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Prominin-1 controls stem cell activation by orchestrating ciliary dynamics

Donald Singer, Kristina Thamm, Heng Zhuang, Jana Karbanová, Yan Gao, Jemma Victoria Walker, Heng Jin, Xiangnan Wu, Clarissa R. Coveney, Pauline Marangoni, Dongmei Lu, Portia Rebecca Clare Grayson, Tulay Gulsen, Karen J. Liu, Stefano Ardu, Angus Wann, Shouqing Luo, Alexander C. Zambon, Anton M. Jetten, Christopher Tredwin, Ophir D. Klein, Massimo Attanasio, Peter Carmeliet, Wieland B. Huttner, Denis Corbeil & Bing Hu

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Transaction Report:

(Note: Please note that the manuscript was previously reviewed at another journal and the reports were taken into account in the decision making process at The EMBO Journal. Since the original reviews are not subject to EMBO's transparent review process policy, the reports and author response cannot be published here. With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 29th Jun 2018

Thank you again for your interest and the submission of your manuscript (EMBOJ-2018-99845) to The EMBO Journal. My apologies for getting back to you with delay due to delayed input from the arbitrating expert as well as detailed discussions in the team. We have carefully assessed your manuscript and the point-by-point response provided to the referee concerns that were raised during review at a different journal. In addition, and as mentioned before, we decided to involve an arbitrating expert to evaluate the revised version of your work. This advisor has been instructed to focus solely on the technical robustness and conclusiveness of the new data added as well as on the suitability of your work for publication in The EMBO Journal.

As you will see from the report provided below, the arbitrating advisor states the high interest and is in light of your additional input supportive of further consideration at The EMBO Journal.

Based on the support provided by the advisor and our own assessment, we realise that you would be potentially able to address the issues raised by the referees in a revised version of the manuscript along the lines of information provided in the point-by-point response.

I judge the comments of the referees to be generally reasonable and can - based on your sensible

preliminary response - offer to invite you to revise your manuscript experimentally to address the referees' concerns. I agree that in particular the aspect of functional causalities between Prom1 and Arl13b-HDAC6 - ciliary dynamics or alternatively recapitulating the tooth development phenotype in an independent way by changing cilia number or size would need to be conclusively addressed in a revised version of the manuscript to move towards publication.

Please submit a revised version of the manuscript using the link enclosed below, addressing the reviewers' comments.

ARBITRATING ADVISOR'S COMMENTS:

'In general, I agree that the performance of the suggested experiments would go a considerable way to addressing the reviewers' critiques; I think the reviewers did a good job, and agree showing causality and anchoring the idea that the Hedgehog pathway is affected is the key to this being a really impactful study.'

1st Revision – authors' response 12th Sep 2018

Arbitrating advisor

Arbitrating advisor's comments:

'In general, I agree that the performance of the suggested experiments would go a considerable way to addressing the reviewers' critiques; I think the reviewers did a good job, and agree showing causality and anchoring the idea that the Hedgehog pathway is affected is the key to this being a really impactful study.'

Answer from the authors:

We thank for the advisor's comments and now have intensively revised our manuscript based on the advisor and reviewers' comments. We now can prove that indeed the Hedgehog pathway is affected, particularly through Glis2. In addition, we have introduced the other two transgenic lines to delete IFT88, the key intrinsic cilia component and showed the impact on incisor epithelial stem cells are significant that further proved our findings. For details please see our below answers to the reviewers and the revised manuscript.

2nd editorial decision 18th Oct 2018

Thank you for submitting your revised manuscript for consideration by The EMBO Journal and your patience with our response. Your revised study was sent back to the arbitrating advisor for re-evaluation, and we have received his/her comments, which I enclose below. As you will see the expert finds that the major concerns have been sufficiently addressed and is in favour of publication, pending a number of minor remaining points are conclusively considered.

Thus, we are pleased to inform you that your manuscript has been accepted in principle for publication in The EMBO Journal, pending the minor issues regarding data interpretation and annotation, as well as manuscript formatting, as outlined below, which need to be adjusted at re-submission.

ARBITRATING ADVISOR'S COMMENTS:

In this extensively revised study, Singer et al. investigated the role of Prominin-1 (Prom1)/CD133 and the primary cilia in the process of the stem cells (SCs) activation. As a model for stem cell progression, the authors, for the first time, used the mouse incisor tooth, which represents a quite interesting and useful tool for studying the activation and progression of the SCs into transit amplifying cells (TACs). Using this model, Singer et al. showed that the cholesterol-binding glycoprotein Prom1 plays a role in SC activation by regulating ciliary dynamics through an interaction with Arl13b and Hdac6, in which Prom1-Arl13b interactions are associated with SCs and Prom1-Hdac6 with TACs. It is known that the fate of SCs is highly dependent on the proper coordination of multiple signaling pathways, including cilia-associated Hedgehog signaling (HHS). Thus, the authors report that the Prom1 control of ciliary dynamics also affects the response of SCs to the HHS. One of the most interesting observations from the current study is of the translocation of Prom1-Arl13b into the nucleus, although the mechanisms and the role of this translocation were not completely investigated. Additionally, the authors also suggested that Prom1 could regulate Hedgehog signaling in the SCs through the interaction with the transcription factor Glis2 and activation of its downstream target Stat3.

In general, the authors very significantly improved the present study by addressing reviewers' comments, most

importantly, by showing the direct interaction between Arl13b and Prom1. Overall, the conclusions are made based on high quality data.

There are some minor points that would be good to address:

1. Figure 5C. The authors say that Prom1 overexpression leads to an "increased proportion of cells at the G0 and G2/M phases'. Could the authors explain why there is a simultaneous increase of both of these cell fractions?

2. Figure 5 D and E. The authors say: "only in the presence of SHH, we found the cell ciliary dynamics were correctly associated with cell cycle". Could you please explain and provide images describing the "incorrect" association of the ciliary dynamics with the cell cycle (in the absence of SHH)?

3. Figure 7 F and G. Could the authors explain why there is an increase of secretion of Prom1+ vesicles from the Prom1-mutated cells?

2nd Revision - authors' response 22nd Oct 2018

Reviewer's comment:
There are some minor points that would be good to address:

1. Figure 5C. The authors say that Prom1 overexpression leads to an "increased proportion of cells at the G0 and G2/M phases'. Could the authors explain why there is a simultaneous increase of both of these cell fractions?

Answer from the authors:
The findings indicate that Prom1 is important for stem cell maintenance and proliferation, which fit with the function of SHH in parallel. We have also inserted a sentence to explain it at the beginning of page 12: "…and Prom1 overexpression in the CLESCs significantly increased the proportion of cells at G0 and G2/M phases (Fig 5C and Appendix Fig 5A), similar to the effect of SHH treatment (Appendix Fig 5B), suggesting that Prom1 was indeed critical for stem cell self-renewal and proliferation."

Reviewer's comment:
2. Figure 5 D and E. The authors say: "only in the presence of SHH, we found the cell ciliary dynamics were correctly associated with cell cycle". Could you please explain and provide images describing the "incorrect" association of the ciliary dynamics with the cell cycle (in the absence of SHH)?

Answer from the authors:
We have now included a new panel (D) in Figure 5, where we show clearly that only when SHH was added into the medium the Ki67-FUCCI CLESCs produced primary cilia.

Reviewer's comment:
3. Figure 7 F and G. Could the authors explain why there is an increase of secretion of Prom1+ vesicles from the Prom1-mutated cells?

Answer from the authors:
The fact that Hdac6 does not interact with K138Q Prom1 leads to the deacetylation of tubulin, and hence the cilium disassembly. Given that the microtubules are essential to support the structure of the cilium and the strong preference of Prom1 for membrane with a high curvature, membrane vesicles are formed along the cilium. The "pearling" morphology of remaining long cilia in cells expressing K138Q mutant illustrated well the phenomenon. We have previously demonstrated that membrane cholesterol, i.e. an interacting partner of Prom1, is also involved in such mechanism (Marzesco et al., FEBS Lett. 2009).

We added one sentence in the revised manuscript to explain it:

"Thus, the reduction of AcTub observed in primary cilia of K138Q mutant-expressing cells might result in the fission of $Prom1^{+}$ vesicles concomitant with the reduction of ciliary length."

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Dr Denis Corbeil and Dr Bing Hu
Journal Submitted to: the EMBO Journal
Manuscript Number: EMBOJ-2018-99845R

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- 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.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs 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 and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship 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.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	A minimum of 3 mice were used as is standard practice in other published studies
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	A minimum of 3 mice were used as is standard practice in other published studies
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No animals were excluded during analyses.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	No
For animal studies, include a statement about randomization even if no randomization was used.	No randomization was used.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Quantification of results was done by a minimum of 2 people and compared.
4.b. For animal studies, include a statement about blinding even if no blinding was done	No blinding was done.
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Normal distribution was verified using Mann-whitney test. The appropriate test was used when the assumption of normal distribution was not achieved.
Is there an estimate of variation within each group of data?	yes, SEM was used where appropriate.

USEFUL LINKS FOR COMPLETING THIS FORM

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http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

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http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is the variance similar between the groups that are being statistically compared?	Yes

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	A full antibody list including catalog number, supplier, lot number and reference is presented in Supplemental Table 1
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	CLE cells were generated and authenticated using mRNA profiling and differentiation assay in Dr. Bing Hu's lab. MDCK cells were obtained from Dr. Denis Corbeil.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	CD1, Prom1 KO, Glis2 KO, BMI1 KO and C57BL/6 (P7). CL characterization of SC and TAC: Prom1 KO, Glis2 KO and C57BL/6 (P7); IFT88-flox/flox and ROSA-cre x IFT88-flox/flox (P31). Animals were housed under standard laboratory conditions with 12h/12h light/dark cycle with ad libitum access to food and water.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All experimental procedures were performed in accordance with the guidelines for the care and handling of laboratory animals, as described in the NIH guidelines, ASPA or the European Union Directive 2010/63/EU.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We confirm compliance with the guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
12. 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.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N/A
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	N/A
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	N/A
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	N/A