Breasi-CRISPR: an efficient genome-editing method to interrogate protein localization and protein-protein interactions in the embryonic mouse cortex

Brandon L. Meyerink, Pratiksha KC, Neeraj K. Tiwari, Claire M. Kittock, Abigail Klein, Claire M. Evans and Louis-Jan Pilaz
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Original submission

First decision letter

MS ID#: DEVELOP/2022/200616

MS TITLE: Breasi-CRISPR: an efficient genome editing method to interrogate protein localization and protein-protein interactions in the embryonic mouse cortex

AUTHORS: Brandon L Meyerink, Pratiksha KC, Neeraj K Tiwari, Claire M Kittock, Abigail Klein, Claire M Evans, and Louis-Jan Pilaz

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

As you will see, the two referees express great interest in your work, but they also have significant criticisms and recommend a substantial revision of your manuscript before we can consider publication. In particular, referee 1 comment on the need to perform controls for the genome editing experiments. If you are able to revise the manuscript along the lines suggested by the referees, which may involve further experiments, I will be happy to receive a revised version of the manuscript. Your revised paper will be re-reviewed by the original referees, and its acceptance will depend on your addressing satisfactorily all their major concerns. Please also note that Development will normally permit only one round of major revision.

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

Reviewer 1

Advance summary and potential significance to field

Meyerink et al. report a CRISPR/Cas9-based method to tag proteins by in utero electroporation. They combine two approaches previously used in the field: tagging proteins via in utero electroporation (Mikuni et al., 2016) with the in utero electroporation of recombinant Cas9 in a complex with gRNA (Kalebic et al., 2016).
They deliver the sequence of the tag as a separate ss DNA as reported previously in the Easi-CRISPR approach (Quadros et al., 2017) and they name their method Breasi-CRISPR. The approach seems to have a high efficiency of genome editing. The authors show several different applications of Breasi-CRISPR which nicely displays the potential of the technique. However, several key controls are missing to fully appreciate the possibilities of the technique.

Comments for the author

1. The authors typically only report genome editing without any control. They should instead use control gRNAs (such as scrambled) and/or use incorrect gRNA/ssDNA pairs.
2. The authors generally report the effects of their genome editing only by showing the immunostaining for the tag. Only once do they show staining for the actual protein that has been tagged (actin). It is important to show immunostaining for the protein itself also to understand if the protein is still functionally expressed and to be able to assess the level of expression in targeted vs non-targeted cells (see also next comment).
3. The authors should address a scenario that one allele has been successfully tagged and the other allele has a frameshifting mutation, which is common with genome editing and can lead to undesired phenotypes.
4. The authors claim that apoptosis and cell distribution are not affected.
These data should be shown at least in the supplemental figures.
5. The authors suggest that the cells that were MYC+ and GFP- might be a consequence of the dilution of the plasmid over cell division. Such a high percentage of these cells is indeed perplexing. The authors should perhaps check at an earlier time point post electroporation, taking into consideration the cell cycle length of neural progenitors which has been well studied. That could make their suggestion stronger

Reviewer 2

Advance summary and potential significance to field

In this paper, the authors adapted to the developing mouse brain easi-CRSPR, a technology first developed by Quadros et al for one-step generation of transgenic mice.
I do acknowledge that this technique adds to the panels of IUP tools available to developmental neurobiologists. However, I am not fully convinced that the advancement and novelty are such to justify the publication of the manuscript in its present form.

Comments for the author

- I did find surprising that the authors cite Quadros, but did not cite Artegiani et al with CRISPR-HOT. The work done in the Clevers lab is dealing with something very similar to what the authors show here, that is the CRISPR-mediated one step generation of edited organoids (tagging is also shown). Although the system is different, one cannot help noticing the obvious overlap in the conceptual approach. I urge the authors to cite Artegiani et al,. and to discuss similarities and differences between the two approaches.
- Since one of the claim is that this approach makes easier to visualize tagged proteins for imaging purpose, the authors should offer the reader better images with better resolution, and higher magnification. In its present form, panels are not very.
- Figure 5 E-G: the authors should show images of a convincing INM, spanning the the entire VZ. In their present form, the panels show a very small area of the VZ.

If the authors want to draw attention to INM and mitosis, then they must show a more convincing movie, or the same movie, but a larger area. A quantification of the nuclear trajectory would not hurt and would make the data more convincing.

- The authors mentioned that upon Breasi-CRISPR, the tagged protein show "surprising" localization in the basal process.
The authors must (i) comment on that, and (ii) check if the tagging did generate mislocalization.

First revision

Author response to reviewers' comments

Dear reviewers,
We thank you for taking the time to evaluate our manuscript “Breasi-CRISPR: an efficient genome editing method to interrogate protein localization and protein-protein interactions in the embryonic mouse cortex”. We are pleased that you found this study as potentially suitable for the journal Development. We are also grateful for your comments and suggestions, which will help us improve our manuscript. We have addressed each of these points as follows.

Responses to reviewer 1
1.The authors typically only report genome editing without any control. They should instead use control gRNAs (such as scrambled) and/or use incorrect gRNA/ssDNA pairs.
Response: We agree with the reviewer that this is an important control. We added a cohort of animals in which Breasi-CRISPR was performed with scrambled negative control crRNA (IDT 1079138) replacing target crRNA with all other conditions held the same. Breasi-CRISPR surgeries were performed with this control condition and Myc-Lmnb1 Breasi-CRISPR showing similar findings as the GFP-only experiments with no appreciable MYC signal appearing in the control tissues. These results were added to Figure 1. We also used these controls in immunoblot experiments with lysates collected two days after electroporation. This also showed absence of Myc-signal in control brain lysates (Figure 7B).

2. The authors generally report the effects of their genome editing only by showing the immunostaining for the tag. Only once do they show staining for the actual protein that has been tagged (actin). It is important to show immunostaining for the protein itself also to understand if the protein is still functionally expressed and to be able to assess the level of expression in targeted vs non-targeted cells (see also next comment).
Response: We performed additional immunofluorescence experiments using antibodies targeting endogenous proteins. We added images showing overlapping signal detected with both the MYC and HA antibody and antibodies detecting LMNB1 and FMRP, respectively (Fig S1). These indicate the tagged proteins are not only being made but retain localization of the endogenous protein. Protein expression concerns are addressed in the response to the following comment.

3.The authors should address a scenario that one allele has been successfully tagged and the other allele has a frameshifting mutation, which is common with genome editing and can lead to undesired phenotypes.
Response: In the initial submission, we reported that frameshift mutations were detected by amplicon sequencing at a frequency of 5.4% (Fig. 3K). We performed additional immunofluorescence experiments using antibodies targeting endogenous LMNB1 and quantified LMNB1 immunofluorescence signal in EGFP+ cells, comparing control and Myc-Lmnb1 Breasi-CRISPR-treated brains. These showed reduced expression of LMNB1 in EGFP+ cells. However, this decrease was minimal, 7% on average compared to control, and consistent with our amplicon sequencing results. We also looked at undesirable phenotypes and we did not observe mis-localization of the protein (Fig. S1) or indications of apoptosis and aberrant cellular migration (Fig. S2). Finally, in the discussion we now mention the scenario evoked by the reviewer.

4.The authors claim that apoptosis and cell distribution are not affected. These data should be shown at least in the supplemental figures.

Response: We now report this in Figure S2.
5.The authors suggest that the cells that were MYC+ and GFP- might be a consequence of the dilution of the plasmid over cell division. Such a high percentage of these cells is indeed perplexing. The authors should perhaps check at an earlier time point post electroporation, taking into consideration the cell cycle length of neural progenitors which has been well studied. That could make their suggestion stronger.
Response: We followed the reviewer's suggestion and measured the fraction of MYC+ GFP- cells within the MYC+ population one day after electroporation. In Figure S1A, we now report this data together with that previously shown two days and five days after electroporation. This shows a gradual increase in this fraction over time, which is in line with our initial suggestion.

Responses to reviewer 2
1. I did find surprising that the authors cite Quadros, but did not cite Artegiani et al with CRISPR-HOT. The work done in the Clevers lab is dealing with something very similar to what the authors show here, that is the CRISPR-mediated one step generation of edited organoids (tagging is also shown). Although the system is different, one cannot help noticing the obvious overlap in the conceptual approach. I urge the authors to cite Artegiani et al,. and to discuss similarities and differences between the two approaches.
Response: We would like to mention that the omission of the CRISPR-HOT study was entirely unintended. We agree that mentioning the CRISPR-HOT is relevant to ours and in particular when it comes to potential utilization of Breasi-CRISPR in brain organoids. Therefore we now mention and reference the CRISPR-HOT study in the discussion.

2. Since one of the claim is that this approach makes easier to visualize tagged proteins for imaging purpose, the authors should offer the reader better images, with better resolution, and higher magnification. In its present form, panels are not very.
Response: We agree with the reviewer that the reader could benefit from high-resolution images and this is probably mostly relevant for figure 2. We have now split the initial figure 2 into two figures (Fig. 2 and 3), in order to provide larger images.

3. Figure 5 E-G: the authors should show images of a convincing INM, spanning the the entire VZ. In their present form, the panels show a very small area of the VZ. If the authors want to draw attention to INM and mitosis, then they must show a more convincing movie, or the same movie, but a larger area. A quantification of the nuclear trajectory would not hurt and would make the data more convincing.
Response: In order to show better evidence of INM, we highlighted several EGFP-LMNB1+ nuclei in Movie S1 and we agree that quantifying the nuclear trajectory would be a valuable addition to the study. We show this in Fig. 6H and these results are consistent with those from the literature.

4. The authors mentioned that upon Breasi-CRISPR, the tagged protein show "surprising" localization in the basal process. The authors must (i) comment on that, and (ii) check if the tagging did generate mislocalization
Response: We assume that the reviewer is referring to the comments related to HA-EMD signal localization. Upon further search of the literature, we discovered that EMD was previously shown as a resident of the endoplasmic reticulum, which is consistent with our observed localization in apical radial glia processes, and within migrating neurons' leading processes, both known to be rich in endoplasmic reticulum. Therefore, we revised our manuscript to reflect this. Of note, as discussed in response to reviewer 1's points 2 and 3, we performed additional immunofluorescence experiments (for LMNB1 and FMRP) to show that Breasi-CRISPR has no overt effect on endogenous protein localization (see Figure S1).

Second decision letter

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

The reviewers' evaluation is positive and we would like to publish your manuscript in Development. However, before I formally accept your manuscript, could you address the remaining minor comments of the two referees. Please attend to these comments in your revised manuscript and detail them in your point-by-point response. If you do not agree with any of these suggestions, explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

The authors have addressed all my comments and I support the publication of this manuscript in Development. I only add a minor suggestion below.

Comments for the author

I have one minor comment for the authors to evaluate. In response to my previous comment #5 and the percentage of MYC+GFP- cells, the authors followed my suggestion and checked the % of these cells 24h after electroporation. This nicely corroborates the idea of a gradual dilution, but it still remains perplexing such a low co-expression of MYC and GFP at the earliest time point (Fig. S1A, seems around 35% of MYC+ are GFP-) at which a maximum of one cell division could have taken place. I was wondering if in the first place the amount of cells that is targeted with RNP complex is greater than the number of cells targeted with the GFP plasmid, in which case increasing the concentration of the GFP plasmid could be beneficial as it could be used as a bona fide proxy of the targeted cells. This is something the authors could potentially mention in the main text or methods section.

Reviewer 2

Advance summary and potential significance to field

I am satisfied with the present revised version of the manuscript and I think all main caveat/points have been addressed.

Comments for the author

A minor point that nonetheless must be addressed/changed before publication.
Please check page 9, line 217-218 and edit the following sentence.
"To demonstrate potential applications for Breasi-CRISPR, we quantified the interkinetic nuclear migration of EGFP-218 LMNB1+ neurons (Fig. 6H)."
Neurons dont undergo INM, only APs do.
Please edit the sentence accordingly

Second revision

Author response to reviewers' comments

We thank the reviewers for their careful reading of the manuscript and for helping us improve it.

In response to reviewer 1:
“I have one minor comment for the authors to evaluate. In response to my previous comment #5 and the percentage of MYC+GFP- cells, the authors followed my suggestion and checked the % of these cells 24h after electroporation. This nicely corroborates the idea of a gradual dilution, but it still remains perplexing such a low co-expression of MYC and GFP at the earliest time point (Fig. S1A, seems around 35% of MYC+ are GFP-) at which a maximum of one cell division could have taken place. I was wondering if in the first place the amount of cells that is targeted with RNP complex is greater than the number of cells targeted with the GFP plasmid, in which case increasing the concentration of the GFP plasmid could be beneficial as it could be used as a bona fide proxy of the targeted cells. This is something the authors could potentially mention in the main text or methods section.”
=> Updated manuscript, line 160, we added the sentence: “However, it is possible that increasing the amount of the co-transfected EGFP-expressing plasmid would increase that proportion.”

In response to reviewer 2:
"To demonstrate potential applications for Breasi-CRISPR, we quantified the interkinetic nuclear migration of EGFP-218 LMNB1+ neurons (Fig. 6H)."
Neurons don’t undergo INM, only APs do. Please edit the sentence accordingly.”
=> We apologize for this typo. Updated manuscript, line 216 we changed “neurons” with “radial glia”.

Third decision letter

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ARTICLE TYPE: Techniques and Resources Article

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