Switching of RNA splicing regulators in immature neuroblasts: a key step in adult neurogenesis

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Summary

The lateral wall of the subventricular zone harbors neural stem cells (NSC, B cells) which generate proliferating transient-amplifying progenitors (TAP, C cells) that ultimately give rise to neuroblasts (NB, A cells). Molecular profiling at the single cell level struggles to distinguish these different cell types. Here, we combined transcriptome analyses of FACS-sorted cells and single-cell RNAseq to demonstrate the existence of an abundant, clonogenic and multipotent population of immature neuroblasts (iNB or D cells) at the transition between TAP and

migrating NB (mNB). iNB are reversibly engaged in neuronal differentiation. Indeed, they keep molecular features of both undifferentiated progenitors, plasticity and unexpected regenerative properties. Strikingly, they undergo important progressive molecular switches, including changes in the expression of splicing regulators leading to their differentiation in mNB subdividing them into 2 subtypes, D1 and D2. Due to their plastic properties, iNB could represent a new target for regenerative therapy of brain damage.

Key words: Subventricular zone, adult neurogenesis, transcriptomic analyses, immature neuroblasts

Introduction

New neurons are constantly produced from neural stem cells (NSC) throughout life in two restricted neurogenic areas of the adult mammalian brain: the sub-granular zone (SGZ) of the hippocampus and the subventricular zone (SVZ) [1]. The lateral wall (LW) of the SVZ, adjacent to the striatum, is the largest germinal zone of the adult mouse brain, in which NSC (type B1 cells) are produced during the embryonic development and are mostly in a quiescent state thereafter[2,3]. Upon activation, NSC divide and give rise to rapidly dividing transit-amplifying cells (TAP, type C cells), which ultimately produce neuroblasts (NB, type A cells). These neuroblasts migrate out of the SVZ anteriorly along blood vessels and the rostral migratory stream (RMS) to reach the olfactory bulb (OB) where they integrate and differentiate into various subtypes of interneurons required to fine-tune odor discrimination [1]. The diversity of the OB interneurons depends on the intrinsic regional identity of NSC in the SVZ along the anterior-posterior and medial-lateral axes[1,4].

Besides their neurogenic fate, NSC and neural progenitors present in the SVZ are also proficient to produce astrocytes and oligodendrocytes that migrate toward the corpus callosum, the RMS and the striatum in the normal[5,6,7] and injured adult brain[8,9].

Transcriptomic analyses using different combinations of cell surface markers and transgenic mice models to isolate SVZ cells have provided important initial insights into stem cell quiescence and have highlighted consecutive transitions during neuronal lineage progression [2,10–13]. Since then, the single cell RNA-sequencing has revolutionized the field and has made it possible to precisely elucidate the transcriptome of SVZ cells in the LW[14–17] and in the septal wall which also harbours NSC niches[18].

We have previously developed a FACS-based method, using a combination of three surface markers (LeX, EGF receptor, CD24), allowing to sort five distinct SVZ cell populations: quiescent NSC (LeX-EGFR-CD24-, hereafter referred to as sorted-qNSC, s-qNSC), activated NSC (LeX+EGFR+CD24-, referred to as sorted, s-aNSC), transit amplifying cells ($LeX^{-}EGFR^{+}CD24^{-}$, referred to as sorted, s-TAP), immature ($LeX^{-}EGFR^{+}CD24^{low}$, referred to as sorted, s-iNB) and migrating neuroblasts ($LeX^{-}EGFR^{-}CD24^{low}$, referred to as sorted, s-mNB) from the adult mouse brain[19,20]. Importantly, whereas s-iNB presented neuroblast markers, e.g. CD24 and doublecortin, s-iNB gathered the most abundant population among SVZ cycling progenitors (representing approximately 4.3% of total SVZ cells *vs* 1% s-aNSC and 2.5% s-TAP)[20].

We have previously shown that following a genotoxic stress (exposition to ionizing radiation) inducing the depletion of cycling progenitors, s-qNSC exit quiescence restoring successively s-aNSC, s-TAP, s-iNB and finally s-mNB[20].

Transcriptomic studies revealed that age-related SVZ neurogenesis decline is associated with TGFß signaling and specific cell cycle regulation changes in s-aNSC. A progressive lengthening of aNSC G1 phase with age leads to a decrease in the production of s-TAP, s-iNB and s-mNB [20–22]. However, despite the decrease of neurogenesis with aging the s-iNB remained more abundant than s-aNSC and s-TAP in the aged brain [21].

Studies of SVZ neural progenitors have been essentially focused on the characterization of the molecular mechanisms regulating quiescence, activation and the regenerative potential of aNSC. Here, we combined transcriptome analyses using DNA microarrays on FAC-sorted cells and single-cell RNAseq to demonstrate that iNB form a molecularly distinct class of SVZ cells, exhibiting molecular features of both neural progenitors and neuroblasts while keeping unexpected regenerative capacity and plasticity. We identified important and sequential molecular switches occurring in these cells before their final differentiation in migrating neuroblasts. Altogether, these data led us to propose a revision of the current model of adult neurogenesis.

Results

Regenerative potential and multipotency of sorted iNB *in vitro and in vivo*

We have previously shown that s-iNB had a clonogenic potential and were able to proliferate *in vitro* for weeks in normoxic conditions (20%O2) unlike s-mNB [20]. However, s-

iNB exhibited reduced clonogenic potential compared to that of s-aNSC and s-TAP (Fig.1A). It has been previously reported that hypoxic conditions promoted SVZ NSC proliferation and neurogenesis[23,24]. Hypoxic conditions (4%O2) had no effects on s-mNB, but increased the proliferation capacities of s-aNSC, s-TAP and s-iNB, which reached similar growth rates, making them undistinguishable based on this endpoint (Fig.1A). Hypoxic conditions also increased the clonogenic potentials of both s-TAP and s-iNB but not that of s-aNSC (Fig. 1B). In these conditions, the clonogenic potential of s-TAP reached similar values as s-aNSC, but that of s-iNB was still lower (Fig.1B).

Quite surprisingly, freshly isolated s-iNB had the capacity to generate the three neural lineages, neurons, astrocytes and oligodendrocytes, when plated for 5–7 days in the appropriate differentiation media, similarly as s-aNSC and s-TAP (Fig.1C).

Thus, to assess the regenerative potential of these immature neuroblasts *in vivo*, eGFP$^+$ iNB were sorted from β–actin:eGFP mice, transplanted into the left striatum of recipient C57Bl/6 mice and compared to eGFP$^+$s-mNB and eGFP$^+$s-NSC/TAP. Five weeks after transplantation, eGFP$^+$ cells were detected in different regions of interest (Table 1) in the brains of mice transplanted with eGFP$^+$s-NSC/TAP or eGFP$^+$s-iNB, whereas no eGFP+ were observed after transplantation of eGFP$^+$s-mNB even at the injection site (IS) (Table 1). Excepted in one out of 3, animals transplanted with eGFP$^+$s-iNB presented high levels of eGFP$^+$ cells in the OB with levels equivalent or superior to animals transplanted with eGFP$^+$s-NSC/TAP or eGFP$^+$s-iNB (Fig. 1D). EGFP$^+$ cells were detected in the rostral migratory stream (Fig. S1A) as well as in the granular cell layer (GCL) and the external plexiform layer (EPL) of the OB where they differentiated into NeuN$^+$ neurons in 2/3 animals transplanted with eGFP$^+$s-iNB (Fig.1Da) and in 3/3 animals transplanted with EGFP$^+$s-NSC/TAP (Table 1).

Importantly, eGFP$^+$ cells were present in the SVZ of all the animals transplanted with eGFP$^+$s-iNB and eGFP$^+$s-NSC/TAP (Fig. 1Db, Fig. 1Dc), some of them expressing GFAP suggesting a possible conversion of eGFP$^+$s- iNB into NSC (Fig. 1Db3, Fig. 1Dc2). Plasticity of s-iNB was also shown at the IS by the detection of eGFP$^+$ cells expressing markers of astrocytes (GFAP) (Fig. S1Ba1) and oligodendrocytes (CNPase) (Fig. S1Bb2).

Overall, these results demonstrate the plasticity of s-iNB *in vitro*, making them very similar to s-NSC/TAP, questioning the exact nature of this abundant SVZ cell populations that express neuroblast markers.

s-iNB are a molecularly distinct class of proliferating SVZ progenitors

We characterized the transcriptomic profiles of each SVZ populations sorted from 2 month-old C57Bl/6 mice using Clariom™ D mouse microarrays that provides intricate transcriptome-wide gene- and exon-level expression profiles (Fig. 2A).

Pseudotime analysis (TSCAN) [25] based on 3679 highly dispersed genes between the five sorted cell populations confirmed the lineage progression from s-qNSC (cluster1), then s-aNSC and s-TAP within a single cluster (cluster2), followed by s-iNB (cluster3) and finally s-mNB (cluster4) (Fig. 2B). Another pseudotime analysis excluding s-qNSC, based on 1481 highly dispersed genes, delineated two distinct clusters for s-aNSC and s-TAP (Fig. 2C), highlighting their molecular differences.

Venn Diagram comparison of data sets revealed 2950, 315, 129, 395 and 1693 genes unique to s-qNSC, s-aNSC, s-TAP, s-iNB and s-mNB respectively (Supp_data_1). G:Profiler functional profiling of these transcriptome signatures with KEGG and reactome databases uncovered significant stage-dependent changes (Fig.S2, Supp_data_1). S-aNSC overexpressed genes involved in metabolism of glyoxylate and dicarboxylate metabolism. S-qNSC rather overexpressed genes related to metabolism of lipids and Rho GTPase cycle. S-TAP significantly displayed genes involved in Mitotic, G1 phase and G1/S transition, Activation of the pre-replicative complex. S-mNB specifically overexpressed genes involved in axon guidance and nervous system development. Conversely, s-iNB signatures contained many genes linked to cell cycle, prometaphase, M phase, and cell cycle checkpoints, clearly distinguishing them from the other cell populations including s-mNB.

Alternative splicing (AS) plays various functions in brain development including neural cell differentiation and neuronal migration [26–30]. Using the splicing index (SI) corresponding to a value assigned to each exon by estimating its abundance with respect to adjacent exons using TAC software (ThermoFisher Scientific), we identified differentially spliced genes (DSG, adjusted p-value ≤ 0.05) between the successive cell transitions along neurogenesis. Results show a very large number of DSG between s-qNSC *vs* s-aNSC (9484) then no DSG at the s-aNSC and s-TAP transition, only 169 between the s-TAP and s-iNB transition, and finally 3759 between s-iNB and s-mNB (Supp_data_2). Functional profiling analysis with g:Profiler linked the DSG between s-TAP and s-iNB to the nervous system development (GO:0007399) and those between s-iNB *vs* s-mNB to cellular component organization or biogenesis (GO:0071840).

We then focused on the differentially expressed genes (adjusted p-value ≤ 0.05) belonging to the nine families of RNA splicing regulators (RSR) that play essential roles in the neurogenic pathway (*Ptbp, Nova, Rbfox, Elavl, Celf, Dbhs, Msi, Pcbp, and Mbnl)* [26]. Strikingly,

a clear down-regulation of most RSR gene expression distinguishes s-qNSCs from the other populations (Supp_data_3). Interestingly another shift discriminates s-aNSC and s-TAP from s-mNB (Supp_data_3). This led us to identify several groups of RSR genes in function of their variation of expression along with neurogenesis: the groups 1 and 6 were overexpressed in s-aNSC and s-TAP and downregulated in s-mNB; the group 2, 3 and 5 were up-regulated in mNB as compared to s-aNSC and s-TAP. All these groups were up-regulated in s-iNB, suggesting that the switch in RSR expression between cycling progenitors and s-mNB occurred in these cells (Fig. 2D). Interestingly, *Ptbp1*, a major splicing repressor [31], was downregulated in s-mNB as compared to s-iNB, consistently with the large increase in DSG at the transition between s-iNB and s-mNB.

Altogether, these data confirm at the molecular level that s-iNB comprises cycling progenitors at the transition between s-TAP and s-mNB that undergo important molecular switches, which include major changes in the expression of some RSR genes leading to a major increase in the number of DSG occurring at the final maturation of s-mNB.

s-iNB correspond to specific SVZ cell clusters identified by single cell RNA sequencing (scRNAseq)

We compared our microarray datasets to published scRNAseq datasets from adult mouse SVZ [14,17,32]. Violin plots show that s-qNSC and s-mNB perfectly matched with the corresponding populations identified in these studies, which was less obvious for s-aNSC and s-TAP (Fig. S3). s-iNB matched with the clusters termed “Mitosis” (Class VI) in the study of Llorens et al. [32] and “Mitotic TAP” in that of Zywitza et al.[17], and with clusters 8, 10, 17, 16 linked to “Mitosis”, but also with clusters 6 and 15 described as $DCX^{+}Ki67^{+}$ neuroblasts in the study by Cebrian_Silla et al.[14]. These comparisons thus confirm that s-iNB comprise cycling SVZ cells exhibiting both TAP and NB features, which illustrates the difficulties to determine the exact nature of cell clusters identified by scRNAseq.

We have previously reported[20] that after brain exposure to ionizing radiation, proliferating progenitors are highly radiation-sensitive and rapidly eliminated after irradiation. On the contrary qNSC that are radiation resistant repopulate the different SVZ neurogenic cell populations successively the following days after irradiation. We therefore used this model and our transcriptomic signatures of SVZ stem and progenitor cells to gain new insights on the nature and the hierarchical ordering of neural progenitor clusters identified by sc-RNAseq.

TenX Genomics droplet-based single-cell transcriptomic analyses were performed on SVZ cells from 2 mice, 5 days after irradiation (4 Gy, SVZ only) and from 2 non-irradiated control

mice after removal of myelin and red blood cells (Fig. 3A). Based on a filtering criterion of 1,500 genes/cell to exclude low-quality sequenced cells, 17,343 cells were retained for analysis, divided into 11,529 non-irradiated cells and 5,814 irradiated cells (Fig. S4).

Non-irradiated and irradiated samples were combined and integrated using the SCTransform workflow. After integration, PCA was performed and 50 PCs were used for Uniform-manifold approximation (UMAP). Leiden graph-based clustering, with a resolution of 1.2, identified 33 clusters (Fig. S5A). These clusters were first annotated based on the expression of known cell markers (Fig. 3B and C, Fig. S5B). Nineteen clusters gathering more than half SVZ-residing cells corresponded to non-neurogenic cells (ordered by decreasing abundance): microglia (expressing *P2ry12*[33]), ependymal cells (expressing both *S100β* and *Slc1a3*[34]), endothelial cells (expressing *Flt1*[35]), oligodendrocyte progenitors (OPC, expressing *Pdgfrα*[36]), Myelin forming oligodendrocytes (MFOL, expressing *Mog*[17]) and pericytes (expressing both *Vtn* and *Pcam1* [7,37]), and dopamine neurons (expressing *Vglut2*[38]). The 14 other clusters corresponded to astrocytes and neurogenic cells (Fig. 3B, C, D). The clusters 4 and 13 that expressed *S100β* were further identified as qNSC (cluster 4) and astrocytes (cluster 13) based on the transcriptomic signatures described by Cebrian-Silla et al., 2021[14] (Fig S5C, Supp_data_4). Cluster 17, was identified as aNSC based on *Gfap, Thbs4, S100α6*, *Egfr*^{low} and *Ascl1* expressions[14], and cluster 20 as TAP based on *Egfr* and *Ascl1* expressions [39]. Clusters 10, 5, 12, and 8 were defined as cycling progenitors based on the expression of proliferative markers such as *Top2a*, *Mki67*, *Ascl1*. Clusters 1, 3, 7 and 9 were identified as mNB due to the loss of *Mki67* and *Ascl1* expressions and the expression of *Robo2* and *Dcx*.

We then applied the UCell workflow to further characterize the neurogenic clusters with the transcriptomic signatures of each of our five sorted SVZ cell populations (Supp_data_1), calculating individual cell scores for each signature based on the Mann–Whitney U statistical analysis, and attributing cells to populations based on the highest score amongst signatures[40]. This score allowed us to unequivocally attribute some clusters to the sorted cell populations (Fig. 3E,F and G): cluster 4 and 13 matched with s-qNSC, cluster 17 with s-aNSC, cluster 20 with s-TAP, clusters 5, 15 and 12 with s-iNB and finally clusters 7, 9, 1 and 3 to s-mNB. Reflecting the continuous nature of the SVZ neurogenic process, the cluster 10 matched both with s-TAP and s-iNB and clusters 8 and 19 both to s-iNB and to s-mNB. Cell-Cycle Scoring[36] indicated that the clusters related to s-iNB were enriched in cells in S or G/2M phase (Fig. 3H).

As the consequence of radiation-induced cell death, the relative abundances of each cluster were different in controls (Fig. 3I) and irradiated (Fig. 3J) brains and dependent on the

progressive repopulation of the different types of progenitors. Normalized to the numbers of s-qNSC (cluster 4), which are supposed not to be affected by irradiation[20], the ratios of the number of cells per clusters in irradiated brains over that in unirradiated controls gave an index of the progressive reconstitution of each cluster (Fig. 3K). As expected, clusters 17 and 20, corresponding to s-aNSC and s-TAP respectively, were almost entirely regenerated. The decreasing values of the ratios evidence a temporal ordering of the regeneration of each cluster corresponding to s-iNB along with neurogenesis as follows: 10, 5, 15, 12, and 8, the latter being the one at the iNB vs mNB transition as anticipated above.

We next addressed the expression of RSR genes in each cluster (Fig. 4). Not all genes could have been investigated since some of them were below the detection threshold in all the cell populations studied. The expression of RSR genes *(Kdrbs3, Kdrbs1 Elavl3 and Elavl2, Elavl4, and Celf1*) that were shown to increase along with neurogenesis in microarrays, also increased in cluster 10, remained stable in the other clusters related to s-iNB and reached a maximum value in the s-mNB clusters (Fig. 4A). Conversely, the expressions of *Mbnl1* and *Msi2* that were shown to decrease along with neurogenesis in microarrays (Fig. 4B) peaked in the clusters related to s-aNSC (17) and s-TAP (20), decreased in the cluster 10, remained low in the clusters related to s-iNB (5, 15, 12, 8) and were not detected in the clusters related to s-mNB (19, 1, 3, 7, 9). Altogether, these data corroborate our microarray data and show that a major switch in the expression of RSR genes occurs in the clusters related to s-iNB. Interestingly, the splicing repressor *Ptbp1* was similarly expressed in s-aNSC, s-TAP and s-iNB-related clusters but was downregulated progressively in the successive s-mNB clusters, suggesting that changes in *Ptbp1* expression constitute an important molecular step for the final maturation of mNB, which involves a major increase of DSG.

The *Dcx* status identifies two molecularly distinct subpopulations in s- iNB

We have previously shown by immunofluorescence that not all the s-iNB were positive for doublecortin (*Dcx*) expression, a common marker of neuroblasts[20]. Violin plot (Fig. 3D) and feature plot (Fig. 5A) show that *Dcx* expression was restrained to s-mNB and to clusters related to s-iNB in which it increased consistently with their hierarchical ordering we defined above. Variations in *Dcx* expression among iNB related clusters led us to analyse separately Dcx^{low} and Dcx^{high} cells in these clusters (Fig. S6). The ratios of numbers of cells per cluster indicated a greater recovery of Dcx^{low} cells as compared to Dcx^{high} cells in s-iNB related clusters from irradiated brains (Fig. 5B), reflecting their temporal ordering.

Characterization of differentially expressed genes according to the *Dcx* status in these clusters showed 588 unique genes expressed in the *Dcx*^{low} cells and 98 genes specifically expressed in the *Dcx*^{high} cells. Functional enrichment profiling using the g:Profiler software illustrates biological differences between both populations : genes related to Metabolism of RNA were overrepresented in the *Dcx*^{low} cells whereas genes linked to GABAergic synapse, Neuronal System were rather overexpressed in the *Dcx*^{high} cells (Supp_data_5). Notably, 55% of *Dcx*^{high} cell specific genes were s-mNB genes (*e.g. Robo2, Gap43, Nav3*). Further analysis of RSR genes expression in function of the *Dcx* status, clearly confirms that progressive transcriptomic changes occurred between *Dcx*^{low} and *Dcx*^{high} cells (Fig. 5C and D). However, RSR gene profiles of *Dcx*^{low} cells in clusters related to s-iNB were obviously different from that related to both s-TAP and s-NSC, and that of *Dcx*^{high} cells from that of s-mNB. Similar results were obtained with cells from irradiated brains (Fig. S7).

These data clearly show that the clusters related to s-iNB contained cells engaged in neuronal differentiation that are molecularly distinct from the other types of SVZ neurogenic cells already characterized This suggest that s-iNB correspond to a new type of cycling neurogenic progenitors progressively engaged in neuronal differentiation.

We next took advantage of the DCX-CreERT2::CAG-floxed-eGFP mice model, allowing the expression of eGFP in cells expressing *Dcx* after the administration of Tamoxifen to sort eGFP^{+}CD24^{+}EGFR^{+} cells corresponding to *Dcx*^{high} iNB and eGFP^{-}CD24^{+}EGFR^{+} cells containing the *Dcx*^{low} iNB to distinguish further these two populations at the functional level. As shown in Fig. 6A and B, recombinant eGFP^{+} iNB exhibited a clonogenic potential and a rate of population doublings quite similar to eGFP^{-} iNB and total iNB, contrary to eGFP^{+} mNB, which did not show clonogenic potential and did not proliferate at long-term *in vitro*. Moreover, we showed that both eGFP^{+} iNB and eGFP^{-} iNB have the capacity to generate the three neural lineages, neurons, astrocytes and oligodendrocytes, when plated for 5–7 day in the appropriate differentiation media (Fig. 6C), similarly as s-iNB (Fig. 1C).

By contrast, when eGFP^{+}iNB were transplanted into the brain of C57Bl/6J mice, no eGFP^{+} cells persisted at long-term (5 weeks) (Fig. S8), suggesting that the *in vivo* plasticity and regenerative properties of s-iNB was rather associated to *Dcx*^{low} iNB.

Discussion

This study bridges the cellular and molecular characterizations of SVZ neural progenitor populations. We combined the transcriptional profiling using DNA microarrays of SVZ cells that were sorted by an efficient FACS strategy[19] and large-scale scRNAseq to decipher the progressive molecular and cellular changes involved in SVZ neurogenesis. Our model of regeneration of the neurogenic niches after brain irradiation[20] allowed a unique way to determinate the sequential regeneration of the different types of neurogenic cell clusters identified by scRNAseq that were precisely characterized using the respective molecular signatures of sorted-SVZ cells, providing new insights onto the progression of SVZ neurogenesis. We show that iNB is an abundant population of cycling progenitors, which is more advanced towards neuronal differentiation than TAP, while retaining unexpected stem cell properties unlike mNB. We suggest that major splicing regulations in iNB might be critical for the final commitment to the neuronal fate.

Our results constitute a valuable resource for delineating the molecular transitions occurring during neurogenesis that are orchestrated by numerous differentially expressed genes and alternative splicing variants. The transition from qNSC to aNSC has been abundantly investigated in the literature[15,32], but the transcriptional shift leading to the differentiation of neural progenitors into neuroblasts has not been thoroughly analysed to date. We showed that it occurs in immature neuroblasts, which form a relatively abundant subpopulation of SVZ cells, which exhibit specific transcriptional features distinguishing them from TAP and mNB.

Consistently with previous works showing that a subpopulation of newly generated neurons continues to divide in the SVZ[41,42], we formerly described the isolation of s-iNB as a subset of cycling SVZ cells that expressed both CD24, a neuroblast marker, and EGFR, a marker of cycling progenitors[19–21]. This was consistent with prior findings describing a subset of neuroblasts co-expressing Ascl1 and Dcx or CD24^{low} expressing low levels of EGFR in the adult SVZ[13,39,43]. Here, we evidenced that s-iNB comprise a molecularly distinct population of cycling cells at the transition between TAP and mNB, and exhibiting molecular characteristics of both these cell types.

The capacity of neuroblasts to reorient towards the glial lineage in pathological contexts have been reported previously[44–46]. Consistent with the molecular findings showing that s-iNB kept transcriptional features of NSC and TAP, we showed that s-iNB also kept *in vitro* and *in vivo* a regenerative potential similar to that of s-aNSC and s-TAP. Importantly, this may also include the capacity to dedifferentiate *in vivo* and generate NSC as suggested by the detection of GFAP-positive engrafted cells persisting in the SVZ.

333 Interestingly, s-iNB corresponded to several scRNAseq clusters, which were not clearly
334 characterized in the literature. Our model of SVZ regeneration established the hierarchical
335 ordering of these clusters, in which we showed progressive molecular changes leading to the
336 differentiation into migrating neuroblasts. This includes the increase in *Dcx* expression. *Dcx*^{high}
337 cells in these clusters have transcriptional features confirming they are more engaged in the
338 differentiation than their *Dcx*^{low} counterparts. Importantly, we showed that *Dcx*^{high} and *Dcx*^{low}
339 cells in these clusters remained molecularly distinct from both TAP and mNB. We thus propose
340 a revision of the current model of SVZ neurogenesis by introducing iNB or D cells, as highly
341 cycling progenitors that undergo progressive neuronal differentiation but kept multipotency
342 (Fig. 7). D cells could be further divided in two subclasses of cells depending on their
343 progression into the neuronal differentiation process: D1 cells, corresponding to *Dcx*^{low} iNB,
344 immediately succeeding the stage C (TAP) and D2 cells, corresponding to *Dcx*^{high} iNB and
345 preceding the stage A (mNB). Importantly, we showed that both D1 and D2 cells kept
346 transcriptomic features that differentiated them from both TAP and mNB, whereas D1 cells
347 kept a regenerative potential both *in vitro* and *in vivo*.

348 One of the most important findings of this study is the first characterization of AS events
349 and RSR expression variations occurring along with neurogenesis. AS events are known to play
350 important roles in neural cell differentiation, neuronal migration, synapse formation, and brain
351 development[27,30,47–49]. Neurons are characterized by a pervasive use of alternative exons[50,51].
352 Consistently, we showed a dramatic increase in DSG at the transition between s-iNB and s-
353 mNB. These alternative splicing events are clearly related to major changes in RNA splicing
354 regulators (RSR) expressions occurring in s-iNB. Indeed, we identified RSR genes whose
355 expression increased with neurogenesis and others whose expression decreased with
356 neurogenesis. Consistently, some genes upregulated in s-aNSC, s-TAP, such as *Msi1* and *Msi2,*
357 have a known role in maintenance and renewal of stem cells[52–54], while some others upregulated
358 in s-mNB, such as *Elavl3 and 4,* have known roles in many neuronal processes[54,55].
359 Interestingly, genes of both groups of genes were overexpressed in scRNAseq clusters related
360 to s-iNBs, which thus undoubtedly distinguished these cells from both s-TAP and s-mNB and
361 confirmed that important molecular switches for neuronal differentiation occurred in D cells.

362 Further analysis of scRNAseq clusters showed that these transcriptomic switches
363 occurred sequentially from D1 cells to D2 cells. Indeed, the expression of *Elavl4* progressively
364 increased from the *Dcx*^{low} iNB/D1 cells to the *Dcx*^{high} iNB/D2 cells fractions, confirming their
365 progressive commitment into neuroblasts, and overall, the interest of discriminating these two
366 cell types in the model of SVZ neurogenesis we propose (Fig.7). Strikingly, the splicing

repressor, *Ptbp1*, was expressed in D1 cells at levels similar to those in s-aNSC and s-TAP, but decreased in D2 cells, and was undetectable in migrating neuroblasts. This suggests that expression of *Ptbp1* may play an important role in maintaining the regenerative properties in D1 cells, which should be further investigated.

Our study has been performed on cells collected from the SVZ the lateral walls of the ventricles of young adult mice. The transcriptional signatures we provide here may thus be useful for further characterization of neurogenic cells present in the septal wall of the SVZ[18].

To conclude, we have characterized the molecular features of iNB/D cells, a relatively abundant population in the adult SVZ that kept unexpected plasticity and multipotency that persist in the aged brain[21]. They may thus represent a new target in regenerative medicine. We have shown that these cells undergo major transcriptomic changes involving AS and associated to switches in RSR genes expression. Dysregulations of many of these RSR genes have been involved in cancer and neurodegenerative diseases[56]. Further characterization of iNB/D cells using the tools we developed may be useful to determine the potential therapeutic targeting of these cells in various brain pathologies.

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Author contributions

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Mathieu Daynac: Collection and/or assembly of data.

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Véronique Ménard: Irradiation of animals.

Léa Bellenger: Analysis of scRNAseq datasets.

Christophe Antoniewski: Analysis of scRNAseq datasets.

Alexandra Chicheportiche: Conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing.

François D. Boussin: Supervision of the study, conception and design, financial support, data analysis and interpretation, manuscript writing.

Figure legends

Figure 1: Plasticity of iNB *in vitro* and *in vivo*

s-aNSC, s-TAP, s-iNB, and s-mNB were sorted from the SVZ of 2-month old C57Bl/6 mice. **(A)** Population doublings (PD). Data were obtained from 3 independent experiments (n=8). **(B)** Clonogenicity assay. Results were obtained from 2 independent experiments. The number inside the bars indicated the number of microplate wells analysed (mean ± SEM). **(C)** Differentiation assay. Representative images of immunofluorescence of freshly sorted iNB cells cultured in oligodendrocyte, astrocyte, or neuronal differentiation medium, and stained for NG2 and CNPase, GFAP and CD133 or βIII-Tubulin and doublecortin (DCX) expressions, respectively. *$p<0.05$, **$p<0.01$, ***$p<0.001$. Scale bar: 20µm.

(D) EGFP-positive s-iNB were isolated from β–actin:eGFP mice and transplanted unilaterally at 3 injection points at proximity of the dSVZ/RMS of recipient C57Bl/6J mice. Transplanted brains were analysed five weeks later by immunostaining. **(a)** Detection of eGFP+ cells in the granule cell layer and the external cell layer of the olfactory bulb of a mouse transplanted. **(a1-a2)** a2 high magnification of the inset (dotted line in a) showing eGFP+ NeuN+ cells (white arrows). **(b-c)** Detection (white arrows) of eGFP+ GFAP+ cells in the dorsal (b2) and lateral (c2) SVZ. GCL:granule cell layer, EPL : external plexiform layer, GL: glomerular layer, lSVZ: lateral SVZ, dSVZ: dorsal SVZ, Scale bars= 40µm or 100µm.

Figure 2: Transcriptomic analysis of SVZ neural progenitor cells

433 **A)** Schematic illustration of our strategy of flow cytometry cell sorting of the five major
434 neurogenic populations (s-qNSC, s-aNSC, s-TAP, s-iNB, s-mNB) of SVZ microdissected from
435 adult mouse brains. **(B)** Pseudotime analysis showing the lineage progression of sorted cell
436 populations based on the highly dispersed genes between s-qNSC, s-aNSC, s-TAP, s-iNB and
437 s-mNB. **(C)** Pseudotime analysis after exclusion of s-qNSC based to further discriminate s-
438 aNSC and s-TAP. **(E)** Heatmap representation of RSR genes differentially expressed among s-
439 aNSC, s-TAP, s-iNB, s-mNB (FDR p-value<0.05).
440
441 **Figure 3: scRNA-seq analysis of the lateral SVZ**
442 **(A)** Experimental design. **(B)** Uniform Manifold Approximation and Projection (UMAP) of the
443 17343 high-quality sequenced cells annotated with corresponding cell type, combining scRNA-
444 seq datasets of non-irradiated mice and 4Gy irradiated mice. Clustering analysis at resolution
445 1.2 segregated cells into 33 clusters that were matched with corresponding cell types determined
446 with marker expression in (**C**). Among them, 14 clusters (dotted lines rectangle) corresponded
447 to the clusters of astrocytes and neurogenic cells (ANC). **(C)** Violin diagrams illustrating the
448 expression level of known cell markers in the 33 clusters according to the literature. **(D)** Violin
449 diagrams representing the expression level of selected markers specific to neurogenic cells and
450 astrocyte from ANC clusters at the resolution 1.2. **(E)** UMAP focusing on the ANC subset. **(F)**
451 UMAP of the top UCell score for the top 100 highly expressed genes of each neurogenic cell
452 sorted population in the ANC clusters (Supp_data_1). **(G)** Chord diagram illustrating the
453 correspondence of cell clusters with cell top score. Cluster 10 matched with both s-TAP and s-
454 iNB and clusters 8 and 19 both to s-iNB and to s-mNB. **(H)** Feature plot of cell cycle scoring
455 **(I, J)** UMAP representation of ANC clusters in the unirradiated (I) and irradiated (J) samples.
456 **(K)** Barplot showing the recovery ratios of each ANC cluster in irradiated brains calculated as
457 the number of cells per clusters from irradiated brains /control brains normalized to the
458 respective numbers of s-qNSC (cluster 4). Taking into account UCell score results, s-iNB
459 clusters overlapped the cluster 10 and 8 containing s-TAP and s-mNB, respectively.
460
461 **Figure 4: Expression of RSR genes in scRNA-seq neurogenic clusters**
462 Violin diagrams illustrating the expression of the RSR genes increasing **(A)** or decreasing **(B)**
463 with neurogenesis.
464
465 **Figure 5: *Dcx* expression in the neurogenic cell clusters**

(A) Feature plot. **(B)** Barplot representations of the recovery ratios of *Dcx*^{low} (left) and *Dcx*^{High} (right) in the iNB clusters of the irradiated brains, calculated as in Figure 3K.
Violin diagrams illustrating the expression level of RSR genes decreasing **(C)** and increasing **(D)** with neurogenesis in *Dcx*^{low} and *Dcx*^{High} in neurogenic cell clusters.

Figure 6: Plasticity *of Dcx*^{High} iNB *in vitro*

(A) Clonogenic potential of FACS-isolated eGFP^{+}iNB and eGFP^{-}iNB, and recombined eGFP^{+}mNB isolated from DCX-CreERT2::CAG-floxed-eGFP mice, 7 days after the first injection of tamoxifen. **(B)** Population doublings (PD) of FACS-isolated eGFP^{+}iNB and eGFP^{-}iNB, eGFP^{+}mNB cells and total iNB cells (regardless of eGFP status). No statistical difference was found in the growth rate of the different isolated populations. (n = 8) **(C)** Representative images of immunofluorescence of freshly sorted eGFP^{+}iNB cells cultured in oligodendrocyte,astrocyte, or neuronal differentiation medium, and stained for NG2 and CNPase, GFAP and CD133, βIII-Tubulin and DCX, respectively. Scale: 10µm.

Figure 7: Proposed model of adult mouse SVZ neurogenesis involving major changes in RSR genes expression

Table 1: Transplantations of eGFP^{+}s-iNB, eGFP^{-}s-iNB and eGFP^{+}s-mNB freshly isolated from β–actin:eGFP mice model

Table recapitulating the immunohistological analyses of the numbers of eGFP^{+} cells in different regions in recipient C57Bl6/J mice brains, 5 weeks after transplantation.

METHOD

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice

All experimental procedures complied with European Directive 2010/63/EU and the French regulations. The protocols were approved by our institutional ethical committee (CEtEA 44) and authorized by the « Research, Innovation and Education Ministry » under registration number APAFIS#25978-2020061110358856 v1. Adult male C57BL/6J were obtained from Janvier Laboratories. Adult male β–actin:eGFP (Okabe et al., 1997) and DCX-

501 CreERT2::CAG-CAT-eGFP mice kindly given by Pr Couillard-Despres Sebastien [57].
502 Experiments were performed with male animals aged 8 and 10 weeks. Tamoxifen (TAM, T-
503 5648, Sigma-Aldrich) was dissolved in corn oil (C-8267, Sigma-Aldrich) at 40 mg/ml. To
504 analyse the expression of the *Dcx* reporter gene, 20 mg TAM/g body weight was administered
505 twice daily by gavage over a period of five consecutive days.
506
507 **Cell sorting**
508 Lateral ventricle walls of the SVZ were microdissected and dissociated as previously described
509 [19–21] with the modification that cells were centrifuged at 250g for 20 min at 4ºC without brake
510 on a 22% Percoll gradient (GE Healthcare) to remove myelin prior to single cell suspension
511 labelling. The antibodies to distinct cell populations were anti-CD24 phycoerythrin [PE]-
512 conjugated (Rat IGg2b; BD Biosciences, 1:50, RRID:AB_2034001), CD15/LeX flurorescein
513 isothiocyanate-[FITC] conjugated (clone MMA, mouse IgM; BD Biosciences, 1:50,
514 RRID:AB_400103), CD15/LeX- Brilliant violet-421-[BV421] (Mouse IgG1, κ ; BioLegend,
515 1:50, RRID:AB_2566519) and Alexa647-conjugated epidermal growth factor (EGF) ligand
516 (Clone 30H45L48, Thermo Fisher Scientific, 1:200, RRID:AB_2662334). Cells were gated
517 following the fluorescence minus one (FMO) control. Immediately prior to FACs sorting,
518 propidium iodide (PI) or Hoechst 33258 was added to a final concentration of 1µg/ml to label
519 the dead cells. Cells were sorted on an INFLUX cell sorter equipped with an 86 µm nozzle at
520 40 psi (BD Biosciences). All the data were analysed using FlowJo software (Tree Star, Ashland,
521 OR).
522
523 **Cell culture expansion and differentiation**
524 Clonogenicity assay and population doublings: FACs-purified populations were collected in
525 NeuroCult medium complemented with the proliferation supplement (STEMCELL
526 Technologies), 2 µg/mL heparin, 20 ng/mL EGF and 10 ng/mL FGF-2, at a density of
527 $1x10^3$ cells/well in 96-well tissue culture plates coated with poly-D-lysine (Merck Millipore).
528 Five days after plating, the neurospheres were counted to determine the clonogenicity of each
529 cell population.
530 Neurospheres were then mechanically dissociated and sub-cultured in 24-well plates over 3
531 weeks to measure the population doublings at each passage (1 passage/week)..
532 Differentiation assays: just after sorting, cells were placed on Poly-L-ornithine (P4957, Merck
533 Millipore) coated 24-well culture plates at 40,000 cells/ml in DMEM:F12 with several
534 supplementation according to the lineage: astroglial : 2% B27 MAO (minus antioxidant,

535 Thermo Fisher Scientific) and 2% fetal bovine serum (10082139, Thermo Fisher Scientific),
536 oligodendroglial : 10 ng/mL FGF-2, a defined hormone mix including Glucose (G-7021,
537 Sigma-Aldrich 50gm/l) /$NaHCO_3$ (S-5761, Sigma-Aldrich, 10mg/l) / HEPES (H-3784, Sigma-
538 Aldrich, 1.3mM), insulin (I-1882, Sigma-Aldrich, 22.5mg/l), Apo transferrin (T-2252, Sigma-
539 Aldrich, 0.09g/l), progesterone (P-6149, Sigma-Aldrich, 18µM), putrescine (P-7505, Sigma-
540 Aldrich, 8.7mg/l), and sodium selenite (S-9133, Sigma-Aldrich, 4.7µM), and neuronal : 2%
541 B27 MAO. Cultures in hypoxic conditions (4%O2) at 37°C for 5 (oligodendroglial or astrocytic
542 differentiations) or 7 days (neuronal differentiation)
543
544 **Transplants**
545 Three injections of 1µl of freshly $eGFP^+$ cells from β–actin:eGFP or DCX-CreERT2:CAG-
546 CAT-eGFP mice were performed in the striatum at proximity of SVZ of wild-type C57Bl6/J
547 recipient mice using the following coordinates: (1) AP, 0.0; L, 1.4; V, -2.1; (2) AP, 0.5; L,1.1;
548 V, -2.2; (3) AP, 1.0; L, 1.0; V, -2.5mm relative to bregma, as described before by Codega et al.
549 2014[2]. For CD1 mouse transplants with freshly $eGFP^+$ cells from DCX-CreERT2:CAG-CAT-
550 eGFP mice the injection coordinates were redefined from the ratio of the distance bregma to
551 lambda for the C57Bl6/J model over the distance bregma to lambda for the CD1 model.
552 The transplantations were performed using a small animal stereotaxic apparatus (Kopf model
553 900) with a 2.5µl Hamilton syringe (Hamilton, Bonaduz, Switzerland). Recipient mice were
554 sacrificed 5 weeks after transplantation.
555
556 **Tissue processing, immunolabeling and microscopy**
557 Deeply anaesthetized animals received a transcardial perfusion of 4% paraformaldehyde.
558 Brains were post-fixed overnight in 4% PFA and cryoprotected in 30% sucrose/PBS. Serial
559 coronal cryostat sections were made at 14µm of thickness (Leica CM3050S). For each brain,
560 all the sections from the hippocampus to olfactive bulb were deposited on a set of 14 slides with
561 a 150µm step. Sections underwent permeabilisation in phosphate buffered saline (PBS)
562 containing 0.3% Triton X100 and 1% of Bovine Serum Albumin 1h at room temperature.
563 Sections were incubated with primary antibodies PBS containing 0.1% Triton X100 overnight
564 at 4°C. Secondary antibodies used were Alexa 594, 488 and 647 and were applied at 1:400
565 (Thermo Fisher Scientific) for 2h at room temperature. Each staining was replicated in at least
566 3 slides from different mice.

567 Cells were fixed with 4% PFA for 15 minutes at room temperature and rinsed three times with
568 PBS. Staining was performed as above except that antibodies were applied for 2h at room
569 temperature.
570 The following antibodies used were: rabbit anti-eGFP (Abcam, ab290, 1:300), goat anti-GFP
571 (Abcam, ab6673, 1:300), mouse anti-O4 (Millipore, MAB345, 1:100), rabbit anti-NG2
572 (Millipore, AB5320, 1:100), goat anti-Olig2 (RαD, AF2418, 1:200), mouse anti-NeuN
573 (Millipore, MAB377, 1:100), rabbit anti-Dcx (Cell Signaling, 4604, 1:400), goat anti-Dcx
574 (Santa Cruz, sc-8066, 1:100), mouse anti-GFAP (Millipore, MAB3402, 1:400), rabbit anti-
575 Gfap (Sigma-Aldrich, G9269, 1:400), mouse anti-CNPase (Millipore, MAB326, 1:100), rabbit
576 anti-β-III tubulin (Covance, PRB-435P, 1:200), rat anti-CD133 (Millipore, MAB4310, 1:100).
577 Brightfield and fluorescent images were captured through Plan Apo X20/ Numerical Aperture
578 (NA):1.3 oil objective and Plan Apo X40/ NA:0.75 dry objective using hybrid detection
579 technology on a laser scanning confocal (Leica Microsystems SP8), Nikon A1R confocal laser
580 scanning microscope system attached to an inverted ECLIPSE Ti (Nikon Corp., Tokyo, Japan)
581

582 **SVZ irradiation**

583 Animals were anesthetized with isoflurane (3% induction for 5 minutes) and 1.5-2% when the
584 mice were placed in Small Animal Radiation Research Platform (SARRP, XSTRAHL, LTD
585 Company). First, a Cone Beam Computer Scanning (CBCT) was performed to target the SVZ
586 and to set up the treatment planning system. Mice were placed on their stomach and two fields
587 (-45° and + 45°) were used to be homogeneous on the irradiation target. The size of the
588 irradiation field was adapted with a multivariable collimator 10*6 mm. The SVZ received a
589 4Gy dose distributed 50% by each beam and the dose output was around 3.64 Gy/min
590 depending of the size of the field. The X-ray configuration was 220 kV, 13 mA and 0.15 mm
591 of Copper in these conditions.

592 **Single cell library preparation for single cell RNA sequencing**

593 SVZ lateral ventricle walls were dissected from two non-irradiated mice brains (2 month old)
594 and two 5 days post-irradiation 4Gy-irradiated mice brains (2-month old).
595 Protocol of SVZ digestion with Papain (Worthington, LK003150) to obtain single cell
596 suspensions were strictly identical to those performed in the whole genome microarray
597 experiment described above, including the Percoll gradient step to remove myelin. Cells were
598 resuspended in 0.04% BSA in PBS passed through a 70µm cell strainer (ThermoFisher
599 Scientific).

Blood cells were lysed using the Red blood Cell lysis solution (Milteny Biotec, 130-094-183) and dead cell Removal using the Dead Cell Removal Kit (Miteny Biotec, 130-090-101) were performed following the manufacter's instructions.

Single cell suspensions of two replicates of each sample (unirradiated, 4Gy) were adjusted to 2000 cells/µl and 10,000 non-irradiated cells and 6,000 irradiated cells were loaded per channel onto Chromium Next GEM Chip Single Cell kit (ref: 1000127). Library preparation was performed using the Dual Index Kit TT (ref: 1000215) according to manufacturer's recommendations (10x Genomics, Pleasanton, CA). Quality and quantification of libraries were done using the High Sensitivity DNA LabChip kit (ref: 5067-4626). Sequencing NGS Standard Illumina was carried out with the NovaSeq6000-S1 flow cell (ICM platform, Paris).

METHOD DETAILS

Whole-genome Microarrays

mRNA were isolated with the RNeasy Micro Kit with DNase treatment (Qiagen) and sample quality was controlled using the Agilent Bioanalyser. MTA-1.0 arrays (Clariom D Affymetrix technology) were performed according to the manufacturer's protocol (Thermoisher). PCA and pseudotime ordering was performed using TSCAN online user interface (Ji and Ji, 2016).The data were analysed with the freeware software Transcriptome Analysis Console (TAC) applying the RMA algorithm normalization. Functional profiling were generated using REACTOME and KEGG datasets obtained from the g:Profiler interface (https://biit.cs.ut.ee/gprofiler/gost).

Signatures of cell sorted populations were delineated using Venn diagrams comparing the five populations of neural progenitors (s-qNSC, s-aNSC, s-TAP, s-iNB and s-mNB) (https://bioinformatics.psb.ugent.be/cgi-bin/liste/Venn/calculate_venn.htpl).

sc-RNAseq and microarray dataset comparisons:

Published genelists were matched with microarray data raw data using aggregated Z-scores. For each gene in a genelist, gene expression amongst all samples was normalized between 0 and 1, then mean expression among samples was calculated for each population. Plots represent the distribution of mean normalized gene expression for all genes in the genelist.

Single-cell RNA control quality and analysis

ScRNA-seq data were aligned to the GRCm38 - mm10 reference genome (cellranger-6.1.2). Data were analysed in R (version 4.1.0) using Seurat (v4.1.1). Quality control removed outlier

cells with fewer than 1200 to 1500 genes per cell (nFeatures_RNA), 2350 to 2700 UMI (nCount_RNA) and cells less than 10% mitochondrial content. Gene expression was normalized using the regularized negative binomial regression to normalize UMI count data (SCTransform) using 4000 variable features and the different samples (controls and irradiated). The features to use in the downstream integration procedure were determined using SelectIntegrationFeatures. After a pre-computed AnchorSet (FindIntergrationAnchors), the Seurat object were integrated using IntegrateData with the normalization method SCT. CellCycleScoring was applied to calculate the S.Score, G2M.Score and Phase. RunUMAP runs the Uniform Manifold Approximation and Projection (UMAP) dimensional reduction technique using 50 PCs. FindNeighbors computes the k.param nearest neighbors using dims = 1:50. Clusters of cells were identified by a shared nearest neighbor (SNN) modularity optimization bases clustering algorithm using FindClusters with resolutions from 0.2 to 2. The resolution 1.2 that segregates cells into 33 clusters was chosen based on Clustree graph showing the relashionship between clusterings at the different resolutions. UMAP plots were generated with UMAPPlot with default parameters.

The PrepSCTFindMarkers function was used to normalize gene expression for differential gene expression analysis among clusters, which was performed using FindAllMarkers on the SCT assay, with significance determined using a Wilcoxon rank sum test (p_val_adj < 0.05) and log(Fold-Change) threshold of 0.1. A histogram representation of *Dcx* (ENSMUSG00000031285) expression in the clusters corresponding to astrocytes and neural progenitors allowed us to place a threshold expression at 1. Cells with an expression lower than or equal to 1 correspond to Dcx^{Low} cells and cells with an expression strictly higher than 1 to Dcx^{High} cells. Then, we reapplied PrepSCTFindMarkers and FindAllMarkers to find differentially expressed genes between Dcx^{Low} and Dcx^{High} iNB.

Gene expression score:

We used AddModuleScore_Ucell (Package *UCell* version 1.3.1) to score cells based on the expression of predefined specific gene lists of sorted s-qNSC, s-aNSC, s-TAP, s-iNB and s-mNB determined by microarrays using the Mann-Whitney U statistic [40]. Each cell was then assigned an identity corresponding to the highest score amongst the signatures tested. Repartition of classes among clusters was visualized using the Circlize package [58].

For astrocyte and B-cell classification, we used the lists of Astrocyte and B-cell markers provided by Cebrian-Silla et al. (2021) [14] to score cells from the clusters 4 and 13 between 0 (min) and 1 (max). Cells were assigned to the highest score amongst both signatures.

Gene enrichment analysis:

Comparisons of the specific gene lists of *Dcx*^{High} s-iNB and *Dcx*^{Low} s-iNB were performed with Biological pathways using the g:Profiler interface (https://biit.cs.ut.ee/gprofiler/gost).

QUANTIFICATION AND STATISTICAL ANALYSIS

Non-parametric Mann-Whitney test was conducted using Prism 8.1.2 (GraphPad Software Inc., La Jolla, CA, RRID:SCR_002798). The statistical significance was set at $p<0.05$. The data are expressed as the mean ± SEM.

DATA AVAILABILITY

The datasets generated for this article were deposited online on the ANNOTARE database (E-MTAB-12265, E-MTAB-12495). Scripts used for analysis are available on https://github.com/AChicheportiche/Bernou2023.

Supplemental informations

Figure S1: eGFP$^+$ s-iNB cells in the rostral migratory stream

Immunofluorescence of mouse brains transplanted unilaterally in 3 injection points near the dSVZ/RMS with eGFP$^+$iNB cells isolated from β-actin:eGFP mice. Transplanted brains were analysed five weeks later. **(A)** EGFP$^+$iNB cells reached the rostral migratory stream and expressed DCX (insets A2 and A3, white arrow). Insets are high magnification panels. **(B)** eGFP$^+$ cells persisting at the injection site expressing astrocyte or oligodendrocyte markers. High magnifications of the inset1 (dotted line in A) showing eGFP$^+$ GFAP$^+$ cells (white arrow) **(a2)** and eGFP$^+$DCX$^+$ cells (white arrow) **(a3)**. High magnifications of the inset2 (dotted line in B) showing eGFP$^+$ CNPase$^+$ (white arrow) **(b2)**. Scale bars= 100 µm.

Figure S2: Functional enrichment analyses of the individual transcriptome signature of neurogenic populations

Comparisons of individual transcriptome signatures with various Gene Ontology gene sets and Biological pathways using the g:Profiler software highlight significant stage-dependant changes. S-qNSCs expressed genes related to Metabolism of lipids, Transport of small molecules. S-aNSC expressed genes involved in Glyoxylate and decarboxylate metabolism. S-TAP significantly displayed genes involved in Mitotic G1 phase and G1/S transition and S

Phase. S-iNB specifically expressed Cell Cycle and Mitotic genes in contrast to s-mNB which are characterised by the expression of Axon guidance and Nervous system development genes.

Figure S3: Correspondence between the molecular signatures of sorted neural progenitor populations in our study and available sc-RNA-seq datasets.

Violin diagrams representing the expression of selected clusters from published sc-RNAseq analysis in our Clariom microarray[14,17,32]. S-qNSC perfectly matched the expression of Glial markers (Class III)[32], qNSC[17] and Quiescent B cells (clusters 5 and 14)[14]. In contrast, s-aNSC poorly matched with Lipid biosynthesis (Class III) and Cell cycle (Class IV)[32], aNSC[17] and Activated B cells (Cluster 13)[14]. Similarly, s-TAP poorly matched with TAP[17] and C cells (Cluster 12)[14]. S-iNB perfectly fit with Mitosis (Class VI), mitotic TAP (mTAP) (Zywitza et al., 2018) and Mitosis (Cluster 8, 10,16 and 17)[14]. s-mNB overlapped genes related to Neuron differentiation (Class VII), Late NBs [17] and A cells (clusters 0, 1 and 4).

Figure S4: Quality controls of scRNA-seq on whole SVZ tissues

(A) Violin diagrams of the nCount_RNA, the nFeature_RNA and the mitochondrial percentage (Percent.mito) before applying cut-offs adapted to each non-irradiated and irradiated samples. **(B)** Proportion of cells from the 4 samples in the 33 clusters.

Figure S5: Annotation of sc-RNAseq clusters

(A) UMAP at the resolution 1.2 combining scRNA-seq datasets of unirradiated and 4Gy irradiated cells segregated into 33 clusters. **(B)** Violin diagrams illustrating the expression level of known cell markers in the 33 clusters according to the literature. **(C)** UMAP of top normalized UCellScore Astrocyte- and the B-cell specific genes described by Cebrian-Silla et al [14] for cells from clusters 4 and 13.

Figure S6: Expression of *Dcx* in scRNA-seq neurogenic clusters

Violin diagram of the expression of *Dcx* in cells from neurogenic clusters illustrating two populations of iNB: iNB Dc^{Hiigh} and iNB Dcx^{Low} cells.

Figure S7: Expression of RSR genes in scRNA-seq neurogenic clusters in irradiated brains

Violin diagrams illustrating the expression of the RSR genes increasing **(A)** or decreasing **(B)** with neurogenesis.

Figure S8: Transplantations of eGFP^{+}s-iNB, eGFP^{-}s-iNB and eGFP^{+s-}mNB freshly isolated from DCX-CreERT2::CAG-floxed-eGFP mice model

Table recapitulating the immunohistological analyses of the numbers of eGFP^{+} cells in different regions in recipient C57Bl6/J mice brains, 5 weeks after transplantation.

Supp_data_1: Transcriptomic signatures of s-qNSC, s-aNSC, s-TAP, s-iNB and s-mNB (Clariom assay) and corresponding g:Profiler Gene Ontology analyses.

Supp_data_2: Differentially spliced genes (DSG) in each transition along SVZ neurogenesis.

Supp_data_3: Differentially expressed genes (DEG) among RNA splicing regulators (RSR) genes in each transition along SVZ neurogenesis.

Supp_data_4: Identification of astrocytes and qNSC among clusters 4 and 13 from our scRNAseq dataset using the transcriptomic signatures of Astrocytes and B_cells described by Cebrian et al. 2021.

Supp_data_5: Differentially expressed genes of s-INB *Dcx*^{High} and s-iNB *Dcx*^{Low} cells and functional profiling using g:Profiler interface.

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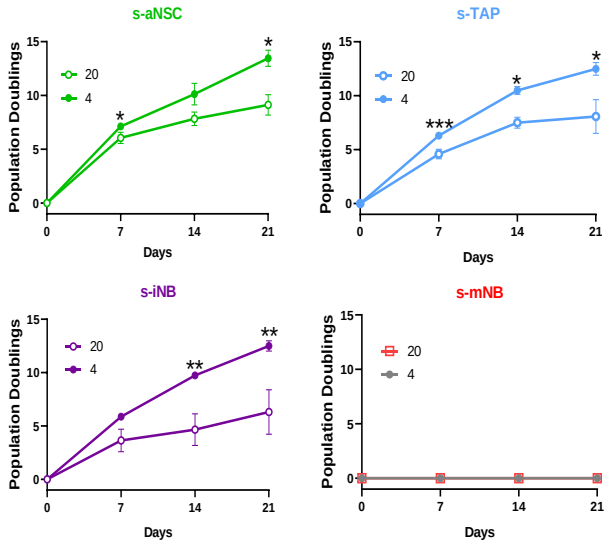
Figure 1: Plasticity of iNB *in vitro* and *in vivo*

A



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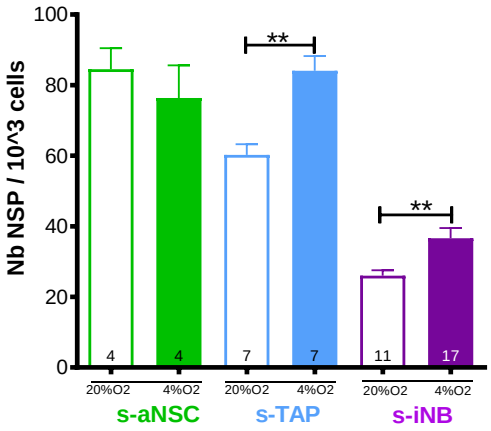

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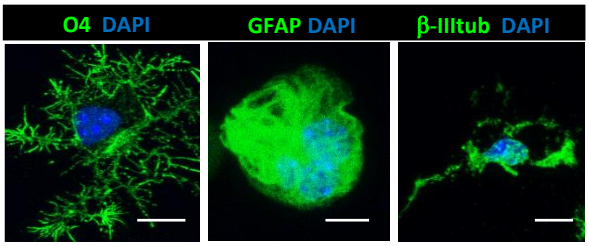

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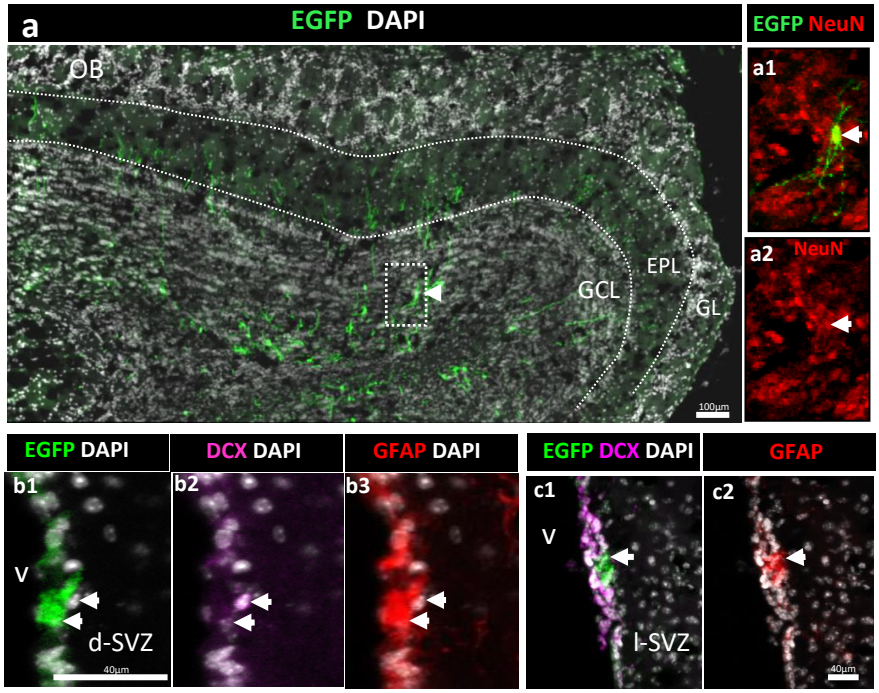
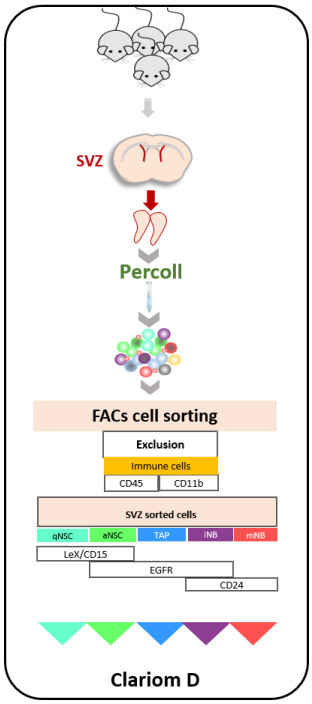
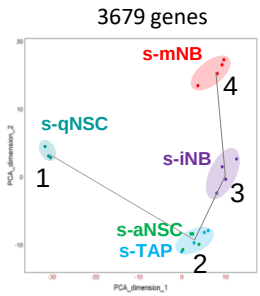
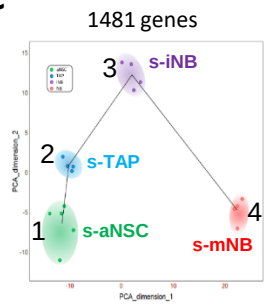


Figure 2: Transcriptomic analysis of SVZ neurogenic cells







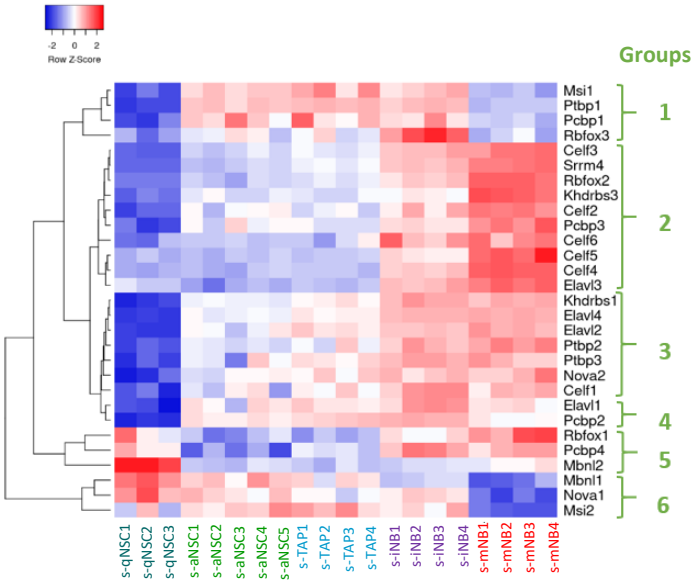


Figure 3: scRNA-seq analysis : whole SVZ

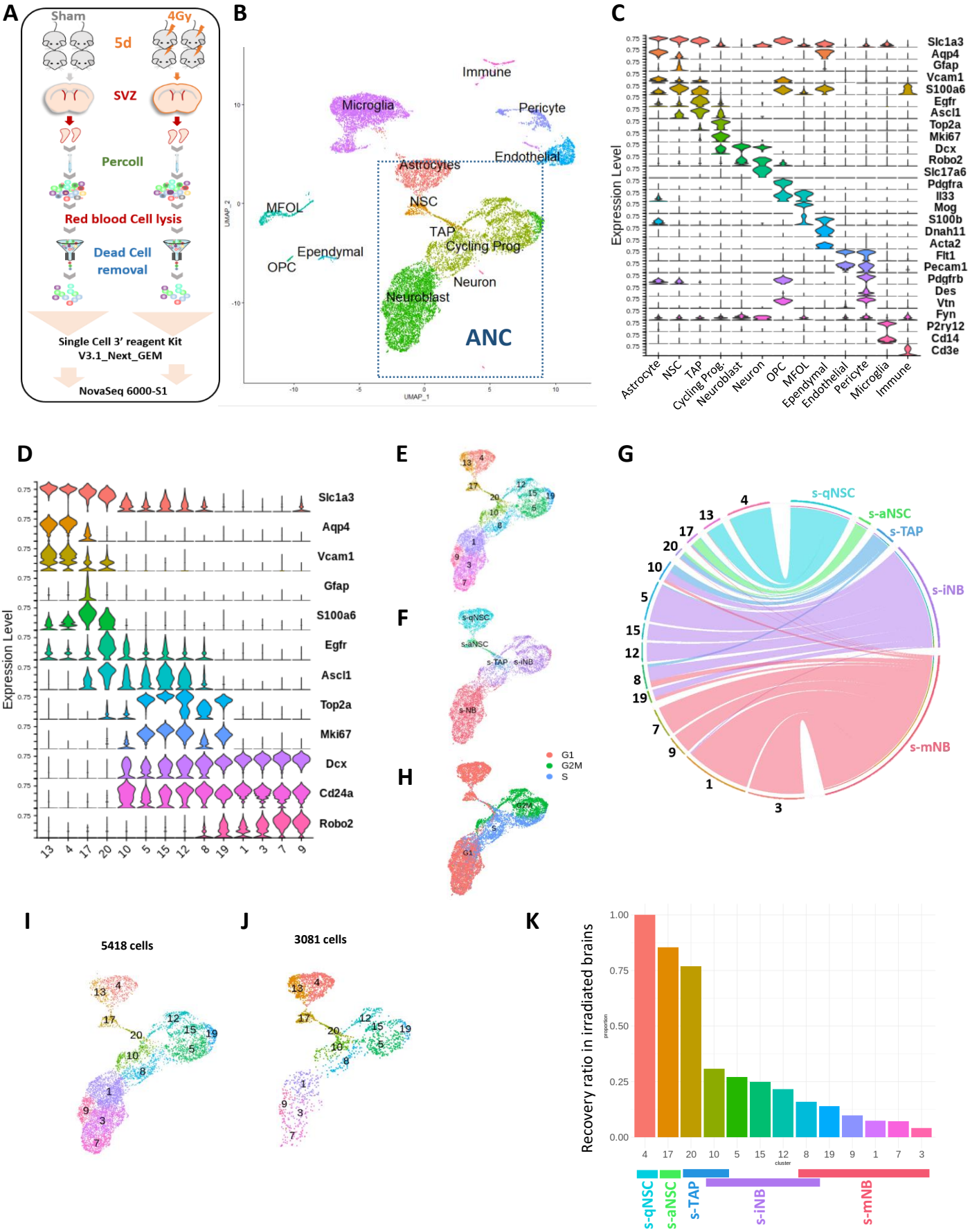
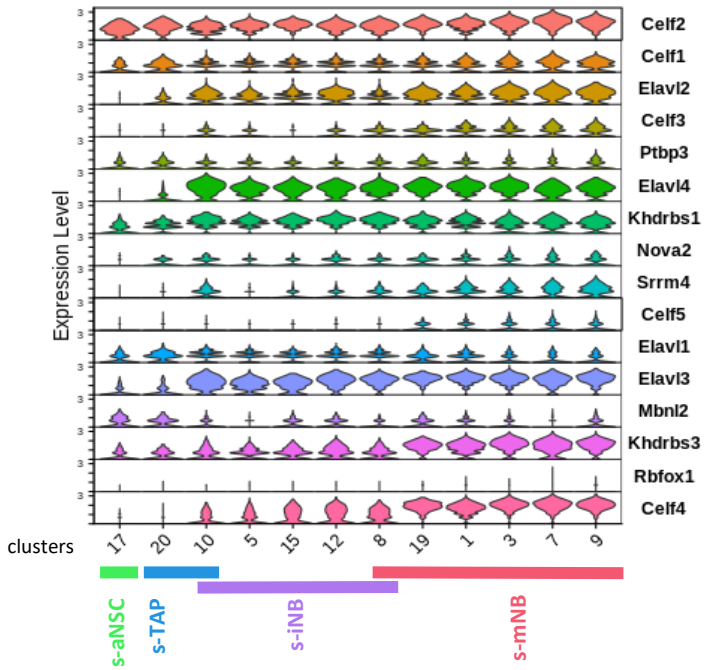


Figure 4: RSR gene expression in scRNA-seq neurogenic clusters

A

RSR genes increasing with neurogenesis



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RSR genes decreasing with neurogenesis

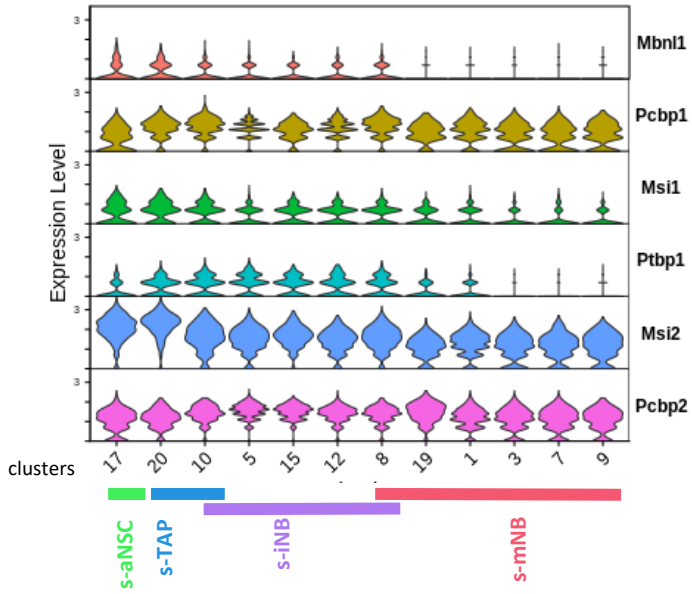
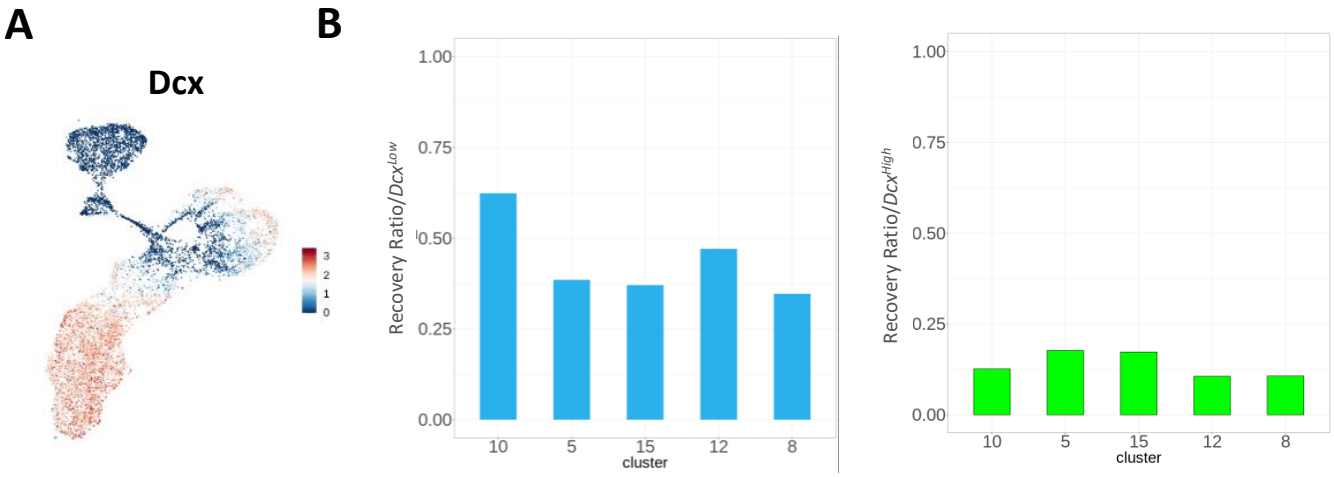
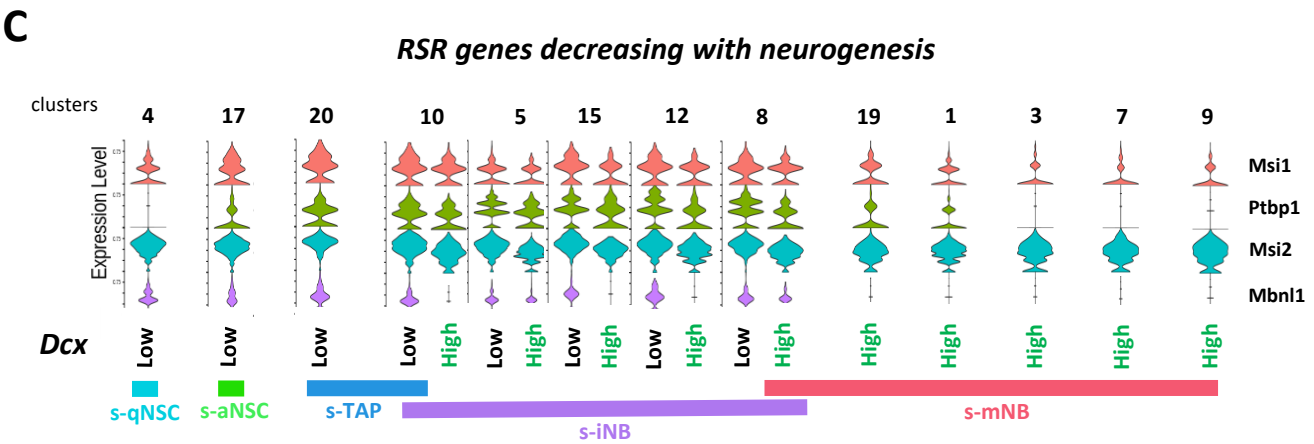


Figure 5: *Dcx* expression in the neurogenic cell clusters





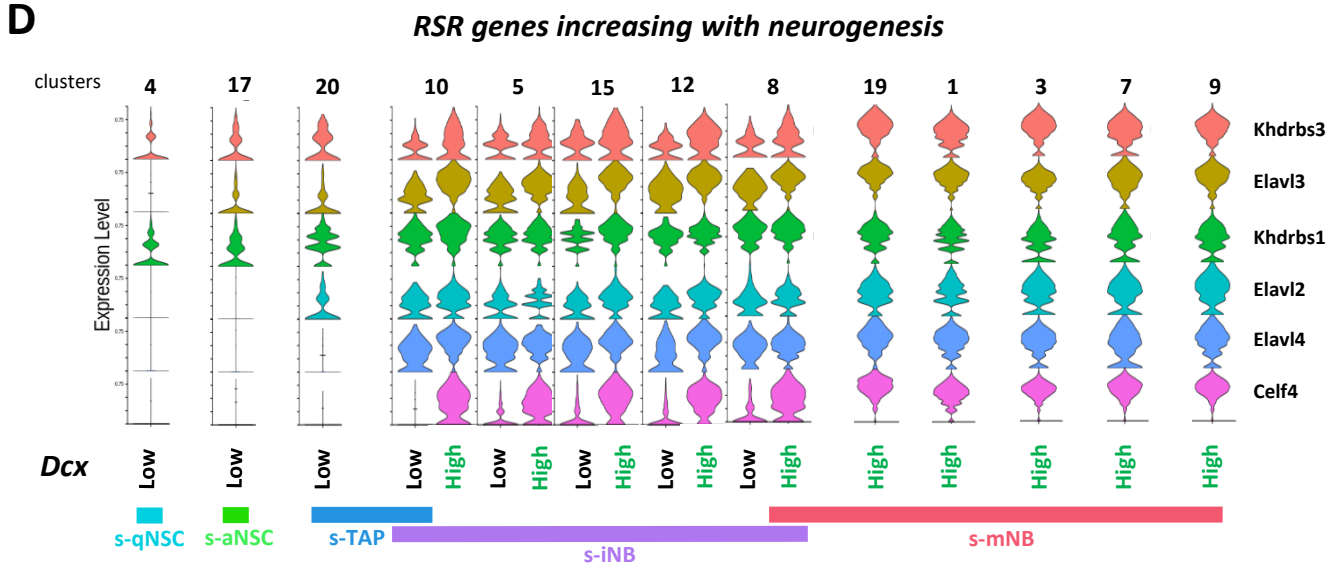
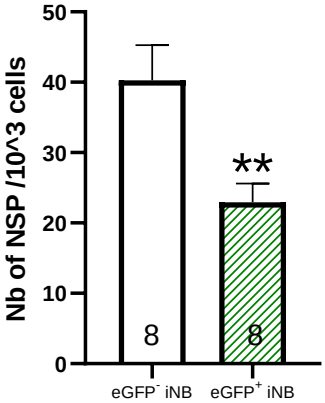


Figure 6: Plasticity of iNB *Dcx+ in vitro*

A



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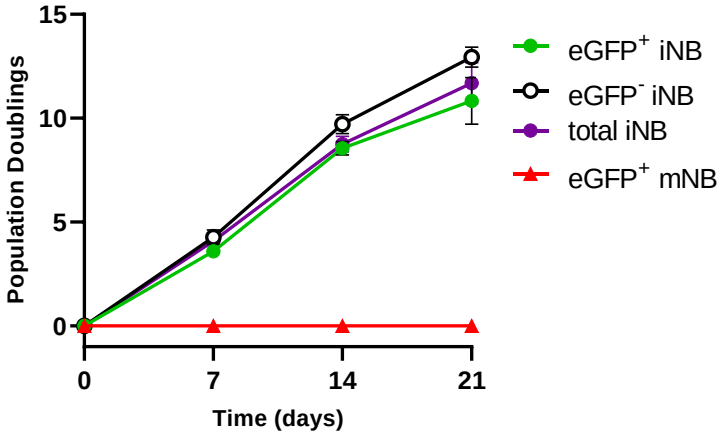

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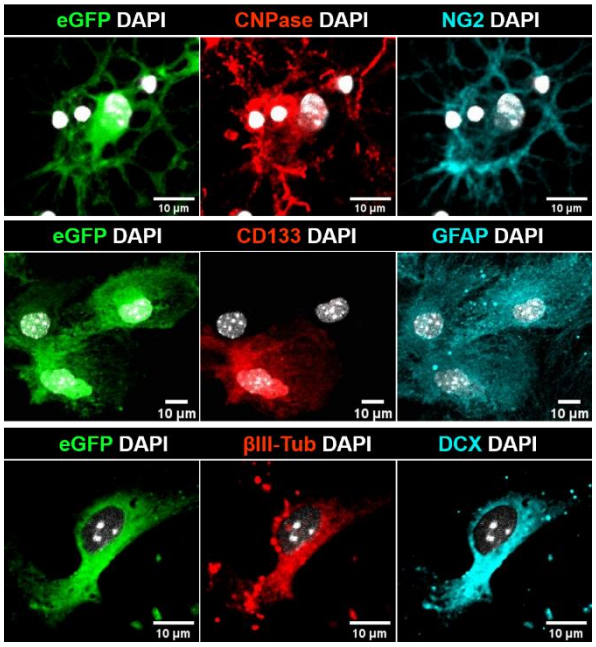


Figure 7: Proposed model of adult mouse SVZ neurogenesis involving major changes in RSR genes expression

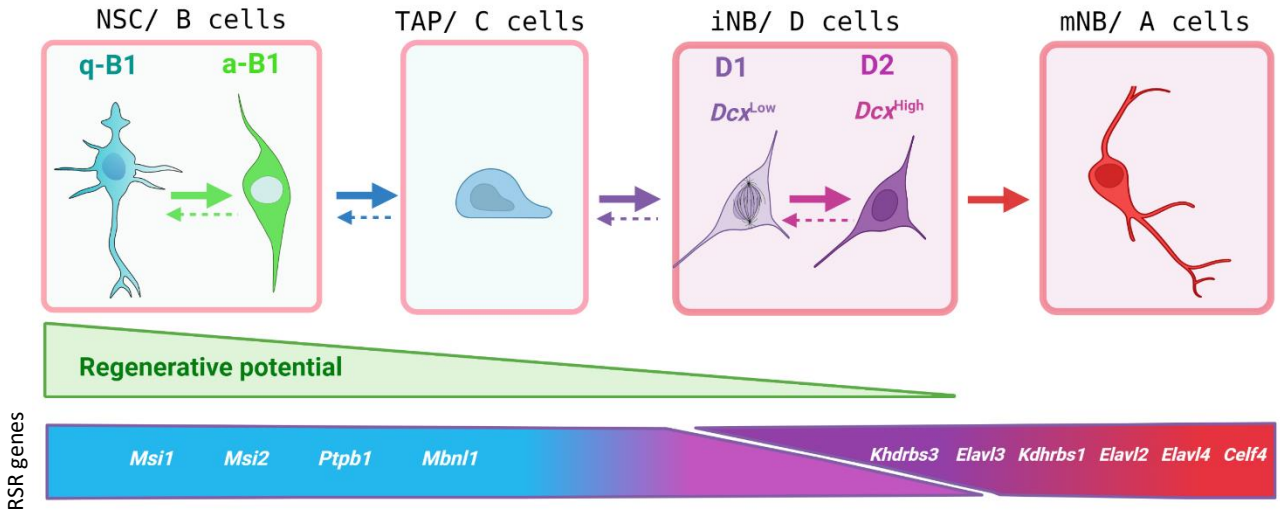
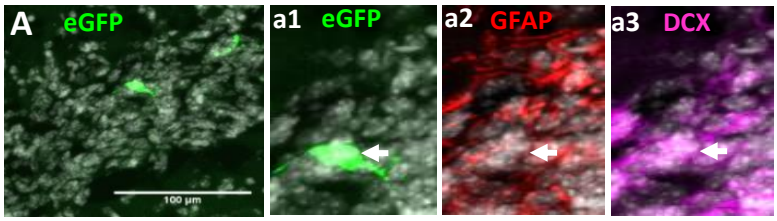


Figure S1:

A EGFP+ s-iNB cells in the rostral migratory stream



B Regenerative potential of eGFP+ s-iNB *in vivo*

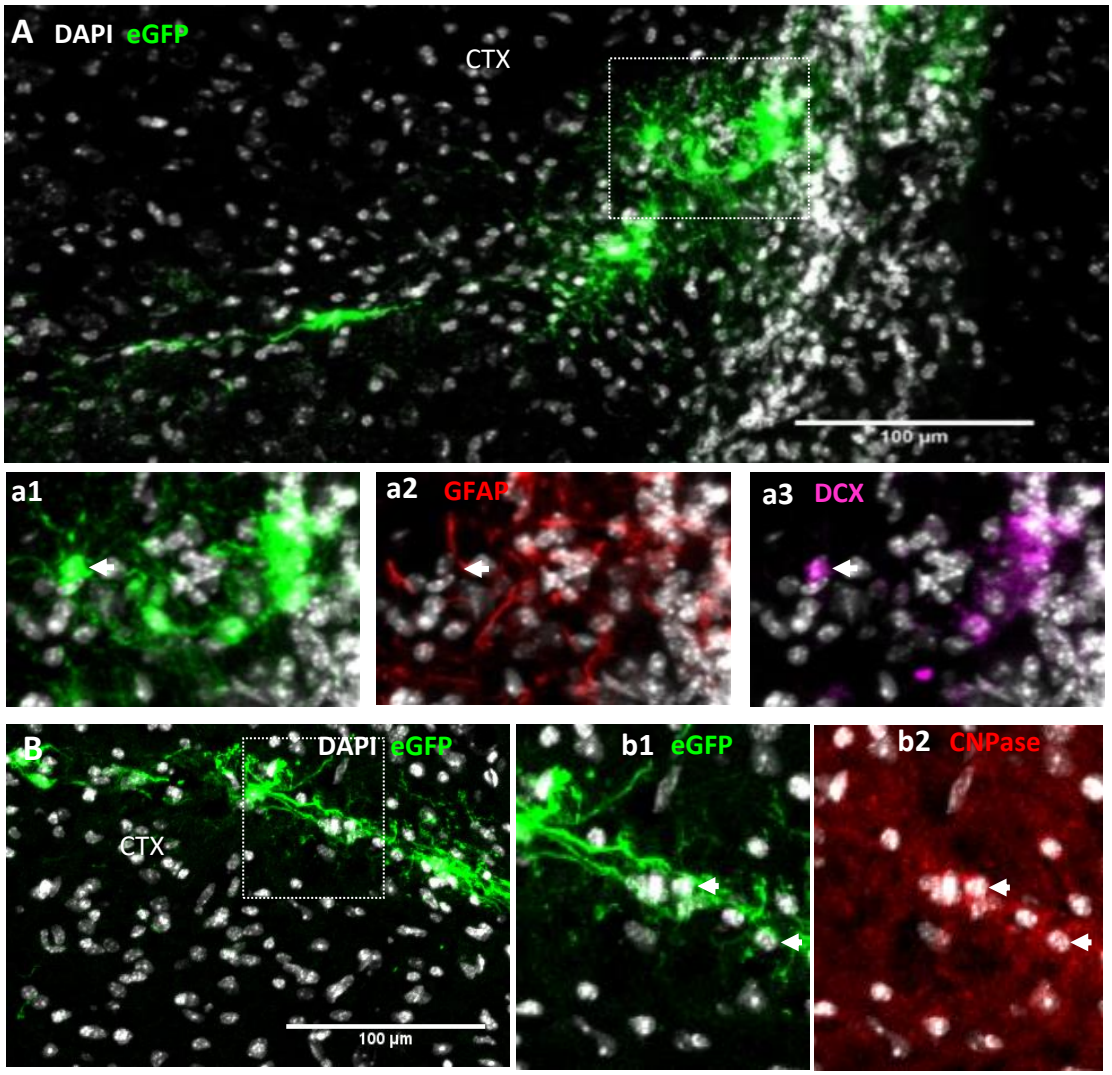


Figure S2: Functional enrichment analyses of individual transcriptome signature of neurogenic populations

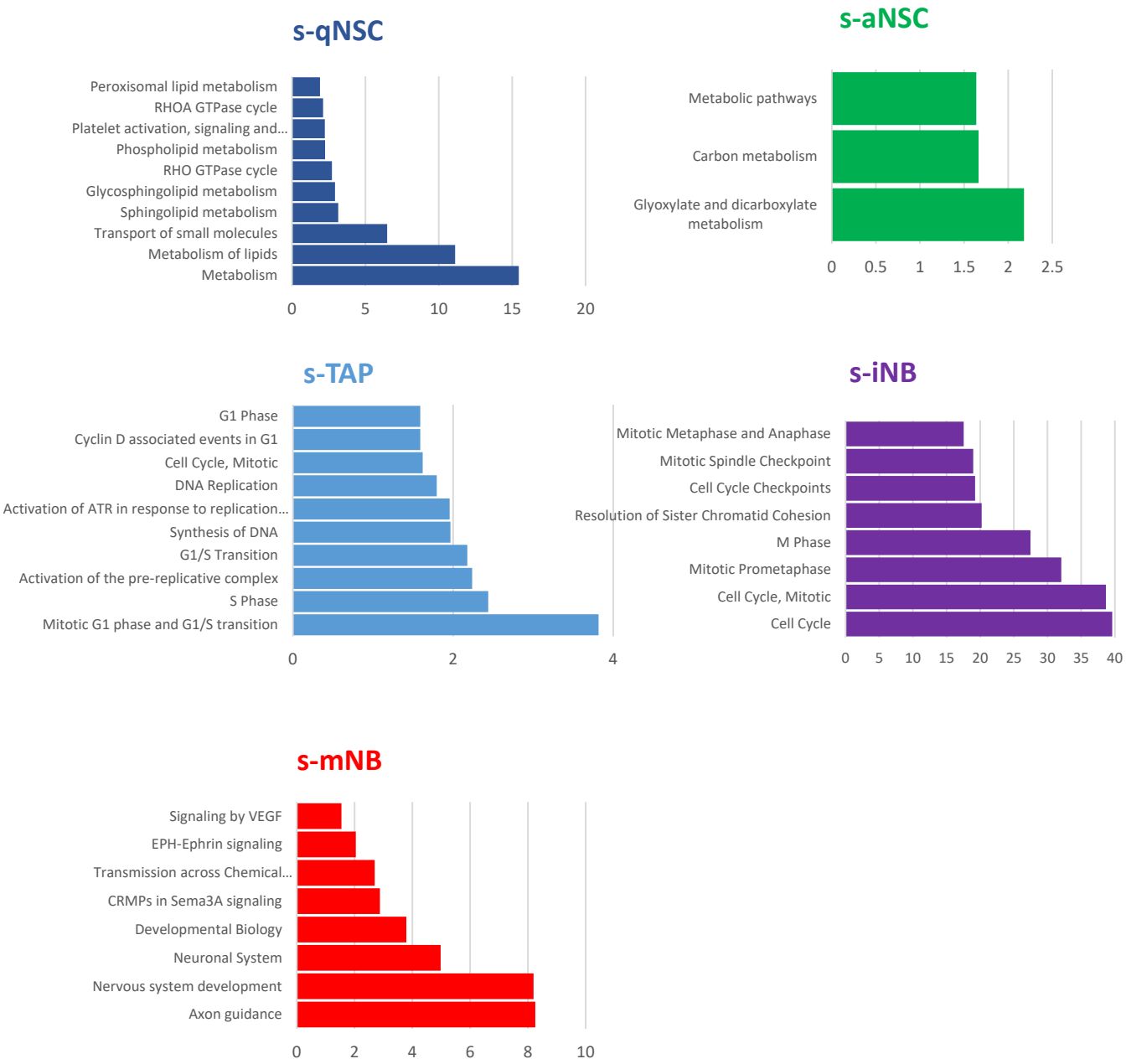
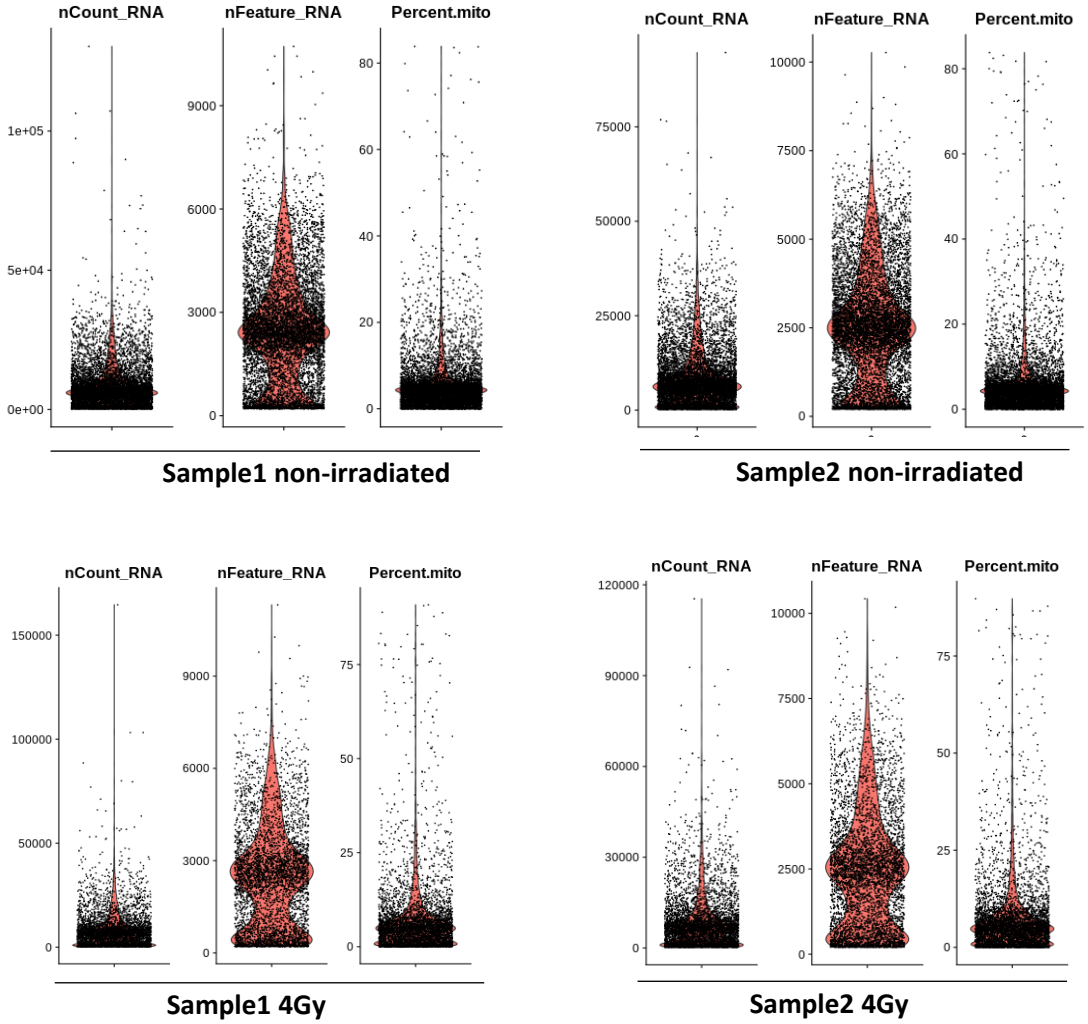


Figure S4: Quality controls of scRNA-seq on whole SVZ tissues

A



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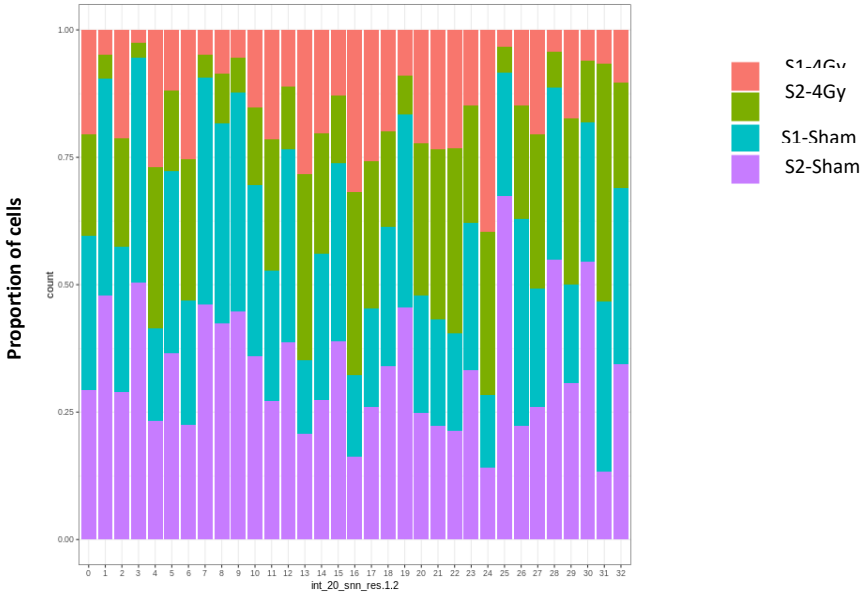
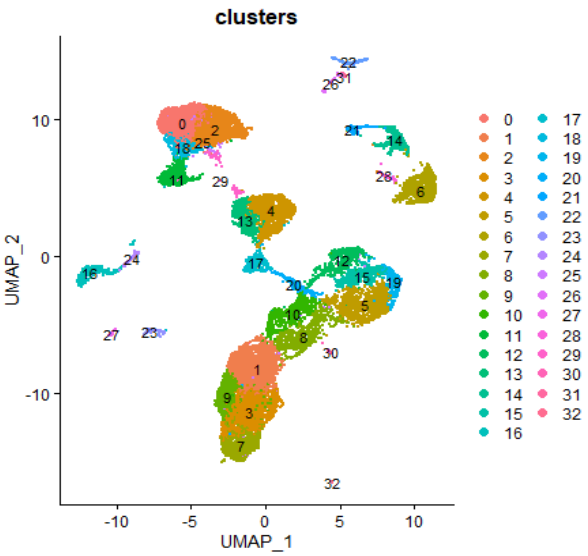


Figure S5: Annotation of sc-RNAseq clusters

A



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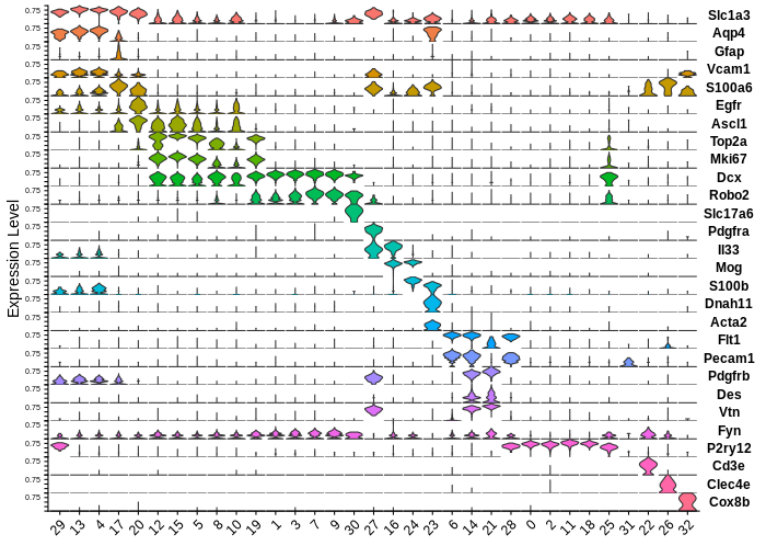

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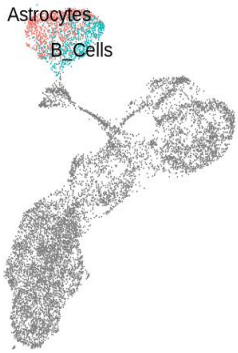


Figure S6: Expression of *Dcx* in scRNA-seq neurogenic clusters

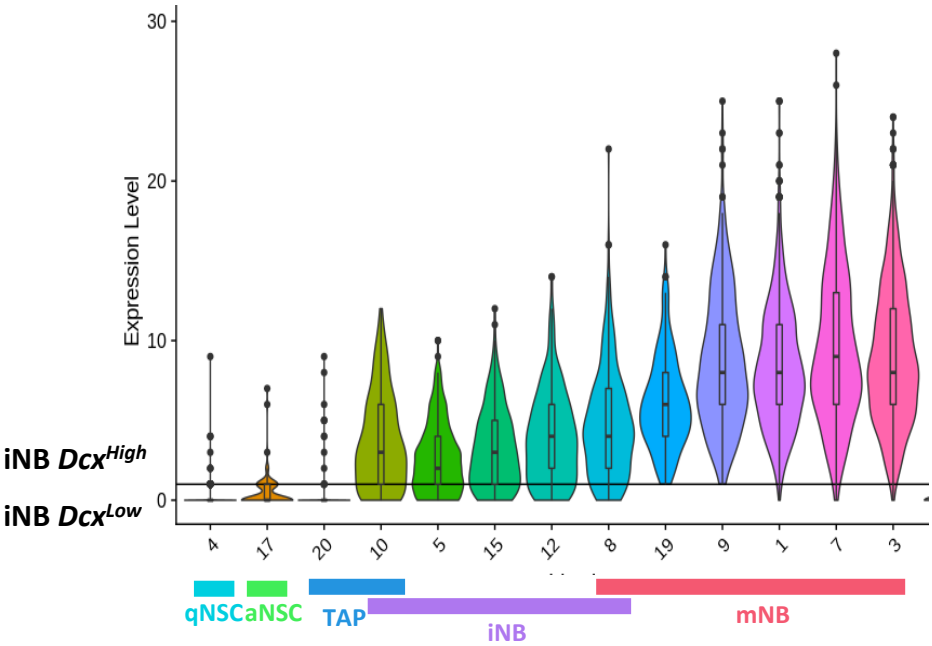
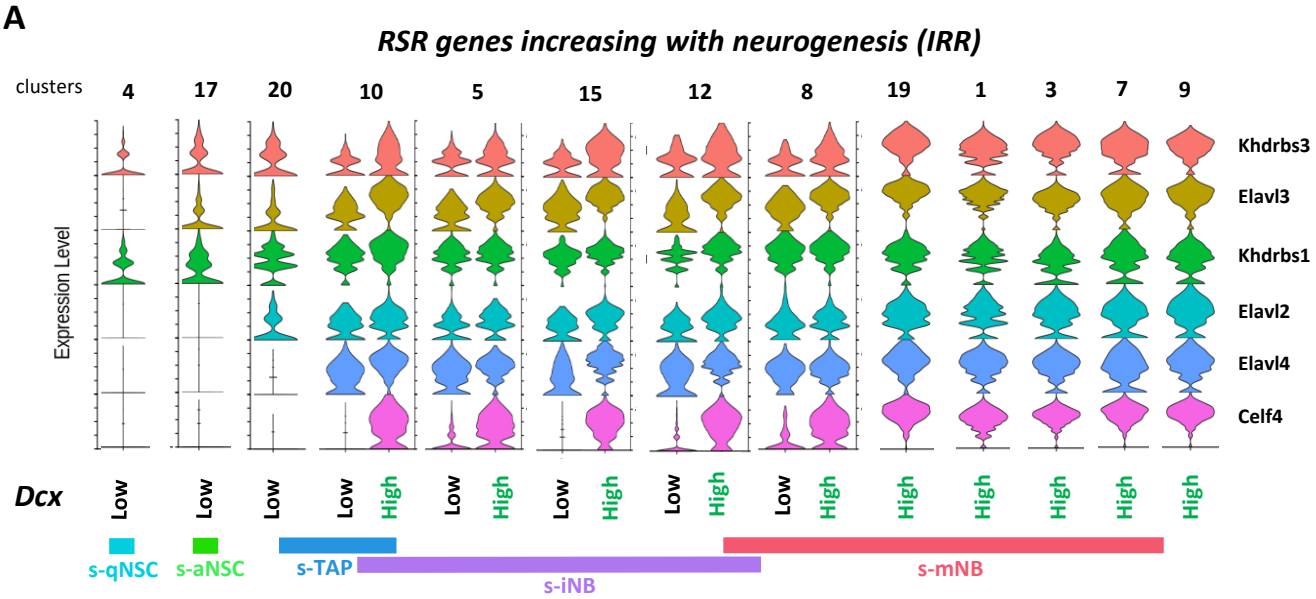


Figure S7: Expression of RSR genes in scRNA-seq neurogenic clusters in irradiated brains



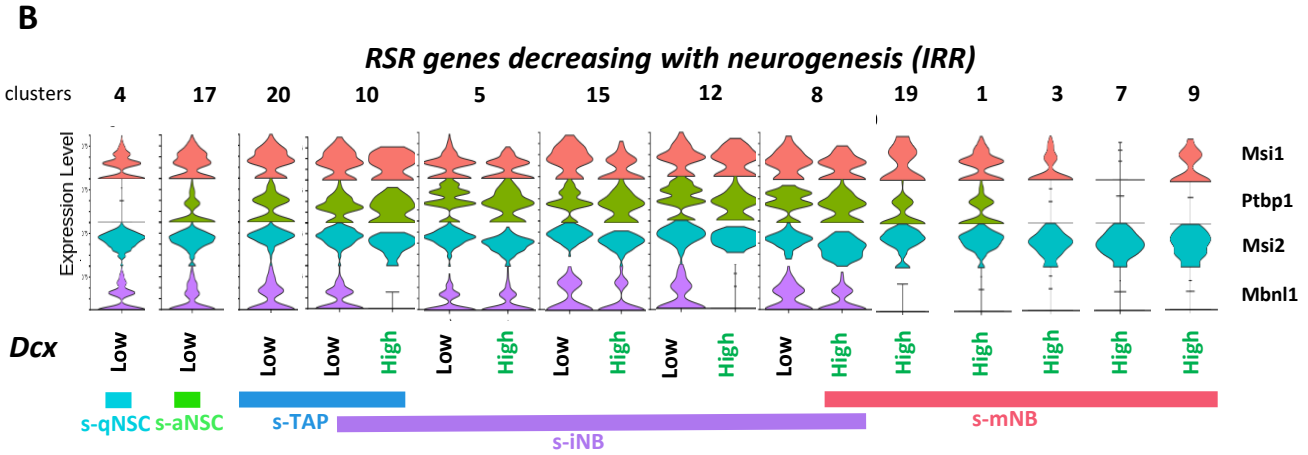


Figure S8: Table recapitulating the immunohistological analyses of eGFP^{+} s-iNB, eGFP^{-}s-iNB and eGFP^{+}mNB transplantations into recipient mouse brains

			IS		SVZ		CC		OB		
		eGFP+ engrafted cells	Nb eGFP+ cells	%	Nb eGFP+ cells	%	Nb eGFP+ cells	%	Nb eGFP+ cells	%	Total eGFP+ cells
s-iNB eGFP+	Animal 1	$1x10^4$	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0
s-iNB eGFP+	Animal 2	$1x10^4$	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0
s-iNB eGFP+ /eGFP-	Animal 1	$2x10^4$ / $8.1x10^4$	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0
s-iNB eGFP+ /eGFP-	Animal 2	$2x10^4$ / $8.1x10^4$	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0
s-iNB eGFP+ /eGFP-	Animal 3	$2x10^4$ / $8.1x10^4$	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0
s-mNB eGFP+	Animal 1	$2x10^4$	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0
s-mNB eGFP+	Animal 2	$2x10^4$	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0
s- mNB eGFP+	Animal 3	$2x10^4$	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0

IS: Injection Site
SVZ: Subventricular Zone
CC: Corpus Callosum
OB: Olfactory Bulb

Table 1:

		IS		SVZ		CC		OB		
		Nb eGFP+ cells	%	Nb eGFP+ cells	%	Nb eGFP+ cells	%	Nb eGFP+ cells	%	Total eGFP+ cells
s-aNSC/s-TAP	Animal 1	271	62.6%	17	3.9%	55	12.7%	90	20.8%	433
1.3-1.5x10^4	Animal 2	300	31.9%	79	8.4%	47	5.0%	515	54.7%	941
eGFP+ engrafted cells	Animal 3	*95*	37.1%	*73*	28.5%	*22*	8.6%	*66*	25.8%	256
s-iNB	Animal 1	1025	65.2%	16	1.0%	49	3.1%	483	30.7%	1573
2x10^4	Animal 2	196	15.5%	41	3.2%	41	3.2%	989	78.1%	1267
eGFP+ engrafted cells	Animal 3	240	84.8%	43	15.2%	0	0.0%	0	0.0%	283
s-mNB	Animal 1	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0
2x10^4	Animal 2	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0
eGFP+ engrafted cells	Animal 3	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0

***IS** = Injection Site*

***CC** = Corpus Callosum*

SVZ = Subventricular zone

OB = Olfactive Bulb