Supplemental Materials

Altering 5-hydroxymethylcytosine modification impacts ischemic brain injury

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Supplemental Figure 1. The Tet2 inhibitor SC1 reduced Tet2 expression. (A) RT-PCR analysis of the mRNA levels of Tet2 in mouse brain after treatment with SC1. (B) Dot blotting analysis with anti-5hmC. ***$P < 0.001$

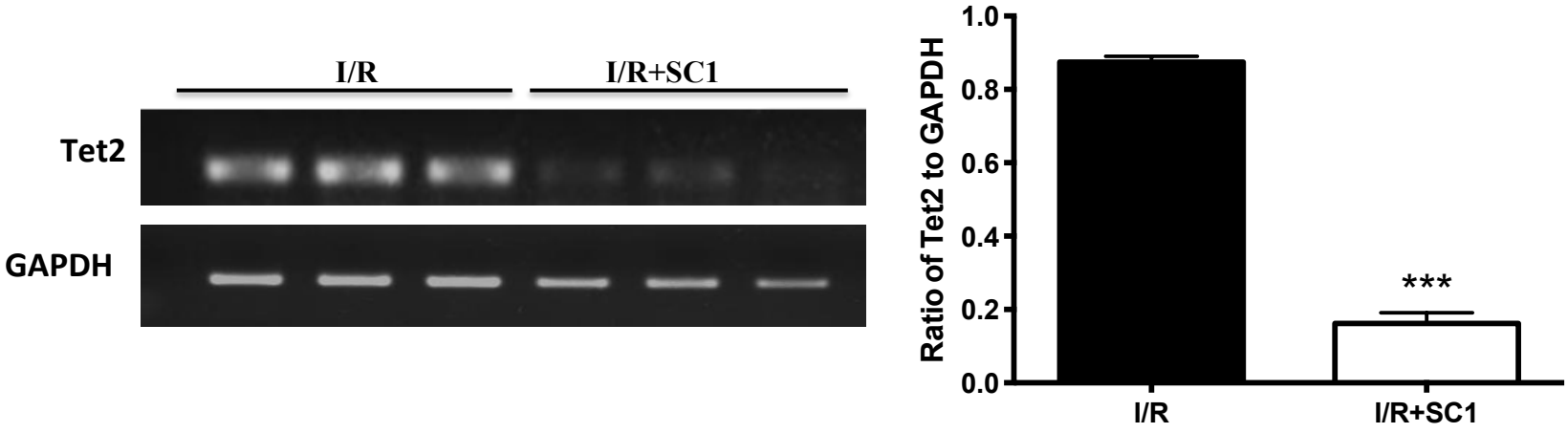
Supplemental Figure 2. Representative genes of 5hmC after stroke. Differential 5hmC distributions on gene bodies were visualized by IGV browser. ZRF-1, SOSC-3, HO-1, Galanin, Caspase3, and APP showed higher 5hmC abundance after stroke, while VGAT, SNAP-25B, TIMP-1, HIP-1, CNTF, and GluR3 showed lower 5hmC abundance.

Supplemental Table 1. Correlation analysis of 5hmC abundance and gene expression after stroke. Gene expressions after stroke in the literature and 5hmC abundance in genes were compared in this study. Up means increase; down means decrease or unchanged.

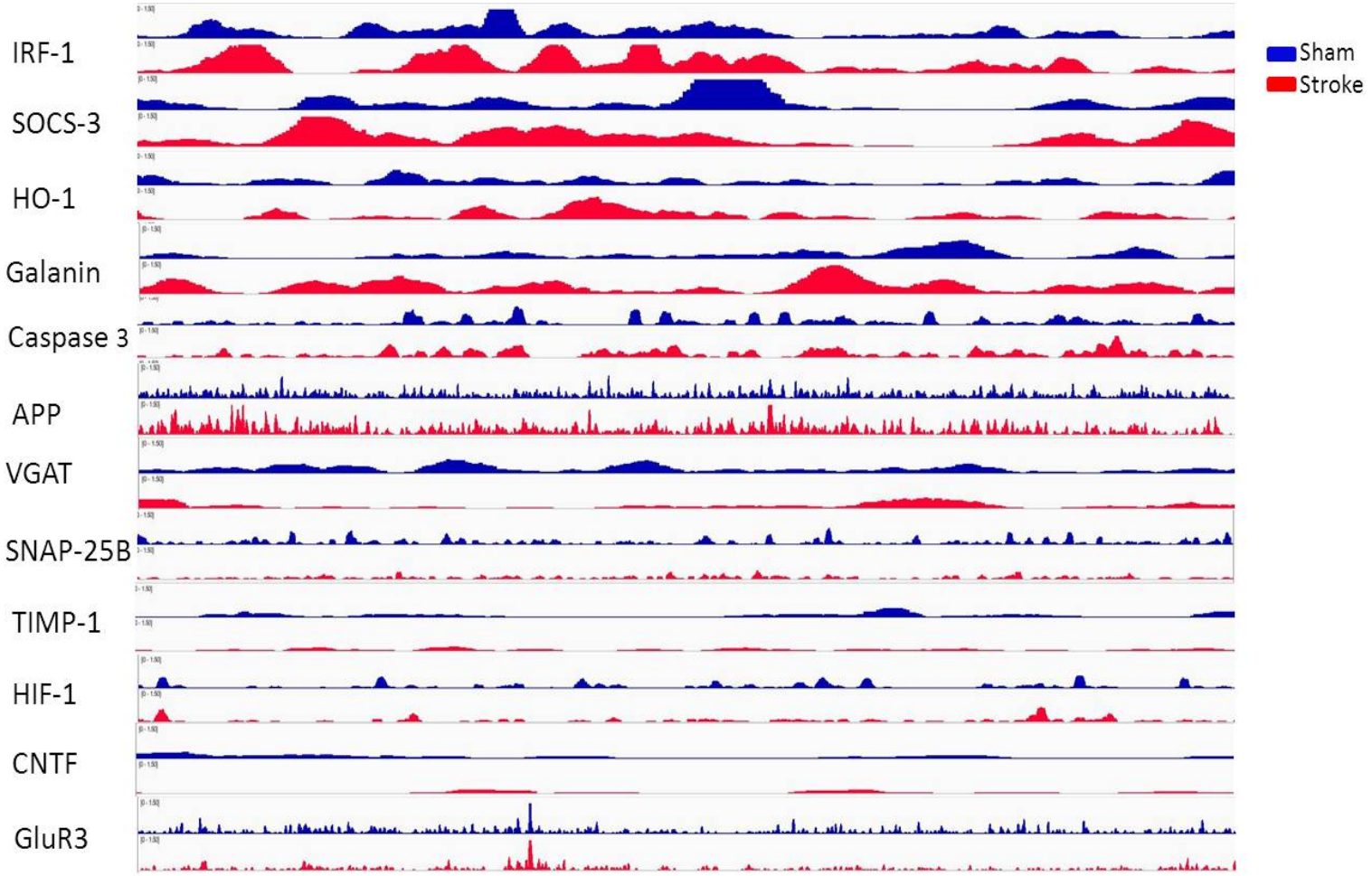
Supplemental Table 2. Correlation analysis of 5hmC abundance and lncRNA after stroke. Gene expressions of lncRNA after stroke in the literature and 5hmC abundance on the genes encoding lncRNA were compared. Up means increase; down means decrease or unchanged.

Supplemental Table 3. Primer sequences for quantitative PCR. The primers of GAPDH, BDNF, Tet1, Tet2, and Tet3 are listed.

Supplemental Figure 1



Supplemental Figure 2



Supplemental Table 1. Correlation analysis of 5hmC abundance and gene expression after Stroke

Gene	Expression after Stroke	5hmc after Stroke	Gene	Expression after Stroke	5hmc after Stroke
Casp3[18]	Up	Up	*Gja1*	Up	Up
Gdnf[20]	Up	Up	*Parp1*	Up	Up
Bdnf[19]	Up	Up	*Mlkl*	Up	Up
Vegfa[21]	Up	Up	*Rbp1*	Unclear	Up
Lif[23]	Up	Up	*Icam1*	Down	Down
Hmox1[17]	Up	Up	*Grm3*[17]	Down	Down
Socs3[17]	Up	Up	*Slc32a1*[17]	Down	Down
Sod2	Up	Up	*Snap25*[17]	Down	Down
Irf1[17]	Up	Up	*Slc8a1*[28]	Down	Down
Ccl3[30]	Up	Up	*Tgfb1*[29]	Down	Down
Hspa1b[17]	Up	Up	*Crem*[17]	Up	Down
Gal[17]	Up	Up	*Cntf*[22]	Up	Down
Ccl2[24]	Up	Up	*Timp1*[26]	Up	Down
Nos2[25]	Up	Up	*Hif1a*[27]	Up	Down
Mmp9[26]	Up	Up	*Il6*[17]	Up	Down
App	Up	Up	*Ptgs2* [25]	Up	Down
Cst3	Up	Up			

Supplemental Table 2 . Correlation analysis and 5hmC abundance and lncRNA expression after Stroke [31]

lnc ID	Pubmed ID	Mimicked Gene	Change after stroke	5hmc after stroke
MRAK077719	499497	Mef2c	Up	Down
XR_006073	362726	Atxn7l4	Up	Down
U77626	361029	Ktn1	Up	Down
BC158779	501747	Qrich2	Down	Up
uc.236	306254	Pbrm1	Down	Down
S39217	311029	Neb	Down	Down
MRAK079854	671517	Megf11	Up	None
MRAK078894	29715	Slc8a1	Up	Down
XR_008501	63881	Rapgef1	Up	Up
XR_009527	100912235	Mast4	Up	None
BC086373	501546	Huwe1	Down	Down
BC103647	363767	Abi3bp	Down	Down
uc.308-	686098	Sorbs1	Down	None
uc.408+	498952	Zfp1	Down	Down
MRAK159688	022197	Fos	Up	Up

Supplemental Table 3. Primer sequences for quantitative RT-PCR

Primer	Sequences
Gapdh forward	5’-CAT GGC CTT CCG TGT TCC TA-3’
Gapdh reverse	5’-CTT CAC CAC CTT CTT GAT GTC ATC-3’
Tet1 forward	5’-CCA GGA AGA GGC GACTAC GTT-3’
Tet1 reverse	5’-TTA GTG TTG TGTGAA CCT GAT TTA TTG T-3’
Tet2 forward	5’-ACT TCT CTG CTC ATT CCC ACA GA-3’
Tet2 reverse	5’-TTA GCT CCG ACT TCT CGA TTG TC-3’
Tet3 forward	5’-GAG CAC GCC AGA GAA GAT CAA-3’
Tet3 reverse	5’-CAG GCT TTG CTG GGA CAA TC-3’
BDNF forward	5’-GCCTCCTCTACTCTTTCTGC-3’
BDNF reverse	5’-ATGGGATTACACTTGGTCTC-3’