Olig2 defines a subset of neural stem cells that produce specific olfactory bulb Interneuron subtypes in the subventricular zone of adult mice

Ángela del Águila, Mike Adam, Kristy Ullom, Nicholas Shaw, Shenyue Qin, Jacqueline Ehrman, Diana Nardini, Joseph Salomone, Brian Gebelein, Q. Richard Lu, Steven S. Potter, Ronald Waclaw, Kenneth Campbell and Masato Nakafuku
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Original submission

First decision letter

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MS TITLE: Olig2 Defines a Unique Subset of Neural Stem Cells That Produce Specific Olfactory Bulb Interneuron Subtypes in the Subventricular Zone of Adult Mice

AUTHORS: Ángela del Águila, Mike Adam, Kristy Ullom, Nicholas Shaw, Shenyue Qin, Jacqueline Ehrman, Diana Nardini, Joseph Salomone, Brian Gebelein, Q. Richard Lu, Steve Potter, Ron Waclaw, Kenneth Campbell, and Masato Nakafuku

I have now received all the referees' reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, the referees express considerable interest in your work, but have some significant criticisms and recommend a substantial revision of your manuscript before we can consider publication. The referees have many constructive suggestions that will help add clarity to your presentation. In addition to the citations and text changes suggested, I would draw your attention to the questions about the interpretation of the Olig2 lineage tracing results and of marker gene expression.

If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision.

We are aware that you may be experiencing disruption to the normal running of your lab that make experimental revisions challenging. If it would be helpful, we encourage you to contact us to

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discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

del Aguila et al. investigate the identity of Olig2-expressing neural stem cells in the ventricular-subventricular zone of the mouse brain from embryogenesis to adulthood. Single cell RNA-sequencing suggested that the cell cycle kinetics and proliferative capacities of the cells changed throughout development, consistent with previously published studies. The authors identified a cross-repression mechanism between ventrally-expressed Olig2 and dorsally-expressed Gsx2 that is established embryonically. Additionally through lineage tracing studies, the authors showed that tamoxifen administration in an Olig2-CreER system resulted in labeling of progeny cells in multiple layers of the olfactory bulb (ie from multiple dorsal-ventral subregions of the stem cells). When OLIG2 is conditionally ablated in the adult mouse, there is a decrease in the proportion of CalR+ neuronal progeny in the olfactory bulb – a population that is largely dervid from the medial subdomain of the stem cell niche. Collectively, these data underscore and extend existing work identifying OLIG2 as an important factor in the neuronal lineage in addition to its function in oligodendrocyte development. However more information needs to be added to place this work in the context of the field and discuss potential points of divergence from what might be expected based on prior studies.

This manuscript would be of interest to researchers focused on mechanisms of olfactory bulb specification and those studying the trajectories of neural stem cells from the subventricular zone. The genetic manipulation experiments would be of interest to Olig2 specialists and those studying developmental diseases in which neuronal developmental trajectories are altered.

Comments for the author

Major Critiques:

1. The manuscript should include additional references that identify the markers the authors used to delineate different cell populations, as understanding of these markers and the cell types they identify have evolved over time. It would also be of value to see more context on how the sequencing data shown here aligns with the many other recent studies within this niche (among others, Cebrian Silla et al Elife 2021, Dulken et al Cell Reports 2017 Core et al Elife 2020, Mizrak et al Cell Reports 2019, Zywitsa et al Cell Reports 2018). Do any of these studies contain Olig2-positive events that distribute in a manner consistent with what is described here?

2. For the initial lineage tracing studies (Olig2CreER; tdTomato labeling) - given that (a) the tamoxifen is dosed once daily for 10 days and (b) the dissection and observation period is long after initial dose (46 days), there is concern that the expression of Olig2 could be transient or oscillatory as is the case in earlier development, thus labeling or affecting a much broader population than cells that durably express this factor. Given that approximately half of the Tomato+ cells in the SVZ have OLIG2 protein but relatively few express markers of immediate progeny (TAPs or NBs) – what are these other cells? A similar examination at a shorter timepoint (for example 2-4 days), after a single dose of tamoxifen, would help shed light on this pattern. Alternatively, if the authors believe that all of these cells are in fact NSCs, examination of OLIG2+ cells in a GFAP:GFP reporter animal or in acutely dissociated single cells, could strengthen this point.

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3. Several of the stated patterns of expression seem to be in disagreement with what has been previously published: embryonic radial glia cells have not been broadly shown to express GFAP (although the hGfap-Cre reporter line expresses prior to the presence of GFAP protein), Pax6 has been shown to be predominant in the dorsal region of the stem cell niche Mash1/Ascl1 is expressed in TAPs as well as aNSCs, ventral NSCs have been shown to give rise predominantly to deep granule layer interneurons and CalB+ periglomerular cells in the OB. The manuscript should be edited to more thoroughly discuss the divergence of the presented data from expectations. It is surprising, given the mapping in Figure 1 and the text indicating that Olig2 is present in ventral subdomains, that there appears to be labeling of nearly all V-SVZ-derived neuronal populations, not just those derived from the more lateral and ventral portions of the niche. To this point, the language describing this finding is unclear. In page 5 (Results), the authors indicate that “since both the density and total number of cells are much higher in the vSVZ than in the lateral and medial subdomains, Olig2+ cells are most enriched in the vSVZ compared with other subdomains.” This seems, to this reader, to be confusing absolute stem cell number at a given spot with location-specific enrichment within the stem cell fraction. It’s also concerning that only the dorsolateral domain appears to be considered – when the callosal roof of the SVZ is also neurogenic and indeed produces the TH+ cells that are discussed in later figures (see for example domains iiC, iiiC, ivC in Merkle et al Science 2007, which the authors reference). Can the authors comment on OLIG2+ NSCs in the callosal roof – their presence or absence?

4. The title of the manuscript should potentially be tempered as it’s not clear Olig2 is truly defining a unique subset of neural stem cells – rather much of the data suggest that Olig2 is broadly expressed in multiple subdomains. The scRNA-seq studies, while informative and interesting as an examination of this population, do not really show us that OLIG2+ cells are unique versus other cell subgroups, more that OLIG2+ populations vary in their properties over time.

Minor Critiques:
1. In the introduction, to the discussion of regionalization in embryonic and adult SVZ, suggest also including Fuentealba et al Cell 2015 – which links the two stages.
2. The specific statistical tests used and displayed in the figures should be moved from the supplement to the figure legends.
3. The figures should be edited to more clearly distinguish which genetic model is being used in each image/graph and whether data from the olfactory bulb or vSVZ is being shown, especially figure 7 F-I.
4. In figure 1, seeing more staining of the dorsal and/or whole SVZ either en face or in cross section, would help the reader to appreciate the claimed ventral enrichment of Olig2.
5. In Figure 3H/I – it is quite difficult to see the Tomato+ cells in the merged image; can this also be shown as an accompanying grayscale image?
6. The authors should provide rationale for how the tamoxifen and BrdU dosing schemes were decided.
7. The method used to determine whether cells were positive for a marker should be clarified in the methods section.

Typographical:
1. The wording of the sentence in the first Results section, second paragraph that begins with “Given that immunoreactivity of GFAP…” is confusing and should be clarified.
2. There is a typo in the second Results section, the final sentence should read “These results suggest that the expression of Olig2 and Gsx2 proteins in NSCs is regulated…”
3. In the last Results section, the second to last sentence should read
“Given that Olig2 is not expressed in either…”
4. In the first paragraph of the first Results section, the authors state that “few Olig2+ cells were detected in the most anterior or posterior portions of the SVZ,” but Figure 1 shows Olig2+ cells in all 4 fields. The text should be updated to match the figure legend that says Gsx2+ and Olig2+ cells were not found.

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Reviewer 2

Advance summary and potential significance to field

This is a beautifully executed paper that provides novel information about the expression and function of Olig2 in mouse olfactory bulb progenitors. The combination of excellent histochemical, genetic, fate mapping, single cell RNA seq analyses provides the field with rich data sets and insights into the generation of mouse olfactory bulb progenitors, glia and neurons over developmental and adult time frames.

Comments for the author

Introduction
Please reference:
RE: Gsx function:
Loss of Gsx1 and Gsx2 function rescues distinct phenotypes in Dlx1/2 mutants.
Wang B, Long JE, Flandin P, Pla R, Waclaw RR, Campbell K, Rubenstein JL. J Comp Neurol. 2013 May 1;521(7):1561-84.
RE Ascl1 function:
Modulation of the notch signaling by Mash1 and Dlx1/2 regulates sequential specification and differentiation of progenitor cell types in the subcortical telencephalon.
Yun K, Fischman S, Johnson J, Hrabe de Angelis M, Weinmaster G, Rubenstein JL.
Development. 2002 Nov;129(21):5029-40.
RE: Olig2 function:
Dlx1 and Dlx2 control neuronal versus oligodendroglial cell fate acquisition in the developing forebrain. Petryniak MA, Potter GB, Rowitch DH, Rubenstein JL.
Neuron. 2007 Aug 2;55(3):417-33.

Results organized by Figures
Figure 1. Clearly describes the expression of Olig2 in the early postnatal and adult SVZ, and its relationship to Gsx2, Ascl1, Ki67, Dlx2 and Dcx, as cells progress from qNSC to NBs. I have never liked the term neuroblasts (NBs) as it implies that they are dividing – aren't these postmitotic immature neurons?
whereas Dlx2 is expressed in late-stage TAPs and NBs and promotes neurogenesis (Kohwi et al., 2007; Brill et al., 2008; Andersen et al., 2014)
Please reference:
Dlx-dependent and -independent regulation of olfactory bulb interneuron differentiation.
Long JE, Garel S, Alvarez-Dolado M, Yoshikawa K, Osumi N, Alvarez-Buylla A Rubenstein JL. J Neurosci. 2007 Mar 21;27(12):3230-43

Figure 2. scRNA-seq analysis of Olig2-expressing NSCs compared with Gsx2+ cells in the adult and P14 brain from manually dissected SVZ. Very useful single cell transcriptomic data that add precision to the immunohistochemical results in Figure 1. The data will also be useful to the field going forward towards understanding more of the molecules controlling OB interneuron development.

Figure 3. Lineage tracing of Olig2+ NSCs and their neuronal progeny in the the adult OB using Olig2-CreER. Adult mice carrying Olig2CreER/+;Rosa-tdTomato+/- or +/+ were treated with the CreER activator tamoxifen (Tx) once daily for 10 days and then analyzed 56 days later by co-staining for tdtomato with Olig2, Ascl1 Ki67, Dlx2, Dcx, and Pax6. Adult-born neurons were detected using 5-bromo-2'-deoxyuridine (BrdU) (injected twice a day for 5 days (from D35 to D39) 3 weeks prior to histological analysis.

"We detected a significant number of tdTomato+ cells in the SVZ of Olig2-CreER mice at D56, and about a half of these cells expressed Olig2 (Fig. 3A, G)." I would replace or element the word "significant".

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In addition, many tdTomato+ cells also co-expressed Dlx2, Dcx, and Pax6, which mark late-TAPs and NBs (Fig. 3D-G) (Kohwi et al., 2005; Brill et al., 2008; de Chevigny et al., 20212).
Please reference Long et al., 2007, and I am not sure that Dlx2 marks late-TAPs – I bet it labels most/all TAPs, at least based in its prominent expression is most/lab SVZ cells and many cells in the VZ (prenatally).
It is hard for me to consolidate the big picture from detailed description – can you send the sections with some major take home points?

Figure 4. Olig2+ NSCs generate a variety of neuronal subtypes in the adult OB.
They provided a clear description for the fates. About 60% of Olig2-CreER fate-mapped cells belonged to the GCL, whereas the remaining 30% and 10% were detected in the GL and PL; not much specificity was identified, except for perhaps few PV+ cells.

Figure 5. Developmental changes in the molecular properties of Olig2+ NSCs from early embryonic to the adult stages revealed by scRNA-seq studies.
PLEASE PROVIDE MORE DETAIL ON THE DESCRIPTION OF THE DISSECTIONS USED AT THE 4 AGES. COMPARING TRANSCRIPTOMES BETWEEN E12 AND E18 OF THE ENTIRE VENTRAL TELENCEPHALON IS A BIT LIKE APPLES AND ORGANGES BECAUSE OF THE HUGE INCREASE IN POSTMITOTIC NEURONS AT E18 (STRIATUM AND PALLIDUM) AND THE RELATIVE REDUCTION OF PROGENTIORS.

scRNA-seq data on the whole ventral telencephalon at embryonic day (E) 12.5 and E18.5 using the 10X Genomics platform and compared these datasets with those from P14 and adult SVZ. Olig2+ NSCs/progenitors at E12.5 and E18.5 are divided into four (0, 1, 3, and 5) and three (3, 4, and 7) distinct subclusters. Among these subclusters, subcluster 3 is the only population that shares common features between cells at E12.5 and E18.5, and the remaining majority of cells at E18.5 belong to unique subclusters that are not present at E12.5. By contrast, a significant fraction of cells at the adult stage form a unique cluster (subcluster 6 in Fig. 5D), which is featured by the enriched expression of genes related to qNSCs and astrocytes such as Rorb (Retinoid-Related Orphan Receptor-Beta), Slc1a2 (Solute Carrier Family 1 Member 2 or EAA2) and Gja1 (Gap Junction Protein Alpha 1)A significant fraction of cells at the adult stage form a unique cluster (subcluster 6 in Fig. 5D), which is featured by the enriched expression of genes related to qNSCs and astrocytes such as Rorb (Retinoid-Related Orphan Receptor-Beta), Slc1a2 (Solute Carrier Family 1 Member 2 or EAA2) and Gja1 (Gap Junction Protein Alpha 1).
Cell cycle properties showed that more than 90% of cells at E12.5 were either in the S or G2/M phase. Olig2+ NSCs/progenitors at E18.5, cells in the S phase were much fewer, and about 35% of cells are in the G0/G1 phase

FIGURE 6. Cross-repression between Olig2 and Gsx2 in the embryonic and adult brains.
"In the developing telencephalon, both Olig2 and Gsx2 are expressed in broad progenitor domains, including the lateral ganglionic eminence (LGE), medial ganglionic eminence (MGE),
preoptic area (POA), and septum":
please also reference regarding olig2 expression in detail in the progenitor zones: Dlx1 and Dlx2 control neuronal versus oligodendroglial cell fate acquisition in the developing forebrain. Petryniak MA, Potter GB, Rowitch DH, Rubenstein JL. Neuron. 2007 Aug 2;55(3):417-33.

The folowing paper showed the complementary expression of gsx2 and olig2 in the LGE and MGE – but it shows that Gsx2 promotes Olig2 expression in the E12.5 GEs and septum. This argues against a simple model of Gsx2-Olig2 cross repression – at least at E12.5 Loss of Gsx1 and Gsx2 function rescues distinct phenotypes in Dlx1/2 mutants.
Wang B, Long JE, Flandin P, Pla R, Waclaw RR, Campbell K, Rubenstein JL. J Comp Neurol. 2013 May 1;521(7):1561-84.

First revision

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Author response to reviewers' comments

Responses to Reviewers' Comments

Please note that reviewers' comments are all in italic below.

Reviewer 1
Advance Summary and Potential Significance to Field: *del Aguila et al. investigate the identity of Olig2-expressing neural stem cells in the ventricular-subventricular zone of the mouse brain from embryogenesis to adulthood. Single cell RNA-sequencing suggested that the cell cycle kinetics and proliferative capacities of the cells changed throughout development, consistent with previously published studies. The authors identified a cross-repression mechanism between ventrally-expressed Olig2 and dorsally-expressed Gsx2 that is established embryonically. Additionally, through lineage tracing studies, the authors showed that tamoxifen administration in an Olig2-CreER system resulted in labeling of progeny cells in multiple layers of the olfactory bulb (ie from multiple dorsal-ventral subregions of the stem cells). When OLIG2 is conditionally ablated in the adult mouse, there is a decrease in the proportion of CalR+ neuronal progeny in the olfactory bulb – a population that is largely derived from the medial subdomain of the stem cell niche. Collectively, these data underscore and extend existing work identifying OLIG2 as an important factor in the neuronal lineage in addition to its function in oligodendrocyte development. However, more information needs to be added to place this work in the context of the field and discuss potential points of divergence from what might be expected based on prior studies.*

This manuscript would be of interest to researchers focused on mechanisms of olfactory bulb specification and those studying the trajectories of neural stem cells from the subventricular zone. The genetic manipulation experiments would be of interest to Olig2 specialists and those studying developmental diseases in which neuronal developmental trajectories are altered.

> We appreciate the reviewer's overall supportive view on our study. We have addressed all issues raised by the reviewer as follows:

Major Critiques:
1) The manuscript should include additional references that identify the markers the authors used to delineate different cell populations, as understanding of these markers and the cell types they identify have evolved over time. It would also be of value to see more context on how the sequencing data shown here aligns with the many other recent studies within this niche (among others, Cebrian Silla et al Elife 2021, Dulken et al Cell Reports 2017, Core et al Elife 2020, Mizrak et al Cell Reports 2019, Zywitsa et al Cell Reports 2018). Do any of these studies contain Olig2-positive events that distribute in a manner consistent with what is described here?

> We have included additional references to describe the properties of molecular makers of various cell types in the postnatal SVZ at appropriate places (For example, see 1st paragraph of page 6; Also see our response to Reviewer 2's comment #1). We have also added recent studies on single-cell RNA-seq analysis of postnatal NSCs to the 1st paragraph of page 7. As a note, we also identified the occurrence of Olig2-expressing NSCs in two previously reported scRNA-seq datasets (Coré et al., 2020; Cebrian-Silla et al., 2021; data not shown) which are consistent with the analysis using our own datasets.

2) For the initial lineage tracing studies (Olig2CreER; tdTomato labeling) - given that (a) the tamoxifen is dosed once daily for 10 days and (b) the dissection and observation period is long after initial dose (46 days), there is concern that the expresssion of Olig2 could be transient or oscillatory as is the case in earlier development, thus labeling or affecting a much broader population than cells that durably express this factor. Given that approximately half of the Tomato+ cells in the SVZ have OLIG2 protein, but relatively few express markers of immediate progeny (TAPs or NBs) – what are these other cells? A similar examination at a shorter timepoint (for example 2-4 days), after a single dose of tamoxifen, would help shed light on this pattern. Alternatively, if the authors believe that all of these cells are in fact NSCs, examination of OLIG2+ cells in a GFAP:GFP reporter animal, or in acutely dissociated single cells, could strengthen this

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point.

> Olig2 expression in postnatal NSCs could be oscillatory and transient as the reviewer suggest. In fact, our data support the idea that Olig2 expression is transient during the lineage progression of NSCs and mostly confined to the stage of activated NSCs and TAPs and lost when cells differentiate into NBs (Figure 1M, N). Thus, we are not claiming that ALL Olig2+ cells are NSCs. Therefore, analysis of Olig2 expression in GFAP:GFP reporter animals would not add any further information. Such a possibility of oscillatory and transient expression of Olig2, however, is NOT inconsistent with our main conclusion that Olig2-expressing cells represent a subpopulation of NSCs. In fact, our data show that the expression of Olig2 and Gsx2 is mutually exclusive in the P16 and adult SVZ, and that Olig2 and Gsx2 play distinct roles in the specification of neuronal subtypes. Thus, Olig2 expression indeed marks a subpopulation of NSCs at any given timepoints, and those Olig2-expressing NSCs play distinct roles compared with Gsx2-expressing NSCs.

Regarding Olig2-expressing and non-expressing cells among tdTomato$^+$ cells in *Olig2-CreER* mice, we examined *Olig2-CreER* mice at day 3 after four Tx injections as suggested by the reviewer. We found that all tdTomato$^+$ cells detected in the SVZ of these animals expressed Olig2. Thus, even if Olig2 expression is oscillatory/transient, our *Olig2-CreER* system faithfully captures Olig2-expressing cells within the 3-day time window. By contrast, in D56 animals, about 45% of Olig2-CreER-labled cells do not express Olig2, and a smaller but significant fraction tdTomato$^+$ cells were Ascl1$^+$ TAPs and Dlx2$^+$ and Dcx$^+$ NBs (Figure 3A-G). These data are consistent with the idea that a fraction of Olig2$^+$ NSCs lose Olig2 expression and become Olig2-negative TAPs and NBs within the 8-week period between D3 and D56 in our studies.
These behaviors of Olig2$^+$ cells also consistent with known properties of adult NSCs: They are mostly quiescent or slowly diving, and only a small fraction of them undergo rapid cell divisions and progress to TAPs and NBs. Thus, it is formally possible that Olig2 expression is oscillatory/transient, but genetic labeling using *Olig2-CreER* is still a useful tool to reveal the identity of Olig2$^+$ NSCs and their progeny. We have revised the text in the 2nd paragraph of page 9 to clarify these points as follows: "Thus, tdTomato$^+$ cells derived from Olig2$^+$ cells include not only NSCs, but also TAPs and NBs, the two major progeny of NSCs in *Olig2-CreER* mice at D56."

3.-1 Several of the stated patterns of expression seem to be in disagreement with what has been previously published: embryonic radial glia cells have not been broadly shown to express GFAP (although the hGfap-Cre reporter line expresses prior to the presence of GFAP protein), Pax6 has been shown to be predominant in the dorsal region of the stem cell niche, Mash1/Ascl1 is expressed in TAPs as well as aNSCs,

> The statement on page 3 regarding GFAP was indeed misreading, and we have modified this part in the revised text. As for Pax6, although its expression is indeed enriched in the dorsal half of the SVZ (Kohwi et al., 2005; Brill et al., 2008; de Chevigny et al., 20212), it is NOT absolutely confined to NBs in the dorsal region, and, in fact, we detect Pax6$^+$ cells in the vSVZ, as well as in other SVZ subregions (Fig. 7E, F and data not shown). Regarding Ascl1, we repeatedly state that it is expressed in both aNSCs and TAPs in the original manuscript (For example, see 1st paragraph of page 6 and 1st paragraph of page 9).

3.-2 ventral NSCs have been shown to give rise predominantly to deep granule layer interneurons and CalB+ periglomerular cells in the OB. The manuscript should be edited to more thoroughly discuss the divergence of the presented data from expectations. It is surprising, given the mapping in Figure 1 and the text indicating that Olig2 is present in ventral subdomains, that there appears to be labeling of nearly all V-SVZ-derived neuronal populations, not just those derived from the more lateral and ventral portions of the niche. To this point, the language describing this finding is unclear. In page 5 (Results), the authors indicate that "since both the density and total number of cells are much higher in the vSVZ than in the lateral and medial subdomains, Olig2+ cells are most enriched in the vSVZ compared with other subdomains." This seems, to this reader, to be confusing absolute stem cell number at a given spot with location-specific enrichment within the

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stem cell fraction.

> This is an important point in this study, and if the reviewer is confused about our conclusions, we should have better described our findings. It is true that "*ventral NSCs have been shown to give rise predominantly to deep granule layer interneurons and CalB+ periglomerular cells in the OB*" as the reviewer stated. It should be noted, however, that published studies do NOT prove that ventral NSC do NOT produce any other neuronal subtypes. For instance, Merkle et al. (2007) have clearly shown that many CB$^+$ glomerular neurons are indeed produced by ventral NSCs, but also by NSCs in the anterior and medial SVZ as well. Moreover, the same study has shown that CB$^+$ cells comprise only 30% of ventral NSCs-derived cells. In fact, the data presented in this study and other related studies demonstrate a large fraction of fate-mapped cells derived from particular regions of the SVZ subdomains remain marker-negative unidentified neurons. In addition, our data clearly show that Olig2-CreER fate-mapped cells do NOT include CB$^+$ neurons in the OB despite the fact that a significant number of Olig2$^+$ NSCs exist in the vSVZ. Thus, these data demonstrate that NOT all NSCs in the vSVZ generate CB$^+$ neurons.

Likewise, data reported in Merkle et al. (2007) show that CR$^+$ glomerular neurons derive not only from cells in the anterior and medial SVZs, but also from those in dorsal and ventral SVZs. Our previous study has also demonstrated that Gsx2$^+$ NSCs enriched in the dorsolateral SVZ are responsible for generation of a significant fraction of CR$^+$ glomerular neurons (López-Juárez et al., 2013). This study also shows that Olig2$^+$ cells, which are enriched in the vSVZ and do not overlap with Gsx2$^+$ cells, also produce CR$^+$ neurons.

As such, the idea that particular subtypes of neurons marked by existing markers such as CB and CR are generated SOLELY by a specific group of NSCs that exist ONLY in particular SVZ subdomains is NOT supported by any published studies and certainly an oversimplification. Rather, existing data support the idea that neurons expressing the same known molecular markers are produced by multiple distinct NSC populations. In addition, we clearly state that Olig2$^+$ cells are enriched in the vSVZ, but also exist in other SVZ subdomains (Fig. 1F). Thus, we do NOT claim anywhere in this study that "ventral Olig2$^+$ NSCs are the sole source of CR$^+$ neurons." These results together support the idea that the same types of OB interneurons are generated by multiple distinct subpopulations of NSCs that exist in multiple SVZ subdomains. We discuss these points on pages 21 and 22.

3.-3 It's also concerning that only the dorsolateral domain appears to be considered – when the callosal roof of the SVZ is also neurogenic and indeed produces the TH+ cells that are discussed in later figures (see for example domains iiC, iiiC, ivC in Merkle et al Science 2007, which the authors reference). Can the authors comment on OLIG2+ NSCs in the callosal roof – their presence or absence?

> We indeed detect Olig2$^+$ cells right above the ependymal layer in the dorsal roof (callosal) region of the SVZ in the P16 and adult animals. However, this SVZ subdomain is immediately adjacent to the overlaying corpus callosum where Olig2$^+$ oligodendrocytes are very abundant. Therefore, it is extremely difficult to distinguish Olig2$^+$ NSCs and Olig2$^+$ oligodendrocytes in this region. We have mentioned this issue in the 1st paragraph of page 5 in the revised manuscript.

4. The title of the manuscript should potentially be tempered as it's not clear Olig2 is truly defining a unique subset of neural stem cells – rather much of the data suggest that Olig2 is broadly expressed in multiple subdomains. The scRNA-seq studies, while informative and interesting as an examination of this population, do not really show us that OLIG2+ cells are unique versus other cell subgroups, more that OLIG2+ populations vary in their properties over time.

> We understand the reviewer's concern, but it seems that it is all up how to define the "uniqueness" of NSCs. Many of the previous studies have emphasized the notion that the regional specificity dictates the identity of NSCs. As discussed in the above point #3-1 and 3-2, it is clearly

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an oversimplification and not supported by existing data at all. Our data rather show that the regionally biased distribution pattern of NSCs only partially explanations their heterogeneity, and we need a more thorough understanding of its molecular underpinnings.

As discussed in the point #2 above, it is formally possible that Olig2 expression is transient and oscillates in broad NSC populations so that it marks a transient state of all NSCs rather than a defined subpopulation of NSCs. However, our data clearly show that Olig2$^+$ NSCs are mostly non-overlapping to Gsx2$^+$ NSCs in the P16 and adult brain, and that Gsx2$^+$ NSCs are known to be a specific subpopulation of NSCs that have defined functions, not represented by all NSCs (López-Juárez et al., 2013). Moreover, our lineage-tracing study demonstrate that Olig2$^+$ NSCs generate a specific subset but not all of OB interneurons that are clearly distinct from the known NSC repertoires. Thus, as a whole, our data indeed show that Olig2$^+$ NSCs correspond to a specific subpopulation of postnatal NSCs.

Minor Critiques:
1. In the introduction, to the discussion of regionalization in embryonic and adult SVZ, suggest also including Fuentealba et al Cell 2015 – which links the two stages.

> We have added this important study in pages 4 and 22.

2. The specific statistical tests used and displayed in the figures should be moved from the supplement to the figure legends.

> We have added this information to the legends of Figs. 6 and 7.

3. The figures should be edited to more clearly distinguish which genetic model is being used in each image/graph and whether data from the olfactory bulb or vSVZ is being shown, especially figure 7 F-I.

> The regions examined in Figures 1-6 are very obvious so that we did not change them. We have added the information on the SVZ in Fig. 7F and the OB in Figs. G-I.

4. In figure 1, seeing more staining of the dorsal and/or whole SVZ, either en face or in cross section, would help the reader to appreciate the claimed ventral enrichment of Olig2.

> We understand the reviewer's suggestion. We collected the recommended images, but, unfortunately, these images do not well reveal the overall distribution pattern of Olig2$^+$ cells along the dorsoventral and mediolateral axis of the SVZ well due to their scatted distribution patterns, not highly condensed in one small region. Thus, we show high-magnification images of individual Olig2$^+$ cells in the vSVZ as representative cells.

5. In Figure 3H/I – it is quite difficult to see the Tomato+ cells in the merged image; can this also be shown as an accompanying grayscale image?

> We tested grayscale images for Fig. 3H and 3I, but they do not look different at all from the original versions. The purpose of these images are to show the wide distribution of tdTomato$^+$ cells in multiple layers of the OB, and the images of individual cells are depicted in Fig. 3J-N".

6. The authors should provide rationale for how the tamoxifen and BrdU dosing schemes were decided.

> Previous studies have shown that it takes 3-6 weeks for newly generated neurons in the SVZ to reach the OB and express mature markers (For example, see Kohwi et al., 2007, Sakamoto et al., 2014, and references therein). Thus, to allow for many tdTomato$^+$ cells derived from Olig2$^+$ NSCs to settle in their final destinations within the OB and express subtype-specific molecular markers, we chose D56 after the first Tx injection followed by a 3-week chase period after BrdU injections as the point of analysis. This information is now described on page 4 of the Supplemental Information.

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7. The method used to determine whether cells were positive for a marker should be clarified in the methods section.

> This information was already included on page 4 of the Supplemental Information section. Marker expression in individual cells was evaluated using a series of 62 z-plane confocal images of vibratome sections with an optical resolution of 0.325 mm or 5 z-plane images of cryosections with an optical resolution of 0.80 mm using a 40x lens.

Typographical:
1. The wording of the sentence in the first Results section, second paragraph that begins with "Given that immunoreactivity of GFAP…" is confusing and should be clarified.

> We have revised the corresponding section on page 6 to clarify the point as follows: We did not detect Olig2^{+}/GFAP^{+} cells, however, this may be due to a technical issue since unlike clear nuclear staining detected using the Ascl1 antibody, weak immunoreactivity of GFAP in aNSCs is often difficult to detect in conventional immunochemistry (Pastrana et al., 2009; López-Juárez et al., 2013).

2. There is a typo in the second Results section, the final sentence should read "These results suggest that the expression of Olig2 and Gsx2 proteins in NSCs is regulated…"

> We have added "of" between "expression" and "Olig2."

3. In the last Results section, the second to last sentence should read "Given that Olig2 is not expressed in either…"

> We have added "in" between "expressed" and "either."

4. In the first paragraph of the first Results section, the authors state that "few Olig2+ cells were detected in the most anterior or posterior portions of the SVZ," but Figure 1 shows Olig2+ cells in all 4 fields. The text should be updated to match the figure legend that says Gsx2+ and Olig2+ cells were not found.

> We apologize for this confusing description. Images in Fig. 1B and 1E show Olig2^{+} cells in the most anterior and posterior parts of the SVZ where Olig2^{+} cells can be detected. However, the actual SVZ encompass more anterior and posterior regions beyond these regions. We describe this information in the legend of Fig. 1 and revised the text on page 5.

Reviewer 2:
Advance Summary and Potential Significance to Field: *This is a beautifully executed paper that provides novel information about the expression and function of Olig2 in mouse olfactory bulb progenitors. The combination of excellent histochemical, genetic, fate mapping, single cell RNA seq analyses provides the field with rich data sets and insights into the generation of mouse olfactory bulb progenitors, glia and neurons over developmental and adult time frames.*

We greatly appreciate the reviewer's positive view on our study. We have revised the manuscript according to her/his suggestions as described below:

1) Please reference:
RE: Gsx function:
Loss of Gsx1 and Gsx2 function rescues distinct phenotypes in Dlx1/2 mutants. Wang B, Long JE, Flandin P, Pla R, Waclaw RR, Campbell K, Rubenstein JL. J Comp Neurol. 2013 May 1;521(7):1561-84.

RE Ascl1 function:
Modulation of the notch signaling by Mash1 and Dlx1/2 regulates sequential specification and

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differentiation of progenitor cell types in the subcortical telencephalon. Yun K, Fischman S, Johnson J, Hrabe de Angelis M, Weinmaster G, Rubenstein JL. Development. 2002 Nov;129(21):5029-40.

RE: Olig2 function:
Dlx1 and Dlx2 control neuronal versus oligodendroglial cell fate acquisition in the developing forebrain. Petryniak MA, Potter GB, Rowitch DH, Rubenstein JL. Neuron. 2007 Aug 2;55(3):417-33.

> We have cited these studies in appropriate sections in the revised manuscript (pages 3, 4, and 15).

2) Figure 1. Clearly describes the expression of Olig2 in the early postnatal and adult SVZ, and its relationship to Gsx2, Ascl1, Ki67, Dlx2 and Dcx, as cells progress from qNSC to NBs. I have never liked the term neuroblasts (NBs) as it implies that they are dividing – aren't these postmitotic immature neurons?

> Previous studies have indeed demonstrated that nascent neurons or neuroblasts (NBs) are proliferative cells (For example, see Pastrana et al., *Proc Natl Acad Sci USA* 106(15): 6387- 6392, 2009; Ponti, G., et al. *Proc Natl Acad Sci USA* 110(11): E1045-1054, 2013). In addition, this information is clearly described in the review article on neural stem cells cited in the manuscript (Götz et al., *Cold Spring Harb Perspect Biol* 8(7):a018853, 2016).

3) whereas Dlx2 is expressed in late-stage TAPs and NBs and promotes neurogenesis (Kohwi et al., 2007; Brill et al., 2008; Andersen et al., 2014)
Please reference: Dlx-dependent and -independent regulation of olfactory bulb interneuron differentiation. Long JE, Garel S, Alvarez-Dolado M, Yoshikawa K, Osumi N, Alvarez-Buylla A, Rubenstein JL. J Neurosci. 2007 Mar 21;27(12):3230-43.

> There are a number of previous studies that have revealed the role of Dlx2 and other Dlx family members in neurogenesis during development, including the one mentioned in the reviewer's comment. However, citing all these studies is certainly out of scope of this study, and we believe that we should focus on studies on the role of Dlx2 in neurogenesis in the postnatal SVZ in this particular context. Meanwhile, the reference mentioned by the reviewer is indeed cited elsewhere in the manuscript where the role of Dlx factors in development is discussed.

4) Figure 2. scRNA-seq analysis of Olig2-expressing NSCs compared with Gsx2+ cells in the adult and P14 brain from manually dissected SVZ. Very useful single cell transcriptomic data that add precision to the immunohistochemical results in Figure 1. The
data will also be useful to the field going forward towards understanding more of the molecules controlling OB interneuron development.

> We appreciate the reviewer's supportive view on our study.

5) Figure 3. Lineage tracing of Olig2+ NSCs and their neuronal progeny in the the adult OB using Olig2-CreER. Adult mice carrying Olig2CreER/+;Rosa-tdTomato+/- or +/+ were treated with the CreER activator tamoxifen (Tx) once daily for 10 days and then analyzed 56 days later by co-staining for tdtomato with Olig2, Ascl1, Ki67, Dlx2, Dcx, and Pax6. Adult-born neurons were detected using 5-bromo-2'-deoxyuridine (BrdU) (injected twice a day for 5 days (from D35 to D39) 3 weeks prior to histological analysis. "We detected a significant number of tdTomato+ cells in the SVZ of Olig2-CreER mice at D56, and about a half of these cells expressed Olig2 (Fig. 3A, G)." I would replace or element the word "significant".

> We have removed the term "significant" here in the revised manuscript to avoid any possible confusions.

6) In addition, many tdTomato+ cells also co-expressed Dlx2, Dcx, and Pax6, which mark late-TAPs and NBs (Fig. 3D-G) (Kohwi et al., 2005; Brill et al., 2008; de Chevigny et al., 20212).
Please reference Long et al., 2007, and I am not sure that Dlx2 marks late-TAPs – I bet it labels most/all TAPs, at least based in its prominent expression is most/lab SVZ cells and many cells in the VZ (prenatally).

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> As described in the response to point #3 above, we focus our citations here on studies regarding postnatal neurogenesis. As for the expression of Dlx2 in the postnatal SVZ, previous studies have demonstrated that it is expressed in most, if not all NBs, but not in all TAPs. For example, only a very small fraction (<2%) of Dlx2-expressing cells co-express Gsx2 in the adult SVZ. However, Gsx2 is expressed in a majority of aNSCs and TAPs in the dlSVZ and mostly overlaps with Ascl1 (see Doetsch et al., Neuron 36(6):1021-1034. 2002; López-Juárez et al., *Genes Dev* 27(11):1272-1287. 2013; Andersen et al., *Neuron* 83(5):1085-1097. 2014). Moreover, our immunohistochemical analysis shows that a significant fraction of Ascl1-expressing cells, which correspond to aNSCs and early-stage TAPs, do not express Dlx2, whereas most Dcx^{+} NBs express Dlx2. In addition, genetically Gsx2 is upstream of Ascl1, and Ascl1 is upstream of Dlx2 (Our unpublished data and the studies cited above). Altogether, these results support the notion that Dlx2 is expressed mostly in late-stage TAPs and NBs, but not in early-stage TAPs or aNSCs. We also see similar results for Pax6 as described in the above cited studies.

7) It is hard for me to consolidate the big picture from detailed description – can you send the sections with some major take home points?

> To better clarify the take home message of this particular section, we have added to following description to the second paragraph of page 9: “Thus, tdTomato^{+} cells derived from Olig2^{+} cells in the SVZ of *Olig2-CreER* mice at D56 include not only NSCs, but also TAPs and NBs, the two major progeny of NSCs” in the 2nd paragraph of page 9.

8) Figure 4. Olig2+ NSCs generate a variety of neuronal subtypes in the adult OB.
They provided a clear description for the fates. About 60% of Olig2-CreER fate-mapped cells belonged to the GCL, whereas the remaining 30% and 10% were detected in the GL and PL; not much specificity was identified, except for perhaps few PV+ cells.

> With due respect, we would argue against the reviewer’s notion “*not much specificity was identified*.” Our data in Fig. 4K demonstrate that Olig2-CreER fate-mapped cells become CR+, NC+, and TH+ cells, but not CB+ or PV+ in the GCL. These results clearly demonstrate the neuronal subtype specificity of the progeny of Olig2^{+} NSCs.

9) Figure 5. Developmental changes in the molecular properties of Olig2+ NSCs from early embryonic to the adult stages revealed by scRNA-seq studies.
PLEASE PROVIDE MORE DETAIL ON THE DESCRIPTION OF THE DISSECTIONS USED AT THE 4 AGES. COMPARING TRANSCRIPTOMES BETWEEN E12 AND E18 OF THE ENTIRE VENTRAL TELENCEPHALON IS A BIT LIKE APPLES AND ORGANGES BECAUSE OF THE HUGE INCREASE IN POSTMITOTIC NEURONS AT E18 (STRIATUM AND PALLIDUM) AND THE RELATIVE REDUCTION OF PROGENTIORS.

> The method used for tissue dissection is described in the supplementary method section. In addition, the reviewer’s concern about differences of the cell type composition between tissues at distinct developmental stages is somewhat irrelevant in this context. Our single-cell analysis identifies cell populations that exhibit clear properties as stem/progenitor cells at each stage and focus on comparisons between those cells. In fact, as shown in Fig. 2 and Supplementary Fig. 1, many different cell types are detected in cell preparations at distinct developmental stages, and their numbers greatly vary between stages. Most importantly, however, stem/progenitor cells, the cells of our interest in this study, are clearly distinguishable from those other cells in each stage, and their numbers are large enough to reveal representative transcriptome profiles of Olig2^{+} stem/progenitors among them.

10) scRNA-seq data on the whole ventral telencephalon at embryonic day (E) 12.5 and E18.5 using the 10X Genomics platform and compared these datasets with those from P14 and adult SVZ. Olig2+ NSCs/progenitors at E12.5 and E18.5 are divided into four (0, 1, 3, and 5) and three (3, 4, and 7) distinct subclusters. Among these subclusters, subcluster 3 is the only population that shares common features between cells at E12.5 and E18.5, and the remaining majority of cells at E18.5 belong to unique subclusters that are not present at E12.5. By contrast, a significant fraction of cells at the adult stage form a unique cluster (subcluster 6 in Fig. 5D), which is

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featured by the enriched expression of genes related to qNSCs and astrocytes such as Rorb (Retinoid-Related Orphan Receptor-Beta), Slc1a2 (Solute Carrier Family 1 Member 2 or EAA2) and Gja1 (Gap Junction Protein Alpha 1) A significant fraction of cells at the adult stage form a unique cluster (subcluster 6 in Fig. 5D), which is featured by the enriched expression of genes related to qNSCs and astrocytes such as Rorb (Retinoid-Related Orphan Receptor-Beta), Slc1a2 (Solute Carrier Family 1 Member 2 or EAA2) and Gja1 (Gap Junction Protein Alpha 1).

Cell cycle properties showed that more than 90% of cells at E12.5 were either in the S or G2/M phase. Olig2+ NSCs/progenitors at E18.5, cells in the S phase were much fewer, and about 35% of cells are in the G0/G1 phase

> We were unable to decipher a question of concern from the reviewer in these passages. However, the reviewer summarizes clearly the main points we were trying to convey.

11) FIGURE 6. Cross-repression between Olig2 and Gsx2 in the embryonic and adult brains. "In the developing telencephalon, both Olig2 and Gsx2 are expressed in broad progenitor domains, including the lateral ganglionic eminence (LGE), medial ganglionic eminence (MGE), preoptic area (POA), and septum":
please also reference regarding olig2 expression in detail in the progenitor zones: Dlx1 and Dlx2 control neuronal versus oligodendroglial cell fate acquisition in the developing forebrain. Petryniak MA, Potter GB, Rowitch DH, Rubenstein JL. Neuron. 2007 Aug 2;55(3):417-33.

> We have cited this study in the second paragraph of page 15.

12) The folowing paper showed the complementary expression of gsx2 and olig2 in the LGE and MGE – but it shows that Gsx2 promotes Olig2 expression in the E12.5 GEs and septum. This argues against a simple model of Gsx2-Olig2 cross repression – at least at E12.5 Loss of Gsx1 and Gsx2 function rescues distinct phenotypes in Dlx1/2 mutants. Wang B, Long JE, Flandin P, Pla R, Waclaw RR, Campbell K, Rubenstein JL. J Comp Neurol. 2013 May 1;521(7):1561-84.

> The published data mentioned in the reviewer's comment actually demonstrate that inactivation of Gsx2 on top of Dlx1/2 KO partially attenuates the otherwise elevated expression of Olig2 in the MGE in Dlx1/2 KO embryos, which the authors argue as an indicator of elevated oligodendrogenesis, to a level closer to but still higher than the wild-type level. In fact, the stated increased expression of Olig2 in Dlx1/2 KO embryos described in this study is detected almost exclusively in the mantle zone, but NOT in the adjacent germinal zones (VZ and SVZ). Rather, the impact of Gsx2 inactivation alone on the expression of Olig2 in the VZ of Dlx1/2 KO embryos is not overtly evident in this study. Therefore, the paper that the reviewer refers to does NOT appear to reveal the relationship between Gsx2 and Olig2 in stem/progenitor cells within the VZ. On the other hand, our data on the cross-regulation between Olig2 and Gsx2 is focused on their expression within the embryonic VZ where the most primitive NSCs exist and importantly, we do not observe a similar cross-regulation in the postnatal SVZ.

Second decision letter

MS ID#: DEVELOP/2021/200028

MS TITLE: Olig2 Defines a Unique Subset of Neural Stem Cells That Produce Specific Olfactory Bulb Interneuron Subtypes in the Subventricular Zone of Adult Mice

AUTHORS: Ángela del Águila, Mike Adam, Kristy Ullom, Nicholas Shaw, Shenyue Qin, Jacqueline Ehrman, Diana Nardini, Joseph Salomone, Brian Gebelein, Q. Richard Lu, Steve Potter, Ron Waclaw, Kenneth Campbell, and Masato Nakafuku

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I have now received all the referees reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

The overall evaluation is positive and we would like to publish a revised manuscript in Development, provided that the referees' comments can be satisfactorily addressed. As you will see, Reviewer 1 has several points that should be addressed to help clarify your experiments and the interpretation. Please attend to all of the reviewers' comments in your revised manuscript and detail them in your point-by-point response. If you do not agree with any of their criticisms or suggestions explain clearly why this is so.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Reviewer 1

Advance summary and potential significance to field

The authors have addressed many of my questions and provided additional data which strengthen the paper. The added discussion paragraphs are very helpful in placing their work within the context of the field – it is reasonable to point out that stem cell microdomains as a model may imply stricter fate choice than is observed for the OLIG2+ population, and the data and discussion here will advance our understanding of neural stem cell identity.

Comments for the author

There are a few points of concern that remain, which can largely be addressed through textual revisions or inclusion of data that are referenced but not shown.
1) The title should be revised to remove the word “unique” – as exemplified by the discussion in review, this is a subjective term. If the cells are defined by the expression of OLIG2, then the title stands well without this adjective.
2) The sentence on page 5 (Results) that reads as follows: “since both the density and total number of cells are much higher in the vSVZ than in the lateral and medial subdomains, Olig2+ cells are most enriched in the vSVZ compared with other subdomains.” - while the authors’ response regarding the overlapping populations of NSCs and the types of neurons they produce is a solid point and worth discussing – that was not the point of concern. Rather, the specific phrasing of this sentence reads as if OLIG2+ cells are most enriched in the ventral SVZ within the stem cell fraction (ie if all GFAP+ type B1 stem cells were counted within this region, a high percentage would be OLIG2+, but in other regions the OLIG2+ percentage within the stem cell fraction would be lower). Though this may be true, this is NOT the scoring that was performed –
OLIG2+ cell abundance is scored in the DAPI-positive fraction (ie all cells in or near the niche), and then marker expression is scored within the OLIG2+ fraction only. Because stem cells in total are less abundant in the lateral and medial subdomains, a decreased OLIG2+ percentage in the DAPI+ fraction could be due to fewer stem cells expressing OLIG2+, or fewer total stem cells of which the same percentage are OLIG2+. It would be more correct to say that, because the density of B1 cells and other immature progeny is higher in the dorsal-most and ventral-most portions of the SVZ, OLIG2+ cell abundance can be most readily detected and compared between these two regions (although it is also present in other subregions, as the authors note).
3) Please indicate clearly, within Figure 4, which mouse model is used.
4) Grayscale images of tdTomato+ cells (without DAPI signal) to complement Figure 3H and 3I would be extremely helpful to experts in visualizing the “broad distribution” of OB progeny – in the present figures, the DAPI signal is so high that tdTomato positive cells in the granular layer are very difficult to see.
The authors assert that “they were not different” from what is shown but we are given no evidence.

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5) Details on image acquisition, as the authors note, were indeed detailed in the Methods section. However, no description of image thresholding criteria were given, and this should also be included. How did the authors determine which cells were positive for a given marker – expert identification by hand?
Automated nuclear segmentation and identification of an intensity value above which cells were scored as positive?
6) The revised text on page 6 explaining why GFAP and OLIG2 were not found together – is it truly “weak GFAP immunoreactivity”? GFAP is typically an abundant and intense stain, but has a cytoplasmic localization that is difficult to co-locate with nuclear stains. Please clarify if the signal is low-intensity or simply has differing localization.

Reviewer 2

Advance summary and potential significance to field

This paper provides novel information about the expression and function of Olig2 in mouse olfactory bulb progenitors. The combination of histochemical, genetic fate mapping, single cell RNA seq analyses provides the field with rich data sets and insights into the generation of mouse olfactory bulb progenitors, glia and neurons over developmental and adult time frames.

Comments for the author

I am satisfied with the revisions and support publication at this point.

Second revision

Author response to reviewers' comments

Responses to Reviewers’ Comments

Please note that reviewers’ comments listed below are all shown in italic, and the revised sections of the text is highlighted in yellow in the manuscript file.

Reviewer 1
Advance Summary and Potential Significance to Field:
The authors have addressed many of my questions and provided additional data which strengthen the paper. The added discussion paragraphs are very helpful in placing their work within the context of the field – it is reasonable to point out that stem cell microdomains as a model may imply stricter fate choice than is observed for the OLIG2+ population, and the data and discussion here will advance our understanding of neural stem cell identity.

> We appreciate the reviewer’s overall supportive view on our study. We have addressed all issues raised by the reviewer as described below:

Reviewer 1 Comments for the Author:
There are a few points of concern that remain, which can largely be addressed through textual revisions or inclusion of data that are referenced but not shown.

1) The title should be revised to remove the word “unique” – as exemplified by the discussion in review, this is a subjective term. If the cells are defined by the expression of OLIG2, then the title stands well without this adjective.

> We took out the term “unique” from the title according to the reviewer’s suggestion.

2) The sentence on page 5 (Results) that reads as follows: “since both the density and total

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number of cells are much higher in the vSVZ than in the lateral and medial subdomains, Olig2+ cells are most enriched in the vSVZ compared with other subdomains." - while the authors' response regarding the overlapping populations of NSCs and the types of neurons they produce is a solid point and worth discussing – that was not the point of concern. Rather, the specific phrasing of this sentence reads as if OLIG2+ cells are most enriched in the ventral SVZ within the stem cell fraction (ie if all GFAP+ type B1 stem cells were counted within this region, a high percentage would be OLIG2+, but in other regions the OLIG2+ percentage within the stem cell fraction would be lower). Though this may be true, this is NOT the scoring that was performed – OLIG2+ cell abundance is scored in the DAPI-positive fraction (ie all cells in or near the niche), and then marker expression is scored within the OLIG2+ fraction only. Because stem cells in total are less abundant in the lateral and medial subdomains, a decreased OLIG2+ percentage in the DAPI+ fraction could be due to fewer stem cells expressing OLIG2+, or fewer total stem cells of which the same percentage are OLIG2+. It would be more correct to say that, because the density of B1 cells and other immature progeny is higher in the dorsal-most and ventral-most portions of the SVZ, OLIG2+ cell abundance can be most readily detected and compared between these two regions (although it is also present in other subregions, as the authors note).

> We understand the reviewer's concern. To better clarify our data, we have revised the corresponding texts in page 5 as follows: Similar to its embryonic expression (Takebayashi et al., 2002; Chapman et al., 2013, 2018), Olig2 expression was found broadly along the dorsoventral (DV) and mediolateral (ML) axes of the LV. However, the density of Olig2^{+} cells among total cells in the dorsolateral (dl) SVZ is significantly lower than other subdomains (Fig. 1F).
Moreover, since both the density and total number of cells are much higher in the vSVZ than in the lateral (l) and medial (m) SVZ subdomains (for details, see López-Juárez et al., 2013), the actual number of Olig2^{+} cells is the highest in the vSVZ among the four subdomains examined (Fig. 1F: The locations of distinct SVZ subdomains are indicated in the lower panel of C).

3) Please indicate clearly, within Figure 4, which mouse model is used.

> We have added this information to Figure 4 as the reviewer requested. To maintain consistency across figures, we have also added the same information to Figure 3.

4) Grayscale images of tdTomato+ cells (without DAPI signal) to complement Figure 3H and 3I would be extremely helpful to experts in visualizing the "broad distribution" of OB progeny – in the present figures, the DAPI signal is so high that tdTomato positive cells in the granular layer are very difficult to see. The authors assert that "they were not different" from what is shown but we are given no evidence.

> We have added a grayscale image of tdTomato+ cells to Figure 3 as 3J according to the reviewer's request.

5) Details on image acquisition, as the authors note, were indeed detailed in the Methods section. However, no description of image thresholding criteria were given, and this should also be included. How did the authors determine which cells were positive for a given marker – expert identification by hand? Automated nuclear segmentation and identification of an intensity value above which cells were scored as positive?

> We have added the following descriptions to the Supplementary Information: Immunoreactivity of individual cells for particular antigens was assessed by evaluating serial z plane images that encompassed the cell body of each cell after setting an appropriate threshold of signal strength for each antigen. The nuclei of individual cells were first identified with an aid of DAPI signals. As for immunoreactivity of transcription factors such as Olig2 and Dlx2, clear overlaps between nuclear immunoreactive signals and DAPI signals were judged as positive.
Regarding cytoplasmic and cytoskeletal antigens such as Dcx and GFAP, cells that clearly showed diffuse and/or fibrous immunoreactive signals that surrounded their DAPI^{+} nuclei were considered to be immune-positive.

6) The revised text on page 6 explaining why GFAP and OLIG2 were not found together – is it

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truly "weak GFAP immunoreactivity"? GFAP is typically an abundant and intense stain, but has a cytoplasmic localization that is difficult to co-locate with nuclear stains. Please clarify if the signal is low-intensity or simply has differing localization.

> As the reviewer knows well, the strength of GFAP immunoreactivity in the postnatal SVZ is quite variable among cells, and some cells have fibrous intense signals that cover the entire cell body, whereas others have weak and diffuse signals that mark only a portion of the cytoplasm. In the latter case, it is often difficult to ascertain which $DAPI^{+}$ signals among very tightly clustered cells overlap with GFAP immunoreactivity. Moreover, the overall GFAP immunoreactivity of aNSCs is weaker than that in qNSCs. We have revised the corresponding text in page 6 as follows to better clarity this point: Although we detected only a few $Olig2^{+}/GFAP^{+}$ cells, it may be due to a technical issue since weak and/or diffuse cytoplasmic signals of GFAP staining in the postnatal SVZ often make it difficult to distinguish individual immunoreactive cells in conventional histology unlike clear nuclear signals detectable by Ascl1 staining (Pastrana et al., 2009; López-Juárez et al., 2013)."

Reviewer 2:
Advance Summary and Potential Significance to Field: This paper provides novel information about the expression and function of Olig2 in mouse olfactory bulb progenitors. The combination of histochemical, genetic, fate mapping, single cell RNA seq analyses provides the field with rich data sets and insights into the generation of mouse olfactory bulb progenitors, glia and neurons over developmental and adult time frames.

Reviewer 2 Comments for the Author:
I am satisfied with the revisions and support publication at this point.

> We greatly appreciate the reviewer's supportive view on our study.

Third decision letter

MS ID#: DEVELOP/2021/200028

MS TITLE: Olig2 Defines a Subset of Neural Stem Cells That Produce Specific Olfactory Bulb Interneuron Subtypes in the Subventricular Zone of Adult Mice

AUTHORS: Ángela del Águila, Mike Adam, Kristy Ullom, Nicholas Shaw, Shenyue Qin, Jacqueline Ehrman, Diana Nardini, Joseph Salomone, Brian Gebelein, Q. Richard Lu, Steve Potter, Ron Waclaw, Kenneth Campbell, and Masato Nakafuku
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.

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