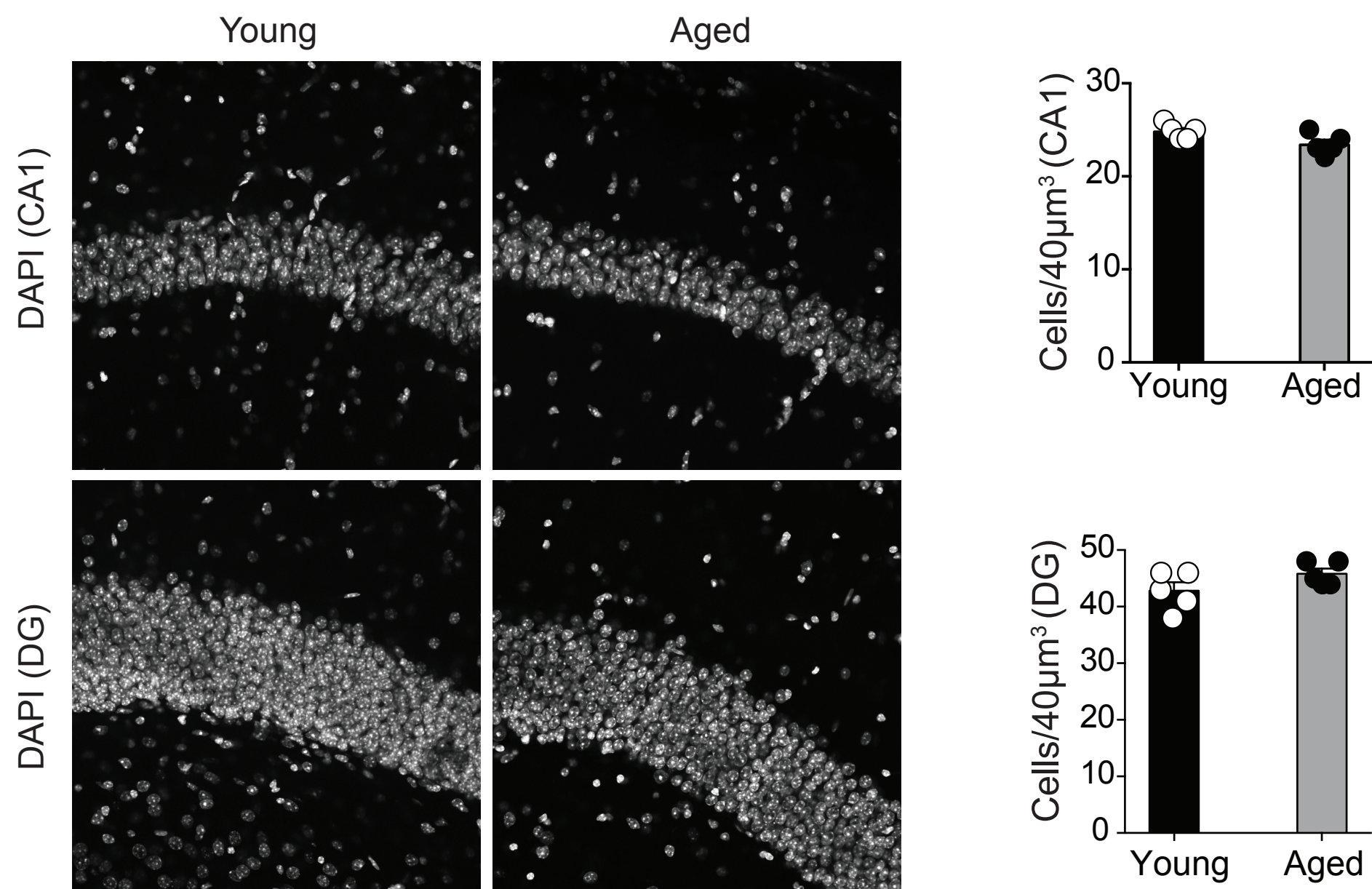
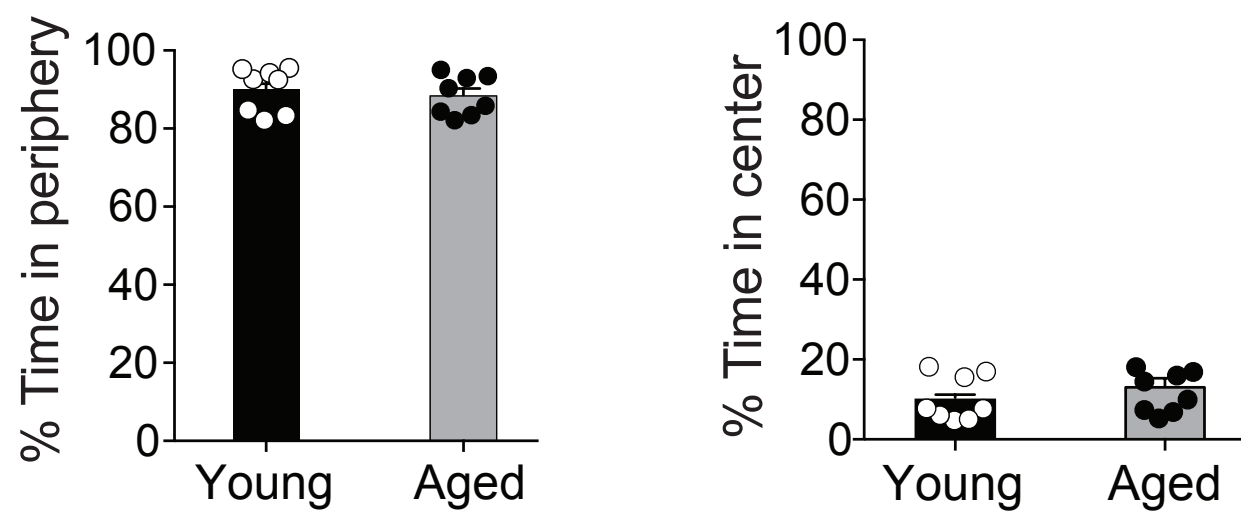
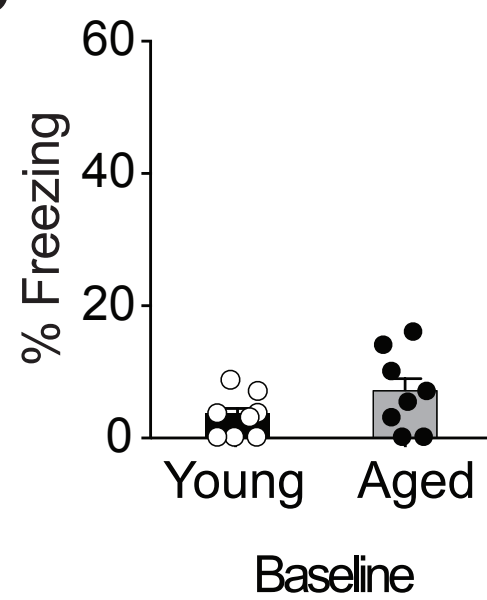


Current Biology, Volume 29

Supplemental Information

**Neuronal O-GlcNAcylation Improves
Cognitive Function in the Aged Mouse Brain**

Elizabeth G. Wheatley, Eddy Albarran, Charles W. White III, Gregor Bieri, Cesar Sanchez-Diaz, Karishma Pratt, Cedric E. Snelthage, Jun B. Ding, and Saul A. Villeda

A**B****C**

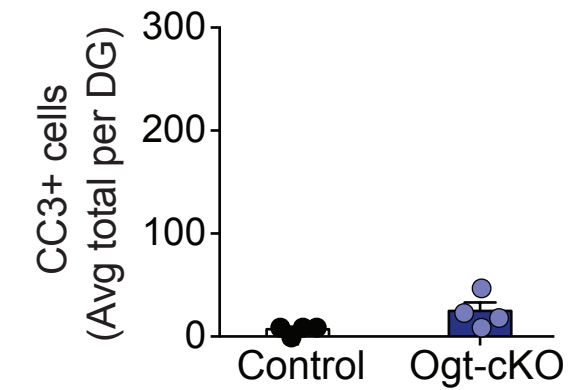
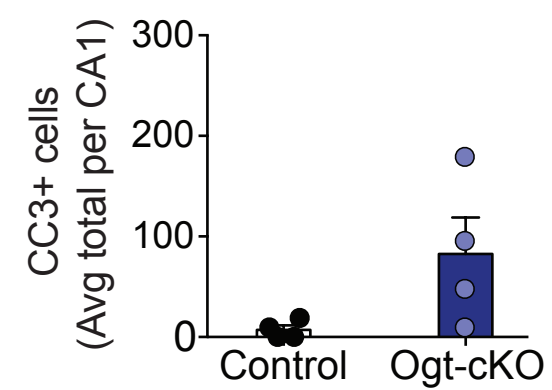
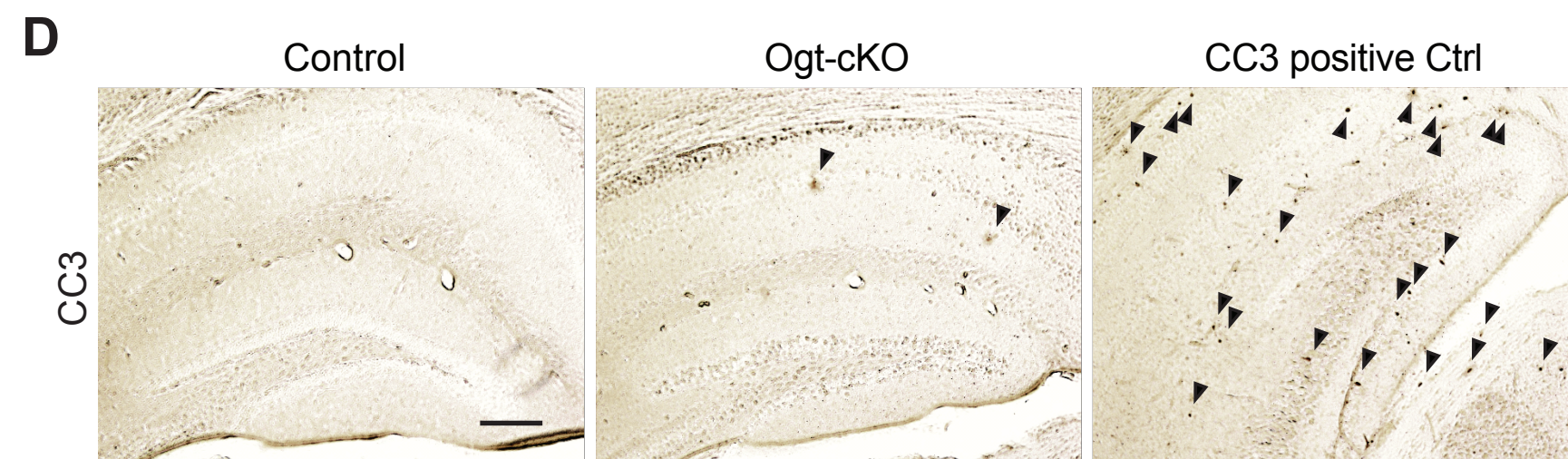
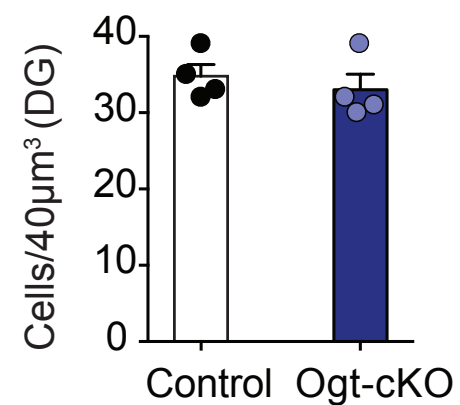
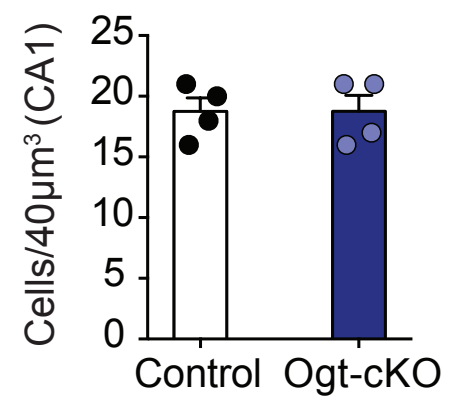
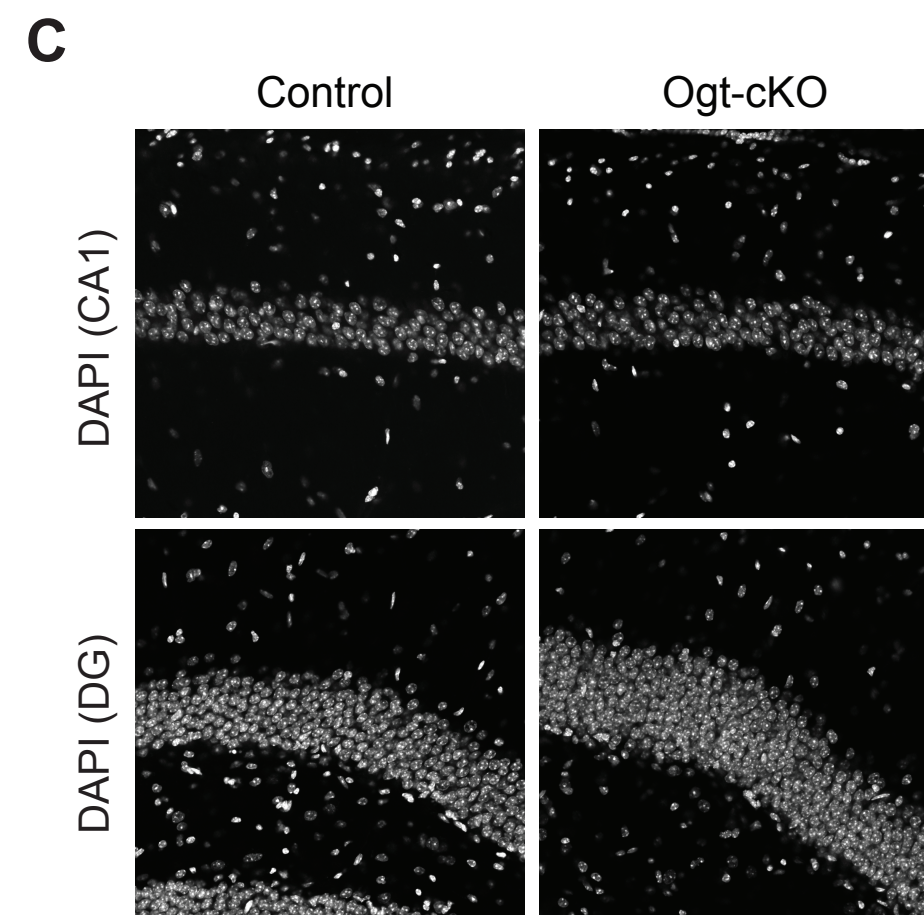
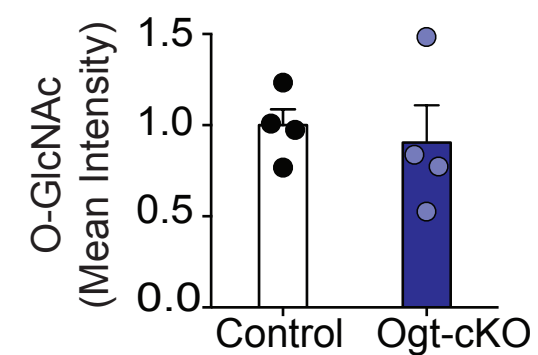
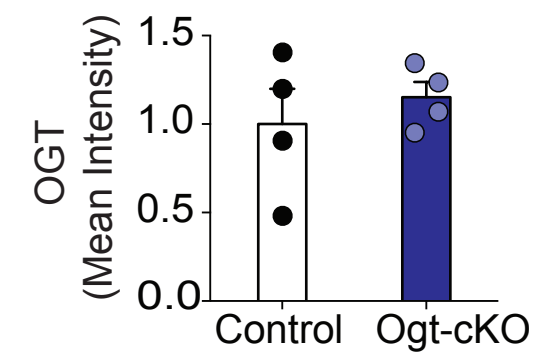
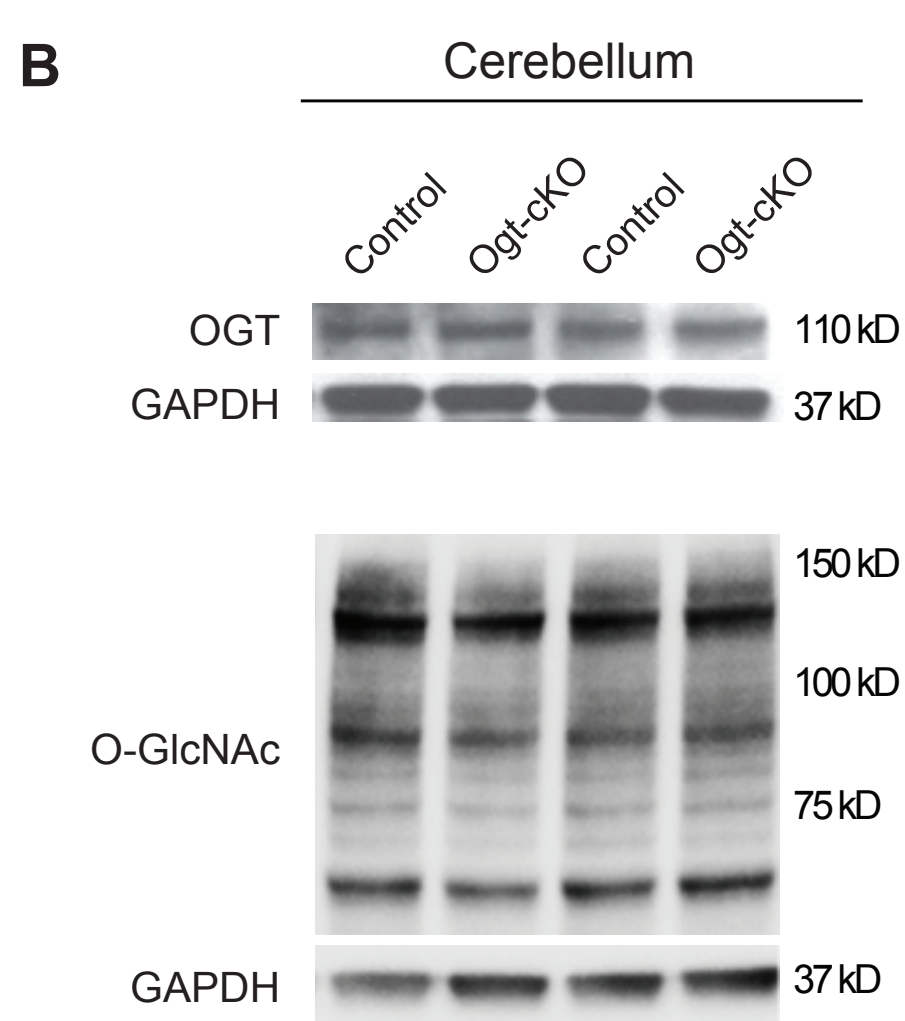
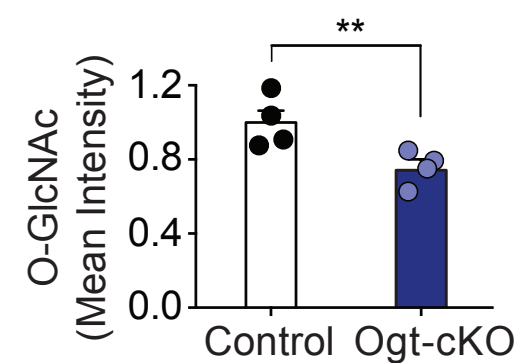
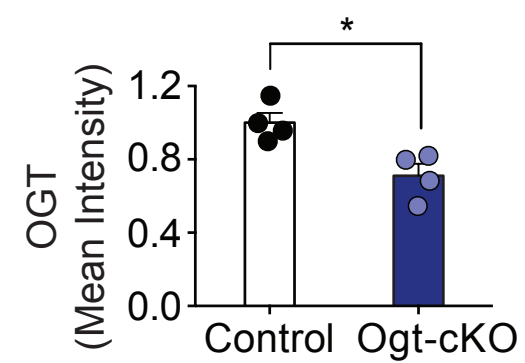
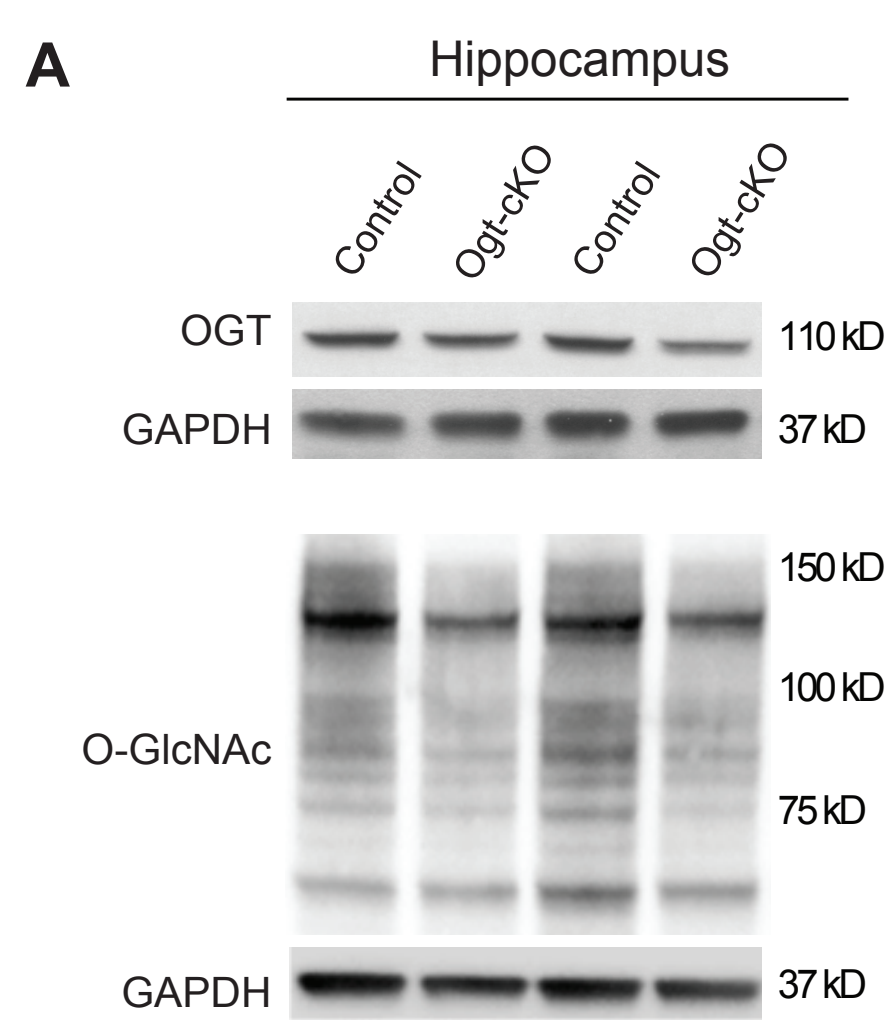
**Figure S1. Cellular and behavioral characterization of young and aged mice.
Related to Figure 1.**

(A) Representative images of DAPI stain in the CA1 and dentate gyrus (DG) regions of the hippocampus of young (3 months) and aged (21 months). Scale bar=40 μ m. Quantification of number of DAPI-positive cells per 40 μ m³. $n = 4$ mice per group.

(B) Data from the open field paradigm depicting percent time spent in periphery and center of the field of Ogt-cKO and control mice. Data shown are cumulative from ten minutes of testing, measured using the MotorMonitor software. $n = 8$ mice per group.

(C) Baseline freezing was measured during the first two minutes of training during fear conditioning. $n = 8$ mice per group.

Data represented as mean \pm SEM; t-test (**A-C**).



**Figure S2. Molecular and cellular characterization of OGT-cKO mouse model.
Related to Figure 2.**

(A) Representative Western blot of OGT protein and O-GlcNAc levels from whole hippocampal lysates of young Ogt-cKO and control mice (4 months). Quantification represented as mean optical intensity normalized to GAPDH. $n = 4$ mice per group.

(B) Representative Western blot of OGT protein and O-GlcNAc levels from whole cerebellum lysates of young (4 months) Ogt-cKO and control mice. Quantification represented as mean optical intensity normalized to GAPDH. $n = 4$ mice per group.

(C) Representative images of DAPI stain in the CA1 and dentate gyrus (DG) regions of the hippocampus of young (4 months) Ogt-cKO and control mice. Scale bar=40 μ m. Quantification of number of DAPI-positive cells per 40 μ m³. $n = 4$ mice per group.

(D) Representative images of cleaved caspase 3 (CC3) immunostain in the hippocampus of young Ogt-cKO and control mice. A positive control mouse known to have CC3-positive cells in the hippocampus was included. Quantification of average total CC3-positive cells in CA1 and DG regions of the hippocampus. $n = 4$ mice per group.

Data represented as mean \pm SEM; t-test (A-D).

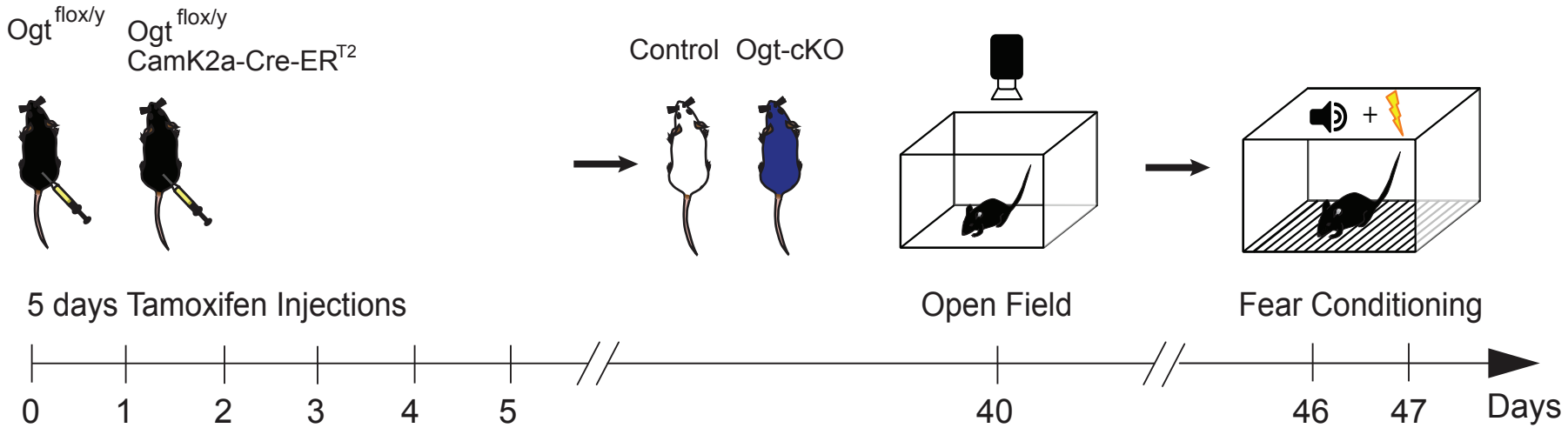
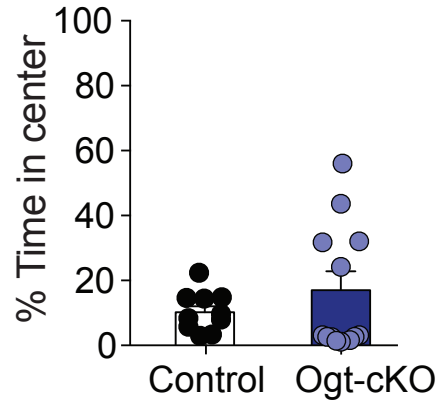
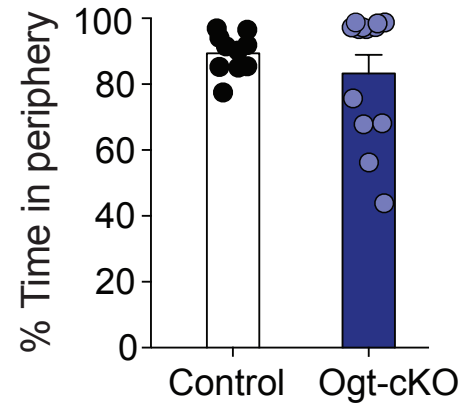
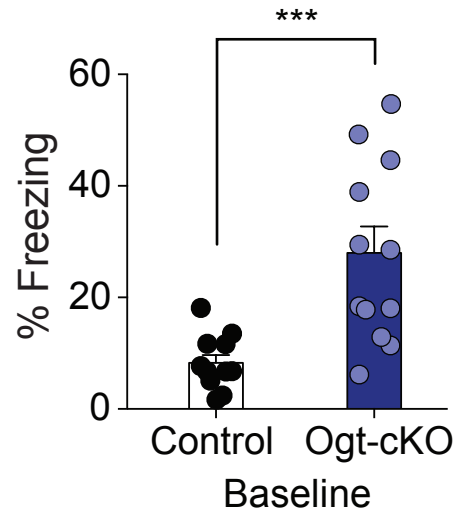
A**B****C**

Figure S3. Behavioral assessment of Ogt-cKO mouse model. Related to Figure 4.

(A) Schematic depicting generation of Ogt-cKO mice and subsequent cognitive testing using open field, radial arm water maze (RAWM), and fear conditioning behavioral paradigms.

(B) Data from the open field paradigm depicting percent time spent in periphery and center of the field of young (4 months) Ogt-cKO and control mice. Data shown are cumulative from ten minutes of testing, measured using the MotorMonitor software.

(C) Baseline freezing was measured during the first two minutes of training during fear conditioning. $n = 12$ Ogt-cKO and 10 control mice.

Data represented as mean \pm SEM; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; t-test (**B,C**).

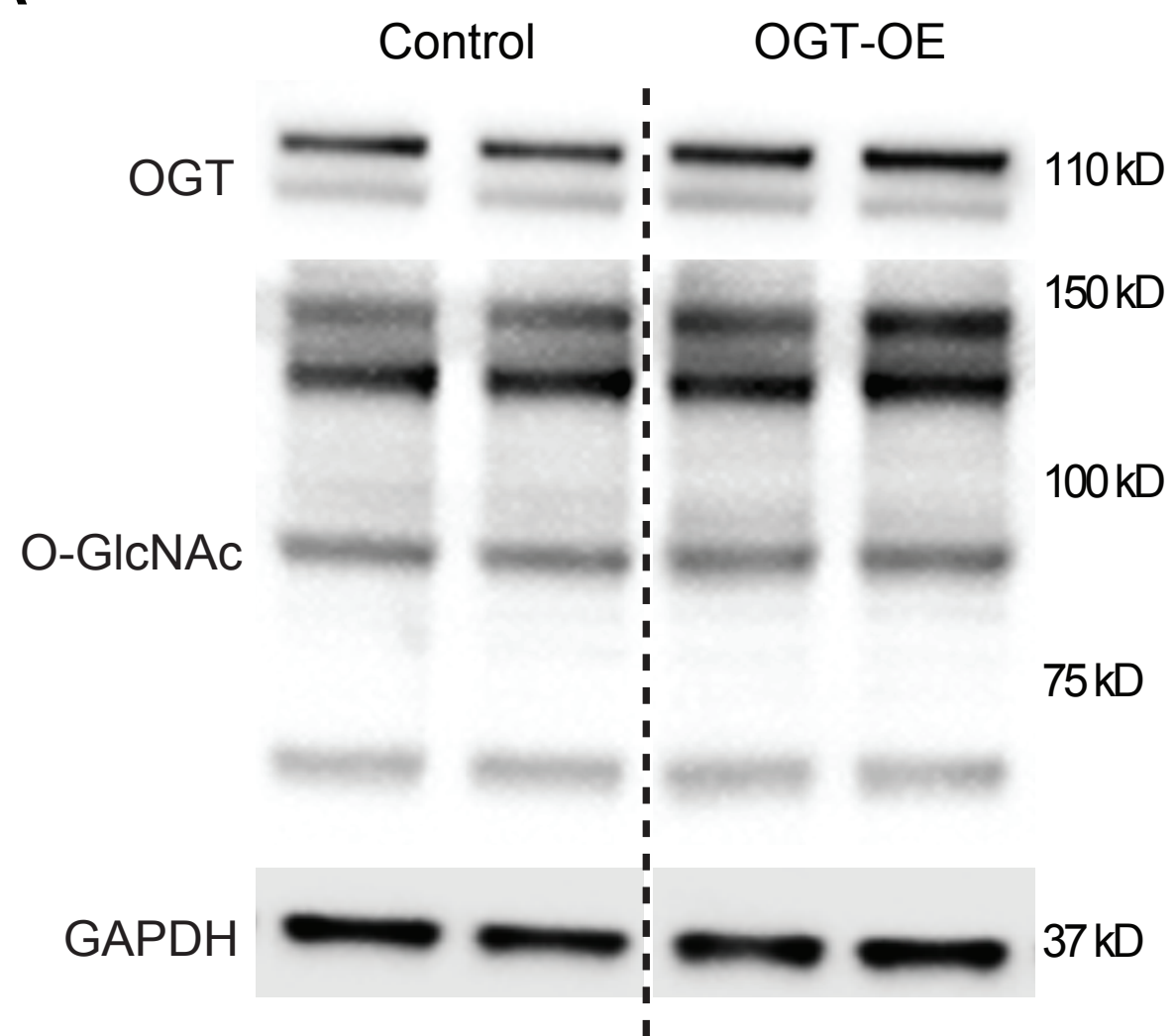
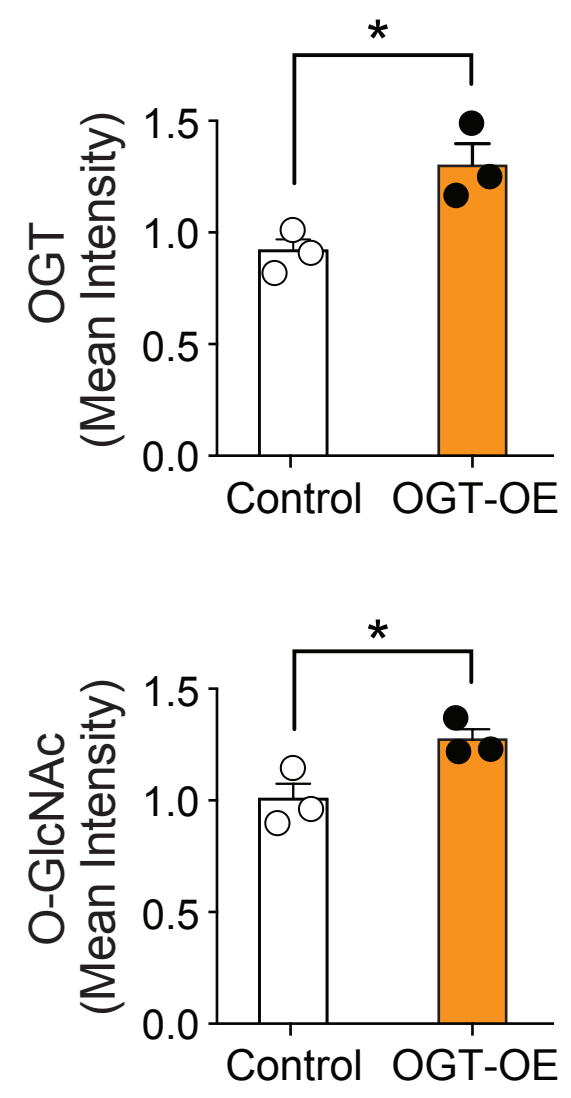
A**B**

Figure S4. Validation of lentiviral construct overexpressing neuronal OGT. Related to Figure 5 and Figure 6.

(A,B) Lentiviral constructs encoding Ogt or green fluorescent protein (GFP) under the neuron-specific Synapsin promoter were generated for overexpression (OE). Young (3 months) wild type mice were given stereotaxic injections of high-titer virus encoding Ogt or GFP into their hippocampi. Representative Western blots of OGT protein and O-GlcNAc levels from whole hippocampal lysates (A). Quantification represented as mean optical intensity normalized to GAPDH (B). $n = 3$ mice per group. Data represented as mean \pm SEM * $P < 0.05$; t-test.

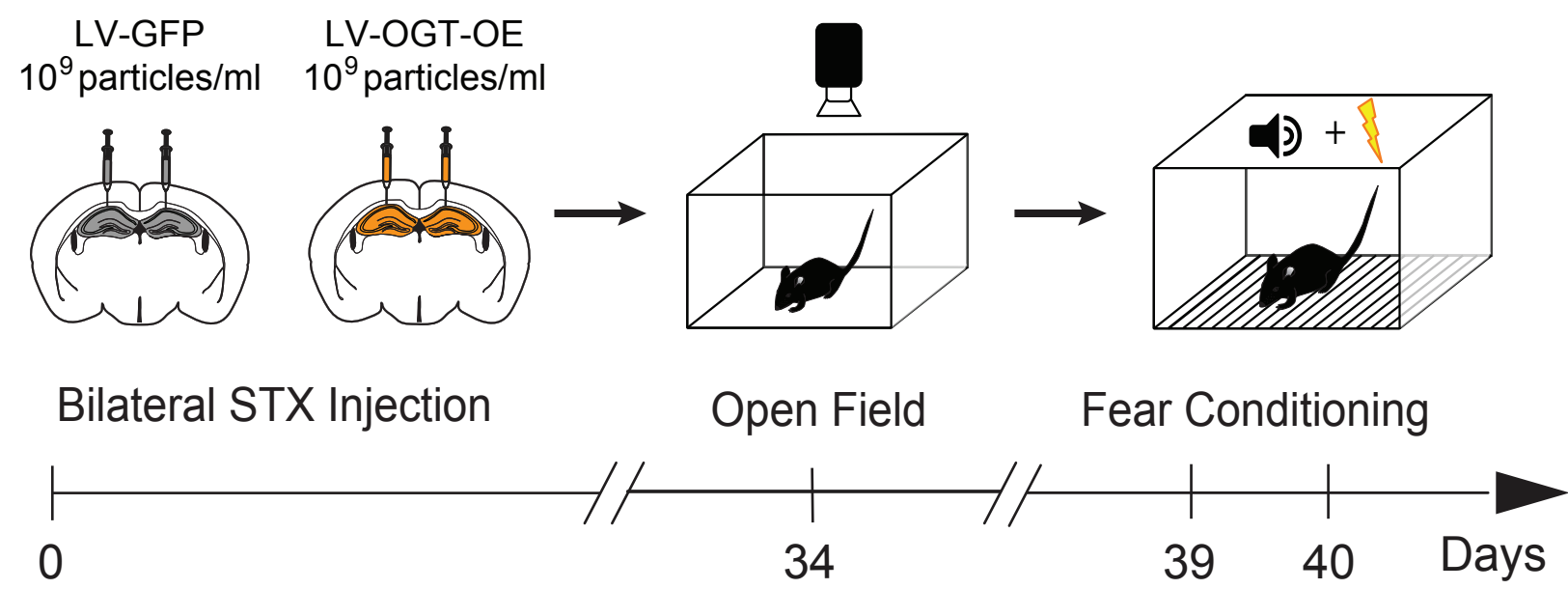
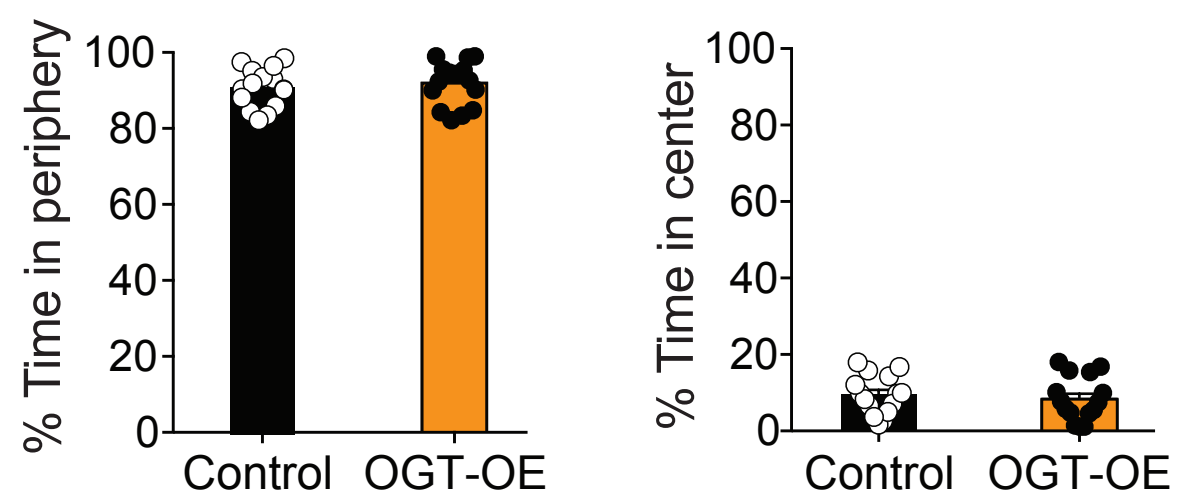
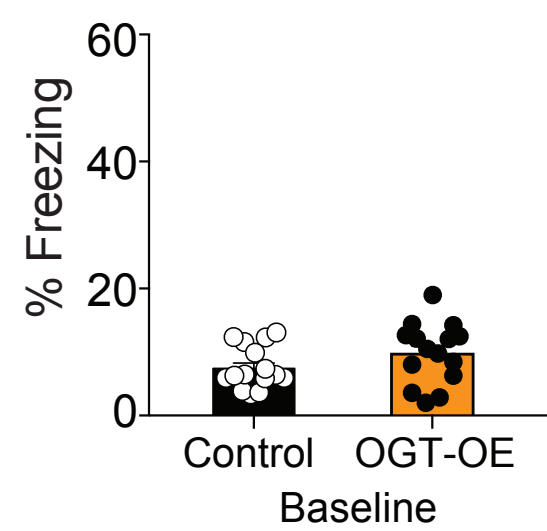
A**B****C**

Figure S5. Behavioral assessment of young mice in which OGT is overexpressed in mature hippocampal neurons. Related to Figure 5.

(A) Schematic depicting experimental paradigm and timeline for cognitive testing using open field, radial arm water maze (RAWM), and fear conditioning behavioral paradigms. Young (3 months) wild type mice were given bilateral stereotaxic injections of lentivirus (LV) encoding either OGT (OGT-OE) or green fluorescent protein (GFP) control sequences driven by the neuron-specific Synapsin promoter into the hippocampus.

(B) Data from the open field paradigm depicting percent time spent in periphery and center of the field of OGT-OE and control mice. Data shown are cumulative from ten minutes of testing, measured using the MotorMonitor software. $n = 15$ mice per condition.

(C) Baseline freezing was measured during the first two minutes of training during fear conditioning. $n = 15$ mice per condition.

Data represented as mean \pm SEM; t-test.

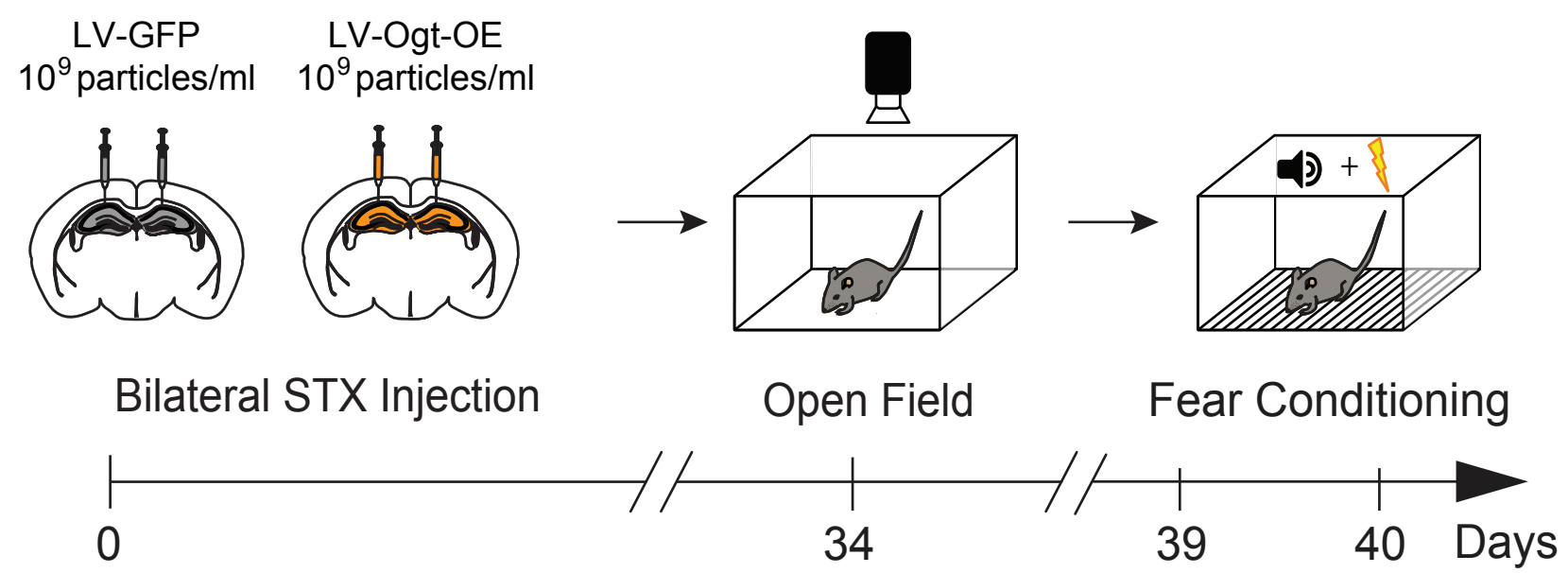
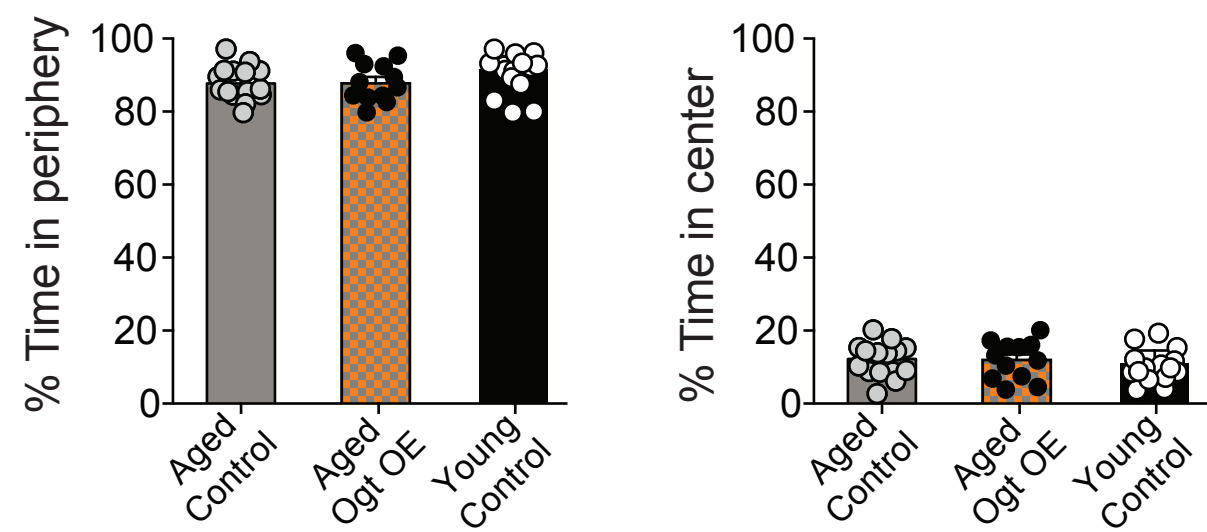
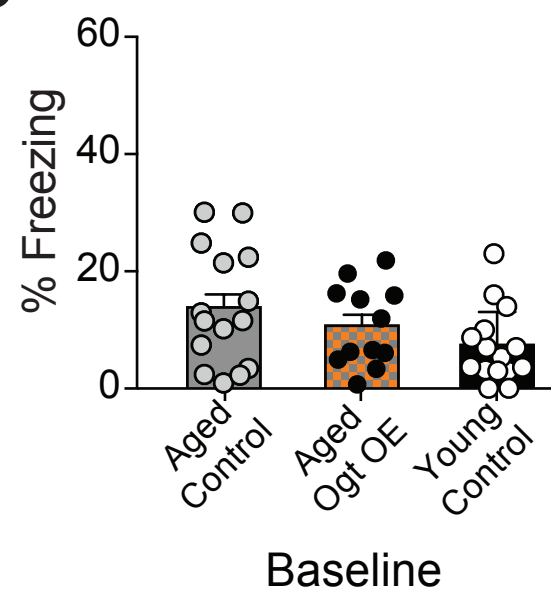
A**B****C**

Figure S6. Behavioral assessment of aged mice in which OGT is overexpressed in mature hippocampal neurons. Related to Figure 6.

(A) Schematic depicting experimental paradigm and timeline for cognitive testing using open field, radial arm water maze (RAWM), and fear conditioning behavioral paradigms. Aged (21 months) wild type mice were given bilateral stereotaxic injections of lentivirus (LV) encoding either OGT (OGT-OE) or green fluorescent protein (GFP) control sequences driven by the neuron-specific Synapsin promoter into the hippocampus.

(B) Data from the open field paradigm depicting percent time spent in periphery and center of the field of aged OGT-OE, aged control mice, and naïve young control mice. Data shown are cumulative from ten minutes of testing, measured using the MotorMonitor software. $n = 12$ aged OGT-OE, 15 aged control, and 15 young control mice.

(C) Baseline freezing was measured for aged OGT-OE, aged control mice, and naïve young control mice during the first two minutes of training during fear conditioning. $n = 12$ aged OGT-OE, 15 aged control, and 15 young control mice.

Data represented as mean \pm SEM; one-way ANOVA, Tukey's post-hoc test (B,C).