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Supplementary Information for

AGE-RELATED LOSS OF NEURAL STEM CELL O-GLCNAC PROMOTES A GLIAL FATE SWITCH THROUGH STAT3 ACTIVATION

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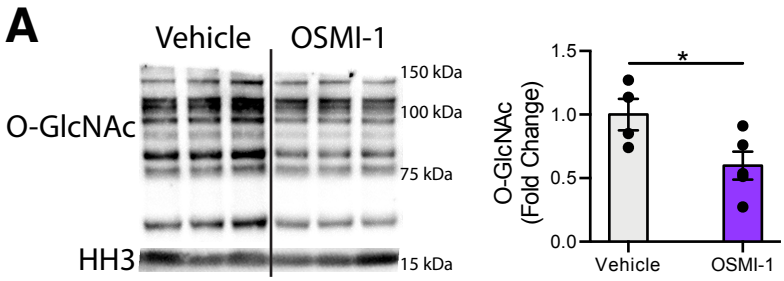
A
Vehicle
OSMI-1
O-GlcNAc
HH3
150 kDa
100 kDa
75 kDa
15 kDa
O-GlcNAc (Fold Change)
1.5
1.0
0.5
0.0
*
Vehicle
OSMI-1

Figure S1

Figure S1. Pharmacological inhibition of Ogt by OSMI-1 treatment reduces O-GlcNAc levels in NSCs *in vitro.*

(**A**) O-GlcNAc levels were assessed by Western blot in primary hippocampal neural stem cells following 24-hour vehicle or OSMI-1 treatment. Data are represented as mean ± SEM; *p<0.05; t test; n=4-5 per group.

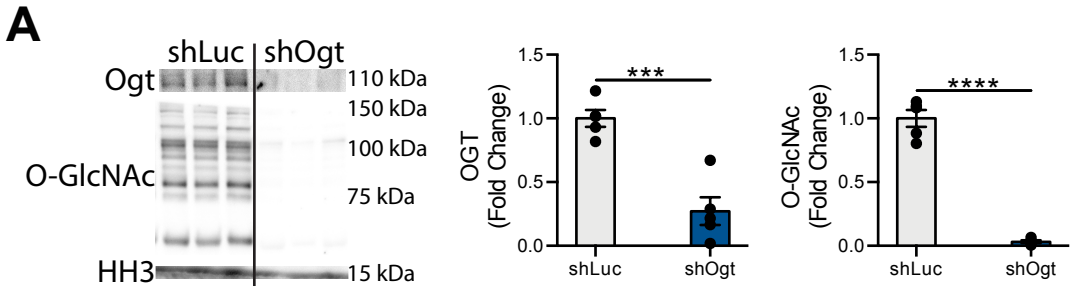
A
shLuc
shOgt
Ogt
O-GlcNAc
HH3
110 kDa
150 kDa
100 kDa
75 kDa
15 kDa
OGT (Fold Change)
1.5
1.0
0.5
0.0
***
shLuc
shOgt
O-GlcNAc (Fold Change)
1.5
1.0
0.5
0.0
****
shLuc
shOgt

Figure S2

Figure S2. Abrogation of Ogt reduces O-GlcNAc levels in NSCs *in vitro*

(**A**) O-GlcNAc and Ogt levels were assessed by Western blot in primary hippocampal neural stem cells following infection with shLuc or shOgt. Data are represented as mean ± SEM; *p<0.05; t test; n=4-5 per group.

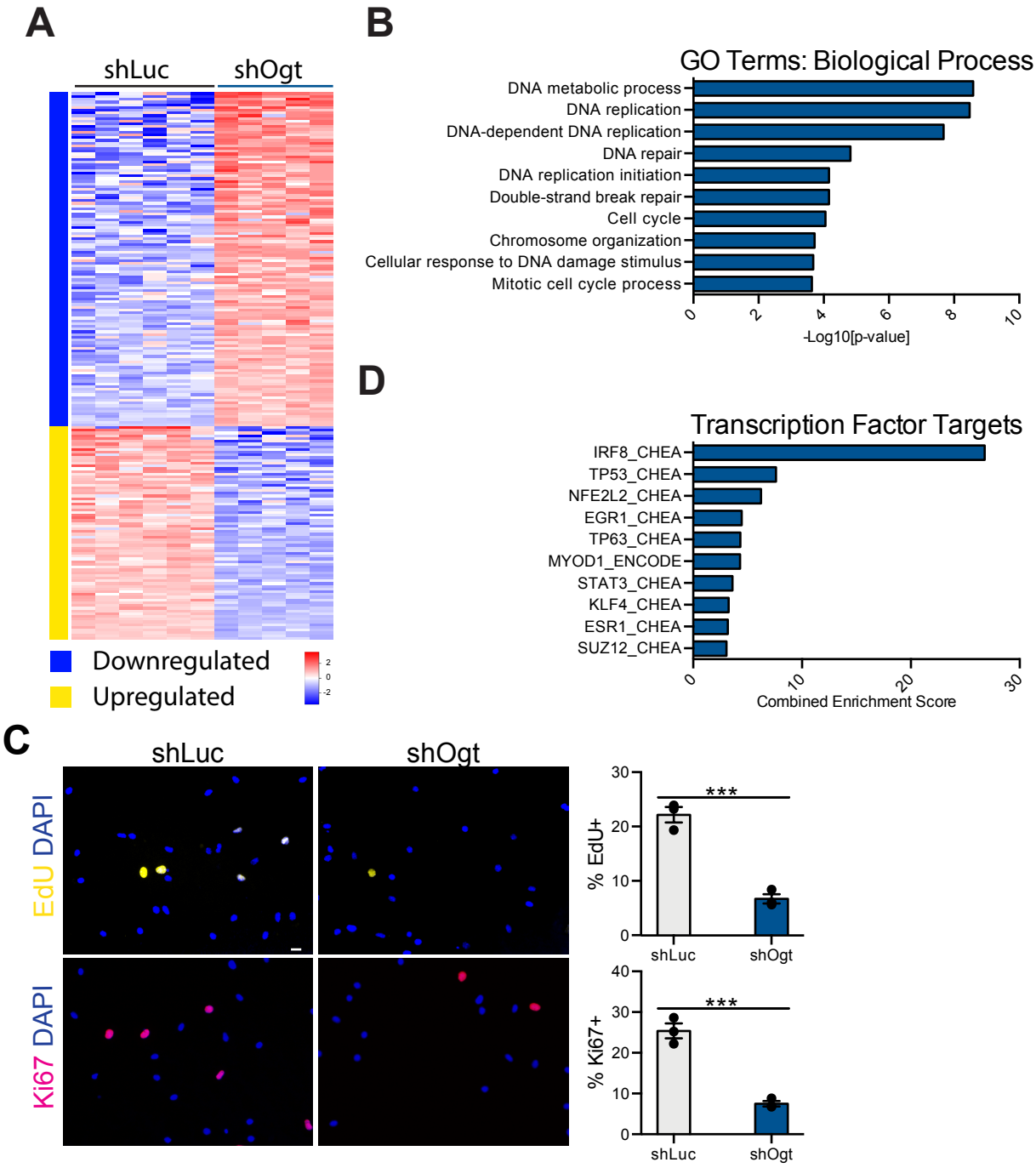
A
shLuc
shOgt
Downregulated
Upregulated
B
GO Terms: Biological Process
DNA metabolic process
DNA replication
DNA-dependent DNA replication
DNA repair
DNA replication initiation
Double-strand break repair
Cell cycle
Chromosome organization
Cellular response to DNA damage stimulus
Mitotic cell cycle process
0
2
4
6
8
10
-Log10[p-value]
D
Transcription Factor Targets
IRF8_CHEA
TP53_CHEA
NFE2L2_CHEA
EGR1_CHEA
TP63_CHEA
MYOD1_ENCODE
STAT3_CHEA
KLF4_CHEA
ESR1_CHEA
SUZ12_CHEA
0
10
20
30
Combined Enrichment Score
C
shLuc
shOgt
EdU DAPI
Ki67 DAPI
% EdU+
% Ki67+
***
***
shLuc
shOgt
shLuc
shOgt

Figure S3

Figure S3. Abrogation of Ogt impairs NSC proliferation *in vitro*.

(**A**) Heatmap of significantly differentially expressed genes with fold change>2 and p<0.05 in primary hippocampal neural stem cells (NSCs) following 72-hour shOgt infection compared to vehicle shLuc control *in vitro*. (**B**) Biological Process Gene Ontology terms associated with genes downregulated following shOgt infection. (**C**) Representative field and quantification of primary mouse hippocampal NSCs following 72 hours of shLuc (left) or shOgt (right) treatment, labeled with DAPI and an 8-hour EdU-pulse (top), or DAPI and anti-Ki67 (bottom) (n=3 per group; scale bar, 10μm). (**D**) Quantification of enriched transcription factor targets as identified by ChEA or ENCODE based on upregulated genes after shOgt treatment compared to shLuc control. Data are represented as mean ± SEM; ***p<0.001; t test; n=3 per group. Scale bar, 10μm.

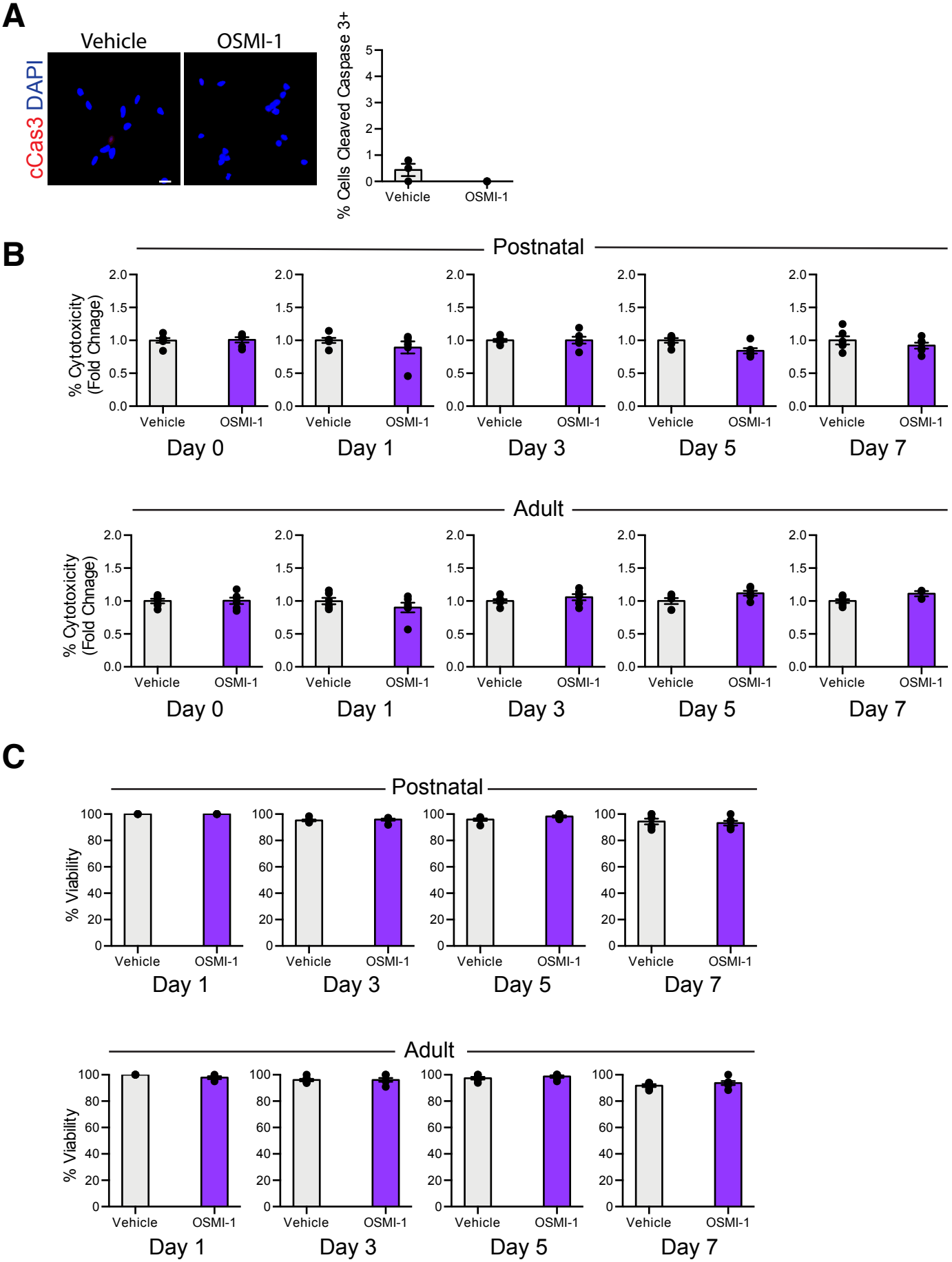
A
Vehicle
OSMI-1
cCas3 DAPI
% Cells Cleaved Caspase 3+
Vehicle
OSMI-1
B
Postnatal
% Cytotoxicity (Fold Chnage)
Day 0
Day 1
Day 3
Day 5
Day 7
Adult
C
Postnatal
% Viability
Day 1
Day 3
Day 5
Day 7
Adult

Figure S4

Figure S4. Pharmacological inhibition of Ogt by OSMI-1 treatment does not increase NSC cell death or cytotoxicity.

(**A**) Primary hippocampal neural stem cells (NSCs) were treated with OSMI-1 or vehicle for 24 hours. Cell death was assessed by immunostaining for Cleaved Caspase 3 (n=3 per group; scale bar, 10μm). (**B**) Primary postnatal and adult hippocampal NSCs were treated with OSMI-1 or vehicle and differentiation was induced by withdrawing growth factors. Cytotoxicity was assessed by lactate dehydrogenase activity at 0, 1, 3, 5, and 7 days post growth factor withdrawal (n=6 replicates per group). (**C**) Primary postnatal and adult hippocampal NSCs were treated with OSMI-1 or vehicle and differentiation was induced by withdrawing growth factors. Cell viability was assessed by trypan blue exclusion at 1, 3, 5, and 7 days post growth factor withdrawal (n=6 replicates per group). Data are represented as mean ± SEM; ns; t test.

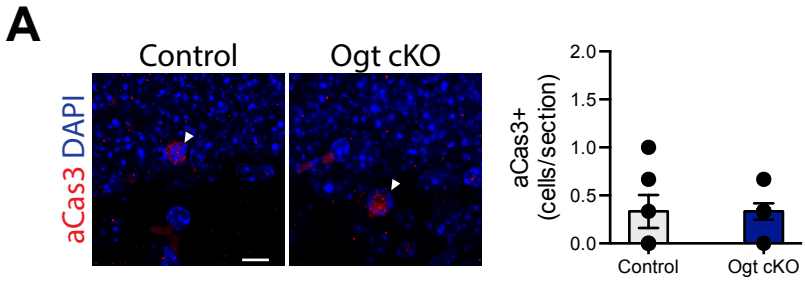
A
Control
Ogt cKO
aCas3 DAPI
aCas3+
(cells/section)
2.0
1.5
1.0
0.5
0.0
Control
Ogt cKO

Figure S5

Figure S5. Abrogation of Ogt does not induce cell death *in vivo*.

(**A**) Representative maximum intensity projections of a confocal image z-stack of control or Ogt cKO hippocampus, labeled with DAPI and anti-cleaved caspase 3 (left). Quantification of cleaved caspase-3-positive cells in control or Ogt cKO littermate mice (n=3 per group). Data are represented as mean ± SEM; ns; t test. Scale bar, 10μm.

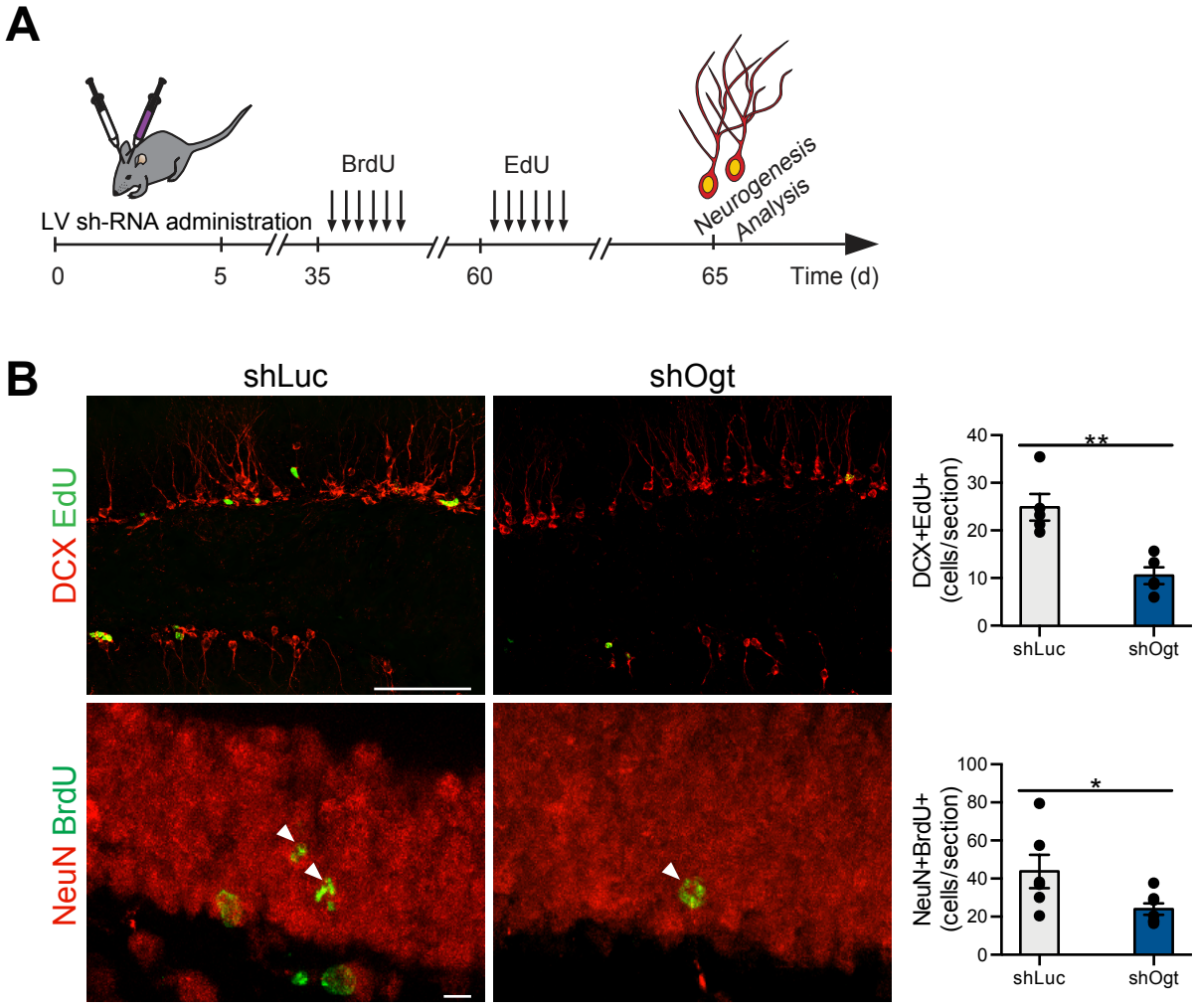
A
BrdU
EdU
LV sh-RNA administration
Neurogenesis Analysis
0
5
35
60
65
Time (d)
B
shLuc
shOgt
DCX EdU
NeuN BrdU
DCX+EdU+ (cells/section)
NeuN+BrdU+ (cells/section)
**
*
shLuc
shOgt

Figure S6

Figure S6. Abrogation of Ogt impairs hippocampal neurogenesis *in vivo*.
(**A**) Schematic of experimental paradigm. 3-month old mice were stereotaxically injected with lentivirus encoding shLuc or shOgt into the dentate gryrus of contralateral hippocampi. One month later, mice were given 6 daily i.p. injections of BrdU (50mg/kg). One month later, mice were given 6 daily i.p. injections of EdU (50mg/kg). 24 hours after the final EdU injection, mice were sacrified. (**B**) Representative maximum intensity projections of a confocal image z-stack of shLuc or shOgt hippocampus, labeled with anti-Doublecortin (DCX) and EdU (top) or or anti-NeuN and anti BrdU (bottom). Quantification of DCX+/EdU+ neuroblasts and NeuN+/BrdU+ newborn mature neurons (n=5-6 per group). Data are represented as mean ± SEM; *p<0.05, **p<0.01; t test; scale bar, 100μm (top) 10μm (bottom).

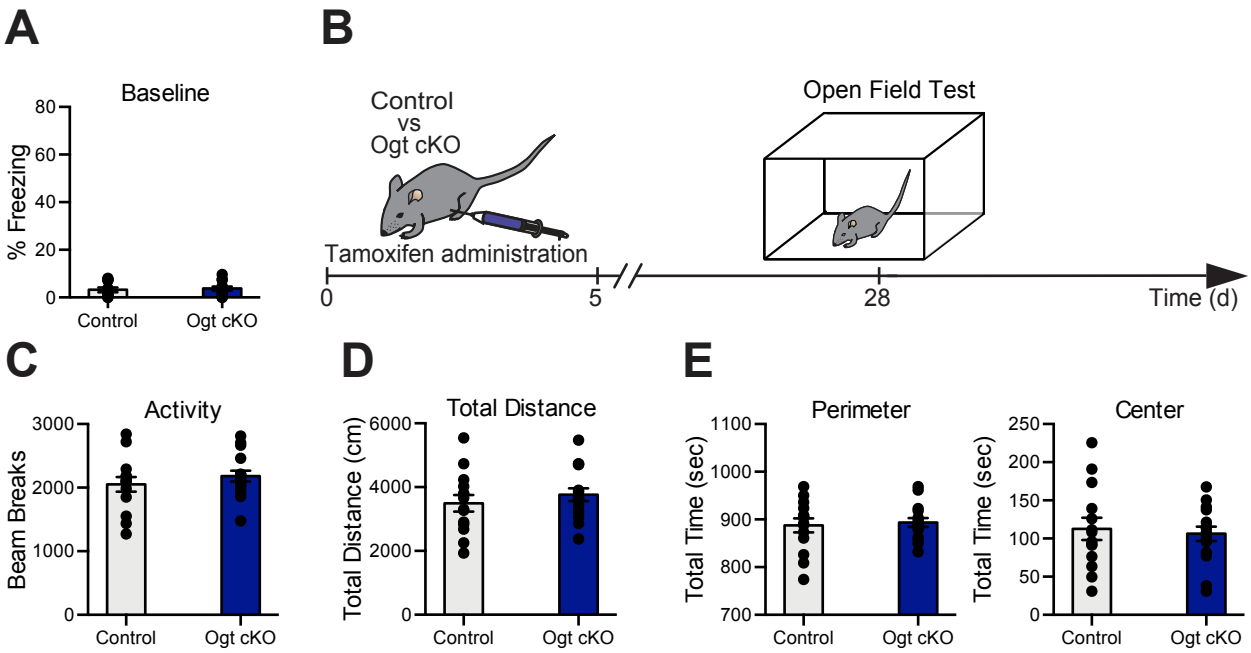
A
Baseline
% Freezing
80
60
40
20
0
Control
Ogt cKO
B
Control
vs
Ogt cKO
Tamoxifen administration
Open Field Test
0
5
28
Time (d)
C
Activity
Beam Breaks
3000
2000
1000
0
Control
Ogt cKO
D
Total Distance
Total Distance (cm)
6000
4000
2000
0
Control
Ogt cKO
E
Perimeter
Total Time (sec)
1100
1000
900
800
700
Control
Ogt cKO
Center
Total Time (sec)
250
200
150
100
50
0
Control
Ogt cKO

Figure S7

Figure S7. Loss of adult NSC Ogt does not affect motor function or anxiety.
(**A**) Baseline freezing was evaluated prior to training stimulus in the fear conditioning paradigm. (**B**) Schematic of cognitive testing timeline. 3-month old *Ogt*$^{flox/y}$ (control) or *NestinCre-ERT*$^{2+/-}$*; Ogt*$^{flox/y}$ (Ogt cKO) littermate mice were administered 5 daily i.p. tamoxifen injections (75mg/kg). One month later, anxiety and motor function were assessed by the open field test (OFT). (**C**) Quantification of activity as number of beam breaks during OFT. (**D**) Quanitifcation of total distance traveled during OFT. (**E**) Quantification of time spent in the perimeter and center of the chamber during OFT. Data are represented as mean ± SEM; ns; t test; n=12-16 per group.

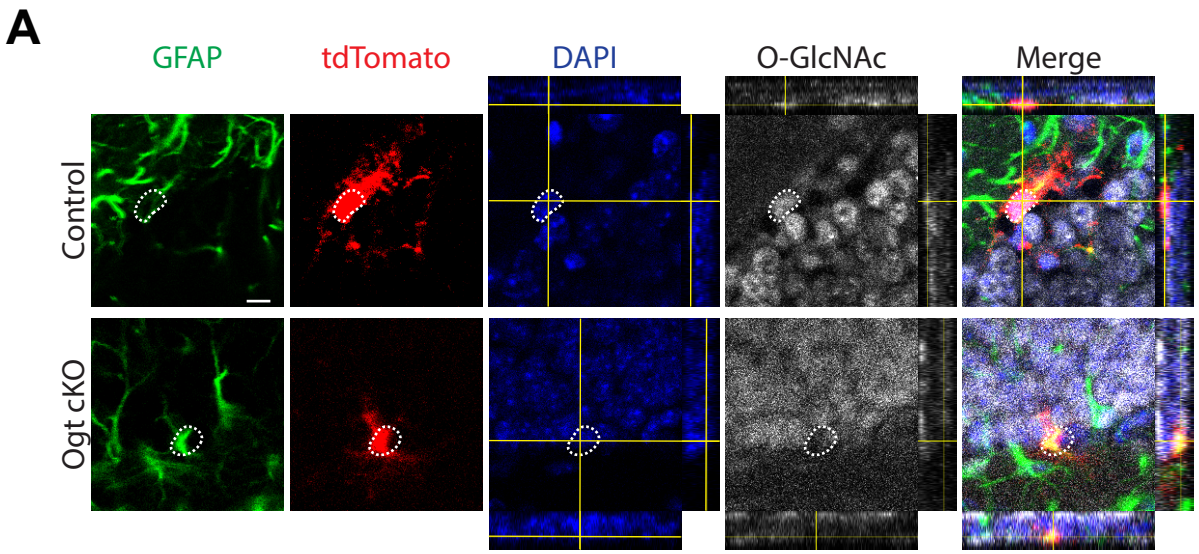

Figure S8

Figure S8. NSC-specific ablation of Ogt reduces astrocyte O-GlcNAc levels *in vivo*. (**A**) Representative single z-plane images and corresponding orthogonal views of tdTomato+ astrocytes in the DG of Ogt cKO mice and littermate controls labeled with anti-GFAP, anti-O-GlcNAc and DAPI. Scale bar, 10μm.

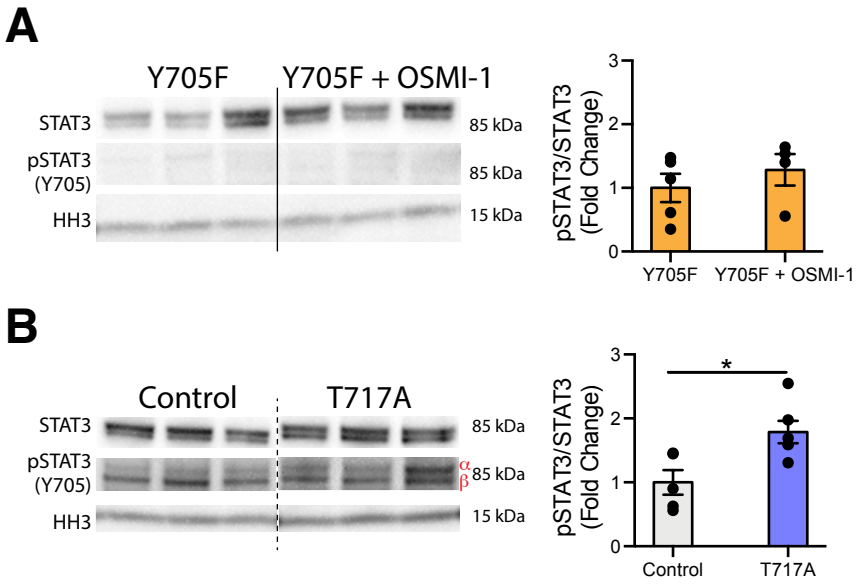
A
Y705F
Y705F + OSMI-1
STAT3
pSTAT3
(Y705)
HH3
85 kDa
85 kDa
15 kDa
pSTAT3/STAT3
(Fold Change)
3
2
1
0
Y705F
Y705F + OSMI-1
B
Control
T717A
STAT3
pSTAT3
(Y705)
HH3
85 kDa
α
β
85 kDa
15 kDa
pSTAT3/STAT3
(Fold Change)
*
3
2
1
0
Control
T717A

Figure S9

Figure S9. Lentiviral-mediated expression of STAT3 point mutants alters STAT3 phosphorylation in NSCs.

(**A**) Primary hippocampal neural stem cells (NSCs) were infected with a lentiviral construct expressing a non-phosphorylatable STAT3 (Y705F). 3 days after infection, NSCs were treated with OSMI-1 or a vehicle control for 24 hours. STAT3 phosphorylation at Y705 and STAT3 expression were quantified following treatment (n=4-5 per group) (**B**) Primary hippocampal NSCs were infected with a lentiviral construct expressing an O-GlcNAcylation-deficient STAT3 (T717A) or wildtype STAT3 (Control). 3 days after infection, phosphorylation of the STAT3 α isoform at Y705 and STAT3 expression were quantified following treatment (n=4-5 per group). Data are represented as mean ± SEM; *$p<0.05$; t test.

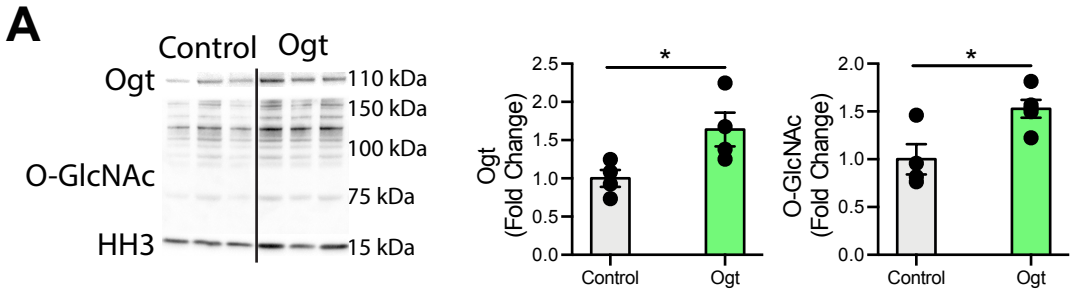
A
Control
Ogt
Ogt
O-GlcNAc
HH3
110 kDa
150 kDa
100 kDa
75 kDa
15 kDa
Ogt (Fold Change)
2.5
2.0
1.5
1.0
0.5
0.0
*
Control
Ogt
O-GlcNAc (Fold Change)
2.0
1.5
1.0
0.5
0.0
*
Control
Ogt

Figure S10

Figure S10. Lentiviral-mediated overexpression of Ogt increases O-GlcNAc levels in NSCs *in vitro*.

(**A**) O-GlcNAc and Ogt levels were assessed by Western blot in primary hippocampal neural stem cells following infection with a lentiviral construct overexpressing GFP or Ogt. Data are represented as mean ± SEM; *p<0.05; t test; n=4-5 per group.

Dataset S1. Quantification of Age-Related Site-Specific Decreases in O-GlcNAcylation in the Adult Hippocampus.
Dataset S2. List of Variable Modifications

DATA AVAILABILITY

- RNA-Seq Data: Gene Expression
  Omnibus GSE143388 Token code: unmxoqwojjclzwx
  (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE143388)
- Mass Spectrometry Data: Raw Data
  MassIVE key: MSV000084825
  (https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?task=633978a3da404371acd5cc0b954cac31)
- Mass Spectrometry Data: Spectra MS-Viewer
  (http://msviewer.ucsf.edu/prospector/cgi-bin/mssearch.cgi?report_title=MS-Viewer&search_key=qcchi2to8l&search_name=msviewer)