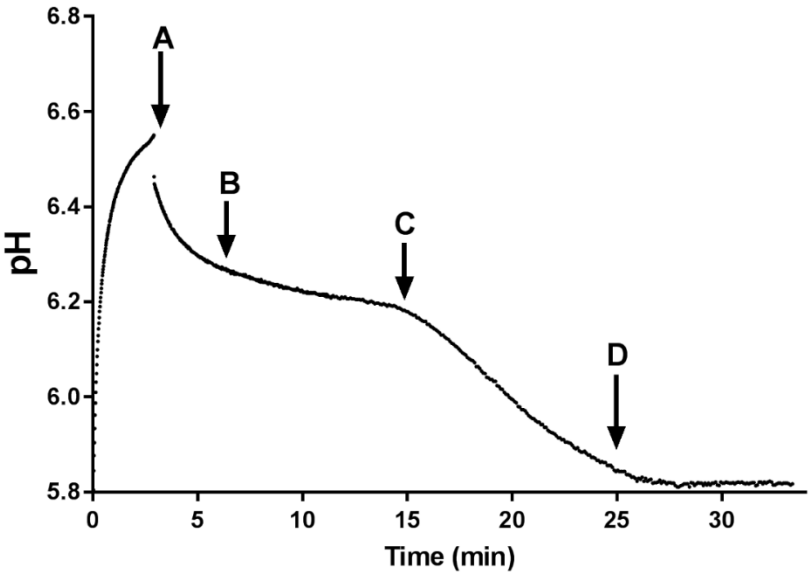
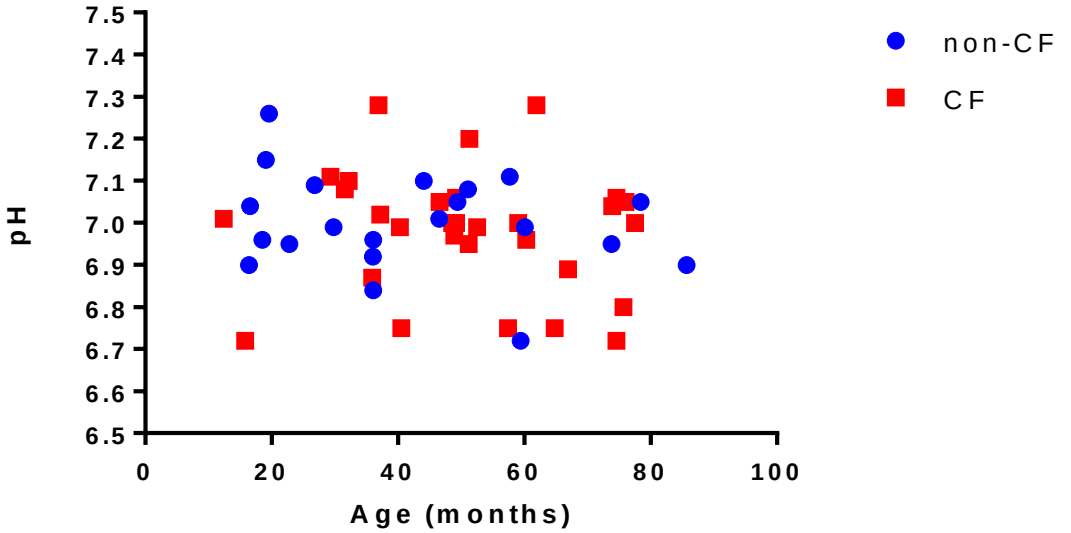
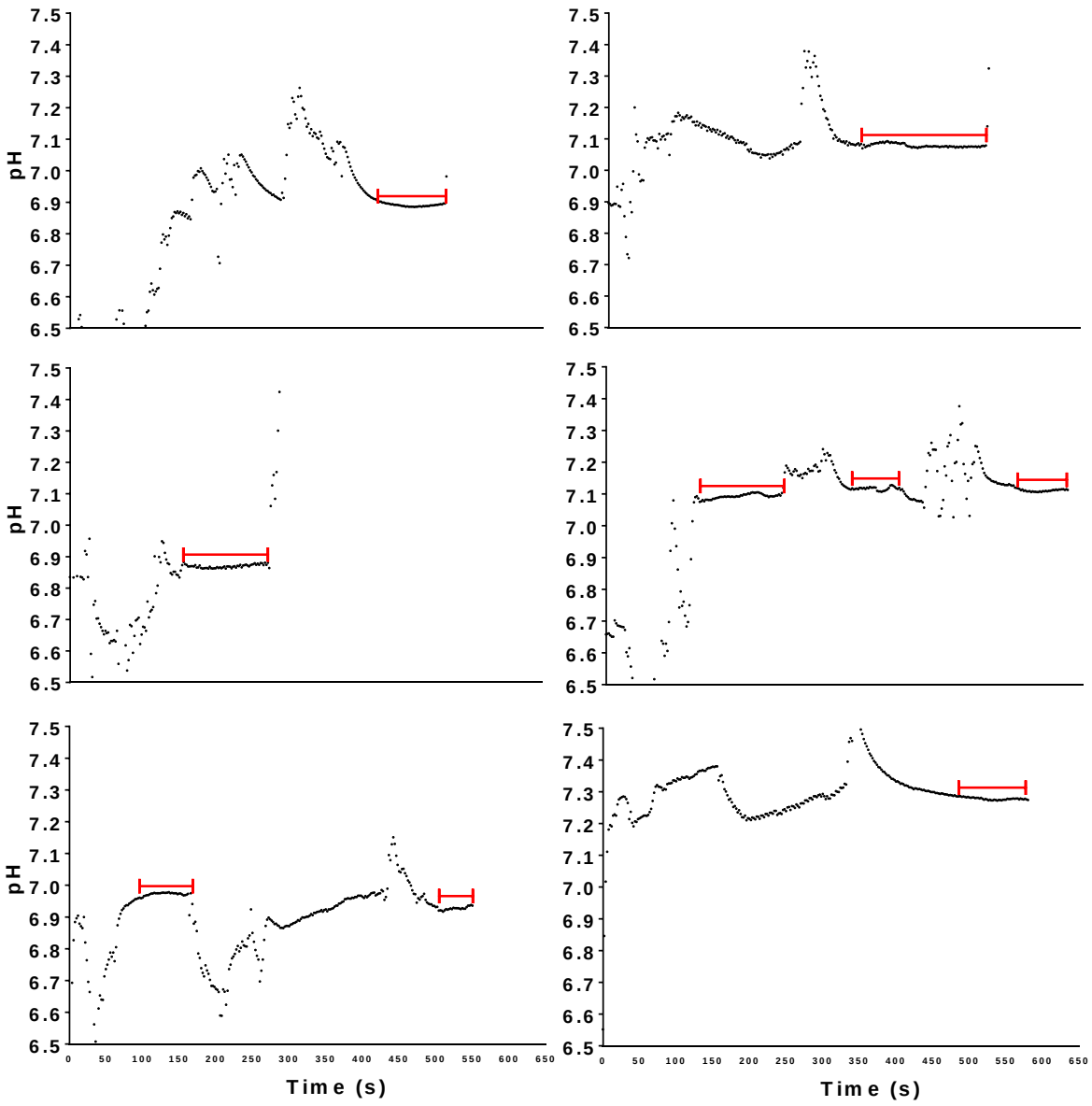
File name: Supplementary Information
Description: Supplementary figures and supplementary tables.



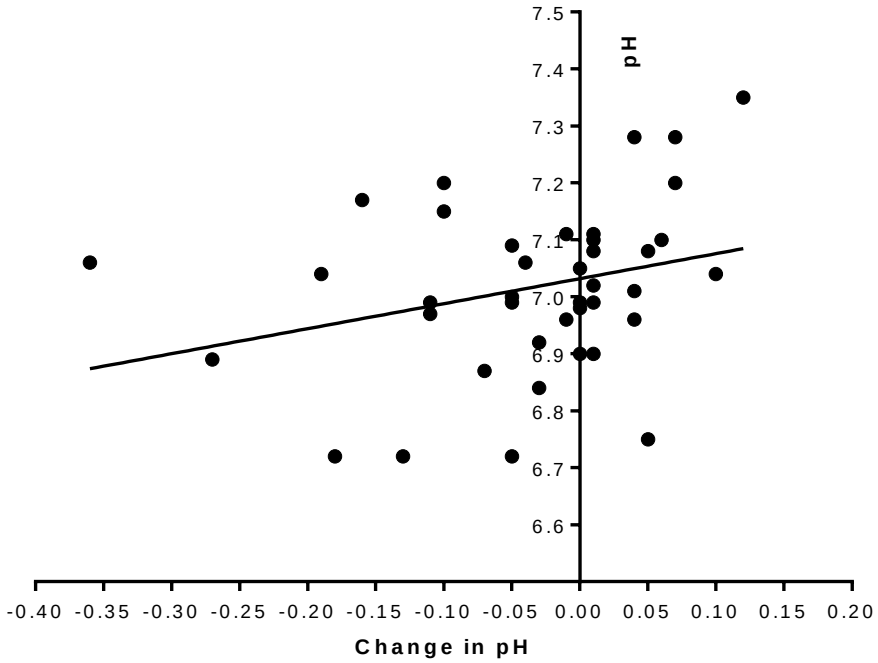
Supplementary Figure 1. *In vitro* ASL pH measurement trace vs. change in ambient CO_2 over time. Sensor hits ASL of cell culture at point A. CO_2 level reaches equilibrium at 5% at point B. CO_2 level set point of incubator changed to 15% at point C, leading to CO_2 injection. CO_2 level reaches equilibrium at 15% at point D.



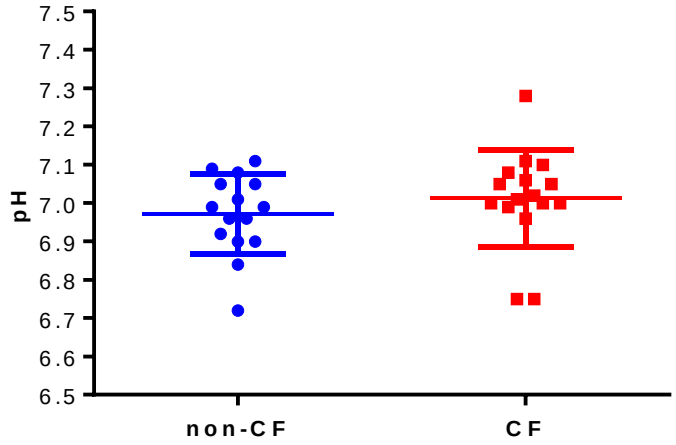
Supplementary Figure 2. *In vivo* measurements of ASL pH vs. age of the patient at the time of measurement. Linear regression analysis demonstrated no significant relationship between ASL pH and the age of the patient for either children with (r^2=0.01; p=0.58; n=30) or without CF (r^2=0.05; p=0.31; n=21).



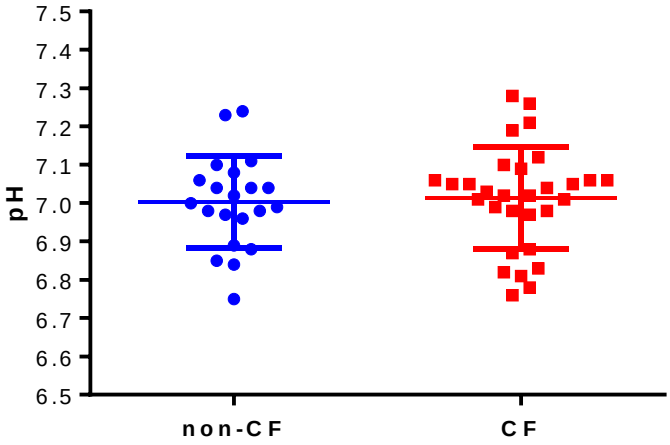
Supplementary Figure 3. Examples of *in vivo* transbronchoscopic pH measurements in 6 different patients. Solid lines correspond to probe contact with the airway surface. Red markings depict plateaus that an individual investigator identified and averaged to determine airway surface liquid (ASL) pH.



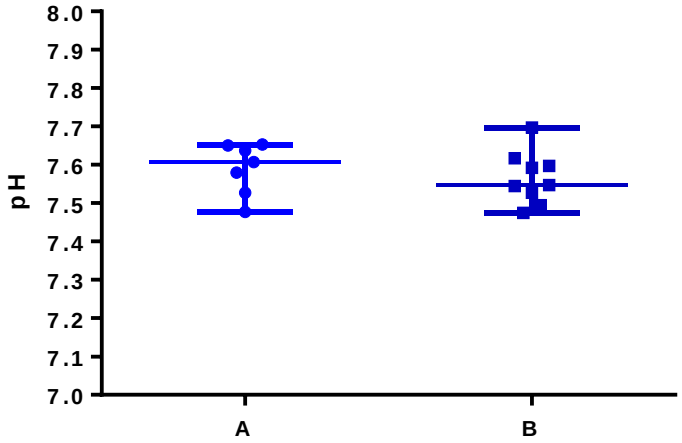
Supplementary Figure 4. *In vivo* pH measurement versus change in pH from 7.6 post-measurement. After *in vivo* pH readings were obtained, fibre-optic probes were returned to the pH 7.6 buffer used in the calibration and the difference from pH 7.6 calculated. Linear regression analysis demonstrated a relationship between the change in pH at the validation step and the *in vivo* measurement recorded (r^2 =0.18, p= 0.001, n=56).



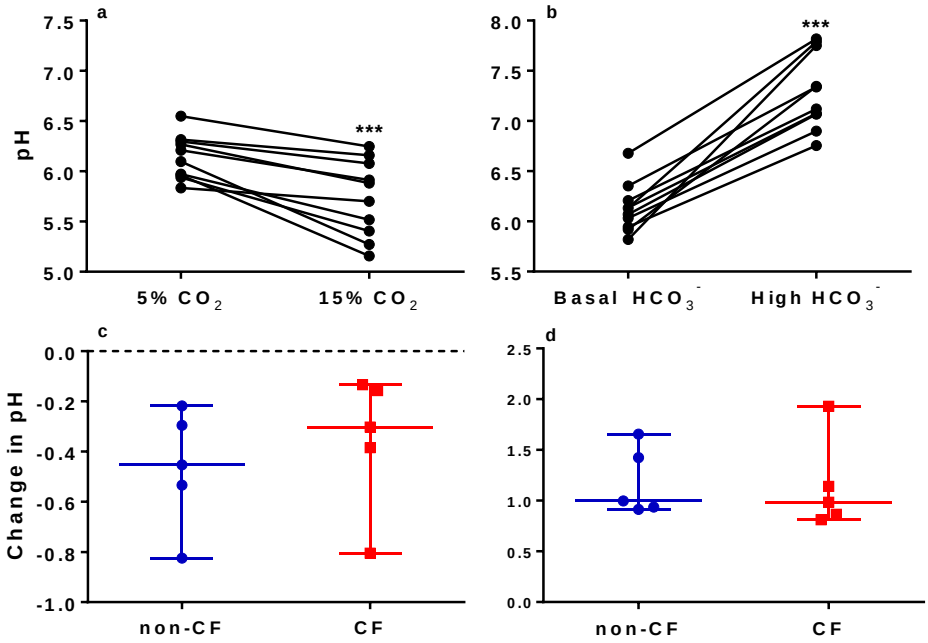
Supplementary Figure 5. Comparison of lower airway surface liquid pH measurements in children with and without cystic fibrosis after exclusion of measurements from pH probes that failed the post-measurement validation step. No difference was found in lower airway pH between non-CF (n=15) and CF (n=16) groups with mean±SD pH in controls vs. CF, 6.97±0.10 and 7.01±0.13 respectively, p =0.33. Two-sided t-test was used to compare CF and non-CF groups. Data presented as individual measurements and mean±SD.



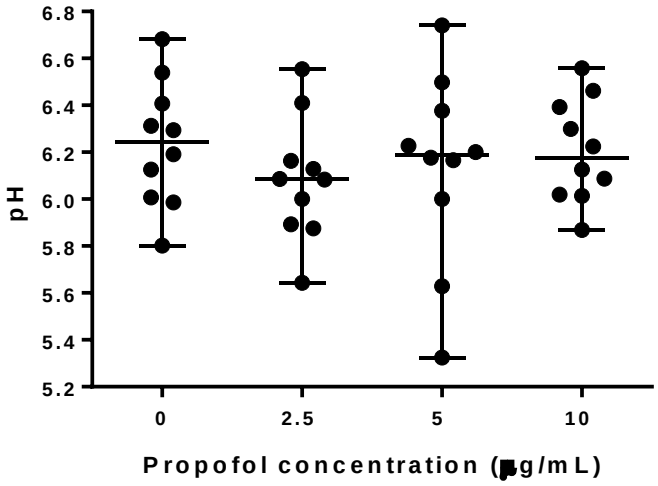
Supplementary Figure 6. Comparison of lower airway surface liquid pH measurements in children with and without cystic fibrosis after correcting for probe drift using corrected calibration point. No significant difference was found between the groups with mean±SD pH in controls (n=21) and CF (n=30), 7.00±0.12 and 7.01±0.13 respectively, p=0.78. Two-sided t-test was used to compare CF and non-CF groups. Data presented as individual measurements and mean±SD.



Supplementary Figure 7. Measurement of a pH 7.6 buffer using fibre-optic probes placed at (A) a 45^{o} angle (n = 7) and (B) a perpendicular angle relative to the mucosal tracheal surface (n = 9). Median (range) of pH measurements were 7.61 (7.48-7.65) and 7.55 (7.47-7.70) in groups A and B respectively. All measurements were performed in triplicate. A two-sided Mann-Whitney test demonstrated that the positioning of the probe had no significant effect on the pH reading (p=0.46). Data presented as median (range).



Supplementary Figure 8. Airway surface liquid pH in airway epithelial cells cultures. **(a)** Paired t-test demonstrated a significant decrease in ASL pH when epithelial cell cultures (n=10; 5 CF and 5 non-CF) were exposed to 15% CO_2 ($p<0.001$). **(b)** Paired t-test comparing epithelial cell culture (n=10; 5 CF and 5 non-CF) at basal HCO_3^- and high HCO_3^- media concentrations showed a significant increase ($p<0.001$) in ASL pH. There were no significant differences in the effect of high CO_2 **(c)** or HCO_3^- **(d)** levels on ASL pH between CF (n=5) and non-CF (n=5) epithelial cell cultures. Figures (a) and (c) describe the same experiment while figures (b) and (d) reflect the same experiment. All data presented as median (range), ***$p<0.001$. CF cell cultures obtained from children with clinical CF disease -genotypes: three of the cultures were of the p.Phe508del/p.Phe508del genotype with cultures generated from different patients. Two of the cultures were of the p.Gly85Glu/p.Arg 352Gln genotype with cultures generated from the same patient at different sampling time points. These experiments also demonstrate that baseline ASL pH in a cell culture model is highly dependent on experimental factors such as the HCO_3^- concentration in the culture medium.



Supplementary Figure 9. Airway surface liquid pH after the addition of propofol to the basal media of airway epithelial cell cultures. No differences between 0 µg/mL propofol (n=10) and 2.5 µg/mL (n=10; p=0.23), 5 µg/mL (n=10; p=0.62) or 10 µg/mL (n=10; p=0.77) were determined by Wilcoxon tests. Data presented as median (range).

Sample	Cohort	Age (months)	Sex	CFTR mutation
1	AREST CF	34.9	M	p.Phe508del/ p.Phe508del
2	AREST CF	3.4	M	p.Phe508del/ p.Phe508del
3	AREST CF	23.4	F	p.Phe508del/ p.Phe508del
4[#]	AREST CF	5.9	F	p.Gly85Glu/p.Arg 352Gln
5[#]	AREST CF	11.4	F	p.Gly85Glu/p.Arg 352Gln
6	WAERP	36.4	F	n/a
7	WAERP	30.1	M	n/a
8	WAERP	33.0	F	n/a
9	WAERP	35.5	M	n/a
10	WAERP	19.9	F	n/a

Supplementary Table 1. Patient demographics for primary airway epithelial cell cultures used in fibre-optic probe ASL pH experiments. [#]Cultures generated from the same patient at different sampling times.

Experimental condition	Basolateral media HCO_3^- concentration (mM)	Atmospheric CO_2 concentration (%)	Basolateral media Propofol concentration (μg/mL)
Basal	27.95	5	0
High HCO_3^-	83.85	5	0
High CO_2	27.95	15	0
Propofol treatment	27.95	5	10

Supplementary Table 2. Experimental conditions for *in vitro* pH experiments in human bronchial epithelium air-liquid interface cultures.