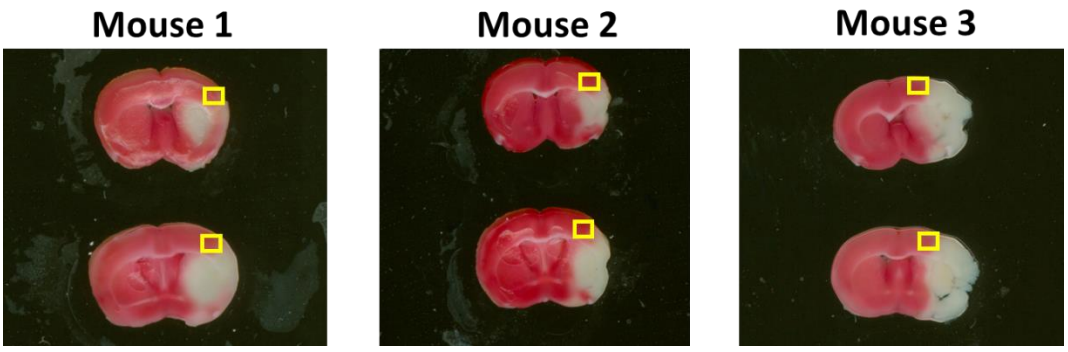
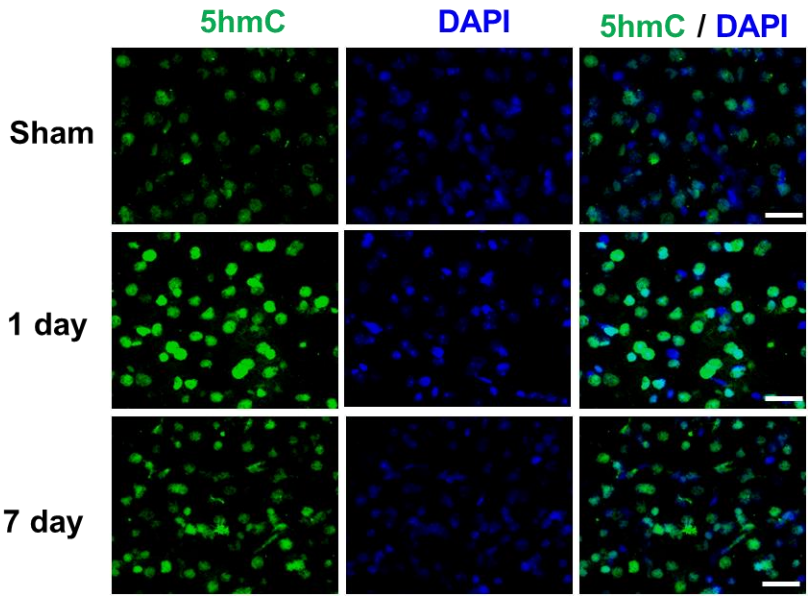
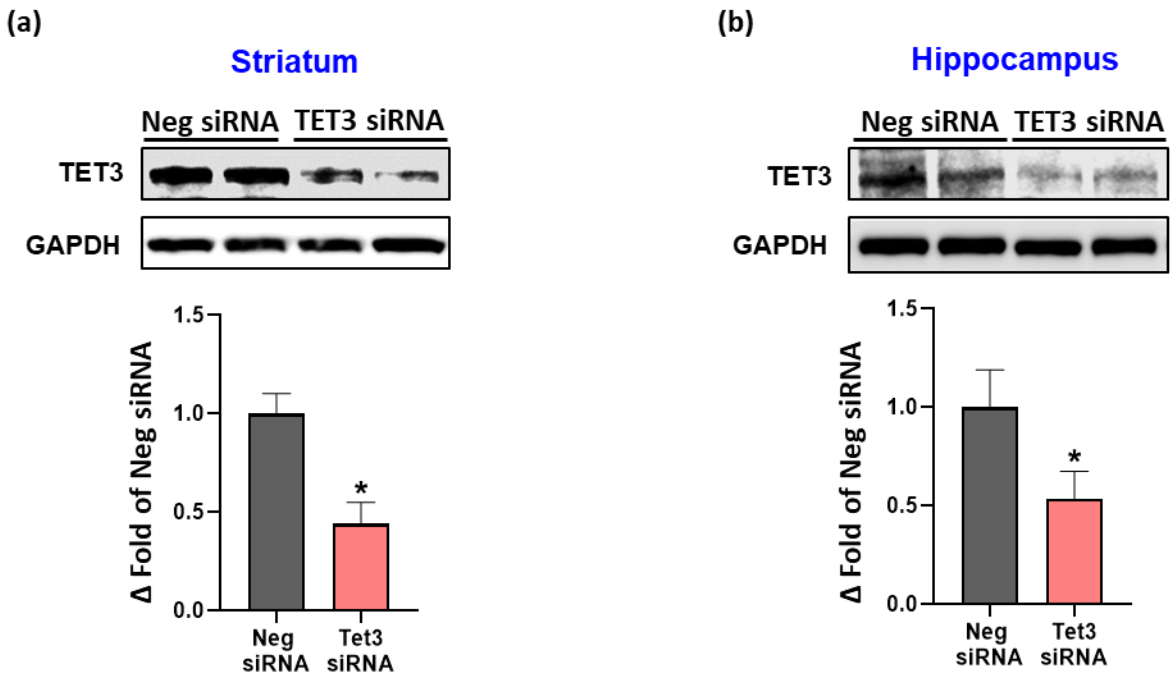


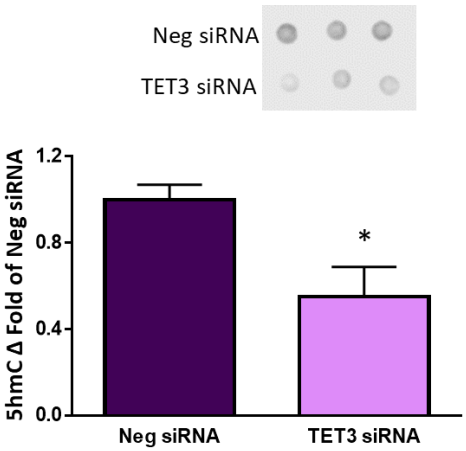
Supplementary Figure 1. Examples of the peri-infarct region. The yellow boxes indicate the peri-infarct region in the representative TTC-stained brain sections with varying sized infarcts.



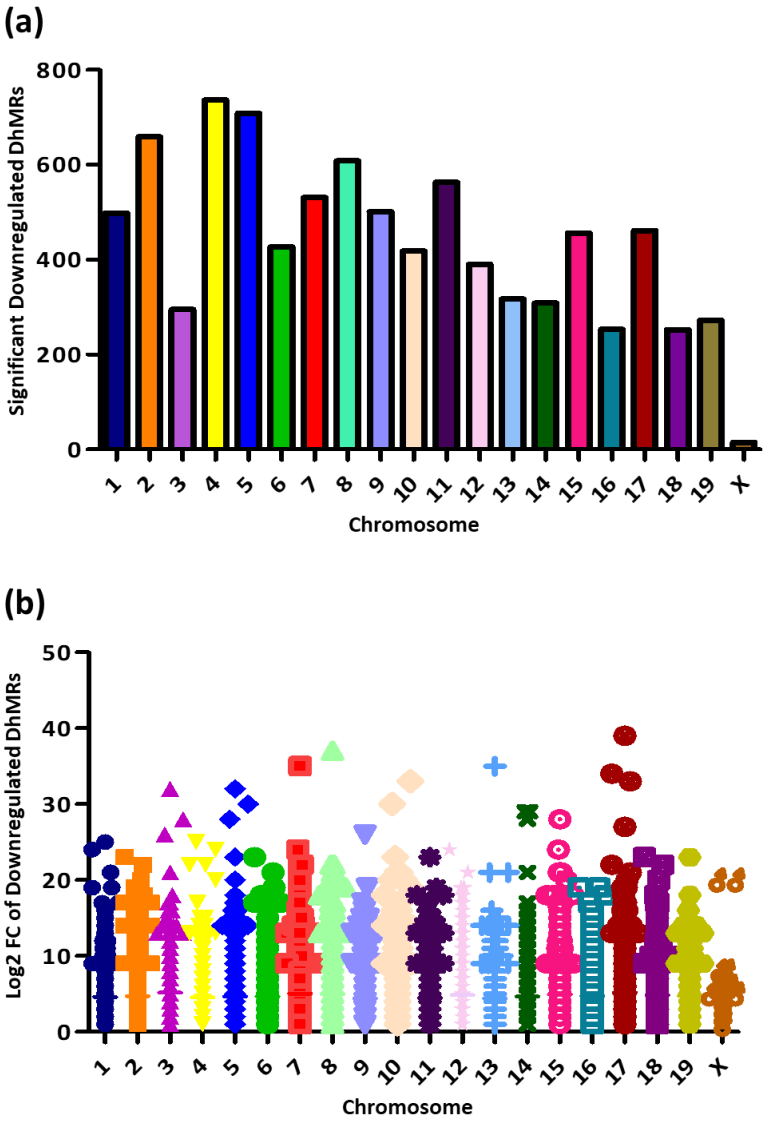
Supplementary Figure 2. Focal ischemia increases 5hmC in the peri-infarct cortex. Increased 5hmC in the peri-infarct cortex at 1 day of reperfusion, but not at 7 days of reperfusion following transient MCAO (n = 3/ group). Scale bars, 30 μm.



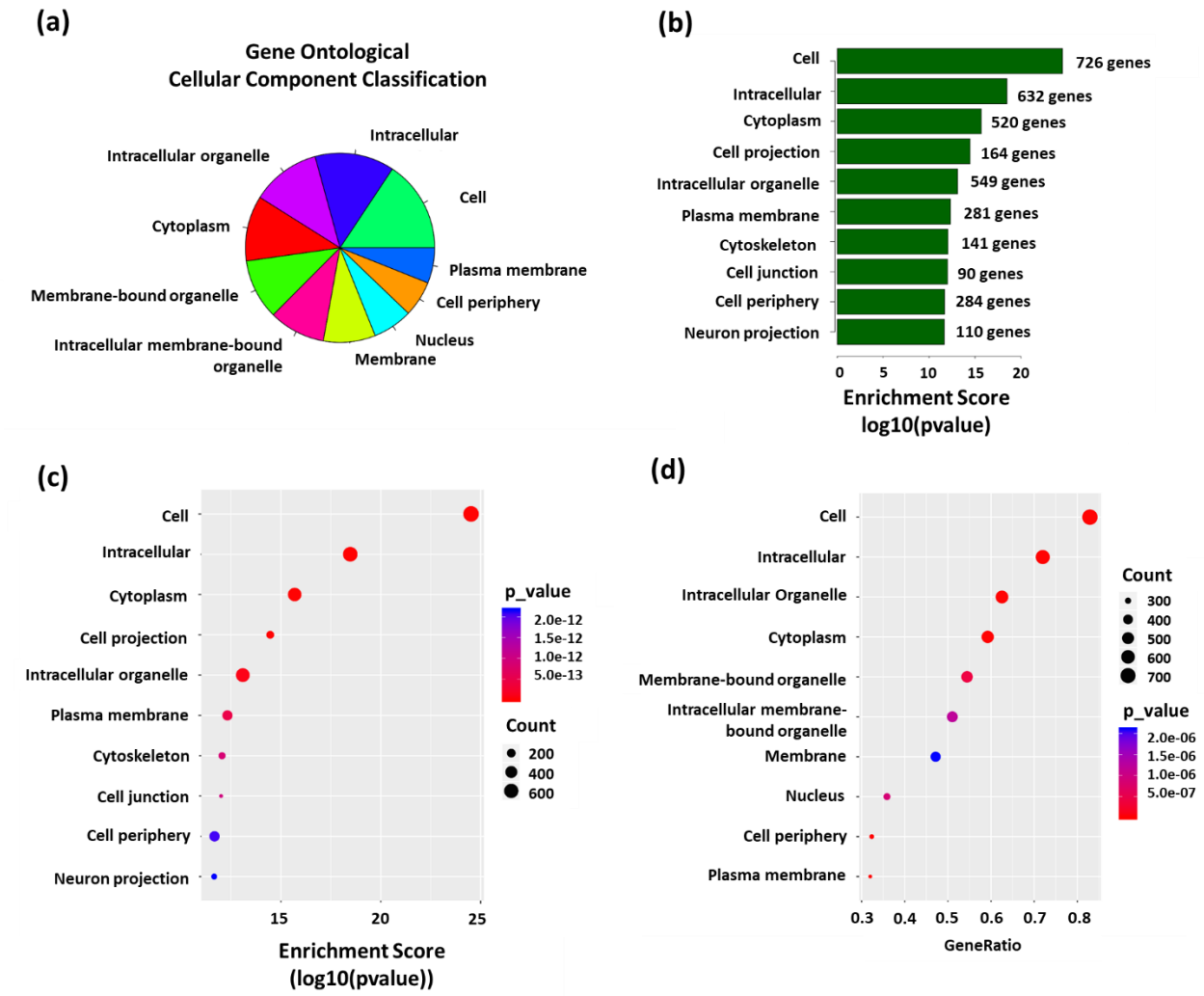
Supplementary Figure 3. TET3 knockdown reduces TET3 Levels in the striatum and hippocampus. Intracerebral injection of TET3 significantly reduced the striatal (a) and hippocampal (b) TET3 protein levels 2 days after treatment compared with negative control siRNA (Neg siRNA)-treated cohort (n = 3/group). *p<.05 by Mann Whitney U test. Values in the histograms are mean ± SD.



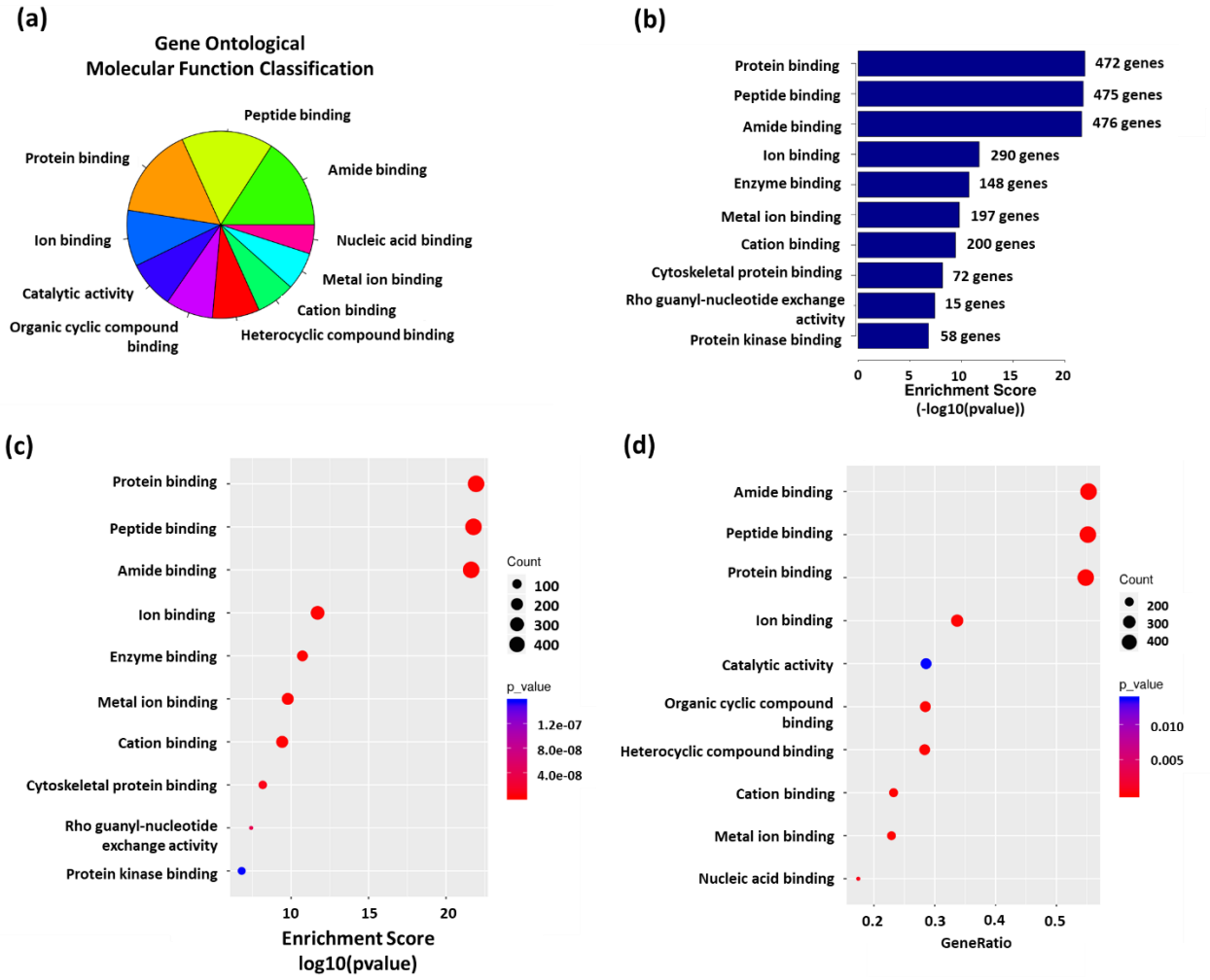
Supplementary Figure 4. Levels of 5hmC following TET3 knockdown in sham. Knockdown of TET3 significantly reduced 5hmC compared to the negative control siRNA (Neg siRNA) group in sham-treated mice (n = 3/group). Values in the histograms are mean ± SD.



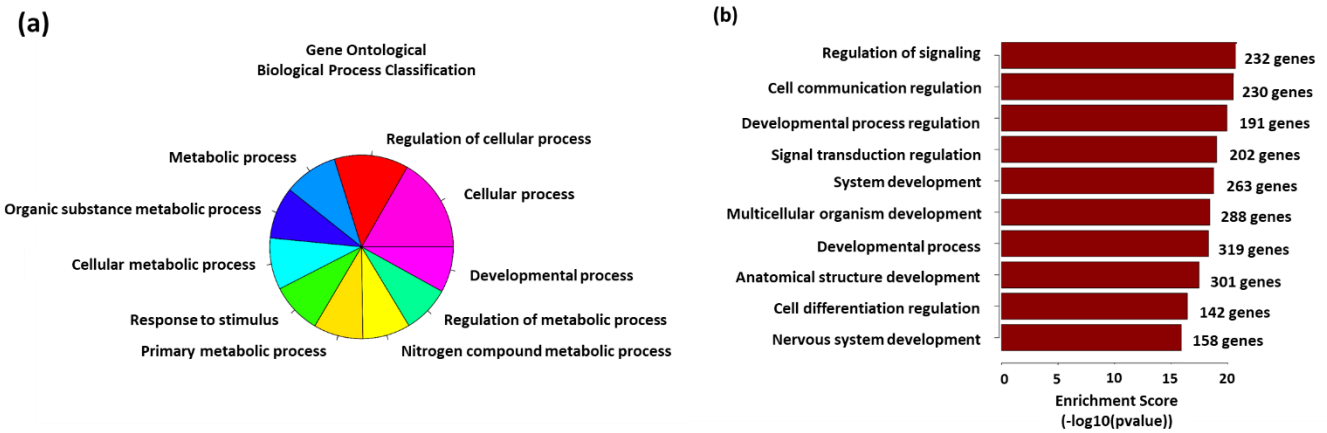
Supplementary Figure 5. TET3 knockdown led to loss of DhMRs in gene promoters in mouse chromosomes. TET3 siRNA group compared to Neg siRNA group at 6h reperfusion. Number of downregulated DhMRS by chromosome (a). Downregulated DhMRS were classified by their Log2 fold change (FC) by chromosome (b). Significant peaks were identified using q value cutoff of 10^{-4}, n = 3/group.



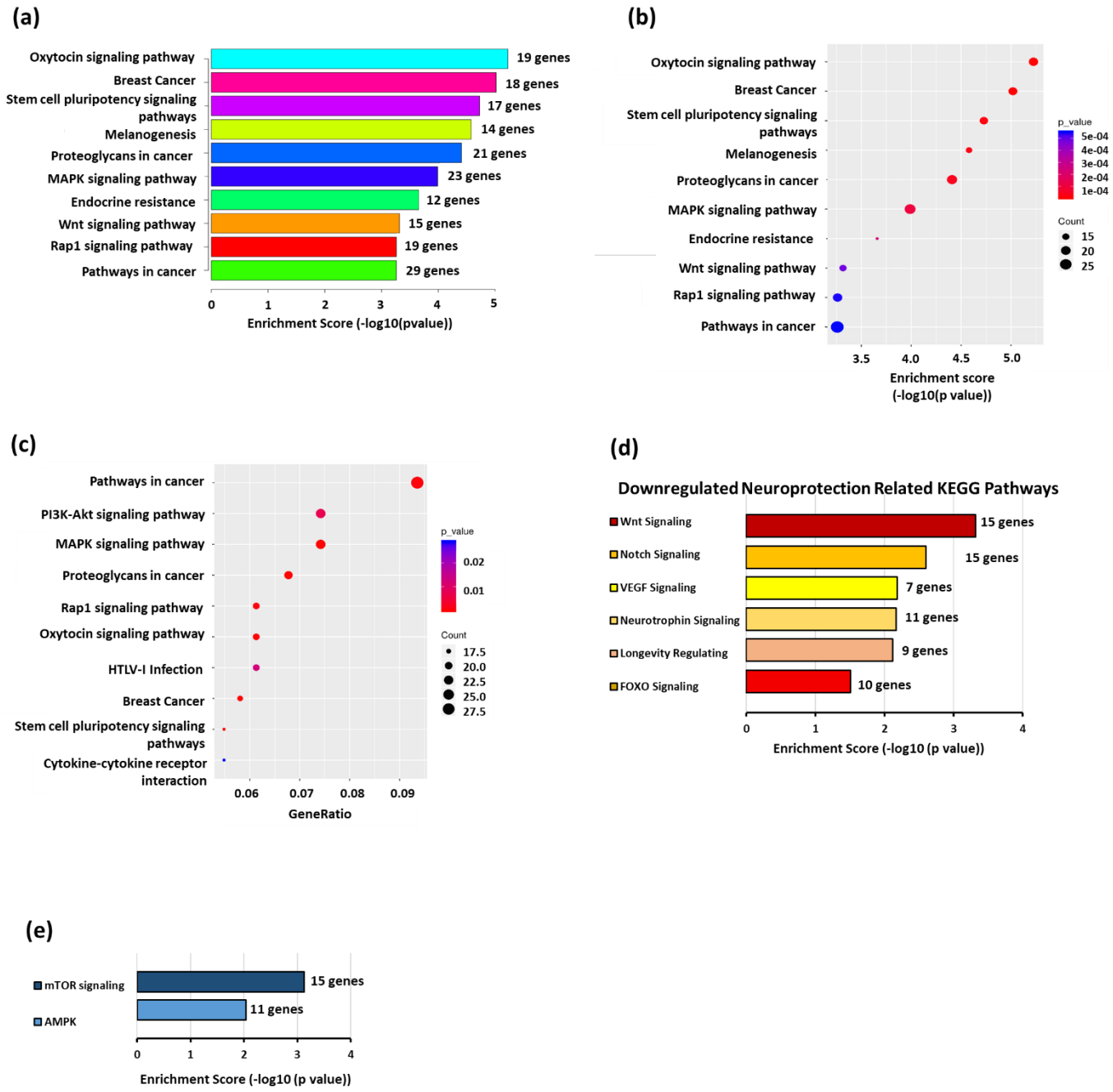
Supplementary Figure 6. Gene ontology analysis of associated cellular components significantly downregulated with TET3 knockdown after transient MCAO. TET3 siRNA group compared to Neg siRNA group at 6h reperfusion. Pie chart denoting composition of each cellular component classification (a). Top 10 cellular components ranked by enrichment score (b, c). Top 10 cellular components by gene abundance in each pathway (d). n = 3/group



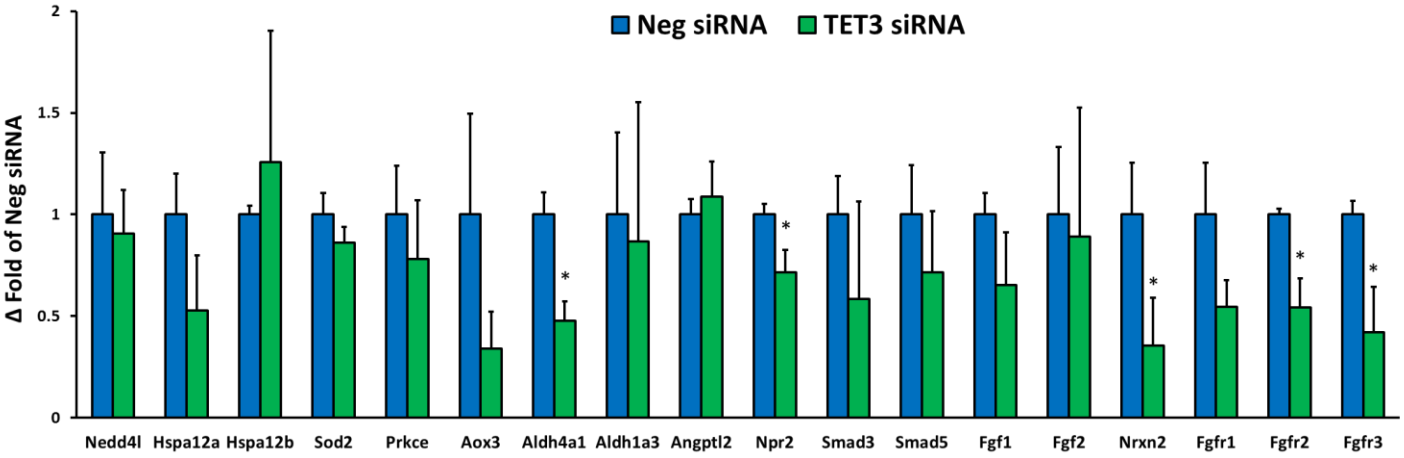
Supplementary Figure 7. Gene ontology analysis of associated molecular functions significantly downregulated with TET3 Inhibition following transient MCAO. TET3 siRNA group compared to Neg siRNA group at 6 h reperfusion. Composition of each molecular function classification (a). Top 10 molecular functions ranked by enrichment score (b, c) and by gene abundance (d). n = 3/group



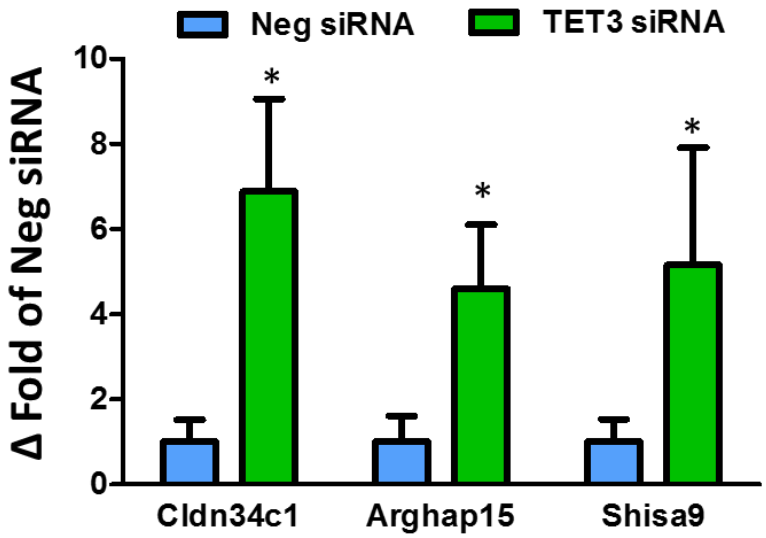
Supplementary Figure 8. Gene ontology analysis of associated biological pathways significantly downregulated with TET3 Inhibition following transient MCAO. TET3 siRNA group compared to Neg siRNA group at 6h reperfusion. Composition of each biological pathway classification (a). Top 10 biological pathways ranked by enrichment score (b). n = 3/group



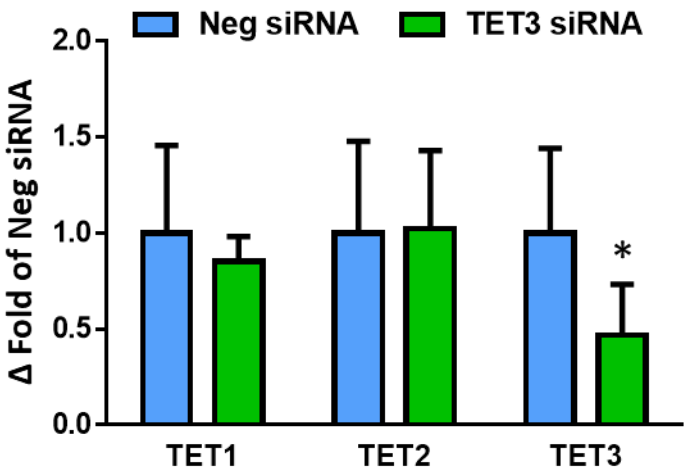
Supplementary Figure 9. KEGG pathways downregulated with TET3 inhibition following transient MCAO. TET3 siRNA group compared to Neg siRNA group at 6h reperfusion. Enrichment scores showing number of genes involved in each of the top 10 pathways (a). Enrichment score dot plot comparing gene count and p-value of top 10 pathways (b). Gene ratio dot plot plotting pathways based on the highest gene counts in top 10 pathways (c). Neuroprotection-related pathways (d). Enrichment scores and gene count of the mTOR signaling and AMPK pathways (e). n = 3/group



Supplementary Fig. 10. TET3 knockdown decreased gene expression of neuroprotective genes in adult male mice. Expression of selected neuroprotective genes displaying downregulated DhMRs with TET3 inhibition at 6h of reperfusion was assessed by real-time PCR analysis. Values in the histograms are mean ± SD (n = 3/group). *p < 0.05 compared to neg siRNA by Mann Whitney test. Abbreviations: Nedd4l (Neural precursor cell expressed developmentally downregulated gene 4-like), Hspa12a,b (Heat shock 70kDa protein 12A,B), Sod2 (Superoxide dismutase 2), Prkce (Protein kinase C epsilon), Aox3 (Aldehyde oxidase 3), Aldh4a1,3 (Aldehyde dehydrogenase 4 family member A1,3), Angptl2 (Angiopoietin like 2), Npr2 (Natriuretic peptide receptor 2), Smad3,5 (Mothers against decapentaplegic homolog 3,5), Fgf1,2 (Fibroblast growth factor 1,2), Nrxn (Neurexin), Fgfr1-3 (Fibroblast growth factor receptor 1,2,3).



Supplementary Figure 11. Increased 5hmC levels in promoter regions correlates with upregulated gene expression. Top three Log2 fold change (FC) upregulated DhMRs, Cldn34c1 (Log2(FC) = 9), Arghap15 (Log2(FC) = 4) and Shisa9 ((Log2(FC) = 4), show increased mRNA levels at 6h of reperfusion following transient MCAO in TET3 siRNA group compared to Neg siRNA group (*$p < 0.05$; n = 3/group; Mann Whitney U test). Values are mean ± SD. Abbreviations: Cldn34c1 (claudin 34C1), Arghap15 (Rho GTPase-activating protein 15), Shisa9 (Shisa Family Member 9).



Supplementary Figure 12. TET3 siRNA treatment reduced TET3 expression in the female mice cortex. Expression of TET1, TET2, and TET3 in the cortex was assessed at 6h of reperfusion. Values in the histograms are mean ± SD (n = 3-4/group). *p < 0.05 compared to neg siRNA by Mann Whitney U test.

Supplementary Table 1. Primers used for quantitative PCR

Gene Name	Forward Primer (5'→3')	Reverse Primer (5'→3')
TET1	CCTCTACTTGGTTGGTCTAC	CTACCACTGTCCGGTTGTATTT
TET2	ACTTCTCTGCTCATTCCCACAGA	TTAGCTACTTCTCGATC
TET3	GAGCACGCCAGAGAAGATCAA	CAGGCTTTGCTGGGACAATC
18s	CGCCGCTAGAGGTGAAATTCT	CGAACCTCCGACTTTCGTTCT
Nedd4l	TAGCCTCAGCTCGCCAACAGTA	GGAGTTGTAAGGTGATGGCTGAG
Hspa12a	GCAGAGTCGGACCTTTTTGGTG	CTGGTAACCGTATCTGATGGACG
Hspa12b	ACCTGCCATCTGGAAACAACCG	GTTTCCGACAGTAGACAGAGGC
Sod2	TAACGCGCAGATCATGCAGCTC	AAGCTGAAGAGCGACCTGACTT
Prkce	TGGCTGACCTTGGTGTTACTCC	GCTGACTTGGATCGGTGCTCTT
Aox3	GAGATGTGCCTGGTGACAATGG	GGCATGAGCATAAGAGTCAGCG
Aldh4a1	AACTTCACCGCGATTGGAGGCA	TAGCCAGCATGGCAGTGTCACT
Aldh1a3	ACTGGAGCTAGGAGGCAAGAAC	GTAGACCTGTTCCACGAAC
Angptl2	GCGACTCCTTTAGGCACAA	GTTGGAGTGAGCGGCGTTA
Npr2	TCGCTTCATGCCAGCCTGCATA	CGCGTAGCGAAACATGTCC
Smad3	GCTTTGAGGCTGTCTACCAGCT	GTGAGGACCTTGACAAGCCACT
Smad5	CAGGAGTTTGCTCAGCTTCTGG	ACGTCCTGTCGGTGGTACTCTG
Fgf1	CCAAGGAAACGTCCACAGTCAG	ACGGCTGAAGACATCCTGTCTC
Fgf2	AAGCGGCTCTACTGCAAGAACG	CCTTGATAGACACAACTCCTCTC
Nrxn2	AGATGAGGCTCACCGTCAACCT	CCTCACCGTGTGCCATTCATTG
Fgfr1	GCCTCACATTCAGTGGCTGAAG	AGCACCTCCATTTCCTTGTCGG
Fgfr2	GTCTCCGAGTATGAGTTGCCAG	CCACTGCTTCAGCCATGACTAC
Fgfr3	ACAGGTGGTCATGGCAGAAGCT	CTCCATCTCAGATACCAGGTCC