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Supplemental information

Loss of neuronal Tet2 enhances hippocampal-dependent cognitive function

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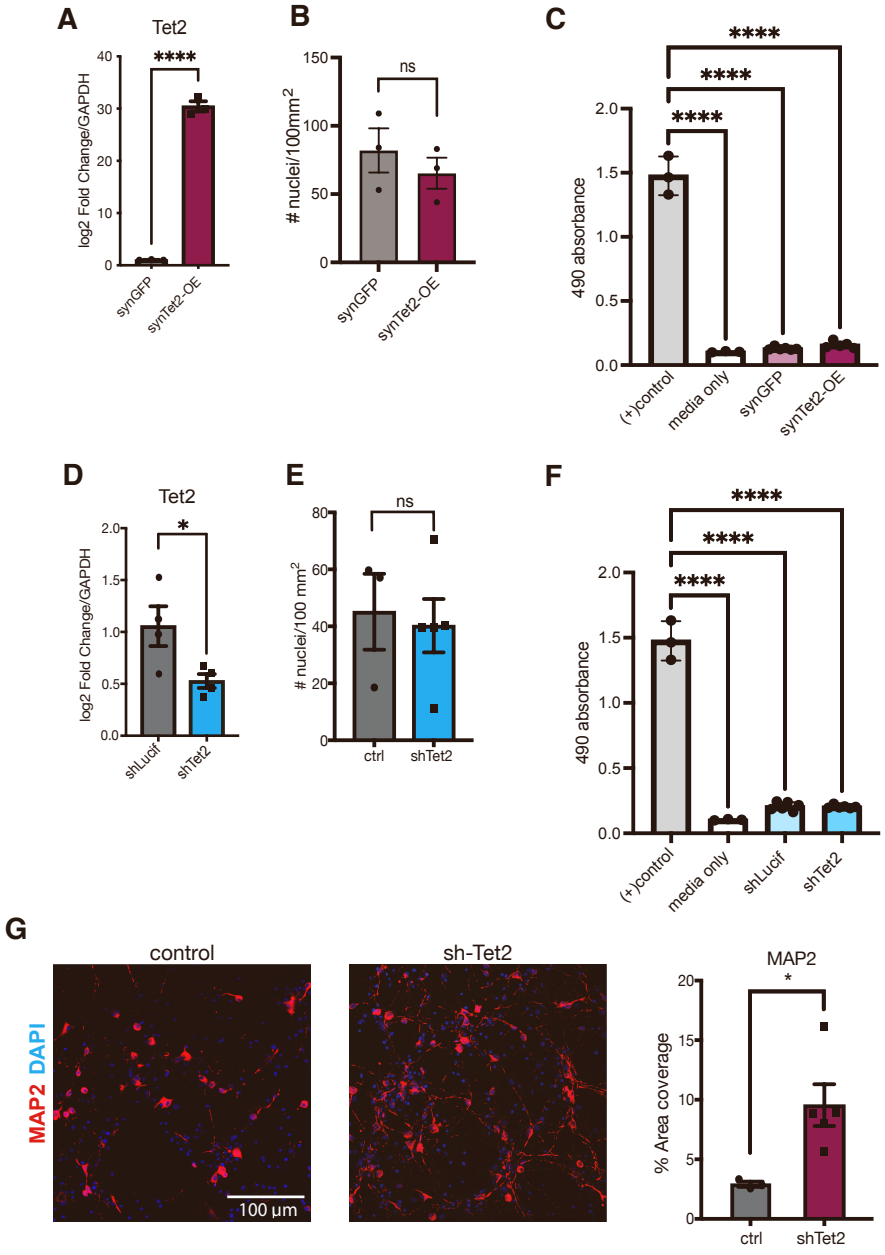

Figure S1. Tet expression and cell viability in primary neurons following neuronal Tet2 overexpression and abrogation. Related to Figure 1.

A, Quantitative reverse-transcription PCR of Tet2 mRNA from primary neurons infected with lentivirus encoding Tet2 (synTet OE) or GFP control (synGFP) under the control of the neuron-specific Synapsin1 promoter. Log2 fold change normalized to GAPDH. (n=3 per group; t-test; *p<0.05).

B, Quantification of number of Hoescht-positive nuclei following Tet2 overexpression (n=3 per group, t-test).

C, Cytotoxicity was measured by lactate dehydrogenase (LDH) activity as relative absorbance following Tet2 overexpression (n=3 per group, t-test).

D, Quantitative reverse-transcription PCR of Tet2 mRNA from primary neurons infected with lentivirus encoding shRNA targeting Tet2 (shTet2) or luciferase control (shCtrl). Log2 fold change normalized to GAPDH. (n=3-4 per group; t-test; *p<0.05)

E, Quantification of number of Hoescht-positive nuclei following Tet2 abrogation (n=3-5 per group, t-test).

F, Cytotoxicity was measured by lactate dehydrogenase (LDH) activity as relative absorbance following Tet2 abrogation (n=5-6 per group, t-test).

G, Representative images and quantification of Immunocytochemistry (ICC) for Map2 expression normalized to number of DAPI-positive nuclei. (n=5 per group; t-test; *p<0.05).

Data are represented as mean +/- SEM.

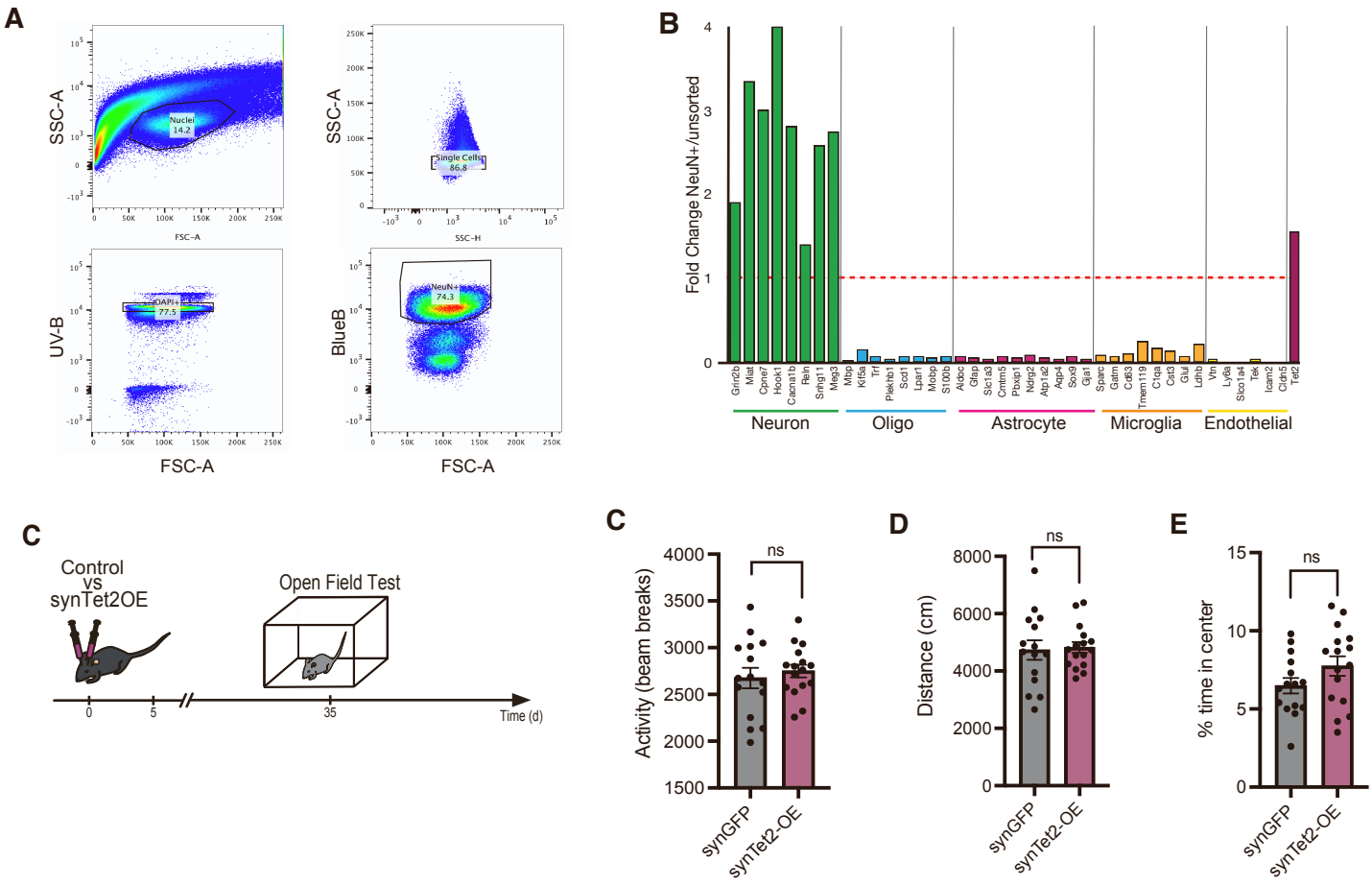

Figure S2. Validation of neuronal nuclei isolation for cell-type specific sequencing analysis and overall health analysis following in vivo viral-mediated neuronal Tet2 overexpression. Related to Figure 2.

A, Representative fluorescence-activated cell sorting (FACS) gating paradigm for isolation of NeuN-positive neuronal nuclei.

B, Fold change of differentially expressed cell-type specific genes between NeuN-positive nuclei and unsorted lysate from RNA sequencing data (all genes adjusted p value with Bonferroni's multiple testing hypothesis correction, *p<0.05)

C, Schematic of experimental paradigm. Adult (3-4 months) wild type mice were given bilateral stereotaxic injections of lentivirus (LV) encoding either Tet2 (Tet2 OE) or GFP control sequences driven by the neuron-specific Synapsin1 promoter into the hippocampus and subject to behavioral analysis 5 weeks later

D, Overall activity was assessed by open field testing and quantified as distance traveled over 10 minutes. (n=15-16 mice per group; t-test)

Data are represented as mean +/- SEM.

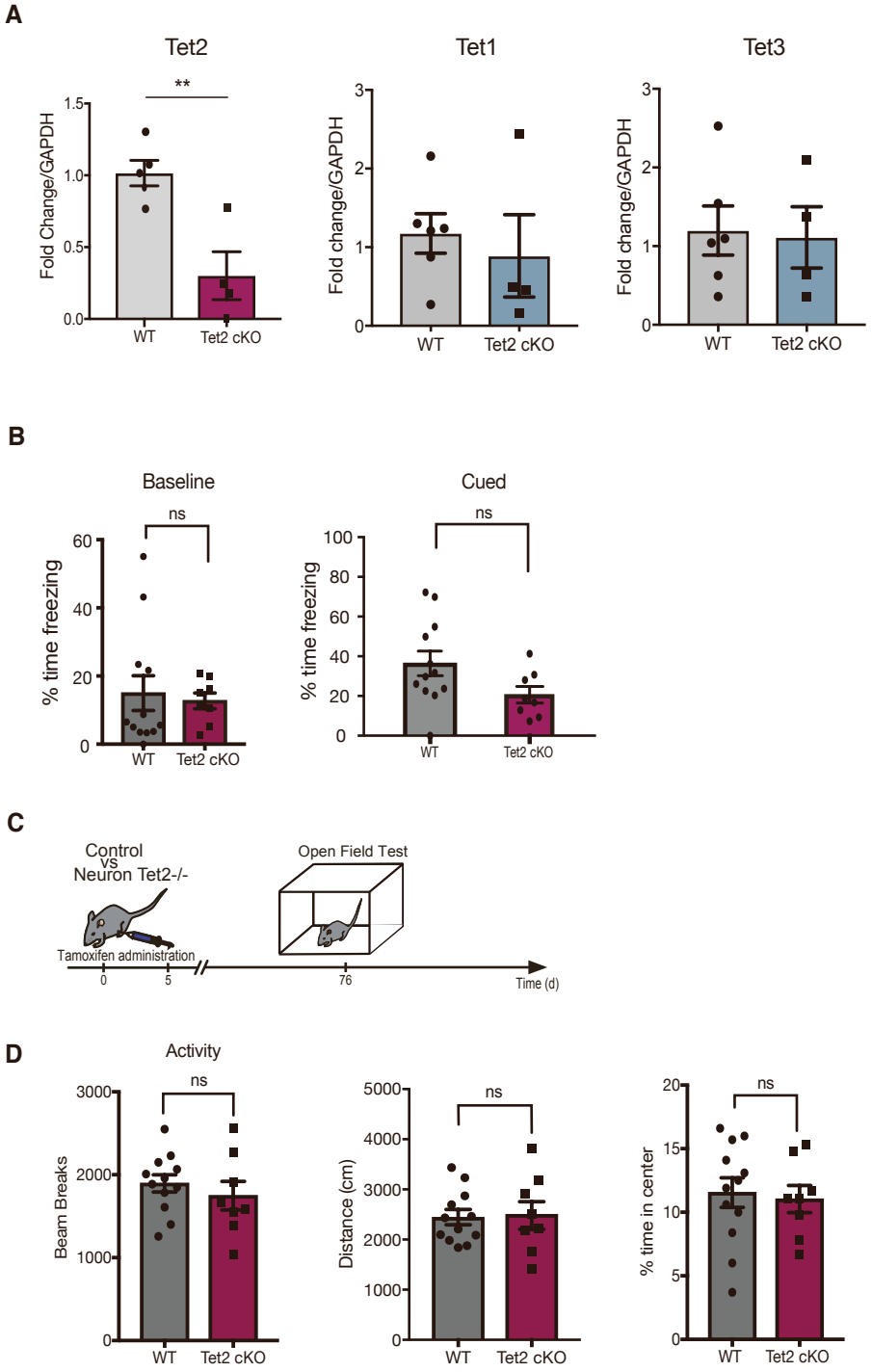

Figure S3. Tet expression and overall health analysis following temporally controlled in vivo loss of neuronal Tet2. Related to Figure 3.

A, Quantitative reverse-transcription PCR of Tet1, Tet2, and Tet3 mRNA from the hippocampus of adult CamK2aCre-ERT2;Tet2 flox/flox (Tet2 cKO) and littermate Tet2 flox/flox control (WT) mice following tamoxifen administration. Log2 fold change normalized to GAPDH. (n=4-5 mice per group; t-test; **p<0.01)

B, Associative fear memory was assessed in Tet2 cKO and WT mice using fear conditioning paradigms. Baseline freezing was quantified as percent freezing in the first two minutes of training. (n=8-12 mice per group; t-test)

C, Tet2 cKO and littermate WT control mice were administered tamoxifen and subject to behavioral assays after four months.

D, Overall activity was assessed by open field testing and quantified as distance traveled over 10 minutes. (n=8-12 mice per group; t-test)

Data are represented as mean +/- SEM.