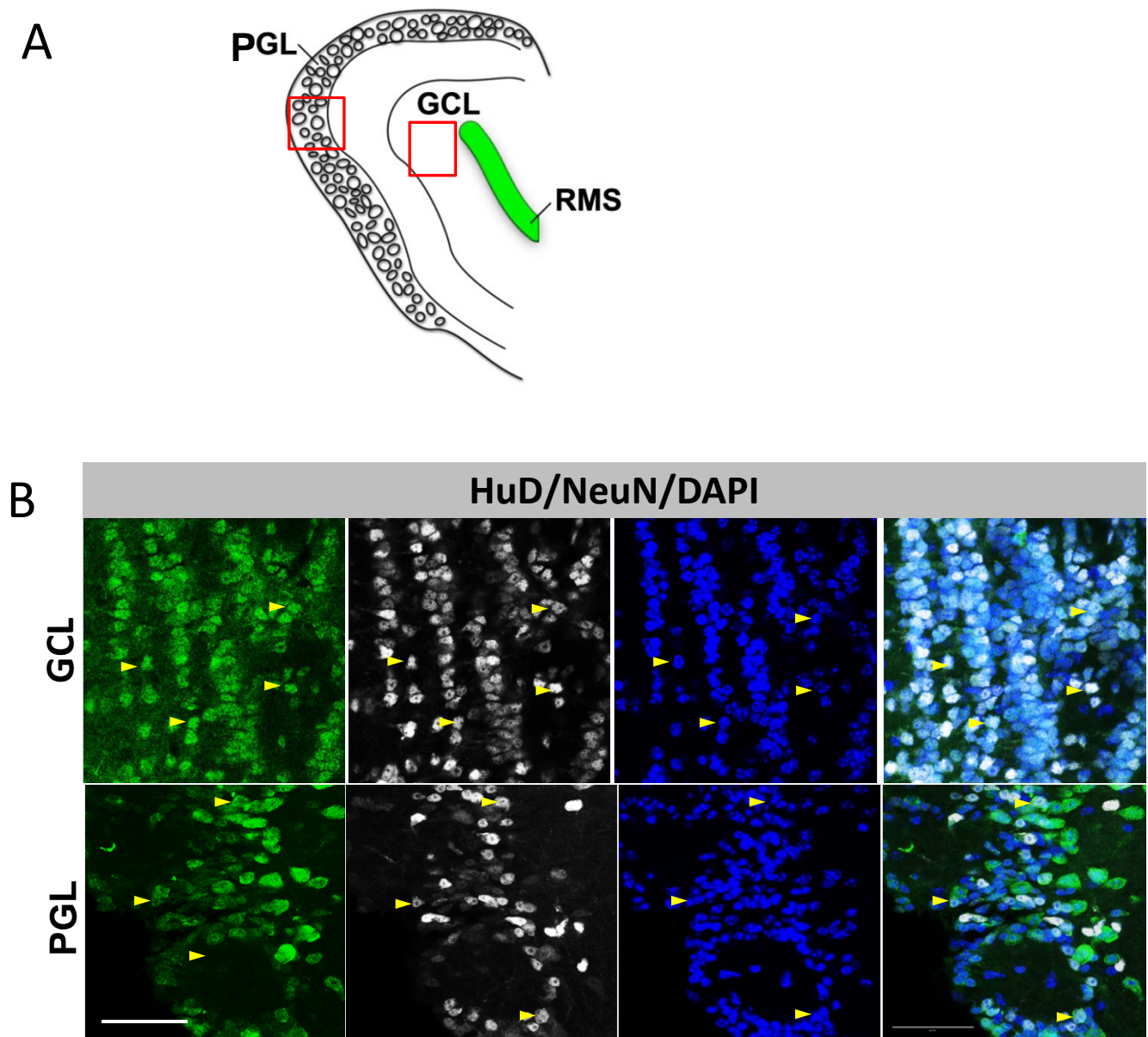


Positive Feedback between RNA-binding protein HuD and transcription factor

SATB1 promotes neurogenesis

(Wang et al 2015)

**Supplementary Figures**



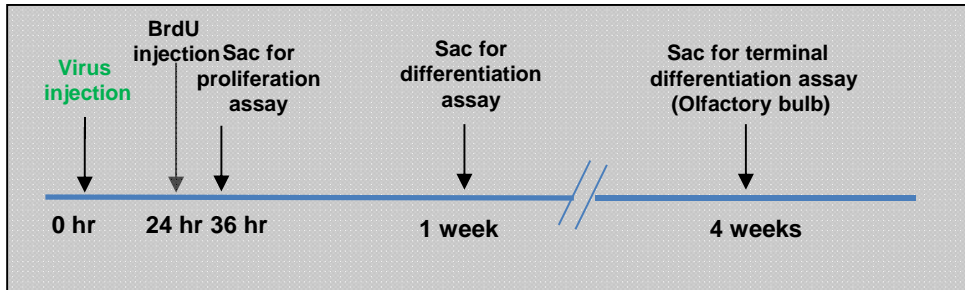
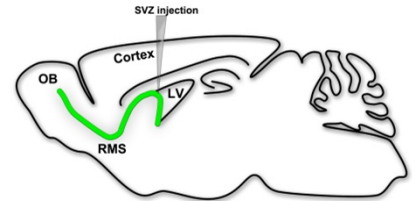
**Figure S1. HuD localization in the adult olfactory bulb.**

- A. Schematics of the olfactory bulb structure. Boxed regions indicates where the images were taken from for B. PGL: Periglomerular layer; GCL: granule cell layer; RMS: the end of rostral migratory stream.
- B. Brain section containing the olfactory bulb from adult mice were co-stained with antibodies for HuD and for a neuronal marker NeuN. Images were taken from PGL and GCL of the Olfactory bulb. Arrows indicated the colocalized cell. HuD: Green; NeuN: Gray; DAPI, Blue. Scale bar, 50  $\mu\text{m}$

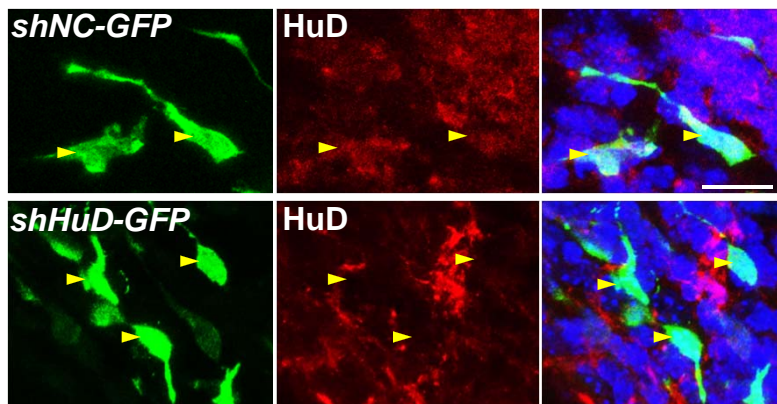
A



B

Retro- *shRNA*-GFP

C

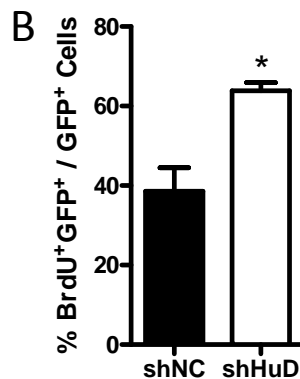
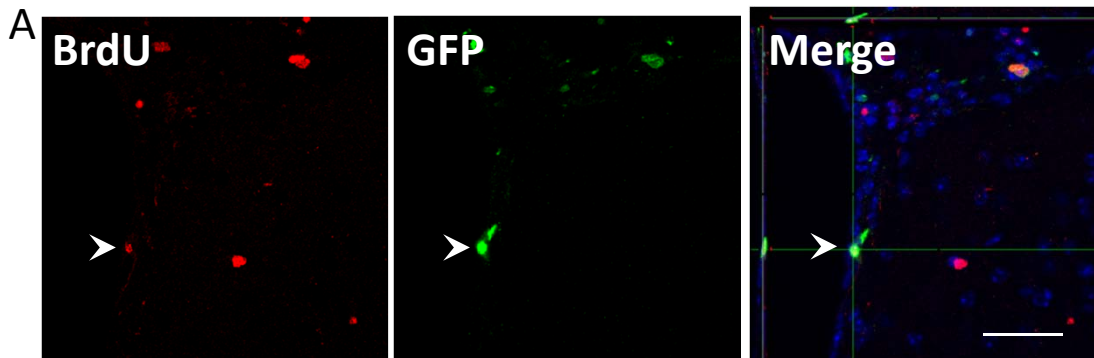


**Figure S2. Strategy for investigating the role of HuD in adult SVZ neurogenesis using retrovirus targeting.**

A Schematic drawing of the retrovirus expressing shRNA and GFP.

B. Experimental scheme for analyzing proliferation of retrovirus infected NPCs in the adult SVZ.

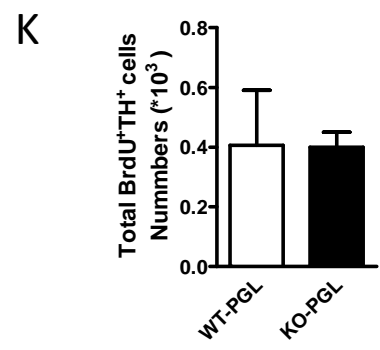
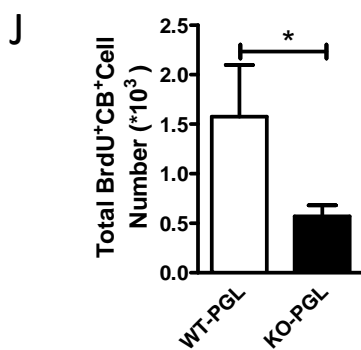
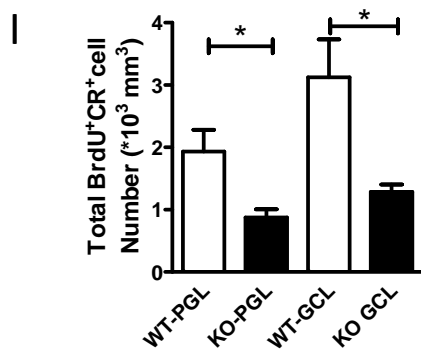
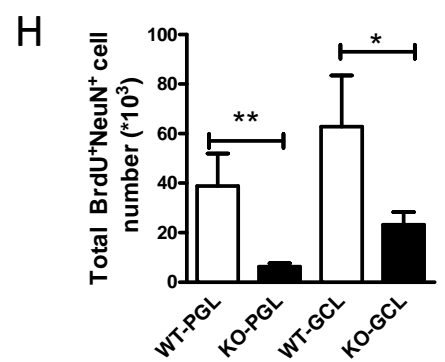
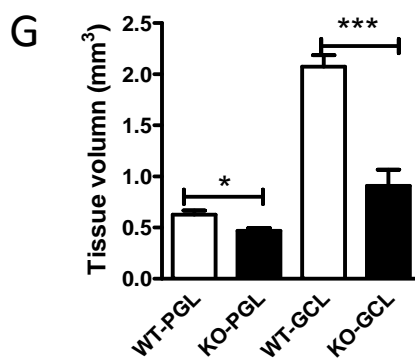
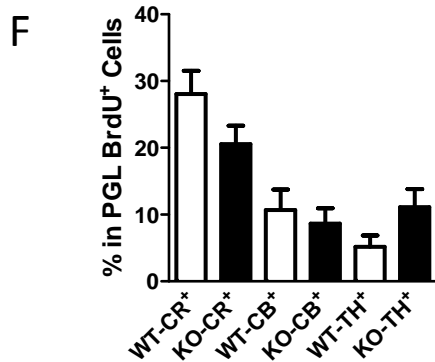
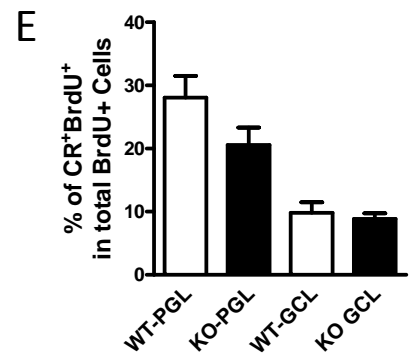
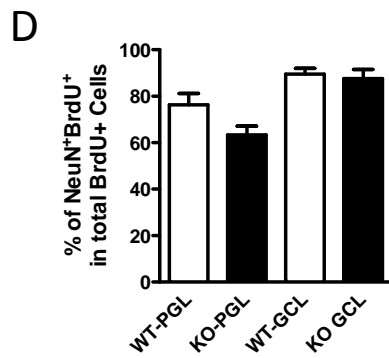
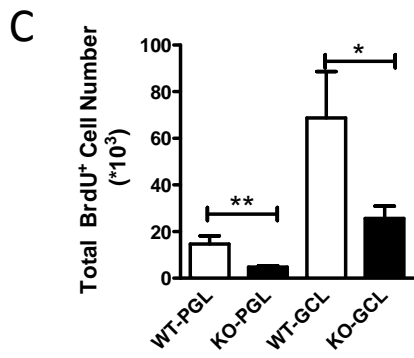
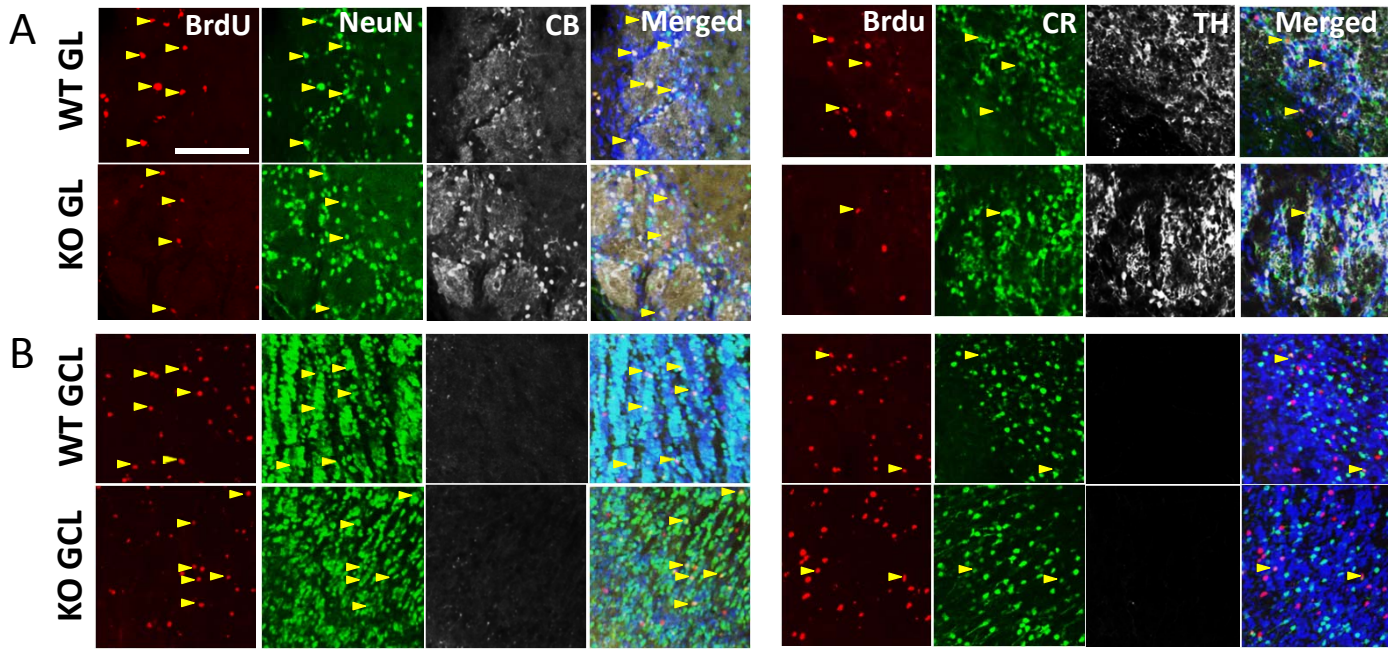
C Mice were injected with retro *shNC*-GFP or retro-*shHuD*-GFP and stained with HuD antibodies (G-2). At 1 weeks post-viral injection, Retro-*shHuD*-GFP infected cells in the OB exhibit significantly reduced HuD expression compared to control *shNC* virus infected cells. Arrowheads point to viral transfected GFP-positive cells in RMS. Scale bar: 20  $\mu$ m



**Figure S3. Acute knockdown of HuD increased the proliferation of adult SVZ NSCs.**

A. A representative image of BrdU and GFP double-labeled cells in that adult SVZ. Scale bar: 50  $\mu$ m.

B. Quantification of the percentage of BrdU<sup>+</sup> cells among cells with HuD acute knockdown (shHuD) compared to control (shNC) cells. n=4 mice/condition. \*, p < 0.05. Student's *t* test. Data are expressed as mean  $\pm$  SEM.



**Figure S4. HuD mutant mice exhibit reduced production of adult-born new neurons in the olfactory bulb.**

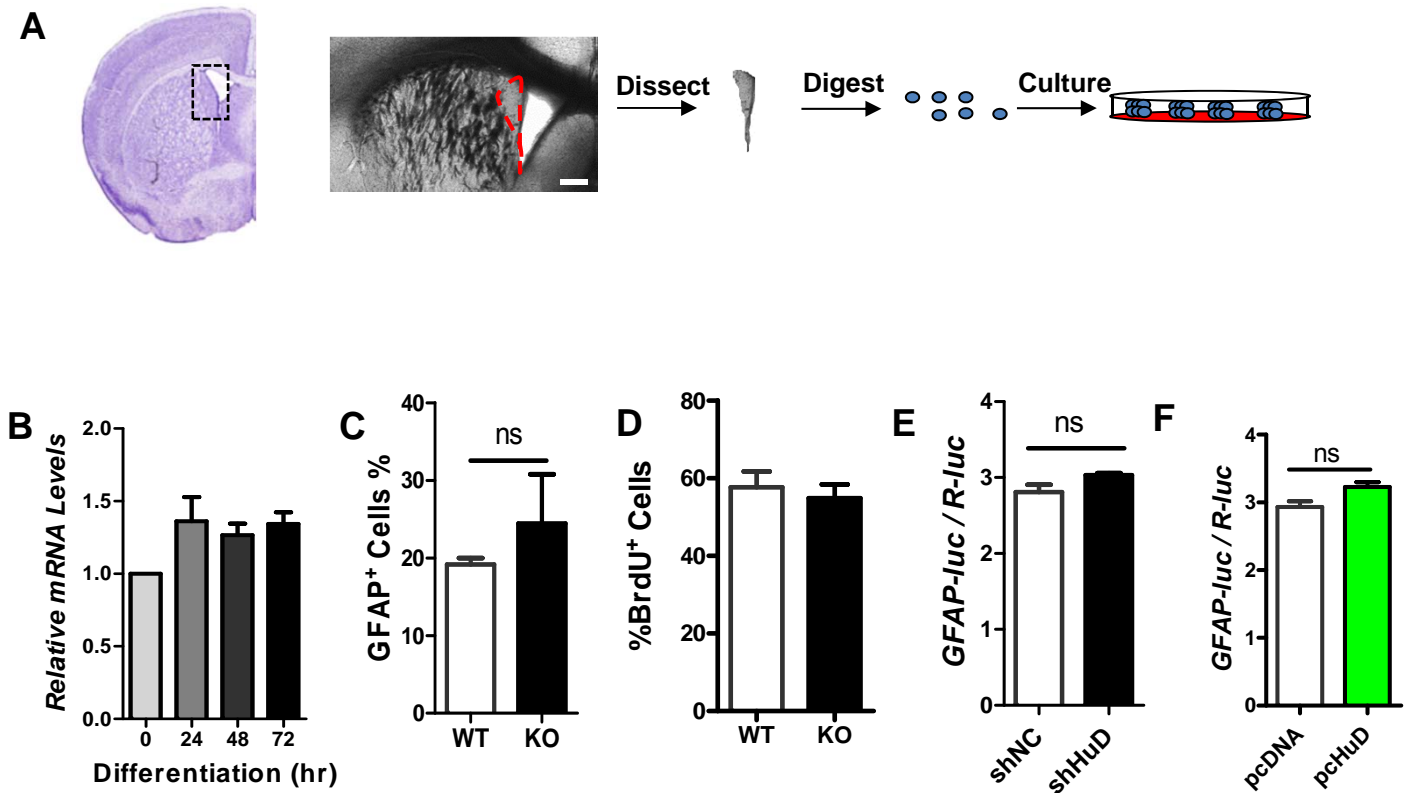
A-B. Sample confocal images showing the expression patterns of various neuronal markers, NeuN (pan-neuronal marker), calbindin (CB), Tyrosine hydroxylase (TH), and calretinin (CR) among BrdU- labeled new neurons in the periglomerular layer (PGL) and granule cell layer (GCL) of olfactory bulb of both HuD WT and KO mice. NeuN, pan-neuronal marker; z-stack projections of confocal series. Yellow arrows point to double-labelled cells (Merge, yellow). BrdU, Red; TH and CB, Grey; NeuN and CR, green; DAPI, blue. Scale bar : 50  $\mu$ m.

C. Quantitative results of the total number of BrdU<sup>+</sup> cells in the PGL and GCL of HuD KO (n=7) and WT (n=4) mice.

D-G. Quantitative results of the percentage of NeuN (D), CR (E), TH and CB (F) positive cells among total BrdU<sup>+</sup> cells in the GL or GCL of HuD KO (n=7) and WT (n=4) mice. Note, same data for CR cells were used in E and F for comparisons with other data. WT vs KO, \*,  $p < 0.05$ ; \*\*\*,  $p < 0.001$ . Student's *t* test. Data are expressed as mean  $\pm$  SEM.

G. Quantification of the volumes of the GL and GCL in HuD KO (n=7) and WT (n=4) mice.

H-K. Quantitative results of the total number of BrdU<sup>+</sup> cells co-expressing NeuN (H), CR (I), CB (J), and TH (K) in the GL or GCL of HuD KO (n=7) and WT (n=4) mice.



### Figure S5. HuD deficiency has no significant effect on glia differentiation

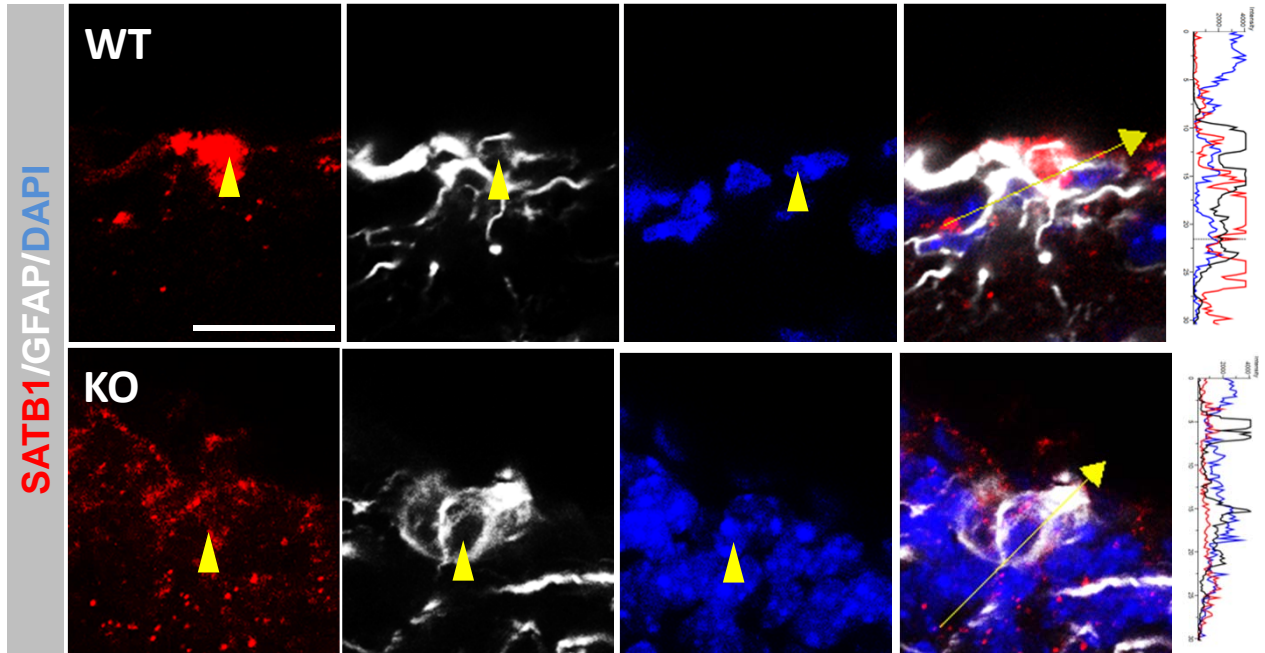
A Schematic drawing showing the isolation of adult SVZ-NSCs.

B Quantitative RT-PCR analysis for HuD mRNA expression at different time points of differentiation; GAPDH mRNA was used as a control.  $n=3$ . RM-ANOVA.  $p < 0.05$ .

C Quantitative analysis showing no significant change in the percentage of HuD KO aNSCs differentiated GFAP<sup>+</sup> positive astrocytes as compared with WT control cells ( $n=6$ ).

D Quantitative analysis showing no significant difference in BrdU incorporation between HuD KO and WT control aNSCs ( $n = 5$ ;  $p = 0.6$ ). Data are expressed as mean  $\pm$  SEM.

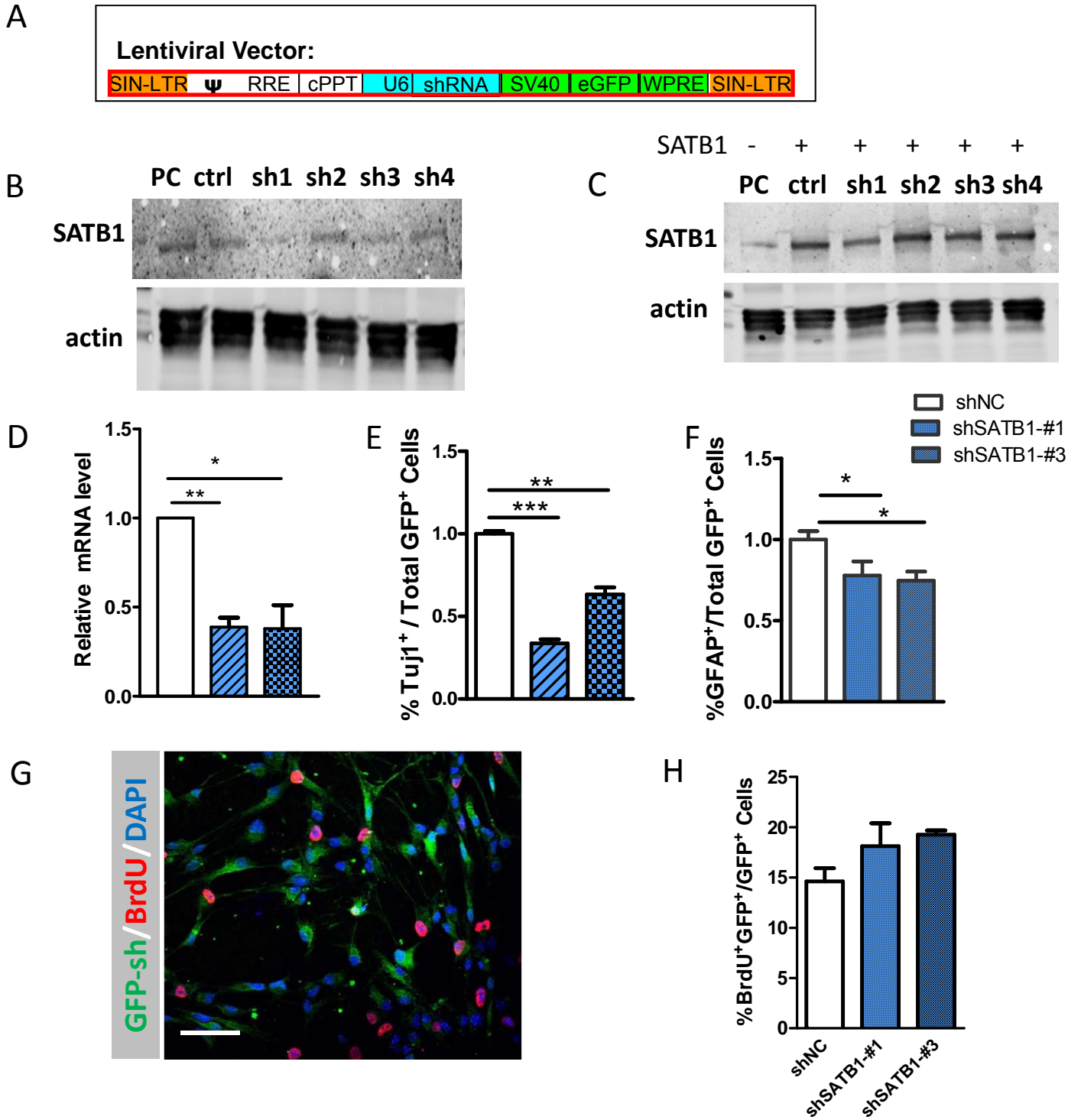
E-F Quantitative analyses showing that, under differentiation conditions, HuD knockdown by shRNA (E) or overexpression by pc-HuD (F) has no significant effect on the promoter activity of transfected GFAP promoter-driven firefly luciferase (GFAP-Luc) construct ( $n = 3$ ).



**Figure S6. Reduced SATB1 levels in HuD-deficient cells.**

- A. SATB1 protein expression in SVZ type A Cell of HuD KO and WT mice were detected by using antibodies against SATB1 (red) and GFAP (grey) (Dapi in blue) followed by confocal imaging. Scale bar: 20  $\mu\text{m}$ .
- B. Fluorescence intensity profiles for red (SATB1), Grey (GFAP) and Blue (DAPI) channels measured along yellow lines shown in the merged channel. Arrows indicated the GFAP<sup>+</sup> cells.





**Figure S7: SATB1 deficiency does not affect NSC Proliferation or astrocyte differentiation**

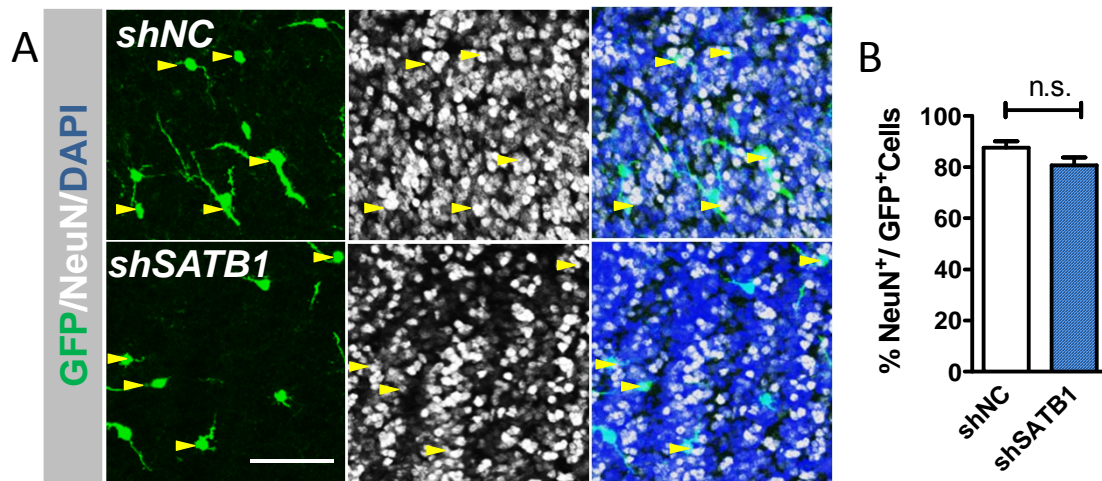
A. Lentiviral vector expressing shRNA also expressed eGFP.

B-C. Selection of shRNA against SATB1 (*shSATB1*): Neuro-2a cells (B) or HEK293 cells (C) were transfected with indicated four different *shSATB1* (sh1, sh2, sh3, sh4) and control (Ctrl). Cells were lysed 48 hours after transfection and the protein level of SATB1 was analyzed by Western blotting with SATB1 antibodies.  $\beta$ -Actin was used as a loading control.

D. NSCs infected with Lentivirus *shNC* or *shSATB1* (1# and 3#) were collected 60 hours later. RT-PCR identified the SATB1 knockdown efficiency from three independent experiments. *Paired t*-test. \*\*,  $p < 0.01$ .

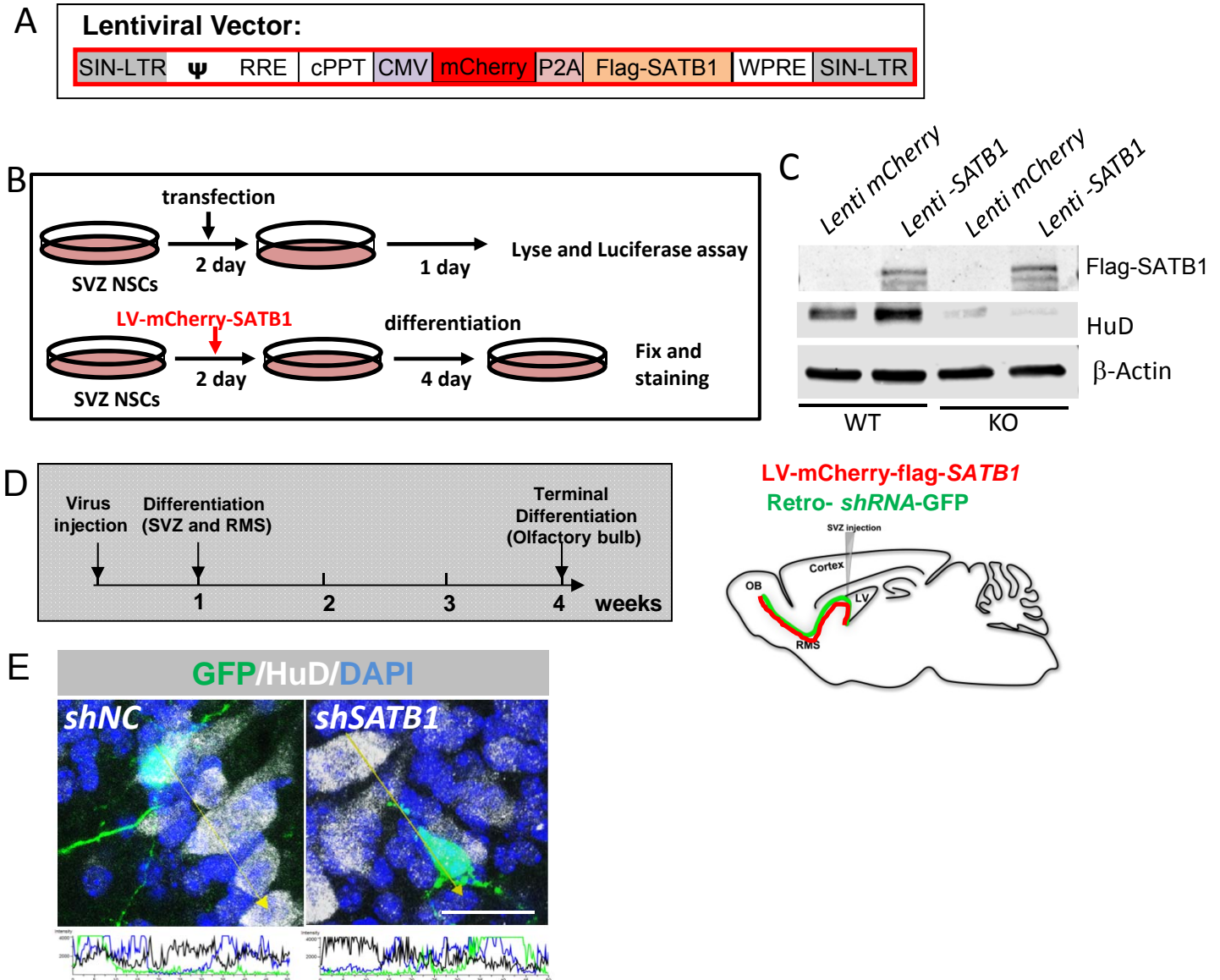
E-F . High-content image analysis by Operetta indicated that Lenti-*shSATB1* (1# and 3#) infected WT aNSCs differentiated into fewer neurons (E) and astrocytes (F).

G-H. Lenti-*shSATB1*-infected cells exhibit no difference in proliferation. (G) Representative images used for quantification (BrdU in red, DAPI in blue, GFP, Green). Bars: 50  $\mu$ m. The results were normalized to control. (H) Quantitative analysis showing no significant change in the percentage of Lenti-*shSATB1*-infected aNSCs incorporating BrdU compared with Lenti-*shNC*-infected aNSCs ( $n = 3$ ). *shSATB1* vs *shNC*. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ . Student's *t* test. Data are expressed as mean  $\pm$  SEM.



**Figure S8: SATB1 deficiency does not affect SVZ NSC terminal differentiation**

(A) Representative confocal images of immunohistological analysis showing that *Retrovirus*-infected cells in the granule cell layer (GCL) of olfactory bulb differentiated into NeuN<sup>+</sup> neurons at 4 weeks post-virus injection. GFP, Green; NeuN, Gray; DAPI, Blue. Arrows indicate cells that were positive for both *shRNA* (GFP) and NeuN. Scale bar, 50  $\mu$ m. (B) Quantitative analyses show that acute knockdown of SATB1 in adult SVZ NSCs had no effect on the terminal differentiated into NeuN<sup>+</sup> mature neurons (n = 5 for *shNC*, n=4 for *shSATB1*, Student's *t*-test.). Data are expressed as mean  $\pm$  SEM. (\*), p = 0.12, student t-test.



**Figure S9. Overexpression and knockdown of SATB1 in adult NSCs from SVZ.**

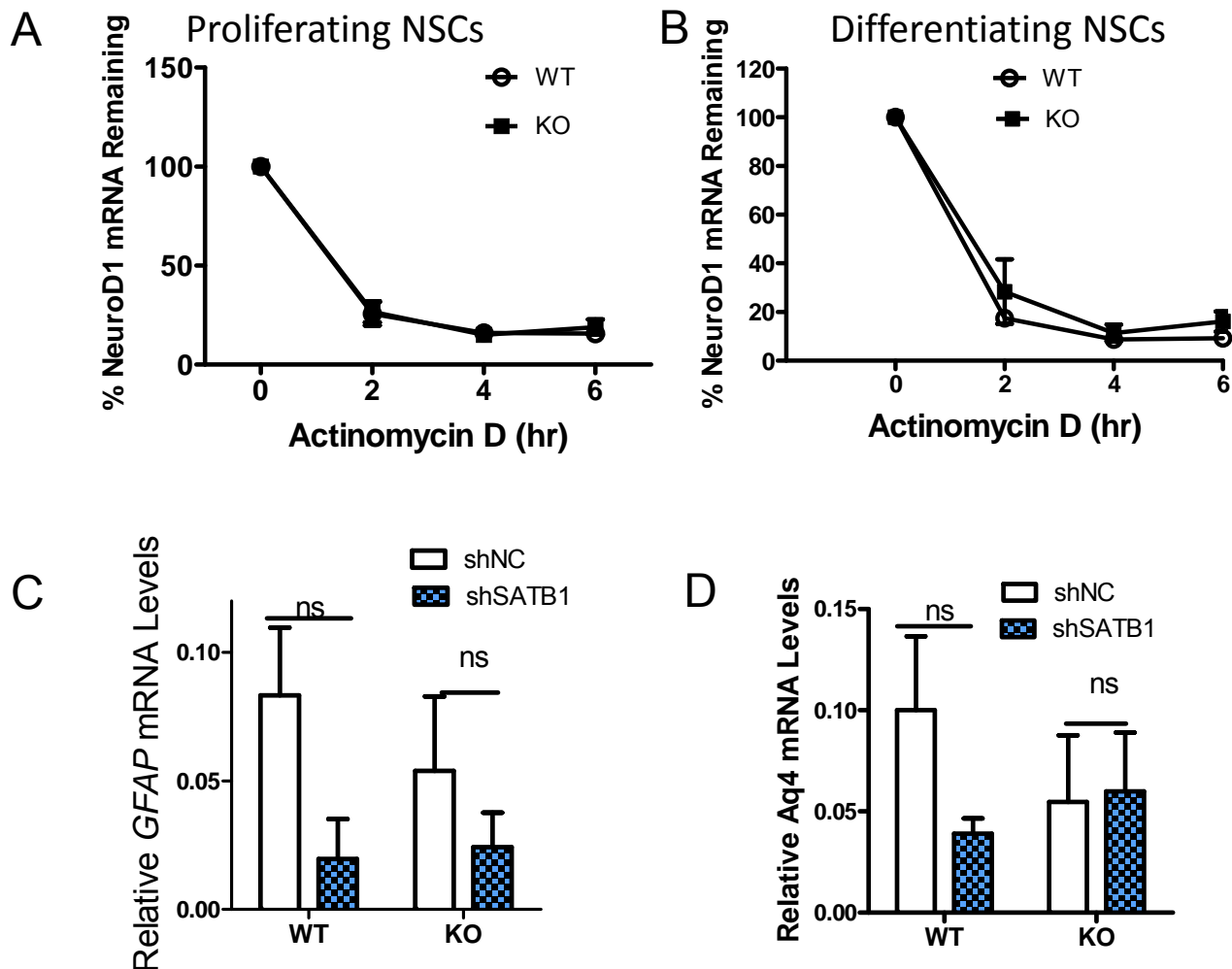
A. Schematic diagram of the Lentiviral vector used for overexpression of Flag-SATB1 and mCherry.

B. Experimental scheme for assessing the effect of exogenous SATB1 on NSCs differentiation in vitro using both promoter reporter assays and neuronal lineage gene expression analysis.

C. Western blotting showing Flag-SATB1 protein in HuD KO and WT aNSCs at 48 hours after virus infection.  $\beta$ -Actin was used as the loading control.

D. Experimental scheme for assessing the effects of exogenous SATB1 on NSC differentiation in vivo by using retrovirus and Lentivirus co-grafting into the adult SVZ

E. Acute knockdown of SATB1 using shRNA (ShSATB1) led to reduced HuD protein levels compared to neurons infected with a control shRNA (shNC). Fluorescence intensity profiles for Green (shRNA), Grey (HuD) and Blue (DAPI) channels measured along yellow lines shown. Scale bar: 20  $\mu$ m.



### Figure S10. NeuroD1 mRNA is not a direct target of HuD

A-B. Adult HuD KO and WT NSCs in proliferating (A) and differentiating (B) conditions were treated with actinomycin D to inhibit gene transcription. The percentage of NeuroD1 mRNA in aNSCs was quantified using real-time PCR at the indicated time points ( $n = 3$ ).

HuD deficiency had no significant effect on NeuroD1 mRNA stability in HuD KO cells compared to WT cells. Two-way ANOVA, Bonferroni post-hoc test.

C-D. Acute knockdown of SATB1 using a Lenti-*shSATB1* virus had no effect on astrocyte differentiation as assessed by GFAP (C) and Aquaporin 4 (D, Aq4) mRNA levels in differentiating cells.  $n = 3$ . Two-way ANOVA, Bonferroni post-hoc test. Data are expressed as mean  $\pm$  SEM.









**Table S1 Predicted HuD binding sites in NeuroD1 3'UTR**

>ENSMUSG00000034701 | ENSMUST00000041099

1 AGGCACGTCAGTTTCACTATTCCCGGGAAACGAATCCACTGTGCGTACAGTGACTGTCCCT  
61 GTTTACAGAAGGCAGCCCTTTTGCTAAGATTGCTGCAAAGTGCAAATACTCAAAGCTTCA  
121 AGTGATATATGTATTTATTGTCGTTACTGCCTTTGGAAGAAACAGGGGATCAAAGTTCCCT  
181 GTTCACCTTATGTATTGTTTTCTATAGCTCTTCTATTTTAAAAATAATAATACAGTAAAG  
241 TAAAAAAGAAAATGTGTACCACGAATTCGTGTAGCTGTATTCAGATCGTATTAATTATC  
301 TGATCGGGATAAAAAAATCACAAGCAATAATTAGGATCTATGCAATTTTTAACTAGTA  
361 ATGGGCCAATTAATAATATATATAAATATATATTTTTCAACCAGCATTTTACTACCTGTGA  
421 CCTTTCCCATGCTGAATTTTGTGTGATTTTGTACAGAATTTTAAATGACTTTTTAT  
481 AACGTGGATTTCCCTATTTTAAAACCATGCAGCTTCATCAATTTTATACATATCAGAAAA  
541 GTAGAATTATATCTAATTTATACAAAATAATTTAACTAATTTAAACCAGCAGAAAAGTGC  
601 TTAGAAAGTTATTGCGTTGCCCTTAGCACTTCTTCTCTCTAATTGTAAAAAAGAAAAGA  
661 AAAGAAAAAACAACAAATTGCACAATTTGAGCAATTCATCTCACTTTAAAGTTTTTC  
721 CTGCTCGCTCCCTAAAATAGAAACCAGACCCATAACACTCAAGAGGATGAAAACCGAAAT  
781 GCATTCCTTATCAAAACACATCAATTCATTACTTGCACAAGCTTGAAATACATATTATA  
841 AATAAATGCCAACACACACTCCTTTAAATCAAAGCTGCTTGACTATCACATACAATTTG  
901 CACTCTTCTTTTTAGTCTTTTACTTCTTTGAATTCCATGATTTTACGGAGTGTGTTGAAG  
961 ATATTGATGTTTCCAGAAAATATAAATGCATGATTTTATACATAGTCAAACAAATGGTGG  
1021 TTTGTCATCTATTCATGTAATAAATCTGAGCCTAAATTTATTTCAGGTTGTTAATGTTGGG  
1081 TTTTATACCTGTGTAGTCAGTTAGTACAGTAGTTTAAATAAAATTCAAACCATCGAATT  
1141 CATAATTAGAACAATAGCTGTTGCATGTAAAATGCAGTCCAGAATAAGTGCTGTTTGAGA  
1201 TGTGATGCTGGTACTACTGGAATTGACATGTAATCTTGTGTTGTAATCCTGTGTAT  
1261 TATGGTGTAATGCACAATTTAGAAAACCTCCATGCAGTTGCAATAAAAATAGTATGGAAA  
1321 ATCAAAACAATTGTTTCATTTCTTTGAAAAGTGGATTTTAAAGAGTAAAATTGTAGCGAG  
1381 GAGGATCAACGTCTGTATTTGCTGAGTTAAATGTTAAACATTAGGAATAAAATATAGAGT  
1441 TGA

AUUUA	132	555	569	578	1055	1276	
ARED							
U-RICH	436	902	903	905	906	909	910
GRE							
MOTIF 1							
MOTIF 2							
MOTIF 3							

## Supplementary Methods

### Mice

All animal procedures were performed according to protocols approved by the University of Wisconsin-Madison Care and Use Committee. The *HuD* KO mice used in this study were created by Akamatsu et al [1], a 2.5-kb fragment containing intronic sequences upstream and a 6.1-kb fragment containing intronic sequences downstream of the second exon that contains the second RNA recognition motifs (25–265 bp of cDNA) were inserted into the target plasmid vector to delete a 1.0-kb genomic DNA fragment and induce a frame shift. The mice were backcrossing to C57BL6 background for 9 generations. Mice were group housed up to 4 per cage with the same gender and maintained on a 12-h light/dark cycle with food and water available ad libitum. For in vivo cell proliferation and terminal differentiation analyses using BrdU labeling, mice were given 4 injections of BrdU (50mg/kg) within 12 hours to label all dividing cells in adult germinal zones within this time period and sacrificed at 4 hours post-last injection based on published paradigm [2]. Mice were then euthanized either at 12 hours or 4 week after the last BrdU injection, by intraperitoneal injection of sodium pentobarbital followed by transcardially perfusion with saline followed by 4% paraformaldehyde (PFA). Brains were dissected out, post-fixed overnight in 4% PFA, and then equilibrated in 30% sucrose. Forty- $\mu$ m brain sections were generated using a sliding microtome and stored in a -20°C freezer as floating sections in 96-well plates filled with cryoprotectant solution (glycerol, ethylene glycol, and 0.1M phosphate buffer, pH 7.4, 1:1:2 by volume).

## DNA Plasmids

The pcHuD plasmid contained DNA fragments corresponding to coding sequence (CDS) of human **HuD** was clones in pcDNA3 as described in Anderson et al, [3]. The plasmid expressing a small hairpin inhibitory RNA (shHuD) under U6 promoter for HuD knockdown was a gift of Dr. Antonia Ratti and previously used in Allen, Bird et al [4]. To create retroviral vector expressing *shHuD*, we amplified the U6-*shRNA* cassettes using the following primers: forward: 5'-CTCTCGAGGTCGACGGTATC-3', reverse: 5'-GCGGTTAACAGACTGCCTTGGGAAAAGC-3'. We then cloned the PCR product between the HapI and ClaI restriction sites of a retrovirus vector that is also expressing EGFP driven by CAG promoter [2, 5]

The plasmid containing DNA fragments corresponding to coding sequence (CDS) of mouse *SATB1* was purchased from Genecopoeia (Rockville, MD, USA), and P2A-FLAG-SATB1 was subcloned into a lentiviral vector expressing red florescent protein mCherry using the following primers: forward (containing P2A-FLAG): 5'-TGGACGAGCTGTACAAGGGAAGCGGAGCTACTAACTTCAGCCTGCTG AAGCAGGCTGG AGACGTGGAGGAGAACCCTGGACCTGATTACAAGGATGACGACGATAAGGATT ACAAGGATGACGACGATAAGGATTACAAGGATGACGACGATAAGATGGATCATTGTAACGAGGC-3', reverse: 5'-CAGGTTTAACTCGAG TCAGTCTTTCAAGTCGG-3'.

The mouse Lenti-*shSATB1* (SureSilencing) plasmids were purchased from Genecopoeia. The efficiency and specificity of shRNA knockdown were determined by transfecting into Neuro2A using Lipofectamine 2000 (Invitrogen, #11668-027) or HEK293T cells using calcium phosphate method, and analyzed at 60-hr post-transfection. The targeted sequence were: GGAAAGGAACCATGTTACC (*shSATB1-1*) and ACAGCACGTACTATGCAAAA (*shSATB1-3*). We then cloned the *shSATB1-1* targeting sequence into the retrovirus vector that is also expressing EGFP driven by CAG promoter

3'-UTR sequence of *Satb1* was PCR amplified directly from purified mouse cortical genomic DNA

using AccuPrime™ Pfx DNA Polymerase according to the manufacturer's protocol (Invitrogen, # 12344024, Carlsbad, CA, USA). The primers contain XhoI and NotI restriction sites for aiding infusion into digested psiCHECK-2 dual luciferase vector (Promega, # C8021, Madison, WI, USA) using In-Fusion HD Cloning Kit (Takara, #011614, Otsu, Shiga, Japan). The primers used are:

<i>Satb1</i> 3'UTR 1 forward:	TAGGCGATCGCTCGAGGAGAACAGTATTCTTTTCAGC
<i>Satb1</i> 3'UTR 1000 reverse:	TTGCGGCCAGCGGCCGCTTTCACATTAATTGCATA
<i>Satb1</i> 3'UTR 1001 forward:	TAGGCGATCGCTCGAGATAATTGATGTTTGCAAT
<i>Satb1</i> 3'UTR 1897 reverse:	TTGCGGCCAGCGGCCGCCACGGGAACCCCTAACAAAG
<i>Satb1</i> 3'UTR 2001 forward:	TAGGCGATCGCTCGAGTTTCCTGGTGGTCTAAAT
<i>Satb1</i> 3'UTR 3000 reverse:	TTGCGGCCAGCGGCCGCCTAGTCACCTTTGTTTTTC
<i>Satb1</i> 3'UTR 3001 forward:	TAGGCGATCGCTCGAGTATGTGTGCCCTACCTGTGT
<i>Satb1</i> 3'UTR 3555 reverse:	TTGCGGCCAGCGGCCGCTTGTCCAAAGACATGCAAG
<i>Satb1</i> 3'UTR 3709 reverse:	TTGCGGCCAGCGGCCGCTCATGTTTTAAACATTAATTCC

1.3 kb HuD Promoter-reporter plasmids cloned into the MCS of the PGL4.14 vector were kindly provided by Dr Bernard J. Jasmin (University of Ottawa, Ottawa, Canada) [6]. *NeuroD1*-luciferase, a gift from Dr. F.H. Gage, the 2.5 kb of glial fibrillary acidic protein (*Gfap*) promoter-firefly luciferase reporter gene and an internal control plasmid containing *Renilla luciferase* (R-Luc) driven by human elongation factor (*EF-1 $\alpha$* ) promoter, gifts from Dr. Kinichi Nakashima (Kyushu University, Japan) were described previously [7, 8].

### Isolation and Analyses of aNSCs

Adult -derived NSCs were isolated from 6- to 8-week-old *HuD* KO mice and wild-type (WT) littermates as described before [9] with modifications. Briefly, SVZ were dissected using forceps and 27 gauge needle (BD, #305109) and place in Hank's balanced salt solution (HBSS; Invitrogen, # 14025-126) on ice. Tissue was spin down and digested using MACS Neural Tissue Dissociation kit (Miltenyi Biotech, # 130-090-753, San Diego, CA, USA). After dissociation with a fire-polished glass pipette,

cells were filtered through a 70- $\mu$ m cell strainer (BD Falcon, #252350, CA) and washed with HBSS, the single-cell suspension from each sample was collected and cultured in proliferating medium (Neurobasal containing B27 serum-free supplement (Invitrogen, # 17504-044) 20 ng/ml basic fibroblast growth factor (FGF-2; PeproTech, #K1606, Rocky Hill, NJ, USA), 20 ng/ml epidermal growth factor (EGF, PeproTech, #A2306), 1% Antibiotic-Antimycotic, and 2 mM L-glutamine, in a 5% CO<sub>2</sub> incubator at 37°C. Half of the medium was replaced every two days. Proliferation and differentiation analyses were performed as described in our publications [2, 10]. To study cell proliferation, we dissociated neural stem cells with trypsin and plated them on 24 well plates with poly-L-ornithin (Sigma-Aldrich, #P-3655, St. Louis, MO, USA) and laminin (BD Biosciences, #354232, San Jose, CA, USA)-coated coverslips at a density of 10,000 cells per well in proliferation medium (see above). At 20 h post-plating, 5  $\mu$ M 5-bromo-2'-deoxyuridine (BrdU, SigmaAldrich, #B5002) was added into the culture medium for 8 h. NSCs were then washed with PBS and fixed with 4% paraformaldehyde for 30 min at room temperature, followed by immunohistochemical analysis. To detect BrdU incorporation, fixed cells were pretreated with 1M HCl for 30 min at 37°C, washed with borate buffer, pH 8.5, for 30 min, and followed by standard immunocytochemical protocol. For the differentiation assay, at 24 h post-plating, cells were changed into differentiation medium, which is Neurobasal medium containing 5  $\mu$ M forskolin (Sigma-Aldrich, #F-6886) and 1  $\mu$ M retinoic acid (Sigma-Aldrich, #R-2625) for 4 d. Upon fixation with 4% paraformaldehyde, the coverslips were subjected to our standard immunohistochemistry protocol.

### **Immunocytochemistry**

Immunocytochemistry staining was carried out as described [7, 11, 12]. Briefly, cells were fixed with 4% paraformaldehyde for 20 min, and then washed with Tris-buffered saline (TBS, 50 mM Tris-Cl, pH 7.5, 150 mM NaCl) for 30 min. Cells were preblocked using TBS containing 2% normal goat serum

(VECTOR, #S-1000, Burlingame, CA, USA) and 0.1% Triton X-100 for 30 min, followed by overnight incubation with primary antibodies. After washing with TBS, cells were incubated with fluorescent secondary antibodies, followed by counterstaining with the fluorescent nuclear dye 4',6-dimidino-2'-phenylindole dihydrochloride (DAPI, Roche Applied Science, Indianapolis, IN). The coverslips were mounted with polyvinyl alcohol (PVA) mounting medium with DABCO (Sigma-Aldrich, # 10981) and stored in cold and dark before and during analysis. The numbers of marker-positive cells (BrdU<sup>+</sup>, TuJ1<sup>+</sup>, or GFAP<sup>+</sup>, etc), as well as total cells (DAPI<sup>+</sup>) and total Lenti- -infected cells (shRNA-GFP<sup>+</sup>, mCherry<sup>+</sup>, or SATB1<sup>+</sup>) were quantified using an Olympus BX51 microscope equipped with a MicroFire digital camera (Optronics) and a motorized stage using a 20X objective lens. The quantification was carried out using an unbiased stereology method with assistance from StereoInvestigator software (MBF Biosciences, Williston, VT, USA). The percentage of differentiated cells was calculated as the number of marker-positive cells divided by the total number of DAPI, GFP<sup>+</sup>, GFP<sup>+</sup>/mcherry<sup>+</sup> or GFP<sup>+</sup>/SATB1<sup>+</sup> positive cells. At least 3 independently viral infected cells were analyzed for statistical analysis.

### **Immunohistology**

Immunohistology of brain tissue sections was performed as published in our papers [5, 12, 13]. For staining, floating brain sections were first washed in TBS to remove cryoprotectant and then blocked in the TBS buffer containing 2% normal serum and 0.2% triton-X 100, followed by incubation with primary antibodies diluted in TBS overnight in 4 °C. After washing 3 times, secondary antibodies were incubated 1 h at room temperature. All sections were counterstained with DAPI.

The primary antibodies used were: chicken anti-GFP (1:1000, Invitrogen, #A10263), mouse anti-Tuj1 (1:1000, Covance, #MMS-435P, Princeton, NJ, USA), rat anti-BrdU (1:3000, Abcam, #ab-6326, Cambridge, UK), rabbit anti-GFAP (1:2000, DAKO, #Z0334, Carpinteria, CA, USA), chicken anti-Nestin (1:500, Aves Labs, #NES0407, Tigard, OR, USA), mouse anti-NeuN (1:1000, MAB377), mouse

anti-calretinin (1:500, MAB1568), mouse anti-Mash1 (1: 200, BD Biosciences, #556604), rabbit anti-tyrosine hydroxylase (TH) (1:250, AB152), and rabbit anti-calbindin (1:500, AB1778) were from Millipore, rabbit anti-doublecortin (1:500, Cell Signaling Technology, #4604S, Beverly, MA, USA), goat anti-SATB1 (1:100, Santa Cruz Biotechnology, #sc-5989, Dallas, CA, USA), mouse anti-HuD (1:200, Santa Cruz Biotechnology, #sc-28299), goat anti-HuD (1:100, Santa Cruz Biotechnology, #sc-5977), rabbit anti-flag (1:1000, Cell Signaling Technology, #2368S) antibodies. Fluorescent secondary antibodies were from Invitrogen (Carlsbad, CA, USA) and used by 1:500 dilution: goat anti-chicken-488 (#A11039), goat anti-mouse-488(#A11029), goat anti-mouse Alexa Fluor 568 (#A11031), goat anti-rabbit 568 (# A11011), goat anti-rat Alexa Fluor 568 (#A11077), goat anti-rabbit Alexa Fluor 647 (#A21245), donkey anti-goat 568 (# A11057), donkey anti-rabbit 647 (#A31573).

To analyze the fate of the cells generated from SVZ, 1 in 6 serial sagittal sections, containing SVZ, RMS and olfactory bulb (relative to bregma, lateral: 1.0 mm to 1.4 mm) were analyzed by Image J. For Brdu injected mice, at least 100 Brdu<sup>+</sup> cells in each slice were randomly selected and their phenotypes (double labeling with TH, CR, CB or NeuN) were determined. For recombinant virus-infected cells in mice injected with both retrovirus (GFP) and lentivirus (SATB1 stained with Flag-antigen labeled with Alexa Fluor 568), GFP<sup>+</sup> single positive (green) and GFP<sup>+</sup>/SATB1<sup>+</sup> double positive (yellow) cells were counted, and at least 50 GFP<sup>+</sup>, GFP<sup>+</sup>SATB1<sup>-</sup> or GFP<sup>+</sup>SATB1<sup>+</sup> cells in each slice were randomly selected and their phenotypes (double labeling with DCX or NeuN) were determined. The data were presented as the percentage of DCX<sup>+</sup> or NeuN<sup>+</sup> cells in the GFP<sup>+</sup>, GFP<sup>+</sup>SATB1<sup>-</sup>, or GFP<sup>+</sup>SATB1<sup>+</sup> populations by Image J. The volumes of periglomerular layer (PGL) and granule cell layer (GCL) (3-dimensional size) in olfactory bulb and the total number of cells were measured using StereoInvestigator (MBF Bioscience, Inc) with a 5- $\mu$ m guard zones at both top and bottom of the sections, as described elsewhere [14].

### **High content image analysis**

To quantify NSC differentiation, NSCs were cultured in 24 well-plates to go through the proliferation or differentiation, after the fixation and staining, cells were incubated in TBS. The images were acquired on an Operetta System (Perkin Elmer) using a 20 X LWD objective in wide-field mode in combination with filters for DAPI (excitation filter: 360–400 nm; emission filter: 410–480 nm), Alexa Fluor 488 (excitation filter: 460–490 nm; emission filter: 500–550 nm), Alexa Fluor 546 (excitation filter: 520–550 nm; emission filter: 560–630 nm), and Alexa Fluor 647 (excitation filter: 620–640 nm; emission filter: 650–760 nm). The laser autofocus was applied and 20 image fields were acquired per well. For quantitative analyses, individual cells were segmented based on the DAPI nuclear stain using the Find Nuclei building block in the Harmony High Content Imaging and Analysis Software (PERKIN ELMER) and GFP intensity was quantified within the DAPI defined boundaries for each cell. The Select population module of Harmony allowed setting a fluorescence intensity threshold in order to identify the sub-population of virus infected GFP<sup>+</sup> cells. Tuj1<sup>+</sup> and GFAP<sup>+</sup> cells were selected in those GFP<sup>+</sup> populations by the Neurite Detection or building blocks.

### **RNA Immunoprecipitation (IP)**

RNA-IP was performed as described [9, 12]. Briefly, WT and HuD KO SVZ NSCs were harvested and homogenized in 1 ml of ice-cold lysis buffer (10 mM HEPES [pH 7.4], 200 mM NaCl, 30 mM EDTA, and 0.5% Triton X-100) with 2X complete protease inhibitors (Roche, #11873580001). Nuclei and debris were pelleted at 3,000 X g for 10 min; the supernatant was collected and raised to 300 mM NaCl, and clarified at 14,000 X g for 30 min. The resulting supernatant was pre-cleared for 1 h with 100 µl recombinant protein G agarose (Invitrogen) (washed with lysis buffer first). An aliquot of pre-cleared input was saved for RNA extraction (200 µl) and protein analysis (100 µl). A monoclonal antibody



against HuD (Santa Cruz Biotechnology, #sc-28299) was incubated with recombinant protein A dynabeads at 4°C for 2 h and washed 3 times with lysis buffer. RNase Inhibitors (Roche) will be added to the remaining lysates. The precleared lysates were immunoprecipitated with antibody-coated recombinant protein G agarose at 4°C for 2 hours. After third wash with the lyses buffer, 10% of immunoprecipitate was saved for protein analysis. The remaining was washed one more time and was re-suspended into Trizol (Invitrogen, #15596018) for RNA isolation.

### **Chromatin Immunoprecipitation (ChIP)**

ChIP was performed according to published method [7, 15]. Briefly, aNSCs grown to 80%-90% confluent in 10cm plates were fixed by adding 1% formaldehyde (Sigma-Aldrich, #S33102) to culture medium for 20 min at room temperature and stop with Glycine buffer (190 mg Glycine in 1ml H<sub>2</sub>O) to final concentration of 0.14 M. After washing with cold PBS, cells were collected with cold PBS, washed, and suspended in 1mL cold cell lysis buffer (5mM PIPES, pH=8.0, 85mM KCl, 0.5% NP40, and 1X Complete Proteinase inhibitor, and incubated on ice for 5 min. Cell lysates were pelleted by centrifugation at 3000rpm for 5 min, resuspended in 1mL cold cell lysis buffer 5min on ice, and then re-pelleted to collect nuclei. Nuclei were lysed at room temperature with 500µL nuclei lysis buffer (50mM Tris pH 8.1, 10mM EDTA, 1%SDS and 1X Complete Protease inhibitor). Nuclear lysates were sonicated using Bioruptor<sup>TM</sup> UCD-200 sonicator (Life Technologies) with 5 cycles in a high poweroutput, each cycle includes 5 pulses of 30 seconds power on within 10 minutes. The size of the sonicated chromatin (average size ~400-700bp) was verified by treating 5 µL aliquots with 1µL 20 mg/mL Proteinase K for 20 min at 50°C and running on a 1.5% agarose gel stained with SYBR Safe dye (Invitrogen, #S33102). The immunoprecipitating and washing were using Chip Assay Kit (Millipore, #17-295, Billerica, MA, USA). Briefly, 500 µL of sonicated chromatin, pre-cleared with salmon

sperm/tRNA blocked Protein A agarose for 60 min at 4°C in 5 ml IP dilution buffer (0.01% SDS, 1.1% TritonX-100, 1.2mM EDTA, 20mM Tris pH 8.1, 500 mM NaCl) was used in immunoprecipitation reactions. Pre-cleared chromatin was rotated at 4°C overnight with 10 µg of the appropriate antibody. Antibodies used were normal anti-Rabbit IgG (Millipore, #12-370), anti-rabbit SATB1 (Santa Cruz Biotechnology, #SC-28676). Antibodies were pulled down with 150 µL blocked Protein A agarose beads overnight at 4°C with rotation. The beads were washed sequentially one wash with low salt immune complex wash buffer, high salt immune complex wash buffer and LiCL immune complex wash buffer for 5 min at 4°C. And two washes with TE Buffer for 5 min at room temperature. Protein-DNA complexes were eluted from the Protein A agarose beads with 500µL fresh prepared IP elution buffer (50mM NaHCO<sub>3</sub>, 1% SDS) for 15 min at 37°C with rotation. Formaldehyde induced protein-DNA crosslinking were heat reversed by incubating protein-DNA complex at 65°C for 4 hours. DNA was purified using Phenol:Chloroform:Isoamyl alcohol (25:24:1) (Life Technologies, #15593-031) isolations and precipitated with 2 volumes 100% ethanol and 10µg linear acrylamide at -20°C overnight. Immunoprecipitated and purified DNA fragments were resuspended in 100 ml nuclease free water, 2 µl DNA was used in 20 µL SYBR Green real-time PCR reactions. Primers sequences spaced at 1kb intervals spanning 6 kb upstream to 1 kb downstream of *HuD* and *NeuroD1* were designed using primer premier 5.0 Software and were (5'-3'):

<i>NeuroD1</i> downstream 0.2 Kb forward:	GCTCCAGGGTTATGAGATCGT
<i>NeuroD1</i> downstream 0.2 Kb reverse:	TGTGGGCAATTACGAGGAGG
<i>NeuroD1</i> upstream 0.8 Kb forward:	GAGCAGGTGACCGTTAGGTT
<i>NeuroD1</i> upstream 0.8 Kb reverse:	GCCTTGACTCAACCTTCCTGA
<i>NeuroD1</i> upstream 2 Kb forward:	GTTCCGGGAATATCCAGGTGT
<i>NeuroD1</i> upstream 2 Kb reverse:	GGAGAGTTATTGGGGAGCGG
<i>NeuroD1</i> upstream 2.5 Kb forward:	GCAGATTTAGGGCATTGCTCG
<i>NeuroD1</i> upstream 2.5 Kb reverse:	TCCAGTTATCTCCGCTTGC
<i>NeuroD1</i> upstream 3.2 Kb forward:	GAGATTTGGGGAGAACGGGT
<i>NeuroD1</i> upstream 3.2 Kb reverse:	GCTGGAATTGCCCGACTAGA

<i>NeuroD1</i> upstream 4.8 Kb forward:	TCCGCGTTTCTTTGATCAATCC
<i>NeuroD1</i> upstream 4.8 Kb reverse:	ACCACATTGGTACACGTGGC
<i>NeuroD1</i> upstream 6 Kb forward:	ATGAGCTGCCTCTTGACACC
<i>NeuroD1</i> upstream 6 Kb reverse:	GGGAGGGATGTGAATGCCAA
<i>HuD</i> downstream 0.5 Kb forward:	GGCGTGTTGAGTTGTTGCTT
<i>HuD</i> downstream 0.5 Kb reverse:	ACCTCCAAGCTGCCTTCTTT
<i>HuD</i> upstream 0.5 Kb forward:	CTGCGAGACCCAATATTTGCC
<i>HuD</i> upstream 0.5 Kb reverse:	GACTGGCCAAGGTGTAGACG
<i>HuD</i> upstream 1.9 Kb forward:	ATGCAGGCCCAAAACATCCT
<i>HuD</i> upstream 1.9 Kb reverse:	GCCTCTCCCTTCGCTTCTTT
<i>HuD</i> upstream 2.5 Kb forward:	TAGAGTGCAGTGCAGCAAGT
<i>HuD</i> upstream 2.5 Kb reverse:	GCGCCCTTCAGGTAGAACAA
<i>HuD</i> upstream 3.5 Kb forward:	CCACTGTGCGGAGTAGGTAG
<i>HuD</i> upstream 3.5 Kb reverse:	AGATATCTCAGCTCGGTCGC
<i>HuD</i> upstream 5 Kb forward:	TCCATCTCTGGGAGATTTCAACT
<i>HuD</i> upstream 5 Kb reverse:	GATGCTGTTTGGGAGGCGTA
<i>HuD</i> upstream 5.3 Kb forward:	GTGGACGTTTCAGAGCTTCA
<i>HuD</i> upstream 5.3 Kb reverse:	CAAAGCAAGCCCATAGCGTT

cDNA from IP and input from 3 independent chromatin preparations was used and all Real-time PCR reactions were carried out in duplicate for each sample on each amplicon. IP readings of both specific SATB1-immunoprecipitations (H-70) or non-specific immunoprecipitations (rabbit IgG only) were normalized Input sample.

### **RT-PCR, Real-Time PCR, and Pathway Arrays**

RT-PCR and real-time PCR were performed using standard methods as described [2, 5, 7]. The first-strand cDNA was generated by reverse transcription with random primers using Transcriptor First Strand cDNA Synthesis Kit (Roche, #04896866001). Standard RT-PCR was performed using GoTaq DNA polymerase (Promega, #M3005). To quantify the mRNA levels using real-time PCR, aliquots of first-strand cDNA were amplified with gene-specific primers and universal SYBR Green PCR supermix (Bio-Rad, #172-5124) using a Step-1 Real-Time PCR System (Applied Biosystems). The PCR reactions

contained 20-40 ng of cDNA (except the cDNA for the IP, for which 5% of the cDNA was used for each gene examined), and 300 nM of forward and reverse primers in a final reaction volume of 20  $\mu$ l. The ratio of different samples was calculated by the data analysis software built in with the Step-1 Real-Time PCR System.

To determine differential gene expression between WT and *HuD* KO mice, cDNA were synthesized from the total RNA from WT and *HuD* KO SVZ NSCs as described above. 10 ng cDNA was added into each well of a 96-well Neurogenesis Pathway Arrays (Qiagen/SABioscience, #PAMM-404Z, Valencia, CA, USA). Each sample was applied to one array and independent duplicates were analyzed for WT and *HuD* KO. Real time PCR and data analyses were performed according to manufacturer's menu. Briefly, the expression level of each gene was obtained by comparing to internal controls on the same array. The relative expression levels of genes in *HuD* KO compared with WT samples were then calculated with WT samples set as "1".

The sequences of primer used are as the following:

<i>Satb1</i> forward:	ACAGACCTCCCCACATCATC
<i>Satb1</i> reverse:	TTCCACGGAAATTTTGGTTC
<i>HuD</i> forward:	CAATACGGTCGCATCATCAC
<i>HuD</i> reverse:	CCTTTGATGGCTTCTTCTGC
<i>NeuroD1</i> forward:	TTAAATTAAGGCGCATGAAGGCC
<i>NeuroD1</i> reverse:	GGACTGGTAGGAGTAGGGATG
<i>Tuj1</i> forward:	TATGAAGATGATGACGAGGAATCG
<i>Tuj1</i> reverse:	TACAGAGGTGGCTAAAATGGG G
<i>Gfap</i> forward:	CCAAGCCAAACACGAAGCTAA
<i>Gfap</i> reverse:	CATTTGCCGCTCTAGGGACTC
<i>Aquaporin 4</i> forward:	CTTTCTGGAAGGCAGTCTCAG
<i>Aquaporin 4</i> reverse:	CCACACCGAGCAAAACAAAGAT
<i>Gapdh</i> forward:	AATGGGAAGCTTGTCATCAACG
<i>Gapdh</i> reverse:	GAAGACACCAGTAGACTCCACGACATA
E1a forward:	GGACCCAGTGAGAAGCGACT
E1a forward:	AGAACAGGAGGCAAGGTCTG

E1a2 forward:	TGAAATCAGCAGGACGCTTA
E1a3 forward:	GGCTGCCTGATATGGGATTA
E1a4 forward:	GACCTGCAGTTGTGACAGGA
E1b forward:	CCACCCCCTCCCAATAATAG
E1c forward:	GAACGTTGAGATGGGCAGTT
E1c1 forward:	ACGCTCCTTTCTGCTTTTGA
E2 reverse:	CCATTCCACTCCATCTGTGA

### Actinomycin D Treatment and mRNA Stability Assay

Culture hippocampus neurons were treated with 10  $\mu\text{g/ml}$  of actinomycin D (Sigma-Aldrich, #A1410) to inhibit gene transcription as described[2] and SVZ NSCs were collected at various time intervals for RNA isolation and real time PCR analysis. *Satb1* and *NeuroD1* mRNA levels were normalized to Gapdh. RNA decay kinetics and half-life were analyzed using a published method [16-18]. Briefly, for single rate decays we used the exponential function  $M_t = M_0 e^{-\lambda t}$  ( $M_t$ : amount of mRNA at t time,  $M_0$ : amount of mRNA t t=0.  $\lambda = (\ln 2)/T_{1/2}$  ( $T_{1/2}$  is the half-life of the mRNA). Since regression analysis indicated that the best fit for *Satb1* mRNA turnover in wild type cultures was obtained using a two-rate decay we calculated the  $T_{1/2}$  separately before and after the inflection point (a t=3 h).

### Western Blotting Analyses

Protein samples were separated on SDS-PAGE gels (Bio-Rad) and then transferred to PVDF membranes (Millipore). After primary and secondary antibody incubations, signals on the membranes were detected and analyzed using a Li-CoR Western blotting system (Li-CoR). Primary antibodies used are: mouse anti-HuD (1:500, Santa Cruz Biotechnology, #sc-28299), goat anti-SATB1 (1:500, Santa Cruz Biotechnology, #sc-5989), rabbit anti-flag (1:2000, Cell Signaling Technology, #2368S),  $\beta$ -Actin (1:1000, Sigma, #A5441) were used as primary antibodies. Blots were washed and incubated with IRDye 800CW-conjugated or 700CW-conjugated antibody (Rockland Biosciences, Gilbertsville, PA,

USA). Infrared fluorescence images were obtained with the Odyssey infrared imaging system (Li-Cor Bioscience). Quantification was performed by using software Image J.

### **Luciferase Reporter Assays**

Transfection of aNSCs was carried out using Fugene HD (Roche, #04709713001) based on the manufacturer's protocol with modification. Briefly, aNSCs were plated into 24-well P/L-coated plate for 24 hours. In a separate tube, 1 µg DNA was added into 50µl opti-DMEM medium (Invitrogen, #11058-021) followed by the addition of 3µl Fugene HD transfection reagent. The mixture was mixed by pipetting 3-5 times, incubated for 10 minute, and then added onto the cells. 24 hours later, the transfected cells were changed into differentiation medium for 24 hours before harvesting. Luciferase activity was detected using the Dual-Luciferase Reporter 1000 System (Promega, #E1980) based on the manufacturer's protocol. Briefly, collected cells were lysed in 100 µl of 1X passive lysis buffer at room temperature for 15 min. Then 20 µL of the lysate was added to 100 µl of Luciferase Assay Buffer II and mixed briefly. Firefly luciferase (F-luc) activity was immediately read using a SpectraMax M2E plate reader (Molecular Devices Corp). Next, 100 µl of Stop & Glo Buffer with Stop & Glo substrate was added and mixed briefly. Renilla luciferase (R-luc) activity was immediately read. For the *HuD*, *NeuroD1* and *Gfap* promotor activity, F-luc activity was normalized to R-luc activity to account for variation in transfection efficiencies. For *Satb1* 3'UTR, R-luc activity was normalized to F-luc activity to account for variation in transfection efficiencies. Each experiment was independently repeated 3-4 times.

### **Production of Lentivirus and Retrovirus**

Lentivirus production was performed as described previous [11, 19, 20]. Retrovirus production was performed as described in our previous publications [5, 7, 19]. Briefly, lenti-viral DNA was co-

transfected with packaging plasmids pMDL, REV and pCMV-Vsvg into HEK293T cells using calcium phosphate method. Retroviral DNA was co-transfected with packaging plasmids pCMV-gag-pol and pCMV-Vsvg into HEK293T cells using calcium phosphate method. The viral transfer vector DNA and packaging plasmid DNA were transfected into 5X15 cm dishes of cultured HEK293T cells using the calcium phosphate method. The medium containing lentivirus was collected at 36 and 60 hours post-transfection, pooled, filtered through a 0.2- $\mu$ m filter, and concentrated using an ultracentrifuge at 19 k rpm for 2 hours at 4°C using a SW27 rotor (Beckman). The virus was washed once and then resuspended in 100  $\mu$ l PBS. We routinely obtained  $1 \times 10^9$  infectious viral particles /ml for lentivirus and  $1 \times 10^8$  infectious viral particles /ml for retrovirus. To study the effects of SATB1 on the proliferation and differentiation of NSCs,  $1 \times 10^7$  viral particles was added to the NSCs cultured in proliferating condition on a 10 cm tissue culture plate. After a 2-day incubation, infected NSCs were either collected for RNA analysis or trypsinized and plated into 24 well plates (Fisher, #87721), at a density of  $1 \times 10^5$  cells/well, for differentiation or proliferation analysis.

### **In vivo Virus Grafting**

In vivo viral grafting using stereotaxic surgery was performed as described [15] with modification. Briefly, 7- to 8-week-old C57B/L6 male mice were anesthetized with isoflurane and placed in a stereotactic instrument (Stoelting, Wood Dale, IL, USA). Microinjections were performed using custom-made injection 33-gauge needles (Hamilton, #776206, Reno, NV, USA) connected to a 10  $\mu$ L syringe (Hamilton, #87930). Virus (1  $\mu$ l with titer greater than  $1 \times 10^8$ /ml for retrovirus, and  $1 \times 10^9$ /ml for lenti virus) was mixed and then stereotaxically injected into the SVZ using the following coordinates relative to bregma, caudal: +1.0 mm; lateral: +/-1.0 mm; ventral: -2.2 mm, and caudal: +0 mm; lateral: +/-1.4

mm; ventral: -1.9 mm. One week post viral grafting, mice were perfused for differential analysis. Mice were deeply anesthetized with pentobarbital and perfused with saline followed by 4% PFA.

### Statistical Analysis

The results were assessed by Student's *t*-test to compare two groups or by one-way ANOVA with *Tukey post-hoc* test for multiple comparisons, or two-way ANOVA with *Bonferroni post-hoc* test by GraphPad Prism. Statistical comparison between 2 genotypes within the same treatment group and between different treatments groups within the same genotype were carried out for each experiment. In all tables and figures, data are expressed as mean with standard error of mean (mean  $\pm$  SEM). \* for  $P < 0.05$ , \*\* for  $P < 0.01$ , and \*\*\* for  $P < 0.001$ .

### Supporting References:

1. Akamatsu, W., et al., *The RNA-binding protein HuD regulates neuronal cell identity and maturation*. Proc Natl Acad Sci U S A, 2005. **102**(12): p. 4625-30.
2. Guo, W., et al., *RNA-binding protein FXR2 regulates adult hippocampal neurogenesis by reducing Noggin expression*. Neuron, 2011. **70**(5): p. 924-38.
3. Anderson, K.D., et al., *Overexpression of HuD, but not of its truncated form HuD I+II, promotes GAP-43 gene expression and neurite outgrowth in PC12 cells in the absence of nerve growth factor*. Journal of neurochemistry, 2000. **75**(3): p. 1103-14.
4. Allen, M., et al., *HuD promotes BDNF expression in brain neurons via selective stabilization of the BDNF long 3'UTR mRNA*. PloS one, 2013. **8**(1): p. e55718.
5. Smrt, R.D., et al., *MicroRNA miR-137 regulates neuronal maturation by targeting ubiquitin ligase mind bomb-1*. Stem Cells, 2010. **28**(6): p. 1060-70.
6. Bronicki, L.M., G. Belanger, and B.J. Jasmin, *Characterization of multiple exon 1 variants in mammalian HuD mRNA and neuron-specific transcriptional control via neurogenin 2*. J Neurosci, 2012. **32**(33): p. 11164-75.
7. Liu, C., et al., *Epigenetic regulation of miR-184 by MBD1 governs neural stem cell proliferation and differentiation*. Cell Stem Cell, 2010. **6**(5): p. 433-44.
8. Barkho, B.Z., et al., *Identification of astrocyte-expressed factors that modulate neural stem/progenitor cell differentiation*. Stem Cells Dev, 2006. **15**(3): p. 407-21.
9. Guo, W., et al., *Isolation of multipotent neural stem or progenitor cells from both the dentate gyrus and subventricular zone of a single adult mouse*. Nat Protoc, 2012. **7**(11): p. 2005-12.
10. Liu, C., et al., *An epigenetic feedback regulatory loop involving microRNA-195 and MBD1 governs neural stem cell differentiation*. PLoS One, 2013. **8**(1): p. e51436.
11. Barkho, B.Z., et al., *Endogenous matrix metalloproteinase (MMP)-3 and MMP-9 promote the differentiation and migration of adult neural progenitor cells in response to chemokines*. Stem Cells, 2008. **26**(12): p. 3139-49.
12. Luo, Y., et al., *Fragile x mental retardation protein regulates proliferation and differentiation of adult neural stem/progenitor cells*. PLoS Genet, 2010. **6**(4): p. e1000898.
13. Guo, W., et al., *Ablation of Fmrp in adult neural stem cells disrupts hippocampus-dependent learning*. Nat Med,



2011. **17**(5): p. 559-65.
14. Guo, W., et al., *Ablation of Fmrp in adult neural stem cells disrupts hippocampus-dependent learning*. Nature medicine, 2011. **17**(5): p. 559-65.
  15. Szulwach, K.E., et al., *Cross talk between microRNA and epigenetic regulation in adult neurogenesis*. J Cell Biol, 2010. **189**(1): p. 127-41.
  16. Bolognani, F., et al., *In vivo post-transcriptional regulation of GAP-43 mRNA by overexpression of the RNA-binding protein HuD*. Journal of neurochemistry, 2006. **96**(3): p. 790-801.
  17. Beckel-Mitchener, A.C., et al., *Poly(A) tail length-dependent stabilization of GAP-43 mRNA by the RNA-binding protein HuD*. The Journal of biological chemistry, 2002. **277**(31): p. 27996-8002.
  18. Perrone-Bizzozero, N.I., V.V. Cansino, and D.T. Kohn, *Posttranscriptional regulation of GAP-43 gene expression in PC12 cells through protein kinase C-dependent stabilization of the mRNA*. The Journal of cell biology, 1993. **120**(5): p. 1263-70.
  19. Smrt, R.D., et al., *Mecp2 deficiency leads to delayed maturation and altered gene expression in hippocampal neurons*. Neurobiol Dis, 2007. **27**(1): p. 77-89.
  20. Li, X., et al., *Epigenetic regulation of the stem cell mitogen Fgf-2 by Mbd1 in adult neural stem/progenitor cells*. J Biol Chem, 2008. **283**(41): p. 27644-52.