SUPPLEMENTARY FILES

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TITLE: ORKAMBI® mediated rescue of mucociliary clearance in CF primary respiratory cultures is enhanced by arginine uptake, arginase inhibition and promotion of nitric oxide signaling to the CFTR channel

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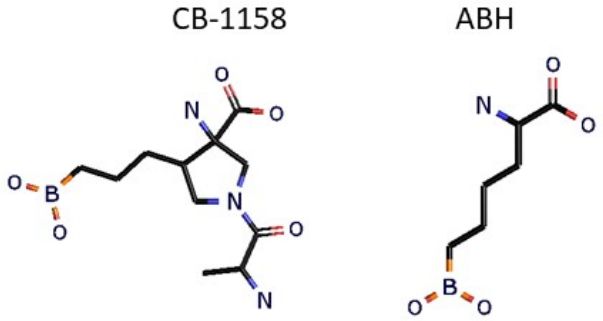
SUPPLEMENTARY TABLE 1

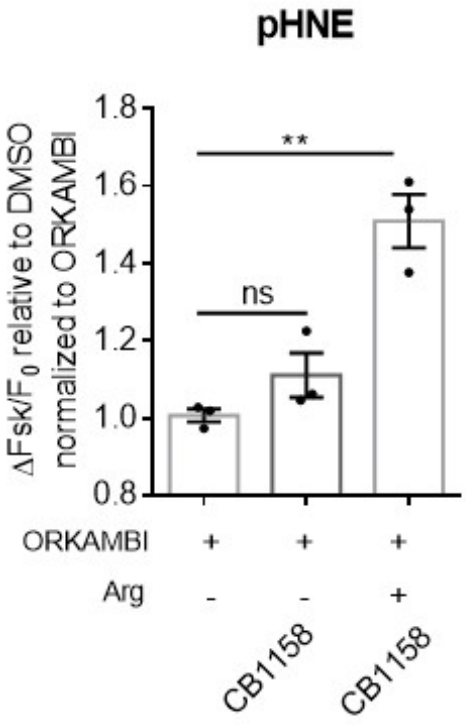
Age and gender listed for each patient, homozygous for F508del and associated with CF code number

Patient ID	Age (years)	Gender
CF030	20	female
CF032	21	male
CF057	25	female

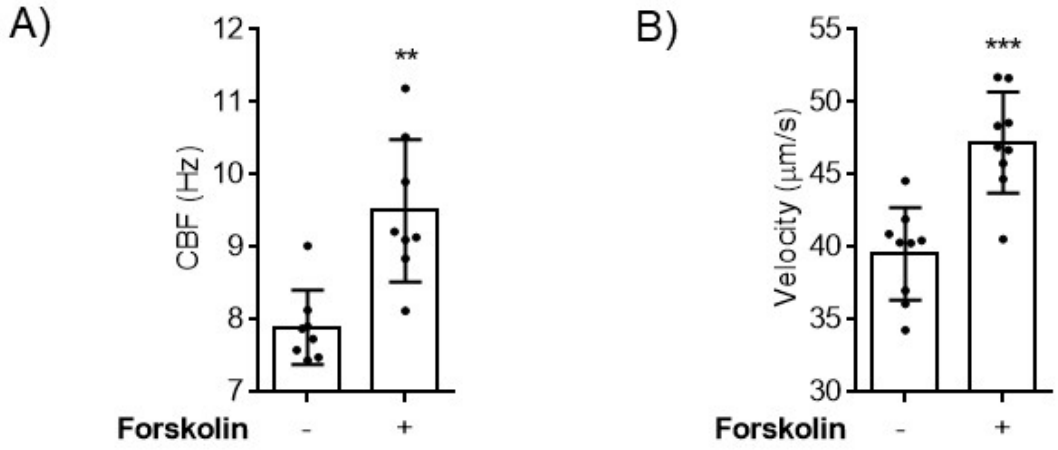
SUPPLEMENTARY FIGURE 1: Structure of arginase inhibitors: CB1158 and ABH



SUPPLEMENTARY FIGURE 2: Addition of arginase inhibitor (CB1158) in the absence of extracellular arginine fails to enhance channel function rescued by ORKAMBI. Each bar in the bar graph shows F508del-CFTR channel activation responses (mean ± SD) measured in nasal epithelial cultures using the FLiPR assay in 3 biological replicates.



SUPPLEMENTARY FIGURE 3: NO modulators influence CBF and MCCV in WT primary human bronchial cells. A. Bar graph represents FSK stimulated CBF (Hz) on WT-CFTR primary bronchial cells (mean ± SD). Cells were pre-treated with HBSS solution following 1 h of equilibration and 30 min of 10 μM FSK or buffer apical addition to the HBSS. One-way ANOVA with Tukey's multiple comparison test was performed (**P= 0.0011, N>7 biological replicates, n=3 technical replicates). **B.** Bar graph represents MCCV (μm/s) of WT-CFTR primary bronchial cells (mean ± SD). Cells were pre-treated with HBSS solution following 1 h of equilibration and 30 min of 10 μM FSK or buffer apical addition to the HBSS containing 1 μm green or red polystyrene microspheres following 1 h of equilibration and 30 min of 10 μM FSK apical addition to the HBSS. One-way ANOVA with Tukey's multiple comparison test was performed ***P=0.0002, N>7 biological replicates, n=3 technical replicates).



SUPPLEMENTARY FIGURE 4: The arginase inhibitor CB-158 does not affect CFTR protein levels in F508del-CFTR nasal epithelial cells. A. Representative immunoblot of CFTR protein in F508del-CFTR nasal epithelial cells treated with 3 μM VX-809 for 48 h +/- 10 μM CB-1158 for 1 h. Calnexin (CNX) protein was blotted as a protein loading control. WT- and knockout (KO) CFTR were used as positive and negative controls, respectively. Band B represents the immature CFTR protein and band C represents the mature band. **B.** Bar graph represents the amount of mature CFTR protein (band C) relative to total amount of CFTR (band B+C) in F508del-CFTR nasal epithelial cells treated with 3μM VX-809 for 48 h +/- 10μM CB-1158 for 1 h. Unpaired t-test was performed (not statistically significant, P=0.87, N=3 biological replicates, n=1 technical replicate).