EMBO reports

RAB6 and Dynein drive post-Golgi apical transport to prevent neuronal progenitor delamination

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1st Editorial Decision 12th Jan 2022

Dear Alexandre,

Thank you for the submission of your research manuscript to our journal. We consider your data on the role of RAB6A/B in aRG cell delamination and in preventing microcephaly of potential interest for EMBO Reports and would like to invite you to revise your study based on the referee reports from Review Commons, as discussed and outlined in your revision plan. I overall feel that the focus of the revised manuscript could lie more on the novel aspects and phenotypes, i.e, that the loss of RAB6A/B causes microcephaly and aRG delamination during interphase, with less emphasis on e.g. the initial characterization of post-Golgi apical trafficking (Figure 1).

Taken together, we would like to invite you to revise your manuscript with the understanding that the referee concerns must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (April 12th, 2022). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions.

You can either publish the study as a short report or as a full article. For short reports, the revised manuscript should not exceed 27,000 characters (including spaces but excluding materials & methods and references) and 5 main plus 5 expanded view figures. The results and discussion sections must further be combined, which will help to shorten the manuscript text by eliminating some redundancy that is inevitable when discussing the same experiments twice. For a normal article there are no length limitations, but it should have more than 5 main figures and the results and discussion sections must be separate. In both cases, the entire materials and methods must be included in the main manuscript file.

IMPORTANT NOTE:
We perform an initial quality control of all revised manuscripts before re-review. Your manuscript will FAIL this control and the handling will be DELAYED if the following APPLIES:

1) A data availability section providing access to data deposited in public databases is missing. If you have not deposited any data, please add a sentence to the data availability section that explains that.

2) Your manuscript contains statistics and error bars based on n=2. Please use scatter blots in these cases. No statistics should be calculated if n=2.

When submitting your revised manuscript, please carefully review the instructions that follow below. Failure to include requested items will delay the evaluation of your revision.

When submitting your revised manuscript, we will require:

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).
Please download our Figure Preparation Guidelines (figure preparation pdf) from our Author Guidelines pages https://www.embopress.org/page/journal/14693178/authorguide for more info on how to prepare your figures.

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) a complete author checklist, which you can download from our author guidelines (). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines
()

6) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online.

A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc... in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here:

- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

- Movies are called "Movie EV#". The legend is provided as a separate text file (README) and zipped together with the movie. The ZIP file is uploaded.

7) Please note that a Data Availability section at the end of Materials and Methods is now mandatory. In case you have no data that requires deposition in a public database, please state so instead of refereeing to the database.
See also < https://www.embopress.org/page/journal/14693178/authorguide#dataavailability>). Please note that the Data Availability Section is restricted to new primary data that are part of this study.

8) Figure legends and data quantification:
The following points must be specified in each figure legend:
- the name of the statistical test used to generate error bars and P values,
- the number (n) of independent experiments (please specify technical or biological replicates) underlying each data point,
- the nature of the bars and error bars (s.d., s.e.m.)
- If the data are obtained from n {less than or equal to} 2, use scatter blots showing the individual data points.

Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied.
See also the guidelines for figure legend preparation:
https://www.embopress.org/page/journal/14693178/authorguide#figureformat
- Please also include scale bars in all microscopy images.

9) We would also encourage you to include the source data for figure panels that show essential data. Numerical data should be provided as individual .xls or .csv files (including a tab describing the data). For blots or microscopy, uncropped images should be submitted (using a zip archive if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available .

10) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at .

11) As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. 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.

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We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

I look forward to seeing a revised form of your manuscript when it is ready and please let me know if you have questions or comments regarding the revision.

You can use this link to submit your revision: https://embor.msubmit.net/cgi-bin/main.plex

Kind regards,

Martina

Martina Rembold, PhD
Senior Editor
EMBO reports

Reviewer #1 (Evidence, reproducibility and clarity (Required)):

In this manuscript, Brault and co-workers characterized the role of the Rab6-dynein-Lis complex in the neuronal progenitor delamination through its role in post-Golgi apical transport. The main finding of this study is that, in aRG cells, post-Golgi apical trafficking occurs in the microtubule minus-end direction via the RAB6-dynein-LIS1 complex and is required for the apical localization of the Crumbs complex. Consequently, genetic inactivation of RAB6A/B or LIS1 leads to CRB3 loss at the ventricular surface and delamination of aRG cells, which adopt features of bRG-like cells, including the ability to proliferate. Due to my expertise, it is difficult for me to identify the importance of the work concerning the development of the neocortex. Therefore, I will focus on the part of the study more related to protein trafficking, which is more my speciality.
Overall it is a job well done, showing the ability to use a neuroepithelial model to study transport events in situ efficiently and powerfully. However, some critical aspects of this work should be addressed before publication:
We thank the reviewer for his/her overall positive evaluation of our work.

-Cells treated with mCherry presented a reduced number of vesicles/cell and reduced time compared to DMSO treated cells, is there any reason for these differences in the controls?
We have observed in the past that DMSO can affect various dynamic processes, and this is why we always control for drug treatments with equivalent amounts of DMSO. Here, more vesicles per apical process and more apical movement could indicate an effect of DMSO on the opposite (presumably kinesin-based) movement. We have added a sentence in the legends highlighting this.

-The authors use p-VIM to detect mitotic RG cells. Is this a good marker for mitotic cells? They should include another marker and a reference for this claim.
Phospho-Vimentin is indeed a classical marker for mitotic RG cells. Vimentin is specific to RG cells, and is only phosphorylated during mitosis. To validate this, we have analyzed P-VIM/PAX6 stainings and measured that 100% of P-VIM+ cells are positive for PAX6. We have also analyzed P-VIM/pH3 stainings and measures that 93.24% (+/- 3.8%) of P-Vim+ cells are positive for pH3. We did not include this data in the manuscript, but have added references for the use of the p-VIM marker.

-Apical trafficking of Crumb3 has been described before using the RUSH system in epithelial cells by some of the authors of the current work. Indeed, the kinetics of Crumb3 transport to the apical membrane is very similar. This reference should be appropriately cited in the manuscript
This critical reference was in a previous version of the manuscript and removed by error. It has now been added back.

-The colocalization analysis performed in HeLa cells should be reproduced in aRG neurons as the authors have the appropriate tools to perform this analysis
We have now analyzed SBP-CRB3/RAB6 colocalization 40 minutes after biotin release in aRG cells. To increase the relatively weak RAB6 signal in the red channel, we used a TagRFP construct instead of the mCherry one (a gift from Yuko Mimori-Kiyosue, Riken Center, Japan). We first performed these experiments in 2D primary cultures of dissociated aRG cells. These experiments revealed a high colocalization 40 minutes after release (~75%) (Figures EV3D & E). We next validated that this colocalization also occurred within the tissue, a much more challenging setting. 40 minutes after release, we observed a substantial colocalization between SBP-CRB3 and TagRFP-RAB6 (~60%) (Figures EV3F &G). The colocalization appeared slightly lower than *in vitro*, likely due to the dim TagRFP-RAB6 signal. Together, these experiments confirm that CRB3 exits the Golgi of aRG cells within RAB6+ vesicles.

-This work is excellent; as mention before, however, it is essential not to draw conclusions that are not derived from the results obtained. One thing is the location of Crb3 in the apical membrane, the steady-state localization, which is manifestly affected by the disruption of genes of the pathway (LIS1 / Rab6 / Dynein), and something else different is how the disruption of these proteins affects their

biosynthetic transport to the apical membrane, which is only analyzed with the negative dominant of p150. The apical traffic phenotype with the negative dominant of p150 is very striking; however, the effect on the apical location of CRB3 is less clear. Bearing in mind that DN constructs often have unwanted effects, perhaps the authors should reconsider performing some experiment that demonstrates the role of Rab6 / Dynein / lis1 in apical Crb3 traffic.
Indeed, the authors suggested in the discussion that Rab6/dynein/Lis1 might have a role in Crb recycling (Lines 312-315)
We understand the reviewer's comment but point out that we already provide a body of highly-challenging *in vivo* experiments to support our model. We show here that:
- *LIS1* KO affects apical localization of CRB3 at steady-state.
- p150 dominant-negative and dynarrestin affect apical trafficking of RAB6+ vesicles (and now that CRB3 exists the Golgi within these vesicles in aRG cells).
- P150 dominant-negative affects apical transport of CRB3 to the apical surface (using the RUSH system). This latter result is very clear, with a major impairment in CRB3 localization to the apical surface. This is unlikely to represent a recycling defect, as CRB3 does not reach the apical surface in the first place.

-It was proposed that the effect of the neuronal delamination phenotype is associated with defects in the cellular junctions, possibly derived from defects in Crb3 (line 228-229) This correlates very well with the role proposed for Crb3 in othe epithelial models. However, it is not analyzed anywhere if the integrity of the cellular junctions is affected in the Rab6 / Dynein / Lis1 mutants, and although it is likely happening, it should be formally analyzed.
This is indeed a very important piece of evidence that was missing from our study. We generated novel *RAB6 A/B* dKO and *LIS1* KO embryos and analyzed integrity of their apical junctions by staining for N-Cadherin. We show that, similarly to the CRB3 apical localization defects, mutant embryos display frequent interruptions of the N-cadherin staining along the ventricular lining, which moreover appear very disorganized (Figures 5G & H). These results support a model whereby altered apical trafficking of CRB3 leads to a destabilization of the apical junctions, and to cell delamination.

Reviewer #1 (Significance (Required)):
Due to my expertise, it is difficult for me to identify the importance of the work concerning the development of the neocortex. Therefore, I will focus on the part of the study more related to protein trafficking, which is more my speciality.

Reviewer #2 (Evidence, reproducibility and clarity (Required)):

Summary
The authors identify RAB6A/B as a major player of post-Golgi traffic in aRG cells, suggesting that alterations of this traffic route lead to delamination, that is, the detachment of an aRG from the apical junctional belt.

General comment
The mechanisms governing traffic neural stem cells in the developing brain are largely unknown. This applies to (i) the mechanisms responsible for keeping the aRG architecture, as well as to (ii) the mechanisms driving delamination, a crucial cell biological process occurring during fate transition. From this point of view, the authors gather convincing, technically challenging evidences that contributes to elucidate the cell biological and molecular mechanisms governing traffic in aRG during brain development.
We thank the reviewer for his/her overall positive evaluation of our work.

A point where the authors are falling short is the ability to convincingly correlate the intracellular traffic route they dissect with fate transition and cell identity. I find particularly weak the claim that the cells generated upon RAB6A/B KO are bRG. From the data they presented, the basal dividing

cells might as well be intermediate progenitors (IPs) expressing Pax6. If the authors cannot provide more convincing data regarding the bRG identity of the basal-dividing-Pax6-expressing cells, then I think It would be safer to call the ectopic cells just basal progenitors (BPs).
We thank the reviewer for raising this very important point, which we agree needed clarification. We have now performed a number of experiments to better define the ectopic PAX6+ cells:
TBR2 staining: We do not observe an increase in subventricular TBR2+ cells, within *RAB6* dKO or *LIS1* KO cortices. Furthermore, we now show that the majority of ectopic progenitors are negative for TBR2. In *RAB6* dKO, 98.1% of ectopic PAX6+ cells are negative for TBR2 (Figures EV1C & D) and 100% of ectopic P-VIM+ cells are negative for TBR2 (Figures EV1E & F). Finally, we confirm this result in *LIS1* KO by showing that the majority of ectopic P-VIM+ cells are negative for TBR2 (89,1% of ectopic cells) (Figures EV4A & B).
Presence of a basal process at mitosis: Based on the phospho-Vimentin stainings (which highlights RG cell morphology at mitosis), we now quantify the percentage of ectopic p-VIM+ cells displaying a basal process. This analysis confirms that the majority of these cells have lost their basal process (92,38% in *RAB6 A/B* dKO and 95,3% in *LIS1* KO) (Figures EV1G & EV4C).
Occurrence of MST: Because delaminated RG cells largely lack a basal process, MST (which is the translocation of the soma into the basal process) cannot occur in these cells.
In conclusion, our results indicate that delaminated cells are PAX6+, TBR2-, lack a basal process, do not perform MST, and proliferate at normal rates. We therefore now refer to these cells as delaminated aRG cells, basal progenitors or ectopic progenitors throughout the manuscript. We speculate in the discussion about how apical transport may participle in aRG cell delamination and bRG cell generation. We also discuss the putative causes of basal process destabilization.

Below are more specific comments.

Line 185
The authors refer to ectopic Pax6 positive cells as RG cells.
Conceptually, it would be best to call these cells just "ectopic Pax6 expressing cells", or "non-ventricular Pax6 expressing cells".
Because these cells remain TBR2-negative, we still call them ectopic RG cells, though not bRG-like cells anymore.

Line 193 (see also 336-337)
The authors state that cells "appeared to have retracted the basal process as well".
One of the main questions in the field of cell biology of aRG pertains to the mechanism regulating apical and basal process retraction, and if the two polarity cues are regulated separately.
It would be very interesting to show qualitative and quantitative data, to understand if RAB6A/B is governing the apical process only, or the basal one as well.
From a general point of view: if cells have retracted the basal process, then it is highly likely that cells generated upon RAB6A/B ablation are intermediate progenitors (IPs), not bRGs. The authors can refer to the work of Kriegstein, Huttner, Borrell labs to compare their description and dissection of bRG and IPs morphology and features.
We agree that morphology is a critical feature of bRG cells. As described above, we now quantify basal process retraction following P-VIM staining, which reveals that most ectopic cells have lost their basal process, though, surprisingly, remain TBR2-negative. In *RAB6* A/B dKO, we hypothesize that basal process retraction is a consequence of defective post-Golgi transport of integrins towards the basal surface, which we now discuss.

Line 204-206
This paragraph highlights one of the weaknesses of this paper: the authors never fully address the possibility that the changes they see are primarily a fate change or a change in lineage progression that if followed (rather than caused) by delamination.
Since the analysis is carried out 4 days after electroporation (as stated in line 201), this point cannot be fully addressed.
It would be best and more informative to dissect the effects of RABA/B KO after 24h, so that it would

be possible to understand if the primary effect is delamination, or if delamination is a secondary effect of cell fate change.
We agree that this experiment would have been informative if we had detected a cell fate change. However, since our new quantifications reveal no detectable fate difference between apical and basal PAX6+ cells (which are both TBR2-) cell fate change is unlikely to cause delamination. Our data rather suggest that the loss of cell polarity due to altered apical transport of Crumbs is the primary cause of the phenotype, as it leads to defective apical junctions (see new N-Cadherin stainings, as requested by reviewer 3, Figure 5), causing delamination of the cells.

Line 284
" aRG cells, which adopt features of bRG-like cells, including the ability to proliferate"

This statement is extremely interesting, but unfortunately, it is not fully supported by the data.
bRG is defined by a combination of criteria, marker expression, location of mitosis, and (most importantly) morphology at mitosis.
The authors did a great job in showing the first two features but are running short on the third, and in general of the morphological dissection of the RG generated upon genetic manipulation.
Given the type of data set the authors have generated I urge them:
- to look at the imaging data more deeply.
Do they see bRG generated?
Do they see basal diving cells keeping the basal process before and during mitosis?
Do they see any sign of MST (mitotic somal translocation)?
Please refer to Kriegstein's lab work for the definition of MST, the meaning of MST for bRG cells, imaging of MST, and relative quantifications.
- to run a deeper analysis of fixed samples.
In particular: 3-D reconstruct basal cells to check morphological features of basal-dividing Pax6 positive cells.
The authors should consider a better analysis of the p-Vimentin staining.
Using p-Vim, one can look for the presence/absence of the basal process at mitosis.
Regarding this point, the authors should refer to the work of the Huttner, Borrell and Kriegstein lab for the type of experiments and quantification needed to classify a cell as bRG.
Please note that these suggestions pertain to the analysis of already generated data.
Addressing these points would certainly increase the impact and significance of the manuscript, and would help to address its main weakness.
As described above, ectopic P-VIM+ cells were negative for TBR2, but did not display a basal process and therefore did not undergo MST. We have therefore modified this sentence to "*aRG cells, which maintain RG features, including fate and ability to proliferate*".

Reviewer #2 (Significance (Required)):

The reported findings shed light on the contribution of traffic to delamination, the crucial cell biological step responsible for the generation of basal-dividing neural stem cells in the developing brain.
To the best of my knowledge, this is the first report focusing on the functional role of post-Golgi traffic in such a process.
I find the cell biological dissection of traffic in aRG very convincing and informative.
In addition, I certainly appreciate the effort in setting up such a challenging experiment in a primary tissue context.
I do however find weak the claim regarding the bRG identity of the cells generated by RAB6A/B KO.
The author should present better pieces of evidence to support such a claim.

The audience that can benefit from this work
Cell biologists can learn how to apply and develop a basic cell biological question in tissue context.
Cell biology in tissue is certainly gathering more and more attention now. So, this manuscript would

provide valuable technical tools
Developmental Neuroscientist: in particular those interested in bringing cell biological approach to dev neuroscience/ neurogenesis

My field of expertise: cell biology of neurogenesis; brain development; brain evolution; neural stem cells; polarity

Reviewer #3 (Evidence, reproducibility and clarity (Required)):

This report documents evidence that apical membrane trafficking that is Rab6A and Rsb6B-dependent is also dynein-dependent and that compromise of this pathway leads to delamination of radial glia processes from the ventricular surface. One cargo of this pathway is Crumbs3 (an apical determinant). The authors also document that the delaminated radial glia maintain proliferative capacity and reside in the subventricular zone. They interpret these cells as basal radial glia that derive from apical radial glia.
In general, the manuscript has many strengths. It is comprehensive, and it leverages outstanding quantitative vital imaging with multiple independent approaches that include dominant negative approaches, chemical inhibition, multiple knockout mouse lines, sophisticated In utero electroporation technology to assess cell autonomous phenomena, and clever use of the powerful RUSH system to synchronize cargo export from the ER. The experiments are done well and the conclusions are supported by the data for the most part.
We thank the reviewer for his/her overall positive evaluation of our work.

However, there are several important weaknesses that must be addressed. These are outlined below:

(i) The authors use Pax6 and phospho-vimentin as markers to identify radial glial cells and this definition is used in data analyses throughout the MS. However, a significant fraction of intermediate progenitor cells (IPCs) also express these two markers; at least transiently. How do the authors know that a large fraction of their basal radial glial cells aren't in fact IPCs? Tbr2 staining is required to make that distinction to properly fate those cells. That determination must be done as this filter could have a significant effect not only on the quantifications presented throughout, but also on the interpretations of the data. This is a major issue with the MS.
We thank the reviewer for this important comment, which was also raised by reviewer 2. We have now performed these experiments, which show that ectopic PAX6+ cells as well as ectopic P-VIM positive cells are largely negative for TBR2 (Figures EV1C, D, E, F, EV4A & B). However, these cells lack a basal process and as a consequence cannot undergo MST. We therefore refer to these cells as ectopic RG cells, rather than bRG-like cells.

(ii) While Rab6 localization is well established in cell lines, and the RUSH system has been characterized in non-neuronal cells, radial glia are unusual cells. The authors should identify Golgi or ER compartments by co-staining for other Golgi or ER markers and not simply relying of Rab6 localization alone; which is what they do as far as this reviewer can determine. This can be done by post-staining of the brain slices. Minor issue.
We have first validated that the characteristic RAB6+ elongated structures below the nucleus were indeed the Golgi apparatus. *In utero* electroporation of GalNacT2-mCherry and GFP-RAB6A revealed a strong colocalization within these structures, confirming that Golgi positioning can be easily identified using RAB6A localization pattern (Figure 3D).
Next, we validated that CRB3 trafficking through the Golgi could indeed be identified based on its localization pattern. SBP-CRB3-GFP and GalNacT2-mCherry were co-*in utero* electroporated, and release was monitored following biotin incubation. At T0, SBP-CRB3-GFP did not colocalize with the Golgi, but rather had a diffuse perinuclear localization typical of endoplasmic reticulum staining. A T45, strong colocalization between SBP-CRB3-GFP and GalNacT2-Cherry was observed within the characteristic elongated structures localized below the nucleus (Figure EV3A).

(iii) Why monitor Crb3 as cargo when Crb1 is the dominant form in the brain? Crb3 knockout mice do not show delamination. Minor issue.
All three CRB isoforms are expressed in the mouse neocortex and are likely redundant in aRG cells, as demonstrated by the stronger phenotype of *CRB1* and *CRB2* dKOs, as compared to single KOs (Dudok et al, PMID: 26802325). Indeed, more basal mitosis (a sign, though not proof, of delamination) are observed in the dKO. We argue here that the molecular mechanism for apical trafficking of all CRB isoform is likely to be the same, and that the CRB3 construct used here for the RUSH assay can therefore be considered as a general read-out of CBR transport. Subtle differences in the dependency of CRB1, 2 and 3 for the RAB6-dynein apical trafficking pathway could indeed occur, although this is quite unlikely and would not change the overall message of the study.

Reviewer #3 (Significance (Required)):

The reported results outline a clear mechanism for Rab6 and dynein-dependent trafficking of apical determinant cargo in radial glia and show that perturbation of this pathway results in loss of apical contact of these cells with the ventricular surface. The delaminates cells retain proliferative capacity and are interpreted as basal radial glial cells. This work is novel and will be of interest to a broad cell and developmental biology audience.

Referee Cross-commenting

I generally agree with Reviewers 1 and 2 and it seems to me there is consensus regarding two major points that must be addressed. First, the cell lineage analysis is inadequate as it relies on PAX6 alone when both PAX6 band TBR2 must be queried to confidently distinguish RG from IPCs. The latter do express PAX6 transiently in a significant fraction of the population (esp new born IPCs) and I suspect a significant fraction of what they are calling bRGs are in fact IPCs. If true, this will materially change quantification and interpretation.

Second, I also agree the authors need to verify compartmental identities with other markers and not rely on associative arguments based on RAB6 localization patters from other cell types.

1st Revision - Editorial Decision **27th Jun 2022**

Dear Dr. Baffet

Thank you for the submission of your revised manuscript to EMBO reports. We have now received the full set of referee reports that is copied below.

As you will see, all referees are very positive about the study and request only minor changes to clarify the nomenclature.

Browsing through the manuscript myself, I noticed a few editorial things that we need before we can proceed with the official acceptance of your study.

- Your manuscript will be published in our Reports section. To make this possible, please adhere to the format for short reports, i.e., please combine the Results and Discussion section and keep an eye on our character limit (25,000 plus/minus 2,000 characters for the main text).

- Please add up to five keywords.

- Please add a 'Disclosure and competing interests statement' and please note that EMBO members must disclose their relationship with EMBO in the author disclosure statement using the standard phrase, "[Author] is an EMBO Member".
For more information see
https://www.embopress.org/page/journal/14693178/authorguide#conflictsofinterest

- Please note that the 'Data availability' section is restricted to new primary data that are deposited to a public database. Therefore, please remove the statements on 'reagents available on request' and state instead: No data that require deposition in a public database have been generated' (or something along these lines). I note that imaging data can be desposited on e.g. BioImage Archive (https://www.ebi.ac.uk/bioimage-archive/) or the Image Data Resource (https://idr.openmicroscopy.or/).

- 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. See also
https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines.
- In this context we note that the contribution of the co-author Jean-Baptiste Brault has not been defined in the online submission system.
- In the Author contribution section free text, the 2 SBs should be SBal and Sbar. Giuliana Victoria, Richard Belvindrah, Vincent Fraisier, Fiona Francis are missing.

- Please update the references to the alphabetical Harvard style. The abbreviation 'et al' should be used if more than 10 authors. You can download the respective EndNote file from our Guide to Authors
https://endnote.com/style_download/embo-reports/

- Our editorial policies do not permit the citation 'data not shown'. Therefore, please either provide the data for the statement on page 9 (line 287) or remove it.

- Figure callouts should be in alphabetical order. In this context we note that Fig. 1E is called out after 1G and recommend the reorganization of the figure panels, if possible.

- Movies: please remove the legends from the manuscript file and provide them as simple README.txt file. Then ZIP each movie with its legend and upload the zipped files.

- The manuscripts sections are in the wrong order.
Please consult our Guide to Authors for more information.

- Please add the heading 'Expanded View Figure Legends' to the respective section.

- Materials and methods: I recommend specifying the gRNAs used to generate the KO mouse to provide more information on the nature of the RAB6B knockout.

- 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.

- 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

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Referee #1:

The authors have addressed all the points raised by the reviewers.
I think the manuscript in its present form should be accepted for publication.
I still have a comment (that was already raised previously): I find the use of progenitor's name and nomenclature not very consistent. This should not prevent the acceptance of the manuscript, or call for another revision.

Referee #2:

The revised version of the MS adequately addresses the three points this referee raised in the initial review. The authors are to be commended for their positive response to the three reviews and clearly much work has been invested in addressing the collective comments. The authors have met every reasonable bar in the revision process. The original submission was judged to be of high quality, substantial novelty and of potentially high impact. The revised MS only strengthens that view. This is a fine body of work.

Referee #3:

In this new revised version of the manuscript, Brault and co-workers addressed most of the questions and requirements to characterize the role of the Rab6-dynein-Lis complex in the neuronal progenitor delamination through its role in post-Golgi apical transport. In particular, they have better defined the identity of the mitotic RG cells. They have better characterized the colocalization analysis in aRG neurons. Also, they have demonstrated that the effect of the neuronal delamination phenotype is associated with defects in the cell junctions (N-cad), possibly derived from defects in Crb3. Finally, they addressed other minor points I suggested in the previous manuscript version.
Referee Cross-commenting: I generally agree with Reviewers 2 and 3, and there is consensus regarding two significant points that must be addressed. First, the cell lineage analysis was inadequate. Second, I agree that the authors need to verify compartmental identities with other markers and the effect on cellular junctions. In my opinion, they have successfully addressed the reviewers' 2 and 3 comments.

In summary, I believe the manuscript has many strengths. It is comprehensive and combines outstanding quantitative vital imaging with the use of the powerful RUSH system to synchronize cargo export from the ER. This is accompanied by multiple independent approaches to assess a singular cell phenomenon. The experiments are done rigorously, and the data support the conclusions. I believe it is now ready for publication in EMBO R.

Embo Reports

To Dr Martina Rembold,

We wish to submit the revised version of our manuscript, entitled "**Regulation of neuronal progenitor delamination by RAB6 and dynein-driven apical transport**" to Embo Reports. We have addressed all editorial points and slightly modified the title. We have also further addressed the nomenclature by removing the term "bRG-like cells".

Finally, we have prepared the requested information:

Summary of the findings and their significance (1-2 sentences)
Brault et al. identify a RAB6-Dynein-LIS1 complex controlling post-Golgi apical transport of CRUMBS in neuronal progenitors. Impairment of this pathway alters apical junctions, causes a delamination of these epithelial cells, and leads to the formation of basal progenitors

Bullet points (2-3)

- RAB6-Dynein-LIS1 control post-Golgi transport of CRUMBS to the apical surface of apical progenitors.
- This transport pathway is essential for the maintenance of apical junctions.
- Alteration of this transport pathway leads to the generation of basal progenitors.

5 keywords
Neocortex development; Polarized trafficking; Cell polarity; RAB6; Dynein

Sincerely,

Alexandre Baffet, PhD
Group leader, **Institut Curie**
Department of Cell Biology - UMR144
12 rue Lhomond - Pavillon Burg

2nd Revision - Editorial Decision 25th Jul 2022

Dr. Alexandre Baffet
Institut Curie, PSL Research University, CNRS UMR144
12 rue Lhomond
Paris 75005
France

Dear Alexandre,

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

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.

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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.

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.

Kind regards,

Martina

Martina Rembold, PhD
Senior Editor
EMBO reports

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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 n<5, 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 (eg 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 $\chi 2$ 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.

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Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Yes	Material and methods

Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Yes	Material and methods

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Short novel DNA or RNA including primers, probes: provide the sequences.	Not Applicable	

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Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Not Applicable	

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Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
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Include a statement about **sample size** estimate even if no statistical methods were used.	Yes	Material and methods
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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.	Yes	Material and methods
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