nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Microsoft Excel Software (2019) is used for data presenting and processing. ADF software package were used for density functional theory calculations. XZ confocal images under each condition was processed using the custom MATLAB code to generate ASL high thickness.
Data analysis	For each condition, the above-noted MATLAB code exported ASL height data to a Microsoft Excel file for plotting and subsequent analysis. The custom MATLAB codes have been deposited at http://doi.org/10.6084/m9.figshare.23302427.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Data are available at http://doi.org/10.6084/m9.figshare.23302427

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes were chosen based on our previous experiments of 1) the intra- and inter-donor culture variability of ASL height data, and 2) reagents which stimulate chloride secretion (CFTR and CaCC). This assay is well established for measuring water flux from airway epithelial cultures and is used as an FDA endpoint for chloride-mediated flux(ref Vertex Pharmaceuticals). We have a long published history of using this technique and use sample sizes based on our analysis of the technical/experimental error and donor-to-donor variability. The donor and replicate size for this study was selected to statistically differentiate a change in ASL volume of 0.5 µm between groups. Here are some historical references for this approach: 1. Matsui H, et. al. Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. Cell. 1998 Dec 23;95(7):1005–15. PMID: 9875854 2. Tarran R, et. al. The relative roles of passive surface forces and active ion transport in the modulation of airway surface liquid volume and composition. J Gen Physiol. 2001;118(2):223–236. PMID: 11479349
Data exclusions	No exclusions were made.
Replication	Studies were performed replicate cultures from primary cells from three different donor lungs (all non-diseased) Randomization. All studies in different donors and replicate cultures performed here were consistent with our presented findings.
Randomization	Analysis of control vs. test agent was performed in replicate cultures from the same donor. Here, random cultures were assigned to be either control or the actual test.
Blinding	Only ASL height studies require Blinding. Treatment of cultures used for ASL height studies blinded. Here, treatment of cultures with control & test agents were performed by a different person than the one doing the subsequent analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Primary human airway cells from CF donor lungs
Authentication	Cells were obtained directly from the lungs by our group using well established protocols (referenced in manuscript). Prior to the study, histological studies were performed to ensure the correct pheontype of CF airway epithelial cells (i.e., fluid hyperabsorbing was observed.
Mycoplasma contamination	Only primary cell lines are used (i.e., not immortalized cell lines) and our cultures are used for only 30-45 days prior to disposal. However, our incubators are checked for mycoplasma contamination if aberrant results are obtained with our cells or on notification of a positive result with our cell isolation group.
Commonly misidentified lines (See ICLAC register)	N/A