Supplemental Information

Epigenetic activation of neuronal gene expression by JMJD3 is required for postnatal and adult brain neurogenesis

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Supplemental Figures

Figure S1

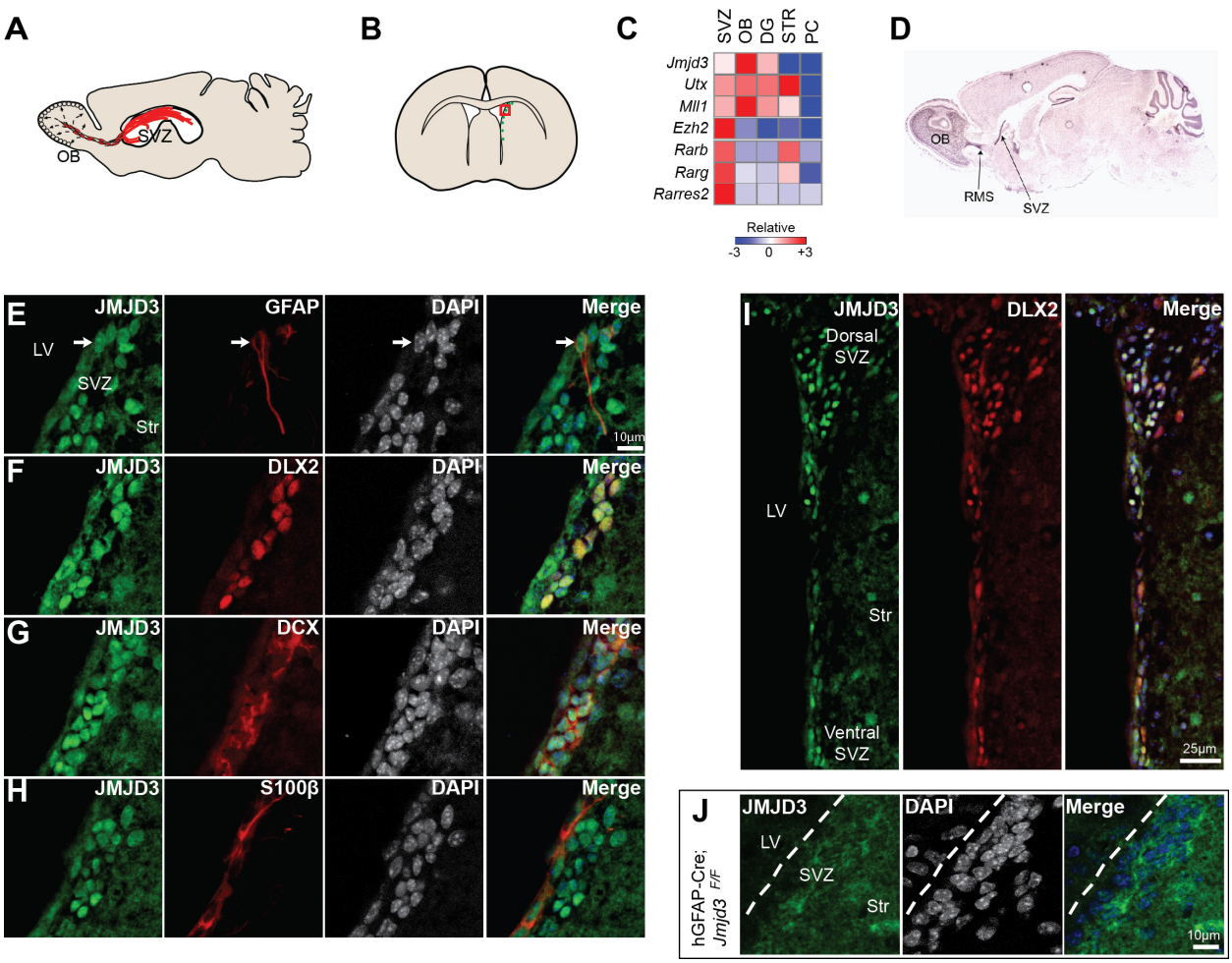

Figure S1. *Jmjd3* expression in the adult SVZ and OB, Related to Figure 1. (A) Schematic sagittal section showing neuroblast migration paths (red) from the SVZ to the OB. (B) Schematic coronal section of the SVZ (green dots); red box indicates regions shown in E-H and J-K. (C) RNA deep sequencing for differential gene expression analysis. SVZ, Subventricular Zone; OB, Olfactory Bulb; DG, Dentate Gyrus; STR, Striatum; PC, Prefrontal Cortex. RNA seq raw data is included in Ramos et al., 2013. (D) *In situ* hybridization data from the Allen Brain Atlas shows prominent expression of *Jmjd3* in SVZ, RMS, and OB. (E-H) JMJD3 (green) expression in the SVZ of P60 mouse brains. Immunohistochemistry (IHC) is shown for GFAP (E), DLX2 (F), DCX (G), and S100β (H). Nuclei are counterstained with DAPI (white). Arrow in (E) indicates example of JMJD3+, GFAP+ cells. (I) IHC for JMJD3 (green) and DLX2 (red) in a wild-type P60 coronal SVZ section. (J) IHC for JMJD3 (green) and DAPI (white) in P60 coronal SVZ section of *hGFAP-Cre;Jmjd3*$^{F/F}$ mice. Nuclear JMJD3 staining was absent in SVZ cells in *hGFAP-Cre;Jmjd3*$^{F/F}$ mice. LV, lateral ventricle; Str, Striatum.

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Figure S2

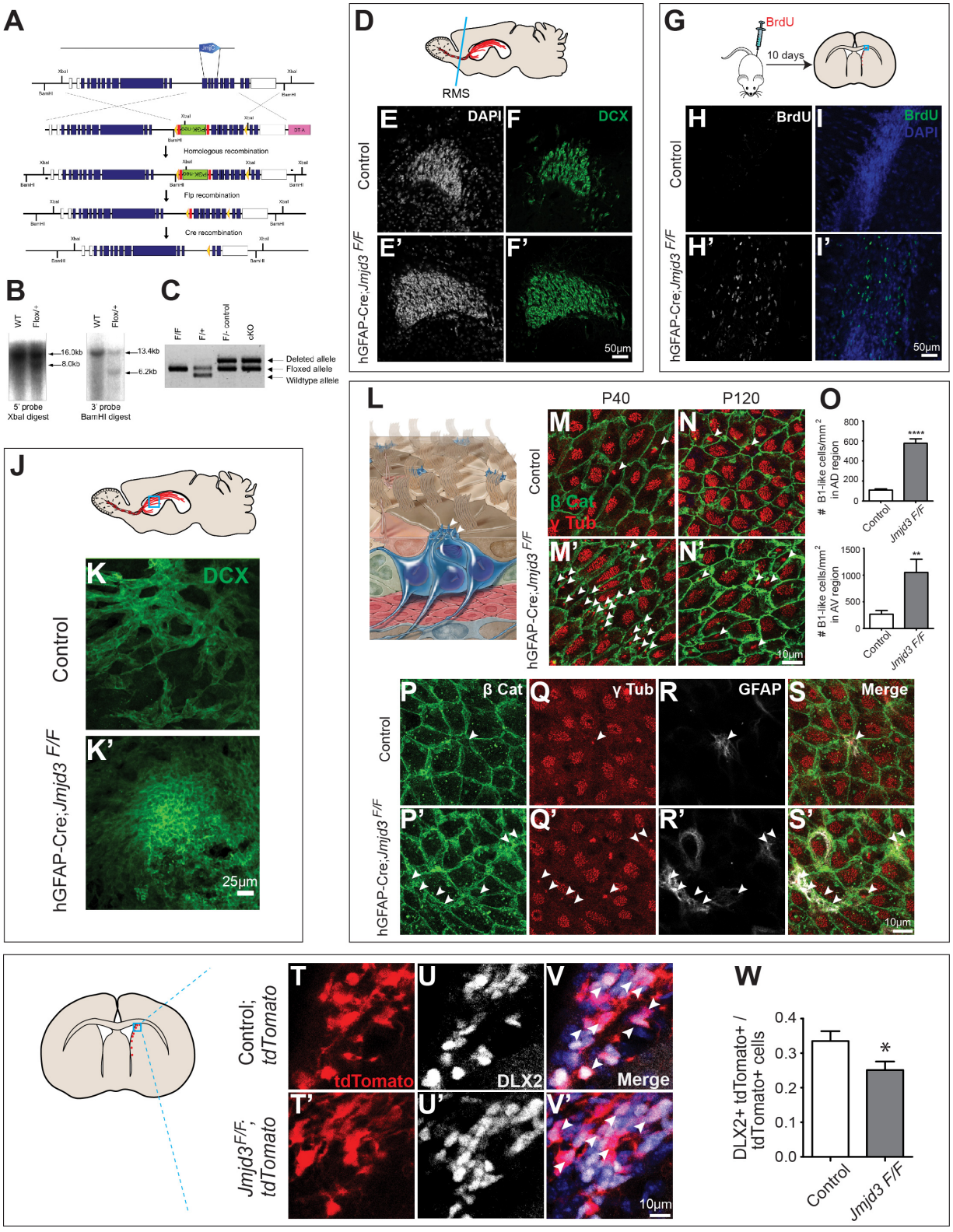

Figure S2. *In vivo* studies of *hGFAP-Cre;Jmjd3$^{F/F}$* mice and *Jmjd3*-deletion targeted to SVZ NSCs in adult mice, Related to Figure 1. (A-C) Conditional allele of *Jmjd3*. (A) Schematic illustration of the generation of the floxed *Jmjd3* allele. A *Frt-PGK1-Neo-Frt-loxP* cassette and a *loxP* sequence were inserted between exon 13 and 14 and between exon 20 and 21 respectively. DTA, diphtheria toxin fragment A. (B) Southern blot analyses using 5' and 3' external probes. Genomic ES cell DNA digested with XbaI or BamHI was hybridized with probes that detect a 16.0 kb WT and 8.0 kb targeted alleles at the 5' end (Left) or a 13.4 kb WT and a 6.2 kb targeted alleles at the 3' end (Right) respectively. (C) Verification of the floxed *Jmjd3* allele by genomic PCR. (D) Schematic sagittal section showing level of coronal section shown in E to F'. (E-F') IHC for DCX (green) in P40 coronal OB sections of control (E and F) and *hGFAP-Cre;Jmjd3$^{F/F}$* mice (E' and F'). (G) Schematic illustration of the experimental design for H-I'. BrdU was injected 10 d before analysis. (H-I') IHC for BrdU (green) in P40 anterior coronal SVZ sections of control (H and I) and *hGFAP-Cre;Jmjd3$^{F/F}$* mice (H' and I'). (J) Schematic sagittal section showing region shown in K and K'. (K and K') Whole-mount IHC of the ventricular surface to visualize DCX+ migrating neuroblasts in control (K) and *hGFAP-Cre;Jmjd3$^{F/F}$* mice (K'). (L) Three-dimensional model of the adult ventricular zone neurogenic niche showing the pinwheel organization composed of B1 cells (blue) surrounded by ependymal cells (light and dark brown). Arrowheads indicate the apical contact of GFAP+ B1 stem cells containing the characteristic single cilium. (M-N') Whole-mount IHC of the ventricular surface to visualize the cell boundaries (β-CATENIN, green) and basal bodies of cilia (γ-TUBULIN, red) in control (M-N) and *hGFAP-Cre;Jmjd3$^{F/F}$* (M'-N') at P40 and P120. Arrowheads indicate examples of Type B1 cell apical contacts. (O) Quantification of B1-like cells /mm^2 at P120 (***P*<0.01 for anterior ventral (AV) regions and *****P*<0.0001 for anterior dorsal (AD) regions, n=3 per group). (P-S') Whole-mount staining of the ventricular surface with β-CATENIN (green), γ-TUBULIN (red), and GFAP (white) in control (P-S) and *hGFAP-Cre;Jmjd3$^{F/F}$* (P'-S') at P120. Arrowheads indicate examples of Type B1 cell apical contacts. (T-W) Analysis of DLX2 expression in the SVZ after co-injection of Ad:GFAP-Cre virus (to delete floxed alleles in GFAP+ SVZ NSCs) and GFP lentivirus (injection control) into the adult SVZ of *tdTomato;Jmjd3^{F+}* or *tdTomato;Jmjd3^{FF}* mice. (T-V') IHC for tdTomato (red) and DLX2 (white) in adult SVZ coronal sections of *tdTomato;Jmjd3^{F+}* (T-V) and *tdTomato;Jmjd3^{FF}* mice (T'-V') 14 d after injection. Arrowheads indicate examples of DLX2+, tdTomato+ cells. (W) Quantification of DLX2+, tdTomato+ cells in SVZ as a % of all tdTomato+ cells (**P*<0.05; n=4 per group; error bars, s.e.m).

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Figure S3

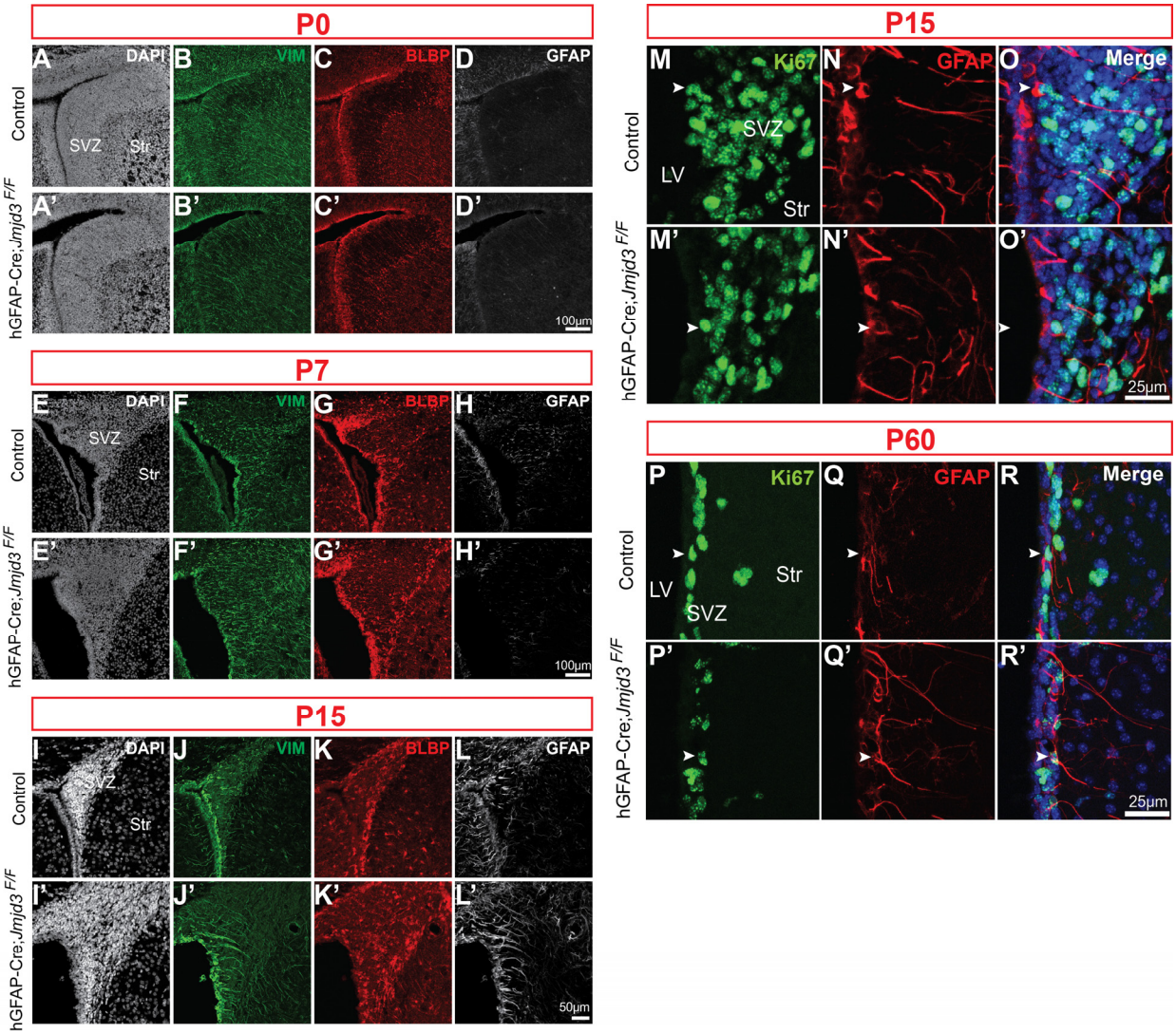

Figure S3. Analysis of postnatal development of the SVZ in *hGFAP-Cre;Jmjd3$^{F/F}$* and control mice, Related to Figure 1. (A-L') IHC for VIMENTIN (green), BLBP (red), and GFAP (white) in P0 (A-D'), P7 (E-H'), and P15 (I-L') coronal SVZ sections. (M-R') IHC for Ki67 (green) and GFAP (red) in P15 (M-O') and P60 (P-R') coronal SVZ sections. Arrowheads indicate Ki67+ GFAP+ SVZ NSCs. LV, lateral ventricle; Str, Striatum.

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Figure S4

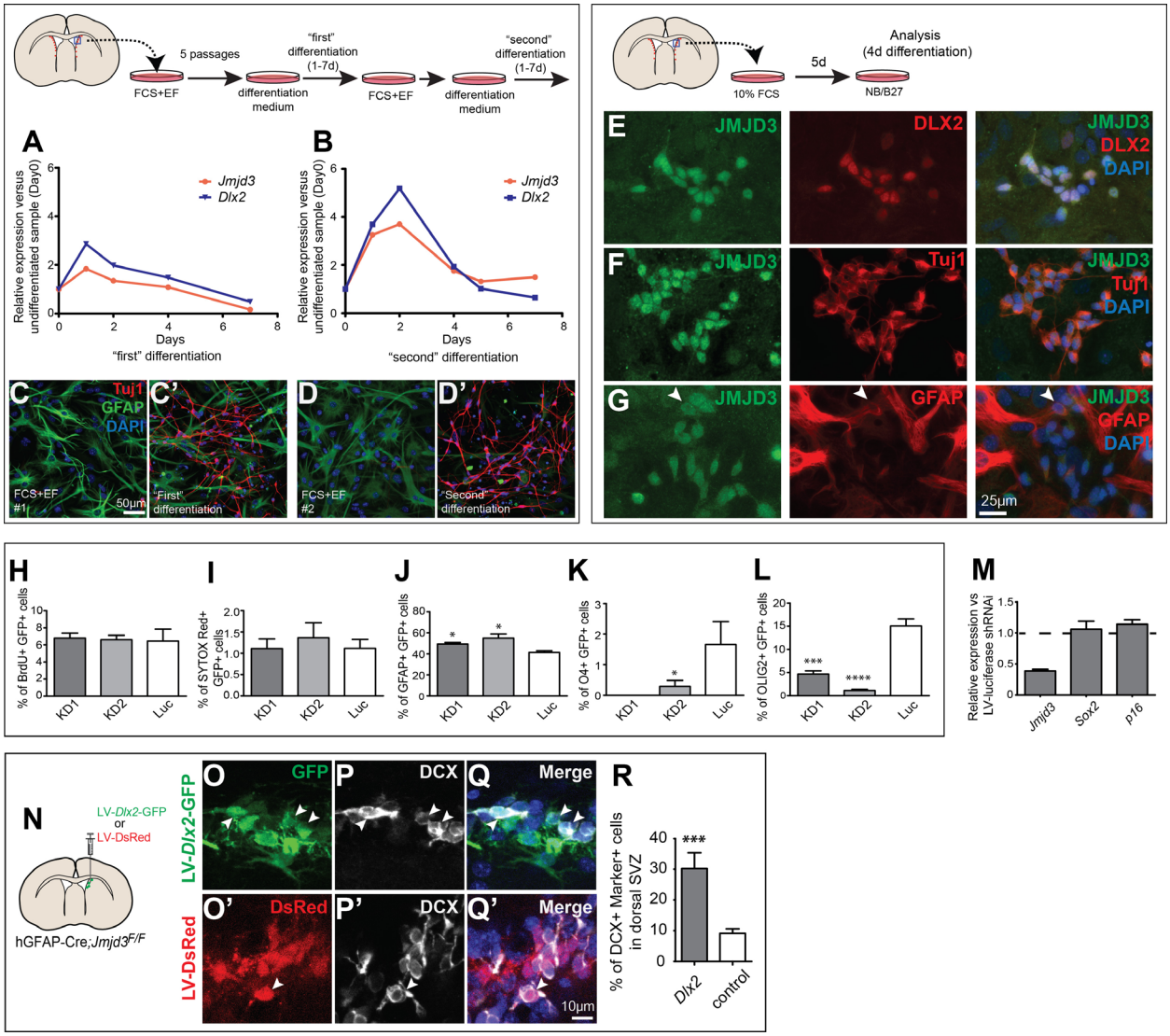

Figure S4. Studies of *Jmjd3* expression and *Jmjd3* shRNA knockdown, Related to Figure 2. (A-D') *Jmjd3* and *Dlx2* expression in SVZ NSC monolayer cultures. Schematic at top illustrates the experimental design. (A and B) RT-qPCR analysis of *Jmjd3* and *Dlx2* expression during first differentiation (A) and second differentiation (B). (C-D') Immunocytochemistry (ICC) for GFAP (green) and Tuj1 (red) for the first differentiation (C and C') and second differentiation (D and D'). (C and D) ICC of SVZ NSC cultures under proliferation conditions. (C' and D') ICC of SVZ cultures after 7 d of differentiation.

(E-G) Primary SVZ NSC cultures from control animals after 4 d of differentiation. (E) ICC for JMJD3 (green) and DLX2 (red). (F) ICC for JMJD3 (green) and Tuj1 (red). (G) ICC for JMJD3 (green) and GFAP (red). The arrowhead indicates an example of JMJD3+ GFAP+ SVZ cells.

(H) Quantification of BrdU incorporation in SVZ NSCs after *Jmjd3* knockdown as a % of all GFP+ cells. BrdU was added to cultures 1hr before fixing cells. (I) Quantification of CYTOX Red incorporation in SVZ NSCs using FACs after *Jmjd3* knockdown. (J) Quantification of GFAP+ cell differentiation from SVZ NSCs after *Jmjd3* knockdown as a % of all GFP+ cells. Error bars, s.e.m of quadruplicate cultures, *P<0.05. (K and L) Quantification of oligodendrocyte differentiation from SVZ NSCs after *Jmjd3* knockdown as a % of all GFP+ cells. Error bars, s.e.m of quadruplicate cultures, *P<0.05, ***P<0.001, ****P<0.0001.

(M) RT-qPCR analysis for *Sox2* and *p16* in SVZ NSCs with LV-*Jmjd3* knockdown. Error bars, s.e.m.

(N-R) Analysis of neuronal differentiation in the SVZ of *hGFAP-Cre;Jmjd3^{FF}* mice after *Dlx2* overexpression. (N) Schematic illustration of the experimental design for O-R. LV-*Dlx2* overexpression or control lentiviral vectors were injected into the adult SVZ of *hGFAP-Cre;Jmjd3^{FF}* mice. (O-Q') IHC for DCX (white) in cells with *Dlx2* overexpression (green in O and Q) and control vector infection (red in O' and Q'). Arrowheads indicate examples of DCX+ cells with lentiviral infection. (R) Quantification of DLX2+ cells as a % of all GFP+ or DsRed+ cells. Error bars, s.e.m. n=3, ***P<0.001.

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Figure S5

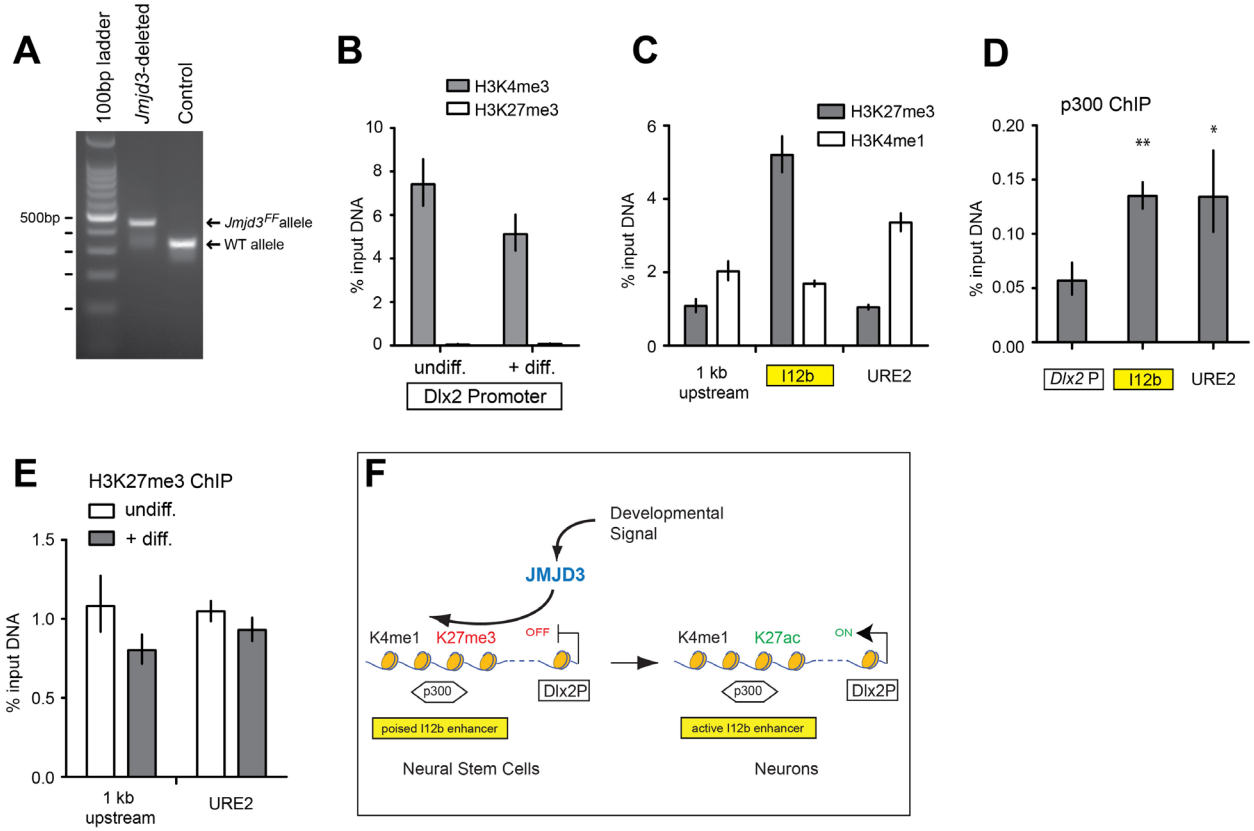

Figure S5. ChIP analysis of the *Dlx2* promoter, I12b enhancer, and control genomic regions, Related to Figure 3 and 5. (A) Genomic PCR of the deleted floxed *Jmjd3* allele in SVZ NSC cultures from UBC-Cre/ERT2;*Jmjd3*$^{F/F}$ mice with 50nM of 4-OHT for 96 hours.
(B) qChIP analysis of H3K4me3 and H3K27me3 levels at *Dlx2* promoter in control cells before and after 30 h of differentiation.
(C) qChIP analysis of H3K27me3 and H3K4me1 at the region 1kb upstream of the *Dlx2* promoter, I12b, and URE2 (a region in the 5' flanking region of *Dlx2*) in proliferating SVZ cells.
(D) qChIP analysis of p300 at *Dlx2* promoter, I12b, and URE2. P value was calculated using *Dlx2* promoter. ***P*<0.01. **P*<0.05.
(E) qChIP analysis of H3K27me3 at URE2 in proliferating and differentiating SVZ cells. Error bars, s.d., n=3. undiff = undifferentiated SVZ NSCs, +diff = differentiated SVZ cells.
(F) Model of JMJD3 activation of chromatin signatures at the enhancer elements of neurogenic genes. Left, schematic of a poised enhancer state. Right, schematic of an active enhancer.