

## **Supplementary materials**

**Title:** Molecular and functional characteristics of airway epithelium under chronic hypoxia.

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**Table S1. List of differentially expressed proteins (DEPs) for hNECs conditional reprogramming cultures. (Excel file)**

**Table S2. List of differentially expressed proteins (DEPs) for hNECs air-liquid interface cultures. (Excel file)**

**Table S3. IPA pathway analysis of differentially expressed proteins for hNECs conditional reprogramming cultures. (Excel file)**

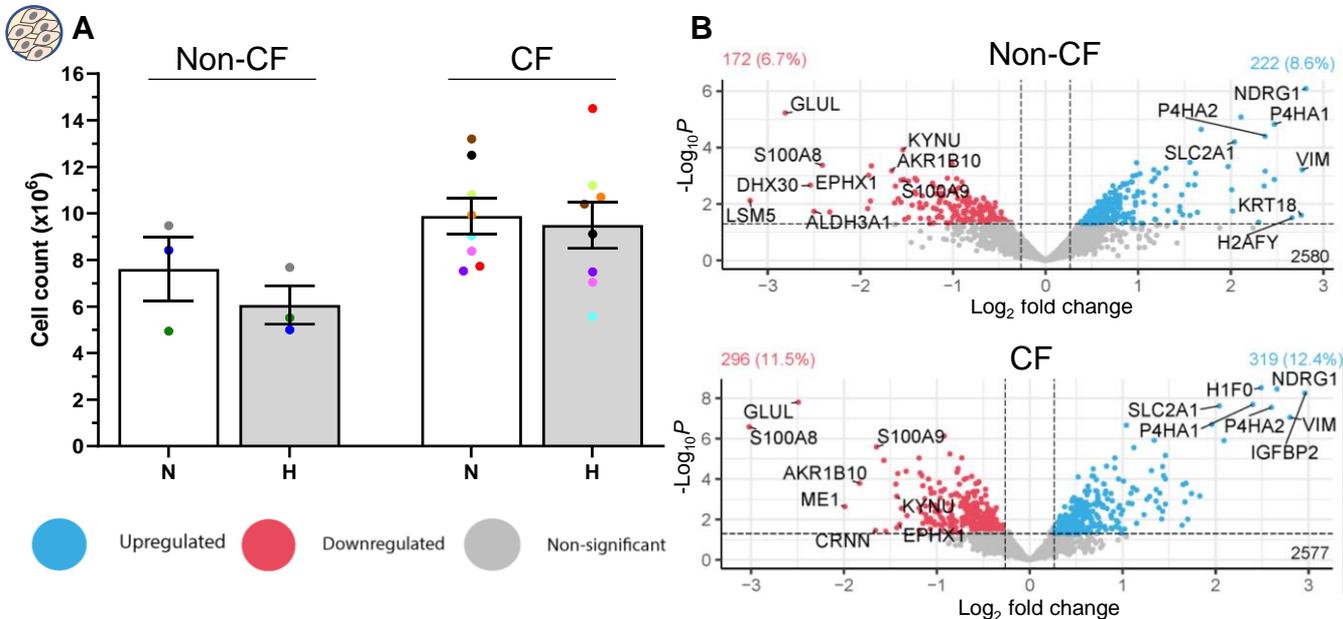
**Table S4. IPA pathway analysis of differentially expressed proteins for hNECs air-liquid interface cultures. (Excel file)**

**Table S5. Details of study participants**

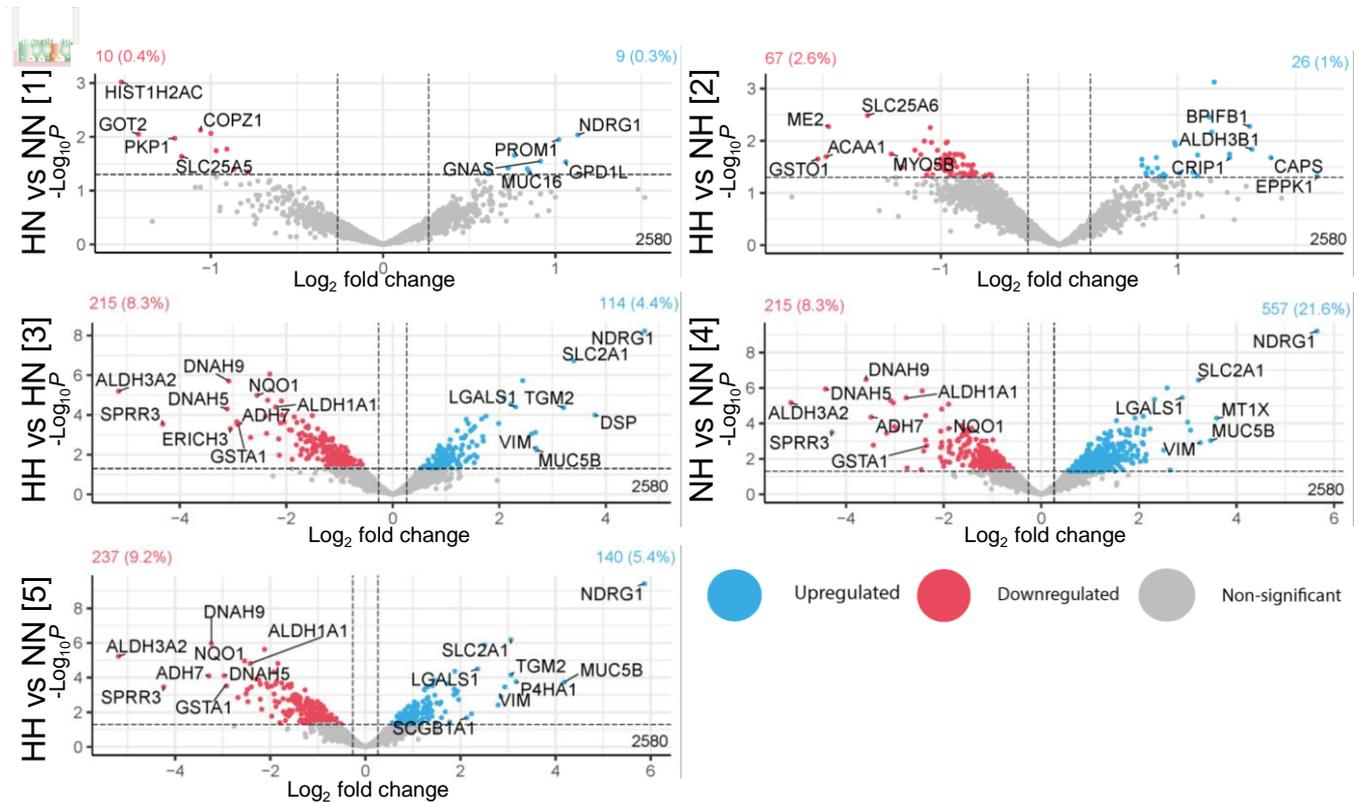
<b>Participant ID</b>	<b><i>CFTR</i> genotype</b>	<b>Age (yr)</b>	<b>Sex</b>
WT1	WT/WT	2.5	M
WT2	WT/WT	1.4	Unknown
WT3	WT/WT	13.3	M
CF1	F508del/F508del	11.2	M
CF2	F508del/F508del	5.0	M
CF3	F508del/F508del	13.8	F
CF4	F508del/F508del	6.4	F
CF5	F508del/F508del	14.2	M
CF6	F508del/F508del	3.7	M
CF7	F508del/F508del	4.8	F
CF8	F508del/F508del	4.5	F

**Table S6. Antibodies for immunofluorescence staining**

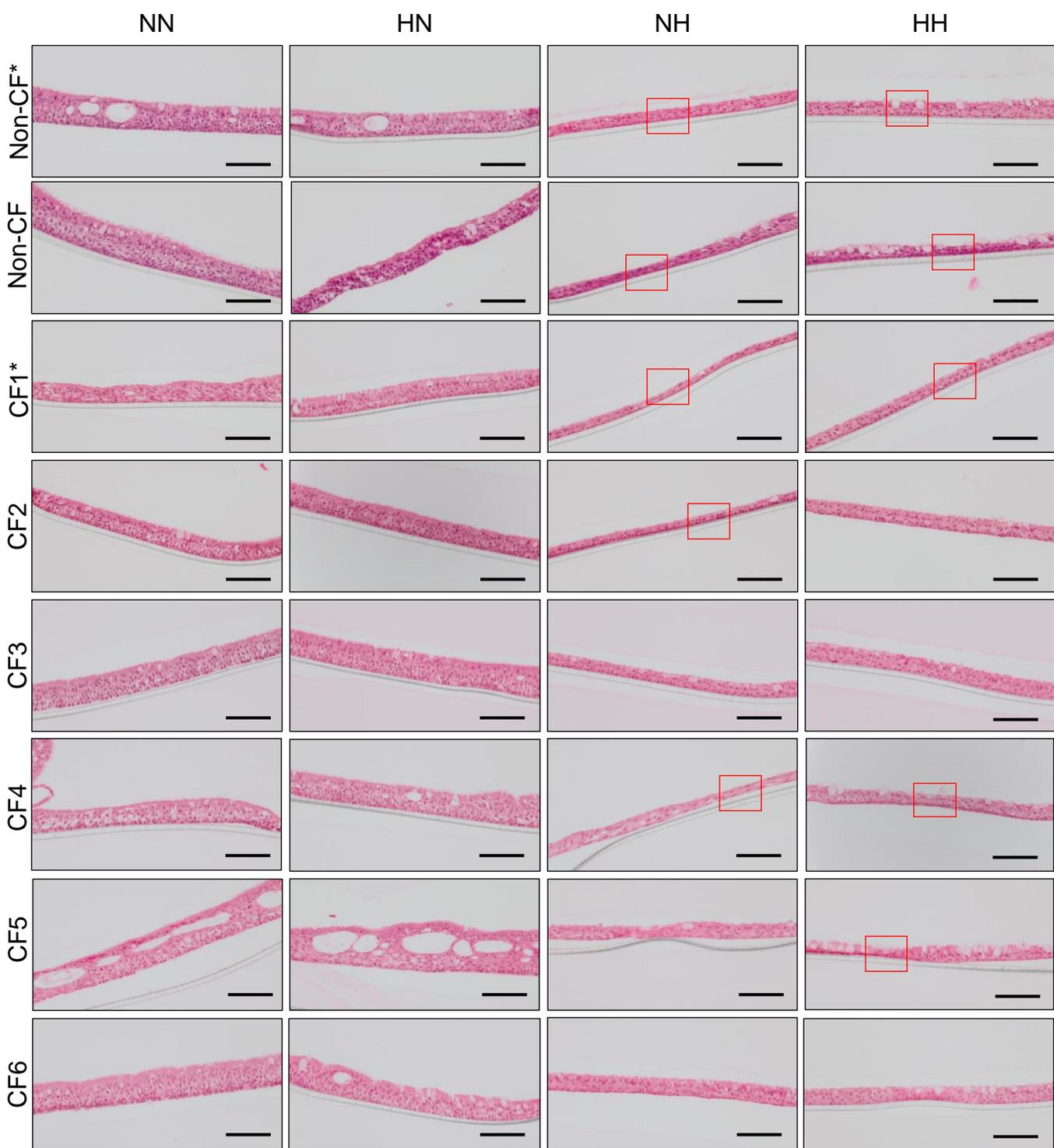
<b>Antibody</b>	<b>Dilution</b>	<b>Supplier</b>	<b>Catalogue number</b>
Rabbit monoclonal anti-E-cadherin (24E10)	1:100	Cell Signalling	3195
Mouse monoclonal anti-MUC5AC (45M1)	1:250	Life Technologies	MA5-12178
Rabbit monoclonal anti-Acetyl- $\alpha$ -Tubulin (Lys40) (D20G3) XP, Alexa Fluor 647 conjugate	1:50	Cell Signalling	81502
Goat polyclonal anti-mouse IgG antibody, Alexa Fluor 555	1:500	Life Technologies	A-21424
Goat polyclonal anti-rabbit IgG antibody, Alexa Fluor 488	1:500	Life Technologies	A-11034



**Figure S1. Effect of chronic hypoxia on the expansion of primary human nasal epithelial cells (hNECs).** (A) Cell count of non-CF ( $n=3$ ) and CF ( $n=8$ ) hNECs following expansion under normoxic and chronic hypoxic conditions. Each coloured circle represents cultures from an individual participant. Data are presented as bar plots with mean  $\pm$  standard error of the mean (SEM). One-way ANOVA was used to determine statistical significance. (B) Volcano plots of differentially expressed proteins (DEPs) in hypoxic cells compared to normoxic cells in non-CF ( $n=3$ ) and CF ( $n=8$ ). Dotted lines indicate significance cut-off ( $p\text{-value} \leq 0.05$ ,  $|\text{fold change}| \geq 1.2$ ). The count of significant upregulated proteins, significant downregulated proteins and total proteins are shown in top right, top left and bottom right respectively. The top 5 to 10 upregulated and downregulated proteins are labelled.



**Figure S2. Effect of chronic hypoxia on the global proteome of differentiated human nasal epithelial cells (hNECs).** Volcano plots of differentially expressed proteins (DEPs) in each oxygen condition in differentiated air-liquid interface (ALI) cultures from three non-CF participants. Dotted lines indicate significance cut-off ( $p\text{-value} \leq 0.05$ ,  $|\text{fold change}| \geq 1.2$ ). The count of significant upregulated proteins, significant downregulated proteins and total proteins are shown in top right, top left and bottom right respectively. The top 5 to 10 upregulated and downregulated proteins (determined based on  $\log_2\text{FC}$ ) are labelled. Comparisons are in pairs; [1] HN compared to NN; [2] HH compared to NH; [3] HH compared to HN; [4] NH compared to NN and [5] HH compared to NN.



**Figure S3. H&E stain of primary hNECS from two non-CF and six CF participants differentiated at ALI in normoxia (NN and HN) and chronic hypoxia (NH and HH) for 21-25 days.** Red rectangle shows squamous cells or cells transitioning towards squamous morphology. \*indicate donor images which are displayed in Figure 4 of the main manuscript. 40x/0.8 objective. Scale bars = 100  $\mu$ m.



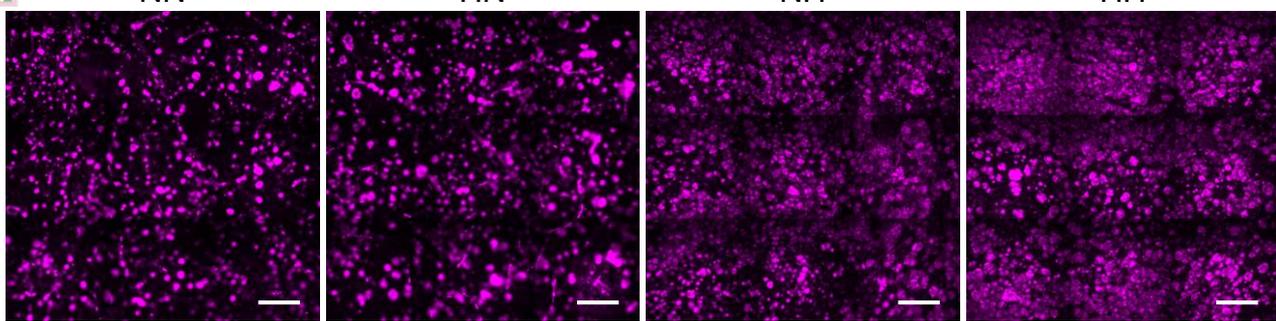
NN

HN

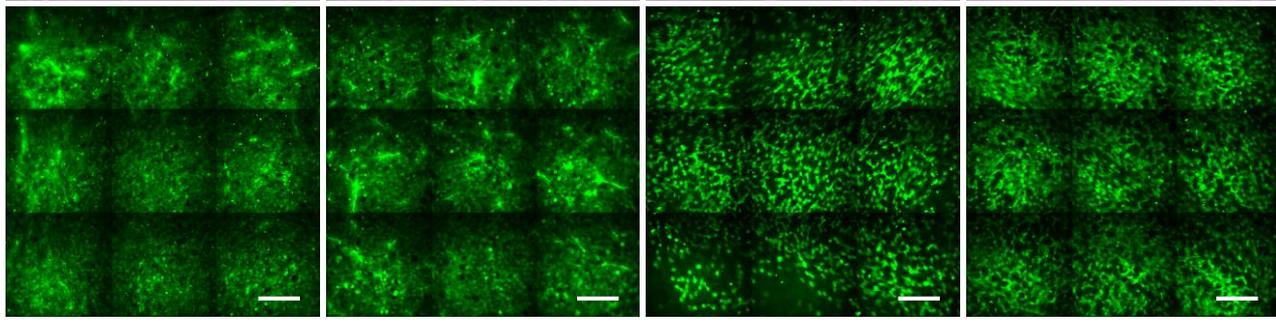
NH

HH

MUC5AC



Actub



**Figure S4. Tiled images of the whole membrane with immunofluorescence staining of MUC5AC (magenta) and acetylated tubulin (actub, green). 20x/0.8 objective. Scale bars = 100 $\mu$ m.**