# Airway surface hyperviscosity and defective mucociliary transport by IL-17/TNF-α are corrected by beta-adrenergic stimulus

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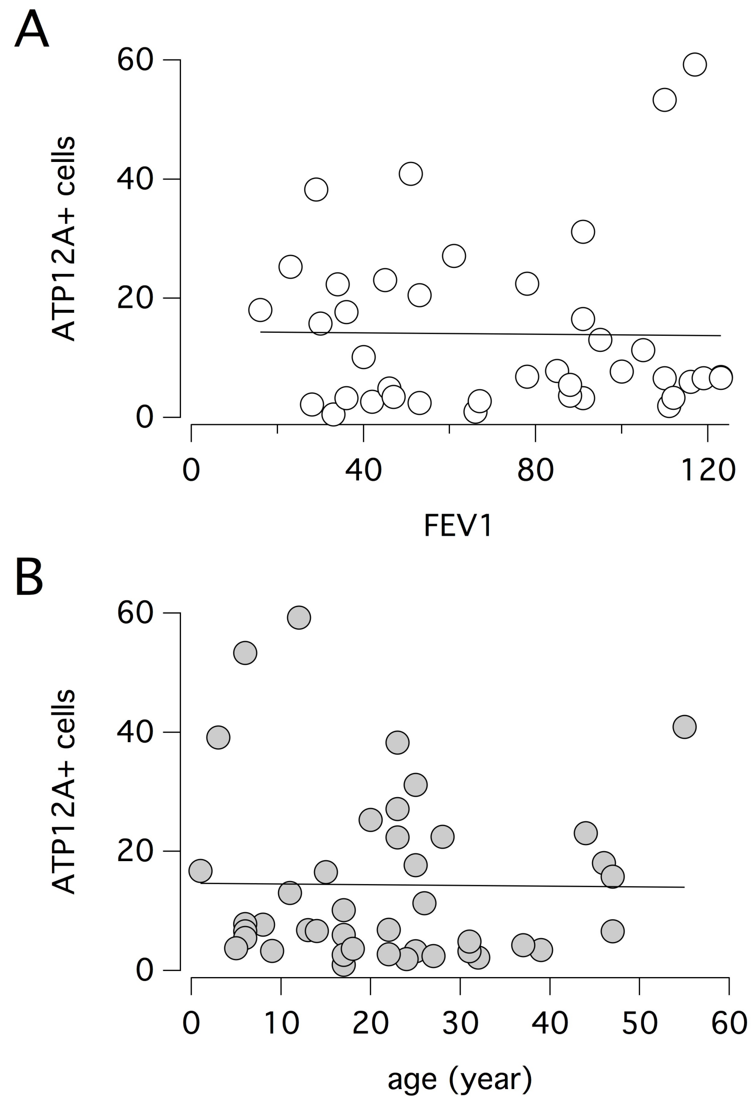
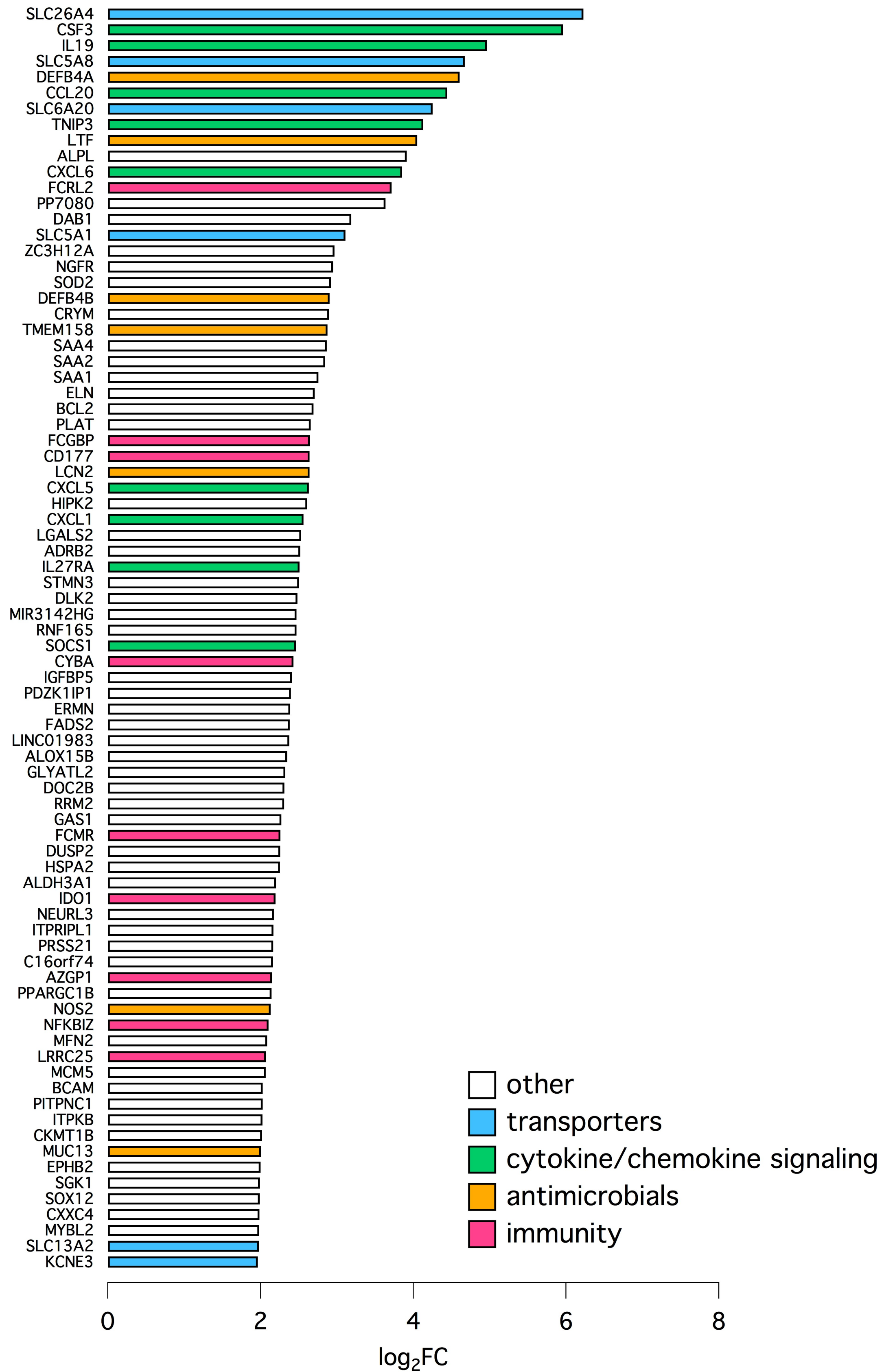
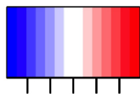
## Supplementary Figures 1-7

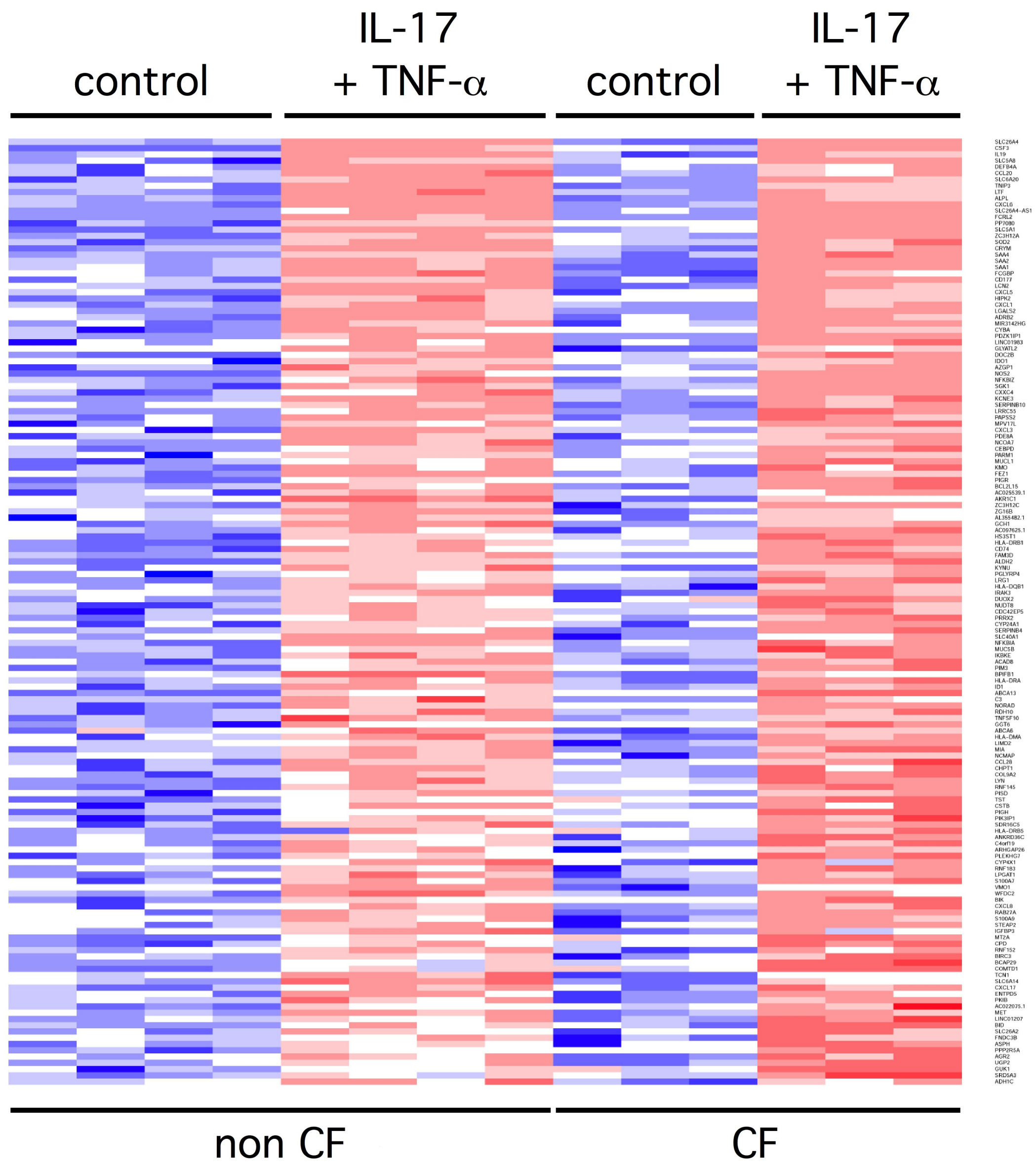



**Supplementary Figure 1. Analysis of ATP12A expression in nasal epithelial cells freshly collected from CF patients.** Each symbol reports the average value of a single patient. (**A**) Percentage of ATP12A+ cells vs. FEV1. (**B**) Percentage of ATP12A+ cells vs. age. Straight lines show the best linear fit of data.

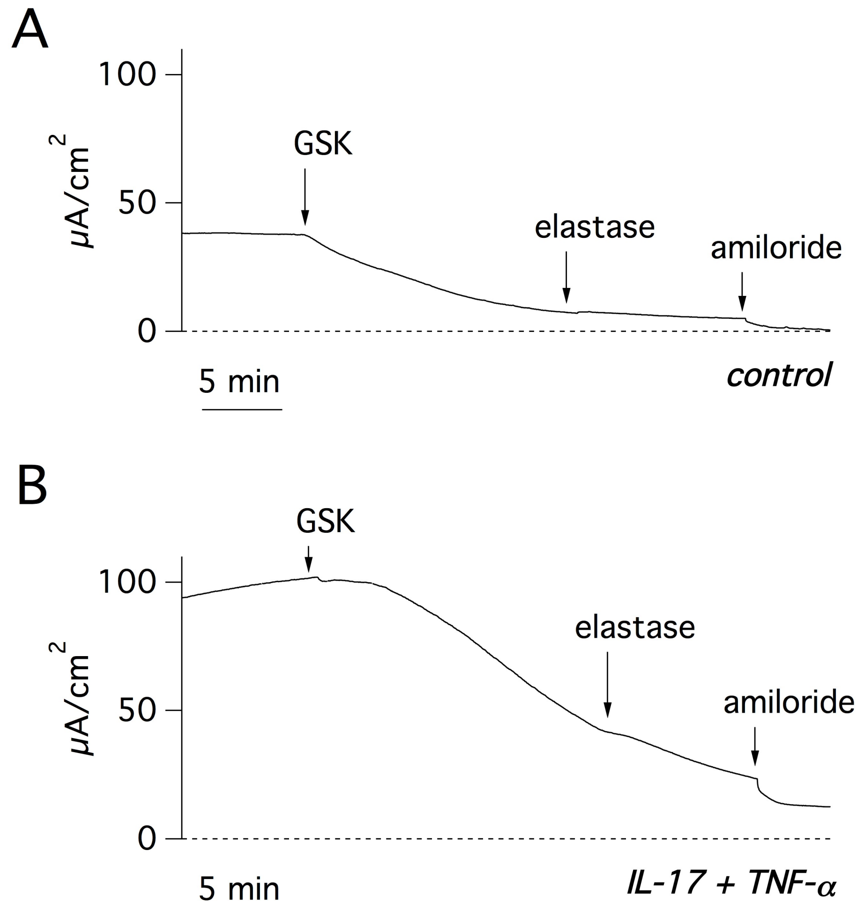



**Supplementary Figure 2. Top 80 genes upregulated by IL-17/TNF-α combination in cultured bronchial epithelia.** The bar graph shows the extent of expression of indicated genes as determined by bulk RNAseq in non-CF cultured bronchial epithelia. Color codes indicate gene function.

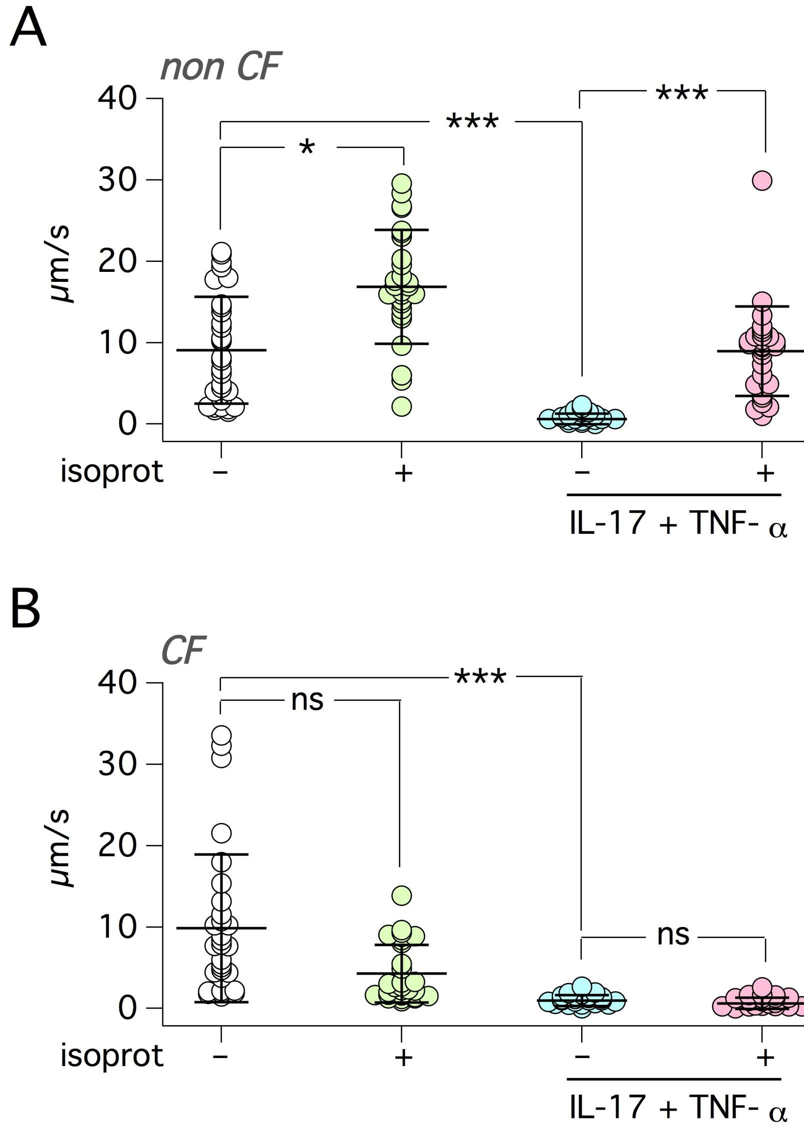


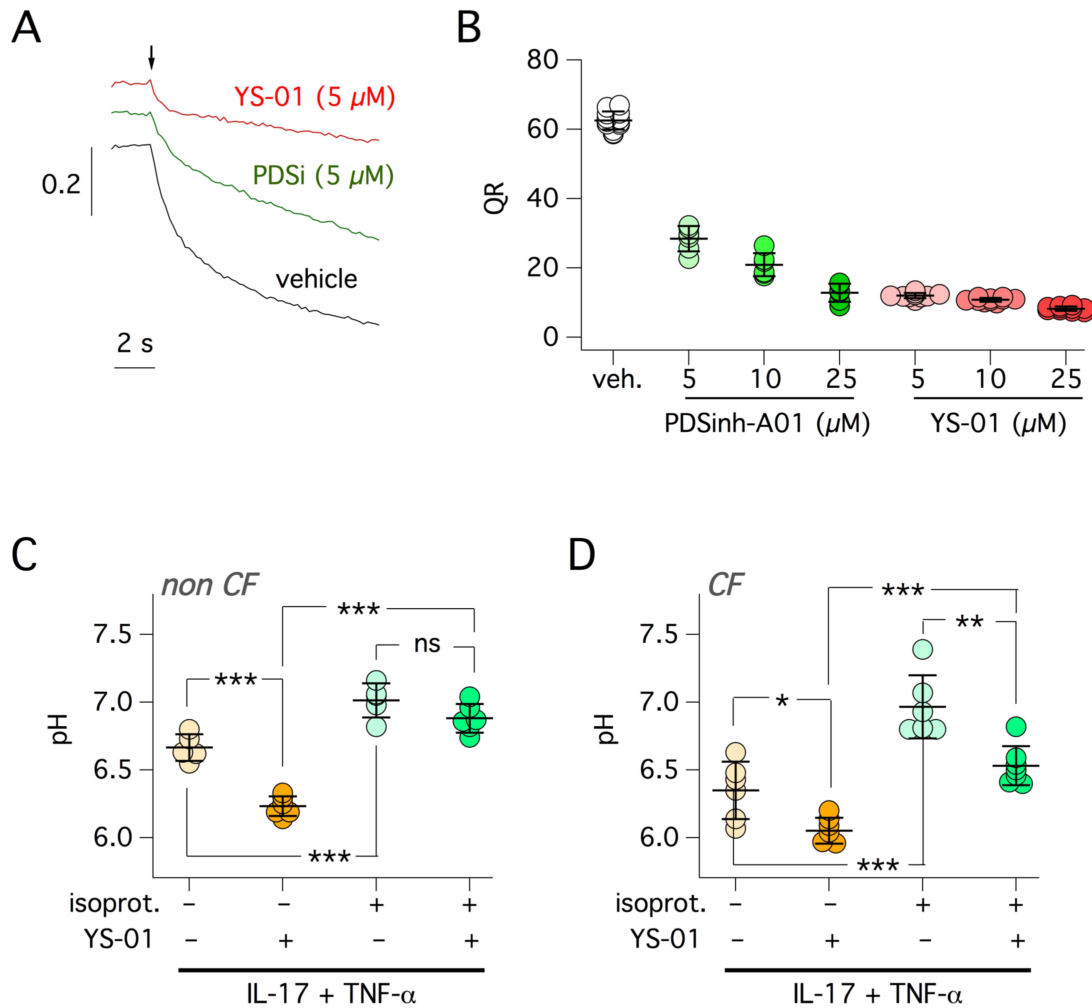

**Supplementary Figure 3. Comparison of gene expression changes induced by IL-17/TNF-α in non-CF and CF cultured bronchial epithelia.** The heat maps show the change in expression for the top 80 upregulated genes.



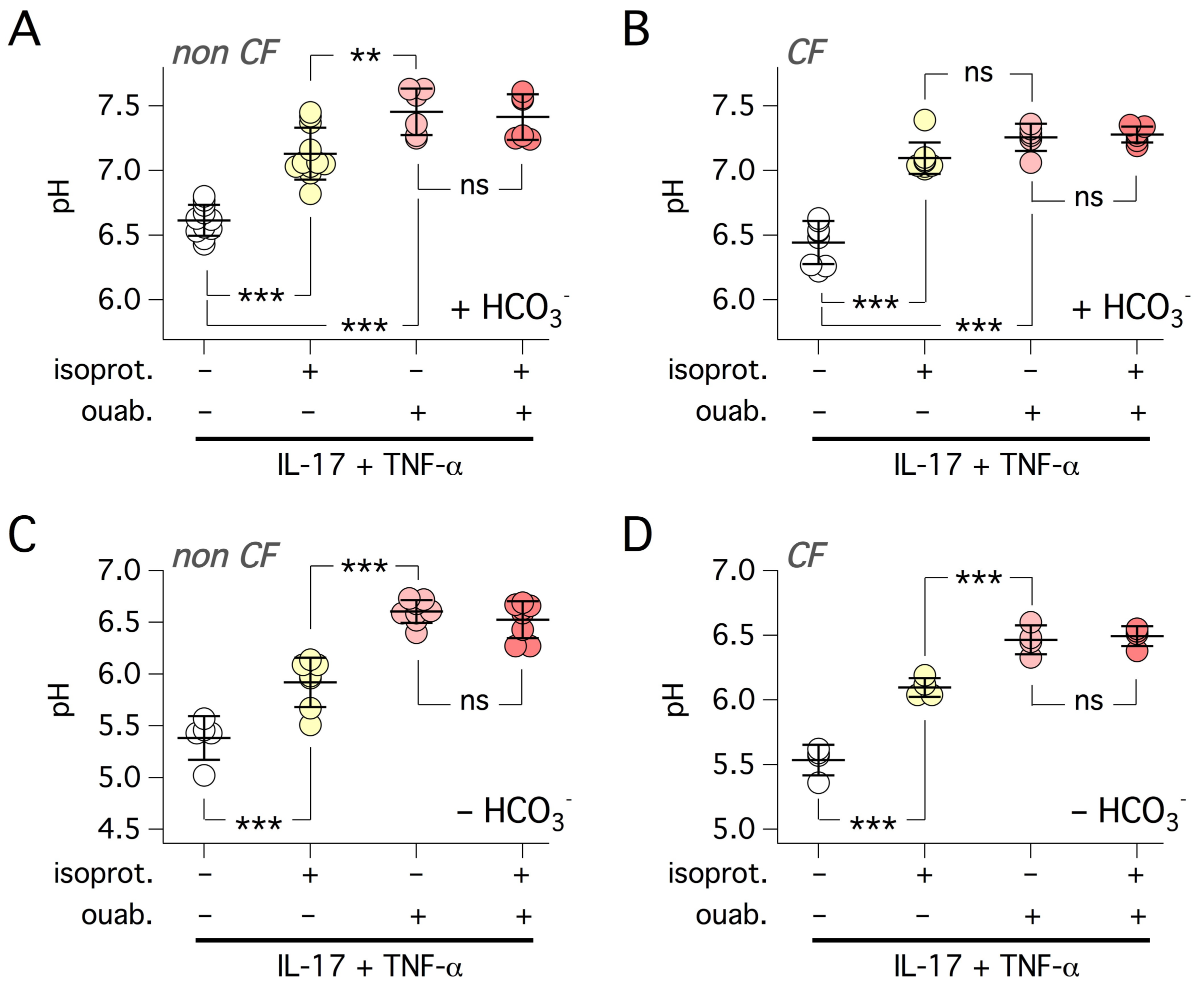

**Supplementary Figure 4. Lack of elastase effect in epithelia treated with GSK650394.** The figure shows representative short-circuit current recordings from cultured bronchial epithelia treated for 72 h in control conditions (**A**) or with IL-17/TNF-α (**B**).




**Supplementary Figure 5. Analysis of mucociliary transport by videomicroscopy.** The graphs report the velocity of microbeads on non-CF (**A**) and CF (**B**) cultured bronchial epithelia. Epithelia were treated for 72 h with/without IL-17/TNF-α and then stimulated for 3 h with isoproterenol (100 nM). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant (Kruskal-Wallis and Dunn's test).




**Supplementary Figure 6. Evaluation of SLC26A4 inhibitors.** (**A**) Representative traces showing quenching of halide-sensitive yellow fluorescent protein (HS-YFP) caused by extracellular addition of $I^-$ (arrow) in FRT cells expressing SLC26A4. SLC26A4 inhibitors (YS-01; $PDS_{inh}$-A01, PDSi) slow down $I^-$ uptake and hence HS-YFP quenching. (**B**) Summary of data showing quenching rate (QR) of HS-YFP with vehicle and SLC26A4 inhibitors at the indicated concentrations. (**C**, **D**) Apical pH measured with the large volume ex situ assay in non-CF and CF cultured bronchial epithelia treated for 72 h with the IL-17/TNF-α combination. Where indicated, in the last 3 h, epithelia were stimulated with/without isoproterenol (100 nM) in the absence/presence of YS-01 (5 μM). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant (ANOVA with Tukey's post hoc test).




**Supplementary Figure 7. Analysis of isoproterenol effect on apical pH.** The graphs report the apical pH (large volume ex situ assay) measured in non-CF and CF bronchial epithelia kept in $HCO_3^-$-rich (**A**, **B**) or $HCO_3^-$-free (**C**, **D**) basolateral media. All epithelia were treated for 72 h with IL-17/TNF-α. Where indicated, epithelia were stimulated with basolateral isoproterenol (100 nM) in the absence/presence of ouabain (200 μM). **, $p < 0.01$; ***, $p < 0.001$; ns, not significant (ANOVA with Tukey's post hoc test).