FIGURE S1

A

F508del/F508del

DMSO

VX-809

VX-661

1mV

5min

ΔIeq FSK ± VX-770 (µA/cm²)

B

ΔIeq CFTRinh (µA/cm²)

C

n.s.

*
FIGURE S1: ORKAMBI and SYMDEKO showed similar rescue of F508del-CFTR in nasal epithelial cultures derived from 6 patients homozygous for F508del/F508del. (A) Representative tracing show Ussing chamber studies measurements of CFTR function in nasal epithelial cell cultures from F508del/F508del patients after pre-treatment with DMSO, VX-809 (3 µM) or VX-661 (3 µM). (B) Bar graphs showing the mean (±SD) of maximal response Ieq (µA/cm²) after stimulation with forskolin (10 µM) + VX-770 (1 µM) (2 inserts for each treatment). (C) Bar graphs showing the IeqCFTRinh-172 (µA/cm²) by CFTRinh-172 (10 µM). Comparative analysis was performed using by one-way ANOVA followed by Turkey’s post-hoc test. *p<0.05.
FIGURE S2

A

B

Δleq FSK+S-VX-445(µA/cm²)

0.9
1.0
1.1
1.2
1.3
1.4
1.5

FSK
FSK+S-VX-445

*
FIGURE S2: VX-445 compound increased channel activation of Wt-CFTR in nasal epithelial cells. (A) Representative trace showing Ussing chamber measurements of CFTR function in nasal epithelial cell cultures from a non-CF donor. (B) Bar graphs showing the fold increased forskolin (0.1 µM) + [S]-VX-445 (3 µM) activated ΔIeq compared to forskolin (0.1 µM) control in 3 technical replicated nasal epithelial cells generated from 1 healthy control. *p<0.05.
FIGURE S3

A

Agonist

CFTRInh

RFU Relative to Baseline (%)

Time (sec)

DMSO+DMSO
DMSO+FSK
VX-661+S-VX-445/FSK/770
VX-661+S-VX-445+VX-770+FSK/770

B

Max Activation (%)

DMSO
VX-809
VX-809+VX-770
VX-661+S-VX-445
VX-661+S-VX-445+VX-770

C

Band C

Band B

CNX

D

C/(C+B)

DMSO
VX-809
VX-809+VX-770
VX-661+S-VX-445
VX-661+S-VX-445+VX-770
FIGURE S3: VX-445+ VX-661+ VX-770 showed functional rescue of N1303K-CFTR in CFF 16HBEge CFTR-N1303K. (A) Representative traces of N1303K-CFTR-dependent chloride efflux by FLIPR assay in HBE cells pre-treated with DMSO, VX-809 (3 µM), [S]-VX-445 (3 µM)+ VX-661 (3 µM), VX-661 (3 µM)+ VX-770 (1 µM) or [S]-VX-445 (3 µM)+ VX-661 (3 µM)+ VX-770 (1 µM) for 24 hrs at 37°C. (B) Bar graphs show the mean (±SD) of maximal activation of N1303K-CFTR after stimulation by FSK (10 µM) +/- VX-770 (1 µM) (n= 4 biological replicates and 4 technical replicates for each experiment) *p<0.05; ****p<0.0001 by one way ANOVA followed by Turkey’s post-hoc test.
FIGURE S4

A

Y569D/Y569D

DMSO

S-VX-445+VX661 +VX-770

1mV

5min

B

ΔIeq FSK+VX-770 (µA/cm²)

C

ΔIeq CFTRinh (µA/cm²)

D

WT DMSO G445S+V661+770

Band C

Band B

CNX

E

C/(C+B)

WT DMSO G445S+V661+770
FIGURE S4: VX-445+ VX-661+ VX-770 failed to rescue Y569D-CFTR channel activity in nasal epithelial cultures derived from 1 patient homozygous for Y569D/Y569D. (A) Representative tracings show Ussing chamber measurements of CFTR function in nasal epithelial cell cultures from 1 CF patient bearing Y596D/Y569D in the absence or presence of the [S]-VX-445+ VX-661+ VX-770. (B) Bar graphs showing the mean (±SD) of maximal response Ieq (µA/cm²) after stimulation by forskolin (10 µM) +/- VX-770 (1 µM) for nasal cultures from 1 patient bearing Y596D/Y569D after pre-treatment (48 hrs at 37°C) with DMSO (0.1%) or [S]-VX-445 (3 µM)+ VX-661 (3 µM)+ VX-770 (1 µM) (2 inserts for each treatment). (C) Bar graphs showing the IeqCFTR inhibition (µA/cm²) by CFTR_{inh-172} (10 µM) (2 inserts for each treatment). (D) Immunoblots of steady-state expression of Wt or Y596D/Y569D following treatments with CFTR modulators. Band C: mature, complex-glycosylated CFTR; Band B: immature, core-glycosylated CFTR; CNX: Calnexin. (E) Bars represent the ratio of band C/( band C+ band B) (n=2). Statistical analysis was performed using paired two-tailed Student’s t-test.
FIGURE S5: VX-445+ VX-661+ VX-770 showed minimal increase in CFTR activity in nasal epithelial cultures derived from patients with G542X/N1303K mutation (n=2). (A) Representative tracings show Ussing chamber measurements of CFTR function in nasal epithelial cell cultures from a CF patient bearing G542X/N1303K in the absence or presence of the small molecule corrector. (B) Bar graphs showing the mean (±SD) of maximal response Ieq (µA/cm²) after stimulation by forskolin (10 µM) +/- VX-770 (1 µM) of 1-2 technical replicates of nasal epithelial cell cultures generated from 2 patients bearing G542X/N1303K. Nasal epithelial cells were treated (48h at 37°C) with DMSO (0.1%), VX-809 (3 µM)+ VX-770 (1 µM), R-VX-445 (3 µM)+ VX-661 (3 µM)+ VX-770 (1 µM) or [S]-VX-445 (3 µM)+ VX-661 (3 µM)+ VX-770 (1 µM) (2-3 inserts for each treatment). (C) Bar graphs showing the mean (±SD) IeqCFTR_{inh-172} (µA/cm²) by CFTR_{inh-172} (10 µM). (D) Immunoblots of steady-state expression of G542X/N1303K following treatments with CFTR modulators. Band C: mature, complex-glycosylated CFTR; Band B: immature, core-glycosylated CFTR; CNX: Calnexin. (E) Bars represent the mean (±SD) of the ratio band C/(band C+ band B) (n=2). Statistical analysis was performed using one-way ANOVA followed by Turkey’s post-hoc test.