SUPPLEMENTAL INFORMATION

Role of TET1-mediated epigenetic modulation in Alzheimer's disease

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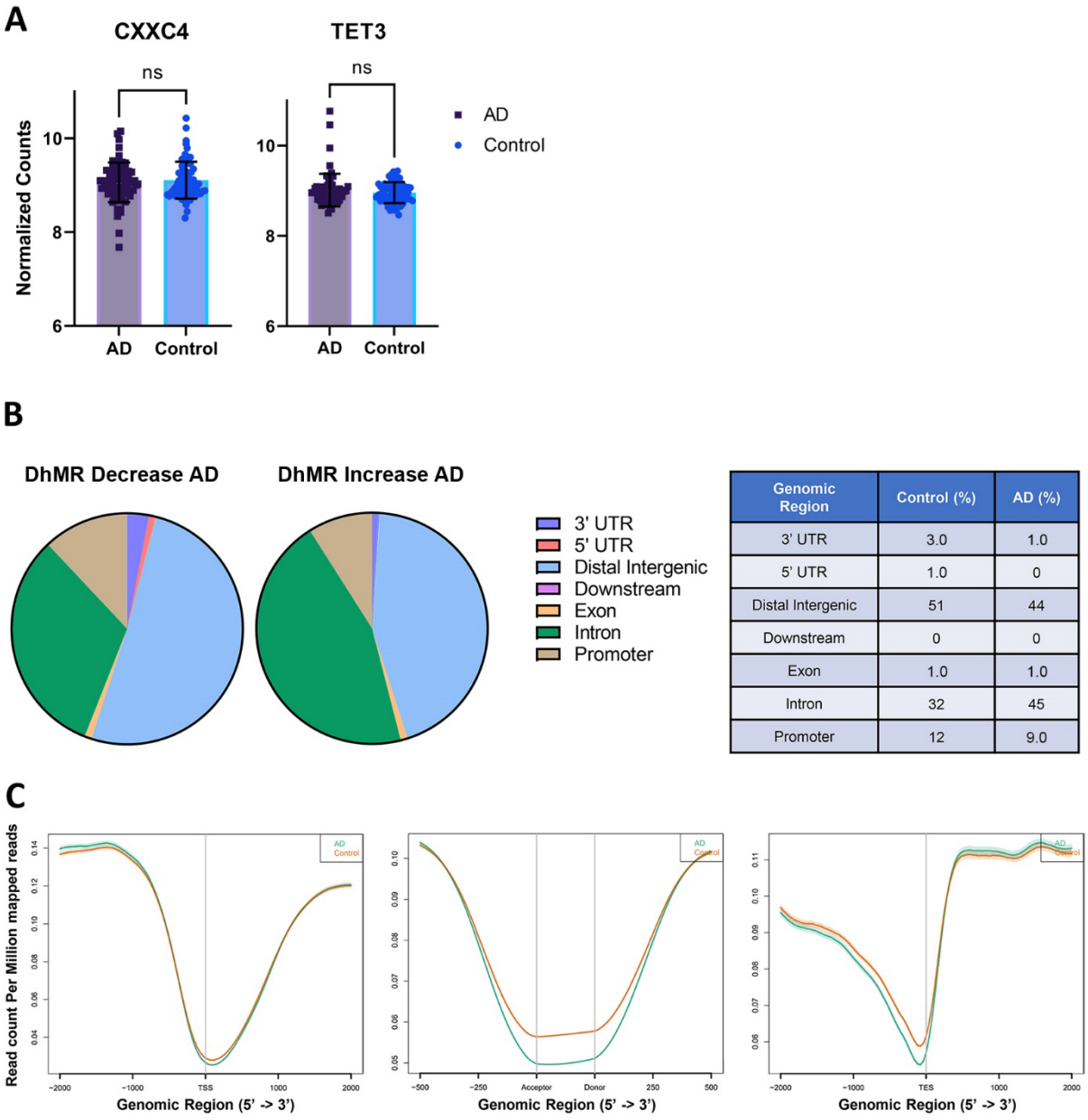


Genomic Region	Control (%)	AD (%)
3' UTR	3.0	1.0
5' UTR	1.0	0
Distal Intergenic	51	44
Downstream	0	0
Exon	1.0	1.0
Intron	32	45
Promoter	12	9.0

Figure S1. Expression of CXXC4 and TET3 is Similar and abundance of 5hmC varies by genomic location between AD and Control.
A) VST normalized expression between ROSMAP AD (n = 68) and control (n = 72) samples. There is no observed difference in the expression of *CXXC4* (p = 0.5033) and *TET3* (p = 0.2682) between AD and control samples. Error bars indicate mean ± SD; two-tailed unpaired t-test; ns, not significant.
B) The difference in genomic localization of 5hmC in AD relative to control. 5hmC peak distribution is significantly altered between AD and control (p = 0.021).
C) Plotted distribution of RPM normalized reads across transcription start sites (TSS), splice junctions, and transcription end sites (TES).

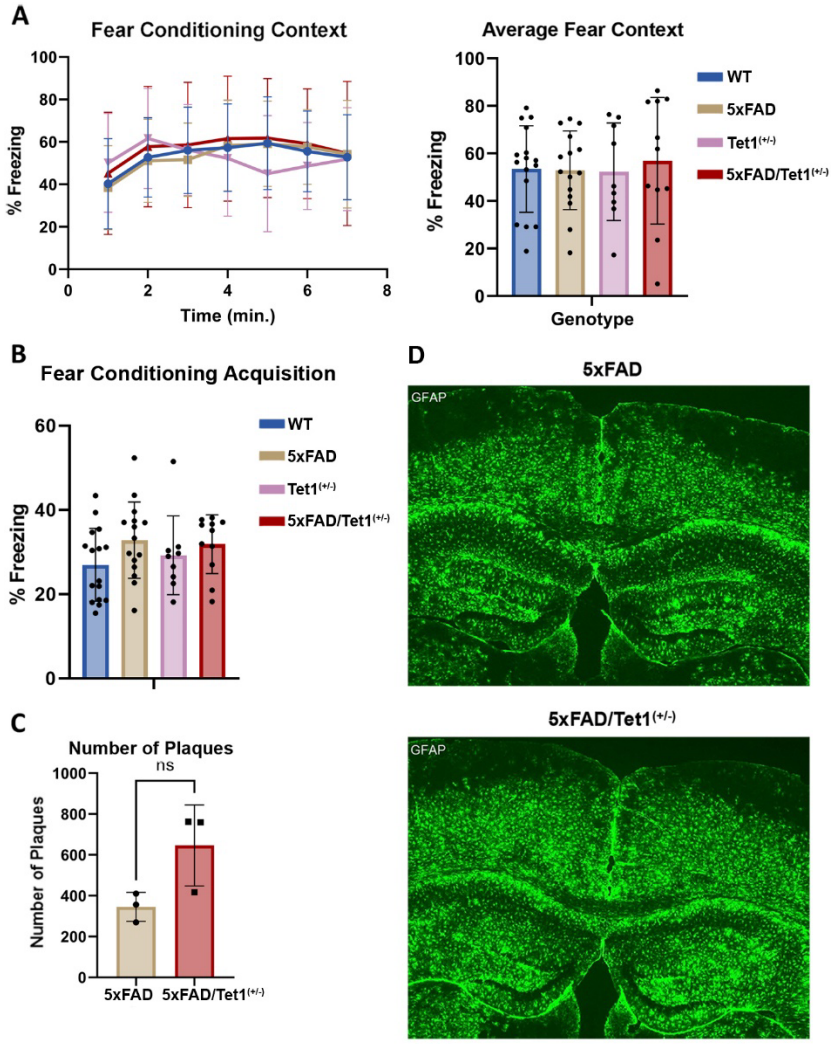
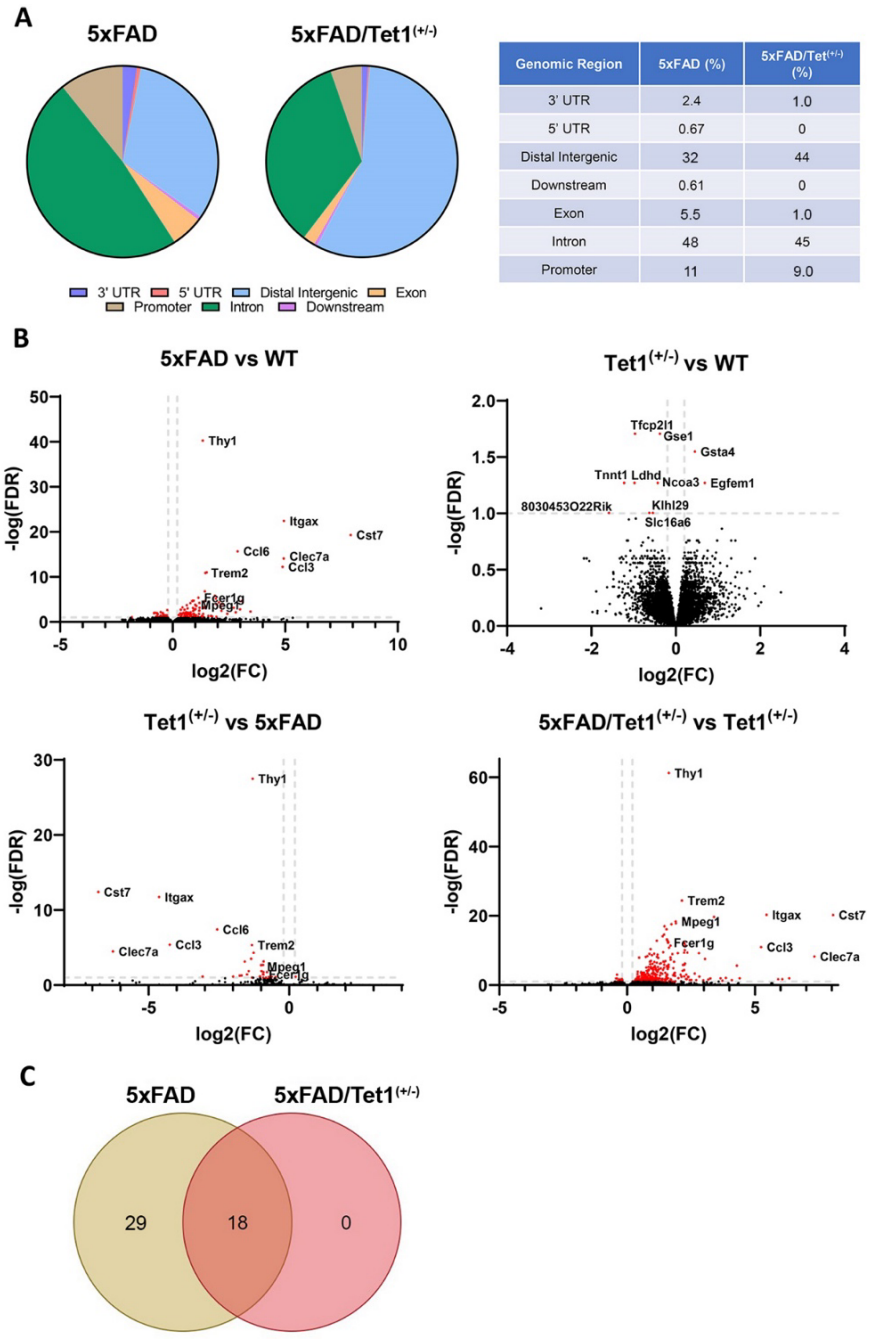


Figure S2. The Partial Loss of Tet1$^{+/-}$ in 5xFAD mice Alters Neuropathology but Not Cognition

A) Fear conditioning context results between WT (n = 16), 5xFAD (n = 15), Tet1$^{(+/-)}$ (n = 9), and 5xFAD/Tet1$^{(+/-)}$ (n = 11) mice. There are no significant differences between the time spent freezing and genotype in the context portion of the fear conditioning assay (p = 0.95); two-way RM ANOVA. There is no measurable difference between the genotypes tested and the average percent freezing during the entire assay. Error bars indicate mean ± SD; two-tailed unpaired t-test; ns, not significant.
B) Fear conditioning performance during the training portion of the assay. There are no significant differences in performance between mice of any conditions. The greatest difference in performance is between WT and 5xFAD mice (p = 0.0759). Error bars indicate mean ± SD; two-tailed unpaired t-test.
C) The total number of amyloid plaques measured for 5xFAD (n = 3) and 5xFAD/Tet1$^{(+/-)}$ (n = 3) mice by the STARDIST machine-learning algorithm (p = 0.0688). Error bars indicate mean ± SD; two-tailed nested t-test; ns, not significant.
D) Visual comparison of Anti-GFAP (ab72600 abundance between 5xFAD and 5xFAD/Tet1$^{(+/-)}$ mice.



Genomic Region	5xFAD (%)	5xFAD/Tet(+/-) (%)
3' UTR	2.4	1.0
5' UTR	0.67	0
Distal Intergenic	32	44
Downstream	0.61	0
Exon	5.5	1.0
Intron	48	45
Promoter	11	9.0

Figure S3. Partial Loss of Tet1 Leads to Dysregulation of 5xFAD-associated Gene Expression and 5hmC Profiles

A) The distribution of 5hmC peak enrichment across genomic features in 5xFAD and 5xFAD/Tet1$^{(+/-)}$ mice is significantly different ($p < 0.0001$).
B) Differential expression results from RNA-Seq analysis between WT (n = 4), Tet1$^{(+/-)}$ (n = 2), 5xFAD (n = 2), and 5xFAD/Tet1$^{(+/-)}$ (n = 4). Between WT vs 5xFAD 137 genes were differentially expressed (-log(FDR) < 1.0), 10 DEGs between WT and Tet1$^{(+/-)}$, 33 DEGs between Tet1$^{(+/-)}$ and 5xFAD, and 336 DEGs between Tet1$^{(+/-)}$ and 5xFAD/Tet1$^{(+/-)}$.
C) Overlap between transcription factor binding motifs between 5xFAD and 5xFAD/Tet1$^{(+/-)}$ mice. There were 29 TF motifs lost in 5xFAD/Tet1$^{(+/-)}$ relative to 5xFAD and 18 shared DNA binding motifs.

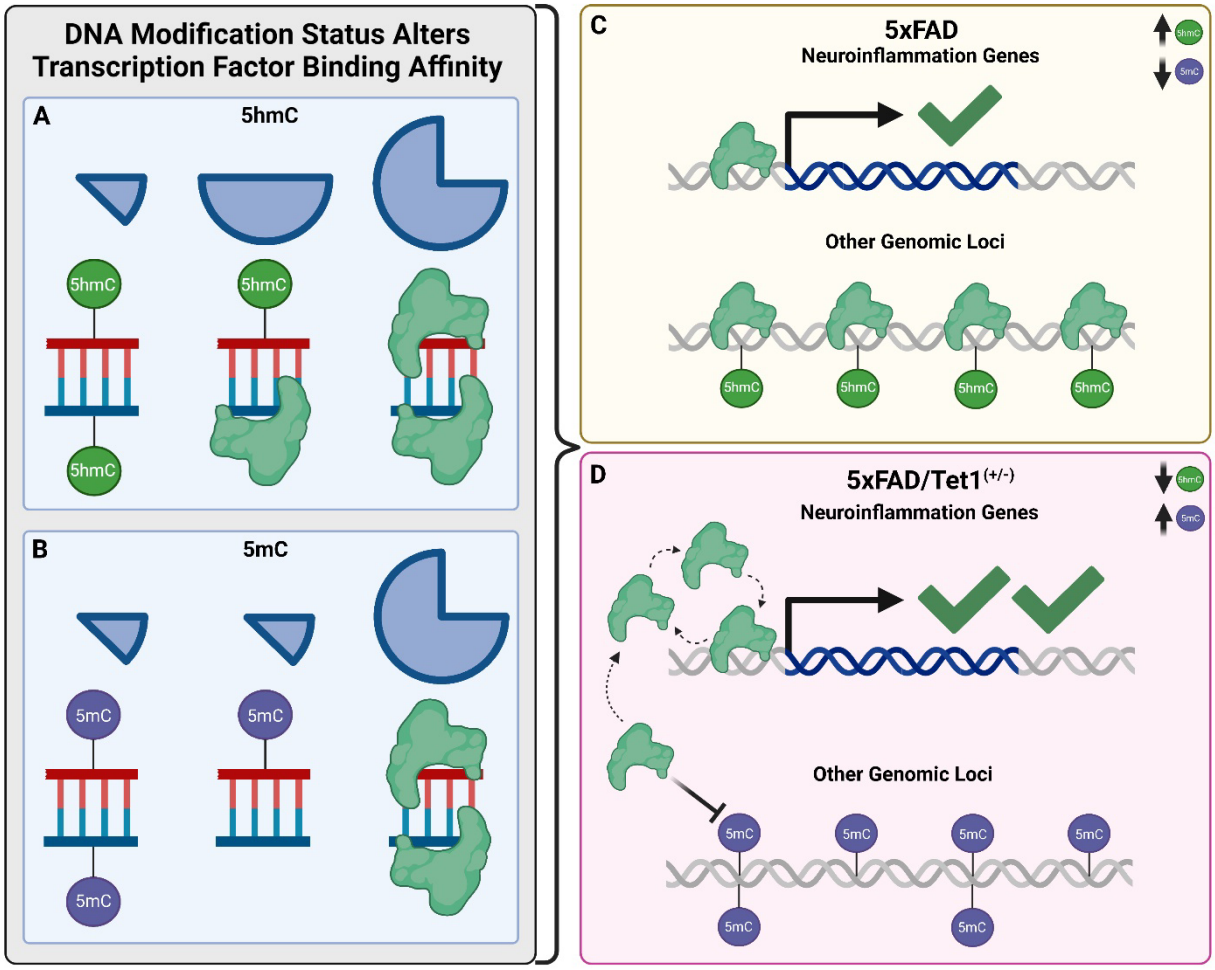


Figure S4. Potential Mechanism for How the Loss of Tet1's Contributes to Increased Inflammation and AD Pathology in the 5xFAD Mouse Model

A) A visual depiction of transcription factor binding affinity for DNA containing differential 5hmC modifications. The TF used in this example is Ets1, which has suppressed binding affinity for symmetrical 5hmC, unaffected binding affinity for hemi-5hmC, and the greatest binding affinity for unmodified DNA.
B) A similar depiction for TF binding affinity for 5mC modification status. There is suppressed binding affinity for both symmetrical and hemi-5hmC modification. Binding affinity is the greatest for unmodified DNA.
C) Relative to 5xFAD/Tet1$^{(+/-)}$ mice, 5xFAD mice have a greater 5hmC and reduced 5mC abundance. This graphic suggests a scenario where the greater affinity for hemi-5hmC DNA for regions not governing the expression of neuroinflammatory genes act as a molecular sponge and sequester Ets1.
D) A potential mechanism for increased abundance unbound Ets1 due to reduced affinity for 5mC sites not converted to 5hmC due to the loss of Tet1. The increased amount of free Ets1 is the able to saturate the promoter region of genes involved in neuroinflammation, increasing their relative expression.