Online Supplementary Data:

IL-1β dominates the pro-mucin secretory cytokine profile in cystic fibrosis

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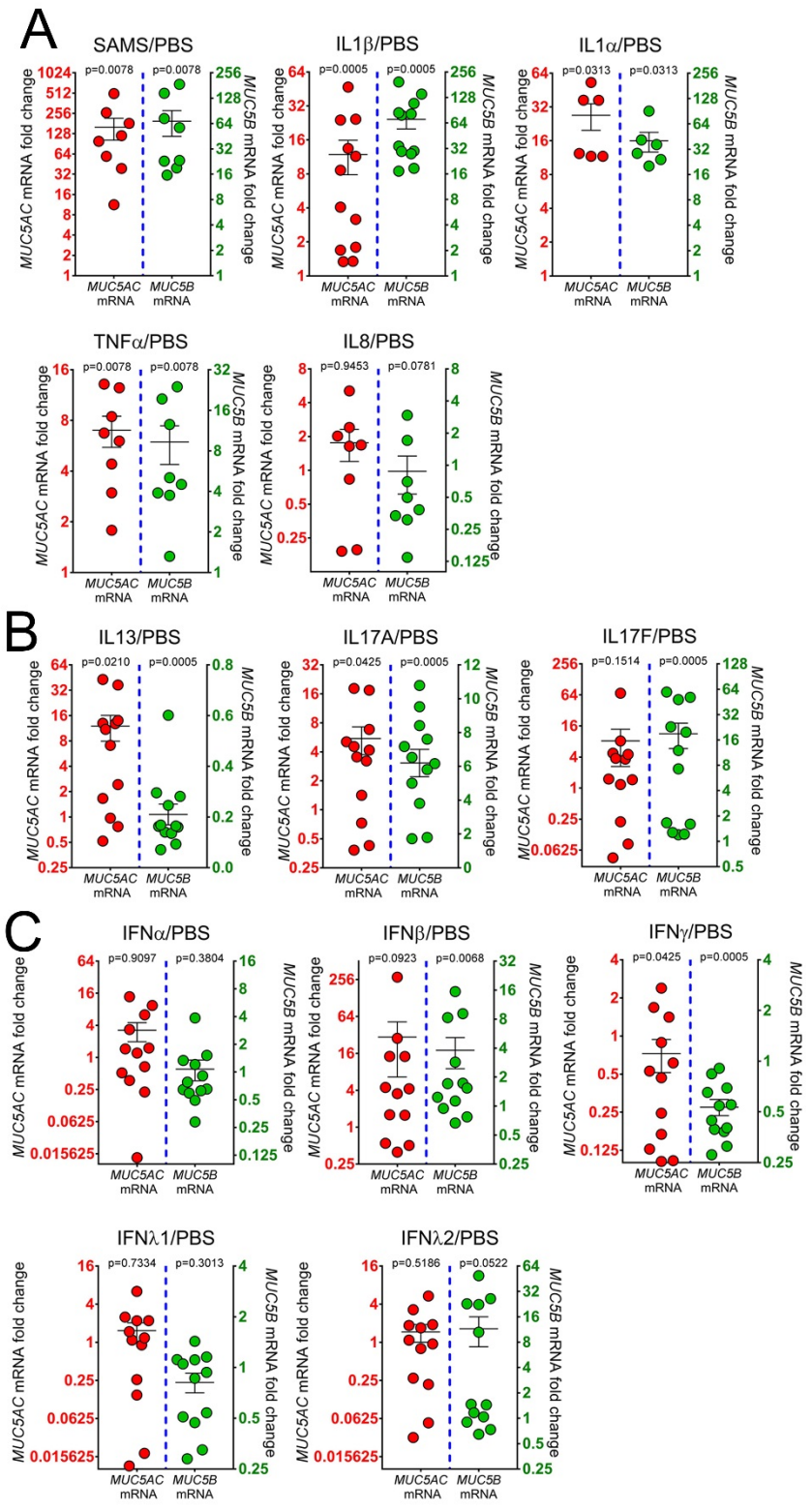
I: Supplementary Table 1 and Supplementary Figures 1-14:

Supplementary Table 1

Treatment	number of codes tested (n=)	*MUC5B* mRNA			*MUC5AC* mRNA		
		Fold Change (mean)	SE	P value	Fold Change (mean)	SE	P value
SAMS	8	68.12	23.00	0.008	159.52	56.96	0.008
IL1α	6	40.25	10.59	0.031	27.03	7.19	0.031
IL1β	12	70.62	16.14	<0.001	11.89	4.01	<0.001
TNFα	8	9.30	2.96	0.008	7.00	1.47	0.008
IL8	8	0.88	0.34	0.078	1.76	0.56	0.945
IL13	12	0.21	0.04	<0.001	12.01	4.07	0.021
IL17A	12	6.19	0.81	<0.001	5.51	1.77	0.043
IL17F	12	18.98	6.32	<0.001	8.22	5.60	0.151
IFNα	12	1.07	0.27	0.380	3.23	1.26	0.910
IFNβ	12	3.79	1.35	0.007	29.50	22.94	0.092
IFNγ	12	0.53	0.06	<0.001	0.73	0.21	0.043
IFNλ1	12	0.82	0.11	0.301	1.53	0.51	0.733
IFNλ2	12	11.53	4.47	0.052	1.46	0.45	0.519

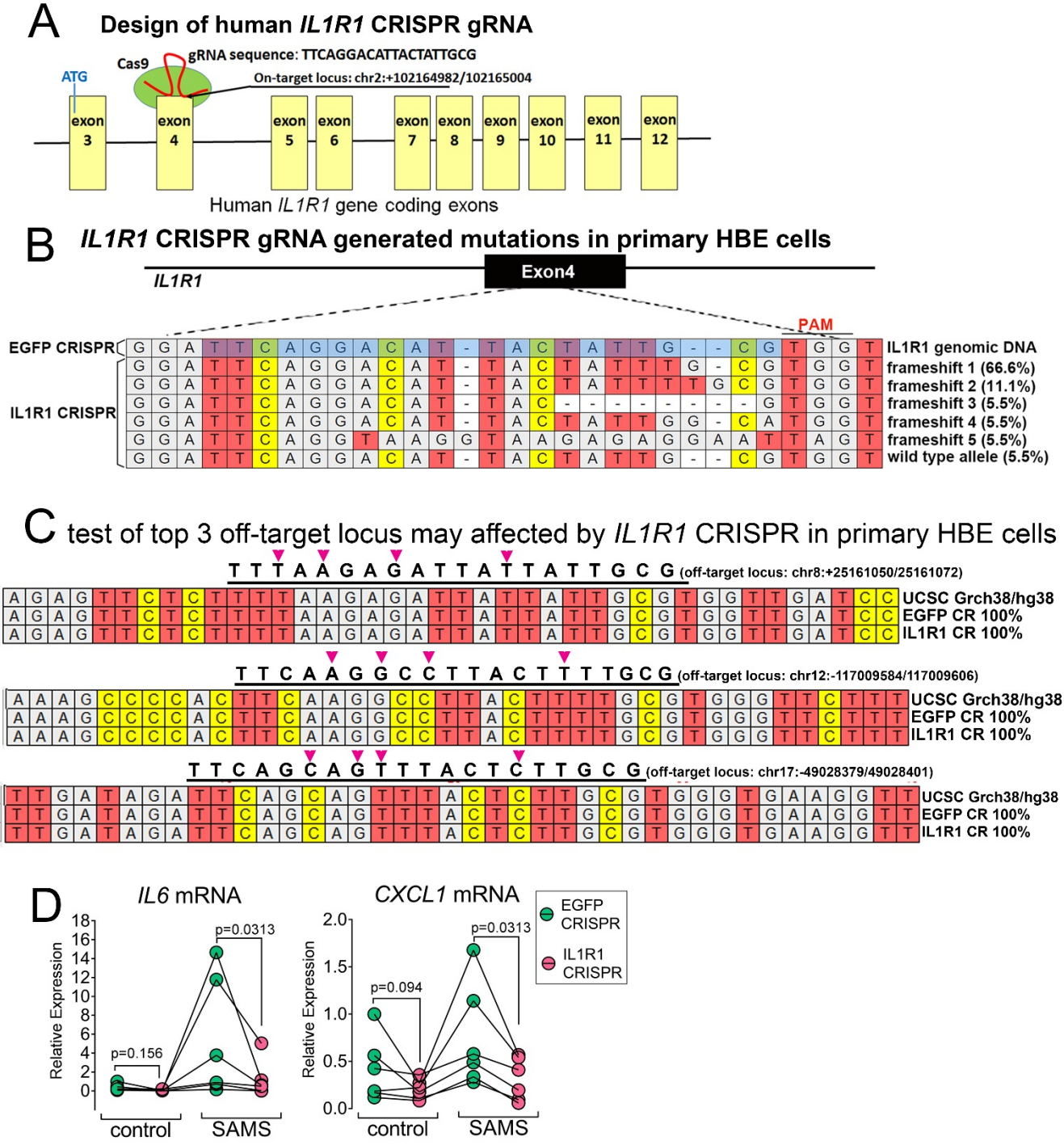
Supplementary Table 1: Expression of *MUC5AC* and *MUC5B* mRNAs in non-CF HBE cells following cytokine exposure. Fully differentiated non-CF HBE cells were cultured under ALI conditions and exposed with cytokines from the basolateral side in ALI media at a concentration of 10ng/ml for 3 days. Undiluted SAMS (50µl) was administered from the apical side of the cells. Expression of *MUC5AC* and *MUC5B* mRNAs was quantitatively measured by Taqman assays. Cytokines present in SAMS: IL1α (n=6 codes), IL1β (n=12 codes), TNFα (n=8 codes) and IL8 (n=8 codes); SAMS (n=8 codes); TH2 and TH17 cytokines IL13, IL17A and IL17F (all tested with n=12 codes); and interferons involved in antiviral responses IFNα, IFNβ, IFNγ, IFNλ1 and IFNλ2 (all tested with n=12 codes). Means of fold changes of *MUC5B* and *MUC5AC* mRNA are presented. Values of relative expression of *MUC5B* and *MUC5AC* mRNAs exposed with inflammatory mediators versus the PBS control were analyzed with 2-tailed paired Wilcoxon tests, and P values are shown. One code means the cells obtained from one individual donor lung. Note, not all treatments were applied to exactly the same codes.

Supplementary Figure 1



Supplementary Figure 1: Fold changes of *MUC5AC* and *MUC5B* mRNAs in responses to SAMS and constituent inflammatory mediators vs. PBS in non-CF HBE cells. Fully differentiated non-CF HBE cells were cultured under ALI conditions and exposed with cytokines or interferons (indicated in the panels) or PBS from basolateral side in the ALI media at the concentration of 10ng/ml for 3 days. Undiluted SAMS (50µl) was administered from the apical side of the cells. Expression of *MUC5B* and *MUC5AC* mRNAs was measured by quantitative RT-PCR. Panel (**A**) shows the fold changes of *MUC5AC* and *MUC5B* mRNAs for SAMS (n=8) and proinflammatory cytokines present in SAMS [IL1β (n=12 codes) , IL1α (n=6 codes), TNFα (n=8 codes), IL8 (n=8 codes)] as compared to PBS exposure in the same code of HBE cells (means±SE). Panel (**B**) shows the fold changes of *MUC5AC* and *MUC5B* mRNAs in responses to [IL13, IL17A and IL17F (all tested with n=12 codes)] and to (**C**) the interferons involved in antiviral responses [IFNα, IFNβ, IFNγ, IFNλ1 and IFNλ2/3 (all tested with n=12 codes)] vs. PBS control (means±SE). Relative expression of *MUC5B* and *MUC5AC* mRNA exposed with inflammatory mediators compared to PBS control were analyzed with 2-tailed paired Wilcoxon tests, and p values of such tests are annotated in the matching panels. One code means the cells obtained from one individual donor lung. Note, not all treatments were applied to exactly the same codes.

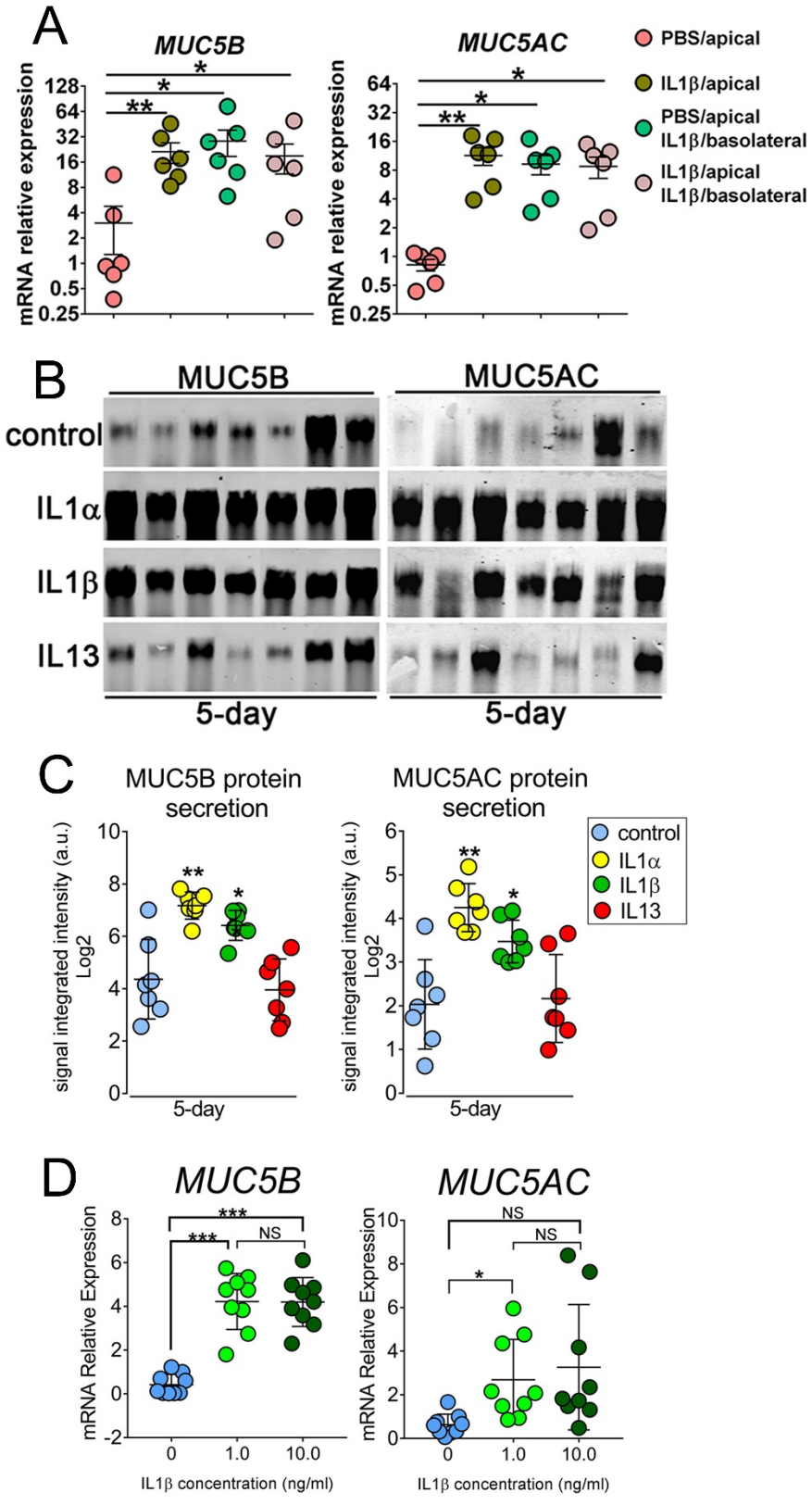
Supplementary Figure 2



Supplementary Figure 2: *IL1R1* CRISPR specifically targets the 4th exon of *IL1R1* gene in primary non-CF HBE cells. On-target and off-target sequences of *IL1R1* CRISPR-Cas9 lentivirus administered

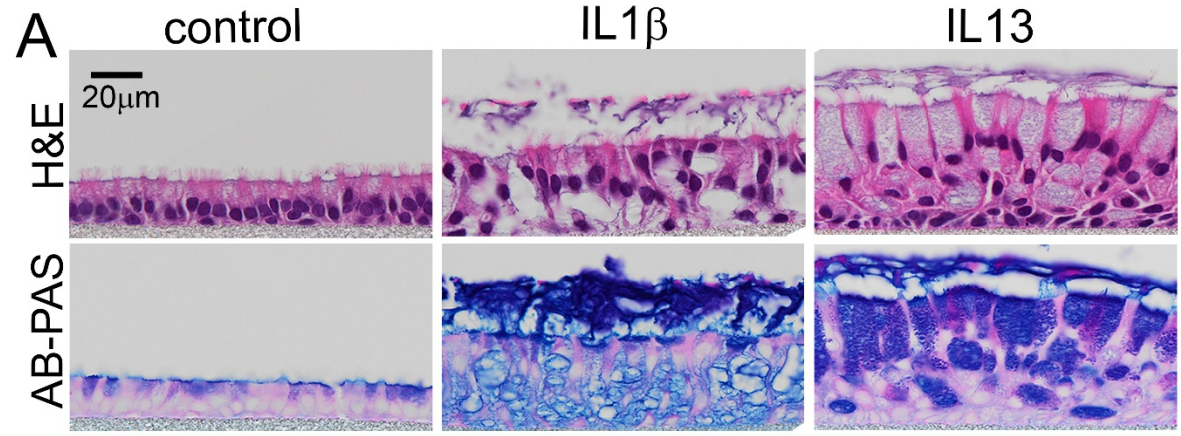
to HBE cells were confirmed by DNA sequencing. Panel (**A**) shows the cartoon illustration of design of *IL1R1* gRNA that targets human *IL1R1* gene. (**B**) Mutations caused by *IL1R1* CRISPR targeting were identified (sequences analyzed from n=3 codes of HBE cells with n=10-12 clones/code). (**C**) The top 3 potential off-target loci were evaluated by DNA sequencing of the regions. The off-target loci were predicted via an online tool: http://crispr.cos.uni-heidelberg.de/index.html), and off-target sequences were analyzed using HBE cells obtained from n=3 non-CF codes, with n=4 clones/code. (**D**) mRNA expression of *IL6* and *CXCL1* was measured quantitatively by Taqman assays in HBE cells targeted with EGFP (control) or IL1R1 CRISPR followed by exposure to SAMS for 3 days (n=6 codes). Scatter plot-line graphs present values of code-matched cells infected with EGFP (control CRISPR) or IL1R1 CRISPRs followed by exposure with vehicle control (PBS) vs. SAMS (50µl of PBS or 50 µl of undiluted SAMS were administered on apical surface). Data were analyzed with 2-tailed paired t test between control and SAMS groups. One code means the cells obtained from one individual donor lung.

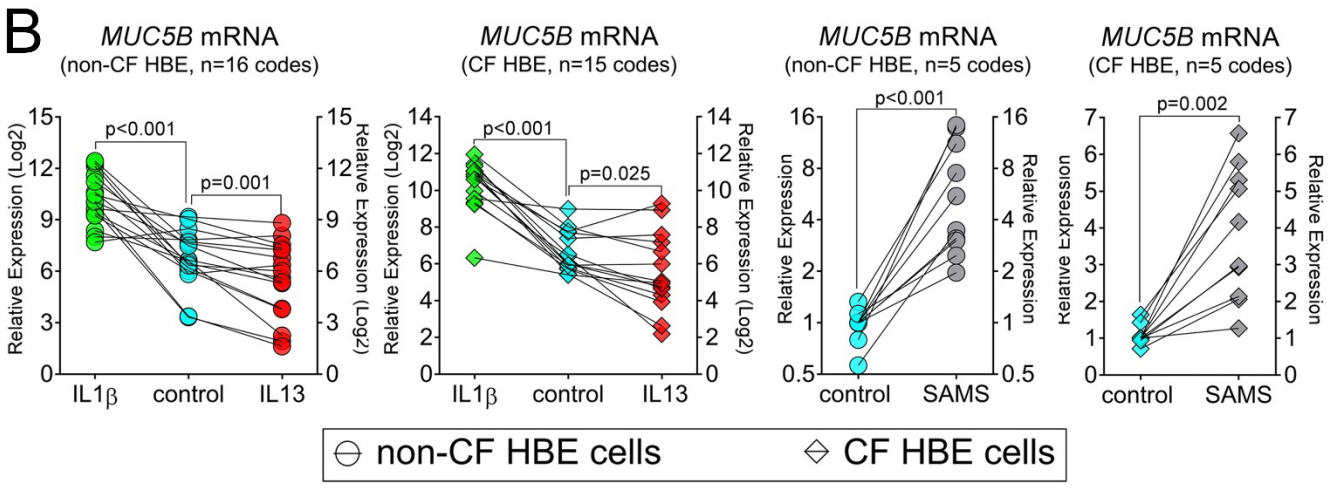
Supplementary Figure 3

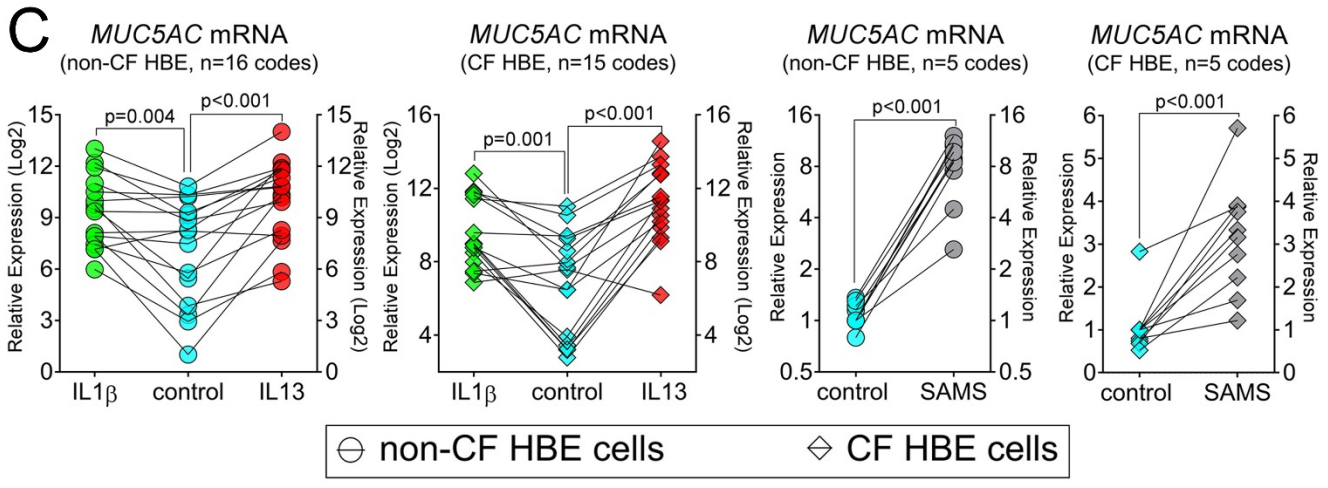


Supplementary Figure 3: IL1α and IL1β induce MUC5B and MUC5AC proteins secretion from HBE cells. (A) IL1β (10ng/ml) or vehicle control (PBS) was added from apical, basolateral, or both sides of 6 codes of non-CF HBE cells (n=6 codes) for 5 days, and mRNA expression of *MUC5B* and *MUC5AC* was quantified by Taqman assays. (**B**) n=7 codes of non-CF HBE cells were exposed with IL1α, IL1β, and IL13 (all at 10ng/ml in ALI media) from the basolateral side for 5 days. Apical secretions were collected by washing with 200µl PBS and subjected to agarose mucin western blot to detect MUC5B and MUC5AC protein expression. (**C**) Secreted MUC5B and MUC5AC protein levels were semi-quantified using Licor Odyssey software. Note, the value was Log2 transformed. (**D**) Non-CF HBE cells were treated with IL1β at the concentration of 1 and 10ng/ml from the basolateral side for 5 days, and *MUC5B* and *MUC5AC* mRNA was quantified by Taqman assays. Graphs present mean±SD, and data were analyzed with one-way ANOVA and Dunnett's test (**C**) and Tukey's test. *P<0.05; **P<0.01; ***P<0.001 compared to controls; NS=not significant.

Supplementary Figure 4







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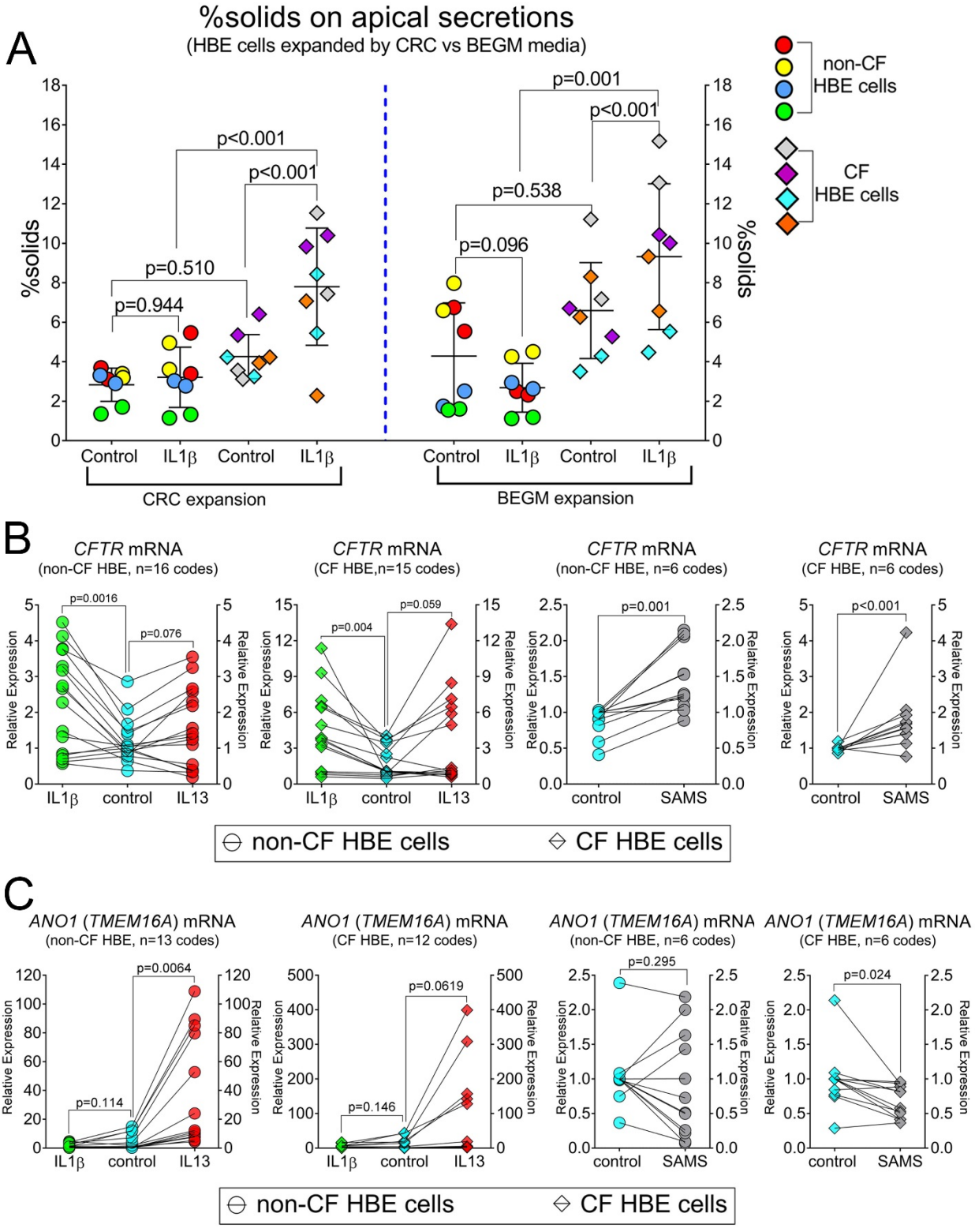
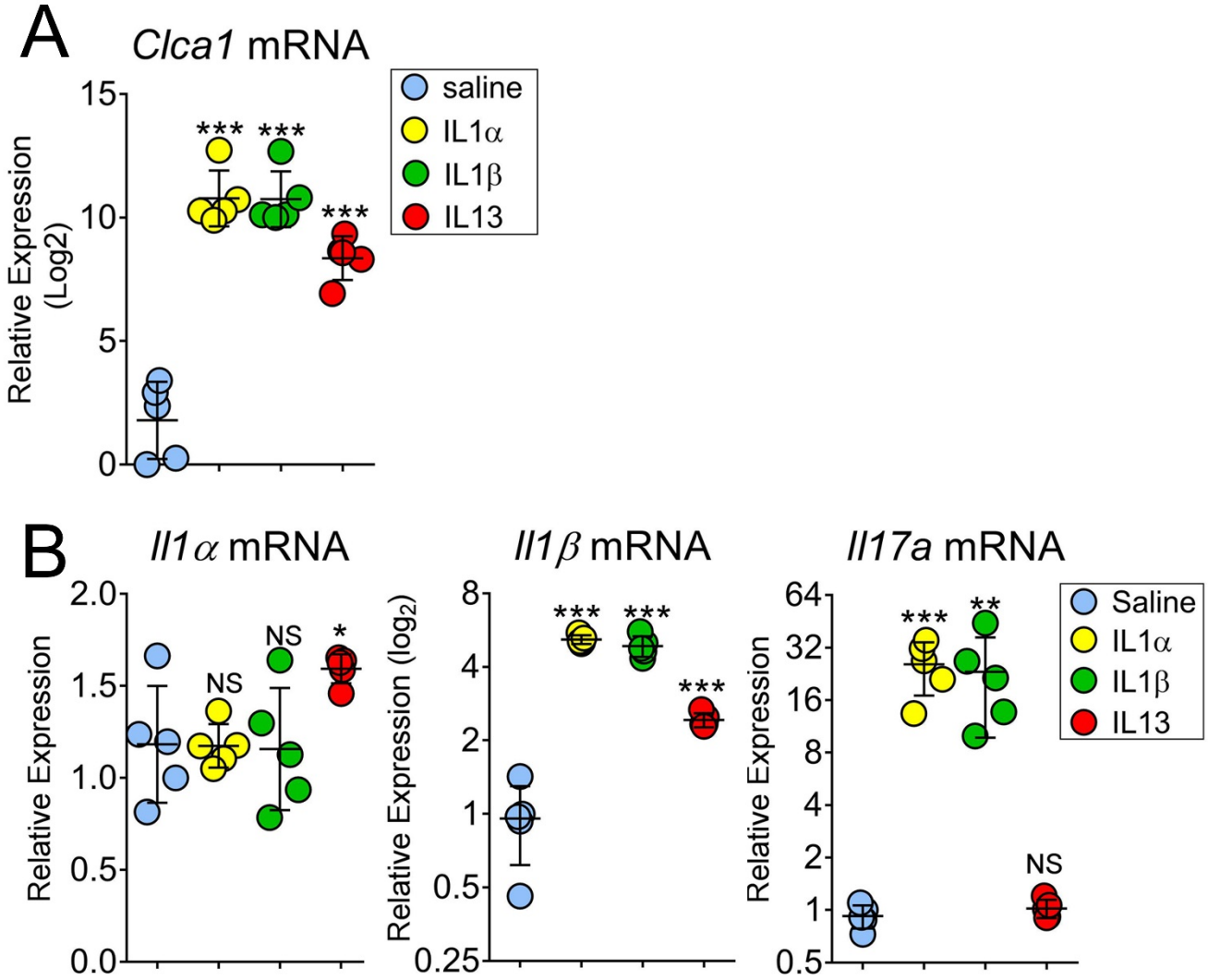
Supplementary Figure 4: IL1β and IL13 cause morphological changes in CF HBE cells; IL1β and IL13 regulate mRNA expression of mucins and ion channel genes in non-CF and CF HBE cells. (**A**) CF HBE cells were exposed to vehicle control (PBS), IL1β or IL13 from the basolateral side (both at 10ng/ml in ALI media) for 7 days. Histological features are shown by H&E staining, and goblet cell differentiation and mucus production are shown by AB-PAS staining. Micrographs are representative of n=3 codes of CF HBE cells/treatment. (**B**, **C**) Non-CF and CF HBE cells exposure with control (PBS), IL1β or IL13, or PBS vs. SAMS for 7 days. *MUC5B* (**B**) and *MUC5AC* (**C**) mRNAs were quantitatively measured by Taqman assays. IL1β and IL13 were both administrated at 1ng/ml from the basolateral side in media; diluted SAMS (containing 1ng/ml IL1β by 1:40 dilution of undiluted SAMS stock) was added on the apical side of HBE cells. Dot-line plots present values of code-matched cultures treated with controls (vehicle, PBS) or cytokines and SAMS. The data were analyzed with one-way ANOVA followed by Dunnett's test in control (PBS), IL1β and IL13 treatment groups (n=16 codes of non-CF and n=15 codes CF HBE cells with n=1 culture/code/treatment were used). Data were analyzed with two-way ANOVA followed by Sidak correction in PBS vs. SAMS treatment group (n=5 codes of non-CF and n=5 codes of CF HBE cells with n=2 cultures/code/treatment were used). One code means the cells obtained from one individual donor lung.

Supplementary Figure 5



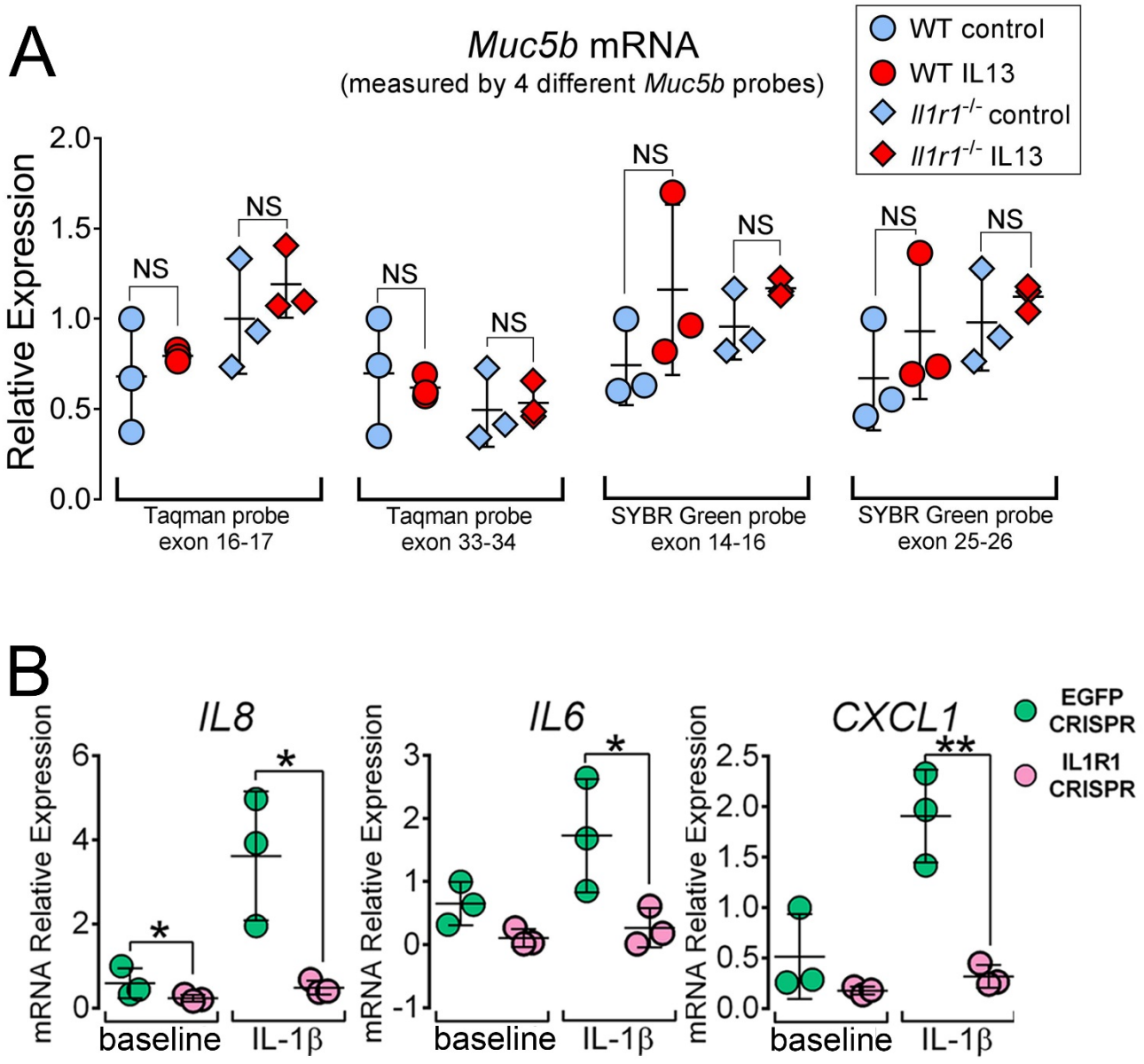
Supplementary Figure 5: IL1β increased mucus concentration in CF HBE cells in both CRC or BEGM expansion protocols. IL1β and SAMS increased *CFTR* mRNA, whereas IL13 induced *TMEM16A* mRNA in non-CF and CF HBE cells. (**A**) n=4 non-CF, non-smoker codes of HBE cells, and n=4 codes of CF HBE cells (all ΔF508 homozygous mutants) were tested for mucin concentration at baseline and after IL1β exposure of HBE cell cultured by conditional reprogrammed culture (CRC) methods or conventional BEGM expansion method. After one week of amplification in CRC or BEGM medium, cells were then seeded onto transwells, and cultured for 4 weeks to allow full differentiation under air-liquid interface (ALI) culture conditions using the exact same culture condition/protocol. The IL1β exposure was started at the 5th week and continued for one week without apical washing during this week before %solids measurements. Two independent cultures of each code/condition were measured for %solids. Each color indicates measurements obtained from the same code. Data represent means±SD, and were analyzed by linear mixed-effects model with subject identification number as random intercept variable. The dot-line plots show *CFTR* mRNA (**B**) and *TMEM16A* (*ANO1*) mRNA (**C**) after exposure with control, IL1β or IL13, or PBS vs. SAMS in non-CF and CF HBE cells. IL1β, IL13 and SAMS administration protocol and data analysis were performed same as described in Supplementary Figure 4 B,C.

Supplementary Figure 6



Supplementary Figure 6: Intratracheal instillation of IL1α and IL1β cytokines induces expression of *Clca1* and endogenous *Il1b* and *Il17a* mRNAs, whereas administration of IL13 cytokine induces expression of *Clac1* and endogenous *Il1a* and *Il1b* mRNAs in the whole lung. Wild type adult (6 weeks old) female C57/B6J mice were exposed with sterile saline, IL1α, IL1β and IL13 via intratracheal instillation. *Clca1* (**A**), *Il1a*, *IL1b* and *Il17a* (**B**) mRNAs were quantitatively measured by Taqman assays. Scatter-plot graphs present means±SD, and data were analyzed with one-way ANOVA followed by Dunnett's test. N=5 mice/treatment group were used to perform cytokine exposures. *P<0.05; **P<0.01; ***P<0.001 compared to saline control group, NS=not significant.

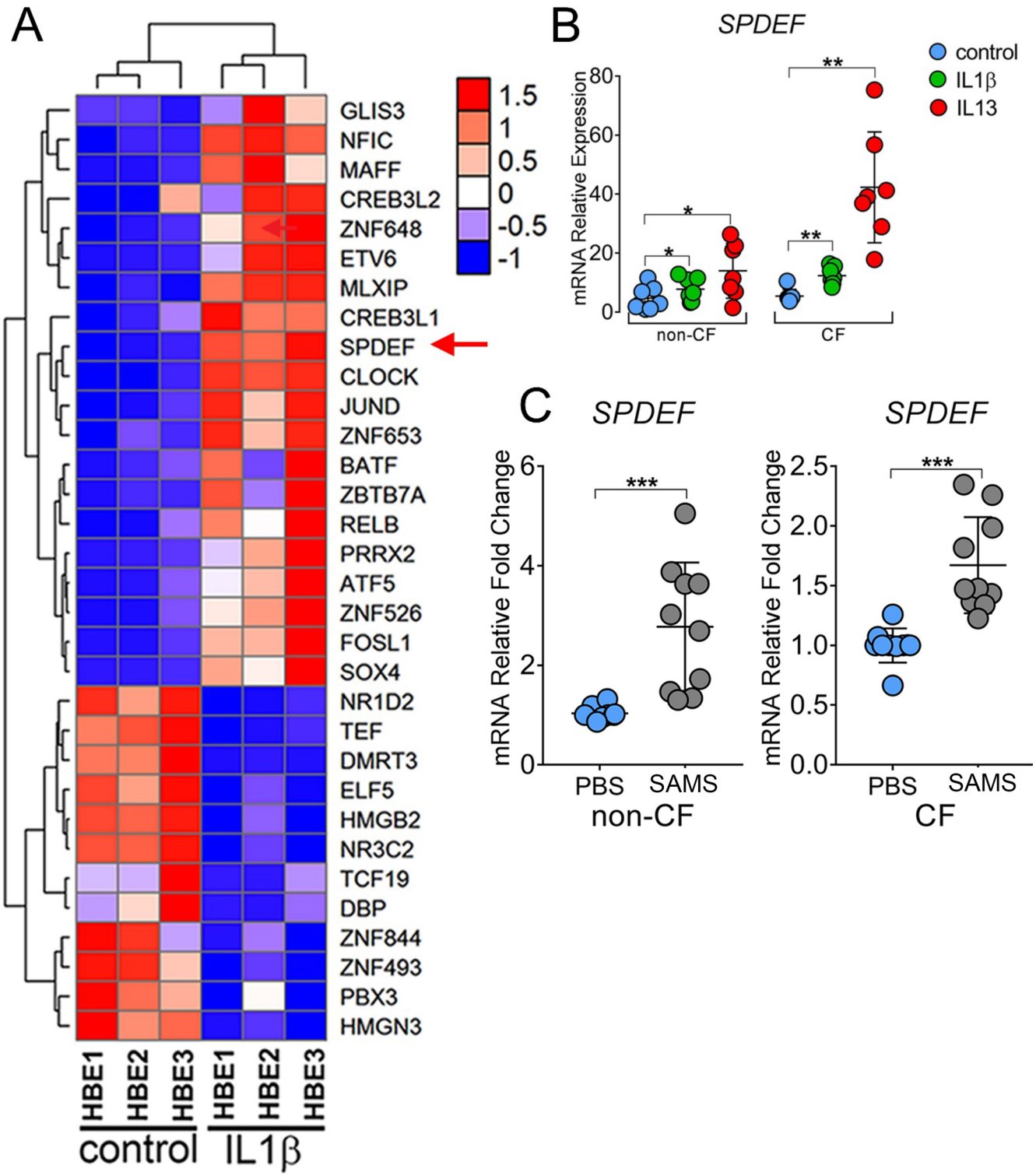
Supplementary Figure 7



Supplementary Figure 7: IL13 does not suppresses *Muc5b* mRNA in mouse tracheal epithelial cells (mTEC) in vitro; IL1R1 CRISPR-Cas9 inhibits IL1β-induced *IL8*, *IL6* and *CXCL1* mRNAs in non-CF HBE cells. mTEC isolated from wild type and *Il1r1*$^{-/-}$ mice were cultured under ALI conditions for 3 weeks to allow full differentiation prior to exposure to murine recombinant cytokine IL13. They were then exposed to murine recombinant IL13 for one week from the basolateral side in media (at 10 ng/ml). mRNA

expression of *Muc5b* was measured by quantitative RT-PCR with n=4 different probes detecting different exon-regions of the mouse *Muc5b* gene (see Supplementary Materials-primers/probes table for probes information). N=3 independent mTEC cultures/treatment/genotype were used for measurements. Scatter plots present mean±SD, and data were analyzed with 2-tailed unpaired t test. NS=not significant. (**B**) mRNA expression of *IL8*, *IL6* and *CXCL1* was measured quantitatively by Taqman assays in HBE cells transduced with control (EGFP) or IL1R1 CRISPR lentiviruses followed by exposure with IL1β (10ng/ml from basolateral in media) for 3 days. Graphs present mean±SD, and data were analyzed with 2-tailed paired t test with non-CF HBE cells from 3 donors. *P<0.05; **P<0.01 compared to EGFP CRISPR groups.

Supplementary Figure 8



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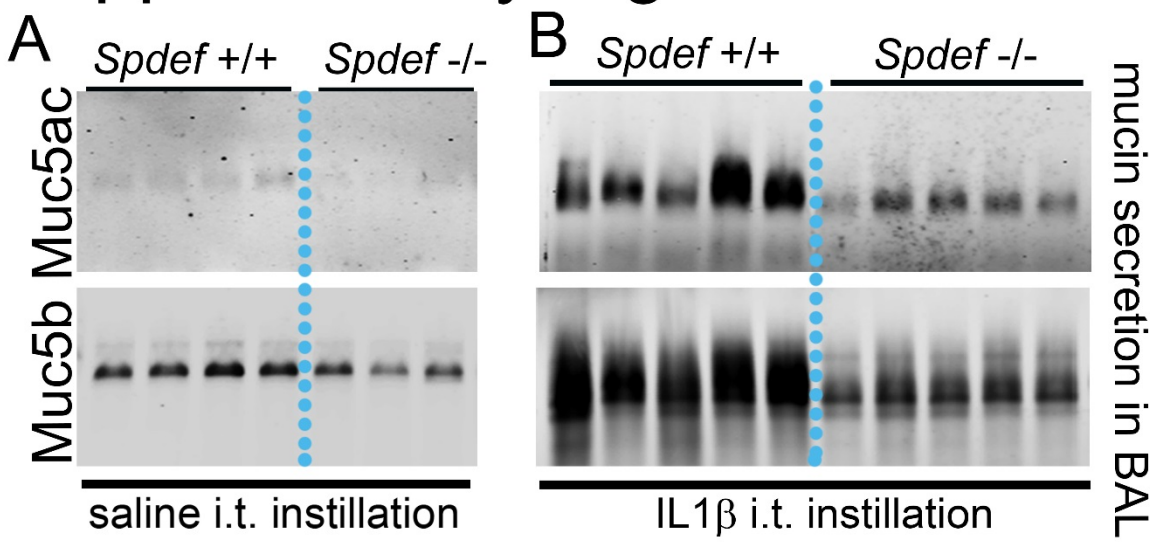
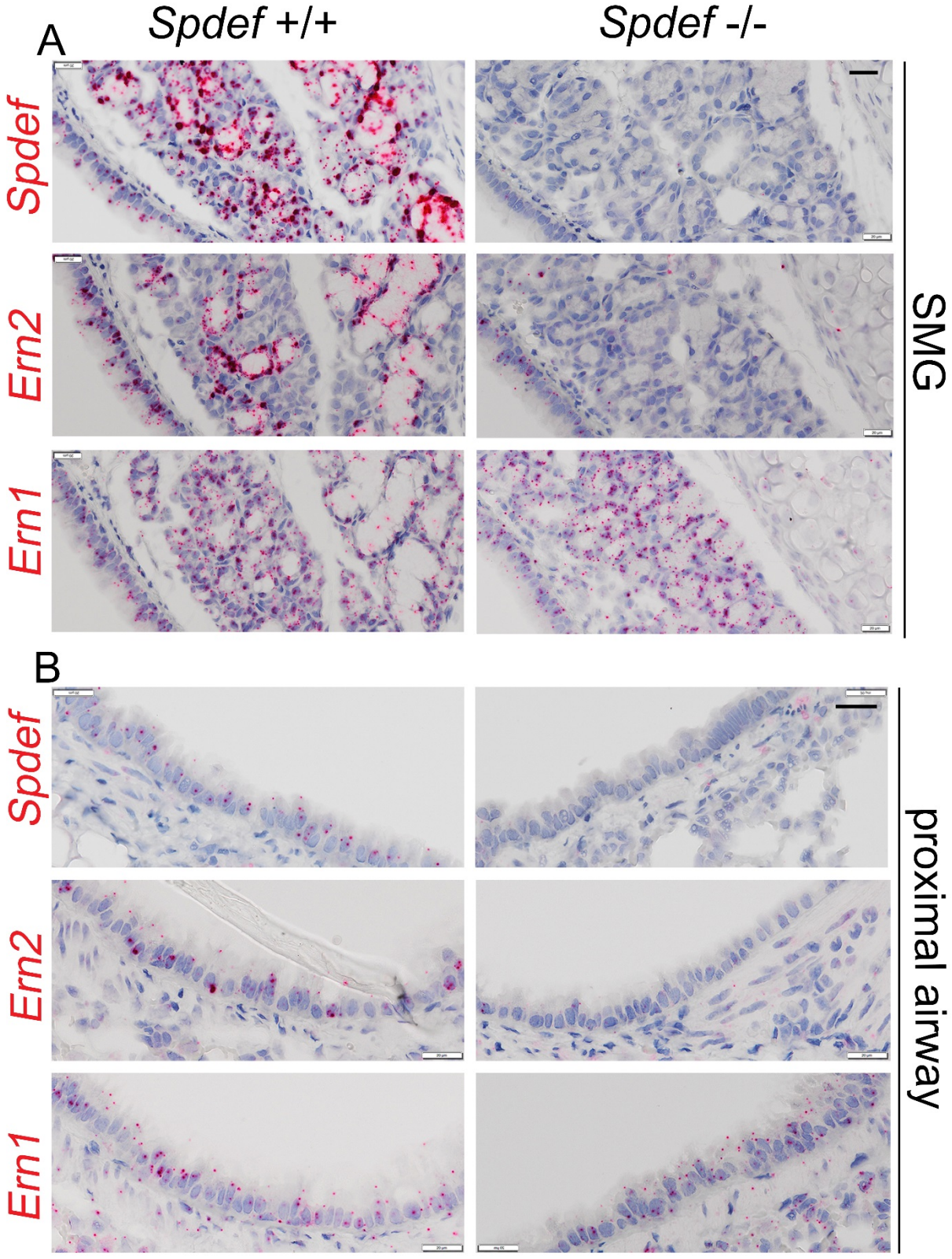
Supplementary Figure 8: IL1β induces *SPDEF* mRNA expression in both non-CF and CF HBE cells. (**A**) A heatmap shows induced (pink, orange to red color) and suppressed (purple to dark blue) transcription factors in n=3 codes of non-CF HBE cells after 24 hours of IL1β exposure (10 ng/ml in media from basolateral side). The red arrow points out SPDEF as one of the upregulated transcription factors. (**B, C**) *SPDEF* mRNA expression was quantitatively measured in non-CF and CF HBE cells following IL1β, IL13 (**B**) and SAMS (**C**) exposure for 1 week by Taqman assays. Graphs present mean±SD. Data were analyzed with one-way ANOVA followed by Dunnett test in control, IL1β and IL13 treatment group (n=7 codes of non-CF HBE cells with n=1 culture/code/treatment were used). Data were analyzed with two-way ANOVA followed by Sidak correction in PBS vs. SAMS treatment group (n=5 codes of non-CF HBE cells with n=2 independent cultures/code/treatment were used). *P<0.05; **P<0.01; ***P<0.001 compared to control or PBS groups. One code means the cells obtained from one individual donor lung.

Supplementary Figure 9



Supplementary Figure 9: Spdef is required for IL1β-induced Muc5ac and Muc5b protein secretion from murine airways in vivo. (**A, B**) Adult (6-week-old) wild type (*Spdef* $^{+/+}$, n=4 for saline, and n=5 for IL1β exposure) and Spdef-deficient (*Spdef* $^{-/-}$, n=3 for saline, and n=5 for IL1β exposure) mice were exposed to saline (**A**) or IL1β (**B**) via intratracheal instillation. Bronchoalveolar lavage (BAL) was collected from the whole lung and subjected to mucin agarose western blot to detect secreted Muc5ac and Muc5b mucin proteins in the BAL.

Supplementary Figure 10



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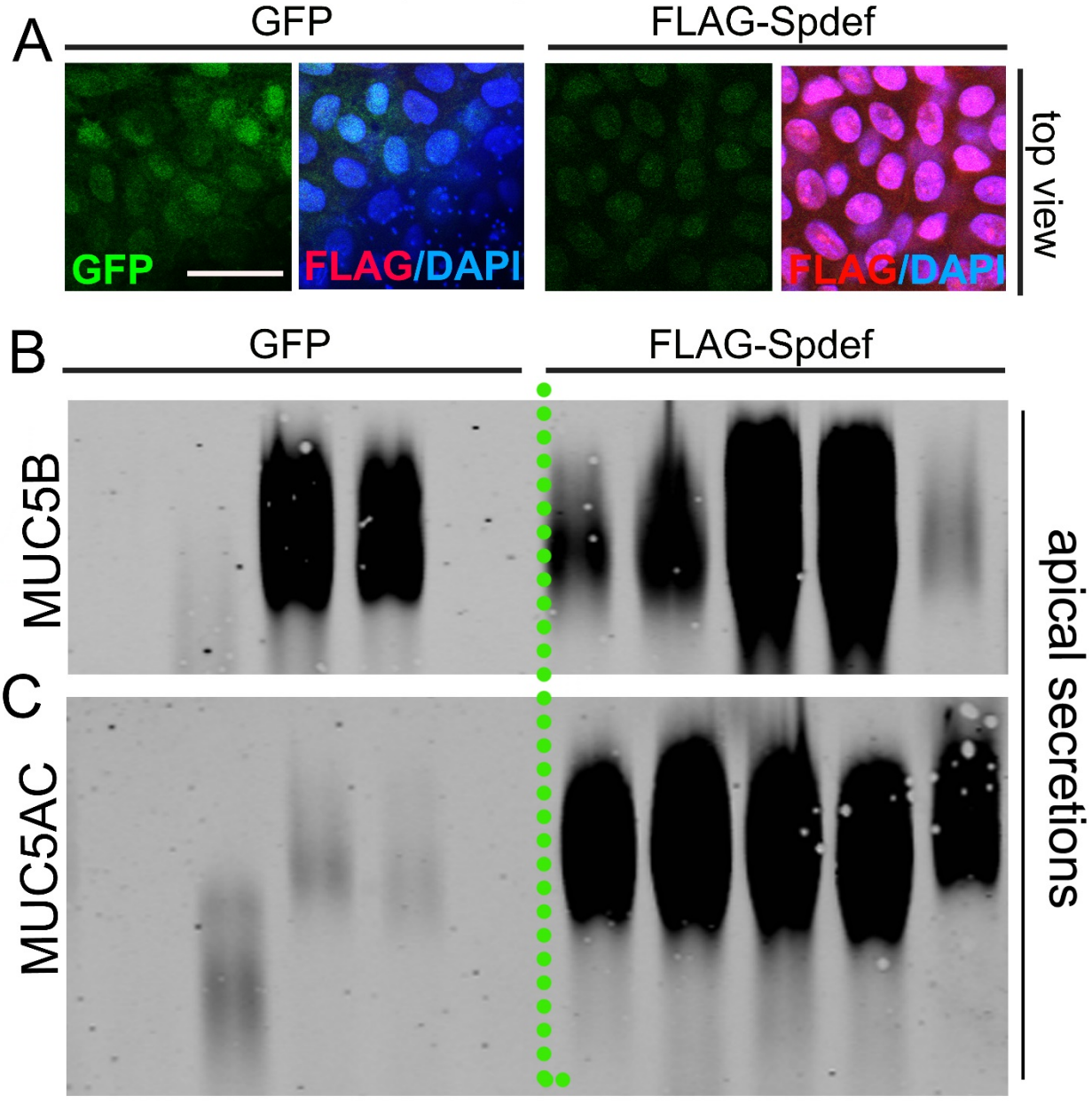
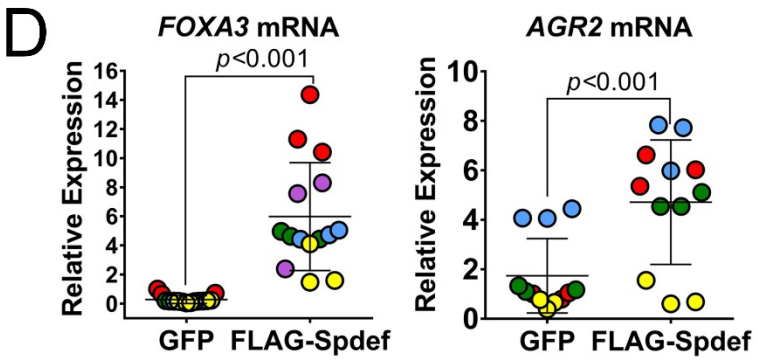
Supplementary Figure 10: mRNA expression of *Spdef*, *Ern2* and *Ern1* in SMGs and proximal intrapulmonary airways in wild type and Spdef-deficient adult mice. (**A,B**) Basal expression of *Spdef*, *Ern2* and *Ern1* mRNAs was detected by Basescope and RNAscope red assays in SMGs (**A**) and in superficial epithelia lining the proximal airways (**B**) of 6-week-old wild type (*Spdef* $^{+/+}$) and Spdef-deficient (*Spdef* $^{-/-}$) mice. Micrographs are representative of n=3 mice/genotype. Scale bar is 20μm in both panels.

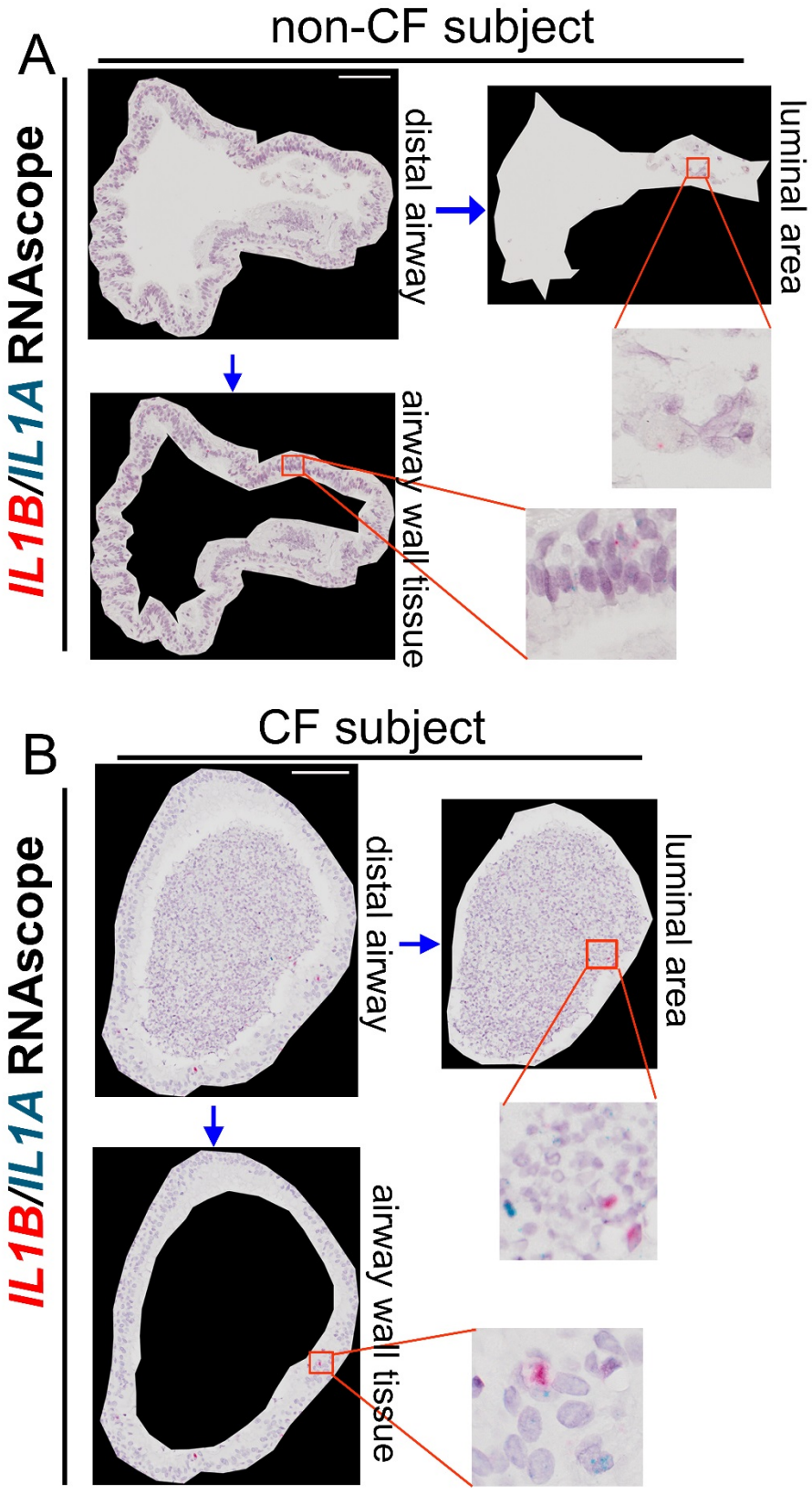
Supplementary Figure 11





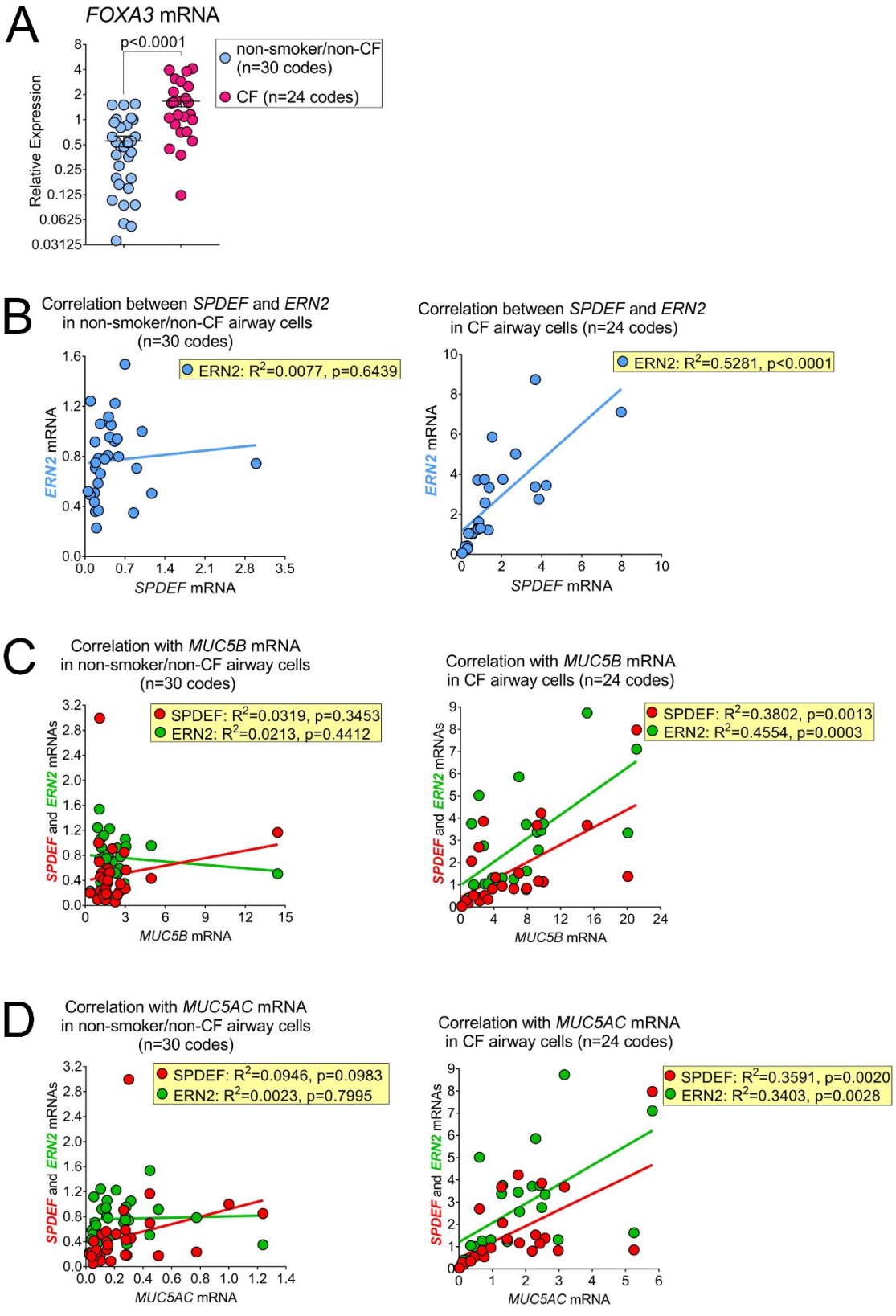
Supplementary Figure 11: Spdef induces MUC5B and MUC5AC protein secretion in HBE cells. Non-CF HBE cells were transduced with lentivirus expressing GFP (control) or FLAG-Spdef (FLAG tag was fused at N-terminus of Spdef protein) and cultured under air-liquid interface conditions for 1 week. (**A**) Expression of GFP and FLAG-Spdef fusion protein was detected by immunofluorescent staining with GFP and FLAG antibodies, counter stained with DAPI to show nuclei. Micrographs are representative of cultures from 3 codes of HBE cells. MUC5B (**B**) and MUC5AC (**C**) protein secretions from the 5 codes of non-CF HBE cells expressing GFP or FLAG-Spdef were detected by mucin agarose gel western blot. (**D**) Non-CF HBE cells were infected with lentiviruses expressing GFP (control) or FLAG-Spdef and cultured under ALI conditions for 1 week. *FOXA3* and *AGR2* mRNA levels were quantitatively measured by Taqman assays. Data were analyzed with 2 way ANOVA followed by Sidak correction. Non-CF HBE cells from n=4-5 donor lungs were used for lentivirus infection, and n=3 independent cultures of each code were used for performing gene expression assays. The cultures from the same code were labeled with the same color dots that were infected with GFP or FLAG-Spdef lentivirus. One code means the cells obtained from one individual donor lung. Scale bar in (**A**)=10μm.

Supplementary Figure 12



Supplementary Figure 12: Morphometry of RNAscope signals in the lung tissue. Representatives of airway epithelial layer tissues and luminal areas were selected for morphometric analyses of *IL1B/IL1A* RNAscope signals from non-CF (**A**) and CF (**B**) subjects. The cells in the luminal areas and airway epithelial layer tissues are shown in high power view from the regions selected. Scale bar=100μm.

Supplementary Figure 13



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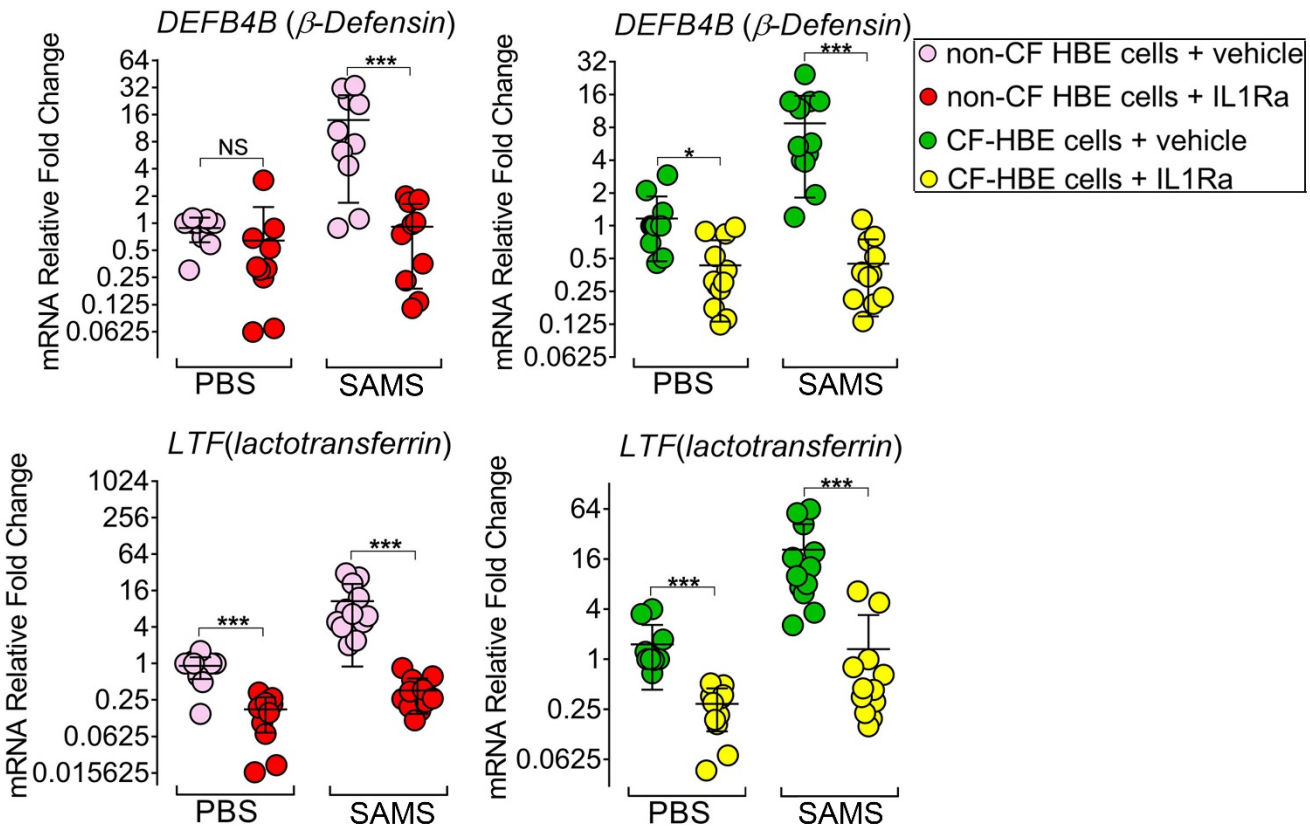
Supplementary Figure 13: Increased expression of *FOXA3* mRNAs in CF airway cells. *SPDEF* and *ERN2* mRNAs are associated with higher level expression of *MUC5B* and *MUC5AC* mRNAs in the freshly isolated airway epithelia from CF compared to non-smoker/non-CF subjects. (**A**) SPDEF-regulated gene *FOXA3* mRNA was quantitatively measured by Taqman assays (normalized to endogenous *GAPDH* mRNA) from the airway epithelial cells (passage #0) freshly isolated from non-CF, non-smoker donors (control, n=30 codes) and CF donors (n=24codes). Scatter plots present means±SE, and data were analyzed with 2-tailed unpaired Mann-Whitney test. The correlations between *SPDEF* and *ERN2* (**B**) mRNAs and the correlation of their expression with *MUC5B* (**C**) and *MUC5AC* (**D**) mRNAs in freshly isolated non-smoker/non-CF (control, n=30 codes) and CF (n=24 codes) airway cells were analyzed by linear regression test. (endogenous *GAPDH* mRNA was used for the normalization of gene expression) The R^2 and p values of the tested genes were annotated in the panel (**B**-**D**). One code means the cells obtained from one individual donor lung.

Supplementary Figure 14



Supplementary Figure 14: IL1Ra inhibits SAMS-induced expression of innate host defense molecules in both non-CF and CF HBE cells. Fully differentiated non-CF and CF HBE cells were pretreated with vehicle (PBS) or IL1Ra (at 2μg/ml on the apical side, and 400ng/ml on the basolateral side) for 24 hours prior to exposure to SAMS (1:40 dilution to reach 1ng/ml of IL1β concentration in SAMS). SAMS+IL1Ra were compared with the control groups that were treated with PBS and IL1Ra that were treated for 3 days. Expression of innate host defense genes *β-Defensin 2* (*DEFB4B*) and *lactotransferrin* (*LTF*) was quantitatively measured by Taqman assays. Graph present means±SD with two cultures of n=5 codes of non-CF and n=5 codes of CF HBE cells. Data were analyzed with 2-way ANOVA followed by Sidak correction. *P<0.05; **P<0.01; ***P<0.001 compared to vehicle treated groups; NS=not significant. One code means the cells obtained from one individual donor lung.

II: Supplementary Materials and Methods:

***Mouse tracheal epithelial cells (mTEC) culture*:** mTEC isolation and culturing followed the procedure previously described (1) with a modified differentiation protocol. To induce differentiation, mTEC were cultured with PluriQ differentiation media (Stem Cell, Inc) on milli-cells (Millipore, PIMC01250) for 3 weeks at the air-liquid interface (ALI) to allow full differentiation. Recombinant mouse IL1α (#400-ML-005), IL1β (#401-ML-005), IL13 (#413-ML-005) and IL17a (#7956-ML-025) cytokines were ordered from R&D System (Minneapolis, MN) and administered in differentiation media from basolateral side at concentration of 10ng/ml for 1 week.

Preparation of the supernatants of airway mucopurulent secretions (SAMS) from CF airways: Mucopurulent material was harvested from the airways of excised human CF lungs and provided by the Tissue Procurement and Cell Culture Core at the UNC at Chapel Hill. The collected material was then centrifuged at 100,000 rpm (60 min, 4 °C), and the supernatant from the mucopurulent material was sterilized by filtration through a 0.2 μm filter and frozen at -80 °C, as previously described (2). Because of the limited volumes of SAMS per patient and the large number of experiments included in the present study, the undiluted stock SAMS was pooled from 8 CF lungs (described in Figure 1B) to ensure sufficient material for the study.

***ELISA determination of IL1α and IL1β concentration in SAMS*:** A pooled SAMS sample collected from 8 CF donors was diluted 1:100, 1:10, 1:2 with sterile PBS. The diluted pooled SAMS and undiluted SAMS collected from individual CF donor lungs (3) were used to for determination and calculation of IL1α and IL1β concentration in each CF airways using the IL1-alpha and IL1-beta Human ELISA kits (BMS243-2 and KHC0012, ThermoFisher) following manufacture instruction.

***Cell and lung tissue collection, total RNA isolation and cDNA preparation*:** The left lobe of the mouse lung was surgically excised after euthanasia and homogenized immediately (Minilys homogenizer, Bertin, Rockville, MD) in Trizol reagent. Freshly isolated HBE cells were collected from airway tissue of non-smoker/non-CF and CF donors right after enzymatic digestion and physical scraping (provide by Tissue Procurement and Cell Core of Marsico Lung Institute, UNC at Chapel Hill), and immediately lysed with Trizol for total RNA purification. The HBE cells growing on the transwell membrane were collected by excision of the whole membrane together with the cells using razor blade, and lysed in Trizol at 37°C shaker (250rpm) for 30 minutes. Total RNA was purified from the Trizol lysates using the Direct-Zol RNA miniprep Kit (cat#R2051, Zymo Research, Irvine, CA), and examined by NanoDrop One Spectrophotometer (ThermoFisher) for its quality and quantity. 1μg of total RNA was reverse transcribed to cDNA by Verso cDNA Kit (cat#AB-1453/B, Thermo Fisher Scientific, Waltham, MA) at 42°C for one hour. Quantitative RT-PCR was performed using Taqman probes (Applied BioSystems, Foster City, CA), or SYBR green primer sets with SsoAdvanced Universal Probes Supermix, Ssoadvanced Univerisal SYBR green Supermix, (cat#1725275, 1725285, Bio-Rad, Hercules, CA) respectively, on QuantStudio6 Real-time PCR machine (Applied Biosystem). The house-keeping gene used for normalization of gene expression for in vitro cultured HBE cells was TATA-binding protein (*TBP*) gene for all the quantitative measurement unless otherwise specified in the figure legend. The house-keeping gene used for normalization of gene expression of mouse lung in vivo was glyceraldehyde 3-phosphate dehydrogenase (Gapdh). See primers/probes table for detailed information.

***Immunohistochemistry, AB-PAS staining and confocal microscopy*:** The surgically excised human lung tissue was dissected from main bronchi to distal airways, lung parenchyma containing bronchi and/or bronchioles and/or terminal bronchioles, followed by fixation with 10% neutral buffered formalin for 24-36 hours in immersion and paraffin-embedding. The mouse lung was inflation fixed with 10% neutral

buffered formalin for 24 hours on a rocker at 4°C. The paraffin-embedded lung tissue specimens were cut at 5μm thickness to produce sections. ALI cultured HBE cells were fixed with 10% neutral-buffered formalin on transwell membrane for 1 hour at room temperature, followed by washing with PBS prior to embedding and sectioning. H&E, AB-PAS and immunohistochemical and immunofluorescent staining were performed as previous described (4, 5). GFP antibody and FLAG antibody was purchased from Abcam (ab5450) and Sigma-Aldrich (cat#F3165-2MG).

***RNA in situ hybridization* (*RNAScope and BaseScope*):** Advanced Cell Diagnostics (ACD) designed and synthesized probes and reagent kits for RNA in situ hybridization used in this study. The probes and reagents were based on ACD proprietary RNAscope® technology which integrates probe design with signal amplification and detection to achieve single-molecule detection. All RNAscope probes consist of a series of individual oligos was called Z probes. Detailed designing information, including the template sequence (GenBank Accession#) used for designing the probe, and the starting and ending positions in the gene sequence where the probes bind, is shown in the "RNAscope and BaseScope Probe Table". The procedure of hybridization with the probes on human and mouse tissue slides were performed following manufacturer's manuals.

***Mass spectrometry*:** A 100ul aliquot of apical secretions from each HBE cells culture chronically exposed to control, IL1β or IL13 was reduced, alkylated, and digested with trypsin as previously described. The resulting peptides were analyzed by liquid chromatography-tandem mass spectrometry (Q Exa]ctive, Thermo Fisher Scientific) using data dependent analysis (6). Proteins were identified from the secretions by searching against the most current human database and quantified with Scaffold 4.4.8 (Proteome Software Inc.) using the total precursor intensity without normalization, including peptides with a minimum of 95% probability by the Scaffold Local FDR algorithm.

***Percentage mucus solids*:** The percentage mucus solids content, an index of hydration, was calculated by measuring dry to wet weight ratio of apical secretions from HBE cells after cytokine treatment using the filter paper technique following the protocol described previously (7, 8).

***Mucin agarose western blot and MUC5B and MUC5AC antibodies*:** MUC5AC and MUC5B agarose Western blot of mouse BAL samples was followed the protocol previously described (4, 9) to detect human and mouse mucins expression. Human MUC5AC and MUC5B protein expression in apical secretions of HBE cells was detected by human MUC5AC (10) and MUC5B (11) antibodies, while mouse Muc5ac and Muc5b protein in the whole lung BAL was detected by Muc5ac (UNC-294) and Muc5b (UNC-222) antibodies (4, 12). Western blot signal detection and densitometry analysis were performed using the Odyssey Infrared Imaging System (LI-COR Biosciences).

***Construction of lenti-CRISPR vectors*:** Single guide RNA (sgRNA) targeting sequences (see primers/probes table) were selected using the online tool: crispr.cos.uni-heidelberg.de, from which *IL1R1* CRISPR was predicated to target the 4th exon of the human *IL1R1* gene. The top 3 potential off-target sequences were also predicted using the same program. Cloning and generation of CRISPR/Cas9 lentiviruses were followed the protocol provided by Dr. Feng Zhang's laboratory at MIT (13). The sgRNA sequence for control CRISPR vector, EGFP CRISPR that did not target mammalian genome was previously described (13).

***Generation of lentivirus and titration*:** To generate the EGFP and IL1R1 CRISPR-Cas9, GFP and FLAG-Spdef (14) (generously gifted by Dr. Jeffery Whitsett laboratory of Cincinnati Children's Hospital Medical Center, Cincinnati, OH) lentiviruses, the transfer plasmids were co-transfected with packaging plasmids pCMV-VSV-G and psPAX2 (cat#8454 and #12260, Addgene) in HEK293T cells (cat#CRL-3216, ATCC). After 6 hours, cell culture media was changed to D10 media, which contained DMEM with 10 % fetal bovine serum with 1 % bovine serum albumin (cat#A9418-50G, Sigma-Aldrich). After continuous

culture for 48 hours without changing media, viral supernatants were collected and harvested by centrifugation at 4,000 rpm at 4 °C for 10 min to pellet cell debris. The supernatant was then filtered through a 0.45μm low protein binding membrane (cat#SLHP033RS, Millipore). The virus titer was determined by quantitative RT-PCR with a kit following manufacture instructions (cat#631235, Takara Bio).

***Primary HBE cell culture, cytokine exposure, and lentivirus infection*:** Primary HBE cells from non-CF (obtained from donors without previously known pulmonary diseases) and CF donors (CFTR mutation genotyping verified) were cultured following the conditional reprogramed cell (CRC) culture protocol described previously (15-17) after isolation from the airways. HBE cells were maintained at an air-liquid interface (ALI). The apical surface was washed with PBS, and ALI medium (18) was replaced only in the basal compartment two-three times per week, and cells were cultured under ALI conditions for 4 weeks to allow full differentiation. Exposure with recombinant human cytokines was administrated 4 weeks after ALI culture (all cytokines were purchased from R&D system, human IL1α: #200-LA, human IL1β: #201-LB-005, human TNFα: #210-TA, human IL8: #208-IL, human IL13: #201-ILB-005, human IL17A: #7955-IL, human IL17F: #1335-IL, human IFNα: #11200-1, human IFNβ: #8499-IF, human IFNγ: #285-IF, human IFNλ1: #1598-IL, human IFNλ2: #8417-IL), and added into basolateral side of ALI media unless otherwise specified. To infect primary HBE cells with lentiviruses, 1 million P1 cells were seeded into Corning 10cm dishes coated with bovine collagen (PureCol model 5005-B; Advanced BioMatrix) in a modified CRC culture (19) media (CRCY): 750ml of DMEM (High Glucose+ Pyruvate) (Gibco #11995-065), 250ml of F12 (Gibco #11765-054), 11 ml of Pen/Strep 100x (Gibco #15140-122), 75ml of FBS (Gibco #16140-071), and the following supplements with their final concentration: Hydrocortisone (25ng/ml, H0888, Sigma), EGF (25ng/ml, #PHG0313, Invitrogen), Insulin (5μg/ml, I5500, Sigma), Amphotericin B (250ng/ml, #BP264550, Fisher), Gentamincin (10μg/ml, #15710-064, Gibco), Cholera

toxin (1nM, C8052, Sigma) and Y-27632 (10μM, ALX-270-333-M025, Enzo life Science). At 30-50% confluence (usually 2nd day after seeding in dish), HBE cells were infected with lentivirus at MOI=3 for 3 hours and grown for 48 hours before passaging to another purecol coated 15cm dish and starting with (the CRISPR/Cas9 lentiviruses) or without (GFP, FLAG-Spdef lentiviruses) puromycin selection (1μg/ml in CRCY) at this time point. Confluent cultures were trypsinized and frozen down in liquid N_2, or seeded directly into Corning Transwell in CRCY-puromycin media at the density of 250k cells/transwell. After confluence in Transwell, cells were cultured under ALI with ALI media containing 1μg/ml puromycin till the end of the culture.

***siRNA transfection of N3T cells*:** Negative control and *SPDEF* specific siRNA (ID:# 4390843 and #S195114, Ambion) were transfected into submerge cultured N3T cells (18) (passage#12) using Lipofectamine™ RNAiMAX Transfection Reagent (#13778150, ThermoFisher) following the protocol previously described (5). Gene expression assays were performed 48 hours after siRNA transfection.

***In vivo administration of cytokines and SAMS in mouse lung*:** In vivo cytokine/SAMS treatment was performed following a protocol previously described (20). Mouse recombinant cytokines IL1α, IL1β, IL13 (same as aforementioned cytokines used in mTEC differentiation were purchased from R&D), and SAMS (diluted by mixing 25μl undiluted pooled SAMS with 15μl sterile PBS) were administrated by intratracheal instillation at 1μg/40μl/mouse on day 1, repeated on day 2 and day3. Mouse lung tissue and BAL was harvested on day 6 for histology, RNA and secreted mucin protein analysis.

***In vitro administration of IL1Ra*:** Non-CF and CF HBE cells were ALI cultured for 4 weeks, and washed 3 times with PBS on apical side prior to treatment with IL1Ra (#280-RA-050, R&D system). Fifty microliter of IL1Ra (2μg/ml diluted in sterile PBS) was added on apical side, and also in ALI media (400ng/ml) for 1 day before SAMS administration to block IL1R1. After 1 day, pretreatment of IL1Ra was removed from both apical side of the HBE cells. Cells were treated with 50μl of SAMS+ vehicle

(1:40 dilution of stock SAMS to achieve 1ng/ml of IL1β in SAMS using sterile PBS) or 50µl of SAMS+IL1Ra (diluted SAMS containing 1ng/ml IL1β and 2µg/ml IL1Ra) at apical surface, and 400ng/ml IL1Ra was kept in ALI media at basolateral side for 3 days.

***Morphometry of MUC5B/MUC5AC, IL1B/IL1A mRNAs in control and CF lung tissues*:** The protocol of performing morphometry studies followed the methods described by Okuda et al. (21). Briefly, all airway sections were scanned and digitized at a magnification at 60X for *MUC5B/MUC5AC, IL1B/IL1A* RNAscope using an Olympus VS120 slide scanner light microscope. For the criteria of selection of distal airways, we selected distal airways based on their sizes, regardless of staining signal intensity. We selected all the distal airways that had luminal diameter <1.5mm or the terminal airways in the non-CF (n=4 donors) and CF (n=3 donors) subjects. Quantification of *MUC5B/MUC5AC* (13.5±2.5 airways/non-CF subject, and 13.6±6.1 airways/CF subject) and *IL1B/IL1A* (11.8±3.1 airways/non-CF subject, and 15.3±4.2 airways/CF subject) mRNA signals in the distal airways tissues was performed following the protocol previously described (21). The length of basement membrane (BM) was measured and used for normalization of stained volumes per the formula below. The image of the target distal airway tissues, which were left after isolating the inside luminal areas and outside regions of the airway epithelial layers, was converted to a gray-scale image followed by quantification of the areas above the optimized threshold values. We evaluated the optimized threshold value by changing the threshold until the threshold (black and white) image accurately represented the red or turquoise signals of the original RNAscope-duplex images. The area above the threshold value was then measured. The volume densities of *MUC5B/MUC5AC* and *IL1B/IL1A* mRNAs in the distal airway epithelial layers were calculated as: airway epithelial layer threshold value / [(BM) (4/π)]. As a result, data are presented as the volume of *MUC5B*, *MUC5AC*, *IL1B* and *IL1A* mRNA per unit surface area of the basement membrane (mm^3/mm^2). To measure the distal airway luminal *IL1B/IL1A* staining contents, we followed the protocol previously

described by Burgel et al (22). We excluded all the areas except the luminal areas (see Supplementary Figure 12). We calculated the *IL1B/IL1A* luminal contents as: luminal threshold value/[$BM^2/(4\pi)$]. As a result, the data are presented as the ratio of *IL1B* and *IL1A* staining contents to the total luminal volume in the airways measured. All the normalized values were then cube-root transformed (23) prior to perform statistical analyses.

Taqman Assay Probes	
Probe Name	**Assay ID**
MUC5B	Hs00861595_m1
MUC5AC	Hs01365616_m1
CFTR	Hs00357011_m1
SCNN1A	Hs00168906_m1
SCNN1B	Hs01548617_m1
SCNN1G	Hs00168918_m1
SPDEF	Hs00171942_m1
ERN1	Hs00980095_s1
ERN2	Hs01086607_m1
AGR2	Hs00982833_m1
CXCL8	Hs00174103_m1
IL6	Hs00174103_m1
CXCL1	Hs00236937 _m1
FOXA3	Hs00270130_m1
DEFB4B	Hs00175474_m1
LTF	Hs00914334_m1
Muc5b (exon 16-17)	Mm00466391_m1
Muc5b (exon 33-34)	Mm00466407_m1
Muc5ac	Mm01276718_m1
Spdef	Mm00600221_m1
Foxa3	Mm00494714_m1
Agr2	Mm01291804_m1
Ern1	Mm00470233_m1
Ern2	Mm00469005_m1
Clca1 (Clca3)	Mm01320697_m1
Il1a	Mm00439620_m1
Il1b	Mm00434228_m1
Il17a	Mm00439618_m1

SYBR Green quantitative RT-PCR primers		
Primers	Forward	Reverse
Muc5b (exon14-16)	5’-CCTACCAAGGCCAGATGTGT-3’	5’-AAC TCC TGA GCT TTC CGT GA-3’
Muc5b (exon25-26)	5’-AAA CCC TTA CCG CAA GTC CT-3’	5’-GAC AAG CAC ACA CCC ACA TC-3’

RNAscope and BaseScope probes					
Probe Name	Catalog No.	ZZ pairs	Accession No.	Target Start	Target Stop
Hs-MUC5B	449881	20	NM_002458.2	3599	4698
Hs-MUC5AC-C2	312891-C2	20	XM_003403450.1	761	2125
Hs-ERN1	497331	20	NM_001433.3	153	1054
Hs-ERN2	497231	20	NM_033266.3	369	1508
Mm-Muc5ac	488471	20	NM_010844.1	4071	5187
Mm-Muc5b	471991	20	NM_028801.2	380	1263
Mm-Ern2	500431	20	NM_001316689.1	2	958
Mm-Ern1	438031	20	NM_023913.2	162	1242
Mm-Spdef-4zz-st	705701	4	NM_013891.4	1251	1478
BA-Hs-SPDEF-E2E3	704211	1	NM_012391.2	843	879

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