing compound metabolic process (GO:0006139)	633	9	2.53	+	3.55	9.51E-04	3.46E-02
Cellular component organization (GO:0016043)	637	9	2.55	+	3.53	9.94E-04	3.54E-02
Cellular nitrogen compound metabolic process (GO:0034641)	779	11	3.12	+	3.53	2.67E-04	2.57E-02
Organic cyclic compound metabolic process (GO:1901360)	714	10	2.86	+	3.5	5.51E-04	2.37E-02
Regulation of cellular process (GO:0050794)	1174	16	4.7	+	3.41	1.41E-05	4.61E-03
Regulation of biological process (GO:0050789)	1221	16	4.89	+	3.28	2.28E-05	6.23E-03
Biological regulation (GO:0065007)	1305	17	5.22	+	3.26	1.31E-05	5.35E-03
Cellular process (GO:0009987)	3293	37	13.18	+	2.81	5.30E-10	2.89E-07
Biological_process (GO:0008150)	4444	46	17.78	+	2.59	1.15E-11	1.88E-08
Primary metabolic process (GO:0044238)	1949	19	7.8	+	2.44	1.99E-04	3.63E-02
Nitrogen compound metabolic process (GO:0006807)	1867	18	7.47	+	2.41	3.49E-04	2.28E-02
Cellular metabolic process (GO:0044237)	1879	18	7.52	+	2.39	3.77E-04	2.20E-02
Organic substance metabolic process (GO:0071704)	2189	20	8.76	+	2.28	4.04E-04	2.20E-02

Metabolic process (GO:0008152)	2363	20	9.45	+	2.12	1.33E-03	4.46E-02
Unclassified (UNCLASSIFIED)	16050	36	64.22	-	0.56	1.15E-11	9.40E-09

Table S13. Overrepresented GO terms in MN hypomethylated genes associated with cellular components using PANTHER.

Analysis Type:	PANTHER Overrepresentation Test (Released 20240807)						
Annotation Version and Release Date:	PANTHER version 19.0 Released 2024-06-20						
Analyzed List:	Client Text Box Input (<i>Ixodes scapularis</i>)						
Reference List:	<i>Ixodes scapularis</i> (all genes in database)						
Test Type:	FISHER						
Correction:	FDR						
PANTHER GO-Slim Cellular Component	<i>Ixodes scapularis</i> - REFLIST (20494)	Input (82)	Expected	Over/under	Fold Enrichment	Raw P-value	FDR
Synaptic vesicle (GO:0008021)	8	2	0.03	+	62.48	4.36E-04	2.18E-02
Lytic vacuole membrane (GO:0098852)	8	2	0.03	+	62.48	4.36E-04	1.97E-02
Lysosomal membrane (GO:0005765)	8	2	0.03	+	62.48	4.36E-04	1.79E-02
Exocytic vesicle (GO:0070382)	10	2	0.04	+	49.99	6.97E-04	2.42E-02
Presynapse (GO:0098793)	11	2	0.04	+	45.44	8.50E-04	2.74E-02
Secretory vesicle (GO:0099503)	13	2	0.05	+	38.45	1.20E-03	3.60E-02
Axon (GO:0030424)	31	3	0.12	+	24.19	2.56E-04	2.31E-02
Plasma membrane bounded cell projection (GO:0120025)	119	4	0.48	+	8.4	1.33E-03	3.54E-02
Cell projection (GO:0042995)	125	4	0.5	+	8	1.60E-03	4.00E-02
Intracellular non-membrane-bounded organelle (GO:0043232)	560	9	2.24	+	4.02	3.97E-04	2.98E-02
Non-membrane-bounded organelle (GO:0043228)	560	9	2.24	+	4.02	3.97E-04	2.56E-02
Cell periphery (GO:0071944)	944	11	3.78	+	2.91	1.31E-03	3.70E-02
Cellular_component (GO:0005575)	4645	41	18.59	+	2.21	7.11E-08	3.21E-05

Intracellular organelle (GO:0043229)	2399	21	9.6	+	2.19	4.16E-04	2.35E-02
Organelle (GO:0043226)	2440	21	9.76	+	2.15	5.08E-04	1.91E-02
Cellular anatomical entity (GO:0110165)	4546	39	18.19	+	2.14	3.63E-07	5.46E-05
Intracellular anatomical structure (GO:0005622)	3236	27	12.95	+	2.09	1.04E-04	1.18E-02
Unclassified (UNCLASSIFIED)	15849	41	63.41	-	0.65	7.11E-08	1.60E-05

Table S14. Overrepresented GO terms in MN hypomethylated genes associated with molecular function using PANTHER.

Analysis Type:	PANTHER Overrepresentation Test (Released 20240807)						
Annotation Version and Release Date:	PANTHER version 19.0 Released 2024-06-20						
Analyzed List:	Client Text Box Input (<i>Ixodes scapularis</i>)						
Reference List:	<i>Ixodes scapularis</i> (all genes in database)						
Test Type:	FISHER						
Correction:	FDR						
PANTHER GO-Slim Molecular Function	<i>Ixodes scapularis</i> - REFLIST (20494)	Input (82)	Expected	Over/under	Fold Enrichment	Raw P-value	FDR
Kinase activity (GO:0016301)	200	7	0.8	+	8.75	1.55E-05	2.87E-03
Transferase activity, transferring phosphorus-containing groups (GO:0016772)	272	7	1.09	+	6.43	1.09E-04	1.51E-02
Catalytic activity (GO:0003824)	2368	21	9.47	+	2.22	3.60E-04	3.99E-02
Molecular_function (GO:0003674)	4727	39	18.91	+	2.06	1.11E-06	3.08E-04
Unclassified (UNCLASSIFIED)	15767	43	63.09	-	0.68	1.11E-06	6.16E-04

Table S15. Overrepresented GO terms in MN hypomethylated genes associated with protein class using PANTHER.

Analysis Type:	PANTHER Overrepresentation Test (Released 20240807)
Annotation Version and Release Date:	PANTHER version 19.0 Released 2024-06-20

Analyzed List:	Client Text Box Input (<i>Ixodes scapularis</i>)						
Reference List:	<i>Ixodes scapularis</i> (all genes in database)						
Test Type:	FISHER						
Correction:	FDR						
PANTHER Protein Class	<i>Ixodes scapularis</i> - REFLIST (20494)	Input (82)	Expected	Over/under	Fold Enrichment	Raw P-value	FDR
Non-receptor serine/threonine protein kinase (PC00167)	118	4	0.47	+	8.47	1.29E-03	4.86E-02
RNA metabolism protein (PC00031)	434	8	1.74	+	4.61	3.44E-04	2.15E-02
Protein modifying enzyme (PC00260)	863	11	3.45	+	3.19	6.32E-04	2.97E-02
Protein class (PC00000)	6682	57	26.74	+	2.13	8.42E-12	1.58E-09
Unclassified (UNCLASSIFIED)	13812	25	55.26	-	0.45	8.42E-12	7.91E-10

Table S16. *Ixodes scapularis* collection, state and location.

State	Location	Year	State	Location	Year	
Texas	Harris county	unknown	Minnesota	Chippewa National Forest	2016, 2017	
				Itasca State Park	2016	
	Beech woods trail, Big Thicket State Park	2019, 2021, 2022, 2023		Saint Croix State Park	2016, 2017	
	Woodlands trail, Big Thicket State Park	2021		William O Brien State Park	2016	
	Kirby Nature trail, Big Thicket State Park			Camp Ripley	2016, 2017, 2021	
	Sam Houston State Park				Lake Elmo Park Reserve	2021
					Deerwood	2022
					Carlos Avery Wildlife Management Area	2023

Table S17. Accession numbers from WGBS, WGS, and nanopore sequences deposited in the GenBank.

	Sample code	State collected	Year of collection	Accession
WGBS	SCSP_M1	Minnesota	2016	SRR28120173
	CHIP_F2	Minnesota	2016	SRR28120172
	WIOBA_M3	Minnesota	2016	SRR28120170
	ITAS_F4	Minnesota	2016	SRR28120169
	ITAS_F4-7	Minnesota	2016	SRR28120168
	TX1	Texas	2019	SRR28120167
	TX2	Texas	2019	SRR28120166
	TX3	Texas	2019	SRR28120165
	TX5	Texas	2019	SRR28120164
	TX7	Texas	2019	SRR28120163
	TX8	Texas	2019	SRR28120171
WGS	MNFem3	Minnesota	2021	SRR29689857
	MNMale4	Minnesota	2021	SRR29689856
	TXFem1	Texas	2021	SRR29689855
	TXMale2	Texas	2021	SRR29689854
Nanopore gDNA	IS_TX_gDNA_reads.fastq.gz	Texas	2023	SAMN48877844
	IS_MN_gDNA_reads.fastq.gz	Minnesota	2023	SAMN48877845
Nanopore 5hmC	IS_TX_5hmC_regions.bed.gz	Texas	2023	SAMN48877844

	IS_MN_5hmC_regions.bed.gz	Minnesota	2023	SAMN48877845
Nanopore 5mC	IS_TX_5mC_regions.bed.gz	Texas	2023	SAMN48877844
	IS_MN_5mC_regions.bed.gz	Minnesota	2023	SAMN48877845

Table S18. BLAST results from *I. scapularis* DNA methyltransferases and de-methylases' sequencing.

Gene	Name	Accession #	Best hit	Query cover (%)	E-value	Identity (%)	Gene size (bp)
DNMT1	1XM13	ON184122	XM_029975414.4	100	5e-71	98.67	5980
	1XM4	ON184120	XM_029975414.4	100	2e-65	96.64	5980
	1XF16	ON184121	XM_029975414	100	5e-71	98.67	5980
	1XF13	ON184123	XM_029975414	100	2e-69	98.64	5980
	1MM10	ON184119	XM_029975414	100	2e-70	98.66	5980
	1MM9	ON184118	XM_029975414	100	6e-70	98.65	5980
	1MF32	ON184117	XM_029975414	100	1e-67	97.96	5980
	1MF25	ON184116	XM_029975414	100	2e-64	96.00	5980
DNMT2	2XM16	ON355322	XM_029992582.4	100	2e-74	100.00	1620
	2XM15	ON355323	XM_029992582.4	100	2e-74	100.00	1620
	2XF5	ON355324	XM_029992582.4	100	2e-74	100.00	1620
	2XF1	ON355325	XM_029992582.4	100	2e-70	99.32	1620
	2MF5	ON355326	XM_029992582.4	100	4e-71	99.32	1620
	2MF1	ON355327	XM_029992582.4	100	9e-73	100.00	1620
	2M9	ON355328	XM_029992582	100	2e-74	100.00	1620
	2M1	ON355329	XM_029992582	100	3e-73	100.00	1620
DNMT3	3M50	ON693356	XM_040208133.3	100	7e-53	99.12	2226
	3MF10	ON693357	XM_040208133.3	100	9e-52	99.11	2226
	3MF11	ON693358	XM-040208133.3	100	2e-49	98.20	2226
	3MM55	ON693359	XM_040208133.3	100	6e-48	99.05	2226
	3XF8	ON693360	XM_040208133.3	100	4e-50	98.21	2226

	3XF15	ON693361	XM_0402081 33.3	98	9e-52	100.00	2226
	3XM12	ON693362	XM_0402081 33.3	100	1e-50	98.23	2226
	3XM14	ON693363	XM_0402081 33.3	100	4e-50	98.21	2226
TET3	DXF53	OR031105	XM_0405043 78.2	100	1e-86	100.00	7173
	XF31	OR031106	XM_0405043 78.2	100	3e-93	99.47	7173
	DXM50	OR031107	XM_0405043 78.2	100	1e-97	100.00	7173
	XM53	OR031108	XM_0405043 78.2	100	2e-95	100.00	7173
	MF36	OR031109	XM_0405043 78.2	100	1e-76	100.00	7173
	MF21	OR031110	XM_0405043 78.2	100	7e-95	100.00	7173
	MM51	OR031111	XM_0405043 78.2	100	2e-89	100.00	7173
	MM9	OR031112	XM- 040504378.2	100	2e-95	100.00	7173
	DXF53	OR031105	XM_0405043 78.2	100	1e-86	100.00	7173
TDG	TXF55	PP747258	XM_2996874 8.4	100	7e-85	100.00	7318
	TXF95	PP747259	XM_2996874 8.4	100	7e-85	100.00	7318
	TXM18	PP747260	XM_2996874 8.4	100	7e-85	100.00	7318
	TXM68	PP747261	XM_2996874 8.4	100	7e-85	100.00	7318
	MNF95	PP747262	XM_2996874 8.4	100	1e-65	100.00	7318
	MNF93	PP747263	XM_2996874 8.4	100	7e-85	100.00	7318
	MNM1	PP747264	XM_2996874 8.4	100	8e-84	100.00	7318
	MNM63	PP747265	XM_2996874 8.4	100	2e-84	100.00	7318

Table S19. Synthesized oligos used for the amplification of enzymes involved in DNA methylation.

Gene	Primer name	Sequence (5' to 3')	T _m (°C)	Product size (bp)	Annealin g temperat ure	Binding position	Length
DNMT1	DNMT1 F5	GAACCTCCTGTAC TACTCGG	53.9	150	54	2406- 2426	20
	DNMT1 R5	TACGCCTCGCTGA AGTAGAA	55.6			2536- 2556	20
DNMT2	DNMT2 F4	TCTACTGCCTCGC TAAACTC	53.9	150	54	527-547	20
	DNMT2 R4	TGTCTTCGTCGGT TTGTTCT	54.2			656-676	20

DNMT3	DNMT3 F13	TTGCAGTGGAGG CGTACTTC	57.5	114	55	236-256	20
	DNMT3 R13	CATCCGTCACACT TTCCACAG	55.9			329-350	21
	Probe	AGGTGGATCAAG GTGCCATTAACGT				263-288	25
TET3	TET3F6	ATGATTCAGCCGT CCTATCTTCC	56.4	195	55	580-603	23
	TET3R6	GGTCATCCTGTAG CAGTTCATTG	55.7			751 -774	23
TDG	899F	ACTTCAAATTGAT CCAGTACGG	52.8	169	52	899-921	22
	1069R	TAGATGCCTTTCC CATTAAACAC	53.1			1067- 1090	23
	Probe	TCTTTCCTTGAGA GGTCAGCACTGC				983-1008	25

Table S20. QRT-PCR reaction conditions for each enzyme.

Gene	Master mix (μ l)	TaqMan probe or primer (μ l)	Template (μ l)	Total volume (μ l)
DNMT3	10	1 (500nM)	2	20
TDG	10	1 (500nM)	4	22
DNMT1, DNMT2 & TET3	10	0.4 (10 μ M)	2	25

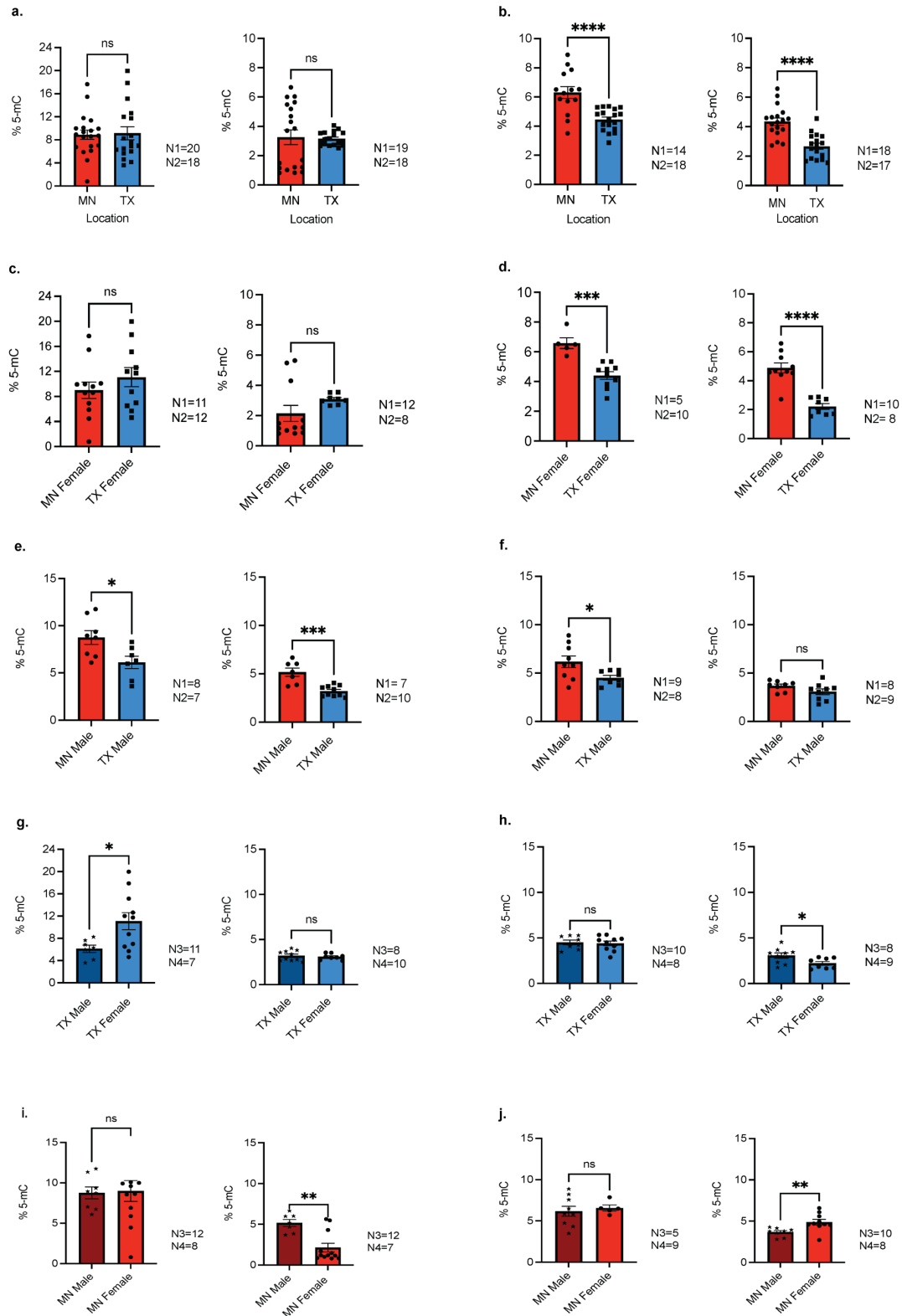


Figure S1. Variation in the percentage of 5-mC among *I. scapularis* populations collected from Minnesota versus Texas. Differences in 5-mC levels between MN and TX were measured across time using ELISA. Data females and males were combined, and differences in methylation levels were

compared by state. Panel A shows results from ticks collected in a) 2021, while panel b presents data from those collected in b) 2022. To discern the differences due to sex between locations females collected in c) 2021 and d) 2022 and males collected in e) 2021 and f) 2022 were compared. To define the differences between sexes within each location, males and females from Texas in g) 2021 and h) 2022 as well as from Minnesota in i) 2021 and j) 2022 were compared. Statistical differences were evaluated with an unpaired two-sided t-test. Asterisks indicated statistically significant differences between samples; **** ≤ 0.0001 , *** $= 0.0003$, ** ≤ 0.0092 , and * ≤ 0.0348 . The “Y” axis represents the percentage of DNA 5-Methylcytosine (%5-mC). Abbreviations TX: Texas and MN: Minnesota, N1: total of MN ticks, N2: total of TX ticks, N3: total of tick males, and N4: total of tick females compared in each graph. The figure includes all the biological replicates for each group including replicates showed in Fig 1.

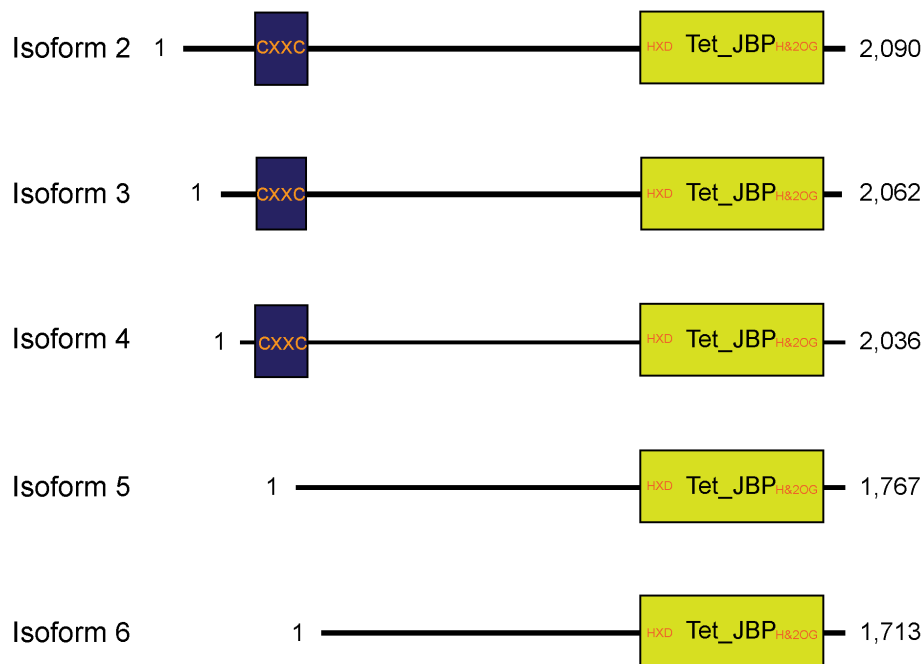


Figure S2. Conserved domains among all TET3 isoforms detected in the *I. scapularis* genome.

Sequence homologs of TET3 were retrieved for GenBank to identify typical domains of enzymes involved in demethylation. The different isoforms identified are listed in Table 1. Domains were determined using SMART v.9. Isoform 2= XP_040075936.1; Isoform 3= XP_040075937.1; Isoform 4= XP_040075938.1; Isoform 5= XP_029838415.4; Isoform 5= XP_040360312.2. CXXC= DNA binding domain, Tet_JBP= Oxygenase domain; HxD= HxD Fe²⁺ binding residues; H&2OG= H Fe²⁺ and 2-Oxoglutarate binding residues.

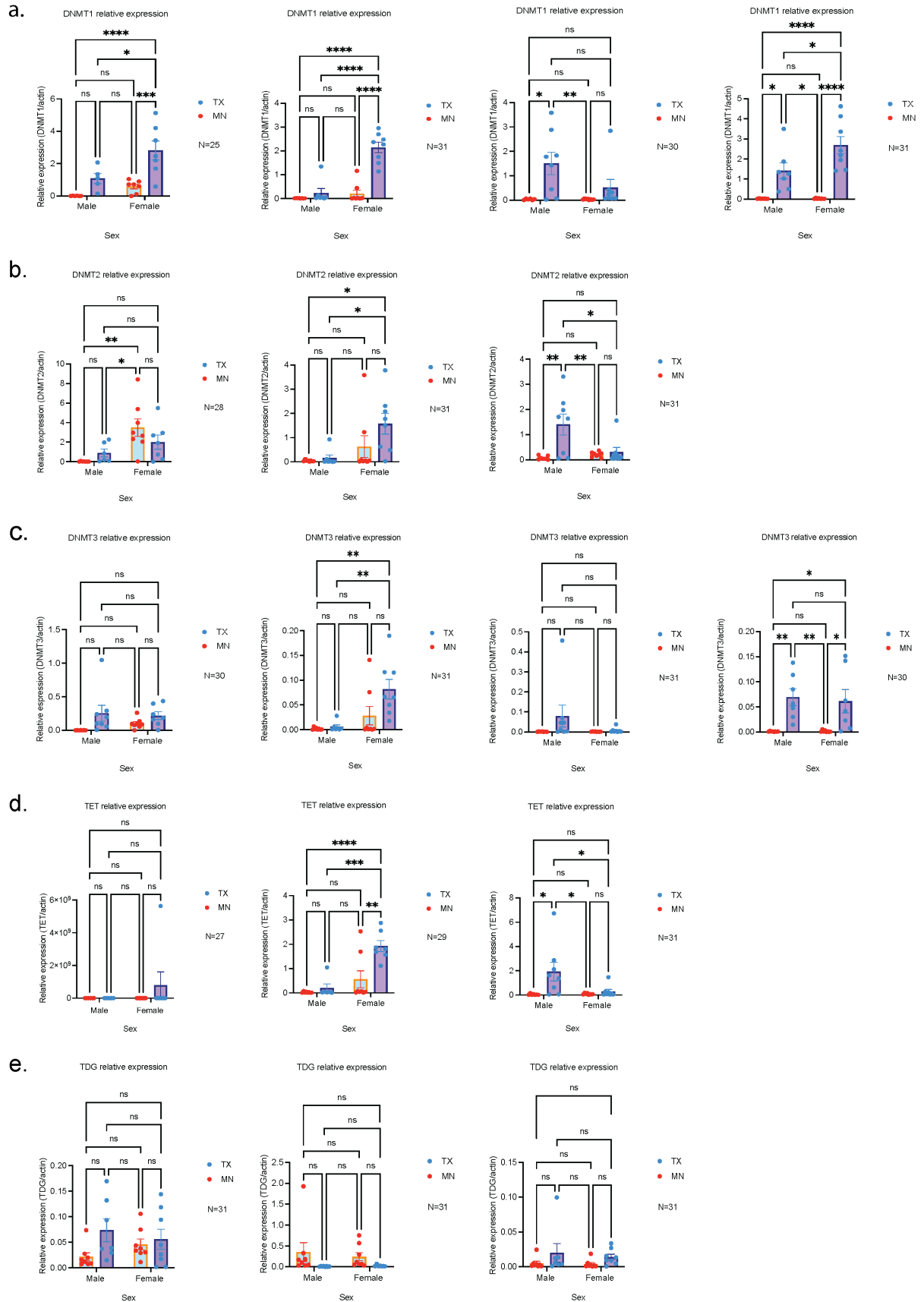


Figure S3. Relative expression of DNMTs and demethylases in *I. scapularis* population collected from MN and TX. a) Relative expression of DNMT1, b) DNMT2, c) DNMT3, d) TET and e) TDG showed no discernable pattern of expression except for DNMT1. Differences in expression were evaluated by Two-way ANOVA followed by Tukey's significant difference for multiple comparisons. ****<0.0001, ***=0.0008, **<0.0082, *<0.05. Abbreviation TX: Texas and MN: Minnesota. The figure includes all the biological replicates for each group. N= total number of ticks compared.

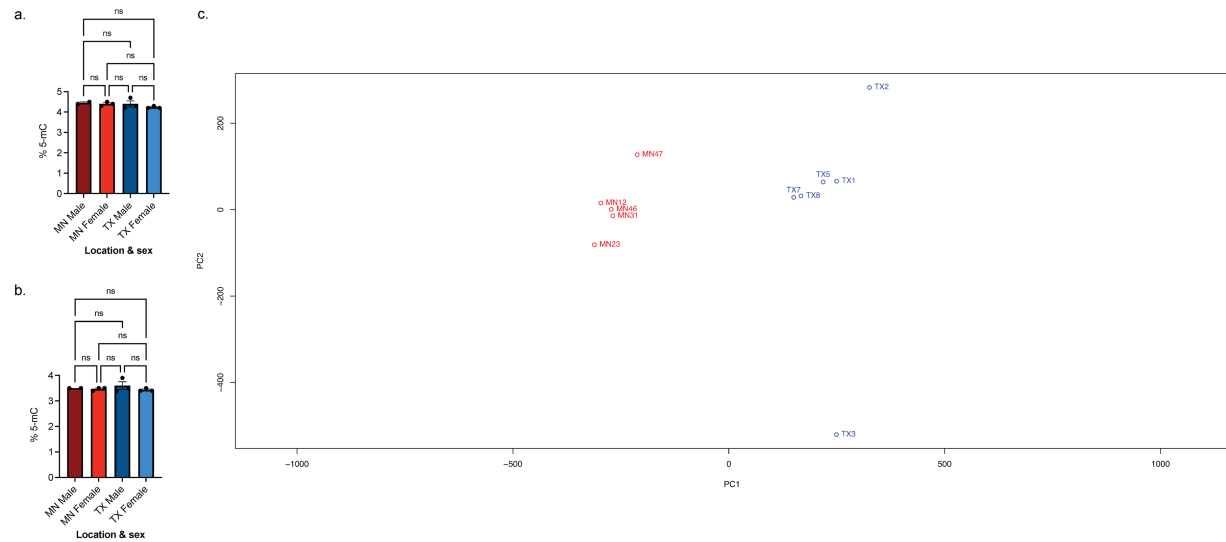


Figure S4. Difference DNA methylation level among MN and TX ticks. Multiple comparisons of the DNA methylation level between MN and TX ticks in two contexts: a) CHH and b) CHG, exhibited no detectable changes in methylation between states, utilizing an ordinary one-way ANOVA. However, a differential analysis of the methylation profiles of MN and TX ticks in CpG sites, using principal component analysis (PCA), showed a distinct separation in their DNA methylation profiles c). The error bars represent the mean $\pm$ SEM of the two variables. The “Y” axis always represents the percentage of DNA 5-Methylcytosine (%5-mC). Abbreviations: TX: Texas, and MN: Minnesota.

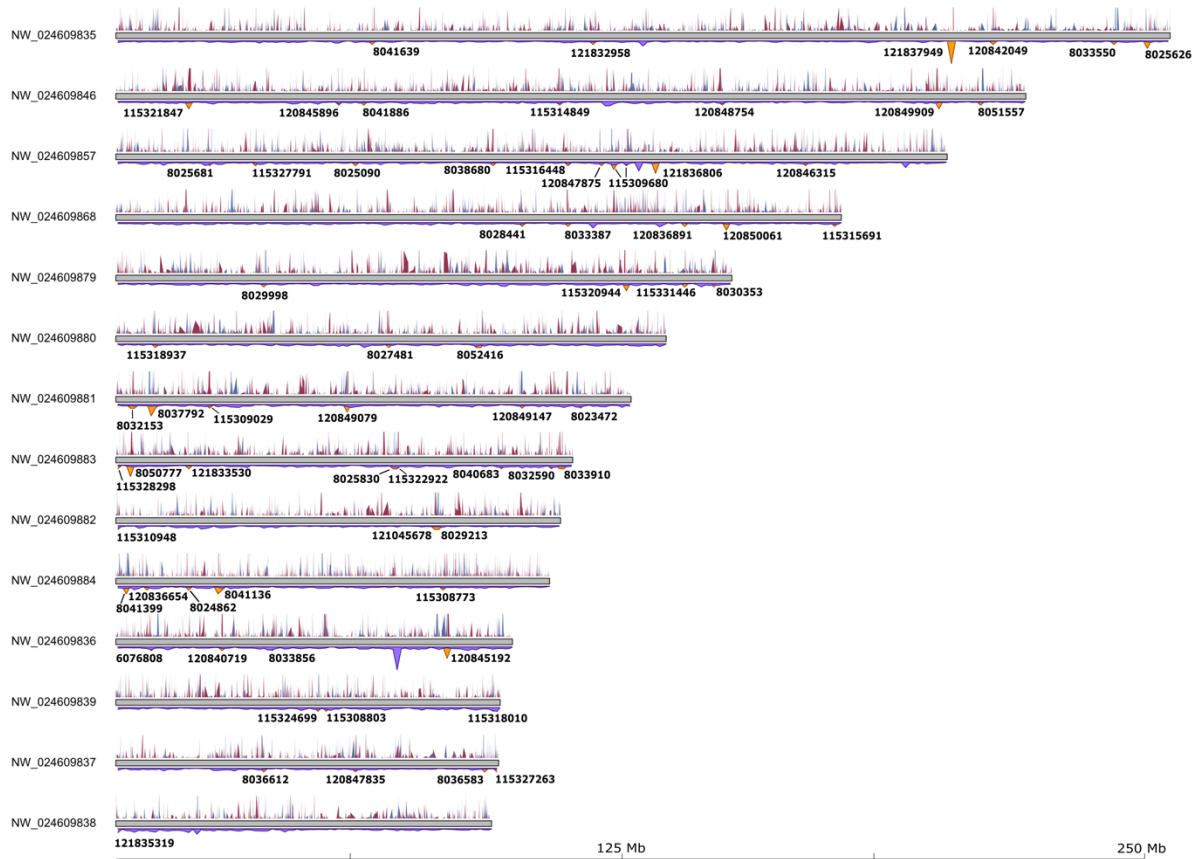


Figure S5. Differentially methylated regions displayed in 12 largest scaffolds between TX and MN *I. scapularis* females detected by nanopore sequencing. The numbers under the DMRs represent the closest gene ID from the PalLabHifi assembly. The blue and red bars above the chromosome scaffold are differentially methylated regions. Red indicates regions that are methylated in the Texas tick and unmethylated in the Minnesota tick, and vice versa for blue bars. The yellow peaks underneath the scaffold are DMRs. The purple histogram underneath the scaffolds is gene density.

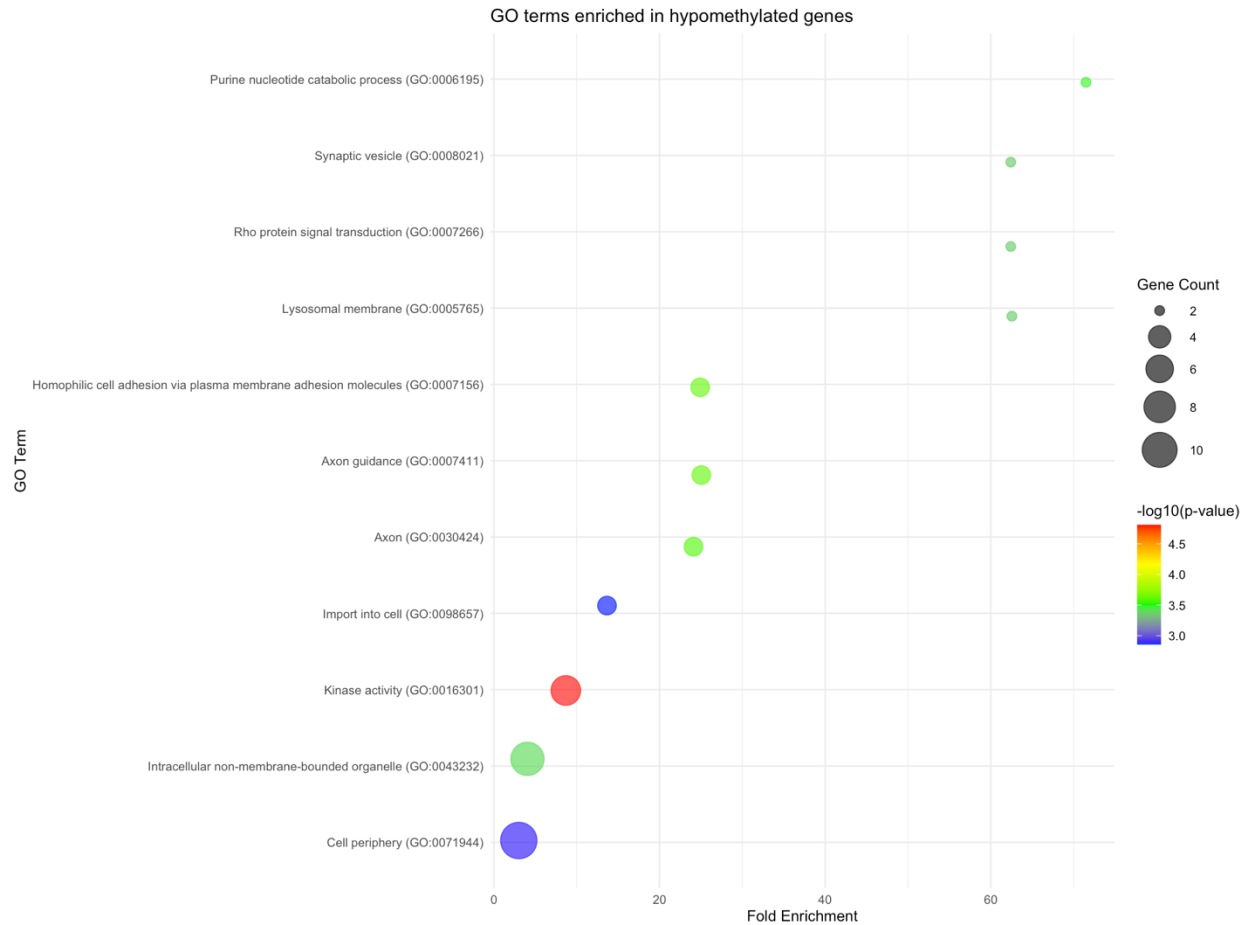


Figure S6. Overrepresented GO terms in MN hypomethylated genes. GO terms are overrepresented in MN ticks using PANTHER overrepresentation test. Only the primary GO terms are presented in the figure. The term “cell periphery” in the GO ontology represents 11 genes, but the actual size of the dot is not displayed in the plot to avoid visual clutter.

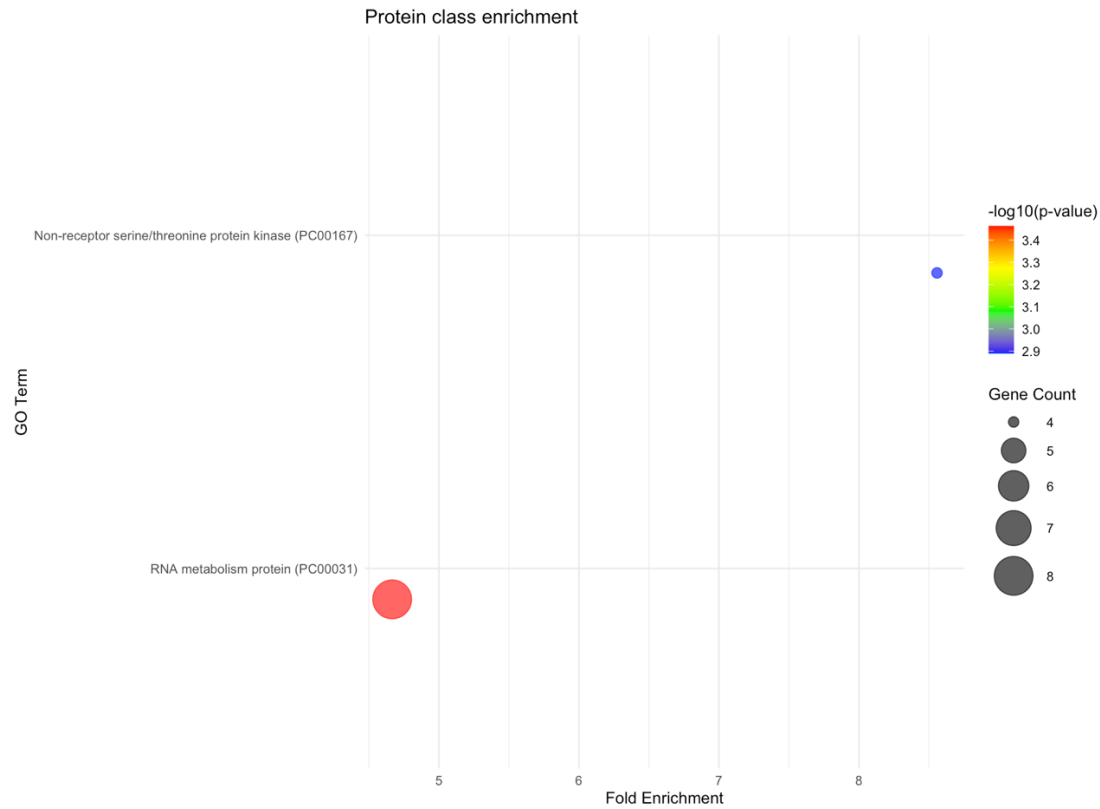


Figure S7. Overrepresented protein class in MN hypomethylated genes. Protein classes that are overrepresented in MN ticks in compared with TX ticks using PANTHER overrepresentation test. Only the primary protein classes are presented in the figure.

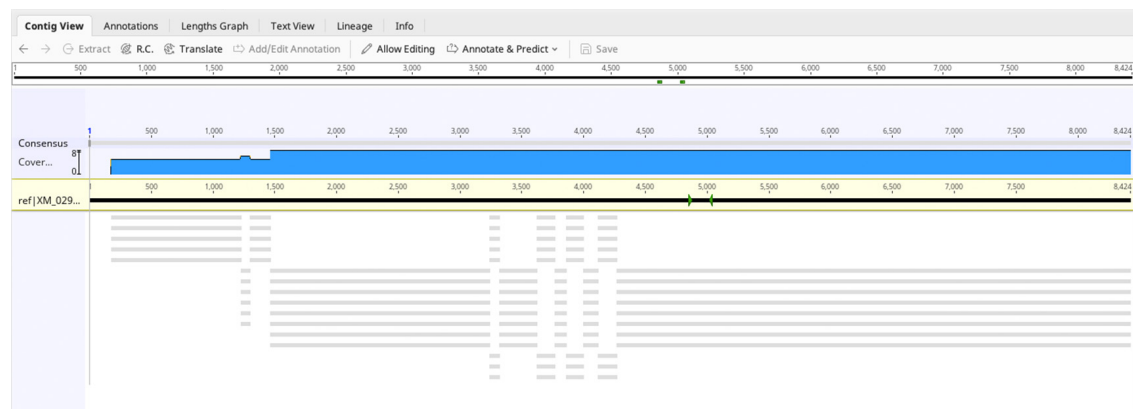


Figure S8. Position of TET3 oligos (TETF6 and TETR6) within the conserved region among TET isoforms. Coding sequences of TET3 isoforms were aligned using Geneious Prime v.2021.2. A conserved region among TET3 homolog isoforms was identified and used to design primers that amplified all potential transcripts expressed in *I. scapularis*. Numbers represent the length in base pair, gray boxes the sequences and green arrow the location of forward (4,849-4,872 position) and reverse (5,020-5,043 position) TET3 primers.