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Long-term expanding human airway organoids for disease modelling

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(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 1st Aug 2018

Thank you for the submission of your manuscript (EMBOJ-2018-100300) to The EMBO Journal. 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.

We found that most concerns to be adequately addressed and concur that the level of novelty provided is sufficient for consideration at The EMBO Journal.

Please note that we editorially decided that, while per se well taken, referee #1's request for additional characterization of the disease applications of the airway organoids is in our view beyond the scope of the current study, given the focus of the current work as a methods resource article.

We are thus pleased to inform you that your manuscript has been accepted in principle for publication in The EMBO Journal, pending minor revision of the following remaining issues, which need to be adjusted in a re-submitted version.

• Re-evaluate the comparison of the current approach with earlier 2D-3D models used in the field and complement accordingly.
• Introduce caveats and relativise where appropriate regarding the claims made on the club cells as the organoid's club cell origin (refs #1, #3).
• Re-discuss Lgr5-dependence as well as newly emerging cell types in the tissue (ref#1, pts.4,5) and their implications for current findings, e.g. in vitro CTFR assays.

• Introduce citations on the four recent studies applying the new protocol and reference additional studies indicated by the referees (Konishi et al, 2016 Stem Cell Reports; McCauley et al, 2017 Cell Stem Cell)

• Revise the model figure summarizing the work (ref#4,pt.2)

1st Revision - authors' response 7th Dec 2018

Reaction to comments of the reviewers.
Re-evaluate the comparison of the current approach with earlier 2D-3D models used in the field and complement accordingly.

We added additional references and outlined differences to earlier methods more clearly in introduction and discussion.

Introduce caveats and relativise where appropriate regarding the claims made on the club cells as the organoid's club cell origin (refs #1, #3).

Well taken, we changed text and figure legend accordingly.

Re-discuss Lgr5-dependence as well as newly emerging cell types in the tissue (ref#1, pts.4,5) and their implications for current findings, e.g. in vitro CTFR assays.

We had never claimed LGR5 or LGR6 to be specifically important for AO growth, but simply used both genes as readouts for WNT pathway activation/block (they are bona fide WNT target genes). Since AOs do not require addition of exogenous WNT to the culture media (as opposed to in testinal organoids), we found it important to come up with an explanation (Extended Data Fig. 2). We included the identification of rare ionocytes in the CFTR paragraph.

Introduce citations on the four recent studies applying the new protocol and reference additional studies indicated by the referees (Konishi et al, 2016 Stem Cell Reports; McCauley et al, 2017 Cell Stem Cell)

We included the respective citations.

Revise the model figure summarizing the work (ref#4,pt.2)

As requested by the reviewer, we have revised the model figure.

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Hans Clevers
Journal Submitted to: EMBO Journal
Manuscript Number: 800100

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 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.

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?	mice: sample size was chosen from results form previous experiments. For RNA sequencing 2 biological replicates were assayed at 2 different time-points to investigate the experssion pattern of biological markers. Cystic fibrosis airway organoid swelling: sample size was not chosen, all available samples (with different mutations) were included.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	≥6 mouse injections per condition were carried out, mice sample size was chosen based on results from previous experiments
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No samples or animals were excluded from analysis, nor were any criteria pre-esthablished.
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.	Swelling; we included all organoid samples, which contain organoids with a range of cftr mutations from severe to mild, and all treatments.
For animal studies, include a statement about randomization even if no randomization was used.	Before starting the experiment mice were randomized on bodyweight. Males and females (aged 8–10 weeks at the start of the experiment; weights, ~30 g for males and ~25 g for females) were used and all animals were included in the analysis.
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.	Organpoid swelling; no blinding, assessing results were calculated automatically.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Exp. on mice (including injection of organoids, measuring IVIS etc) were performed by persons who didn't know which animal was injected with which organoid line. Histopathology sections were analyzed blinded. The origin of the samples was only revealed after the histopathologic eveluation was finished.
5. For every figure, are statistical tests justified as appropriate?	sAll statistical tests are justified as appropriate.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Student's t-test
Is there an estimate of variation within each group of data?	not applicable
Is the variance similar between the groups that are being statistically compared?	not applicable

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

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

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

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

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).	Antibodies APC anti-human EPCAM Biolegend 369810 PE anti-human NGFR Biolegend 345106 488 anti-human CD24 Biolegend 311108 Rabbit anti-Keratin 14 Biolegend 905301 Goat anti-SCGB1A1 Santa Cruz sc-9773 Mouse anti-acetylated α-tubulin Santa Cruz sc-23950 Mouse anti-Mucin 5AC (IF) Santa Cruz sc-21701 Mouse anti-Ki67 Monosan MONX10283 Rabbit anti-Keratin 5 Covance PRB 160P-100 Mouse anti-Mucin 5AC (IHC) Novocastra Leica NCL-HGM-45M1 Mouse anti-p53 Santa Cruz sc-126 Mouse anti-Cytokeratin Becton Dickinson 345779 Rabbit anti-GFP Thermo Fisher A11122 Mouse anti-RSV Abcam ab35958 Mouse anti-β-tubulin IV Biogenex MU178-UC Rabbit anti-GAPDH Abcam ab-9485
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	before entering the animal facility all the organoid lines were tested for (mouse)pathogens by IDEXXThis is provided in the Methods and Protocols section. All lines were tested for mycoplasma contamination.

* 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.	NOD scid gamma (NSG; NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ) mice were used. Mouse experiments spanned over an 8-week period after which lungs were resected for examination. Randomly distributed males and females (aged 8–10 weeks at the start of the experiment; weights, ~30 g for males and ~25 g for females) were used and all animals were included in the analysis. Ear clipping was used for animal recognition. Animals were caged together and treated in the same way.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Experiments on NSG mice were carried out at the Netherlands Cancer Institute according to local and international regulations and ethical guidelines, and were approved by the local animal experimental committee at the Netherlands Cancer Institute . (DEC-NKI OZP: 13.022, WP 5418).
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.	Confirmed

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	The collection of patient data and tissue for the generation and distribution of airway organoids has been performed according to the guidelines of the European Network of Research Ethics Committees (EUREC) following European, national, and local law51. In the Netherlands, the responsible accredited ethical committees reviewed and approved the studies in accordance with the 'Wet medisch-wetenschappelijk onderzoek met mensen' (medical research involving human subjects act)52. The medical ethical committee UMC Utrecht (METC UMCU) approved protocols 07 125/C (isolation and research use of neutrophils from healthy donors), TCBio 15-159 (isolation and research use of broncho-alveolar lavage fluid of CF patients), and TCBio 14-008 (generation of organoids from rectal biopsies of CF patients). The 'Verenigde Commissies Mensgebonden Onderzoek' of the St. Antonius Hospital Nieuwegein approved protocol Z-12.55 (collection of blood, generation of normal and tumor organoids from resected surplus lung tissue of NSCLC patients). The Medical Ethics Committees of the Netherlands Cancer Institute Amsterdam approved PTC14.0929/M14HUP (collection of blood, generation of normal and tumor organoids from resected surplus lung tissue of NSCLC patients), and PTC14.0928/M14HUM (generation of tumor organoids from biopsies of metastatic NSCLC).
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.	All patients participating in this study signed informed consent forms approved by the responsible authority. In all cases, patients can withdraw their consent at any time, leading to the prompt disposal of their tissue and any derived material.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	Not applicable
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	Future distribution of organoids to any third (academic or commercial) party will have to be authorized by the METC UMCU/TCBio at request of the HUB in order to ensure compliance with the Dutch medical research involving human subjects' act.
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	Not applicable
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.	Not applicable
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.	Not applicable

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	Whole genome sequencing data of matching tumor tissue and tumor organoid and RNA sequencing can be found under EGA study ID EGAS00001002899.
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).	Not applicable
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).	Not applicable
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.	Not applicable

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.	