### Positive Feedback between RNA-binding protein HuD and transcription factor

## SATB1 promotes neurogenesis

(Wang et al 2015)

**Supplementary Figures** 



Α



### 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 costained 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 μm

#### Α





# 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  $BrdU^+$  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 ± SEM.

## Wang\_Fig.S4



## 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 hydroxylse (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 ± 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).

## Wang\_Fig. S6



#### 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 μm.
- 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.

Wang\_Fig. S7



## 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<sup>#</sup> and 3<sup>#</sup>) 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-sh*SATB1* (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 ± 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 µm. (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 ± 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 SATB1 3'UTR

>ENSMUSG0000023927|ENSMUST00000144331 mouse SATB1 1 GAGAACAGTATTCTTTTCAGCCACACCACCGGTATTTCCTAACAACATGAGAAGTCCACC 61 TTGTGTTCACTCAGACAAACCTTCATTGTTTATTGTTTGGCCAATGAATCTTCGGAAACT 181 TTTACAAAACTGCTTGGCAGCCCCGGGTGAAGCGTCAAGGACTGTTTGGTAGAATTTGTG 241 TTCAAGGGCTGCACCCAGGTGTTGTACCCTGTCAGCATGATACCAGAAATTGGTTTTCCT 301 TTTGATTATTATTATTCTGGAGCCTCAAATAAGCATTATAACTTCTGTGGTTATGATTGC 361 CTTTTCTTTAGTAATTCTTCTGTAACCCGCCACACTAGTCTTTGGAAACTGGCCCCTTAT 661 AAACACAATTTAGTCCTTGTTATTTATTTGTAGCTCCTGCAGCATCATGTCATAATTAAG 721 TTTTTTGGAGATTTCTGTTAAATGTAATGTTGCTTTCCCATCCTGATTTCCTTTCTATTT 781 ATAACTGTATTTTGATGGGCAGTAAAACAAAGTGTCTTAAAAGTTTTAAATAGAGAAAAA 841 TGTGCTTTACACAGTTGCCTATAAAAAGTCTATGTTATCCAAGCAATTCACTCTAGAAGC 961 CTAATAGATCACCTTAGAAAATTATGCAATTAATGTGAAAATAATTGATGTTTGCAATGT 1021 GTCTTCCTTTGGTTTACAATCAATTTTAAAGCGACATCTGTATAAAGTTTCTGTATAAAG 1081 GTGTATTTCTTTTTTATGAGTTTATGGCTATGAAAACACCAGCTATTTTGTTACAGCTGC 1141 TGTTTTTATAAGTGTATCACAGTTTTCTTTATGCAGAAATGTGCTGATTAGGAGTGGTTA 1201 TTGACTGTAAGTACACGATTAAAATTGTTTGTATGGTATGACATGGCAGGGTTTGTCTGC 1261 TTATGTGAAGTAACTTGAGCCAGGCTCAAGGACGGCTGTCATGTAGGTGCATGCTCACGC 1321 TTTCTTGGTGATAGCTTGTGGGTTCTGAAAAGCAACTGAACACTCCCTGAGATACTCAAT 1381 TCAAGCTACACATATGCTCAGTTTTCAATAGTTATGGAATTCCACAATGCAGATGATAAT 1441 CTGCTTTTGCTTATTACACCAGACAGTCAAAGACTCCAGCAGAAATATTACTGTAGCAGT 1501 TAAGTACTTGAGATGGCCATATCTGCTTTTTCTGTACAATCCTCCAGTTAGGGCAATATT 1561 TTCTTCAAGCTAGGGTACATTTTTTTTAAAAGCCATCAATAGCAGCATGAACAAGGGAAC 1621 GCATGGGCCTTCCGTGCGAGAATCCTTTGCAGGAAACAGTTTAGGGGAGGGGCTTGTTTG 1681 CTGGTGATTTGTTTTACAAGTCATAGGTAGGCAAGGTGAACATGGACTGCCTTTGTCAAA 1741 ACTGAGAACCACATGAAGATAGTAGGAGGCAGAGCCCTGGACTCCAAGTCTAAACTGCTG 1801 CATCATGGCCCAGGCTGGTGTGCTGTGGCTGGCTCCAGAGAAACTTTCGTAGGCT 1861 TTTCTGTCTTTTGTATCCCTTTGTTAGGGTTCCCGTGTTGATGGGGATATTTAGGTTTGT 1921 GGAGCCAGGCTCTGTAGCTACTGTCTTCCGGGGGGGGCATTTCCTCAGGCCTGGGCAGCTC 1981 CATTCTGGTGGCTGCAGGGCTTTCCTGGTGGTCTAAATGGGCTCCTTGAAAACTCCACTG 2041 AGGGATTTACTTCGAAGCACAGCTGAGCACCTTGGAAGGCTGCTGTTCTGCTGCAAACTA 2101 ATTTCTGATTCTTAAGTGAGAGAGAGAAAACAATGCAAACTTTCTTGTTCGCTGGAGTTGAG 2161 CAAAGTAATTACTTGAGCCAAAGGCATTTGAACGGAGATAATGCAAGCTAATTTTAAATG 2221 TTAAAAACAACAACAACAACAAAAACCCCCCAAAACAAGTAACATGCTGCTAACCACAGATT 2341 GCCTTTTTTATTTAAAACAGCCCAACACTTAAGATATCAGATAGGTGTAAAATTCAATAG 2401 TTATTTAGAAGGAGGAAGATTTTTCCTGTTCCTAACAGTCTGAGTTATTTTGAATTGATG 2461 TATCCAAGGAAATGAGTGTGTTGGAAAGCAACACCATTGTGCCAGCAAAATTATCAGATT 2521 TTTAAAATTTCAGGTCAGCATGGTTTTGTTACTTTAGAATAAAGCACATGTTTGCAAGCC 2581 ACGATGGTCACAATGGAGTGGGCAAAACTGTTGAAGACAGCGTATTTCAGAAGATTTCCC 2641 CTAGTGATCCACATTGTCCCCAGCTCCCAACGTCATTGTCACCCATGTGTCTCTGGTTGC 2701 CTATGGCTGTTGGTAAAATTCCTTTAACAAGCTTCATTGTGTCCCAGCGTTTCGGCTGGG 2761 CATGACATAGTGCTTTGTGCTGTTAGAACCTTAGCAGAGTCAGGTAATTTCCGGGTAGTA 2821 GTCAGTTGTGTGCATCTGAGTCTGTCATAGCATCCTACACTCAGCACTTTGCCACTAATT 2881 TGTATTACACTCTGTGGCCATATAATACCTGCACATTATGCAAAAAATGTTGTTGACAGT 2941 ATCTTCTTGCCACACAATATGCAGAGTTGACACATAACCTTTGAAAACAAAGGTGACTAG 3001 TATGTGTGCCCTACCTGTGTGGCACTTAGTAACAAAGTCTGTACATATGTATAAAATGCG 3061 TATTGACTGGCATTTAGTACTATGAAATTCTGAGCTTAAGCGTCTGCCCCTAGTAGTATC 3181 AGAAGTGATTGGAAATGAGTGTCCCACACCTGCTATTATTAAATTCTTCCCTGGTCCCAG 3241 TTGTTCACATTTGGCTAACAAGGTCCTTGATTGTCACAGTATTTTCAATAAATGAGCTAT 3301 TATGAACTGCCTGGTGATATTTTCAGCAAGAGCAACTAAAATGTGTATGGCCTGTGCTAT 3481 ATTCTTGGATTCAGTGTTCCCTCAGAAGCAATCCTTTAAATGTAAATAGTGAATACTTGC 3541 ATGTCTTTGGACAAAAGCTGAGCTACAATCTATTAAAATGCACAATTTTTGATAAAATAT 3601 AAAGTGCCCTCTAACAAAATGTGGTGGAAGGTGTGTGTTAAGAATACTATACCTGTTTAA 3661 CAATACCATATGCTAAATAAAAATAGTTTGGAATTAATGTTTAAAACATGA

#### ARE and HuD binding motifs:

AUUUA 007 081 770 1908 2044 2305 2349 2402 3071 3107				
ARED				
U-RICH 293 564 565 566 567 568 569 570 622 623	1083	1085	1854	1856
GRE				
MOTIF 1 2290				
MOTIF 2				
MOTIF 3 1578 2343 2344				

	HuD shSATB1- 3/WT-MC	267	1 8	5.30	2.06	-1.51	157	1.34	198	1.30	10.7	2.05 1.33	2.68	-154	133	1.33	18.5	1.24	282	-152	-1.58		1.32	2.04	1.36	2.72	. w	1	1.32	2.68	88	1.50	59	51. 25.1	121	2.70	19	132	151		13	130	13	10.64	1.52	132	1.52 2.66	2.66	5	133	13	157	2.68	8 S S	181	121	2.73	58	267	1.53 1.13	1.34	1.32	
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	NUD INSKERT -1	809/109E00	0.00451.0029	1222 2200 000	0.017306204	0.5 7863 0952	2207 3036 0.0	2025 2226 0.0	0.0 0223 4782	0.0 0112 8965	TELO YTTO OD	0.0022555682	0.000562101	0.03600304	1616 5500 010	0.0363582	0.00056144	0.035009532	171821111000	1951 6652 000	0.00415312	00000000000	0.2003 TUT	11 5960 8834	0.001071935	0.071363651	0.011305.000		0.000130437	0.000561764	0.018240804	0.0362.8324	0.01751611 0.287689041	0.035726943	0.004540541	0.000274538	2019105200	0.002266363	0.000030749		0.141472116 0.004501784	0.035578178	0.000566554	0001130064	1/16/20/50	0.584715991	0.017849965	0.004327592	1822 6627 0.0	4.5 9930 7889	11 6095 7877	0.2 8997 5208	01000078172	0.000554382	0071632736	0.00447187	0142039785	0.001126509	0.001129739 0.071180632	4.649495566	4526395525	0.035126458	
ŭ	HAD NO-NC	805 9096 0 0	11469 (2220 010	261011100'0	0.034590742	0.5 8396 2302	0.0 3588 1318	0.0 3604 3827	0.002218906	0.002244148	ACCI VITO OD	0.002249278	0.001128761	0.0 3593 1447	0.001120193	0.01792.6125	0.001131365	1027 7925 0.0	1002 8010 00	0.017635764	10005123-007.0	10001000	0.2878 8666 0	1.162518969	0.0022573333	0.071685558	0.01736362		0.0001 3044	0.000553500.0	0.000000000000000000000000000000000000	0.036018818	0.017736448 0.288329266	0.017814069	0.0000 Web 2019	0.000547848	0.0 3486 1382	0.00225422	0.000337645		0.14154216 0.002164707	0.035874013	0.001137619	0.001126983	22/27/29/20	0.579625383	0.018041566	0.004336942	0072502083	2.2.25039736	1.158413397	0.291128905	111000000000000000000000000000000000000	0.000543154	0.07169559	0.004504850	014549877000	0.002243869	0.001134711 0.03462872	4.627213484 0.583901314	4517472276	0.035223112	
24	WT #MSATB1-3	0.0 3072 2208	9492 1600 0 0	3955 2300 0 0	0.030721274	05 0928 0089	00062763149	0.031601193	1366 6600 0	0.000356453	000000000000000	0.003946395	0.00097114	0.063987/42	0.001963183	0.031582022	Serv. 25100'0	0.0 6277 4933	0.001890575	0.03086127	N801 6260 0 0	0.0000000000000000000000000000000000000	0.252271665	0.997129939	0.0 0383 6019	0.061859782	0.01306000		0.000227018	0.00076576	0.031629155	0.031510205	0.2 5105 7201	0.062623819	2842 0612 00 0	0.000384134	0.029260572	0.003942115	0.00782.8785		0.007745108	7721 02050 0	NOSE 6600010	0.0078520	0.247604714	1.010862557	0.03138618	0.00373077	0.06249629	4.0.1100 9485	2.022527134	0.250205336	1778441210	0.000339497	0.062202007	0.007811013	0.253735051	0.0019568938	0.001976637 0.062059984	4.051991033	3.9 4026 4467	VESS 250E 010	
	WT #54782 -1	0.02 6664 505	100000000000000000000000000000000000000	122 00000 0	0.026487949	0.628875114	0.0 5477 2696	0.027566938	0.003416463	100.01197194	100 101 100 10	0.003424114	0.00084838	0.0 5583 3908	0.001715827	0.02727316	0.001218 988	0.05463419	2 2959 100 10	0.038293137	0.00 1945 00.0	10000000	110722115.0	0.0003952326 0.861342548	0.003 3747 7	0.053940132	0.00.0000100		100 8610 00 0	0.00.0597.858	0.027637459	0.027417054	1M2 0272 0.0	0.054745683	228678900.0	0.000857302	0.025532583	0.003421144	0.000683629		0.218129327 0.006814307	0.054956926	0.001221403	545500000	817.127.202.0	0.617408013	0.027480497	0.003253116	0.077288217	3 49 6963 953	1.769304891	0.310737239	0.106037557	0.00.0824458	0.076653883	0.006839014	0.152023612	0.001710812	0.00171981	3.51.3578593 0.886184745	3 37 1872 881	0.038181871	
	WT-MC	0.02.0582.096	100 0771 107 0	290 9510 00'0	0.02.0487.609	E14 65002 970	0.041420852	0.02.0574 197	0.001260243	0.00129698	THEFT	0.00 1285 816 0.0 10135 388	0.000542558	0.0419993577	Net 00010010	0.020681045	0.00045758	0.041041037	V12 852100'0	0.041405627	105 9915 007 0	0.00000000	10555 525 0	0.000327512	0.002512677	0.041208441	0.010001000		0.00.0150 389	0.000319762	0.0209336	0.041459245	0.020402615	0.04162.26	0.01044559525	0.000157596	0.040568199	0.00.2617.838	0.010367805		0.165353238 0.005198596	0.041675571	0.000654602	0.000545042	218 00162 510	0.674789764	0.02.0850.848	0.00 MM 698	0.08319228	2.63.9656.475	1.338771274	0.335397115	0.082595991	0.00.0305.951	0.082362638	02/ 2000 2.0	0.080626872	0.001283116	0.000548818 0.040798566	5.390302113 0.673163605	2,49,0188,903	0.041518946	
	7		2 8	51	8 13	161	8 8	88		25	4	C 2	a	8 K	8 2	14	8 8	8.8	8 8	8	6		22	× 8	13	2 2	,	2	24	19	5 8	8	10 10	80	38	18	2 51	91	166	1	8.8	S 8	4	121	6	R	88	2.4	2 3 1	88	19 X	9 22 :	28	8 8	18:	8 6	2 6	22	85	នុង	5	ß	
	THIN W SALAT	01 41879119 01000711 10	21 00/11 25	41 10.213661	91 4.1956.21	38 1.16896.32	29 10 1000 000 000 000 000 000 000 000 00	26 5.1762.48 27 A 1.9161.01	48 9.18542.28	05 9.2131156 24 9.2109650	NC 60011 7 6 107	06 81960945 99 6.2139263	280664.16 28	S7 5.1951045 31 9.1772308	12 9.180917	5180862	10 81882509	665464TF 86	75 8.2546215	87 5.194233	36 82351036	10 10 10 10 10	6689/11 66	91 1017659 57 -08168354	1026221.926501	76 3.19193.26 88 4.18955.42	00110010 10		15 12 2962 11	78 10.187393	22 10.192214 59 6.2065753	46 5.1810626	31 3.19/4391	63 51873092	N 7.1785945	36 11.198352	41 11.187896 59 5.2370014	35 8.1727695 ee 6.2017500	7.1818332		37 2.205390 23 7.1768550	41 42070751	81 10.176501	26999811 H9	11 (55177 56	47 0.1640491	52 61916236 73 9.1792011	72 72462940	25 41813392	18083436	28 -0.826139	21738662	23 10.1960.0 26 2.1767599	73 9.1980361	3 191183	N 2.197178	11 2.1855201 66 8.2092018	N 82014045	59 9.172616 45 4.1900844	42 07230258	12 52 52 981	60 41881103	
4K G()	TATA A SUTAT	34 47961538	21.02267.5 17	00 11 11 10 10 10	23 5.8525.06 d8 1.7915851	96 0 2 89 26 96	22 4.7931758	04 4.7839977 36 4.7975554	16 8.8056499	41 9.7909118	00.0076.776 0.	25 87922203 84 6.8275691	17 10.796881	61 4.7957374 01 9.7730457	67 10.804370	26 4.7815753	00 10 7385 70 1	31 4.8 361083	21 5.815/123 56 8.8236438	97 4.796181	64 7.8103676	001000000000000000000000000000000000000	55 1.7840320 56 1.7840320	27 10.720.79	78 9.8655673	94 3.7984935 11 3.8096572	0.09603 10		87 12.904357	53 10.797 M	0 12814105 61 5.776868	62 4.7843143	58.561737 15 1.7974178	81 4.8059477	14 7.7829200	07 11.830180	10 11 202 41 48 3581 27	52 8.785405	11 6.7900385		59 2.821410 73 7.7952873	53 4.8128635 84 9.8660230	64 10.785498	0380816 94	85210080 66	02.61M.20 62	16 58079349 96 10.791758	16 7.8522197	N 3.7890203	73 10.79185 11 2.2014167	0.2153156	00 1.7859085	28 2.7918102	01 10.816832	17 3.8 0323 71	22 1.7 2005050 50 00005057 55	04 2.8156330 36 8.7954799	32 9.7939254	91 9.7897941 31 3.8123744	61 2.2170742 37 0.7829124	47 -21789526	32 48312980	
C8(001) - Ave C1()	3 NUD ND-NC	28 47932552	01 21.029 04	189621816 25	49 4.8534.70 86 1.8.085464	81 0.7760528	46 48006233	82 4.7941040 ec 4.7962322	73 8.8159355	85 8.7996162 33 9.8053878	01000006 00	66 8.796322 04 6.8204692	22 9.7910146	10 9.7 2000 11	51 9.8020366	261085 5801928	0022787201	03 4.8 0885.35	22 9.8454307 23 9.8454307	77 5825520	27 7.78466.02	0.0000	21 1796071	0217540 N 0217542	45 8.791164	31 3.8005641 34 3.8021778	20 CK 20 10		17 12 90825	23 10.8191.05	65 6.8054216 66 6.8054216	58 4.7951053	43 5.8171390 23 1.7942108	90 5.8108390	30 4.79627U7 36 6.7845291	60 10.8339.37	18 9.7940781 24 4.842226	99 87931560 00 A 706740 00	26 6.8058895		88 28206962 56 8.8516124	11 48009170	50 97 NV64	010001/10 1000181818181	13 1.82218.3	53 0.80181.92	73 57924980	83 7.8491062 14 A.7000104	1 7858 3	00 11.822740 76 · 1.15383	S8 0.2121501 22 1.2756711	36 1.7802700	58 10.805.57 02 3.8675557	28 10.84635	65 38019718	06 07 07 00 00 00 00 00 00 00 00 00 00 00	27 2.7809.28	59,000 8 29,000 50	88 9.7834590 71 4.8518871	81 2.2101436 44 0.7762035	60 -21750157	31 48 2733 38	
AVGAC, I	-1 WT INSATB2-	50267	1932661 0	2 11 0967 96	1.9897956	89462.60	78 E66E 6 E 1	12 49838771	189/1861	10.03001	0.0006.000	3 7.0269165	10.0093	3966060	56852668 E	1000000	89821166	X 99666 E	0.040558	5 5.0180587	1 8.079705		36N6961 6	9.9878768 0.0041465	2 8.0261745	3 4.014854 7 3.999126	0.000		12 10/90	7 11.03470	110002869 6	4.9890230	1.9385.4	39971446	100869 5	1 2888 0 6	5 0 9489 80 8	2 7986814v	5669669 8		7 2000M95	5 3990503 8050466	5094V.66 2	70003	6555107 0	0.01558.62	4.9937.20	8.0663106	4.000108	2003962012- 2	10161590	51886.61	3 3.04158	10.0558.24	40068950	x 2000.7 4	5 1.978605 8.0057582	8577968	1 89827362 1 4.0101922	20196305	x 6282 61- 8	5026767	
	WT MSATBE	5 5.22805562	012020515 9	7 10.7190062	8 5.23852005 9 2.186733.22	8 0.66915454	25 6660 6T P I	6 5.18091716 A A 19722021	090622618 2	2 9.70612754	1000000716	4 8.19005336 8 6.72173824	3 102030023	7 9.6717567	50 0017 June 6	4 5.19637432	2 9.68010.082	7 4.19405212	2201M-62.6 8	2 4.067032	4 7.75107040		9 158557083	2 0.21534090	4 8.21099510	9 4.21289713 0 4.19552459	0 5 2 AND 10		4 12300234	4 10.7078851	6 5.1772312U	3 5.1887821.2	9 5.20010414 7 1.690555	5 419111090	7 7.18341102	8 10.1879.095	7 5.29151668	8 8.19130554 8 8.19130554	6.69.023647		1 2.1967434 5 7.19721739	6 41855489	ZS5W21296 1	1011111000 0 00101110000 00	200 1006 DV 1 0	5 0.695N388	3 5.18544807 1 9.18246972	7 8.2.6396.217	3.6936071	502502076 0	0.82318267	1 5 96232 94	3 10.204um 4 3.23755275	6 10.242559	3412850144	2211N2122 0 009691917 6	4 2.71763267 6 8.19920959	9 9191103 96	4 9.18353500 3 4.20485725	7 0.1742060	1.7535914	5 4.71096839	
	WT-WC	5.60246696	8.58727302	12.64277.68	5.60910453	0.57763900	4.59.342861	5 6 000000 A	9.6 32032 36	9.59062805	46 T 1000 T 1	9.60310020	10.6038860	4.57.348861	58 58 658 5 6	2 29 5547 10	10.5967182	4,60,6789.01	9.63376464	4.59 399452	7.5968279	10.0000	1.5753787878	115761646	8 63 6558 91	4.63769187 4.6009162	20 Maee 2 2		12.6900131	11.6107143	5.57803573	4.5 9181 412	5.61227645 1.58694005	4.5854910	6,581,1332	12 6314 891	4.6250692	8.57740821	SL SN 165 9		2.59637680 7.58766212	4.58465423 9.610754390	10.5770954	105983203	78-2515 091	00084.950	5.58375015 10.588788	8.6585315	3.587.4065	1 0000011	-0.4 2090 95	1.5 7605781	3 59 7784 42	11.6744121	3 601 9561	6.60023345	3.63 2505 44	9.60613288	10.58389.75 4.61.5337.75	-2.4 303661	-1.31625515	4.5 90095 36	
	rat -3	NDI	MDI	502	202	10	9476	1731	165	378	0.0	202	152	335	590	8	126	1874	970	815	1216		273	59	1887	100	9	2	1554	195	415	51.45	1012	1802	1382	157	1018		00		006. 1981	388	210	164	60	161	245	1278	100	000	1931	805	301	305	5	664	1813	1.81	504	52	906	1161	
	T82-1 HuD M54	9666 268917	0.947 20.8800 9041 30.8806	2927 32.9174	2881 238877	1249 23.8726	1874 279199	4058 27,8900	2928 31.8892	5472 31.9169	14/16/10 0005.0	6316 308998 1205 289177	2472 3138392	8031 27,8388 8858 31,8310	1297 31.8847	1825 27,894	2 226 30,8923	5125 268835	8673 30.9584	2472 278982	1049 302 309		7401 238801	4196 328803 4928 218869	1025 308966	3643 25.8957. 0014 26.8933	000011 0001		4948 37.0040	9066 328911	2 <i>97</i> 1 28.9103	572 27.8848	1665 289540 6068 259012	9052 27.8910	2380 278870	2321 33.9021	5561 27,9407	31876 6101 30876	8142 298856		5322 249001 3018 29.8906	0639 26.9108	4166 32.8803	2312 298902	1871 249176	3349 228678	5778 288954 0158 31.8826	6263 29.9500	6318 268851	2607 208954	2722 218776	4139 24.8776	7.238 3.25 usu 5.338 2.4.8805	7559 319018	258949	2285 248959 4893 299009	7586 248893 2282 30.9190	6833 30.9051	3701 31.876 0143 26.8938	686 20.8831 5653 22.8758	202 20.968	4092 268918	
	HIC HUD WEN	2937 268907	081.2 258.62 208.02 29.8802	4262 3331200	4436 279482 2058 238872	2699 228849	9745 268888	7814 268796	106.06 206.0	9037 318965	000076 1070	9638 30.8878 4342 289232	1875 328925	8329 268913 1343 318696	7108 329000	6666 268772	9424 328942	2766 269317	4.00 273033	2623 268918	239000	000010 0100	0129 238796	3106 328764 1988 218832	3891 319612	3833 258941 5194 259053	MYOT MYO		3604 370,252	7966 328933	10005 240000 9579 27872	P18.82 26879	1319 279308 8405 238930	1321 269024	4489 203855 0325 298785	1121 339258	0054 269314	3018 30.8810	6364 288865 econ 210112		7039 249170 8661 298909	9119 269085 2912 319625	105 328811	9231 318850	206.82.2 218.0	9336 22.865	7.215 27.903	8035 299478	0787 258836	4303 198942	2304 218803 ACM 218803	4414 233816	0317 325-uso 2986 248874	2423 329124	496	4408 2338290	0217 249112 0017 308911	6967 318896	3322 318854 6126 25,908	3047 198785 7767 22.878	5839 19.91.7	02632 0440	
õ	47.81-3 HuD 10	96701 26888	668100 90550 668100 90550	01610 10580	71397 26948 10151 23908	56461 22871	26892 26896	102311 26889 102311 26891	11608 2882	31368 30895	0.010 0.000	04439 30891 01233 28916	12958 31886	15646 26894 00944 31866	168 12 82 589	64948 27897	21252 31883	70543 2690F	349.00 27358 31941	31546 27920	24.600	0000 00000	0.675 23892	97272 31875 02424 21878	27037 30895	05026 25896 02226 25897	10010 00100		743.71 36983	79813 32914	283000 3338960 289000	06892 85611	ti6527 27912 99035 23889	20612 22062	1931 0 2029L 61443 28880	62676 32929	BELSA 31890 99405 26937	91032 30888 Press 2690	2890175 28901		84541 24916 59468 30947	59914 26896 54274 31946	70139 31.87	12032 31888	21652 01092	50807 2289/	82256 27888 86553 31891	70671 29947	20454 25581	13045 20940	83677 21883 00016 22083	91136 23879	86N 32A7A 6N03 25963	0201 32942	99089 25897	27882 298892 37048 298892	37011 24876 86411 24876	333.98 308.95	83241 31879 52887 26947	46484 19389 94138 22871	02.021 26.008	86356 26923	
	VIBI-T ML WI WE	15422 27.915	260 00 18912	105 US 105 US	279.21 27.919 28.02 20.834	69157 23.968	10210 201201	81441 27.878 7.914 27.878	620.28 30.88	0945 32.925	10010 210110	71542 30.880 41724 29.922	82822 32.903	23111 26.861 26489 31.878	02829 31.887	978.72 MS88	9422 31.877	0.939 26.838	20018 21.942	33913 27.91	82784 30.974	000 M 0000	04901 24882	93842 32.882 39267 22.895	672.91 30.921	78001 26.909 78001 26.894	1000		283.42 37.035	925.29 33.92.9	718.72 27.877	307 M 27.883	99609 27.904 11273 24.888	93454 25.892	5456 Z7.261 16071 29.878	1819 32.883	2002 27,989 1007 27,989	18816 18839	33427 23.892		20636 24205 70314 24207	46371 26.885	N2 N 32.869	2000 2000 2000 2000	206WZ 2016Z	85188 22.879	02667 27.888	47072 30.961	963.96 26.895	11180 212812	650.96 21.878 a.ms 24.878	50761 2A 293	2023 Z 230	56485 32.950	705.05 26.901	700.52 24.902 5746 24.895	51733 24875 51816 30:90.0	8582 31.892	190 <i>6</i> 7 31.877 378.89 26.90	6059 20.876 2529 22.882	0.942 20.916	03532 27.921	
	NC WTINS	45383 27.938	2000 1012 2000 10101	7673 32.906	00914 27.957 09861 24.886	6228.7 22.969	48247 26.891	0096 27.882 27.822 X.842	0605 30.903	60.091 31.887 20123 31.896	0.070 0700	08.02 N.189 11.25.32 N.184	87289 32.902	47548 26.864	03373 31.88.6	53397 27.912	70508 22.850	77588 26.895	75151 31.932	98138 26.900	20.02 20.022	1000 M 0000	36566 23.883	15149 32.875 17361 22.931	54578 30.900	67874 26.91/ 90315 26.896	2000 1000		22811 36.991	1/012 32.885	72147 35.928 902.26 27.876	80128 27.89.4	2002 23002	4797 26.889	201.96 Z7.888 12013 Z9.8888	47604 32.89	116.75 6.000	39508 30.900	73262 28.888		58315 24831 54839 24886	26411 26.885 2012.6 20.888	08228 31.884	3072 31.895	01632 62441	47687 23.911	73701 27.882 26677 31.883	51843 30.966 critic 30.966	20 M 25 801	4/45/2 31.897	07736 21.874	04458 23.878	77129 25.037 77129 25.937	3902 32.937	8301 25.897	Z012 Z012 2088	58231 25.961 1142 3 30.897	11975 31.889	32462 25.904 32462 26.904	62073 20.897 95778 22.896	73167 20.976	07323 26.900	
	τw	27.90.	20.00	L family 31.94	22.904	22.876	v68.35 (6 Mq	20.02	31.935	31.89.	unk 1 34.94	9/12 28.925	disc 32.900	20.87/ 22.872	31.887	0 27.89,	32.897	1a) 26.90.	31.934	26.82	cogene 29.897		23.876	22.927	186 (1 eset) 30.931	105 XZ (01)	mont 1 jpom.	C Jacon	16 % 36 %	motifike 3391	27875	768.92	erria 1 27.91. 23.887	26.85	m) 20.81. 28.822	34.93	3281	3187	28.8%	denue	21880 21880	2682	32.87	a) 32.896	10 m Ib, 2190	above 0	27.85	30.95	or 3 2588	20,900	1 21.89	23.87	df() 33.7L al 25.838	33.97.	22,900	28.501	960n 3 25.93. 31.924	31.90	3281	19.87	state 20.984	268.92	1055.8
	o Marriso	dinesterate Al recentor	A2 a receptor	rahamakinase sorprotein-binding	errber 1 sor oten E	0 precursor probein	c hom dog 1 (Droso)	N/hymphama2	genetic protein 2	ge neti c protein 4 wee tic ante in Sta	e 5, regulatory sub	p 36) wit as so dated prote	, muscarivic 2, card	ment binding prote e.c. motifi leand 1	fecort in	olog 4 (Drosoph La)	receptor D2	Interest (Drosophil	growth factor	3 pr 006 n p30 0	Heubernia viral onco antorna deri ved onco	f install for	r, alpha	d neurotrophic fact hate isomerate 1	not ropic, NMDAL (2	eacetylase 4 of split1 (Drosoph	related with YRPW i	AND DATING THE POST POST		Habod with WBP Win	Kulkin 3 Kaline	ancer factor 2C	rixed-line agel extros so diated priotein 2	ectin	rubigiona) (numa Merensiation 1	ogonin 1	ogen n 2 rometo sis 1	oggin ottor i morecetativi	olog 2 (Drosophia)	of cella design mo	egdin 1	oplin 1 main 2	trophin 3	molog 1 (prosophile	zans cription factor. scetyfhydr das e, i so	unit1	nvome?	aox gene 5	, transcription facto	t transcription fact of for othin	Rulinum substrate 1	cuton 4	g prote in Ab (carrys polypeptide, neuro	ve dijeti og	nutstel, soluble	taining gene 2 taining gene 3	ctivator of transcrip meth factor heeat	hydroxylase	ascin R dial growth factor A	n, beta icroglobulin	sightate dehydroge	idase, beta	alpha (cytosolic) a
	Gen	Adenticia	Admostra	Anadastich Xdbeta (M) preur	Andino Andino	Amyloid beta (Ak	the terson te complex	B-cell leuker. Brain der keef oor	Bone morpha	Bone morphy, Bone morphose	Independentions.	n on sho non she she	Cholinergic receptor	AMP responsive ek Chemotore (C-X	C) allocation of the country of the	Discs, Large horn.	Docertine	Dishevelie d3, dsh.	Epidemal <sub>1</sub>	ElAbirdin	stb-tiz erythroblists dog 2, neur o/głobi		Flam	Gial cel live derive Glucose phosel	"am aber eceptor, io.	Histoner Hairy and enhancer	Ferhancer of split i	facts new or of odds	Autororation V	ferhancer of split r.	M	Myocyte ert.	which Avriation or Microsoft and a set	4	Navine disease (ps. Neurogenic d	Neur	Neurofb.	A Note to a constraint	No tch gene hom	on of a Caterrate	Neur	Pleu.	Neuro	0.dd 0t/ten-mha	Chigodiendrocyte Xadiivading factoria	50 States of advantageous	Paired	Paired	OUdomain, class 5	Pleio	RAS related C3 by	Ret	S100 caki umbrirum. S100 protein, beta	Sonk:	Super code dia	SRY-box con	with another and a Transforming on	Tyrosine	Vacular endothe	Act. Beta-2 m.	1yosrakl etyde 3-ph	Câuarra	ot shock protein 9 tu
'stb	Desic riptio n	Adreat	AdoraZa	Ak Aptibit Amyli	ADP	400	Acti Act	Bcl2 Bdvf	8mp2	8mp4 8mo8b	onteou OdiSr1 Oyc	CdkSrap 2 CC	01m2	Coult C	Do X	100	Drd2	Dv8	EØ4	E p300	Erbit2 hom	100	fha	Gold	Grint Glu	Hdac4 Hest	Hey1 Hair	Hey2 Law	2	Wild Hand	MAK	Mello	MIL M	Ndin	Neurod1	Ne uro g1	Ne'L	Nog	Notch2	Nr.com Mar	Nrg1	Nep 1 New 2	Naf3	11PO	Paranth1 Parak	Pard3	Pand	Pac5	Pougia I	- Land	Ract	RbM	Stutue Stote	94	2001	2010	Sat3 Sigr	i∉,	Yogfa	Kth BDw	Colpun G		Han Stadd 1
dina y Array Arah	Rofford L	005 000 WAN	009 000 VAN	NM_007409	NW 009 685	NW 007488	ESS 800 WAV	166 N.1 WIN	152 600 WAV	NM 007554	100 JON 100	NM 002559	NW 025876	NW 145 990	160 203 dat	0/1800 Mill	NW 007 865	710010 Mill	NW 007889	MM_010110	01010 <sup>T</sup> WeV	100000	VM_001003817	NW 183171	NW 008005	New 010 227	001010		NM_008155	NW_008169	019 572 May	NW 008 235	NW 010423	New 013 905	000 000 WAV	NW 010 784	VM 001081049	228 010 Min	NW 010 883		New 008 711 New 010 928	008730 MW	165 8/1 WAV	New 010 939	THE STORY AND	MM_011855	New 013 625	NM 021409	NW 008 782	NW 008 900	NM 011143	100 000 May	CIP CID MAN	NW 011313	NW 013660	NUL COD MAN	NM 009 237	NM 022 312	Nev 000 202	NM_010368 NM_013556	NM_008302	NW_008094	
Neu rope nests P.a.	UniGene	Mm-4892.29	Mm.2007.04	Mm.311854	Mm.490092 Mm.375152	Mm 38 594	Mm 1362 17	Mm /43 133	Mm /12 160	Mm 1032 05 Mm 6813	67601184	Mm.439764 Mm.474282	Mm 289427	Mm 379344	Mm. 44365 32	Mm 21 013	Mm. 4875	Mm A1 970	Mm.272443	Mm 3374	Mm 252481	2 00 00 - 1 1	Mm 29082.2 h	Mm 5264	Mm.473689	Mm.205333 Mm.4679	14 co. 761 AVE		Mm589	Mm 278572	Mm 394027	Mm. 30085.9	Mm 29581 Mm 103573	Mm 103615	Mm.9342	Mm906	Mm-401574 Mm 2389 N	Mm 27 592	Mm 5014		Mm 135266 Mm 485843	Mm 5142 Mm 209439	Mm.460675	116992 WW	500 FC U.W.	Mm.327698	Mm 397111	Mm 292834	Mm.43965.9	Mm 483878	Mm 24655.0	Mm.460963	Mm.5107 rz Mm.440639	Mm 1001-44	Mm 33 903	Mm 2897.302 Mm 2897.39	Mm 35784 Mm 249934	Mm.4832.49	Mm 2821 84 Mm 3323 14	Mm 3317 Mm 299381	Mm 2180	Mm.304088	
Table S2	weight	104	100	NON	406 A06	10V	99 F	A10	1	801	70.0	808	908	808	808	608	811	812	58	8	C01		88	58	60	83	55	1	100	D02	88	500	88	800	010	D11	10	88	103	1	88	88	610	3 8 1	Ē	ą	89 89	88	6	22	F10	123	19 G	88	5	55	88	95	612	H01 H02	H03	H04	

Table S3 Neurogenesis Pathway Array Resutls Sorted by shSATB1

well	UniGene	RefSeq	Description	Gene Name	WT shSATB1-1 /WT-NC	WT shSATB1-3 /WT-NC	HuD KO-NC/WT- NC	HuD shSATB1- 1/WT-NC	HuD shSATB1- 3/WT-NC		
D11	Mm.906	NM_010784	Neurog1	Neurogenin 1	4.74	6.24	3.48	1.74	2.70		
A04	Mm.311854	NM_007439	Alk	Anaplastic lymphoma kinase	4.73	3.04	7.10	1.77	5.39		
A08	Mm.56897	NM_009711	Artn	Artemin	4.63	6.00	6.90	1.75	2.66		
C01	Mm.167154	NM_013503	Efnb1	Ephrin B1	2.41	1.53	1.81	1.80	1.35		
G03	Mm.100144	NM_011313	Shh	Sonic hedgehog	2.36	3.07	1.78	1.81	5.56		
C05	Mm.258397	NM_177821	Fgf2	Fibroblast growth factor 2	2.35	3.08	1.76	1.77	2.70		
A11	Mm.1442	NM_007540	Bdnf	Brain derived neurotrophic factor	2.35	3.10	1.77	1.77	2.71		
A12 609	Mm.42160 Mm 249934	NM_009757	Bmp2 Tefh1	Bone morphogenetic protein 2 Transforming growth factor, beta 1	2.35	3.13	1.76	1.77	1.36		
000	NA 270672	NNA 000460	15io1		2.00	5.07	4.72	1.77	2.00		
002	WIII.2/00/2	NNI_008109	неуі	hairy/emilancei-or-split related with the without-like	2.35	1.49	1.75	1.70	2.00		
G01	Mm.310772	NM_019413	\$100a6	S100 calcium binding protein A6 (calcyclin)	2.34	3.03	1.76	1.75	2.67		
E09 E08	Mm.208439 Mm.33870	NM 013627	Pou4f1	POU domain, class 4, transcription factor 1	2.34	2.95	-1.18	-1.19	5.38		
B02	Mm.6813	NM_007554	Bmp8b	Bone morphogenetic protein 8b	2.32	3.06	1.75	1.76	2.64		
F04	Mm.299254	NM_033620	Pax3	Paired box gene 3	2.31	3.04	1.73	-1.15	2.66		
B03	Mm.439764	NM_007559	Cdk5r1	Cyclin-dependent kinase 5, regulatory subunit 1 (p35)	2.31	3.07	1.75	1.75	2.65		
E12	Mm.266341	NM 010939	Odz1	Odd Oz/ten-m homolog 1 (Drosophila)	2.31	12.07	1.75	1.75	10.64		
G11	Mm.282184	NM_009505	Tnr	Tenascin R	2.31	3.05	1.75	1.74	2.67		
C07	Mm.5264	NM_183171	Gdnf	Glial cell line derived neurotrophic factor	2.31	3.01	3.47	1.74	2.64		
E10	Mm.460675	NM_178591	Ntf3	Neurotrophin 3	2.29	1.52	1.74	-1.16	1.32		
C09	Mm.473689	NM 008006	Grin1	Glutamate receptor, ionotropic, NMDA1 (zeta 1)	2.24	2.97	-1.11	-1.17	1.36		
C12	Mm 251445	NNA 010308	Heud	Hain/anhances of colit related with VDDW motif 1	1.10	1.57	1 12	1 1 1	1.40		
C12	WIII.251445	NWI_010308	неут	Hairy/enhancer-of-split related with YRPW motif 1	1.16	1.57	-1.13	-1.11	1.40		
C10	Mm.295533	NM_010227	Hdac4	Histone deacetylase 4	1.17	1.54	1.79	1.79	2.72		
A10 G10	Mm.43133 Mm.483249	NM_174991 NM_022312	BCI2 Th	B-cell leukemia/lymphoma 2 Tyrosine bydroxylase	1.17	1.54	1.75	1.76	1.34		
D06	Mm.29581	NM 010423	MII1	Myeloid/lymphoid or mixed-lineage leukemia 1	1.17	1.52	-1.15	-1.17	-1.56		
B11	Mm.4875	NM 007865	Drd2	Dopamine receptor D2	1.16	3.06	1.75	-1.15	5.31		
G12	Mm.332314	NM 011738	Vegfa	Vascular endothelial growth factor A	1.16	1.52	-1.18	1.74	1.34		
B06	Mm.379344	NM_145990	Creb1	CAMP responsive element binding protein 1	1.16	1.52	-1.17	-1.17	-1.54		
A07	Mm.38594	NM_007488	App	Amyloid beta (A4) precursor protein	1.16	-1.32	-1.15	-1.16	-1.51		
A02	Mm.298908	NM_001008533	Adora1	Adenosine A1 receptor	1.16	1.53	1.77	1.76	1.34		
B12	Mm.41970	NM 010077	Dvl3	Dishevelled 3. dsh homolog (Drosophila)	1.16	1.53	-1.15	-1.17	1.34		
B07	Mm.28297	NM_030248	Cxcl1	Chemokine (C-X-C motif) ligand 1	1.16	3.01	1.74	1.74	2.63		
F10	Mm.246550	NM_011143	Rac1	RAS-related C3 botulinum substrate 1	1.16	1.51	-1.16	-1.15	1.32		
G05	Mm.33903	NM_013660	Sod1	Superoxide dismutase 1, soluble	1.16	-1.32	-1.15	-1.15	1.33		
B01	Mm.103205	NM 007553	Bmp4	Bone morphogenetic protein 4	1.16	-1.36	1.74	-1.15	1.30		
E04	Mm.5014	NM_010883	Notch2	Notch gene homolog 2 (Drosophila)	1.16	-1.32	-1.16	-1.15	-1.51		
B04	Mm.474282	NM_009871	Cdk5rap2	CDK5 regulatory subunit associated protein 2	1.16	-1.32	-1.15	-1.15	1.33		
F09	Mm.489878 Mm 126217	NM_008552	Ptn Accl1	Pleiotrophin Achaete-scute complex homolog 1 (Drosophila)	1.15	1.52	-1.19	1.74	1.33		
AUS	WIIII.130217	14141_00000000		Achaete-scate complex nonitolog 1 (brosophila)	1.15	1.52	-1.15	-1.15	-1.54		
F03	Mm.397111	NM_013625	Pard3	Par-3 (partitioning defective 3) homolog (C. elegans)	1.15	1.51	-1.16	-1.17	-1.52		
D04	Mm.384027	NM_019572	Mdk	Midkine	1.15	1.51	-2.34	-1.15	-1.55		
B05	Mm.289427	NM_025876	Chrm2	Cholinergic receptor, muscarinic 2, cardiac	1.15	1.51	1.76	-1.14	2.68		
B08	Mm.448632	NM 203491	Dcx	Doublecortin	1.15	1.50	-1.16	-2.33	1.33		
E07	Mm.485843	NM_010928	Nrg1	Neuregulin 1	1.15	1.49	-2.40	-1.15	1.33		
E08	Mm.5142	NM_008730	Nrp1	Neuropilin 1	1.15	1.51	-1.16	-1.17	1.30		
D01	Mm.589	NM_008155	Hey2	Hairy/enhancer-of-split related with YRPW motif 2	1.15	1.51	-1.15	-1.15	1.32		
E06	Mm.135266	NM_008711	Nrcam	Neuron-glia-CAM-related cell adhesion molecule	1.15	1.51	-1.17	-1.17	1.31		
F12	Mm.469963	NM_009007	Rtn4	Reticulon 4	1.15	-1.34	-1.15	-1.16	-1.51		
D08	Mm.103615	NM_013905	Ndn	Necdin	1.15	1.50	-2.34	-1.17	-1.52		
F06 F07	Mm.439659	NM 008781	Pax6 Pou3f3	POU domain, class 3, transcription factor 3	1.15	-1.34	-1.15	-1.15	-1.52		
C08	Mm.7995	NM_010200	Gpi1	Glucose phosphate isomerase 1	1.15	1.54	1.79	1.79	2.72		
D07	Mm.103573	NM_013904	Mtap2	Microtubule-associated protein 2	1.15	-1.33	-1.15	-1.16	-3.05		
E05	Mm.4636	NM_010894	Nr2e3	Nuclear receptor subfamily 2, group E, member 3	1.15	1.52	1.75	1.74	2.67		
A03	Mm.333734	NM 009630	Adora2a	Adenosine A2a recentor	1.15	-1.54	-2.33	1.73	1.32		
F01	Mm.39095	NM_008744	Olig2	Oligodendrocyte transcription factor 2	1.15	-1.33	-1.16	1.74	-1.53		
C06	Mm.290822	NM_001003817	Flna	Filamin, alpha	1.15	-1.33	-1.17	-1.16	1.32		
F05	Mm.292834 Mm 271745	NM_021409	Pax5	Paired box gene 5	1.15	1.51	1.75	1.75	2.66		
D12	Mm.451574	NM 025282	Neurog2	Neurogenin 2	1.14	-1.32	1.74	-2.29	-3.02		
F11	Mm.279690	NM_008973	Robo1	Roundabout homolog 1 (Drosophila)	1.14	1.51	1.74	1.74	1.32		
B09	Mm.21013	NM_008176	Dlg4	Discs, large homolog 4 (Drosophila)	1.14	1.53	-1.15	1.76	1.33		
E02 G08	Mm.27592 Mm.35784	NM_019825 NM_009237	Nog Stat3	Noggin Signal transducer and activator of transcription 3	1.13 1.13	1.51 3.15	-1.16 1.80	-1.16 1.76	1.32 2.73		
C04	Mm.252481	NM_010113	Erbb2	homolog 2, neuro/glioblastoma derived oncogene	1.13	-1.40	-1.14	-1.16	-1.56		
A01	Mm.489229	NM_009599	Ache	Acetylcholinesterase	1.12	1.49	1.75	1.75	2.67		
G02	Mm.440639	NM_194053	\$100b	S100 protein, beta polypeptide, neural	1.12	1.47	-1.21	1.75	2.68		
A05	Mm.490092	NM_009685	Apbb1	Amyloid beta (A4) precursor protein-binding, family B, member 1	1.11	1.50	1.69	-1.18	2.66		
A06 G07	Mm.305152 Mm.289739	NM_009696 NM 178804	Apoe Sox3	Apolipoprotein E SRY-box containing gene 3	-1.72 -1.72	-1.31 -1.32	-1.15 -2.29	-1.14 -2.30	1.34 -1.51		
D09	Mm.983	NM 010556	Ndp	Norrie disease (pseudoglioma) (human)	-1.73	-1.31	-1.15	-1.14	-1.50		
D05	Mm.390859	NM_008235	Mef2c	Myocyte enhancer factor 2C	-1.74	-1.32	-1.15	-1.14	-1.50		
B10	Mm.27256	NM_007864	DII1	Delta-like 1 (Drosophila)	-1.74	-1.33	-2.30	-2.30	-3.04		
E03	Mm.400253	NM 0108380	Notch1	Notch gene homolog 1 (Drosonhila)	-1.74	-1.32	-1.15	-2.30	-1.51		
G06	Mm.57202	NM_009170	Sox2	SRY-box containing gene 2	-1.77	-1.33	-1.15	-1.15	-1.51		
F02	Mm.327698	NM 011855	Pafah1b1	Platelet-activating factor acetylhydrolase, isoform 1b,	-1.79	1.50	-1.18	-1.15	1.32		
501	Mm 2200	NM 001091040	NF1	subunit 1	-1.80	-1.20	-1.16	-1.16	-1.52		
H01	Mm.3317	NM 010368	Actb	Actin, beta	-1.82	-1.39	-1.16	-1.10	-1.53		
H02	Mm.299381	NM_013556	B2m	Beta-2 microglobulin	1.16	1.50	-1.15	-1.16	1.32		
H03	Mm.2180	NM_008302	Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	1.16	1.58	1.81	1.82	1.34		
H04	Mm.304088	NM_008084	Gusb	Glucuronidase, beta	1.14	-1.35	-1.18	-1.18	1.32		
H05	Mm.391967	NM_007393	Hsp90ab1	неат snock protein 90 alpha (cytosolic), class B member 1	1.15	-1.32	-1.15	-1.15	-1.53		

#### Predicted HuD binding sites in NeuroD1 3'UTR Table S1 >ENSMUSG0000034701 | ENSMUST00000041099 1 AGGCACGTCAGTTTCACTATTCCCGGGAAACGAATCCACTGTGCGTACAGTGACTGTCCT 61 GTTTACAGAAGGCAGCCCTTTTGCTAAGATTGCTGCAAAGTGCAAATACTCAAAGCTTCA 121 AGTGATATATGTATTTATTGTCGTTACTGCCTTTGGAAGAAACAGGGGATCAAAGTTCCT 181 GTTCACCTTATGTATTGTTTTCTATAGCTCTTCTATTTTAAAAATAATAATACAGTAAAG 301 TGATCGGGATAAAAAAAATCACAAGCAATAATTAGGATCTATGCAATTTTTAAACTAGTA 361 ATGGGCCAATTAAAATATATATATATATATATATTTTTCAACCAGCATTTTACTACCTGTGA 421 CCTTTCCCATGCTGAATTATTTTGTTGTGATTTTGTACAGAATTTTTAATGACTTTTTAT 481 AACGTGGATTTCCTATTTTAAAACCATGCAGCTTCATCAATTTTTATACATATCAGAAAA 541 GTAGAATTATATCTAATTTATACAAAATAATTTAACTAATTTAAACCAGCAGAAAAGTGC 601 TTAGAAAGTTATTGCGTTGCCTTAGCACTTCTTCTTCTTCTAATTGTAAAAAAGAAAAGA 661 AAAGAAAAAAAACCAACAAATTGCACAATTTGAGCAATTCATCTCACTTTAAAGTTTTTC 721 CTGCTCGCTCCCTAAAATAGAAACCAGACCCATAACACTCAAGAGGATGAAAACCGAAAT 781 GCATTCCTTATCAAAACACATCAATTCATTACTTGCACAAGCTTGTAAATACATATTATA 841 AATAAATGCCAACACACACCCCTTTAAATCAAAAGCTGCTTGACTATCACATACAATTTG 901 CACTCTTTCTTTTAGTCTTTTACTTCTTTGAATTCCATGATTTTACGGAGTGTTTGAAG 1021 TTTGTCATCTATTCATGTAATAAATCTGAGCCTAAATTTATTCAGGTTGTTAATGTTGGG 1141 CATAATTAGAACAATAGCTGTTGCATGTAAAATGCAGTCCAGAATAAGTGCTGTTTGAGA 1261 TATGGTGTAATGCACAATTTAGAAAACTCCCATGCAGTTGCAATAAAAATAGTATGGAAA 1321 ATCAAAACAATTGTTTCATTTCTTTCGAAAGTGGATTTTAAAGAGTAAAATTGTAGCGAG 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-µm brain sections were generated using a sliding microtone 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-SATB1was subcloned into a lentiviral vector expressing red florescent protein mCherry using the following primers: forward (containing P2A-FLAG): 5'-TGGACGAGCTGTACAAGGGAAGCGGAGCTACTAACTTCAGCCTGCTG AAGCAGGCTGG AGACGTGGAAGGAGAGAACCCTGGACCTGATTACAAGGATGACGACGATAAGGATT ACAAGGATGACGACGATAAGGATTACAAGGATGACGACGATAAGGATTACAAGGATGACGACGATAAGGATCATTTGAACGAGGC-3', reverse: 5'-CAGGTTTAAACTCGAG 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 ACAGCACGTACTATGCAAA (*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<sup>TM</sup> 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:

Satb1 3'UTR 1 f	forward:	TAGGCGATCGCTCGAGGAGAACAGTATTCTTTTCAGC
Satb1 3'UTR 10	000 reverse:	TTGCGGCCAGCGGCCGCTTTCACATTAATTGCATA
Satb1 3'UTR 10	01 forward:	TAGGCGATCGCTCGAGATAATTGATGTTTGCAAT
Satb1 3'UTR 18	97 reverse:	TTGCGGCCAGCGGCCGCCACGGGAACCCTAACAAAG
Satb1 3'UTR 20	01 forward:	TAGGCGATCGCTCGAGTTTCCTGGTGGTCTAAAT
Satb1 3'UTR 30	000 reverse:	TTGCGGCCAGCGGCCGCCTAGTCACCTTTGTTTTC
Satb1 3'UTR 30	01 forward:	TAGGCGATCGCTCGAGTATGTGTGCCCTACCTGTGT
Satb1 3'UTR 35	55 reverse:	TTGCGGCCAGCGGCCGCTTGTCCAAAGACATGCAAG
Satb1 3'UTR 37	09 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-1a*) 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-um 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% CO2 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 µM 5bromo-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 uM forskolin (Sigma-Aldrich, #F-6886) and 1 µM 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 antityrosine 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 antirabbit 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-dimentional size) in olfactory bulb and the total number of cells were measured using StereoInvestigator (MBF Bioscience, Inc) with a 5-µm guard zones at both top and bottom of the sections, as described elsewhere [14].

#### High content image analysis

To quantify NSC differentiation, NSCs was cultured in 24 well-plates to go through the proliferation or differentiation, after the fixation and staining, cells was 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 describe [9, 12]. Briefly, WT and HuD KO SVZ NSCs were harvested and homogenized in 1ml 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  $\mu$ l recombinant protein G agarose (Invitrogen) (washed with lysis buffer first). An aliquot of precleared input was saved for RNA extraction (200  $\mu$ l) and protein analysis (100  $\mu$ l). 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 H2O) 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 repelleted 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 dve (Invitrogen, #S33102). The immunoprecipitating and washing were using Chip Assav 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 NaHCO3, 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'):

NeuroD1 downstream 0.2 Kb forward: NeuroD1 downstream 0.2 Kb reverse: NeuroD1 upstream 0.8 Kb forward: NeuroD1 upstream 0.8 Kb reverse: NeuroD1 upstream 2 Kb forward: NeuroD1 upstream 2 Kb reverse: NeuroD1 upstream 2.5 Kb forward: NeuroD1 upstream 2.5 Kb reverse: NeuroD1 upstream 3.2 Kb reverse: NeuroD1 upstream 3.2 Kb reverse: GCTCCAGGGTTATGAGATCGT TGTGGGCAATTACGAGGAGG GAGCAGGTGACCGTTAGGTT GCCTTGACTCAACCTTCCTGA GTTCCGGGAATATCCAGGTGT GGAGAGTTATTGGGGAGCGG GCAGATTTAGGGCATTGCTCG TCCCAGTTATCTCCGCTTGC GAGATTTGGGGAGAACGGGT GCTGGAATTGCCCGACTAGA *NeuroD1* upstream 4.8 Kb forward: *NeuroD1* upstream 4.8 Kb reverse: *NeuroD1* upstream 6 Kb forward: *NeuroD1* upstream 6 Kb reverse:

HuD downstream 0.5 Kb forward:
HuD downstream 0.5 Kb reverse:
HuD upstream 0.5 Kb reverse:
HuD upstream 0.5 Kb reverse:
HuD upstream 1.9 Kb forward:
HuD upstream 2.5 Kb forward:
HuD upstream 3.5 Kb reverse:
HuD upstream 3.5 Kb reverse:
HuD upstream 5 Kb reverse:
HuD upstream 5.3 Kb forward:
HuD upstream 5.3 Kb reverse:

TCCGCGTTTCTTTGATCAATCC ACCACATTGGTACACGTGGC ATGAGCTGCCTCTTGACACC GGGAGGGATGTGAATGCCAA

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 firststrand 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:

Satb1 forward: *Satb1* reverse: *HuD* forward: HuD reverse: *NeuroD1* forward: *NeuroD1* reverse *Tuj1* forward: *Tuj1* reverse: *Gfap* forward: *Gfap* reverse: Aquaporin 4 forward: *Aquaporin 4* reverse: *Gapdh* forward: *Gapdh* reverse: E1a forward: E1a forward:

ACAGACCTCCCCACATCATC TTCCACGGAAATTTTGGTTC CAATACGGTCGCATCATCAC CCTTTGATGGCTTCTTCTGC TTAAATTAAGGCGCATGAAGGCC GGACTGGTAGGAGTAGGGATG TATGAAGATGATGACGAGGAATCG TACAGAGGTGGCTAAAATGGG G CCAAGCCAAACACGAAGCTAA CATTTGCCGCTCTAGGGACTC CTTTCTGGAAGGCAGTCTCAG CCACACCGAGCAAAACAAAGAT AATGGGAAGCTTGTCATCAACG GAAGACACCAGTAGACTCCACGACATA GGACCCAGTGAGAAGCGACT 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 µg/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  $M_t=M_0e^{-\lambda t}$  ( $M_t$ : 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  $\frac{1}{2}$  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-28299), goat anti-SATB1 (1:500, Santa Cruz Biotechnology, #sc-5989), rabbit anti-flag (1:2000, Cell Signaling Technology, #2368S), β-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  $\mu$ L of the lysate was added to 100  $\mu$ l 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-µm 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 µl PBS. We routinely obtained 1X10<sup>9</sup> infectious viral particles /ml for lentivirus and 1X10<sup>8</sup> infectious viral particles /ml for retrovirus. To study the effects of SATB1 on the proliferation and differentiation of NSCs, 1X10<sup>7</sup> 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 1X10<sup>5</sup> cells/well, for differentiation or proliferation analysis.

#### In vivo Virus Grafting

In vivo viral grating using stereotaxic surgery was performed as described [15] with modification. Briefly, 7- to 8-week-old C57B/L6 male mice were anesthetized with isofluorane 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 1X10<sup>8</sup>/ml for retrovirus, and 1 X 10<sup>9</sup>/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.
- 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.