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Life Sciences Reporting Summary

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Experimental design

1. Sample size

Describe how sample size was determined.

Mouse study:
The number of animals necessary was based on our previous case-control analysis derived from our population of 102 CFTRtm1Eur CF and 42 WT FVB control population. We used a multivariate discriminant analysis to develop a score to best discriminate between the CF and WT populations.
This led us to determine that for a treatment effect of 40 % toward wild-type values, we needed at least 6 animals per group in a placebo control design to reach a power of 95% for an SD value of 0.1 (Faria da Cunha et al., Int J Biochem Cell Biol. 2016;80:87-97).
As the design of the study was slightly different, (e.g animals acting as their own control, reducing even more the variability of the measurement) we assumed that this number of 6 animals was sufficient, and increased it to 8 for more reliability.

Human respiratory epithelia:
Our previous study (Veit et al., PLOS Biology 2016 14(5):e1002462) showed that HBE isolated from 5 individuals homozygous for ΔF508-CFTR are sufficient to significantly determine an effect corresponding to 25% of the WT-current.
To encompass more of the naturally occurring variation in the residual ΔF508-CFTR current, we used HNE isolated from 17 individuals homozygous for ΔF508-CFTR.

2. Data exclusions

Describe any data exclusions.

No samples, mice or data points were excluded from the reported analyses.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

To verify reproducibility, CFTR modulator effects were recapitulated in cell models, primary human cells and animal models. Mutually supportive results were obtained in three independent laboratories, confirming reproducibility of the key findings of the study. All individual experiments were repeated for the number of times indicated in the manuscript. All the relevant data collected are included in the study and replication experiments were successful.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Randomization was not applicable, since treatment/control were done in parallel (in vitro) on cells form the same individuals or sequentially (in vivo) in the same mice.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Investigators were not blinded, since no subjective analysis were performed.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ 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 any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Most study analysis were performed using Excel or GraphPad Prism. Dose-response fitting was performed with OriginPro 8, SPR data was analyzed with BIACORE T200 evaluation software, BLM recordings were analyzed with Clampfit 10.3, YFP quenching data was analyzed with XLfit 4.3. and immunoblot analysis were done using ImageJ 1.50i.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

All unique materials used in the study are readily available from the authors.
Transduced CFBE cells are joint property of University California San Francisco and McGill University. The IP of the Clontech`s LentiX Tet-On Advanced System, used for transduction of the CFBR belongs to TET Systems GmbH & Co. KG, Germany.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Mouse monoclonal anti–hemagglutinin (HA) antibody was purchased from Covance (clone: 16B12, catalog no: MMS-101R, dilution 1:1000). Monoclonal anti-CFTR antibody clone M3A7 (recognizing aa 1365-1395 at the C terminus of the NBD2, catalog no: MAB3480, dilution 1:500) and clone MM13-4 mouse monoclonal anti-CFTR Ab (specific to the N-terminal 25-36 residues, catalog no: MAB3482, dilution 1:1000) were from Millipore. The 660 anti-CFTR antibody, recognizing the NBD1 has been characterized (Cui, L., et al., J Mol Biol 365, 981-994 (2007)), was kindly provided by Dr. J. Riordan (University of North Carolina, Chapel Hill, NC) and was used at a dilution of 1:2000. Monoclonal mouse anti-Na+/K+-ATPase (Santa Cruz Biotechnology, clone: H3, catalog no: sc-48345, dilution: 1:5000) and monoclonal mouse anti-βactin (abcam, catalog no: ab8226, dilution: 1:2000) were used as loading control.
All commercially available antibodies are validated by the manufactures.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

The CFBE41o- cells were generated by and are a kind gift from Dr. Dieter Gruenert (University of California, San Francisco). BHK-21 cells were purchased from ATCC.

b. Describe the method of cell line authentication used.

BHK-21 cells have been validated by ATCC and were obtained directly from ATCC. CFBE41o- were directly received from Dr. Dieter Gruenert and have not been authenticated in our lab.

c. Report whether the cell lines were tested for mycoplasma contamination.

We routinely test cells for myocoplasma. All cells used in the study tested negative.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

Congenic mice homozygous for the ΔF508 mutation, ΔF508 Cftrtm1EUR (FVB/N background), as well as homozygous knockout Cftr mice, KO CFTRtm1Unc (B6;129 background) and their wild-type littermates were obtained from CDTA (Cryopreservation, Distribution, Typage et Archivage animal, Orléans, France), and housed at Animal Care Facility of Institut Necker Enfants Malades, Paris. Mice were fed with fiber-free diet and a laxative was used in their drinking water (Colopeg 17.14 g/l; Bayer Santé Familiale, France) to avoid intestinal obstruction. Experiments were approved by the local committee for animal experiments (Comité Régional d'Ethique sur l'expérimentation animale Ile de France Sud-MESR N° 01345.03). The sex, age and weight distributions were:
Figure 5h: 2 females and 6 males, 13 to 20 weeks (mean : 15.88), 20 to 26 g (mean 23.13 g).
Supplementary Figure 7a: 1 female and 4 males, 13 to 16 weeks (mean 15 weeks), 20 to 26 g (mean 24 g).
Supplementary Figure 7b: 3 females and 2 males, 12 to 22 weeks (mean 15,75), 13 to 22 g (mean: 18,75 g).

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The isolation of human nasal epithelia (HNE) from healthy and subjects with CF was performed under the protocol and consent form approved by the McGill MUHC Research Ethics Board (14-234-BMB). The age/sex of the individuals homozygous for ΔF508-CFTR from which nasal tissue was obtained by scrape biopsy is listed in Supplementary Table 3. The age range at the time of collection was 20-53 years (median 29) and the female:male ratio was 9:8.