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Supplementary Materials for

DNA methylation regulates neuronal glutamatergic synaptic scaling

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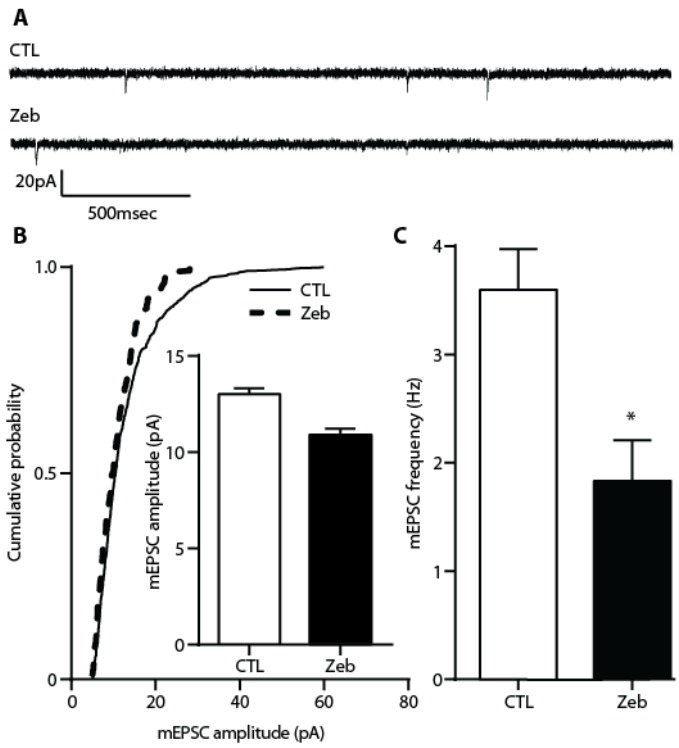

Figure S1. Indirect DNMT inhibition with the nucleoside analog zebularine decreases mEPSC frequency with no effect on amplitude. (**A**) Sample mEPSCs traces from cortical pyramidal neurons after 24 hour control (CTL) or zebularine (Zeb) treatment. (**B and C**) Cumulative probability distributions and mean mEPSC amplitudes (B) and mean mEPSC frequencies (C) from cortical pyramidal neurons exposed to Zeb. Bar graphs are means ± SEM, cumulative of cells pooled from four experiments (CTL, n = 7 cells; Zeb, n = 6 cells); (B) P = 0.0352, Kolmogorov-Smirnov (K – S) test; Inset, P = 0.0352, Mann-Whitney (M – W) test; (C) *$P < 0.05$, Student's unpaired t test.

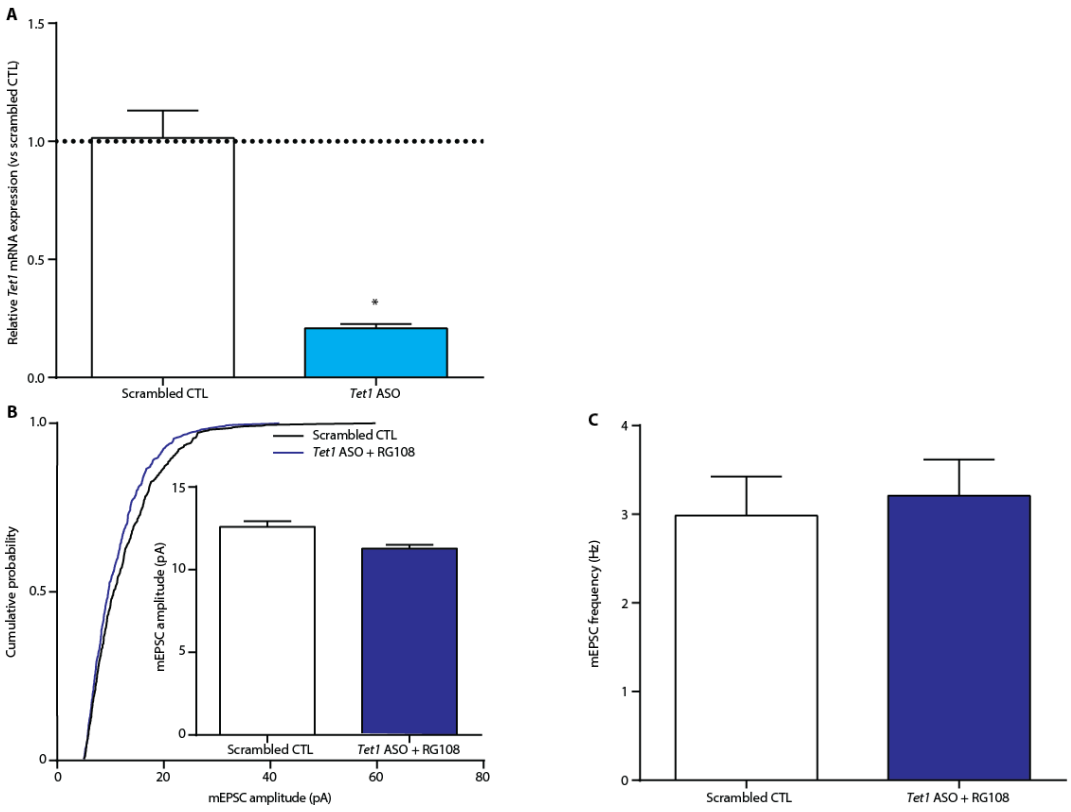

Figure S2. *Tet1* knockdown blocks RG108-induced upscaling of excitatory synaptic strength. An ASO was utilized that selectively targets positions 6059-6078 of the transcript for the TET1 dioxygenase, a known regulator of activity-dependent active de-methylation in neurons. As described in the Materials and Methods, the *Tet1*-targeted ASO nucleotide was a 5-10-5 2'-OMe gapmer. (**A**) Bar graphs show relative expression of *Tet1* mRNA in cultured cortical neurons after *Tet1* ASO (light blue) treatment. Bar graphs are means ± SEM from 3 biological replicates from one experiment, each performed in technical triplicate; **P* < 0.05 compared to scrambled CTL, Student's unpaired *t* test. (**B and C**) Cumulative probability distributions and mean mEPSC amplitudes (B) and mean mEPSC frequencies (C) from cortical pyramidal cells after*Tet1* ASO + RG108 treatment (dark blue). Bar graphs are means ± SEM. Data are cumulative of cells pooled from two independent experiments (scrambled CTL, n = 4 cells; Tet1 ASO + RG108, n = 7 cells); (B) *P* = 0.0182 compared to scrambled CTL, K – S test;) Inset, *P* = 0.0041, M – W test; (C) *P* = 0.7142, Student's unpaired *t* test.

Table S1: Primers used in this study.

Gene	**Sense primer (5'-> 3')**	**Antisense primer (5'-> 3')**
Gapdh	ACCTTTGATGCTGGGGCTGGC	GGGCTGAGTTGGGATGGGGACT
Bdnf IX	GAGAAGAGTGATGACCATCCT	TCACGTGCTCAAAAGTGTCAG
Smug1	TCGCTACTGCCAAGGCCCCA	AGGGGACAGCACAGAGCCCC
Arc/Arg3.1	GCTGAAGCAGCAGACCTGA	TTCACTGGTATGAATCACTGCTG
Apobec1	GCACGGCTTTATCACCACGCA	CCCACAGATGGGGGTACCTTGG
Mecp2	TTGCCTGAAGGTTGGACGCGA	GGGTCCAAGGAGGTGTCTCCCA
Dnmt1	GTGTGCGGGAATGTGCTCGCT	CAGTGGTGGTGGCACAGCGT
Dnmt3a	AGCAAAGTGAGGACCATTACCACCA	TGTGTAGTGGACAGGGAAGCCA
Dnmt3a1	TGCCAAGACTCACCTTCCAG	GGCTTTCCTCCACAGCATTC
Dnmt3a2	CTGTACTGCAGAGGGGCTG	CTGGCTTTCCTCCACAGCAT
Dnmt3b	TGGCAAGGATGACGTTCTGTGGT	CTGGCACACTCCAGGACCTTCC
Tet1	GCCAACCAGGAAGAGGCGACTG	GAGGAAGCCTGCAGGGGACAG
Tet3	GGAGTTGGCTGGAGTCACCACT	CCGAGTAGCTCTCCACCACAGCA