

Supplemental Information

Notch2 Signaling Maintains NSC Quiescence in the Murine Ventricular-Subventricular Zone

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SUPPLEMENTAL DATA AND INFORMATION

SUPPLEMENTAL FIGURES AND LEGENDS

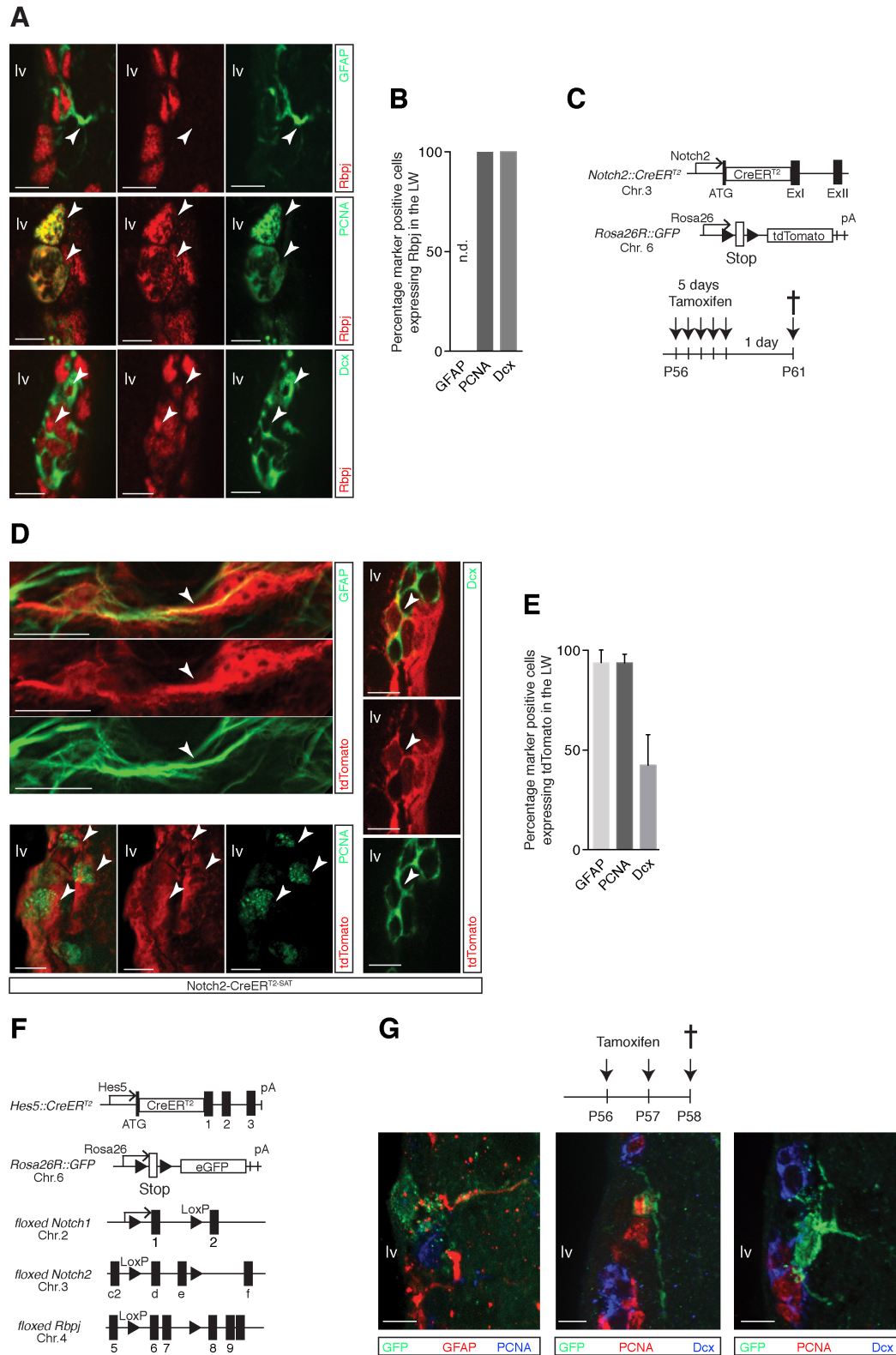


Figure S1. Notch paralogues are broadly expressed in the V-SVZ. Related to Figure 1 and Table S1.

A. Expression of Rbpj with GFAP, PCNA or Dcx showing individual color channels of Figure 1C. Arrows point to Rbpj and marker double-positive cells. **B.** Quantification of coexpression of GFAP, PCNA or Dcx with Rbpj. n.d. – not determined. **C.** Schematic representation of *Notch2-CreER^{T2-SAT}* transgene and *Rosa26R::tdTomato* Cre-reporter allele with chromosome (Chr.), exons (Ex) and poly-adenylation sites (pA). **D.** Expression of tdTomato of *Notch2-CreER^{T2-SAT}* animals with GFAP, PCNA or Dcx with split channels of Figure 1E-F. Arrows

point to tdTomato marker double-positive cells. **E.** Quantification of coexpression of GFAP, PCNA or Dcx with tdTomato. **F.** Schemes of floxed *Notch1*, *Notch2* and *Rbpj* loci, *Hes5::CreER^{T2}* transgene and *Rosa26R::GFP* Cre-reporter allele with chromosome (Chr.), exons, LoxP, and poly-adenylation sites (pA). **G.** Schematic representation of tamoxifen administration regiment and analysis of low-dose recombination and lineage analysis for driver in immunofluorescent costainings of GFP⁺ cells with GFAP, PCNA and Dcx. Values are means \pm SD; Control (C57BL/6J) animals n = 4, *Notch2-CreER^{T2-SAT}* animals n = 3; Low-dose analysis n = 3. Scale bars = 15 μ m in **A** (GFAP and Dcx) and **D** (GFAP and Dcx), 10 μ m in **A** (PCNA), **D** (PCNA) and **G**.

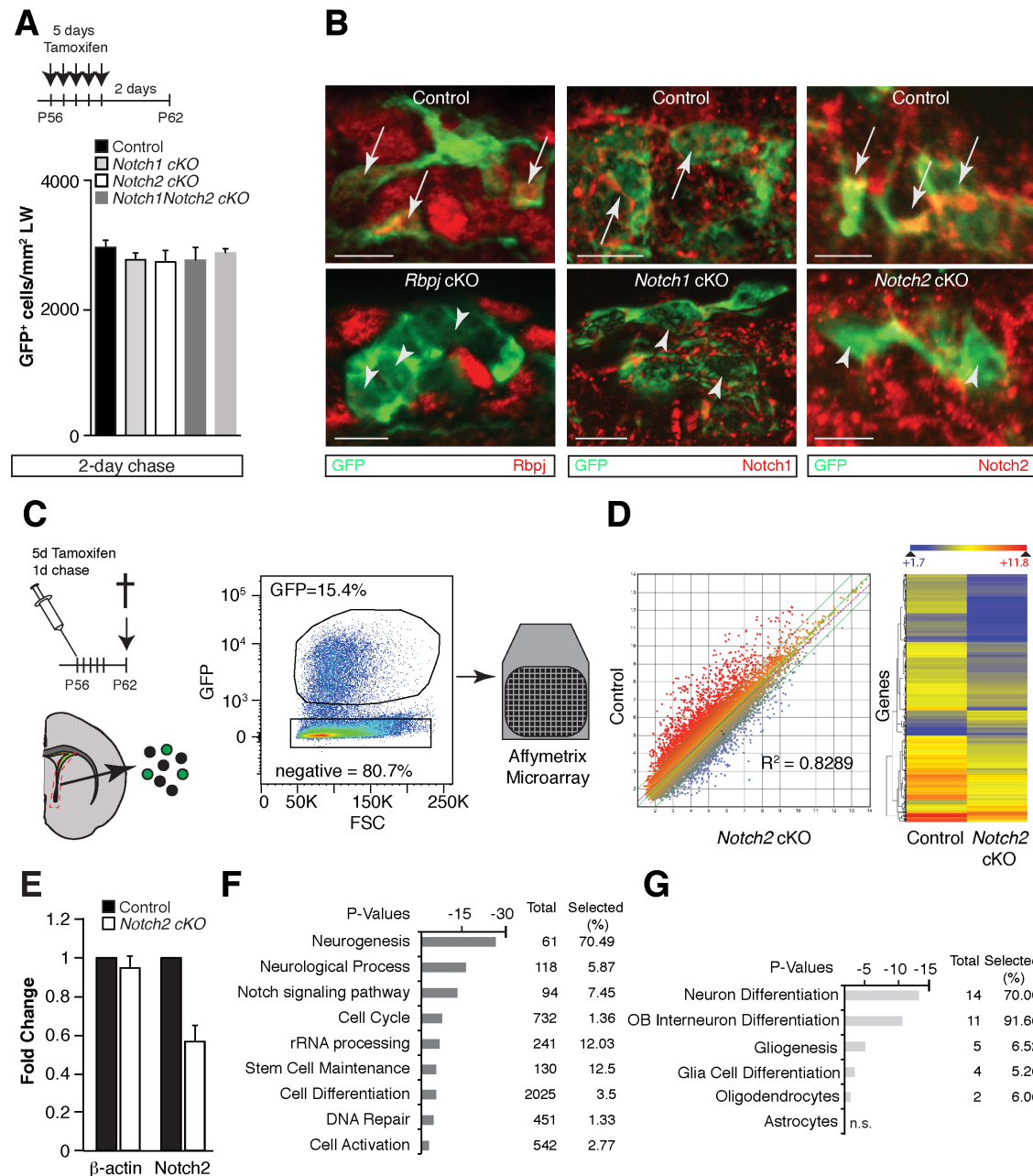


Figure S2. Acute loss of Notch signaling results in activation of quiescent NSCs. Related to Figure 2, Table S2 and Table S3.

A. Schematic representation of tamoxifen administration regiment, analyzed genotypes and levels of recombined GFP⁺ cells 2 days after tamoxifen administration. **B.** Recombination efficiency in Control and (from left to right) *Rbpj* cKO, *Notch1* cKO and *Notch2* cKO animals. Arrows point toward GFP⁺ marker⁺ cells, arrowheads towards GFP⁺, *Notch1* or *Notch2* ablated cells, respectively. **C.** Scheme of experimental setup. Following 5 days of tamoxifen induced mice were sacrificed 1-day later and *Hes5::CreER^{T2}*-derived (GFP⁺). Control or *Notch2* cKO V-SVZ cells were isolated by FACS and RNA prepared for microarray analysis. **D.** Scatter plot of mean Control versus *Notch2* cKO gene expression (log2). **E.** Confirmation of *Notch2* depletion in sorted cells **F.** Gene ontology (GO) analysis of differentially-expressed genes in *Notch2* cKO versus Control with significance, total genes in category and percent differentially expressed. **G.** GO analysis and top targets within Neurogenesis

category in *Notch2* cKO versus Control with significance, total genes in category and percent differentially expressed. Values are means \pm SD. 2-day chase: Control n = 5, *Notch1* cKO n = 6, *Notch2* cKO n = 5, *Notch1Notch2* cKO n = 4, *Rbpj* cKO n = 4. Scale bars = 10 μ m in **B**.

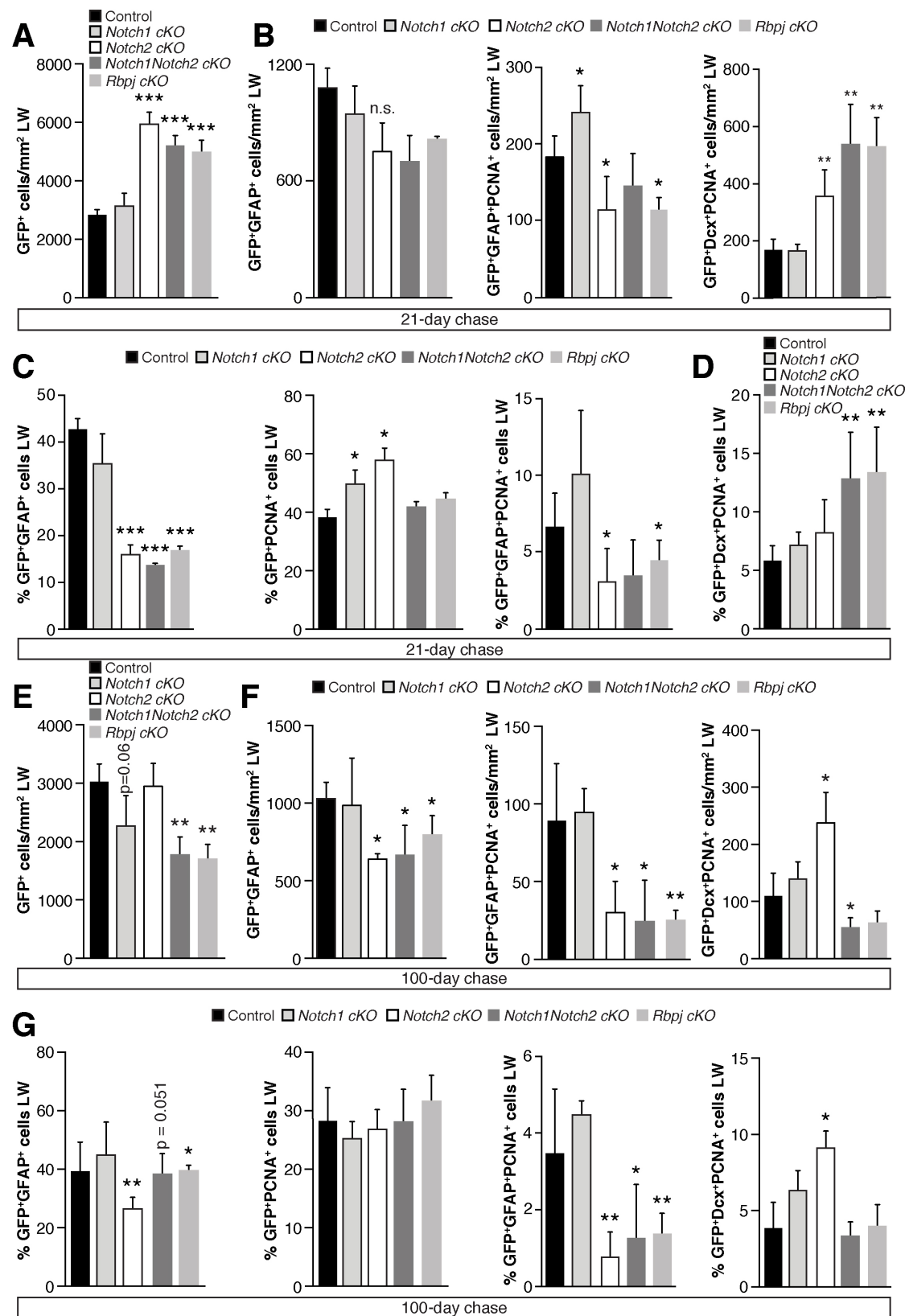


Figure S3. Notch1 and Notch2 have distinct functions in adult neurogenesis. Related to Figure 3 and Table S4.

A. Quantification and analysis of total *Hes5::CreER*^{T2}-derived (GFP⁺) cells. **B.** GFP⁺GFAP⁺ NSCs and GFP⁺GFAP⁺PCNA⁺ proliferating NSCs. **C.** Percentage of GFP⁺GFAP⁺ NSCs, GFP⁺PCNA⁺ proliferating cells and GFP⁺GFAP⁺PCNA⁺ proliferating NSCs. **D.** Percentage of GFP⁺Dcx⁺PCNA⁺ proliferating neuroblasts in the

LW of the V-SVZ in Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO mice 21 days post-tamoxifen induction. **E.** Quantification of the total number of *Hes5::CreER^{T2}*-derived (GFP⁺) cells. **F.** GFP⁺GFAP⁺ NSCs and GFP⁺GFAP⁺PCNA⁺ proliferating NSCs and **G.** Percentage of GFP⁺GFAP⁺ NSCs, GFP⁺PCNA⁺ proliferating cells and GFP⁺GFAP⁺PCNA⁺ proliferating NSCs in the V-SVZ of Control, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO mice 100 days post-tamoxifen induction. Values are means \pm SD; * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$, 21-day chase: Control $n = 6$, *Notch1* cKO $n = 4$, *Notch2* cKO $n = 5$, *Notch1Notch2* cKO $n = 6$, *Rbpj* cKO $n = 4$, 100-day chase: Control $n = 5$, *Notch1* cKO $n = 4$, *Notch2* cKO $n = 4$, *Notch1Notch2* cKO $n = 4$, *Rbpj* cKO $n = 4$.

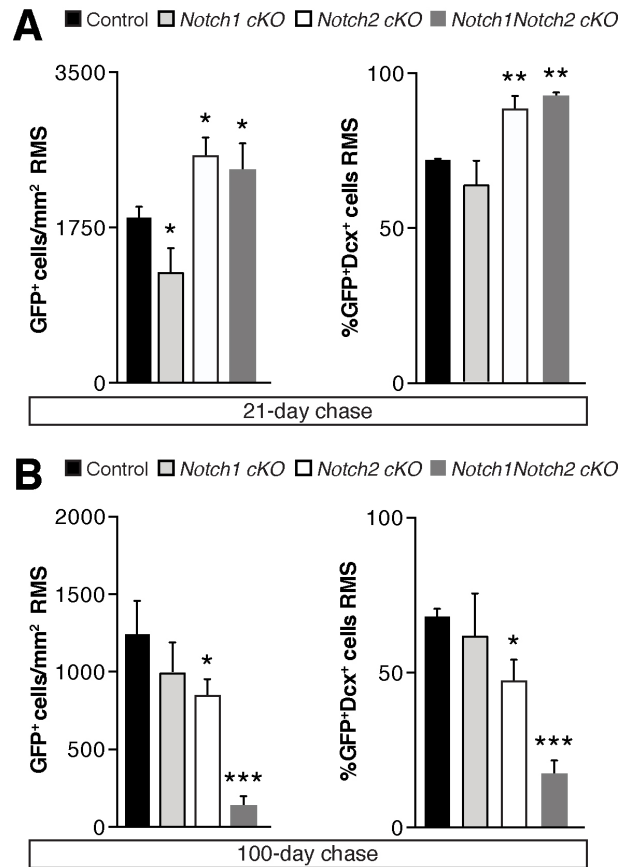


Figure S4. NSC activation projects down the rostral migratory stream. Related to Figure 4 and Table S5.

A. Quantification and analysis of the total GFP⁺ progeny and the percentage GFP⁺Dcx⁺ in the RMS of Control, *Notch1* cKO, *Notch2* cKO and *Notch1Notch2* cKO mice 21 days post-tamoxifen induction. **B.** Quantification and analysis of the total GFP⁺ progeny and the percentage GFP⁺Dcx⁺ in the RMS of Control, *Notch1* cKO, *Notch2* cKO and *Notch1Notch2* cKO mice 100 days post-tamoxifen induction. Values are means \pm SD; * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$, 21-day chase: Control $n = 6$, *Notch1* cKO $n = 4$, *Notch2* cKO $n = 5$, *Notch1Notch2* cKO $n = 6$, 100-day chase: Control $n = 5$, *Notch1* cKO $n = 4$, *Notch2* cKO $n = 4$, *Notch1Notch2* cKO $n = 4$.

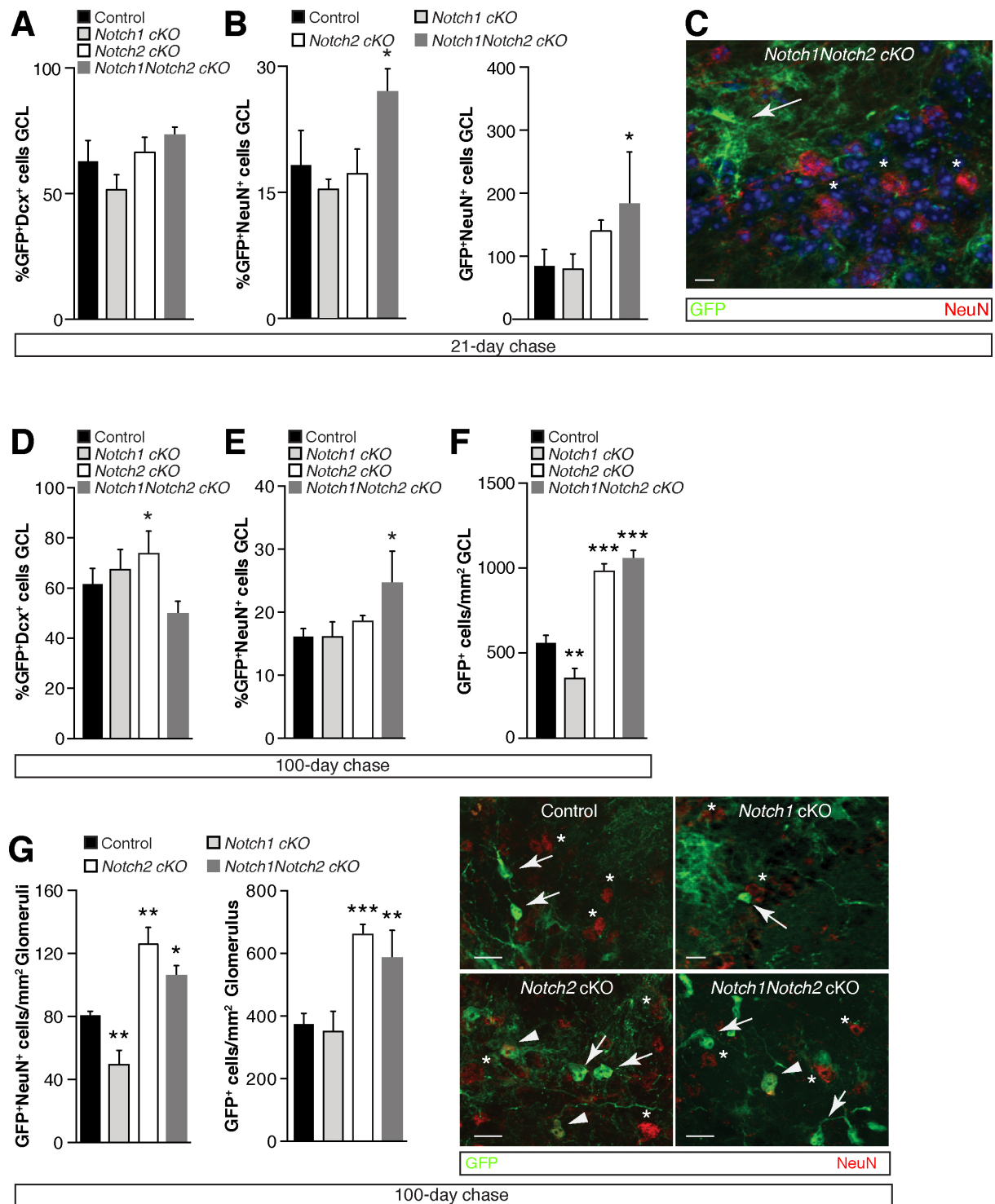


Figure S5. Notch signal manipulation affects the lineage into the OB. Related to Figure 5 and Table S6.

A. Percentage of GFP⁺Dcx⁺ neuroblasts in the GCL 21 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO and *Notch1Notch2* cKO. **B.** GFP⁺NeuN⁺ cells in the GCL 21 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO and *Notch1Notch2* cKO. **C.** At 21 days after tamoxifen administration, no new neurons have integrated into the glomeruli of the OB. Arrows point to GFP⁺ cells, asterisk mark NeuN⁺ cells. **D.** Percentage of GFP⁺Dcx⁺ neuroblasts in the GCL 100 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO and *Notch1Notch2* cKO. **E.** Percentage of GFP⁺NeuN⁺ neurons in the GCL 100 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO and *Notch1Notch2* cKO. **F.** Total number of recombined GFP⁺ cells in the GCL 100 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO and *Notch1Notch2* cKO. **G.** Total number of GFP⁺NeuN⁺ neurons and recombined GFP⁺ cells in the glomeruli 100 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO and *Notch1Notch2* cKO. Arrows point to GFP⁺ cells, asterisk mark NeuN⁺ cells, arrowheads point to GFP⁺NeuN⁺ cells. Values are means ± SD; * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$, 21-day chase:

Control n = 6, *Notch1* cKO n = 4, *Notch2* cKO n = 5, *Notch1Notch2* cKO n = 6, 100-day chase: Control n = 5, *Notch1* cKO n = 4, *Notch2* cKO n = 4, *Notch1Notch2* cKO n = 4, Scale bars = 15 μ m in C and G.

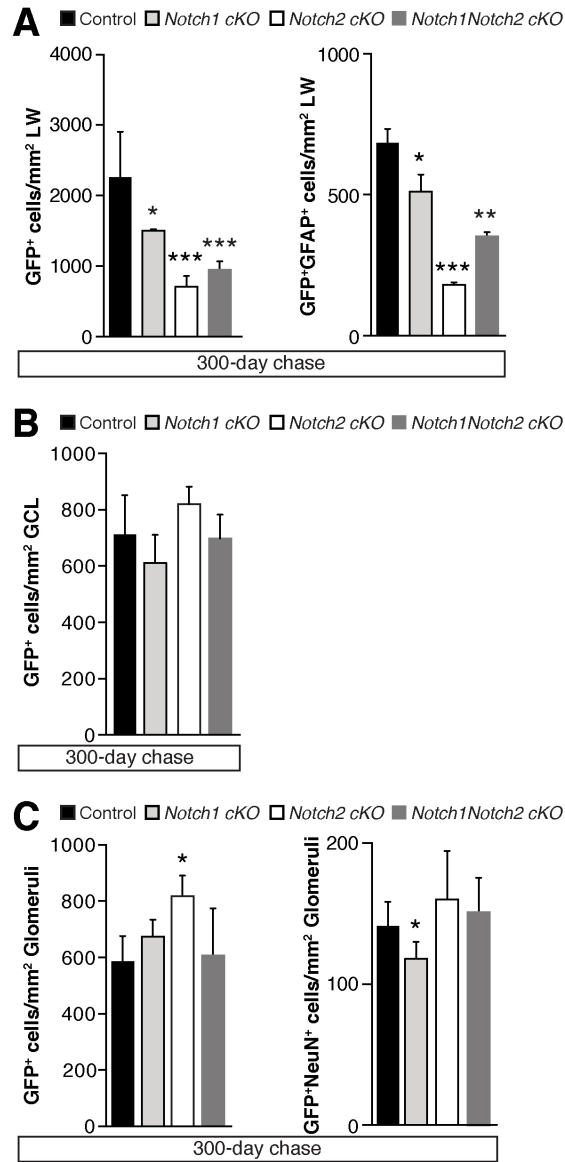


Figure S6. Notch signal manipulation affects the lineage into the OB. Related to Figure 6 and Table S7.

A. Quantification of total number of GFP⁺ cells and GFP⁺GFAP⁺ cells in the LW of the V-SVZ in Control, *Notch1* cKO, *Notch2* cKO and *Notch1Notch2* cKO mice 300 days after tamoxifen administration. **B.** Quantification of total number of GFP⁺ the GCL 300 days after tamoxifen administration. **C.** Quantification of total number of GFP⁺ cells and GFP⁺NeuN⁺ neurons in the glomeruli in Control, *Notch1* cKO, *Notch2* cKO and *Notch1Notch2* cKO mice 300 days after tamoxifen administration. Values are means \pm SD; * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$, 300-day chase: Control n = 4, *Notch1* cKO n = 3, *Notch2* cKO n = 3, *Notch1Notch2* cKO n = 3.

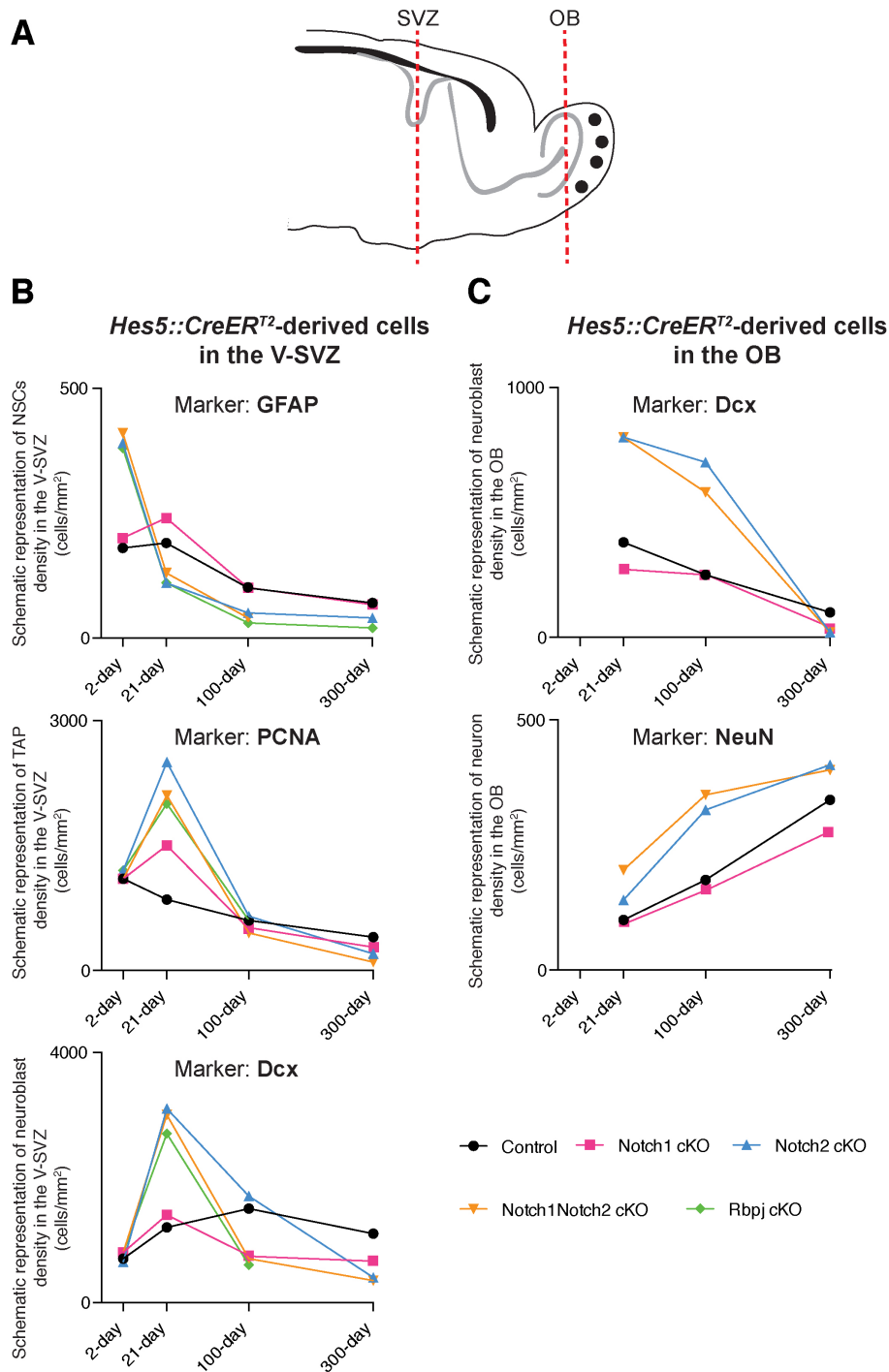


Figure S7. Notch ablation affects the neurogenic lineage. Related to Figure 7.

A. Scheme of a sagittal section of the adult mouse brain showing the plane of section and regions of analysis in the SVZ and OB. **B.** Summary of the changes in cell-type specific marker expression in the V-SVZ in *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO animals over time compared to Control animals. **C.** Summary of the changes in cell-type specific marker expression in the OB in *Notch1* cKO, *Notch2* cKO and *Notch1Notch2* cKO animals over time compared to Control animals.

SUPPLEMENTAL DATA

Table S1, related to Figure 1 and Figure S1.

Table for the coexpression data of Notch signaling components with lineage markers. Coexpression of lineage markers with *Rbpj* or coexpression of lineage marker with tdTomato of *Notch2-CreER^{T2-SAT}* lineage tracing in percent per lineage marker in the lateral wall V-SVZ.

	GFAP	PCNA	Dcx
Rbpj	n.d.	100.0 ± 0.0	100.0 ± 0.0
tdTomato	93.8 ± 5.9	93.8 ± 2.8	42.4 ± 12.7

Table S2, related to Figure 2 and Figure S2.

Tables showing the number of GFP⁺ cells per mm². **A.** Knockout efficiency in recombined, GFP⁺ cells in percentage. **B.** GFP⁺ cells (per mm²) 2 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO in the lateral wall V-SVZ. **C.** Expression of lineage markers by recombined GFP⁺ cells in Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO in the V-SVZ.

A. Knockout efficiency

	Percentage GFP⁺ cells	
	Control	Knockout
Notch1 ⁺	75.2 ± 8.2	19.1 ± 11.2
p-value (t-test)		0.0035 (**)
Notch2 ⁺	81.3 ± 9.7	18.9 ± 12.1
p-value (t-test)		0.0026 (**)
Rbpj ⁺	88.8 ± 8.4	13.8 ± 4.7
p-value (t-test)		< 0.0001 (***)

B. GFP⁺ cells 2 days post-tamoxifen administration

GFP⁺ cells (mm²)	Control	<i>Notch1</i> cKO	<i>Notch2</i> cKO	<i>Notch1Notch2</i> cKO	<i>Rbpj</i> cKO
2 days	2949.3 ± 125.8	2760.1 ± 112.0	2712.9 ± 197.9	2754.8 ± 201.2	2845.5 ± 79.2

C. GFP⁺ cells costained with specific markers, 2 days after tamoxifen administration

5-day TAM + 2 days chase	GFP+GFAP⁺ (mm²)	GFP+GFAP+PCNA⁺ (mm²)	GFP+PCNA⁺ (mm²)	GFP+Dcx⁺ (mm²)
Control	1326.4 ± 157.2	185.3 ± 85.3	1131.0 ± 102.0	713.6 ± 13.7
<i>Notch1</i> cKO	1127.4 ± 43.7	223.6 ± 23.7	1096.7 ± 56.2	880.3 ± 112.9
p-value (t-test)	0.135 (n.s.)	0.421 (n.s.)	0.662 (n.s.)	0.212 (n.s.)
<i>Notch2</i> cKO	903.3 ± 129.7	394.3 ± 28.3	1198.4 ± 294.8	690.3 ± 276.7
p-value (t-test)	0.217 (n.s.)	0.015 (*)	0.296 (n.s.)	0.831 (n.s.)
<i>Notch1Notch2</i> cKO	997.9 ± 24.7	444.2 ± 43.3	1093.7 ± 147.7	939.3 ± 87.2
p-value (t-test)	0.363 (n.s.)	0.046 (*)	0.541 (n.s.)	0.0414 (n.s.)
<i>Rbpj</i> cKO	998.2 ± 48.3	372.0 ± 41.5	1270.8 ± 95.7	937.2 ± 221.6
p-value (t-test)	0.125 (n.s.)	0.044 (*)	0.187 (n.s.)	0.037 (n.s.)

Table S3, related to Figure S2.

See Supplemental Excel table showing results of Microarray analysis of Control vs. *Notch2* cKO animals.

Table S4, related to Figure 3 and Figure S3.

Tables showing the number and marker expression of GFP⁺ cells per mm², 21 days and 100 days post-tamoxifen administration. **A.** GFP⁺ cells (per mm²) 21 and 100 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO in the V-SVZ. **B.** Expression of lineage markers by recombined GFP⁺ cells 21 days post-tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO in the V-SVZ. **C.** Expression of lineage markers by recombined GFP⁺ cells 21 days post-tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO in the V-SVZ.

A. GFP⁺ cells in the V-SVZ.

	GFP⁺ cells (mm²)	Control	<i>Notch1</i> cKO	<i>Notch2</i> cKO	<i>Notch1Notch2</i> cKO	<i>Rbpj</i> cKO
21 days		2827.3 ± 191.2	3127.0 ± 450.3	5942.1 ± 414.3	5212.8 ± 345.9	4998.6 ± 395.7
100 days		3026.7 ± 303.8	2265.9 ± 460.7	2945.2 ± 391.5	1774.3 ± 302.7	1703.2 ± 250.1

B. GFP⁺ cells coexpressing lineage markers 21 days after tamoxifen administration.

5-day TAM + 21 days chase	GFP+GFAP⁺ (mm²)	GFP+GFAP+PCNA⁺ (mm²)	GFP+PCNA⁺ (mm²)	GFP+Dcx⁺ (mm²)	GFP+Dcx+PCNA⁺ (mm²)
Control	1156.5 ± 97.5	185.3 ± 27.3	730.2 ± 193.1	1341.8 ± 447.2	179.2 ± 20.4
<i>Notch1</i> cKO	964.9 ± 101.7	240.4 ± 40.8	1747.3 ± 410.0	1489.8 ± 635.8	174.7 ± 9.8
p-value (t-test)	0.627 (n.s.)	0.038 (*)	0.521 (n.s.)	0.240 (n.s.)	0.358 (n.s.)
<i>Notch2</i> cKO	798.7 ± 121.7	107.6 ± 80.1	2502.1 ± 705.5	3284.5 ± 342.3	374.8 ± 98.7
p-value (t-test)	0.186 (n.s.)	0.044 (*)	0.0032 (**)	<0.001 (***)	0.014 (**)
<i>Notch1Notch2</i> cKO	701.5 ± 132.8	152.1 ± 30.2	2155.7 ± 378.9	3008.5 ± 328.9	521.7 ± 116.2
p-value (t-test)	0.063 (n.s.)	0.056 (n.s.)	0.008 (**)	0.010 (**)	0.003 (**)
<i>Rbpj</i> cKO	842.3 ± 23.8	111.2 ± 12.6	1956.3 ± 13.2	2426.3 ± 147.4	513.6 ± 102.8
p-value (t-test)	0.055 (n.s.)	0.032 (*)	0.043 (*)	0.002 (**)	0.002 (**)

C. GFP⁺ cells coexpressing lineage markers 100 days after tamoxifen administration.

5-day TAM + 100 days chase	GFP+GFAP⁺ (mm²)	GFP+GFAP+PCNA⁺ (mm²)	GFP+PCNA⁺ (mm²)	GFP+Dcx⁺ (mm²)	GFP+Dcx+PCNA⁺ (mm²)
Control	1019.5 ± 75.5	85.5 ± 40.2	610.0 ± 127.4	1583.8 ± 573.3	105.8 ± 42.3
<i>Notch1</i> cKO	972.1 ± 199.2	91.2 ± 20.2	545.5 ± 94.0	648.8 ± 40.3	132.7 ± 29.6
p-value (t-test)	0.46 (n.s.)	0.52 (n.s.)	0.612 (n.s.)	0.009 (**)	0.217 (n.s.)
<i>Notch2</i> cKO	672.4 ± 24.6	30.2 ± 19.2	675.1 ± 120.4	1897.7 ± 327.9	240.2 ± 54.7
p-value (t-test)	0.048 (*)	0.021 (*)	0.126 (n.s.)	0.219 (n.s.)	0.015 (*)
<i>Notch1Notch2</i> cKO	685.9 ± 142.7	24.1 ± 28.8	463.6 ± 71.4	550.1 ± 73.4	48.7 ± 9.7
p-value (t-test)	0.04 (*)	0.018 (*)	0.44 (n.s.)	<0.001 (***)	0.048 (*)
<i>Rbpj</i> cKO	727.8 ± 92.5	25.1 ± 8.1	609.8 ± 167.5	461.7 ± 8.5	50.1 ± 12.2
p-value (t-test)	0.050 (*)	0.008 (**)	0.087 (n.s.)	<0.001 (***)	0.056 (n.s.)

Table S5, related to Figure 4 and Figure S4.

Tables showing the number and marker expression of GFP⁺ cells per mm² 21 days and 100 days post-tamoxifen administration and area of RMS. **A.** GFP⁺ cells (per mm²), marker expression and area 21 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO, and *Notch1Notch2* cKO in the RMS. **B.** GFP⁺ cells (per mm²), marker expression and area 100 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO, and *Notch1Notch2* cKO in the RMS.

A. GFP⁺ cells in the RMS, 21 days post-tamoxifen administration.

5-day TAM + 21 days chase	GFP⁺ (mm²)	GFP⁺Dcx⁺ (mm²)	Area RMS (mm²)
Control	1763.3 ± 52.1	1181.6 ± 47.2	0.21 ± 0.02
<i>Notch1</i> cKO	1150.6 ± 302.1	772.4 ± 227.9	0.18 ± 0.03
p-value (t-test)	0.025 (*)	0.030 (*)	0.11 (n.s.)
<i>Notch2</i> cKO	2602.0 ± 97.6	2204.3 ± 122.3	0.26 ± 0.04
p-value (t-test)	0.028 (*)	0.002 (**)	0.041 (*)
<i>Notch1Notch2</i> cKO	2498.1 ± 245.7	2198.5 ± 141.7	0.28 ± 0.05
p-value (t-test)	0.033 (*)	0.003 (**)	0.035 (*)

B. GFP⁺ cells in the RMS, 100 days post-tamoxifen administration.

5-day TAM + 100 days chase	GFP⁺ (mm²)	GFP⁺Dcx⁺ (mm²)	Area RMS (mm²)
Control	1230.3 ± 257.2	893.9 ± 135.1	0.18 ± 0.01
<i>Notch1</i> cKO	998.4 ± 205.5	625.5 ± 73.4	0.17 ± 0.03
p-value (t-test)	0.14 (n.s.)	0.019 (*)	0.30 (n.s.)
<i>Notch2</i> cKO	867.2 ± 103.7	524.3 ± 45.7	0.16 ± 0.02
p-value (t-test)	0.016 (*)	0.021 (*)	0.089 (n.s.)
<i>Notch1Notch2</i> cKO	98.3 ± 17.3	23.3 ± 7.3	0.11 ± 0.00
p-value (t-test)	<0.0001 (***)	<0.0001 (***)	0.0087 (**)

Table S6, related to Figure 5 and Figure S5.

Tables showing the number and marker expression of GFP⁺ cells per mm², 21 days and 100 days post-tamoxifen administration. GFP⁺ cells (per mm²) and marker expression **A.** 21 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO, and *Notch1Notch2* cKO in the granule cell layer. **B.** GFP⁺ cells (per mm²) and marker expression 100 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO, and *Notch1Notch2* cKO in the granule cell layer. **C.** GFP⁺ cells (per mm²) and marker expression 100 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO, and *Notch1Notch2* cKO in the glomeruli.

A. GFP⁺ cells in the granule cell layer, 21 days after tamoxifen administration

5-day TAM + 21 days chase	GFP⁺Dcx⁺ (mm²)	GFP⁺NeuN⁺ (mm²)
Control	382.5 ± 38.7	85.3 ± 19.4
<i>Notch1</i> cKO	255.2 ± 33.3	80.8 ± 20.3
p-value (t-test)	0.045 (*)	0.38 (n.s.)
<i>Notch2</i> cKO	802.1 ± 103.7	135.8 ± 11.5
p-value (t-test)	<0.0001 (***)	0.141 (n.s.)
<i>Notch1Notch2</i> cKO	791.3 ± 98.6	168.5 ± 89.0
p-value (t-test)	<0.0001 (***)	0.019 (*)

B. GFP⁺ cells in the granule cell layer, 100 days after tamoxifen administration

5-day TAM + 100 days chase	GFP⁺ (mm²)	GFP+Dcx⁺ (mm²)	GFP+NeuN⁺ (mm²)
Control	511.2 ± 15.3	210.8 ± 173.8	85.7 ± 27.1
<i>Notch1</i> cKO	337.3 ± 30.8	231.7 ± 92.7	62.1 ± 24.9
p-value (t-test)	0.002 (**)	0.35 (n.s.)	0.16 (n.s.)
<i>Notch2</i> cKO	999.1 ± 11.2	714.9 ± 75.8	175.3 ± 41.3
p-value (t-test)	<0.0001 (***)	0.006 (**)	0.058 (n.s.)
<i>Notch1Notch2</i> cKO	1003.3 ± 75.2	575.7 ± 21.8	245.3 ± 91.2
p-value (t-test)	<0.0001 (***)	0.022 (*)	0.007 (**)

C. GFP⁺ cells in the glomeruli, 100 days after tamoxifen administration

5-day TAM + 100 days chase	GFP⁺ (mm²)	GFP+NeuN⁺ (mm²)
Control	384.5 ± 21.3	79.2 ± 11.1
<i>Notch1</i> cKO	378.6 ± 29.7	51.3 ± 9.6
p-value (t-test)	0.40 (n.s.)	0.015 (*)
<i>Notch2</i> cKO	660.6 ± 25.6	122.8 ± 15.3
p-value (t-test)	<0.0001 (***)	0.002 (**)
<i>Notch1Notch2</i> cKO	585.8 ± 64.2	105.3 ± 7.7
p-value (t-test)	0.005 (**)	0.021 (*)

Table S7, related to Figure 6 and Figure S6.

Tables showing the number and marker expression of GFP⁺ cells per mm², 300 days post-tamoxifen administration. **A.** GFP⁺ cells (per mm²) 300 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO, and *Notch1Notch2* cKO in the V-SVZ. **B.** GFP⁺ cells (per mm²) and marker expression 300 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO, and *Notch1Notch2* cKO in the V-SVZ. **C.** GFP⁺ cells (per mm²) and marker expression 300 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO, and *Notch1Notch2* cKO in the granule cell layer of the OB. **D.** GFP⁺ cells (per mm²) and marker expression 300 days after tamoxifen administration in Control, *Notch1* cKO, *Notch2* cKO, and *Notch1Notch2* cKO in the glomeruli of the OB.

A. GFP⁺ cells in the V-SVZ

GFP⁺ cells (mm²)	Control	<i>Notch1</i> cKO	<i>Notch2</i> cKO	<i>Notch1Notch2</i> cKO
300 days	2166.7 ± 565.6	1427.3 ± 8.4	710.1 ± 101.7	948.6 ± 71.2

B. GFP⁺ cells in the V-SVZ

5-day TAM + 300 days chase	GFP+GFAP⁺ (mm²)	GFP+PCNA⁺ (mm²)	GFP+Dcx⁺ (mm²)
Control	687.6 ± 20.2	667.1 ± 128.0	1342.3 ± 112.8
<i>Notch1</i> cKO	512.3 ± 35.5	307.1 ± 95.4	623.3 ± 11.8
p-value (t-test)	0.049 (*)	0.022 (*)	0.034 (*)
<i>Notch2</i> cKO	181.6 ± 7.9	209.1 ± 14.5	340.8 ± 62.8
p-value (t-test)	<0.001 (***)	0.006 (**)	<0.001 (***)
<i>Notch1Notch2</i> cKO	352.1 ± 7.2	93.7 ± 2.7	281.7 ± 42.7
p-value (t-test)	0.004 (**)	<0.001 (***)	<0.001 (***)

C. GFP⁺ cells in the GCL of the OB

5-day TAM + 300 days chase	GFP⁺ (mm²)	GFP⁺Dcx⁺ (mm²)	GFP⁺NeuN⁺ (mm²)
Control	707.6 ± 110.8	61.5 ± 39.9	222.3 ± 13.1
<i>Notch1</i> cKO	595.6 ± 107.3	12.3 ± 8.9	183.8 ± 21.3
p-value (t-test)	0.11 (n.s.)	0.047 (*)	0.026 (*)
<i>Notch2</i> cKO	801.6 ± 45.9	11.1 ± 3.1	266.8 ± 61.2
p-value (t-test)	0.534 (n.s.)	0.007 (**)	0.241 (n.s.)
<i>Notch1Notch2</i> cKO	706.1 ± 74.2	14.7 ± 2.9	294.3 ± 39.0
p-value (t-test)	0.431 (n.s.)	0.008 (**)	0.157 (n.s.)

D. GFP⁺ cells in the glomeruli of the OB

5-day TAM + 300 days chase	GFP⁺ (mm²)	GFP⁺NeuN⁺ (mm²)
Control	591.3 ± 98.9	146.6 ± 14.4
<i>Notch1</i> cKO	680.0 ± 21.6	120.5 ± 13.7
p-value (t-test)	0.09 (n.s.)	0.039 (*)
<i>Notch2</i> cKO	800.2 ± 84.2	173.3 ± 26.2
p-value (t-test)	0.042 (*)	0.196 (n.s.)
<i>Notch1Notch2</i> cKO	602.1 ± 193.4	157.6 ± 20.0
p-value (t-test)	0.08 (n.s.)	0.295 (n.s.)

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animals and husbandry according to ARRIVE-guidelines

Hes5::GFP, *Hes5::CreER^{T2}*, *Notch2::CreER^{T2-SAT}*, *Rosa26R::GFP*, *Rosa26R::tdTomato*, floxed *Notch1*, floxed *Notch2*, floxed *Rbpj* mice have been described elsewhere (Basak and Taylor, 2007; Besseyrias et al., 2007; Schouwey et al., 2007; Fre et al., 2011; Basak et al., 2012; Lugert et al., 2012). Mice were maintained on a C57Bl6 genetic background and kept on a 12-hour day/night cycle with food (Kliba Nafag Haltungsextrudat 3436) and water (filtered and autoclaved) *ad libitum* under specified pathogen free conditions and according to Swiss Federal and Swiss Veterinary office regulations under license numbers 2537 and 2538 (Ethics commission Basel-Stadt, Basel Switzerland). Animals were housed in IVC Greenline Techniplast GM500 cages on Aspen bedding (Tapvei). 5-6 animals were cohoused per cage at an ambient temperature of $22 \pm 2^\circ\text{C}$ and $55 \pm 15\%$ humidity. Animals for experiments were selected at random based on genotype. Experimental animals were virgins and all experiments were performed with a mix of genders (43 females, 37 males in total). Animals were genotyped after endpoint analysis.

Administration of tamoxifen and tissue preparation for immunochemical staining

Adult mice 8-10 weeks of age, weighing 20-30 grams, were used in the experiments. *Hes5::CreER^{T2}* mice carrying floxed *Rbpj*, floxed *Notch1* or floxed *Notch2* alleles were injected daily intraperitoneal (i.p.) for optimal recombination with 2 mg tamoxifen in corn oil (100 μl of 20 mg/ml) for five consecutive days, and thereafter housed in home cage and killed 2, 21, 100 or 300 days after the end of the treatment. Conditional gene knockout mutants were necessary as loss of *Rbpj* or *Notch* receptors is embryonic lethal. Conditional deletion allowed for mosaic analysis of gene function. Animals were injected intraperitoneal (i.p.) with a lethal dose of Ketamine-Xylazine and perfused with cold phosphate buffered saline (PBS) followed by 4% PFA in PBS. Brains were excised, fixed overnight in 4% PFA in PBS, cryoprotected with 30% sucrose in PBS at 4°C 48 hours, embedded and frozen in OCT (TissueTEK), and 30 μm floating sections cut by cryostat (Leica).

Ex vivo microarray analysis of tamoxifen-induced, recombined cells

Adult mice 8-10 weeks of age were used in the experiments. *Hes5::CreER^{T2}* mice carrying floxed *Notch2* alleles were injected daily intraperitoneal (i.p.) with 2 mg tamoxifen as stated previously. After five days consecutive administration, animals were sacrificed 24 hours after the end of the treatment. Animals were euthanized in CO_2 , brains were dissected in L15 Medium (GIBCO) and cut into 0.55 mm thick sections using a McIlwains tissue chopper. The SVZ was microdissected under a binocular microscope avoiding contamination from the striatum, and digested using a Papain solution and mechanical dissociation as described previously (Lugert et al., 2010). Cells were resuspended in Leibovitz medium (Life Technologies), filtered through a 40 μm cell strainer (Miltenyi Biotec) and sorted on a BD FACS Aria III. Cells were discriminated by forward and side-scatter (for live cells – from the control animals) and gated for GFP^- (wild-type levels) or GFP^+ populations. Cells were directly sorted into cooled Trizol (Life Technologies). Appropriate amount of Chloroform was added and RNA extraction was performed using Isopropanol with LiCl (0.75M). RNA was immediately frozen to -80°C . RNA quality was tested on a Fragment Analyzer (Advanced Analytical) using a high sensitivity RNA analysis kit (DNF-472). Samples were sent for Expression Profiling with Atlas Biolabs. Samples were subjected to a second quality control on an Agilent 2100 Bioanalyzer, small samples were amplified using the Ovation Picokit (NuGen) and then run on an Affymetric Biochip. GO analysis was done using Lasergene Arraystar (DNASar) software.

Quantitative PCR confirmation of *Notch2* knockout

Ex vivo mRNA was prepared as described above. Isolated RNA was treated with DNaseI (Roche). cDNA was prepared using BioScript (Bioline) and random hexamer primers. qPCR was performed using SensiMix SYBR kit (Bioline). Primers for PCR reactions are as follows:

GAPDH	Fwd: CTCCCACTCTTCCACCTTCG Rev: CCACCACCTGTTGCTGTAG
β -Actin	Fwd: AGGTGACAGCATTGCTTCTG Rev: GGGAGACCAAAGCCTTCATA
<i>Notch2</i> (Exon 26/27)	Fwd: CAGGAGGTGATAGGCTCTAAG Rev: GAAGCACTGGTCTGAATCTTG

Immunofluorescence staining of floating sections and antibodies

Immunostainings on sections was performed as described previously (Giachino and Taylor, 2009; Lugert et al., 2010). Briefly, sections were blocked at room temperature for 30 minutes with 10% normal donkey serum (Jackson ImmunoResearch) in PBS containing 0.5% TritonX-100. Primary antibodies diluted in 2.5% donkey serum blocking solution were incubated overnight. Sections were washed with PBS and incubated at room temperature for 1-2 hours with the corresponding secondary antibodies in 5% donkey serum blocking solution

and counter-stained with DAPI (1 µg/ml). Sections were mounted on glass slides (SuperFrost, Menzel) in DABCO mounting media and visualized using a Zeiss Observer with Apotome (Zeiss). For PCNA detection, the antigen was recovered at 80°C for 20 minutes in Sodium Citrate (10 mM, pH7.4).

Primary antibodies used were as follows: Anti-Doublecortin (goat, 1:500, Santa Cruz, sc-8066), anti-dsRed (rabbit, 1:500, Clontech Takara, 632496), anti-Glial fibrillary acidic protein (mouse, 1:500, Sigma, G3893), anti-Glial fibrillary acidic protein (rabbit, 1:1000, Sigma, G9269), anti-GFP (chicken, 1:250, AvesLab, GFP-1020), anti-GFP (rabbit, 1:500, Invitrogen, A11122), anti-GFP, (sheep, 1:250, AbD Serotec, 4745-1051), anti-Neuronal nuclear antigen (mouse, 1:800, Millipore, MAB377), anti-Notch1 (rabbit, 1:100, (Nyfeler et al., 2005)), anti-Notch2 (rat, H. Robson Lausanne, 1:200), anti-Proliferating cell nuclear antigen (mouse 1:1000, DAKO, M0879), and anti-Rbpj (rabbit, 1:1000, Cell Signaling, 5313).

Secondary antibodies used were as follows: Donkey anti-rabbit Ig Cy3 conjugated (1:500, Jackson ImmunoResearch, 711165152), donkey anti-mouse Ig Cy3 conjugated (1:500, Jackson ImmunoResearch, 715165151), donkey anti-rabbit Ig Cy5 conjugated (1:300, Jackson ImmunoResearch, 711496152), donkey anti-mouse Ig Cy5 conjugated (1:300, Jackson ImmunoResearch, 715175151), donkey anti-rabbit Ig 488 conjugated (1:500, Jackson ImmunoResearch, 711545152), donkey anti-sheep Ig 488 conjugated (1:500, Jackson ImmunoResearch, 713095147), donkey anti-goat Ig Cy3 conjugated (1:500, Jackson ImmunoResearch, 705165147), and donkey anti-rat Ig Cy3 conjugated (1:500, Jackson ImmunoResearch, 712160153).

Quantification and statistical analysis

Stained sections were analyzed with a Zeiss Observer with Apotome (Zeiss). Images were processed with Photoshop or ImageJ. Data are presented as averages of a minimum of three sections per region and multiple animals (n in figure legends). Statistical significance was determined by two-tailed Student's t-test on mean values per animal, percentages were transformed into their arcsin value. Significance was determined at * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$ or P-values are given in the graphs. Deviance from mean is displayed as standard deviation if not otherwise indicated. Complete data tables are provided in supplemental data and information.