Data Supplement

Diabetes-induced lncRNA *Dnm3os* regulates macrophage functions and inflammatory genes via nuclear mechanisms

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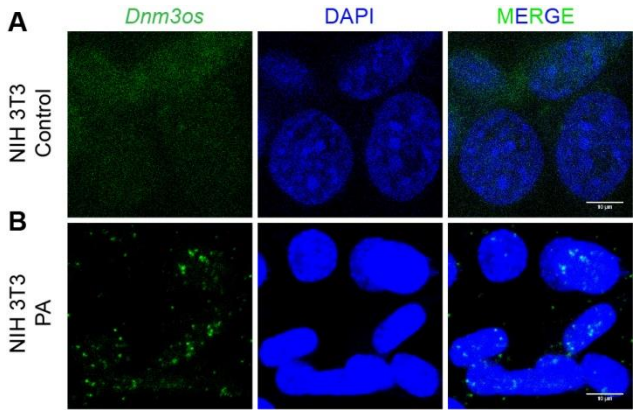
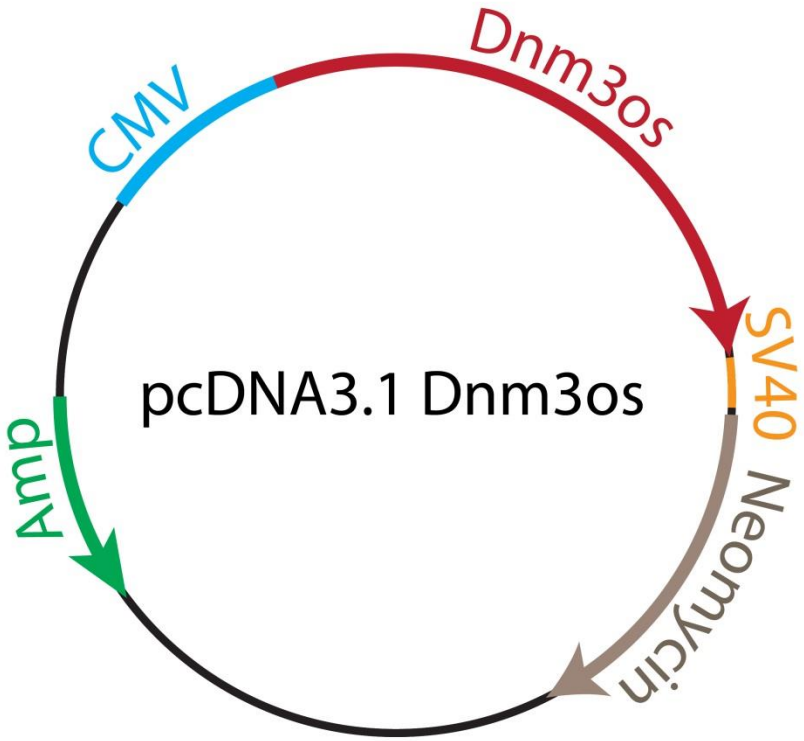
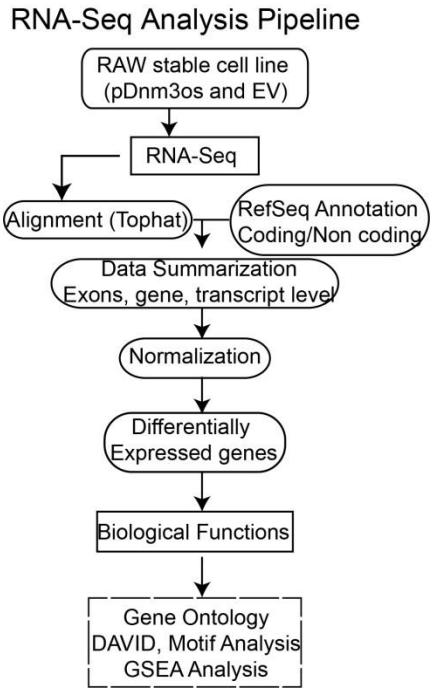


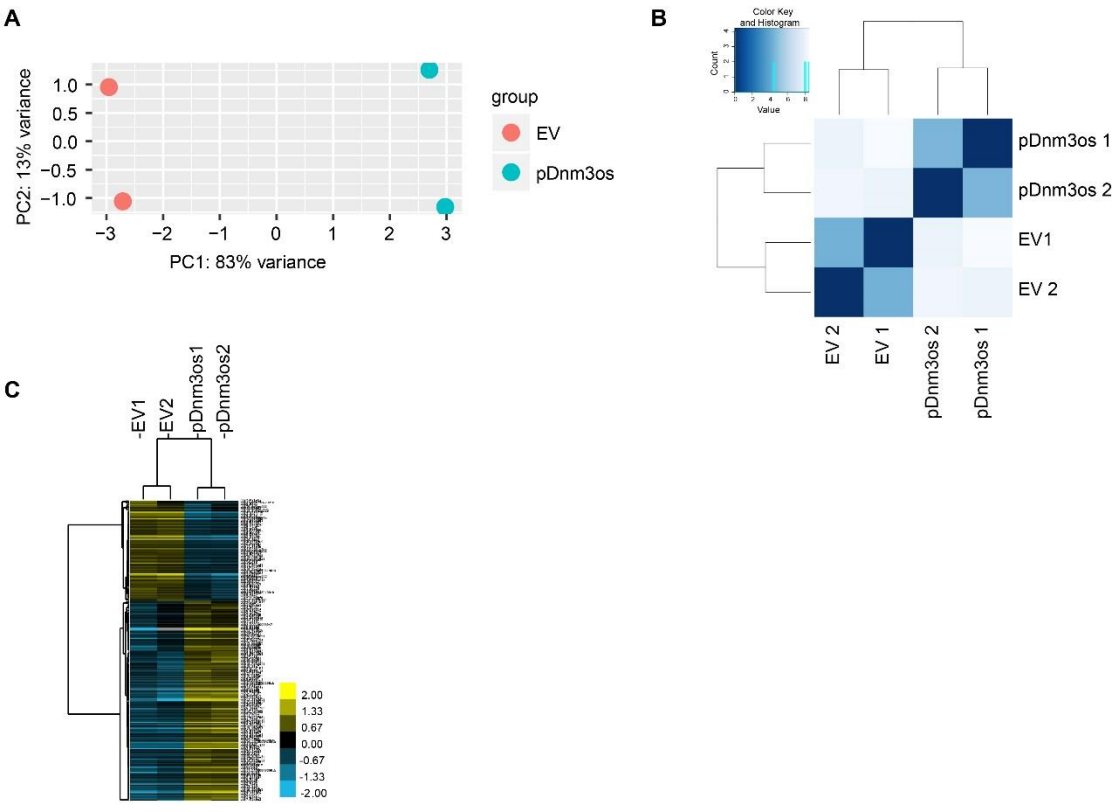
Supplementary Figure I. A-B. RNA fluorescence in situ hybridization (RNA-FISH) analysis of *Dnm3os* in Control (A) and PA (B) treated NIH 3T3 cells. Green dots represent single molecules of *Dnm3os* RNA in the nucleus. Blue color indicates nuclear staining by DAPI. Scale bar: 10 μm.



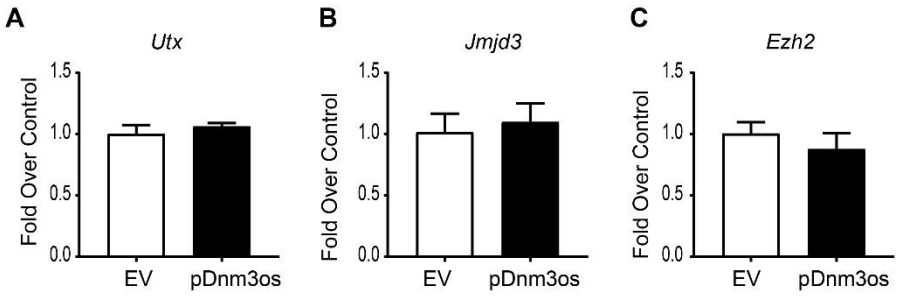
Supplementary Figure II. Map of *Dnm3os* expression vectors. *Dnm3os* was cloned into pcDNA3.1 (+) downstream of the CMV promoter as described in Methods section. To generate *Dnm3os* anti-sense RNA, the same PCR fragment was cloned into pcDNA3.1 (-).



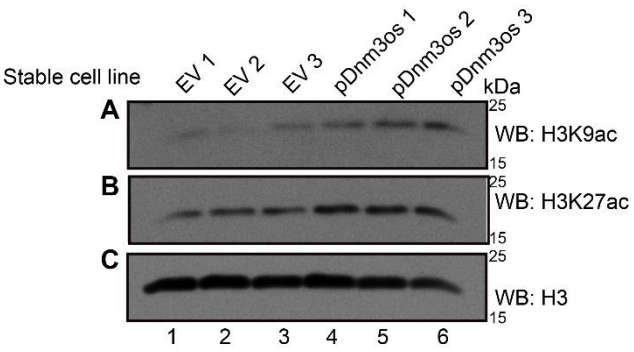
Supplementary Figure III. RNA-seq analysis pipeline used to analyze gene expression in RAW macrophages stably transfected with empty vector pcDNA3.1 (EV) and a vector expressing *Dnm3os (*pDnm3os).



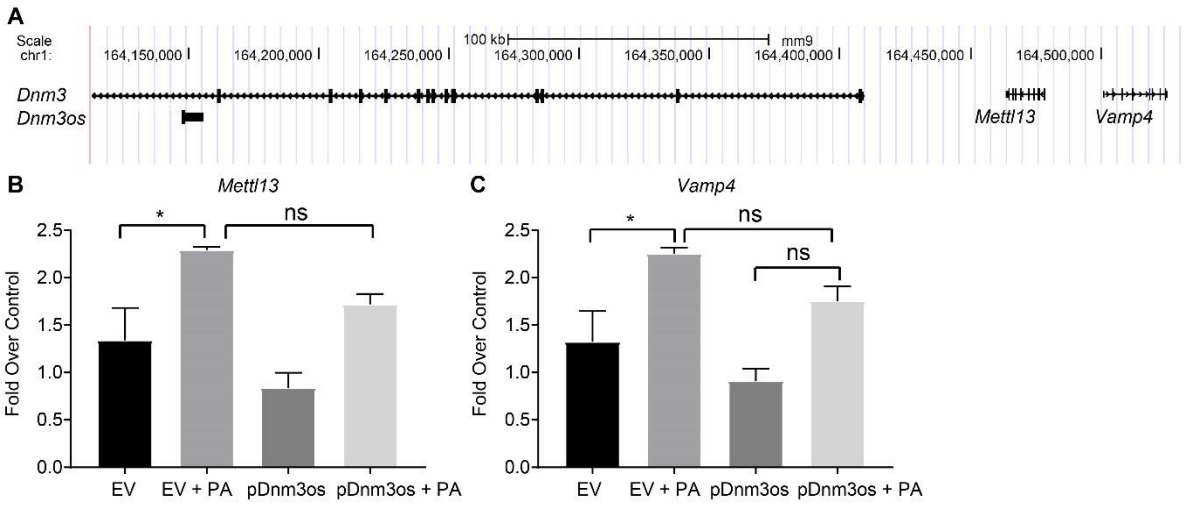
Supplementary Figure IV. A. Principle component analysis (PCA) plot and B. Correlation heatmap showing good replicate consistency. C. RNA-seq results depicted by heat map of differentially expressed genes from RAW macrophages stably expressing *Dnm3os* (pDnm3os) or control empty vector pcDNA3.1 (EV) (n=2).



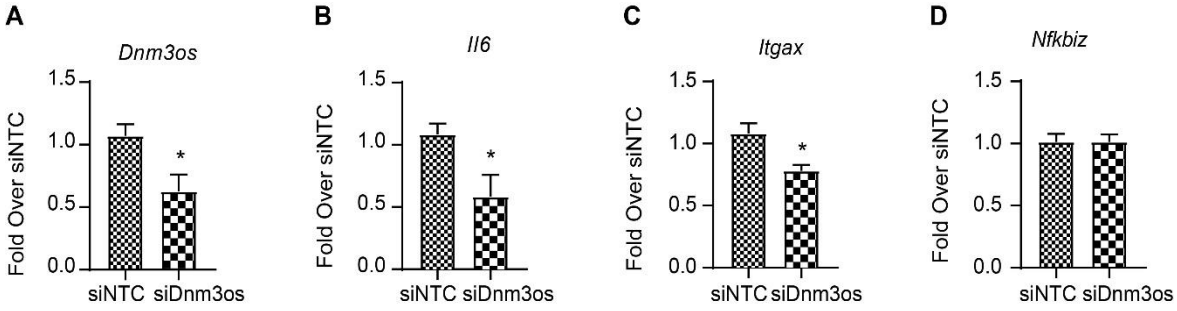
Supplementary Figure V. RT-qPCR analysis of additional differentially expressed genes in RAW-Dnm3os (pDnm3os) and control RAW-EV (EV) macrophages identified in RNA-seq analysis.



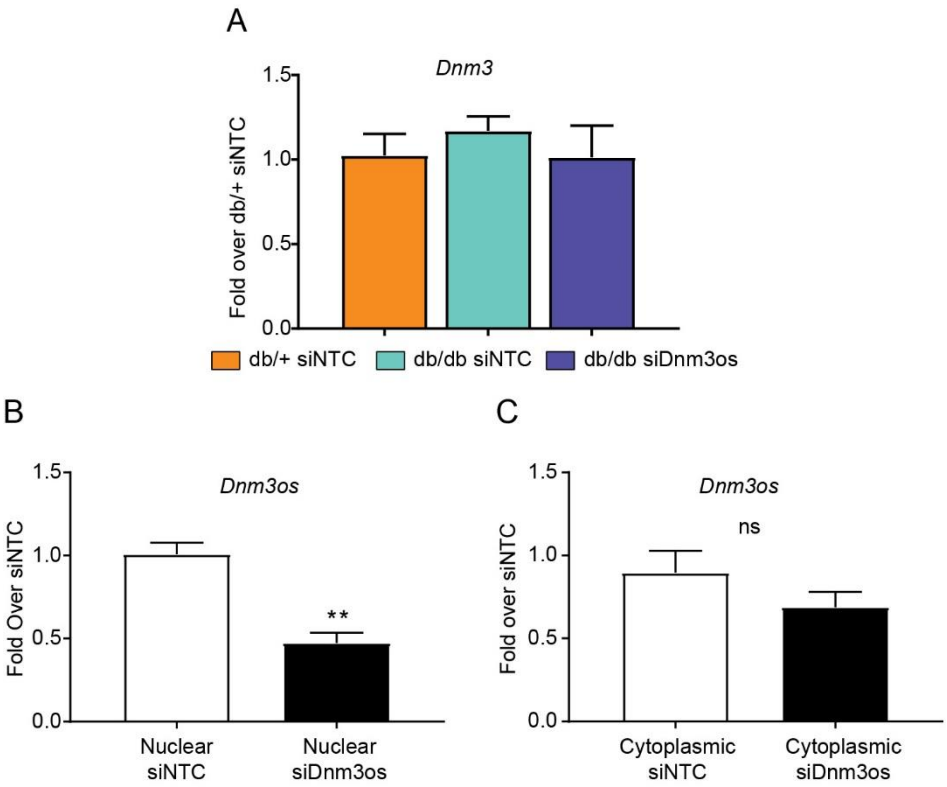
Supplementary Figure VI. Western blots showing levels of global histone modifications in RAW-EV (EV) and RAW-Dnm3os (pDnm3os) macrophages. Total cell lysates were immunoblotted with antibodies against histone modifications H3K9ac (A) and H3K27ac (B). Histone H3 (C) was used as a loading control.



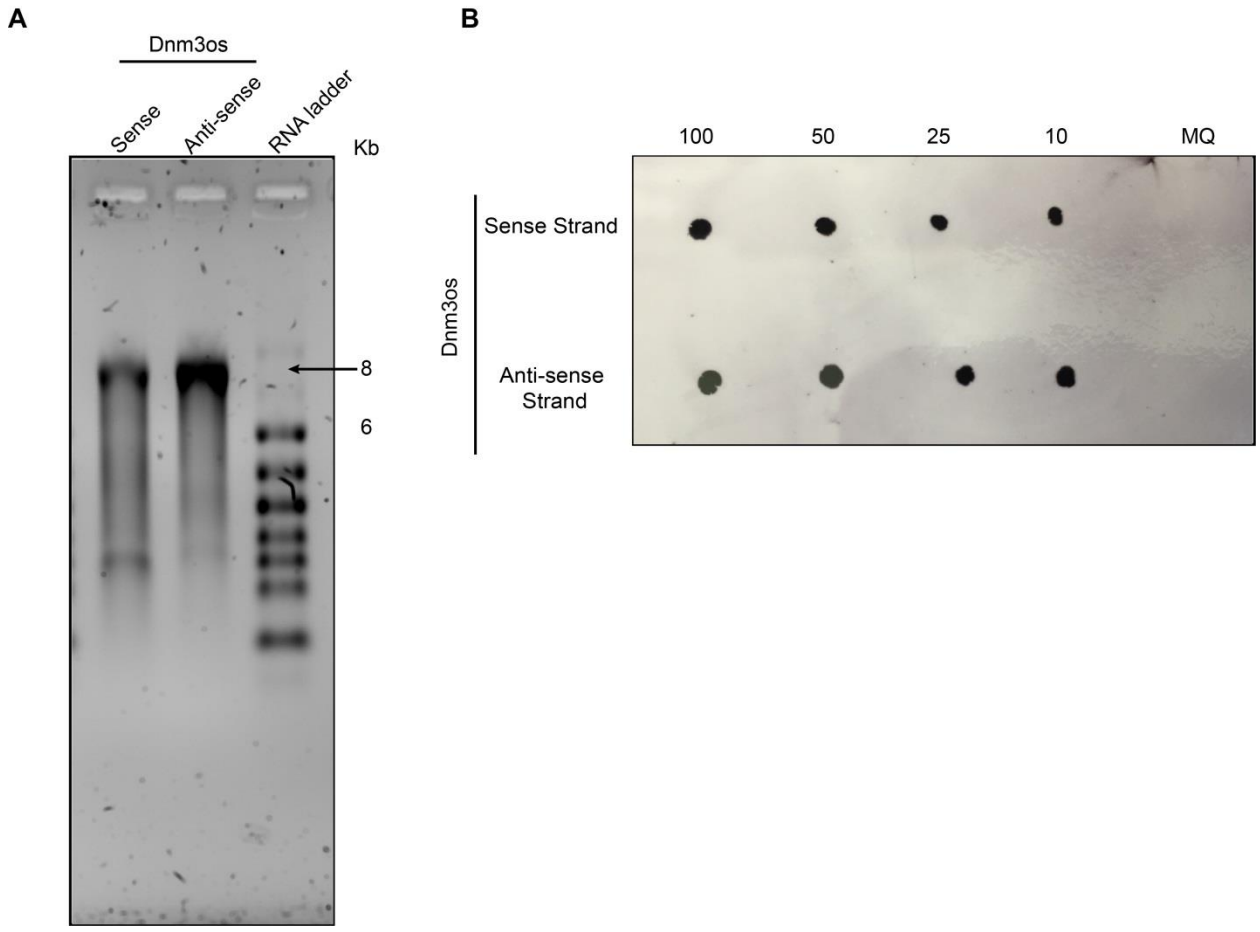
Supplementary Figure VII. *Dnm3os* overexpression does not affect its nearby genes *Mettl13* and *Vamp4* in PA treated cells. A. Genomic locations of *Dnm3os* nearby genes. B-C. Bar graphs showing RT-qPCR analysis of indicated genes in RAW-Dnm3os (pDnm3os) versus RAW-EV (EV) macrophages. Mean +SEM; n=3, **P*<0.05; one way ANOVA, Tukey's multiple comparison test.



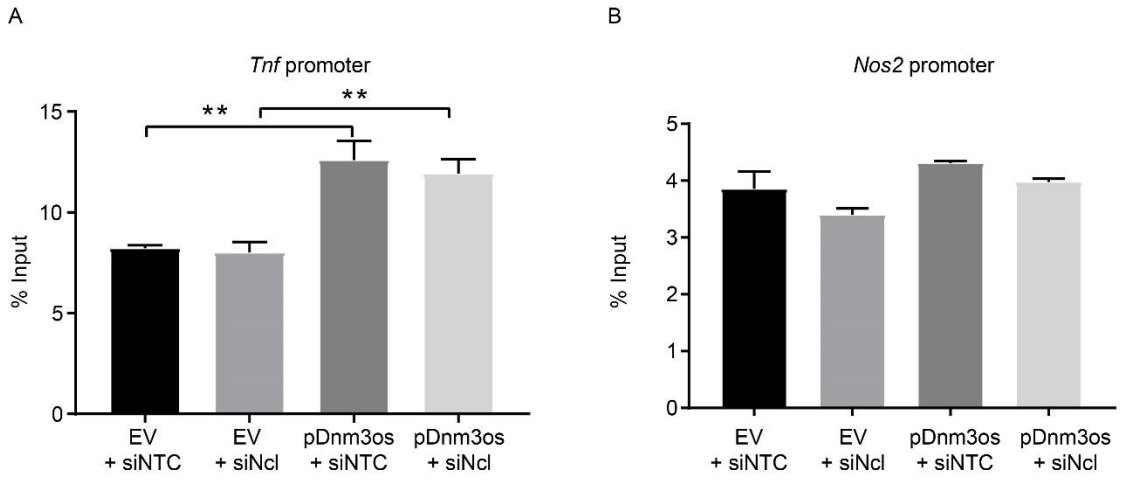
Supplementary Figure VIII. Gene expression in RAW macrophages transfected with siDnm3os versus non-targeting control (siNTC) oligonucleotides. Mean+SEM; n=3, * $P<0.05$, unpaired two tailed t-tests.



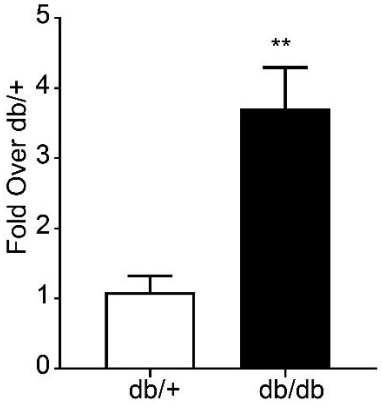
Supplementary Figure IX. A. *Dnm3* gene expression (RT-qPCR) in BMDMs transfected with siDnm3os versus non-targeting control (siNTC) in db/db versus db/+ mice. Mean+SEM, n=4; one way ANOVA, Tukey's multiple comparison test. **B-C.** RT-qPCR analysis of *Dnm3os* from nuclear and cytoplasmic fractions of BMDMs from db/db mice after *Dnm3os* knockdown with siRNAs. Gene expression normalized to *Ppia* is expressed as Fold over siNTC. Mean + SEM; n=3 independent experiments. ***P*<0.01, unpaired two tailed t-tests.



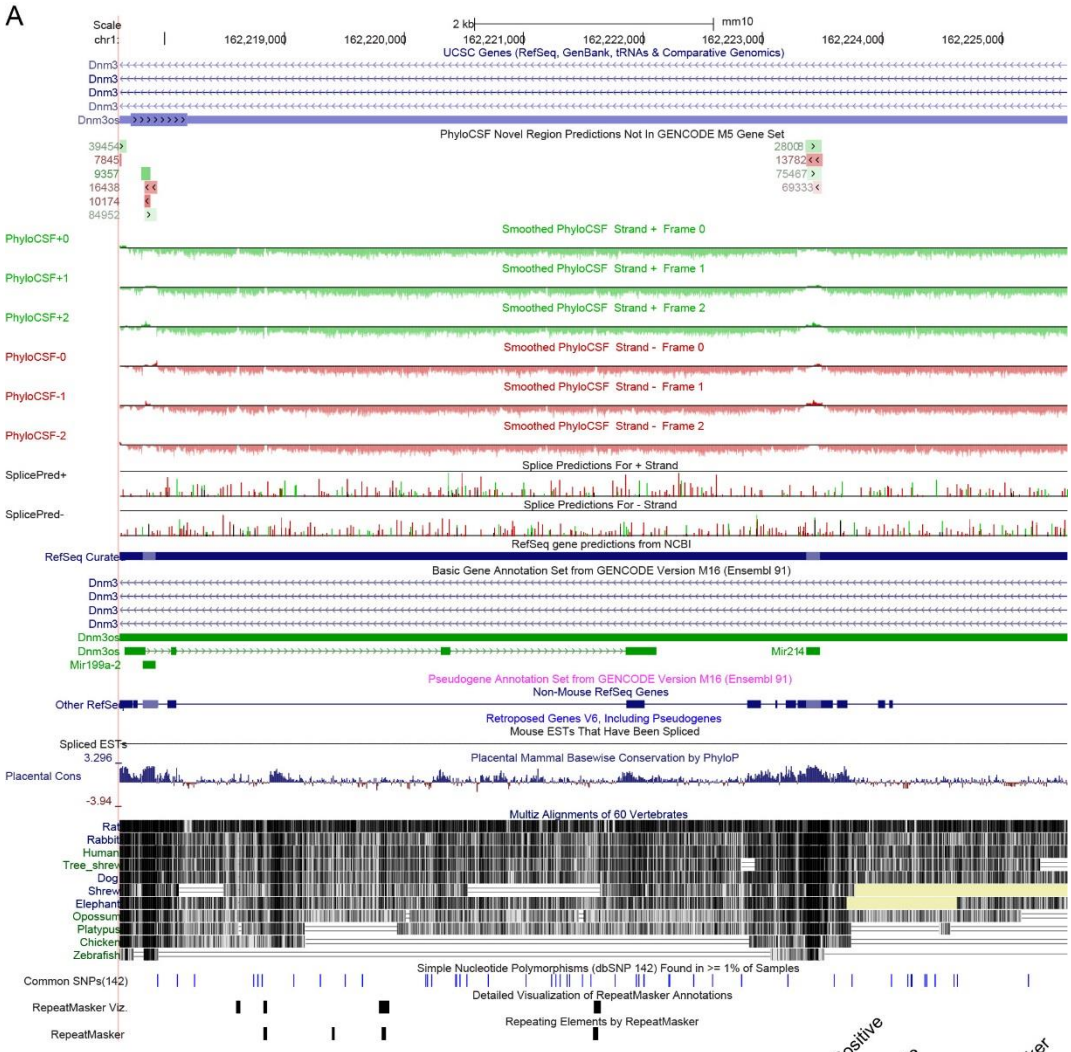
Supplementary Figure X: Synthesis of biotinylated *Dnm3os* RNA probes using *in vitro* transcription. A. Image of denaturing agarose gel showing integrity of biotinylated *Dnm3os* sense and anti-sense RNA probes synthesized by *in vitro* transcription. Arrow indicates bands of sense and anti-sense probes (8 kb). B. Dot blot quantification of indicated *Dnm3os* probes. Serial dilutions of indicated probes were spotted onto nitrocellulose membrane and biotinylation levels were detected with horse radish peroxidase conjugated Avidin.



Supplementary Figure XI: A-B. Bar graphs represent H3K9ac enrichment on the *Tnf* (A) and *Nos2* (B) promoter (at NF-κB binding site) in RAW-EV and RAW-Dnm3os (pDnm3os) macrophages transfected with siNTC or siNcl. Data are shown as percent input. Mean+SEM; n=4, (**P<0.01) one way ANOVA, Tukey's multiple comparison tests.



Supplementary Figure. XII. *Dnm3os* expression in BMDMs from female db/db mice versus control db/+ littermates. Mean+SEM; n=5-6, ***P*<0.01, unpaired two tailed t-tests.


A
Scale
chr1:
2 kb
mm10
162,219,000
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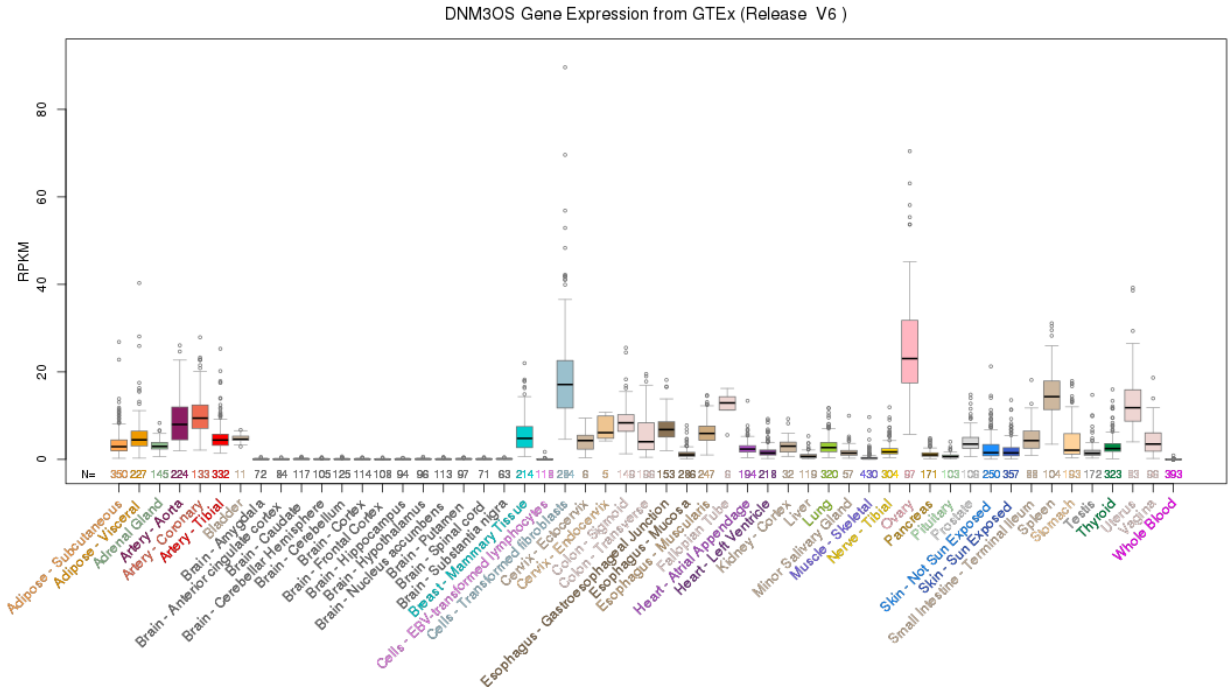
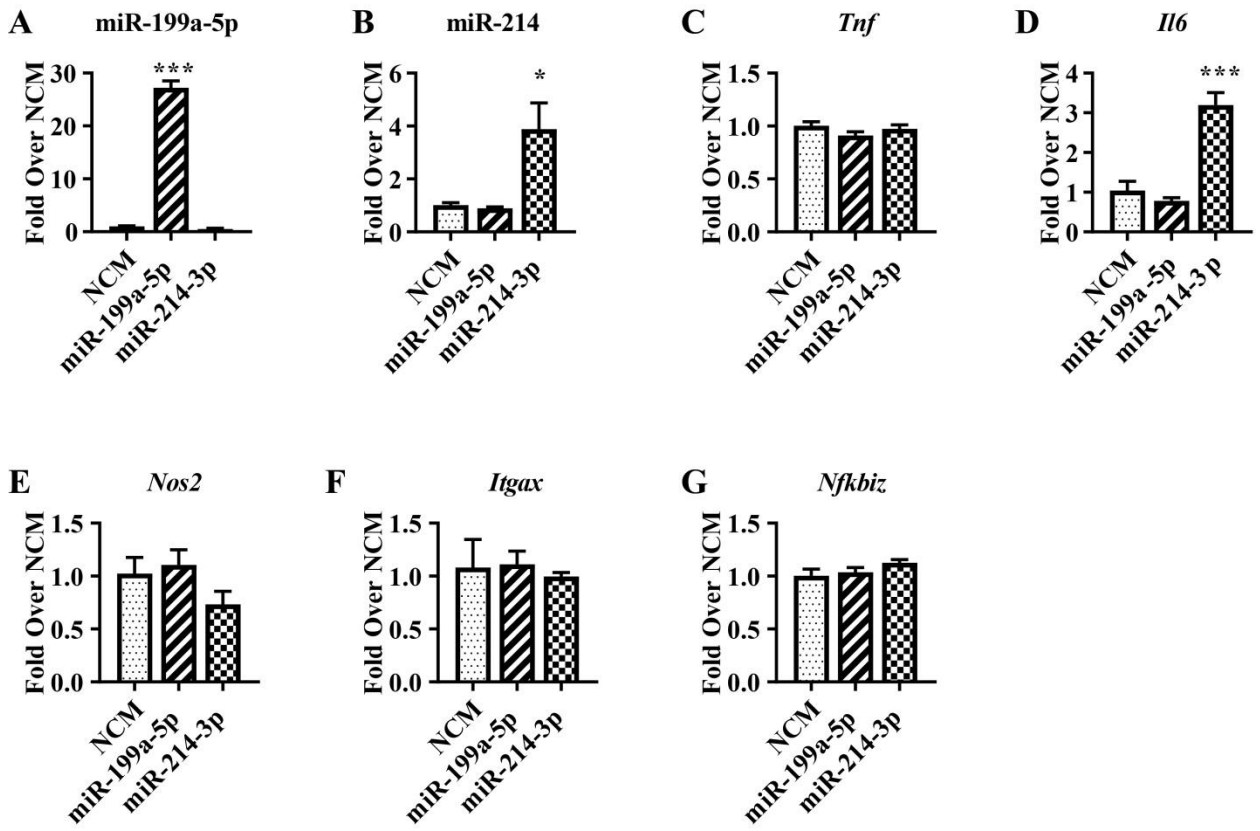
162,221,000
162,222,000
162,223,000
162,224,000
162,225,000
UCSC Genes (RefSeq, GenBank, tRNAs & Comparative Genomics)
Dnm3
Dnm3os
PhyloCSF Novel Region Predictions Not In GENCODE M5 Gene Set
Smoothed PhyloCSF Strand + Frame 0
Smoothed PhyloCSF Strand + Frame 1
Smoothed PhyloCSF Strand + Frame 2
Smoothed PhyloCSF Strand - Frame 0
Smoothed PhyloCSF Strand - Frame 1
Smoothed PhyloCSF Strand - Frame 2
PhyloCSF+0
PhyloCSF+1
PhyloCSF+2
PhyloCSF-0
PhyloCSF-1
PhyloCSF-2
Splice Predictions For + Strand
Splice Predictions For - Strand
SplicePred+
SplicePred-
RefSeq gene predictions from NCBI
RefSeq Curated
Basic Gene Annotation Set from GENCODE Version M16 (Ensembl 91)
Mir214
Mir199a-2
Pseudogene Annotation Set from GENCODE Version M16 (Ensembl 91)
Non-Mouse RefSeq Genes
Other RefSeq
Retroposed Genes V6, Including Pseudogenes
Mouse ESTs That Have Been Spliced
Spliced ESTs
Placental Mammal Basewise Conservation by PhyloP
Placental Cons
3.296
-3.94
Multiz Alignments of 60 Vertebrates
Rat
Rabbit
Human
Tree_shrew
Dog
Shrew
Elephant
Opossum
Platypus
Chicken
Zebrafish
Simple Nucleotide Polymorphisms (dbSNP 142) Found in >= 1% of Samples
Common SNPs(142)
Detailed Visualization of RepeatMasker Annotations
RepeatMasker Viz
Repeating Elements by RepeatMasker
RepeatMasker
B
Coding potential
Ribosome profiling TPM/Input TPM
0
2
4
6
8
Dnm3os
Neat1
Actb
Ppia
Ncl
lncRNA
Coding genes
C
Luciferase Positive Control
no template DNA
Dnm3os
Protein Marker
kDa
250
150
100
75
62
50
37
25
20
15
10
1
2
3
4

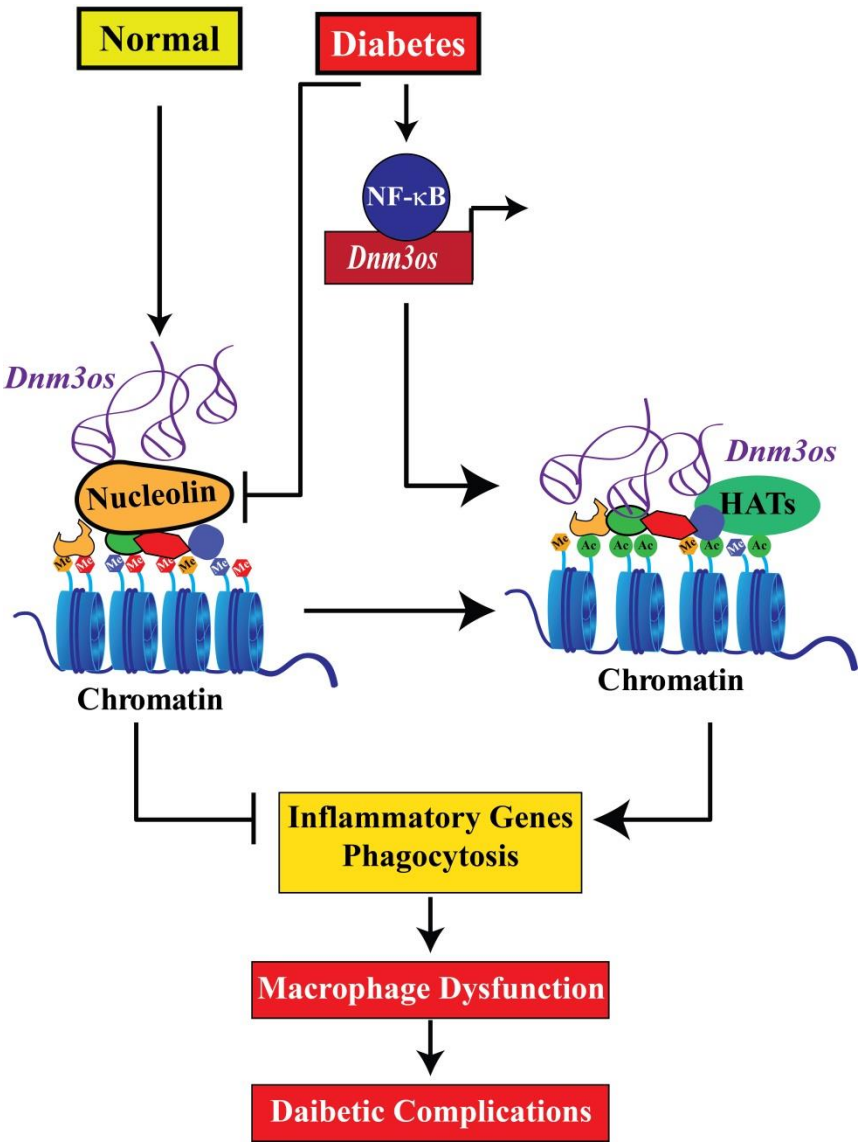
Supplementary Figure XIII: A. PhyloCSF tracks of the lncRNA *Dnm3os* genomic region in UCSC browser. The tracks show scores for each codon in 6 frames. A score greater than 0 indicates protein-coding. For *Dnm3os* loci, all scores were less than 0. B. Ratio of transcripts per million (TPM) values from ribosomal profiling and input-RNA profiling for indicated genes in peritoneal macrophages at basal condition. C. *In vitro* transcription/translation reactions were performed with *Dnm3os,* no template DNA and luciferase control plasmid. The luciferase positive control generated a 62 kDa protein while *Dnm3os* transcript generated no protein products.



Supplementary Figure XIV: Graphs showing expression levels of lncRNA *Dnm3os* in various human tissues (RNA-Seq Expression Data from GTEx (https://www.gtexportal.org/home).



Supplementary Figure XV: Effect of miR-199a and miR-214 overexpression on *Dnm3os* targets. RAW macrophages were transfected with miR-199a-5p and miR-214 mimic oligonucleotides. As control, RAW cells were also transfected with Negative Control mimic (NCM) oligonucleotides. RNA was extracted at 48 hrs post transfection and expression of indicated miRNAs and *Dnm3os* target genes was analyzed by RT-qPCR. *U6* and *Rplp0* were used internal controls for miRNAs and target genes respectively. Normalized data was expressed as Fold over NCM transfected cells. Mean+SEM, n=3. ***, $p<0.0006$ and *, $p<0.05$, ANOVA, Dunnett's multiple comparisons test.



Supplementary Figure XVI: Schematic diagram showing mechanisms of *Dnm3os* induced inflammatory gene regulation in diabetes. Under normal conditions, nucleolin interacts with and prevents the functional ability of *Dnm3os* to increase enrichment of permissive histone modifications like H3K9ac at promoters of inflammatory genes such as *Il6*. But, under diabetic conditions, increased *Dnm3os* and reduced nucleolin levels disrupt such interactions, allowing *Dnm3os* to enhance promoter H3K9ac, likely via recruitment of histone acetyl transferases (HATs), and other unidentified epigenetic mechanisms leading to chromatin relaxation and upregulation of inflammatory genes and macrophage dysfunction associated with diabetic complications.

Sample	Total Reads	Aligned Reads	Exon Reads	rRNA Reads	% Aligned	% Exon	%rRNA
EV1	35,030,107	32,153,451	27,453,044	238,190	91.8%	85.4%	0.7%
EV2	31,152,524	28,548,480	24,481,379	206,047	91.6%	85.8%	0.7%
Dnm3os 1	26,104,459	23,876,409	20,302,743	296,821	91.5%	85.0%	1.2%
Dnm3os 2	30,405,409	27,808,541	23,674,005	255,258	91.5%	85.1%	0.9%

Supplementary Table I: RNA-seq data from RAW macrophages stably expressing *Dnm3os* and EV. RNA-seq run in biological replicates (1 and 2).

Supplementary Table II. List of PCR primer sequences used in this study.

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
Taqman assays		
Dnm3os	ggtgtagatgctatggtgtgag	agaacatccgctcacctgta
DNM3OS	cgacaaacatccaactgaagc	acactctcgacattcactgc
SYBR Green Assays		
U6	gttcttgcttcggcagaac	aaaatgtggaacgcttcacg
Neat1	tggtctcactcttccctactc	ccacctttaccaaaatgccg
Kcnq10t1	cctgaggatggctgtgtattag	taccgtgtgccctttgttag
Ppia	atggtcaaccccaccgtg	ttcttgctgtctttggaactttgtc
Ncl	tctgtttcttggcttcaggg	gaagatgacgaggatgaggatg
Nfkbiz	ggaataagagcctggtagacac	aagaggcgaatgagttccag
Dnm3os	tttcttttccccctcacgg	cagggtaaatctgtcaggagc
Cd74	aagtacggcaacatgaccc	atcttccagttcacgccatc
Irak3	acgaaggccaagttaagacc	aagaacagtggagaaggacac
C5ar1	gagggtttaaaaggcacagc	atccatggttccatagtgatcatag
Ptafr	ctcctacaggcatattcagcg	tcgaaactcagaatccacacg
Cxcl3	tttgagaccatccagagcttg	ttcttgaccatccttgagagtg
Tnfrsf1b	atgttaggactggtgaactgc	tgtggtcaacaggtgctg
Tlr1	agaggcaattgtggacacc	gaagatgagggaatttggtttagtc
Itgal	ctatgtagtgttgacctggacc	cctgtagctctgagaccatttc
Cxcl2	tgctcaccctaaagaaacctc	ctgaagttaaccttgattttccctg
Itgax	ttcaaggagacaaagacccg	agagaaaagttgaggcgaagag
Il6	ctctgaaggactctggctttg	gatgctaccaaactggatataatcag
Tnf	tgttgcctcctcttttgctt	tggtcaccaaatcagcgtta
Nos2	cctggagacccacacactgg	cacagccacattgatctccg
Cd36	gatgacgtggcaaagaacag	tcctcggggtcctgagttat
Ptgs2	gagcaactattccaaaccagca	cggttttgagtttgaagtggtaaccg
Utx	atcccagctcagcagaagtt	ggaggaaagaaagcatcacg
Jmjd3	cccccatttcagctgactaa	ctggaccaaggggtgtgtt
Ezh2	ctaattggtacttactacgataacttt	actctaaactcatacacctgtctacat
Mettl13	gcactcggcagtgacttaata	ggaatagtggcttcgtcagtag
Vamp4	gagggctgtggactgaaatag	aggtgtccggcagtctataa
IL6	caacctgaaccttccaaagatg	acctcaaactccaaaagaccag
TNF	ccctgaaaacaaccctcaga	gtcctttccaggggagagag
NOS2	tgccaagctgaaattgaatgag	cttcgcctcgtaaggaaataca
HPRT1	agatggtcaaggtcgcaag	acagagggctacaatgtgatg
NFKBIZ	aattagatgctgtccgcctg	ctttcccttcaggatacgtcg

ITGAX	gctccacatctgaccttctag	accacgctgtctccaaac
Dnm3	gcctgtcctggtataaagacg	cattcctttgctcggtgttg
ChIP Primers		
Il6 promoter	cgtttatgattctttcgatgctaaacg	gtgggctccagagcagaatgag
Tnf promoter	ccaggattctgtggcaatct	gtttcagttctcagggtcctatac
Nos2 promoter	cacagactaggagtgtccatca	gcagcagccatcaggtattt
Dnm3os promoter	aaaggaggaggaggaggag	ccttagtgaggtcagataagttgg
Cloning primers		
Dnm3os WT Promoter	cgagctcgggccagtttcaacagatttac	ccgctcgagttgtcctgaacaggtagtctgaac
Dnm3os Mut Promoter	cgagctcaacttatctgacctcactaagg	ccgctcgagttgtcctgaacaggtagtctgaac
Dnm3os (FL cloning)	cgggatccttttcctggtcctaaattc	aaggaaaaaagcggccgcttcatacttgtaatttttattc