Supplemental Tables

Table S1. qChIP Primers and Antibodies, Related to Figure 3 and 5.

Primer name	Sequence	Amplicon size (bp)
Dlx2 Upstream F	TTGGCTAAGGAAGGCCTAGA	61 bp
Dlx2 Upstream R	CACCAGGGAGCGTTTCTAAT	61 bp
Dlx2 Promoter F	AAGCACTGCAGAAAGTGGGTA	76 bp
Dlx2 Promoter R	GGAGCCTTATGTCCTGTTGC	76 bp
I12b Enhancer F	AGGCCCGGCCATTGACAAGC	87 bp
I12b Enhancer R	GCGAGCTGCCATCTCTGCAA	87 bp
Slc32a1 Promoter F	CAGCTCGGCAATGAAACTCG	106 bp
Slc32a1 Promoter R	AGATACAGCTGTGGGTTGGC	106 bp
Myt1 Promoter F	TGGTTTGGAGCACCTCTCAC	122 bp
Myt1 Promoter R	ACGCTACCCATGAACCCTTG	122 bp
Gjb6 Promoter F	AGAAGCCACAGGTGGAAACC	97 bp
Gjb6 Promoter R	TACCCTGAGCGTAGGTTGGA	97 bp
URE2 F	GCATCCAGGCCTTTTACTGC	90 bp
URE2 R	TAGTAGGCGGGAGCTCCATT	90 bp

Antibody	Company	Cat#	µg/ml
H3K4me3	Active Motif	39159	1µg/ml
H3K27me3	Active Motif	39155	1µg/ml
H3K4me1	Active Motif	39635	2µg/ml
H3K27ac	Active Motif	39133	2µg/ml
p300	Santa Cruz Biotech	sc-585	2µg/ml
JMJD3	ABGENT	AP1022a	4µg/ml
MLL1	Bethyl	A300-374A	2µg/ml
IgG	Santa Cruz Biotech	sc-2027	2-4µg/ml

Table S2. Selected Gene Ontology categories for 1050 genes upregulated after 30 h of differentiation in control NSCs (Figure 3C, P<0.05).

Category	Term and Description	Count	%	P Value
GOTERM_BP_FAT	GO:0021543~pallium development	9	0.953390	0.002339
GOTERM_BP_FAT	GO:0021761~limbic system development	7	0.741525	0.003136
GOTERM_BP_FAT	GO:0021766~hippocampus development	6	0.635593	0.003745
GOTERM_MF_FAT	GO:0004407~histone deacetylase activity	5	0.529661	0.004541
GOTERM_BP_FAT	GO:0021537~telencephalon development	10	1.059322	0.004757
GOTERM_BP_FAT	GO:0014014~negative regulation of gliogenesis	4	0.423729	0.006172
GOTERM_BP_FAT	GO:0045686~negative regulation of glial cell differentiation	4	0.423729	0.006172
GOTERM_BP_FAT	GO:0050767~regulation of neurogenesis	14	1.483051	0.006377
GOTERM_BP_FAT	GO:0050768~negative regulation of neurogenesis	7	0.741525	0.006468
GOTERM_BP_FAT	GO:0051960~regulation of nervous system development	14	1.483051	0.015834
GOTERM_BP_FAT	GO:0030900~forebrain development	15	1.588983	0.018268
GOTERM_BP_FAT	GO:0048715~negative regulation of oligodendrocyte differentiation	3	0.317797	0.018381
GOTERM_BP_FAT	GO:0045685~regulation of glial cell differentiation	4	0.423729	0.022629
GOTERM_BP_FAT	GO:0014013~regulation of gliogenesis	4	0.423729	0.022629
GOTERM_BP_FAT	GO:0048713~regulation of oligodendrocyte differentiation	3	0.317797	0.036360
GOTERM_BP_FAT	GO:0007413~axonal fasciculation	3	0.317797	0.036360
GOTERM_BP_FAT	GO:0001764~neuron migration	8	0.847458	0.037053

Table S3. Selected Gene Ontology categories for 53 genes in Figure 3C (P<0.05).

Category	Term and Description	Count	%	P Value
GOTERM_MF_FAT	GO:0003677~DNA binding	15	30.61224	5.76E-05
GOTERM_BP_FAT	GO:0006323~DNA packaging	5	10.20408	2.14E-04
GOTERM_BP_FAT	GO:0006334~nucleosome assembly	4	8.163265	0.001274
GOTERM_BP_FAT	GO:0031497~chromatin assembly	4	8.163265	0.001378
GOTERM_BP_FAT	GO:0034728~nucleosome organization	4	8.163265	0.001432
GOTERM_BP_FAT	GO:0065004~protein-DNA complex assembly	4	8.163265	0.001432
GOTERM_BP_FAT	GO:0021766~hippocampus development	3	6.122449	0.002238
GOTERM_BP_FAT	GO:0006333~chromatin assembly or disassembly	4	8.163265	0.003998
GOTERM_BP_FAT	GO:0021761~limbic system development	3	6.122449	0.004211
GOTERM_BP_FAT	GO:0021893~cerebral cortex GABAergic interneuron fate commitment	2	4.081633	0.005879
GOTERM_BP_FAT	GO:0021898~commitment of multipotent stem cells to the neuronal lineage in the forebrain	2	4.081633	0.005879
GOTERM_BP_FAT	GO:0021882~regulation of transcription from RNA polymerase II promoter involved in forebrain neuron fate commitment	2	4.081633	0.005879
GOTERM_BP_FAT	GO:0051276~chromosome organization	6	12.2449	0.006334
GOTERM_BP_FAT	GO:0021543~pallium development	3	6.122449	0.010591
GOTERM_BP_FAT	GO:0006325~chromatin organization	5	10.20408	0.013435
GOTERM_BP_FAT	GO:0021877~forebrain neuron fate commitment	2	4.081633	0.014635
GOTERM_BP_FAT	GO:0048715~negative regulation of oligodendrocyte differentiation	2	4.081633	0.014635
GOTERM_BP_FAT	GO:0021537~telencephalon development	3	6.122449	0.018968
GOTERM_BP_FAT	GO:0021892~cerebral cortex GABAergic interneuron differentiation	2	4.081633	0.020430
GOTERM_BP_FAT	GO:0048713~regulation of oligodendrocyte differentiation	2	4.081633	0.020430
GOTERM_BP_FAT	GO:0006836~neurotransmitter transport	3	6.122449	0.023107
GOTERM_BP_FAT	GO:0014014~negative regulation of gliogenesis	2	4.081633	0.026192
GOTERM_BP_FAT	GO:0045686~negative regulation of glial cell differentiation	2	4.081633	0.026192
GOTERM_BP_FAT	GO:0021895~cerebral cortex neuron differentiation	2	4.081633	0.034773
GOTERM_BP_FAT	GO:0014013~regulation of gliogenesis	2	4.081633	0.040452
GOTERM_BP_FAT	GO:0021879~forebrain neuron differentiation	2	4.081633	0.040452
GOTERM_BP_FAT	GO:0045685~regulation of glial cell differentiation	2	4.081633	0.040452
GOTERM_BP_FAT	GO:0021872~generation of neurons in the forebrain	2	4.081633	0.046100

Supplemental Experimental Procedures

Jmjd3 conditional knockout mice

Jmjd3 $^{F/F}$ mice were generated, maintained and genotyped as illustrated in **Figure S2A-S2C**. The genomic region of *Jmjd3* (10.5 kb) in BAC bMQ73n09 from Welcome Trust Sanger institute was cloned into pBluescript SK. To generate a *Jmjd3* allele containing the catalytic *JmjC* domain flanked by LoxP sites, a *loxP* sequence and a *Frt-Pgk-Neo-Frt -loxP* cassette was inserted between exon 20 and 21 and between exon 13 and 14, respectively. The linearized targeting construct was electroporated into E14Tg2A embryonic stem (ES) cells and selected with G418 (0.18 mg/ml). Targeted clones were screened by Southern blot analysis using 5' and 3' probes, and three of the correctly targeted clones were injected into C57BL/6J blastocysts (**Figure S2B**). Chimeric males were bred to C57BL/6J females, and the *Pgk1-Neo* cassette was removed by mating to Flp expressing mice. Mice were genotyped by genomic PCR with the following primers: WT/KO forward, GCGAGAGACCTGAGGCATGA; WT reverse, CTCGCCTCCACCAGAGTCTT; KO reverse, AGGGGGAAGAGCTTGCACAC (**Figure S2C**).

Antibodies used for immunostaining

Brain sections and fixed SVZ cultures were blocked with 10% normal goat serum, 0.3% Triton-X 100, 1% bovine serum albumin, and 0.3M glycine in PBS for 1hr at 25 °C before primary antibody incubation at 4 °C overnight. For JMJD3 staining, heat-induced epitope retrieval was used with 10mM sodium citrate buffer (pH 6.0) and fluorescence signal was amplified using TSA Plus fluorescence kit (PerkinElmer). EdU was detected with the Alexa Fluor azide per manufacturer's protocol (Invitrogen). The following primary antibodies were used: rabbit anti-JMJD3 (epitope purified, 1:100), mouse anti-GFAP (1:1000, Sigma), guinea pig anti-Doublecortin (1:1000, Millipore), rat anti-BrdU (1:500, ThermoScientific), guinea pig anti-DLX2 (1:1,000, gift from Dr. Kazuaki Yoshikawa), mouse anti-NeuN (1:500, Chemicon), mouse anti-Nestin (1:500, Millipore), chicken anti-Vimentin (1:500, Millipore), mouse anti-S100β (1:100, Sigma), mouse anti-βIII-tubulin (Tuj1 clone, 1:1000, Covance), rabbit anti-Brain lipid binding protein (BLBP, 1:300, Millipore), rabbit anti-β Catenin (1:500), mouse anti-γ-Tubulin (1:1000, Abcam), rabbit anti-DsRed (1:500, Clontech), rabbit anti-Ki67 (1:500, Abcam), mouse anti-O4 (1:100, Millipore), rabbit anti-Olig2 (1:500, Millipore), and chicken anti-GFP (1:500, Aves). Goat Alexa-Fluor secondary antibodies (Invitrogen) were used, and nuclei were counterstained with DAPI (Sigma).

FACS isolation of SVZ cells for ChIP

SVZ NSC monolayer cultures were grown in 10cm dishes, and differentiation was induced for 0 or 3 d. Cells were then dissociated to a single cell suspension with 0.25% Trypsin and mechanical dissociation. To fix chromatin, the cell suspension was treated with 1% paraformaldehyde for 10 min at room temperature. Paraformaldehyde was then quenched with 0.125M glycine on ice for 5 minutes. After rinsing cells with PBS three times, 0 d cells were incubated with Alexa Fluor 488-conjugated GFAP and Alexa Fluor 647-conjugated Nestin antibodies (BD Pharmingen, 1:40) in blocking buffer for 30 min on ice. 3 d cells were treated identically except incubated with Alex Fluor 488-conjugated Tuj1 antibody (BD Pharmingen 1:40). Using a FACSAria (BD Pharmingen) cell sorter with a 100 μm nozzle and relatively low pressure, we first set FSC and SSC gates to eliminate debris and potential cell doublets. Unstained SVZ cells, Alexa Fluor 488 or Alexa Fluor 647 stained cells were individually used to set the sort gates. The fixed, FACS-isolated cells were then pelleted by centrifugation at 4°C and washed three times with ice-cold PBS containing protease inhibitor cocktail (Roche). For chromatin isolation, cells were incubated in 1 ml swelling buffer (0.1 M Tris pH7.6, 10 mM KOAc, 15 mM MgOAc) on ice for 20 min followed by dounce homogenization (20 times on ice). The nuclear pellet was isolated by centrifugating at 2500g for 5 min at 4°C. The pellet was lysed with 320μl lysis buffer (50 mM Tris, pH8, 10 mM EDTA, pH8, 1% SDS, Protease inhibitor cocktail) and incubated on ice for 10 min. Lysed nuclei were sonicated for 20-25 cycles at 30 sec on and 30 sec to achieve 200-700 bp band of sheared chromatin. ChIP qPCR was subsequently performed as previously described in this methods section. qPCR analysis was performed in a Light Cycler 480 machine (Roche), using technical triplicates and ChIP-qPCR signals were calculated as percentage of input. Standard deviations were measured from the technical triplicate reactions and represented as error bars with error propagated using the root sum square of the standard deviation of input and sample. Statistical tests of significance were analyzed using t-Test in GraphPad Prism 5.

Microarray analysis

Samples from 3 biological replicates (P9 UBC-Cre/ERT2;*Jmjd3*$^{F/F}$ and littermate control) were prepared as described in Ramos et al., 2013 and hybridized to MouseRef-8 v2.0 Expression BeadChip arrays (Illumina). Array data were normalized using IlluminaNormalizer v.2 (Normalizing method; Quantile) on the Gene Pattern public server (Reich et al., 2006) and

then processed using Cyber-T web server (http://cybert.ics.uci.edu/) which is based on the Bayesian regularization method for high-throughput differential analysis (Bayesian analysis parameters; Siding window size-101, Bayesian Confidence value-9) (Kayala and Baldi, 2012). Genes with false discovery rate (FDR)-corrected P values (Q values) less than 0.05 and fold changes more than 1.5 were considered to be significant. As a result, 1050 upregulated genes in control cells after 30 h of differentiation and 53 genes in which expression was significantly decreased in *Jmjd3*-deleted cells in 30 h differentiation condition were identified. 18 genes overlapped between two groups were considered as potential JMJD3 targets. For Gene Ontology (GO) annotations, gene groups from microarray analysis were analyzed using DAVID functional annotation tool (http://david.abcc.ncifcrf.gov/) with the default parameter and GO categories significantly enriched in each gene group (P<0.05) were selected. GO categories related to chromatin biology and neurodevelopmental process were listed in Supplementary Table 2 and 3. These GO categories were highly enriched in the 53 gene group (48%, 27/56).

Bioinformatic analysis

SVZ RNA-seq and SVZ NSC ChIP-seq data was analyzed as in (Ramos et al., 2013). ChIP-seq data from GEO series GSE36673, GSE42881, and GSE13845 were analyzed as follows: FASTQ reads were aligned to mm9 using Bowtie2 2.1.0 with default parameters (Langmead et al., 2009). Tracks and peaks were generated using MACS 1.4 with default parameters (Zhang et al., 2008). Co-occupancy analysis was performed using BEDTools against published peaks (Quinlan and Hall, 2010). Genomic Regions Enrichment of Annotations Tool (GREAT) analysis was performed with the following parameters: 5kb upstream and 1kb downstream for proximal sites; 1 Mb extension for distal sites; FDR < 0.05 (McLean et al., 2010). Plots were generated using ngsplot (Shen et al., 2013).

Supplemental References

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