

Fig. S1. Neurons and glial cells are produced from Dbx1⁺ progenitors in the spinal cord and hindbrain.

(A-C) *Dbx1/lacZ* mice faithfully recapitulate Dbx1 expression. Cross sections of *Dbx1/lacZ*⁺ spinal cord at the level of the vz p0 domain at E11.5, E13.5 and E14.5 stained for Dbx1, β -gal and Sox2.

(D-F) Ventromedial and lateral β -gal⁺ cells have a distinctive nuclear shape. (D) Transverse section of E18.5 *Dbx1/lacZ*⁺ spinal cord. Magnifications show β -gal⁺ cells positioned in the ventromedial (D') and lateral (D'') spinal regions. (E) Measures of major and minor axis of β -gal⁺ nuclei in ventromedial and lateral areas (dotted boxes in D). (F) The elongation ratio, calculated as longer over shorter axis, shows different nuclear shapes (n=100 cell each; ***p<0.001, Mann-Whitney test).

(G-L) Spatial distribution of Dbx1-derived cells in the spinal cord. (G,H) Distribution of the 775 β gal⁺,Sox2⁺ and 890 β -gal⁺,NeuN⁺ cells used to produce the density map in Fig. 1 K,L with Rexed's *laminae* reference. (I-L) β -gal⁺,Sox2⁺ cells are found across the spinal cord GM and WM and are included in a 21° slice. The x-y arrangement of cells was transformed and analyzed in angular and radial coordinates. Distribution of β -gal⁺,Sox2⁺ cells along the radial axis in pGM, dGM and WM (K). Angle histogram plot of β -gal⁺,Sox2⁺ cells (L) shows that 95% of cells are included in a slice section spanning 21° (corresponding to 4 SDs).

(M-P) Dbx1-derived cells in the hindbrain comprise both neuronal and glial populations. (M) Transversal section of E18.5 hindbrain of *Dbx1/lacZ*⁺ mouse. (N,O) Density maps of the ventrolateral medulla (VLM, dotted box in M), based on positions of 685 β -gal⁺,Sox9⁺ cells and 1284 β -gal⁺,NeuN⁺, shows that Dbx1-derived neurons and glia occupy similar territories, with 34.3±4.5% of β -gal⁺ cells expressing Sox9. (P) Magnification of 2 Sox9⁺ or NeuN⁺ cells in the VLM.

(Q) Cross section of P6 spinal cord shows that the regional allocation of β -gal⁺ astroglial cells is maintained postnatally. Arrows point the intermediate-lateral β -gal⁺ population settled in the GM and the WM.

(R,S) Dorsal-ventral organization of glial populations reflects vz patterning. E13.5 p0 progenitors are dorsal to Nkx6.1⁺ domains. At E18.5. β -gal⁺ cells occupy different WM territory than Nkx6.1⁺ cells.

Scale bars: 50 μ m in D; 10 μ m in D',D'',P; 300 μ m in M; 100 μ m in Q; 20 μ m in A-C,R,S.

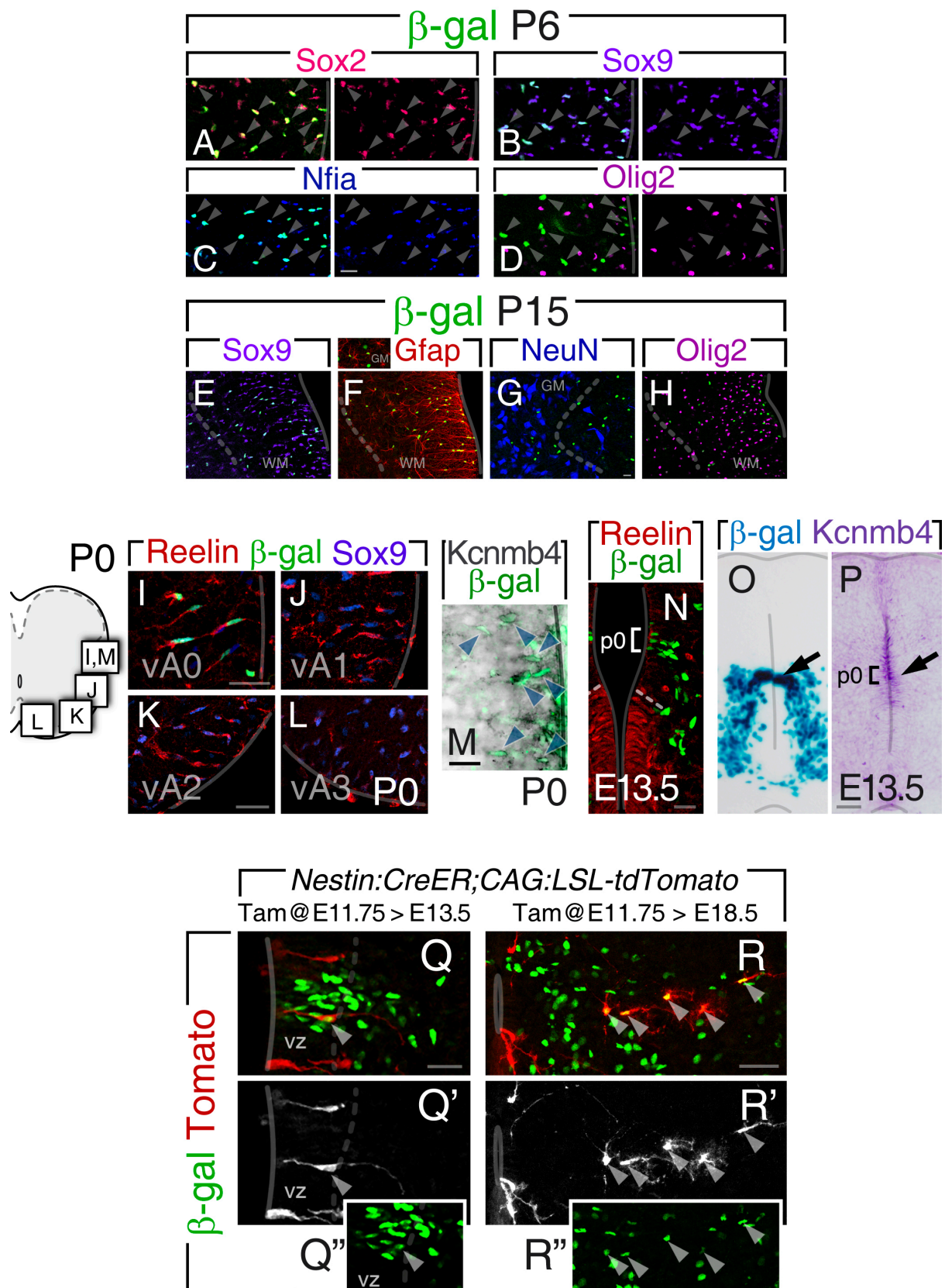


Fig. S2. Dbx1-derived cells express astroglial molecular markers.

(A-D) Magnifications of WM region of P6 spinal cord show that β -gal cells express Sox9, Sox2 and Nfia, and are negative for Olig2. Arrowheads point at β -gal cells.

(E-H) P15 spinal cord immunostaining showing β -gal cells in the GM and WM are Sox9- and GFAP-positive but negative for neuronal NeuN and oligodendrytic Olig2.

(I-P) Expression of Reelin in WM vA0, vA1 and vA2 astrocytes at P0. (M) *Kcnmb4* *in situ* hybridization and β -gal staining in the lateral WM. (N) At E13.5, Reelin is present in ventral vz cells but absent in p0 β -gal⁺ and p1 progenitors. (O-P) *Kcnmb4* is expressed in the p0 vz domain (bracket, arrow) and also in presumed p1 area and dorsal domains. β -gal activity was developed in consecutive sections to identify the p0 region.

(Q-R'') Genetic mosaic labeling strategy. *Nestin:CreER;CAG:LSL-tdTomato;Dbx1^{lacZ}/+* embryos were inoculated with low doses of Tam (37.5 mg/kg b.w) at E11.75 to label a sparse number of vz cells. Tissue was collected at E13.5 (Q) and E18.5 (R) and immunostained against β -gal and Tomato. Dotted line in Q-Q'' delimits vz region. Arrowheads point at β -gal⁺ cells indelibly labeled with Tomato.

Scale bars: 20 μ m in A-N,Q; 50 μ m in P,R.

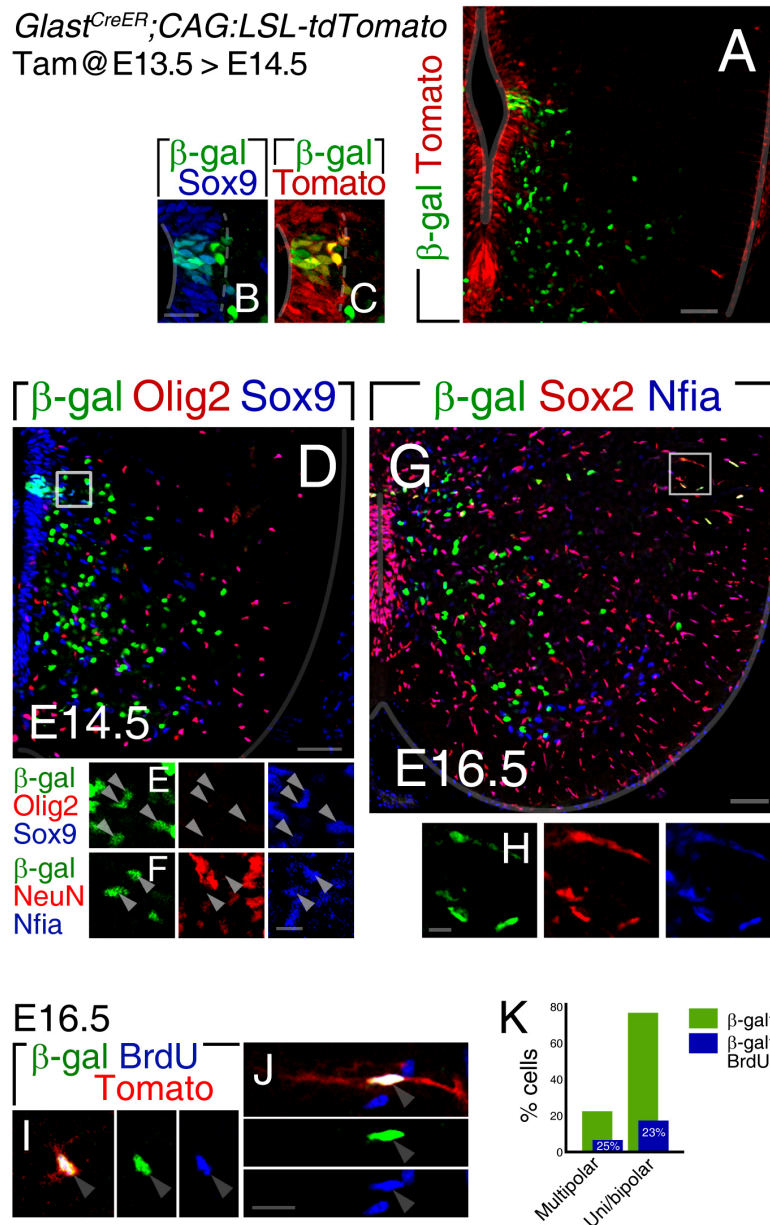


Fig. S3. Late-born Dbx1-derived cells arise from vz radial glia and express astrocytic markers.

(A-C) Analysis at E14.5 of *Glast^{CreER};CAG:LSL-tdTomato;Dbx1^{lacZ/+}* embryos induced with Tam (150 mg/kg b.w.) at E13.5 indicated efficient indelible labeling of β -gal⁺ cells (90±3% tomato⁺) that still reside in the vz (Sox9⁺).

(D-H) During embryonic development Dbx1-derived cells express astroglial markers. (D-F) E14.5 spinal cord section immunostained against β -gal, Sox9, Nfia, Olig2 and NeuN. Magnifications show expression of astroglial markers Sox9 and Nfia but not of oligodendrocytic Olig2 and neuronal NeuN. (G-H) Sox2 and Nfia late-born β -gal⁺ cells at E16.5.

(I-K) Cells with multipolar or uni/bipolar morphologies have similar proliferation rates. (I-J). Representative images of BrdU-labeled cells with each shape. (K) Percentages of β -gal astroglia according to their morphology (green bars) and the proportion labeled with BrdU (blue bars) show that ~23-25% of β -gal⁺ cells at E16.5 were BrdU⁺, irrespective of their shape.

Scale bars: 50 μ m in A,D,G; 20 μ m in B,C; 10 μ m in E,F,H-J.

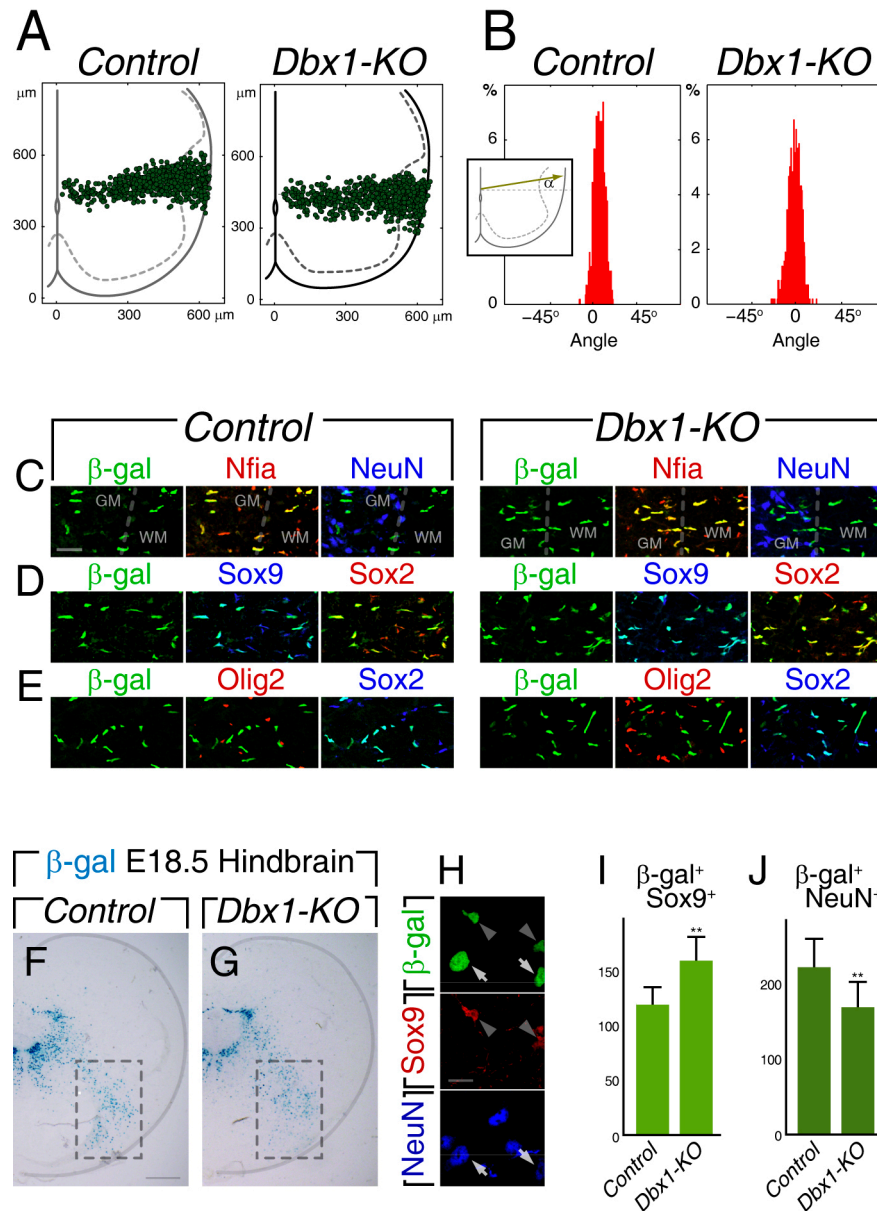


Fig. S4. Dbx1 controls the size of astroglial vA0 population

(A) Analysis of p0-derived cells in the E18.5 spinal cord of *Dbx1* mutants. Scattered plot of 650 βgal^+ , Sox9^+ cells in *control* and 895 cells in *Dbx1-KO* (12 sections, 2 embryos ea.) used to build density maps in Fig. 4E,F. (B) Histograms of angular distributions of βgal^+ astroglial cells rendered a slight ventral shift in mutants ($\alpha=7.0\pm5.5^\circ$ in *control*, $\alpha=-0.5\pm6.5^\circ$ in *Dbx1-KO*).

(C-E) Immunohistochemistry of E18.5 spinal cord from *control* and *Dbx1-KO* with antibodies against *Nfia*, *NeuN*, *Sox2*, *Sox9* and *Olig2* shows βgal cells in the gray and white matter have astroglial identity in both *heterozygous* and *Dbx1* mutants.

(F-J) Dbx1 balances p0-derived astrocytic and neuronal populations in the hindbrain. (F-G) Transverse section of E18.5 hindbrain of control and Dbx1^{-/-} developed for β -gal. (H) Neurons and astrocytic cells were identified by Sox9 or NeuN. (I,J) Quantification of β -gal⁺,Sox9⁺ and β -gal⁺,NeuN⁺ in the ventrolateral medulla (dotted boxes in F,G) showing an increase in astroglial cells in mutants (34 \pm 16% rise, 2 embryos) concomitant with reduction in neurons (24 \pm 6 % decrease), (7 sections, 2 embryos, **p<0.01, Mann Whitney test).

Scale bars: 300 μ m in F,G; 20 μ m in C-E; 10 μ m in H. Data: mean \pm SD.

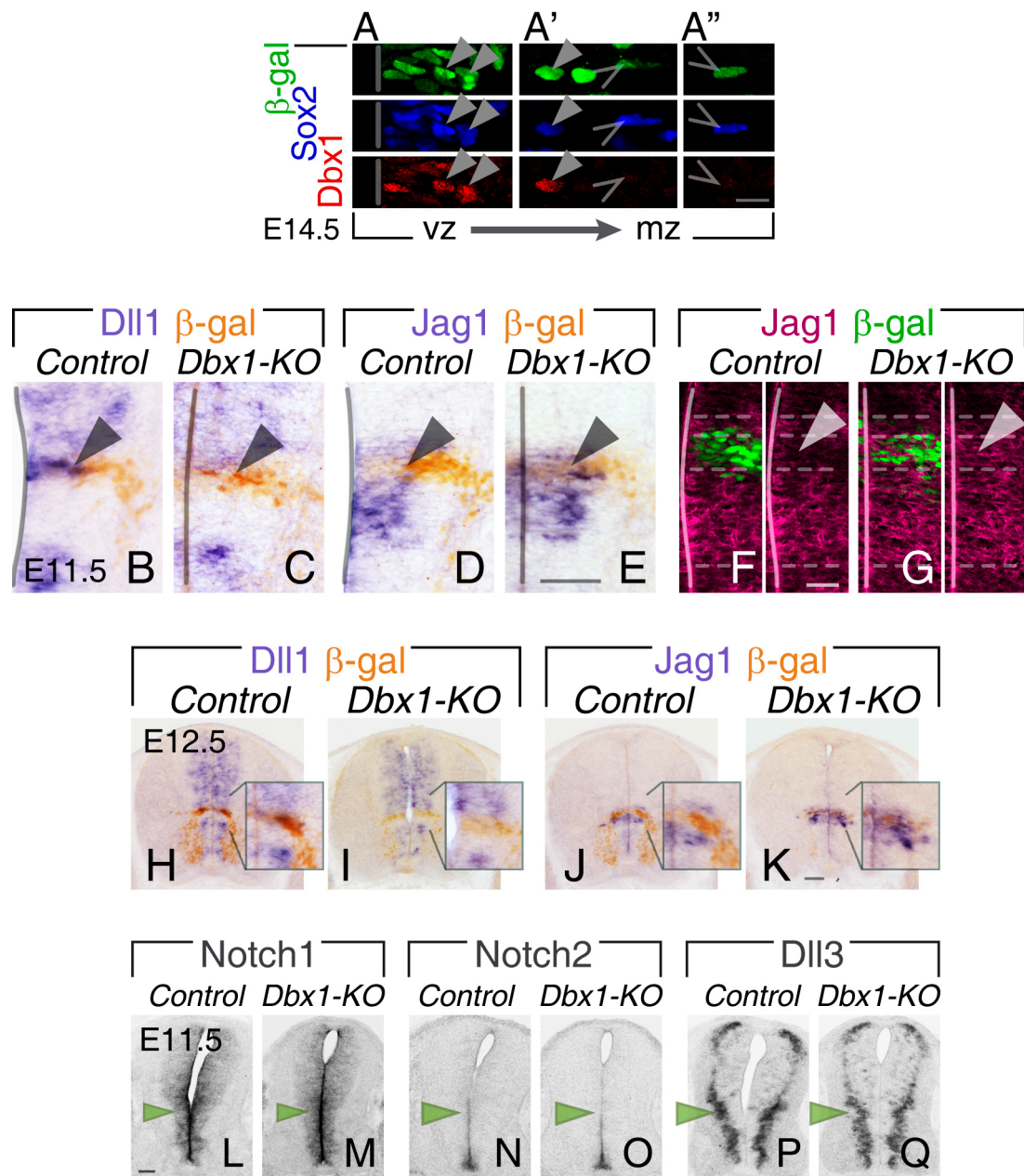


Fig. S5. *Dbx1* regulates Notch signaling factors during the neurogenic developmental phase.

(A-A'') *Dbx1* is expressed in vz progenitors but absent in astrocyte precursors in the mantle zone (mz). Immunostainings for *Dbx1*, β -gal and Sox2 at E14.5 in the vz (A), the transition from the vz to the mz (A') and astrocytic cells in the mz (A''). Filled arrowheads point out *Dbx1*⁺ cells within or leaving the vz, while empty arrowheads mark β -gal⁺, Sox2⁺ cells migrating in the mz lacking *Dbx1*.

(B-K) Dll1 and Jag1 are altered in Dbx1 mutants. In situ hybridization for Dll1 and Jag1 ligands combined with β -gal immunostaining on control and Dbx1^{-/-} neural tube sections at E11.5 (B-E) and E12.5 (H-K). Immunohistochemistry for Jag1 and β -gal in E11.5 control and Dbx1 mutants. Arrowheads in B-G point at the p0 progenitor domain. Insets in H-K are magnifications of the p0 domain.

(L-Q) *In situ* hybridization of Notch1 and Notch2 receptors, showing similar expression patterns in E11.5 *Control* and *Dbx1-KO* embryos. *In situ* hybridization with Dll3 probe shows a reduced expression at the ventricular-mantle zone boundary at the level of the p0 domain in *Dbx1-KOs*. Arrowheads point to the p0 progenitor region determined by β -gal activity in consecutive sections.

Scale bars: 10 μ m in A-A", 50 μ m in B-E, H-Q; 20 μ m in F,G.