A novel stem cell type at the basal side of the subventricular zone maintains adult neurogenesis

Katja Baur, Yomn Abdullah, Claudia Mandl, Gabriele Hölzl-Wenig, Yan Shi, Udo Edelkraut, Priti Khatri, Anna Hagenston, Martin Irmler, Johannes Beckers, and Francesca Ciccolini

DOI: 10.15252/embr.202154078

Corresponding author(s): Francesca Ciccolini (ciccolini@nbio.uni-heidelberg.de)

Review Timeline:

- Submission Date: 1st Oct 21
- Editorial Decision: 9th Nov 21
- Revision Received: 14th Apr 22
- Editorial Decision: 15th Jun 22
- Revision Received: 20th Jun 22
- Accepted: 4th Jul 22

Editor: Esther Schnapp/Martina Rembold

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the referee chooses to sign their reports.)
Dear Dr. Ciccolini,

Thank you for your patience while your manuscript was peer-reviewed at EMBO reports. Unfortunately, referee 1 felt unable to review your manuscript, I paste her/his comments below. We have received the enclosed comments from referees 2 and 3 though.

I am sorry to say that the evaluation of your manuscript is not a positive one. As you will see, both referees point out that the technical approach is not clean. Referee 2 further notes that the stem cell potential is not demonstrated, and that real lineage tracing would have to be performed. Referee 3 notes that the relations of the described cell populations with published populations in the V-SVZ are unclear, which might be along similar lines as referee 1’s concerns. These technical concerns preclude a solid interpretation of the experimental evidence provided, and we can therefore not offer to publish your manuscript.

I am sorry to disappoint you on this occasion, and hope that the referee comments will be helpful in your continued work in this area.

Kind regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

I am sorry to inform you that I feel unable to review this manuscript. The data is presented in an incoherent and unstructured form that makes it impossible for me to understand the content. I can certainly not judge the quality of the presented data and the justification of the experiments that were performed. I asked my colleague, a renown specialist in adult stem cell biology, for an independent opinion. His/her conclusion was the same. This paper is to our eyes not reviewable. It needs complete re-writing or at least major editing, structuring and shortening before it can be judged.

Referee #2:

In their manuscript Baur et al. address putative neural stem cells in the V-SVZ of the mouse brain. Using a hGFAP driven Tet-off system to drive expression of H2B-GFP they identify cells in the ventricular lining with contact to the ventricular lumen and cells in a more basal location lacking luminal contact. By tracing the expression of H2B-GFP under chase and during lactation in females, the authors claim the basal cells have stem cell properties and are potentially a major precursor of olfactory bulb neurons. The authors present some interesting data but most of the observations are correlative rather than definitive. Most of the conclusions are not supported by hard data. In the absence of a clan lineage tracing experiments the results remain speculative.

Major concerns

1. A main concern is the methodology of hGFAP driven Tet-off and H2B-GFP expression as this is neither selective for either the apical or basal cell population that the authors are addressing or a definitive lineage tracing approach. Using the hGFAP enhancer driven Tet-off depends on expression levels of the transgene which is translated into the levels of H2B-GFP expressed, which can cause major differences in the levels of H2B-GFP expression in different cells. In addition, the hGFAP enhancer has been shown not to faithfully reflect mouse GFAP expression and this has not been addressed in the manuscript.

2. The expression of H2B-GFP is affected not only by the levels of hGFAP-Tet expression but also the perdurance of the transgene mRNA and the Tet-protein. The authors need to describe the system better including expression of the transgene relative to known stem and progenitor markers and progression of labelling of cells down the olfactory bulb lineage.

3. If the authors want to claim stem cell potential, they need to perform selective lineage tracing of the apical and basal lineages. The use of a non-selective transgene or relying on the infection of the cells using non-selective viral constructs injected into the lateral ventricles is not sufficient to show fate or potential. The authors need to selectively trace the basal cells.

4. The sorting experiments are difficult to interpret based on the FACS plots shown. The authors need to show how they set the negative populations and positive gates in the experiments and show negative cell plots for comparison. The levels of GFP and Prominin expression are, sometimes, barely above what looks like background which also emphasizes the need to show the gating parameters.
5. Using Sox9 and Ki67 expression is really not sufficient to claim stem cell identity in vivo. Neither is the expression of GFAP or Nestin, none of these markers are definitive. Similarly, generation of primary neurospheres is also not restricted to stem cells and it has been demonstrated that many primary neurospheres are generated by TAPs. The authors need to perform sphere propagation assays and assess the maintenance of sphere formation by the apical and basal populations and the self-renewing potential of sphere-forming cells.

6. The Notch experiments are not convincing at all. The immunostaining for expression of the NICD are not visible. The Hes1 knockdown experiments do not separate the apical and basal cell populations and claiming that Basal cells have lower Notch levels based on the data shown is also not convincing. Do these populations show differences in Notch paralogue receptor expression?

7. In addition, the experiments claiming differential Notch activity in apical and basal cell populations based on examining lactating females with increased TAPs is obscure. There is no evidence that there is a feedback mechanism in this paradigm that goes through Notch. Hence, it is an over interpretation to claim that this shows a higher activation of Notch on the apical cells.

Minor comments
The authors need to show better and high magnification images of the immunostainings in most of the figures. The coexpression of GFP and most of the markers is not visible or not convincing. This is particularly true for Figures 2A, 2C, 3A, 5A, 7A and 7B.

This reviewer is surprised to so little H2B-GFP labelling in the none Dox treated mice. The authors need to discuss this in detail.

The treatment of the mice with Dox is not described in the methods.

The authors have not cited many of the important Notch papers describing the regulation of the stem cells and in adult V-SVZ.

Referee #3:

In this manuscript Baur and colleagues describe their findings on subpopulations of neural stem cells in the V-SVZ of the adult mouse brain and the contribution of these subpopulations to progenitor division in the SVZ and production of OB neurons. The authors apply an array of approaches to support their main hypothesis that a basally-located subpopulation of neural stem cells is the key cell type that contributes to olfactory neurogenesis. They also propose that the role of the apically located ciliated NSCs, traditionally considered as true stem cells, may be to control, in a Notch-dependent manner, the overall stem and progenitor cell homeostasis and proliferation in the walls of the lateral ventricles rather than to directly contribute to the birth of new OB interneurons.

The manuscript present interesting and intriguing data and is clearly written. There are several comments.

1. The relation to other cell populations in V-SVZ with stem and progenitor features remains unclear. This is particularly important since authors' hypothesis present a departure from the commonly held view of olfactory neurogenesis. This refers both to the originally described cells (B1, B2, A, C, E) and to the populations described by others (cited in the manuscript). For example, what is the correspondence to the aNSC and qNSC cells described in Joppe et al 2020, Llorens-Bobbadilla at al 2015, and Obernier et al 2018? For instance, several features of the basal population described by the authors resemble those of the progeny of B1 and non-B1 FoxJ1-labeled cells of Joppe at al. Do they fully or partially overlap? Is there a lineage relation between them? These questions are important for putting the features of the described cells in the proper context.

2. In the same vein, Discussion section should be significantly expanded to include a detailed comparison and discussion of where do the basal and the apical cells described the manuscript belong in the continually expanding universe of stem cells in the adult brain. This is not simply an editorial comment, but a critical point for fully evaluating the novelty and the importance of the findings.

3. A scheme summarizing the findings and the relation between the basal cells, apical cells, and other types of the V-SVZ progenitor populations described so far should be presented. Again, this is not simply a matter of better presentation, but is critical for understanding the message; as it stands now, the true novelty of the finding and the relation between the basal stem cells described here and various classes of neural progenitors described by others is not fully clear.

4. A higher magnification image of the basal and apical cells, along with other cell types, should be presented. At this point it difficult to understand the morphologies of the basal and apical cells in Figs. 1, 2, 3, 7, S1.

5. Could the use of hGFAP promoter exclude certain types of stem and progenitor cells from the analysis or bias away from them? This should be discussed in more detail, despite the broad use of hGFAP promoter for lineage tracing by other groups.

6. If indeed the hGFAP-marked and traced cells encompass the entire population of neural stem and progenitor cells in the V-SVZ, where are the known types - B2, C, and A cells? It seems that quantitatively there is some disconnect between the reported sizes of these subpopulations and the results in the manuscript.

7. How reliable is the Dil staining as a true marker of apically-reaching cells? Does the dye diffuse out of the originally labeled
cell? Can it be transferred to the neighboring cells - if not from stem cells per se then from the numerous ciliated cells which have a very large surface and a large number of contacts with other cells across the entire depth of the V-SVZ?

8. Same refers to the AAV infection - how sure one can be that only the lumen-contacting cells are infected and marked? This is not to argue that these cells aren’t indeed preferentially labeled, but still the question remains of how much leakage would this experimental design tolerate without affecting the conclusions.

9. The authors write about the contribution of the apical and basal NSCs to production of interneurons in the text and the abstract. However, formally they are assessing the numbers and the lineage of neuroblasts, not of the mature interneurons. Corresponding statements should be modified.
Dear Dr Schnapp,

Many thanks for your previous email mentioning that we could resubmit our paper entitled “A novel stem cell type at the basal side of the subventricular zone maintains adult neurogenesis”, provided that we had fully addressed the comments of reviewers 2 and 3.

We have now extensively revised the manuscript to address the reviewers’ comments. The revision includes several editorial changes of text and figures and especially new additional data sets. Besides a more thorough characterization of our genetic mouse model (illustrated in Fig. 2D, F, supplementary Figs. S1A, S3A-F, E), the new experiments include new lineage tracing analyses to directly compare contribution of apical and basal NSCs in the OB (supplementary Fig. S5E, F; Fig. 5E, F and supplementary Fig. S5H). All the major text modifications have been highlighted in our detailed reply to the reviewers below.

We hope that you will find now our manuscript acceptable for publication in “Embo Reports” and we are looking forward to your decision.

Sincerely
Francesca Ciccolini

Referee #2:

General comment: In their manuscript Baur et al. address putative neural stem cells in the V-SVZ of the mouse brain. Using a hGFAP driven Tet-off system to drive expression of H2B-GFP they identify cells in the ventricular lining with contact to the ventricular lumen and cells in a more basal location lacking luminal contact. By tracing the expression of H2B-GFP under chase and during lactation in females, the authors claim the basal cells have stem cell properties and are potentially a major precursor of olfactory bulb neurons. The authors present some interesting data but most of the observations are correlative rather than definitive. Most of the conclusions are not supported by hard data. In the absence of a clan lineage tracing experiments the results remain speculative.

We would like to thank the reviewer for mentioning that our findings are potentially interesting and for the valid suggestions on how to improve them. As detailed in our reply below we have now revised our manuscript to fully address the concerns raised. Our revision includes several new experiments. Besides, a more thorough characterization of our genetic mouse model (illustrated in Figure 2D, F, supplementary Figs. S1A, S3A-F, E), following the reviewer’s indication, we performed also further experiments of lineage tracing to directly compare contribution of apical and basal NSCs in the OB using tagging of each group by AAV-GFP in WT mice (supplementary Figure S5E, F) and with AVV-hGFAP-mycCRE in RiboTag mice (Figure 5E, F and supplementary Figure S5H) in the revised manuscript.

Major concerns

1. A main concern is the methodology of hGFAP driven Tet-off and H2B-GFP expression as this is neither selective for either the apical or basal cell population that the authors are addressing or a definitive lineage tracing approach. Using the hGFAP enhancer driven Tet-off depends on expression levels of the transgene which is translated into the levels of H2B-GFP expressed, which can cause major differences in the levels of H2B-GFP expression in...
different cells. In addition, the hGFAP enhancer has been shown not to faithfully reflect mouse GFAP expression and this has not been addressed in the manuscript.

We apologize for the rather long reply, but this is a very important issue and we would like to clarify it. The hGFAP promoter is one of the most widely used tools to tag different reporter genes to NSCs. This may also be due to the fact that in contrast to mGFAP, it allows to tag radial glia progenitors from embryonic development throughout adulthood ((Kriegstein & Alvarez-Buylla, 2009); See also references within). Supporting that it is a very well-established approach to NSCs tagging countless groups including ours have previously used hGFAP to express not only GFP or CRE but also a doxycycline responsive transactivator (tTA) in NSCs of both adult neural niches. As at length discussed in our replies to the comments below our data are consistent with this body of literature and confirm the specificity of hGFAP promoter to tag NSCs. Indeed, as the reviewer rightly mentions, our mouse model does not allow to specifically tag apical or basal NSCs and therefore it is not suitable to trace the progeny of either population. However, we would like to respectively point out that we take advantage of the genetic model not to trace the progeny of NSCs but mainly to highlight the whole NSC population in the V-SVZ, which was a prerequisite for the identification of basal NSCs. Only by using two additional different stainings, i.e. Dil labelling of the apical surface and prominin immunostaining, we were able to distinguish between apical and basal progenitors present in the V-SVZ of hGFAP;H2B-GFP mice and demonstrate the stemness of the latter.

Instead, for the analysis of the progeny of apical and basal NSCs, and their contribution to OB neurogenesis, we took advantage of both non-selective and selective permanent viral-mediated lineage tagging, see also our reply to point 3 below, using distinct stereotactic injection coordinates to differentially tag the apical and basal NSCs, i.e. by injection intraventricular or at the lateral dorsal corner of the V-SVZ, respectively. A similar strategy of varying sites of viral particle injections was also previously used to tag proliferating progenitors localized at different sites along the ventral/dorsal axis of the V-SVZ to show that they are regionally-specified with respect to their progeny output (Merkle et al, 2007). In the revised manuscript, we have now expanded these analyses to directly compare the generation of labelled new neurons in the OB upon tagging apical or basal NSCs. These new data, now illustrated in supplementary Fig. S5E, F (AAV-GFP in WT mice) and in Fig. 5E, F and supplementary Fig. S5H (AVV-hGFAP-mycCRE in RiboTag mice) of the revised manuscript, show that basal NSCs are the main contributors to OB neurogenesis.

2. The expression of H2B-GFP is affected not only by the levels of hGFAP-Tet expression but also the perdurance of the transgene mRNA and the Tet-protein. The authors need to describe the system better including expression of the transgene relative to known stem and progenitor markers and progression of labelling of cells down the olfactory bulb lineage.

As we mentioned above hGFAP promoter has been extensively used to drive the expression of CRE or tTA and thereby activate reporter expression in NSCs. Moreover, this has been thoroughly investigated with multiple approaches including live imaging. Supporting the specificity of the reporter expression, our new data, illustrated in supplementary Fig. S1A of the revised manuscript, show that H2B-GFP+ cells display nuclear tTA expression and that this is completely downregulated upon Doxycycline administration. Previous extensive analyses of the dynamic of hGFAP activation have shown that the promoter is especially active in self-renewing, slow cycling primary NSCs and that the expression of the reporter is particularly high in NSCs just undergoing activation (Costa et al, 2011). Consistent with these previous observations, we found that many tagged cells co-express stem cells markers like GFAP and especially Sox9. With respect to the levels of GFAP expression, our data are fully consistent
with previously reported observations showing that only a subset of tagged hGFAP display GFAP immunoreactivity. Importantly, we have also now investigated additional lineage (DCX and Mash-1) marker and mitosis (see supplementary Fig.S3I, and relative result section in the revised manuscript). Moreover, in supplementary Fig. S3C-E of the revised manuscript we show that a subset of apical and basal NSCs are actively dividing and that doxycycline administration also leads to an overall decrease in levels of reporter expression (Fig. 3A and C), providing strong evidence that cell division affects levels of reporter expression. By measuring the effect of doxycycline on the number of apical and basal cells expressing high, intermediate and no fluorescence we were able to show that while the treatment reduces fluorescence levels in both populations this results in an overall increase in the number of unlabeled cells only in the basal population, further supporting our conclusion basal but not apical cells divide enough times to cause a significant increase in the number of reporter-negative cells during the course of the treatment (see supplementary Fig. S3C-E), which is consistent with the data reported in Fig. 3B, C. Thus, not only the reporter system used in our study has been extensively characterized, and its specificity is supported by our new analysis of tTA expression, but also can be used to monitor differences in cell divisions.

3. If the authors want to claim stem cell potential, they need to perform selective lineage tracing of the apical and basal lineages. The use of a non-selective transgene or relying on the infection of the cells using non-selective viral constructs injected into the lateral ventricles is not sufficient to show fate or potential. The authors need to selectively trace the basal cells.

We agree with the reviewer that this is an important issue. As mentioned above, we have now used distinct stereotactic injection of different AAV constructs for the non-selective (supplementary Figure S5E, F) and the selective permanent (Fig. 5E, F and supplementary Fig. S5H) tagging of apical and basal NSCs to explore their contribution to neurogenesis. These data show that basal NSCs are the main contributors to OB neurogenesis.

4. The sorting experiments are difficult to interpret based on the FACS plots shown. The authors need to show how they set the negative populations and positive gates in the experiments and show negative cell plots for comparison. The levels of GFP and Prominin expression are, sometimes, barely above what looks like background which also emphasizes the need to show the gating parameters.

We would like to respectively point out that for each marker used we have already described the setting of the sorting gates and an extensive characterization of the sorted populations in previous publications. This is particularly the case for the Prominin immunostaining for which gate-setting was described at length in multiple papers from our laboratory, and the specificity of the staining was confirmed by a several approaches, including cilia analysis, label-retention, proliferation and electrophysiology (Carrillo-Garcia et al, 2010; Cesetti et al, 2011; Khatri et al, 2014; Monaco et al, 2019). Because of space limitations, we have therefore not added this information to the current study. However, we have now modified the “Methods” section to describe the populations used to set the gates of Prominin positive and negative cells (page 16; lines 15-19), and added representative examples of FACS plots of unstained and DiI labelled cells used to set gate parameters (Fig. 1A, D).

5. Using Sox9 and Ki67 expression is really not sufficient to claim stem cell identity in vivo.
Neither is the expression of GFAP or Nestin, none of these markers are definitive. Similarly, generation of primary neurospheres is also not restricted to stem cells and it has been demonstrated that many primary neurospheres are generated by TAPs. The authors need to perform sphere propagation assays and assess the maintenance of sphere formation by the apical and basal populations and the self-renewing potential of sphere-forming cells.

We agree with the reviewer that the establishment of the stemness should be based on multiple factors. Indeed, we take advantage here of multiple approaches like expression markers, gene expression analysis at multiple stages of development and analysis of fundamental stem cell properties. In the revised manuscript, we now also use selective permanent tagging to study the contribution of apical and basal progenitors to OB neurogenesis. Whereas most of these analyses were performed ex vivo, as the reviewer mentions we take advantage also of clonal neurosphere assays, as it is known that active NSCs can be induced to undergo clone formation. These experiments are important not only because they confirm that both apical and basal tagged progenitors can form clonal neurospheres, but we could also show that there is a difference in neurosphere ability between NSCs isolated from untreated mice and mice treated with doxycycline, and therefore enriched for quiescent NSCs. Further supporting the concept that tagged cells include activated as well as NSCs at different stages of quiescence, we show now show that Mash-1 is overexpressed in activated tagged E\textsuperscript{th} precursors (see supplementary Fig. S3I), and a subset of apical and basal tagged NSCs enter quiescence during mid-development (Fig. 3D), which is consistent this the current literature. Moreover, as we now explicitly mention in the revised manuscript (page 7 lines 20-22) not only did tagged cells form neurospheres, but these could also be frozen and thawed for further propagation in culture. Importantly, this process could not be efficiently used for the propagation of apical prominin\textsuperscript{+} cells. Thus, all the collected evidence points at the stemness of basal NSCs.

6. The Notch experiments are not convincing at all. The immunostaining for expression of the NICD are not visible. The Hes1 knockdown experiments do not separate the apical and basal cell populations and claiming that Basal cells have lower Notch levels based on the data shown is also not convincing. Do these populations show differences in Notch paralogue receptor expression?

We would like to respectively point out that the viral construct expressing the short hairpin RNA were delivered directly in the ventricle and therefore we were able to tag selectively apical cells in these experiments, albeit not exclusively apical NSCs, as explicitly shown in supplementary Fig. S5C, D of the revised manuscript. We agree with the reviewer that the immunostaining of NICD results in weak fluorescence levels, for this reason we have taken advantage of unbiased measurement of nuclear fluorescence levels. Moreover, we have also confirmed the data using different NICD antibodies. Since we have previously shown differences between Notch1 and Notch3 expression levels in the apical and basal side of the V-SVZ, we have now also investigated the expression of the latter in the different populations, but we found no significant differences in expression between the various populations. A copy of this analysis is pasted below. Taken together, our data strongly indicate that the differences in NICD immunoreactivity between apical and basal progenitors reflects differences in Notch activation between the two groups.
7. In addition, the experiments claiming differential Notch activity in apical and basal cell populations based on examining lactating females with increased TAPs is obscure. There is no evidence that there is a feedback mechanism in this paradigm that goes through Notch. Hence, it is an over interpretation to claim that this shows a higher activation of Notch on the apical cells.

We agree with the reviewer that essentially our suggestion rests on the interpretation of our data in light of the current literature. To provide more direct evidence of the involvement of Notch signaling in the lactation context, we have now measured levels of NICD in apical and basal NSCs of virgin and lactating littermate dams. This analysis now illustrated in Fig. 7F of the revised manuscript show that Notch signaling is specifically altered in apical but not basal NSCs 7 days after lactation.

Minor comments
1) The authors need to show better and high magnification images of the immunostainings in most of the figures. The coexpression of GFP and most of the markers is not visible or not convincing. This is particularly true for Figures 2A, 2C, 3A, 5A, 7A and 7B.

We would like to apologize for these shortcomings. We have now comprehensively modified all the above-mentioned figures in an effort to improve their quality.

2) This reviewer is surprised to so little H2B-GFP labelling in the none Dox treated mice. The authors need to discuss this in detail.

Indeed, only a few percent of the dissociated V-SVZ cells displayed nuclear fluorescence. Although this is consistent with the expectation that the reporter tags NSCs, as the reviewer mentions, this is not comparable with other studies which use the same driver to tag GFP expression in NSCs. As we discuss in the revised manuscript (page 14 lines 26-37), the most likely explanation for this apparent discrepancy is that the expression of the reporter is actively degraded at each cell division, which reduce the leaking of the reporter expression to the progeny (see also our reply to comments 5, 6 of reviewer 3 below). Supporting this hypothesis, compared to similar studies which have used the hGFAP-CRE system, we found an increased percentage of GFAP+/SOX9+ tagged cells in our population and almost no neuroblasts. Notably, the modality of undergoing slow symmetric self-renewing divisions characteristic of our hGFAP-tagged progenitors is reminiscent of the dynamic of divisions
observed in a subset of hGFAP tagged progenitors in vitro, which were referred to as more primitive NSCs (Costa et al., 2011).

3) The treatment of the mice with Dox is not described in the methods.

We apologize for this oversight. We have now modified the “Methods” section of the manuscript to provide this missing information (page 15 lines 35/37).

3) The authors have not cited many of the important Notch papers describing the regulation of the stem cells and in adult V-SVZ.

The literature is very broad and therefore it is difficult to provide a comprehensive overview. However, in light of the importance of the different notch molecules expressed in the V-SVZ and of Notch signaling in the regulation of apical cell behavior we have now added the following papers.
1) Notch2 Signaling Maintains NSC Quiescence in the Murine Ventricular-Subventricular Zone (Engler et al, 2018)
2) Forebrain ependymal cells are Notch-dependent and generate neuroblasts and astrocytes after stroke (Carlen et al, 2009)

Referee #3:

In this manuscript Baur and colleagues describe their findings on subpopulations of neural stem cells in the V-SVZ of the adult mouse brain and the contribution of these subpopulations to progenitor division in the SVZ and production of OB neurons. The authors apply an array of approaches to support their main hypothesis that a basally-located subpopulation of neural stem cells is the key cell type that contributes to olfactory neurogenesis. They also propose that the role of the apically located ciliated NSCs, traditionally considered as true stem cells, may be to control, in a Notch-dependent manner, the overall stem and progenitor cell homeostasis and proliferation in the walls of the lateral ventricles rather than to directly contribute to the birth of new OB interneurons.

The manuscript present interesting and intriguing data and is clearly written. There are several comments.

We would like to thank the reviewer for the encouraging comments.

1. The relation to other cell populations in V-SVZ with stem and progenitor features remains unclear. This is particularly important since authors' hypothesis present a departure from the commonly held view of olfactory neurogenesis. This refers both to the originally described cells (B1, B2, A, C, E) and to the populations described by others (cited in the manuscript). For example, what is the correspondence to the aNSC and qNSC cells described in Joppe et al 2020, Llorens-Bobbadilla et al 2015, and Obernier et al 2018? For instance, several features of the basal population described by the authors resemble those of the progeny of B1 and non-B1 FoxJ1-labeled cells of Joppe at al. Do they fully or partially overlap? Is there a lineage relation between them? These questions are important for putting the features of the described cells in the proper context.
Concerning the relationship between apical and basal NSCs and the population of activated and quiescent NSCs identified in Llorens-Bobadilla et al (2015), the pattern of marker expression suggests that most of the NSCs identified in this study based on Prominin-1 and GLAST expression are similar to our apical NSCs. Supporting many Prominin-1+/GLAST+ cells displayed an apical surface and consistent with TypeB1/apical NSCs such as expression of Nestin and Tlx. However, underlying the limits of an identification based uniquely on marker expression, Prominin-1+/GLAST+ cells were not and homogeneous stem cell population as they included a subpopulation of progenitors, which based on the transcriptional signature were assigned to the oligodendrocyte lineage. Notably, Prominin-1+/GLAST+ cells upon transplantation in the V-SVZ gave rise to OB interneurons. This is not in contrast with our data, as in this case Prominin-1+/GLAST+ cells were transplanted and therefore taken out of the niche interaction, including Notch-mediated interactions, that regulate their behavior in physiological conditions.

The study from Obernier et al (2018) proposes that neurogenesis is sustained by apical type B1 NSCs, which undergo essentially two types of cell symmetric divisions: either self-renewing or more often consuming to generate two TAPs. In addition, it was also proposed in the study that type B1 give rise to type B2, which in the literature is a terminology used to include progenitors as well as niche astrocytes. Whereas direct imaging was given as proof of the self-renewing and consuming divisions of type B1 NSCs, the evidence for a lineage relationship between type B1 and type B2 progenitors was only indirect and limited by the fact that the tagging approach was not selective for type B1 cells. Although, it is known that type B2 progenitors are capable of proliferating and of quiescence, their stemness or their ability to contribute to neurogenesis were not investigated. Therefore, our study extends these previous observations by thoroughly characterizing type B2 progenitors/basal NSCs providing ample evidence of their stemness and neurogenic potential. In the revised manuscript we have further strengthened these conclusions with new experiments illustrated in Figs. 3D, F; 5E, F and supplementary Fig. S3; S5H, F. Our study contributes essential knowledge on this issue by showing that basal NSCs are the largest NSC population at least from birth onwards and that they are rarely generated from apical type B1 cells in normal circumstances.

Our apical virally tagged cells also share several characteristics with the apical cells identified by Joppe et al (2020), by means of electroporation. Beside the location at the apical side of the niche strictly adjacent to the lateral ventricle, the type B1 like non-ependymal progenitors represented a subset (around 10%) of the total electroporated cells, which underwent a very slow increase in cell number, suggesting a low division rate and in some rare cases displayed neuronal markers after a period of 4 weeks. Underscoring the complexity of the various cell types, apical progenitors identified in this previous study were not homogenous as they included a subset of cells expressing FoxJ1, which were morphologically different from the type B1 and were instead ependymal-like. However, electroporated progenitors were incapable of activating and of forming neurospheres. In contrast, apical NSCs in our study still displayed a subset of cells capable of undergoing EGFR expression and activation, as demonstrated by the increase in Mash-1 expression observed both in apical and basal G’Eh cells (supplementary Fig. S3I in the revised manuscript). A possible cause for these inconsistencies between the two studies could be that we directly measured EGFR expression and used clonal analysis to monitor neurosphere formation. Another source of difference between the hGFAP-tagged progenitors previously analyzed and the H2B-GFP tagged apical progenitors in our study is that despite using a similar driver (hGFAP) to promote the reporter expression the mechanism underlying the expression of the reporter and the reporter itself used
in the two studies both are different, which could affect the progenitor incidence of the tagged populations (see also our reply to comments 5, 6 below).

2. *In the same vein, discussion section should be significantly expanded to include a detailed comparison and discussion of where do the basal and the apical cells described the manuscript belong in the continually expanding universe of stem cells in the adult brain. This is not simply an editorial comment, but a critical point for fully evaluating the novelty and the importance of the findings.*

3. *A scheme summarizing the findings and, the relation between the basal cells, apical cells, and other types of the V-SVZ progenitor populations described so far should be presented. Again, this is not simply a matter of better presentation, but is critical for understanding the message: as it stands now, the true novelty of the finding and the relation between the basal stem cells described here and various classes of neural progenitors described by others is not fully clear.*

We would like to thank the reviewer for pointing out these issues with the manuscript. We have now modified the “Introduction” (page 3, lines 35-42) and the “Discussion” (page 14) of the revised manuscript to include a detailed comparison between the cell types studied here and NSC populations identified in previous studies. We have also added a schematic representation summarizing our findings illustrated in figure 8.

4. *A higher magnification image of the basal and apical cells, along with other cell types, should be presented. At this point it difficult to understand the morphologies of the basal and apical cells in Figs. 1, 2, 3, 7, S1.*

We would like to apologize for these shortcomings. We have now comprehensively modified all the above-mentioned figures in an effort to improve their quality.

5. *Could the use of hGFAP promoter exclude certain types of stem and progenitor cells from the analysis or bias away from them? This should be discussed in more detail, despite the broad use of hGFAP promoter.*

6. *If indeed the hGFAP-marked and traced cells encompass the entire population of neural stem and progenitor cells in the V-SVZ, where are the known types - B2, C, and A cells? It seems that quantitatively there is some disconnect between the reported sizes of these subpopulations and the results in the manuscript for lineage tracing by other groups.*

This are very important points which were raised also by reviewer 2 (see also above our reply to reviewer’s minor comment 2). In our study, independent of the side of the niche only a few percent of the dissociated V-SVZ cells displayed nuclear fluorescence and only few of these cells display markers of type A cells. Although this is consistent with the expectation that the reporter tags NSCs, as the reviewer mentions, this is not comparable with other studies which use the same driver to tag GFP expression in NSCs. As we discuss in the manuscript (page 14 lines 26-35) and explained above (see also our reply to issue 1), the most likely explanation for this apparent discrepancy is that the expression of the reporter is actively degraded at each cell division, which reduces the permanence of the reporter expression to the immediate progeny, i.e. type C and type A cells. This is consistent also with in vitro analysis of the dynamic of the reporter expression showing that that the promoter is especially active in self-renewing, slow
cycling primary NSCs (Costa et al., 2011). In this study, it was shown that the promoter is especially active in self-renewing, slow cycling primary NSCs and that the activity of the promoter is downregulated in rapidly proliferating progeny, even if they maintain astroglial characteristics, which is consistent with the lack of more differentiated progeny in our hGFAP tagged progenitors. Concerning the type B2 cells in the literature, this terminology is used both for progenitors (as mentioned in our reply to point 1 above) as well as niche astrocytes. Whereas our assumption is that basal NSCs represent the type B2 progenitors, our lineage tracing analysis reveals that apical NSCs poorly contribute to the generation of either cell types in physiological conditions, and that at least from one week after birth onwards the mass of these cells is already present in the V-SVZ.

7. How reliable is the Dil staining as a true marker of apically-reaching cells? Does the dye diffuse out of the originally labeled cell? Can it be transferred to the neighboring cells - if not from stem cells per se then from the numerous ciliated cells which have a very large surface and a large number of contacts with other cells across the entire depth of the V-SVZ?

The dye is known to intercalate in the lipid bilayer of the membrane and therefore does not diffuse. It can be only transferred to the neighboring cells by process that involve a membrane exchange like for example during cell division or vesicle exchange. Since the timing of labelling and analysis are very rapid and conditions were optimized, it is extremely unlikely that these processes bias our findings. We have confirmed the specificity of the labelling by confocal analysis of dissociated cells and in whole mounts preparations upon immunostaining to characterize the labeled cells. Consistent with this, our analysis of the labelled cells with respect to Prominin, EGFR and cilia extension, illustrated here (Figure1, supplementary figure S2E, F) and in a previously published paper (Khatri et al., 2014), shows that this is a very effective approach to label monocialiliated and non-ciliated progenitors at the apical side. Instead, it is less efficient to tag multiciliated ependymal cells, as applying our protocol we can only label around 20% of the total cell population. This is likely due to the fact that their apical membrane is covered with multiple motile cilia, which may interfere with the labelling process. Thus, all the available data indicate that the approach is very reliable to label apical progenitors.

8. Same refers to the AAV infection - how sure one can be that only the lumen-contacting cells are infected and marked? This is not to argue that these cells aren't indeed preferentially labeled, but still the question remains of how much leakage would this experimental design tolerate without affecting the conclusions.

We have used this approach in a previous paper to label apical cells in a mutant mouse line (Luque-Molina et al, 2019) and in this study in wild type mice. Independent of the genotype we have found a very specific tagging of apical cells which hardly contained a basal cellular component. This was carefully monitored in particular in this study by extensive quantification shown in supplementary figure S5A-D, and in supplementary figure S8. It is also consistent with our lineage analysis, in which we directly compare OB generation after differential stereotactic injection, i.e. into the lateral ventricle or at the lateral corner of the V-SVZ, of WT and Ribotag mice. These new data now illustrated in figure 5E, F and supplementary figure S5E-H, show clear differences between the amount of tagged OB interneurons with the change of the stereotactic parameters, showing the selectivity of the approach. In fact, the number of tagged basal cells observed upon intraventricular injection of AAV-GFP is so limited that it would be difficult to establish whether they are a result of a fault of the labelling procedure or of a lineage relationship between apical and basal cells based only on these data. However,
several lines of evidence point to the fact that they derive from a lineage relationship: firstly, virtually all tagged basal cells are non-proliferating and DCX negative when analyzed 14 days and 6 weeks after injection, respectively. Secondly, we observed that injection of AAV-Hes1-sh led to a change in the number of tagged basal cells but not in their marker expression, compared to AAV-sc-sh injection. Moreover, the manipulation also increased apical neurogenesis, both in the V-SVZ and in the OB. Taken together, these data show that the approach allows us to effectively distinguish apical and basal NSCs and to study their characteristics.

9. The authors write about the contribution of the apical and basal NSCs to production of interneurons in the text and the abstract. However, formally they are assessing the numbers and the lineage of neuroblasts, not of the mature interneurons. Corresponding statements should be modified.

We would like to thank the reviewer for pointing out this problem. We have now modified the manuscript accordingly.

References

Carlen M, Meletis K, Goritz C, Darsalia V, Evergren E, Tanigaki K, Amendola M, Barnabe-Heider F, Yeung MS, Naldini L et al (2009) Forebrain ependymal cells are Notch-dependent and generate neuroblasts and astrocytes after stroke. Nat Neurosci 12: 259-267
Carrillo-Garcia C, Suh Y, Obernier K, Holzl-Wenig G, Mandl C, Ciccolini F (2010) Multipotent precursors in the anterior and hippocampal subventricular zone display similar transcription factor signatures but their proliferation and maintenance are differentially regulated. Mol Cell Neurosci 44: 318-329
Cesetti T, Fila T, Obernier K, Bengtson CP, Li Y, Mandl C, Holzl-Wenig G, Ciccolini F (2011) GABAA receptor signaling induces osmotic swelling and cell cycle activation of neonatal prominin+ precursors. Stem Cells 29: 307-319
Costa MR, Ortega F, Brill MS, Beckervordersandforth R, Petrone C, Schroeder T, Gotz M, Berninger B (2011) Continuous live imaging of adult neural stem cell division and lineage progression in vitro. Development 138: 1057-1068
Engler A, Rolando C, Giachino C, Saotome I, Erni A, Brien C, Zhang R, Zimber-Strobl U, Radtke F, Artavanis-Tsakonas S et al (2018) Notch2 Signaling Maintains NSC Quiescence in the Murine Ventricular-Subventricular Zone. Cell Rep 22: 992-1002
Joppe SE, Cochard LM, Levrós LC, Jr., Hamilton LK, Ameslon P, Aumont A, Barnabe-Heider F, Fernandes KJ (2020) Genetic targeting of neurogenic precursors in the adult forebrain ventricular epithelium. Life Sci Alliance 3
Khatri P, Obernier K, Simeonova IK, Hellwig A, Hözl-Wenig G, Mandl C, Scholl C, Wolfli S, Winkler J, Gaspar JA et al (2014) Proliferation and cilia dynamics in neural stem cells prospectively isolated from the SEZ. Sci Rep 4: 3803
Kriegstein A, Alvarez-Buylla A (2009) The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci 32: 149-184
Llorens-Bobadilla E, Zhao S, Baser A, Saiz-Castro G, Zwadlo K, Martin-Villalba A (2015) Single-Cell Transcriptomics Reveals a Population of Dormant Neural Stem Cells that Become Activated upon Brain Injury. Cell Stem Cell 17: 329-340
Luque-Molina I, Shi Y, Abdullah Y, Monaco S, Holzl-Wenig G, Mandl C, Ciccolini F (2019) The Orphan Nuclear Receptor TLX Represses Hes1 Expression, Thereby Affecting NOTCH Signaling and Lineage Progression in the Adult SEZ. *Stem Cell Reports* 13: 132-146

Merkle FT, Mirzadeh Z, Alvarez-Buylla A (2007) Mosaic organization of neural stem cells in the adult brain. *Science* 317: 381-384

Monaco S, Baur K, Hellwig A, Hözl-Wenig G, Mandl C, Ciccolini F (2019) A Flow Cytometry-Based Approach for the Isolation and Characterization of Neural Stem Cell Primary Cilia. *Front Cell Neurosci* 12

Obernier K, Cebrian-Silla A, Thomson M, Parraguez JI, Anderson R, Guinto C, Rodas Rodriguez J, Garcia-Verdugo JM, Alvarez-Buylla A (2018) Adult Neurogenesis Is Sustained by Symmetric Self-Renewal and Differentiation. *Cell Stem Cell* 22: 221-234 e228
Dear Dr. Ciccolini

Thank you for the submission of your revised manuscript to EMBO Reports. As my colleague Esther Schnapp is currently travelling, I have temporarily taken over the handling of your manuscript.

We have meanwhile received the full set of referee reports (copied below). As you will see, both referees find that the study has been significantly improved during revision and recommend publication. Please address the remaining concerns from referee #2 with a discussion of the technical limitations regarding lineage tracing and Notch antibody staining in the manuscript.

From the editorial side, there are also a few things that we need before we can proceed with the official acceptance of your study.

1) Please include a ‘Disclosure and competing interests statement’. For more information see https://www.embopress.org/page/journal/14693178/authorguide#conflictofinterest

2) Regarding the Author Contributions, we now use CRediT to specify the contributions of each author in the journal submission system. CRediT replaces the author contribution section. Please enter this information in the submission system and you can also use the free text box to provide more detailed descriptions, if you wish. See also our guide to authors https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines.

3) Our editorial policies do not allow to cite ‘data not shown’. Please either provide the corresponding data for the statement on page 9 or remove the conclusion.

4) Please note that a Data Availability section at the end of Materials and Methods is now mandatory. I note that you have performed transcriptomics using a microarray and these data should be deposited in an appropriate public database. Please see our guide to authors for further information: <https://www.embopress.org/page/journal/14693178/authorguide#dataavailability>

5) APPENDIX:
   a) Please combine all supplementary information into a single pdf file called "Appendix". The Appendix starts with a table of content on the first page (incl. page numbers) and contains all figures, their legends and the tables. Please also include Supplementary table S1 and change its name to Appendix table S1.
   b) Please also change all nomenclature to 'Appendix Figure S#' and 'Appendix Table S#'.
   c) Supplementary table S2 is a complex dataset. Please call it 'Dataset EV1'. Otherwise, the format as .xls file with a legend within the file is fine.

6) APPENDIX FIGURE LEGENDS:
   a) When you describe the results of a quantification, please make sure to specify the number of experiments (n) and whether this relates to biological/independent or technical replicates. This applies to all panels showing quantification in the Appendix (e.g. S2C, S2D, S2F, S3A, B etc).
   b) Please use “Data information” when you describe attributes that apply to more than one panel in the figure. E.g. for Figure S1: “Data information: Bars represent mean ± SEM, * indicate significance *p<0.05”
   c) Please add the nature of the statistical test used to the description.
   d) Figure S9: please remove ‘***p<0.001” as this p-value is not shown in the figure.

7) FIGURE CALLOUTS:
   a) Appendix Figs S3F, S5F+H, S9D callout is missing. Please add this in the relevant section.
   b) The callout to Appendix Fig S10 needs correcting to Appendix Fig S9
   c) Callouts to Supplementary Tables S3 + S4 callouts are missing in the manuscript. They should also be renamed as Appendix Table S2 and S3 (see above) and the note in the Author checklist needs to be updated (Antibodies)
   d) Fig 6B callout is missing.

8) We generally recommend arranging the figures in a manner that the individual panels can be called out in an alphabetical order. In this context we noticed that Fig 1C is called out after 1F. Maybe these two panels can be swapped in the figure.

9) I attach to this email a related manuscript file with comments by our data editors. Please address all comments and upload a revised file with tracked changes with your final manuscript submission. I also introduced some minor changes to the Abstract.

10) Finally, EMBO reports papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is 550x200-600 pixels large (width x height) in .png format. You can either show a model or key data in the synopsis image. Please note that the size is rather small and that text needs to be readable at the final size. Please send us this information along with the revised manuscript.
We look forward to seeing a final version of your manuscript as soon as possible.

With kind regards,

Martina Rembold, PhD
Senior Editor
EMBO reports

*******************

Referee #2:

In their revised manuscript Baur et al. have made an attempt to address most of my concerns in the "response to reviewers" letter and by adding additional data. The manuscript has, in my opinion, improved. The lack of definitive lineage tracing of the basal cell population is still a weak point but it seems that the authors are not able, with the tools they have in hand, to conclusively address this issue. In my opinion, the NICD data differences in lactating females are still questionable but I accept that it is extremely difficult to quantify Notch signaling with antibody staining.

Referee #3:

the authors have answered most of this reviewer's concerns.
Point-by-point response 22.06.22

1. If there is no data to deposit then this text should be added at the end of the Materials & Methods section: "This study includes no data deposited in external repositories." OR "No primary datasets have been generated and deposited."

The section “Data availability” with this statement has been added to the Materials and Methods section.

2. Supplementary Table S2 should be called Dataset EV1.

Dataset EV1 has been renamed in the entire text. The reference to Appendix Table S2 in the Materials and Methods section refers to the previous Supplementary Table S3.

3. Appendix Table S1 callout is missing the 'S'. Also add a callout to Dataset EV1.

S has been added to appendix table callouts. Callout to dataset EV1 is present on page 5, line 9 and page 10, line 21.

4. In the Appendix file please use the naming 'Appendix Figure S#' 'Appendix Table S#

“Appendix” has been added to all figure and table legends.

5. Appendix Fig S2F: does N=20 mean 20 cells were analyzed, in which case the number of independent experiments needs to be added, or was the experiment really repeated 20 times?

N = 20 means that the experiment was performed on cells from 20 individual animals. A single data point is a percentage of ciliated cells of all the sorted cells per population from one animal; the bar represents an average of these values from 20 animals.

6. For Appendix Fig S3I, when N=2 the error bars need to be removed, and only the single data points and their mean can be shown.

The error bars were removed for the bars with N = 2.
Dear Francesca,

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

Please note that under the DEAL agreement of German scientific institutions with our publisher Wiley, you could be eligible for publication of your article in the open access format in a way that is free of charge for the authors. Please contact either the administration at your institution or our publishers at Wiley (emboreports@wiley.com) for further questions.

At the end of this email I include important information about how to proceed. Please ensure that you take the time to read the information and complete and return the necessary forms to allow us to publish your manuscript as quickly as possible.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. As you are aware, this File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: emboreports@embo.org]. If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case." Please note that the author checklist will still be published even if you opt out of the transparent process.

Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

Best,

Esther Schnapp, PhD
Senior Editor
EMBO reports

THINGS TO DO NOW:

Please note that you will be contacted by Wiley Author Services to complete licensing and payment information. The required 'Page Charges Authorization Form' is available here: https://www.embopress.org/pb-assets/embo-site/er_apc.pdf - please download and complete the form and return to embopressproduction@wiley.com

You will receive proofs by e-mail approximately 2-3 weeks after all relevant files have been sent to our Production Office; you should return your corrections within 2 days of receiving the proofs.

Please inform us if there is likely to be any difficulty in reaching you at the above address at that time. Failure to meet our deadlines may result in a delay of publication, or publication without your corrections.

All further communications concerning your paper should quote reference number EMBOR-2021-54078V3 and be addressed to emboreports@wiley.com.

Should you be planning a Press Release on your article, please get in contact with emboreports@wiley.com as early as possible, in order to coordinate publication and release dates.
EMBO Press Author Checklist

Corresponding Author Name: Francesca Ciccioli
Journal Submitted to: Embo Reports
Manuscript Number: EMBO-2021-54078V2

Reporting Checklist for Life Science Articles (updated January 2022)
This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal’s guidelines in preparing your manuscript.

Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for figures
1. Data
The data shown in figures should satisfy the following conditions:
- The data were obtained and processed according to the field’s best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- Ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- Plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- If available, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data Presentation.

2. Captions
Each figure caption should contain the following information, for each panel where they are relevant:
- A specification of the experimental system investigated (e.g. cell line, species name).
- The assay(s) and method(s) used to carry out the reported observations and measurements.
- An explicit mention of the biological and chemical entity(ies) that are being measured.
- An explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- The exact sample size (n) for each experimental group/condition, given as a number, not a range;
- A description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- A statement of how many times the experiment shown was independently replicated in the laboratory.
- Definitions of statistical methods and measures:
  - Common tests, such as t-test (please specify whether paired vs. unpaired), simple χ² tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - Are tests one-sided or two-sided?
  - Are there adjustments for multiple comparisons?
  - Exact statistical test results, e.g., P values = x but not P values < x;
  - Definition of ‘center values’ as median or average;
  - Definition of error bars as s.d. or s.e.m.

Please complete ALL of the questions below. Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

| Newly Created Materials | Information included in the manuscript? | In which section is the information available? |
|-------------------------|-----------------------------------------|-----------------------------------------------|
| New materials and reagents need to be available; do any restrictions apply? | Not Applicable | (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |

| Antibodies | Information included in the manuscript? | In which section is the information available? |
|------------|-----------------------------------------|-----------------------------------------------|
| For antibodies provide the following information: Commercial antibodies: RRID (if possible) or supplier name, catalogue number and clone number. Non-commercial RRID or citation. | Yes | Appendix Tables S2 and S3 |

| DNA and RNA sequences | Information included in the manuscript? | In which section is the information available? |
|-----------------------|-----------------------------------------|-----------------------------------------------|
| Short novel RNA or DNA including primers, probes; provide the sequences. | Not Applicable | (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |

| Cell materials | Information included in the manuscript? | In which section is the information available? |
|----------------|-----------------------------------------|-----------------------------------------------|
| Cell lines: Provide species information, strain. Provide accession number in repository OR supplier name, catalogue number, clone number, and OR RRID. | Not Applicable | (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |

| Primary cultures | Information included in the manuscript? | In which section is the information available? |
|------------------|-----------------------------------------|-----------------------------------------------|
| Provide species, strain, sex of origin, panels modification status. | Not Applicable | Materials and Methods |

| Experimental animals | Information included in the manuscript? | In which section is the information available? |
|----------------------|-----------------------------------------|-----------------------------------------------|
| Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository OR supplier name, catalogue number, clone number, OR RRID. | Yes | Materials and Methods |

| Animal observed in or captured from the field: Provide species, sex, and age where possible. | Not Applicable | Materials and Methods |

| Please detail housing and husbandry conditions. | Information included in the manuscript? | In which section is the information available? |
|-----------------------------------------------|-----------------------------------------|-----------------------------------------------|
| | Yes | Materials and Methods |

| Plants and microbes | Information included in the manuscript? | In which section is the information available? |
|--------------------|-----------------------------------------|-----------------------------------------------|
| Provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens). | Not Applicable | (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |

| Microbes: provide species and strain, unique accession number if available, and source. | Information included in the manuscript? | In which section is the information available? |
|------------------------------------------------|-----------------------------------------|-----------------------------------------------|
| | Not Applicable | (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |

| Human research participants | Information included in the manuscript? | In which section is the information available? |
|----------------------------|-----------------------------------------|-----------------------------------------------|
| If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants. | Not Applicable | (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |

| Core facilities | Information included in the manuscript? | In which section is the information available? |
|----------------|-----------------------------------------|-----------------------------------------------|
| If your work benefited from core facilities, was their service mentioned in the manuscript? | Yes | Acknowledgements |

USEFUL LINKS FOR COMPLETING THIS FORM
- The EMBO Journal - Author Guidelines
- EMBO Reports - Author Guidelines
- Molecular Systems Biology - Author Guidelines
- EMBO Molecular Medicine - Author Guidelines

Design
### Study protocol

Information included in the manuscript: [Material and Methods]

| In which section is the information available? | (Materials and Methods) |
|-----------------------------------------------|------------------------|

If study protocol has been pre-registered, provide DOI in the manuscript. For clinical trials, provide the trial registration number OR cite DOI.

Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.

Laboratory protocol

Information included in the manuscript: [Materials and Methods]

| In which section is the information available? | (Materials and Methods) |
|-----------------------------------------------|------------------------|

Provide DOI or other data details if external detailed step-by-step protocols are available.

### Experimental study design and statistics

Information included in the manuscript: [Material and Methods]

| In which section is the information available? | (Material and Methods) |
|-----------------------------------------------|------------------------|

Include a statement about sample size estimate even if no statistical methods were used.

Were any steps taken to minimize the effects of subject bias when allocating animals/tissue to treatment (e.g. randomization procedure)? If yes, have they been described?

Replicates: Include a statement about blinding even if no blinding was done.

Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?

If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.

For every figure, are statistical tests justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess if there is an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?

### Sample definition and in-laboratory replication

Information included in the manuscript: [Figure legends]

| In which section is the information available? | (Figure legends) |
|-----------------------------------------------|------------------|

In the figure legends: state number of times the experiment was replicated in laboratory.

In the figure legends: define whether data describes technical or biological replicates.

### Ethics

Information included in the manuscript: [Materials and Methods]

| In which section is the information available? | (Materials and Methods) |
|-----------------------------------------------|------------------------|

Studies involving human participants: State details of authority granting ethics approval (IRB or equivalent committee(s)), provide reference number for approval.

Studies involving human participants: Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.

Studies involving human participants: For publication of patient photos, include a statement confirming that consent to publish was obtained.

Studies involving experimental animals: State details of authority granting ethics approval (IRB or equivalent committee(s)), provide reference number for approval. Include a statement of compliance with ethical regulations.

Studies involving specimen and field samples: State if consent was obtained, provide details of authority approving study, if none were required, explain why.

### Dual Use Research of Concern (DURC)

Information included in the manuscript: [Materials and Methods]

| In which section is the information available? | (Materials and Methods) |
|-----------------------------------------------|------------------------|

Could your study fall under dual use research restrictions? Please check biosafety documents and list of select agents and toxins (CDC): [https://www.selectagents.gov/]

If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?

If a study is subject to dual use research of concern regulations, is the name of the authority granting approval and reference number for regulatory approval provided in the manuscript?

### Data availability

Information included in the manuscript: [Materials and Methods]

| In which section is the information available? | (Materials and Methods) |
|-----------------------------------------------|------------------------|

Have primary datasets been deposited according to the journal's guidelines (see "Data Deposited" section) and the respective accession numbers provided in the Data Availability Section?

Have human clinical and genomic datasets deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?

Are computational models that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?

If publicly available data were reused, provide the respective data citations in the reference list.

### Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

Adherence to community standards

Information included in the manuscript: [Material and Methods]

| In which section is the information available? | (Material and Methods) |
|-----------------------------------------------|------------------------|

State if relevant guidelines or checklists (e.g., IMUJE, MIBBI, ARRIVE, PRISMA) have been followed or provided.

For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link at top right). See author guidelines, under "Reporting Guidelines". Please confirm you have followed these guidelines.

For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link at top right) and submit the CONSORT checklist (see link at top right) with your submission. See author guidelines, under "Reporting Guidelines". Please confirm you have submitted this list.

### References

Information included in the manuscript: [References]

| In which section is the information available? | (References) |
|-----------------------------------------------|-------------|

If publicly available data were reused, provide the respective data citations in the reference list.