Chronic β2AR stimulation limits CFTR activation in human airway epithelia

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Supplemental Results

Supplemental Figures and Figure Legends

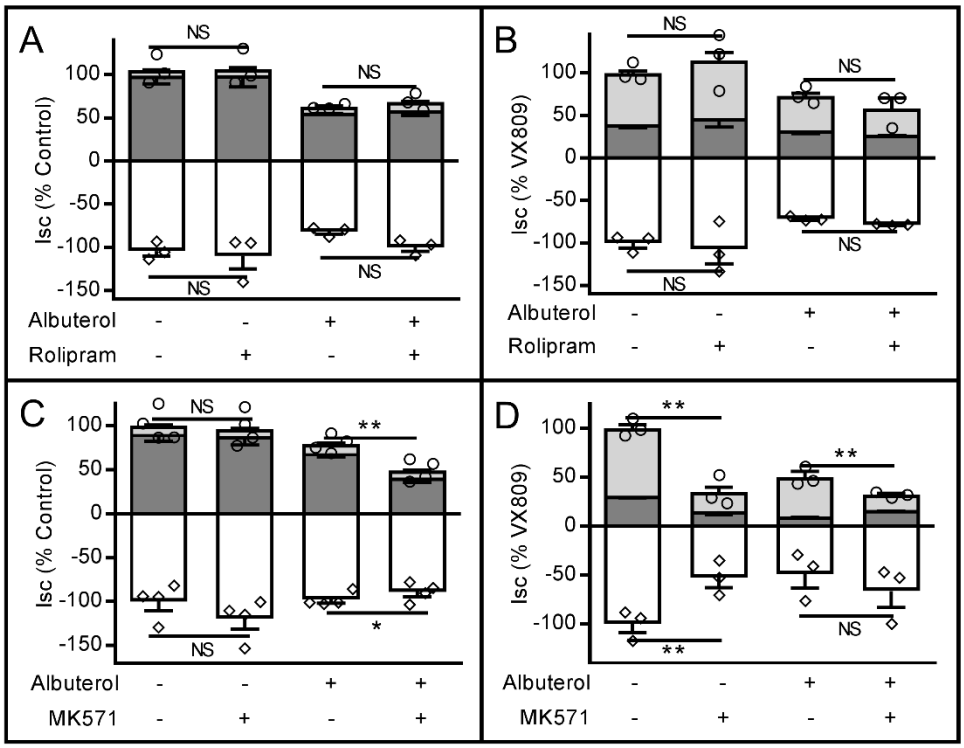

Figure S1:

Chronic albuterol-induced CFTR impairment is not rescued by functional inhibition of the key spatial cAMP regulators Phosphodiesterase, isoform 4 (PDE-4) and the multidrug resistance pump 4 (MRP4). Wild-type and VX809-corrected F508del CFTR+ CFBE41o- cells were exposed to albuterol (10µM) in media for 72 hours, then mounted in Ussing chambers and exposed to inhibitors of PDE-4 (rolipram, 100µM) or MRP4 (MK571, 20µM). CFTR function was then quantified under voltage clamp conditions. The addition of rolipram (100µM, added after forskolin/IBMX) to further inhibit PDE-4 did not rescue albuterol-induced CFTR dysfunction in either wtCFTR+ (**Panel A**, n=3 inserts/condition; circles represent total CFTR activity [cAMP+genistein], diamonds represent inhibited CFTR currents [Inh172]) or VX809-corrected F508del CFTR+ cells (**Panel B**, 3 inserts/condition).
Similarly, the albuterol-induced reductions in CFTR-dependent I_{sc} in pretreated wtCFTR+ (**Panel C**, n=4 inserts/condition) and VX809-corrected F508del CFTR+ (**Panel D**, n=3 inserts/condition) CFBE41o- cells were not rescued following exposure to the MRP4 inhibitor MK571 (20µM, added before amiloride). All studies are internally normalized as indicated to allow for comparisons. Stimulation protocol was as follows: Amiloride (100µM, not shown), cAMP (forskolin 10µM/IBMX 100µM – dark grey bars), CFTR potentiator (genistein 50µM for wtCFTR+; VX770 1µM for F508del CFTR+ cells – light grey bars), and CFTR inhibition (Inh172 10µM – white bars). All experiments are representative of studies repeated in duplicate or triplicate with similar results. Data presented represent mean ± SEM.
*p<0.05; **p<0.01; NS: non-significant by two-way ANOVA with Tukey's multiple comparisons test.

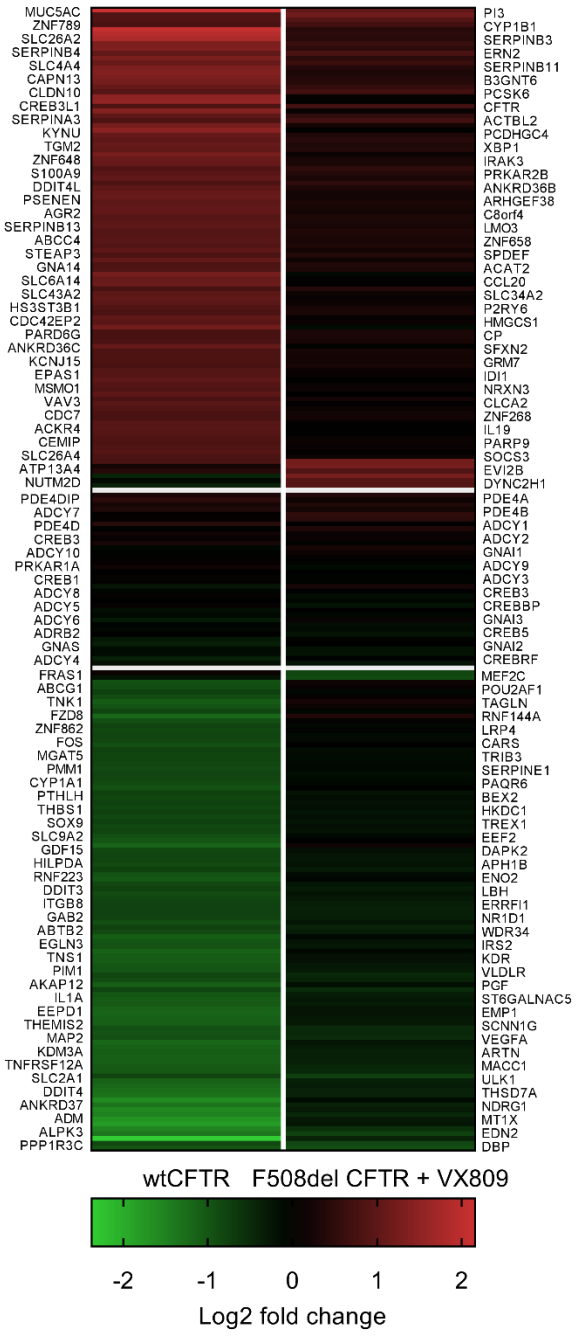

Figure S2:

Transcriptional changes in primary human airway epithelial cells (HAECs) following 72 hours of albuterol exposure.

Heatmap displaying differentially expressed genes in wtCFTR+ (left column) and VX809-corrected F508del CFTR+ primary HAECs following chronic albuterol exposure; each experimental group is compared to its same-donor baseline (untreated wtCFTR+ on the left, VX809-treated F508del CFTR+ on the right). The top 100 significantly up- or down-regulated genes are listed in the top and bottom groups, respectively; expression of genes in these columns was statistically changed with albuterol exposure, though not all reach a 2-fold change. The center group represents key genes of interest (AC isoforms, *CREB*, etc.); no genes in the center row had a significant change in expression. Key genes are noted to the left or right of their respective row (left/right placement is for spacing only and does not carry significance).

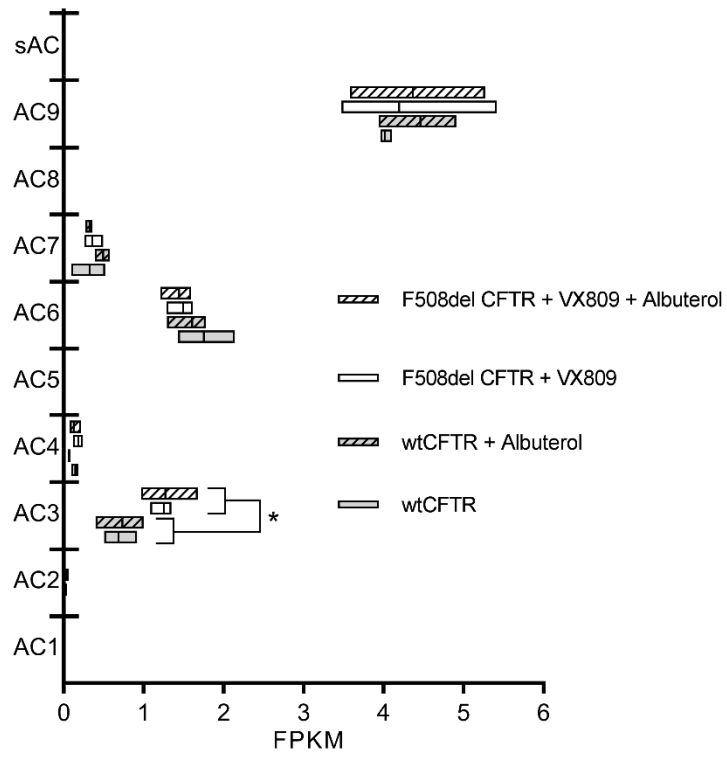

Figure S3:

Expression patterns of Adenylyl Cyclase (AC) isoforms are similar between wild-type and F508del CFTR+ human airway epithelial cell (HAEC) donors, and are not modified by albuterol pre-treatment.
Expression levels, as measured in Fragments per Kilobase of transcript per Million mapped reads (FKPM), of the nine transmembrane AC isoforms (AC1-AC9) and soluble AC (sAC) in albuterol-treated and untreated wt and VX809-corrected F508del CFTR+ primary HAECs. The isoforms of highest expression include AC3, AC6, AC7, and AC9, and expression levels are unchanged by albuterol exposure. Expression of AC3 was slightly higher in VX809-corrected F508del CFTR+ cells compared to wtCFTR+ cells; all other isoforms were similarly expressed in both cell lines.
*$p<0.05$ by two-way ANOVA with Tukey's multiple comparisons test.

Supplemental Tables and Table Legends

Primary Antibody	Secondary Antibody
Rabbit anti-CFTR (Sigma C7491); 1:100	FitC Goat anti-Rabbit IgG (Invitrogen 656111); 1:500
Alexa Fluor 568 Phalloidin (Life Technologies A12380); 1:250	N/A
Chicken anti-β2AR (Abcam ab13989); 1:250	Goat anti-Chicken IgY (Abcam ab150171); 1:500

Table S1:

Antibodies and fluorophores used for immunofluorescence.