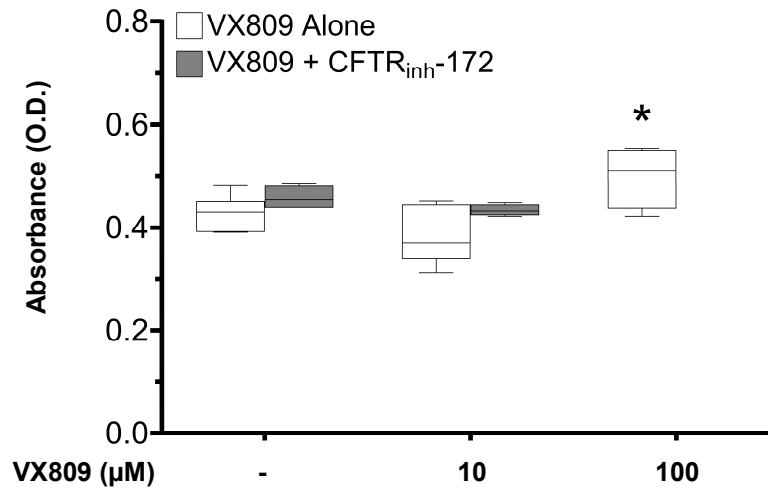
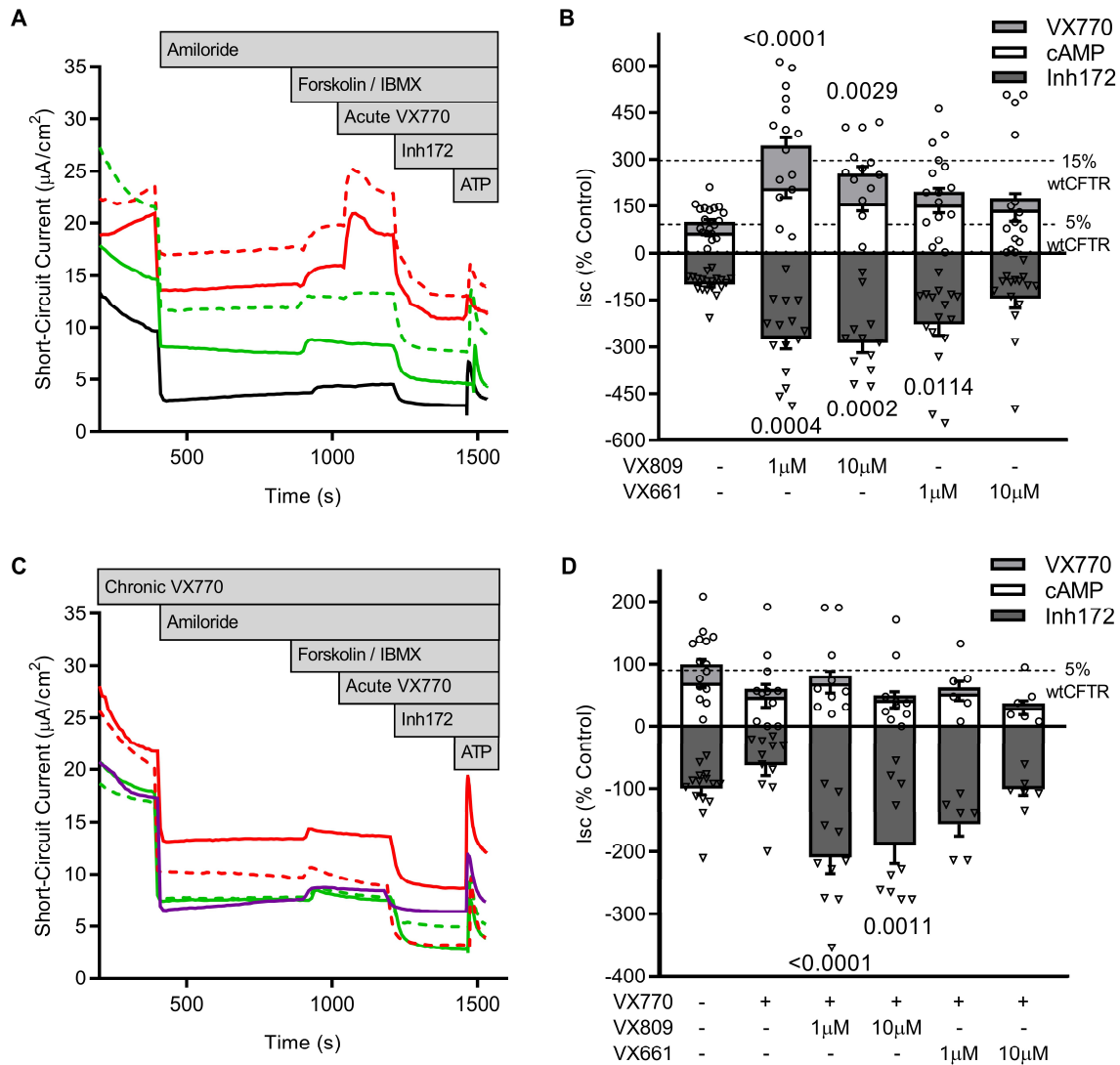


## Cytotoxicity

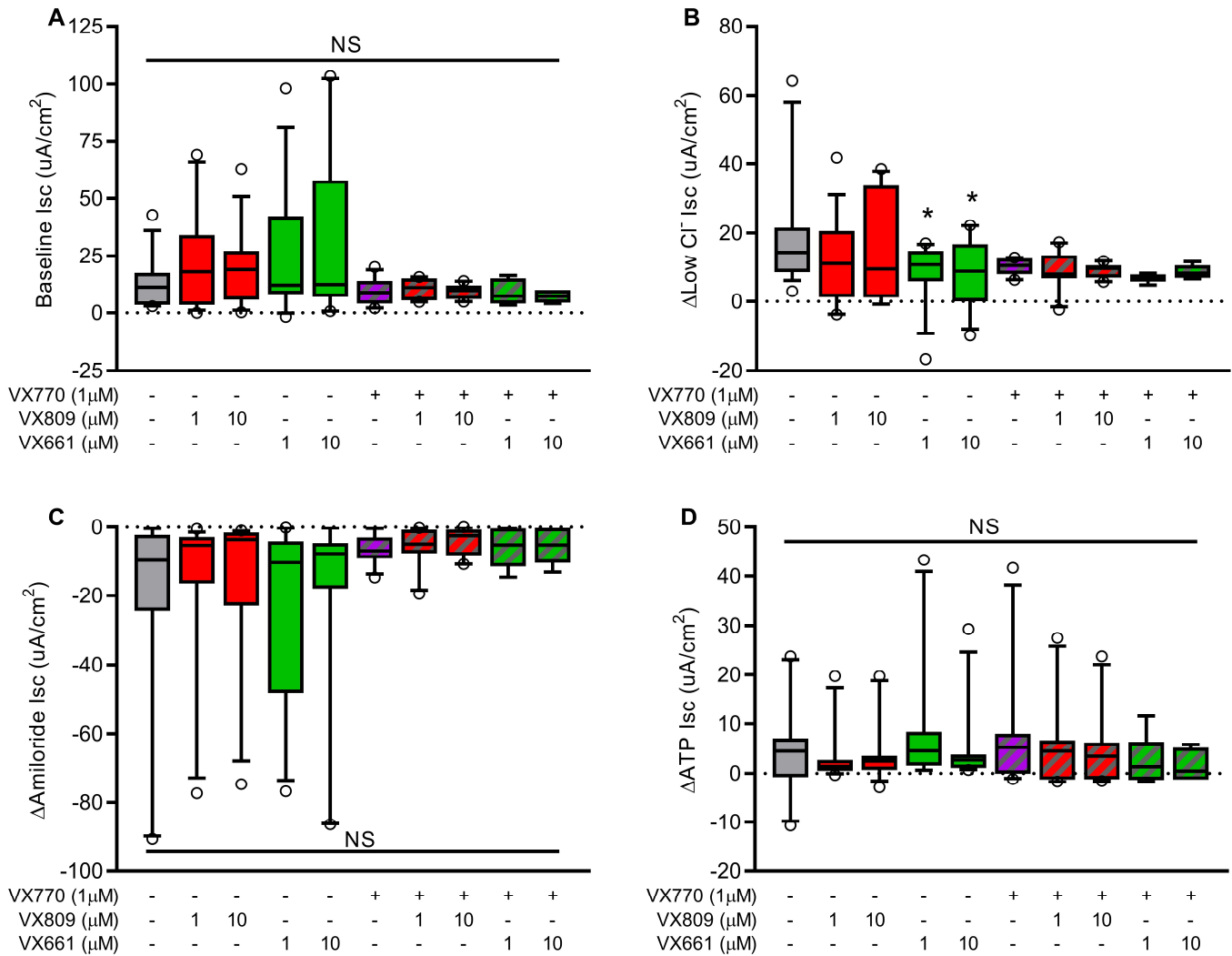


**Supplemental Figure S1. VX809 and CFTR<sub>inh</sub>-172 treatment is not cytotoxic to primary NhBE cells.** NhBE cells were differentiated and then incubated with 1-10 µM VX809 and/or 20 µM CFTR<sub>inh</sub>-172 for 72 hours. Cell viability was determined by MTT assay and absorbance on a plate reader (where significant decrease in OD connotes decreased cell number). Data are expressed as box-and-whisker plots. Horizontal bars indicate the median, box borders indicate 25<sup>th</sup> and 75<sup>th</sup> percentiles, and whiskers indicate 5<sup>th</sup> and 95<sup>th</sup> percentiles. \*p<0.05 vs NhBE control by 1-way ANOVA with Dunnett's multiple comparisons test.

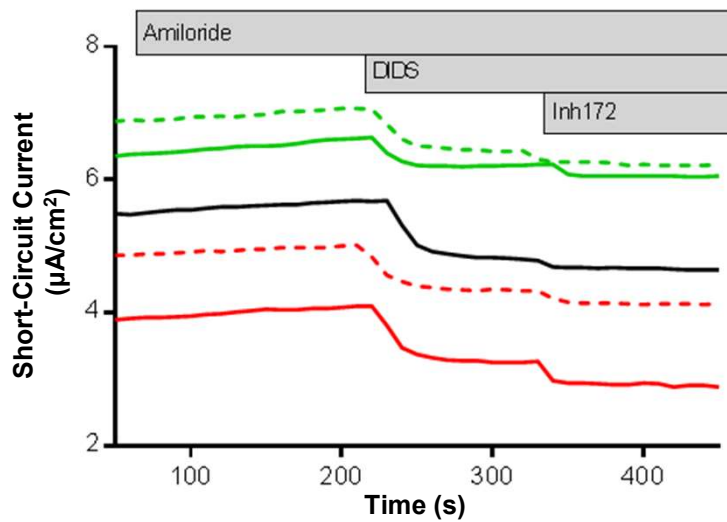


**Supplemental Figure S2. VX809 and VX661 rescue F508del CFTR function in CFhBE cells.**

F508del homozygous CFhBE cells were pretreated as indicated for 48 hours with varying concentrations of VX809, VX661, and/or VX770. (A) Representative short-circuit (ISC) tracings from a single donor (DMSO control: black; VX809: red [1 $\mu\text{M}$  solid line, 10 $\mu\text{M}$  hashed line]; VX661: green [1 $\mu\text{M}$  solid line, 10 $\mu\text{M}$  hashed line]). (B) Aggregate data from all 5 donors for cells pretreated with VX809 or VX661. (C) Representative ISC tracings of filters from the same donor in panel A that were pretreated with VX770 (VX770 alone: purple; VX770+VX809: red [1 $\mu\text{M}$  solid line, 10 $\mu\text{M}$  hashed line]; VX770+VX661: green [1 $\mu\text{M}$  solid line, 10 $\mu\text{M}$  hashed line]). (D) Aggregate data from all 4 donors for cells pretreated with VX770  $\pm$  VX809 or VX661. Mean  $\pm$  SEM for n=2-5 experiments/condition/donor, from 5 unique patient donors. Individual donor data was normalized as percent of DMSO control to allow for aggregate comparison. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001, vs. CFhBE cells incubated with vehicle control (DMSO) by 1-way ANOVA with Dunnett's multiple comparison test.

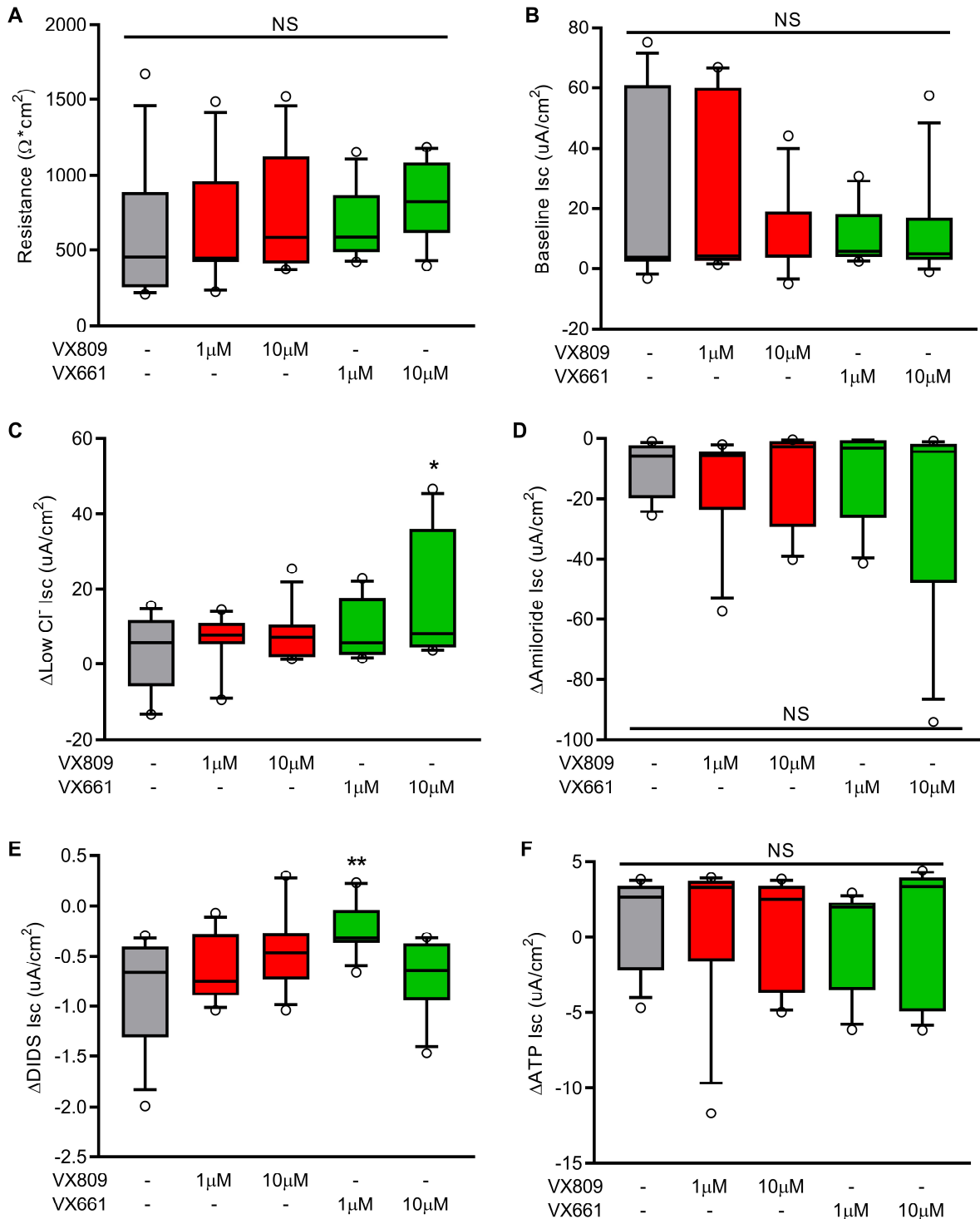


**Supplemental Figure S3. Pre-incubation with VX809, VX661, and/or VX770 does not consistently impact baseline or non-CFTR-dependent ion transport.** F508del homozygous CFhBE cells were pretreated as indicated for 48 hours with varying concentrations of VX809, VX661, and/or VX770. No significant differences were noted between any conditions and DMSO control for (A) baseline current, (C) amiloride-inhibited current, or (D) ATP-induced current, while cells treated with VX661 demonstrated a small reduction in (B) low chloride-induced current. Mean ± SEM for n=2-5 experiments/condition/donor, from 5 unique patient donors. \*p<0.05; NS: nonsignificant (p>0.05) vs. CFhBE cells incubated with vehicle control (DMSO) by 1-way ANOVA with Dunnett's multiple comparison test.

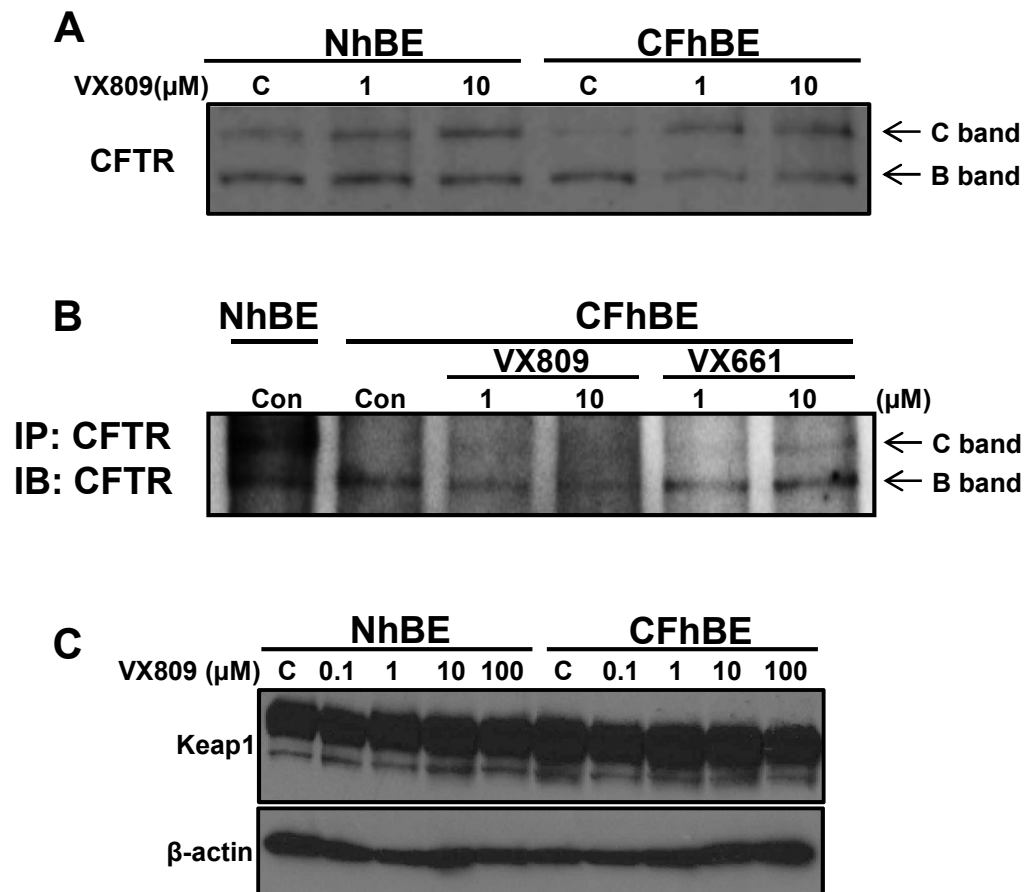


**Supplemental Figure S4. VX809 and VX661 correct unstimulated F508del-CFTR function in CFhBE cells.** F508del homozygous CFhBE cells were pretreated as indicated for 48 hours with varying concentrations of VX809 or VX661. Representative short-circuit current (ISC) tracings from a single donor (DMSO control: black; VX809: red [1 $\mu\text{M}$  solid line, 10 $\mu\text{M}$  hashed line]; VX661: green [1 $\mu\text{M}$  solid line, 10 $\mu\text{M}$  hashed line]).

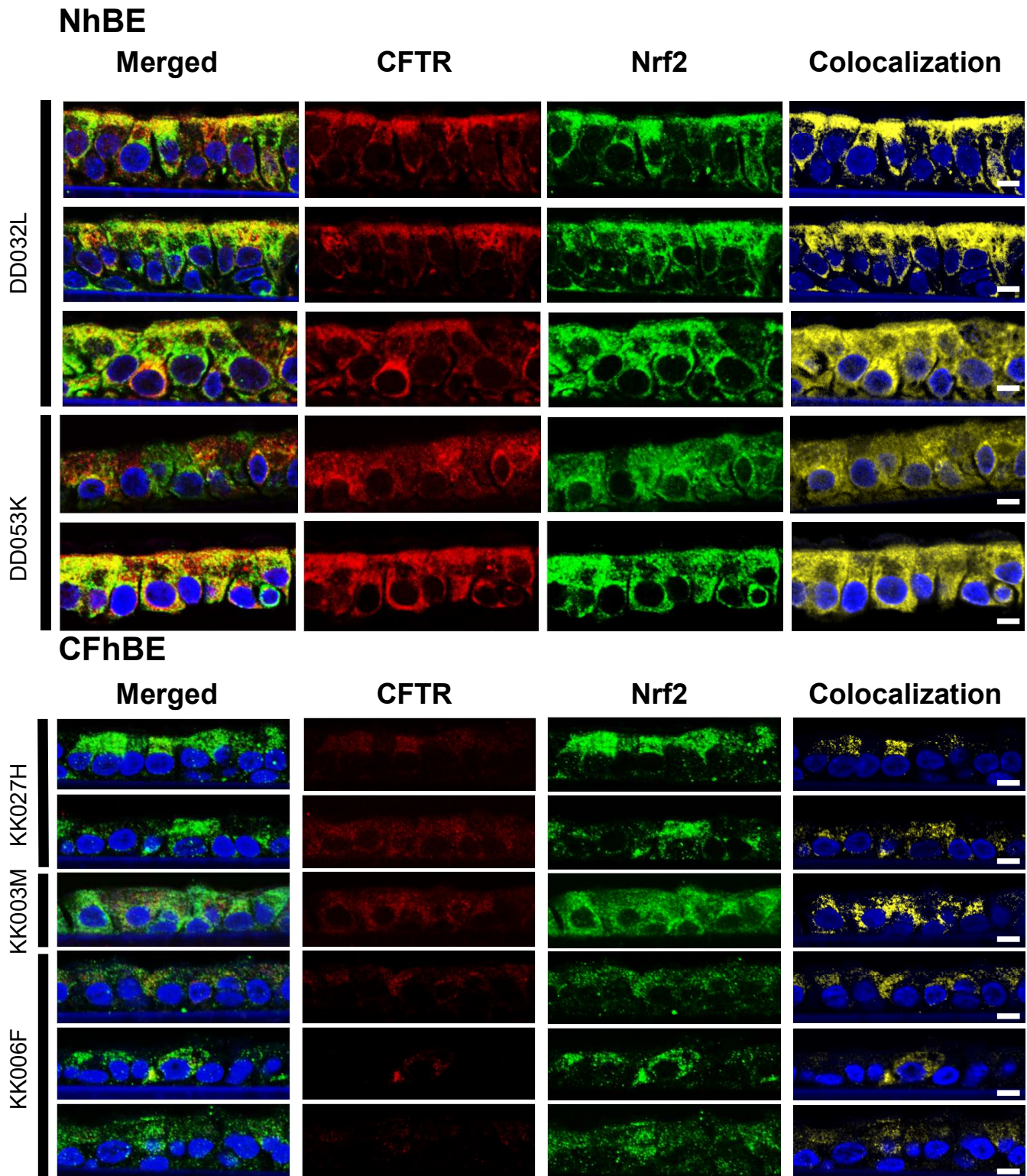




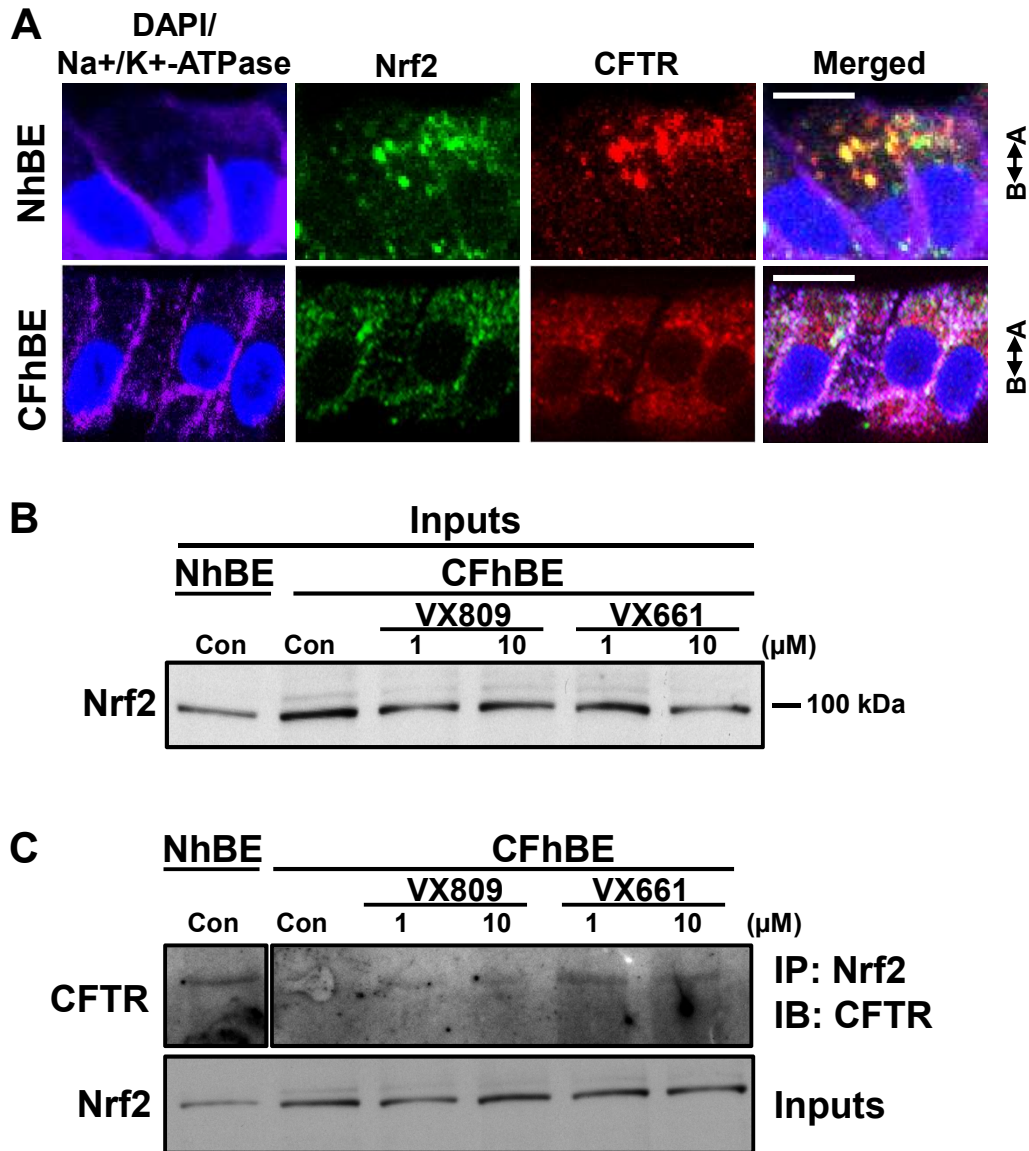
**Supplemental Figure S5. Pre-incubation with VX809 or VX661 does not consistently impact resistance or non-CFTR-dependent ion transport in unstimulated CFTR assays.** F508del homozygous CFhBE cells were pretreated as indicated for 48 hours with varying concentrations of VX809 or VX661. No significant differences were noted between any conditions and DMSO control for (A) baseline resistance, (B) baseline current, (D) amiloride-inhibited current, or (F) ATP-induced current. Cells treated with 10  $\mu\text{M}$  VX661 had an increase in low chloride-induced current, while cells treated with 1  $\mu\text{M}$  VX661 had a small decrease in DIDS-inhibited current. Mean  $\pm$  SEM for n=4 experiments/condition/donor, from 3 unique patient donors. \* $p < 0.05$ ; \*\* $p < 0.01$ ; NS: nonsignificant ( $p > 0.05$ ) vs. CFhBE cells incubated with vehicle control (DMSO) by 1-way ANOVA with Dunnett's multiple comparison test.



**Supplemental Figure S6. VX809 treatment increases mature CFTR protein, but VX809 does not affect Keap1 protein levels.** NhBE and CFhBE cells were treated with the indicated doses of VX809 or VX661 for 48 hours. Cell homogenates were subjected to Western blot analysis with (A) anti-CFTR antibody, or (B) lysates immunoprecipitated with anti-CFTR antibody and blotted for CFTR, or (C) anti-Keap.  $\beta$ -Actin served as a loading control.

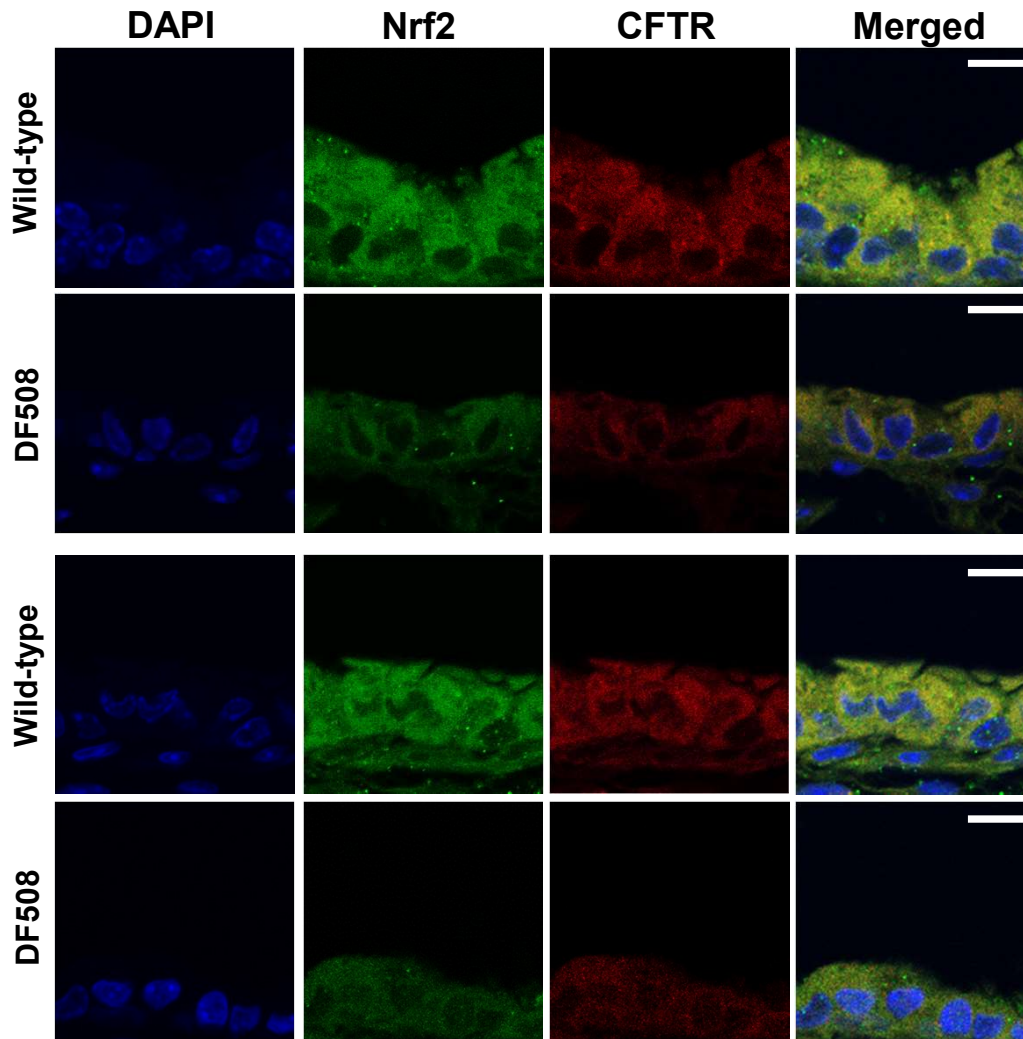


**Supplemental Figure S7. Colocalization of Nrf2 and CFTR in NhBE versus CFhBE cells.** Quantification of Nrf2-CFTR colocalization in NhBE (top panel) and CFhBE (bottom panel) donor (coded on the left) cells. Filters embedded in paraffin. Sections 8  $\mu\text{m}$  thick were stained with anti-CFTR (24:1) and anti-Nrf2, mounted, and imaged at 60x magnification. \*\*Panels for donors DD032L and KK003M that are shown in **Figure 2E**. Merged, CFTR, Nrf2, and colocalization channels are shown for multiple donors. Scale Bar: 5 $\mu\text{m}$ .

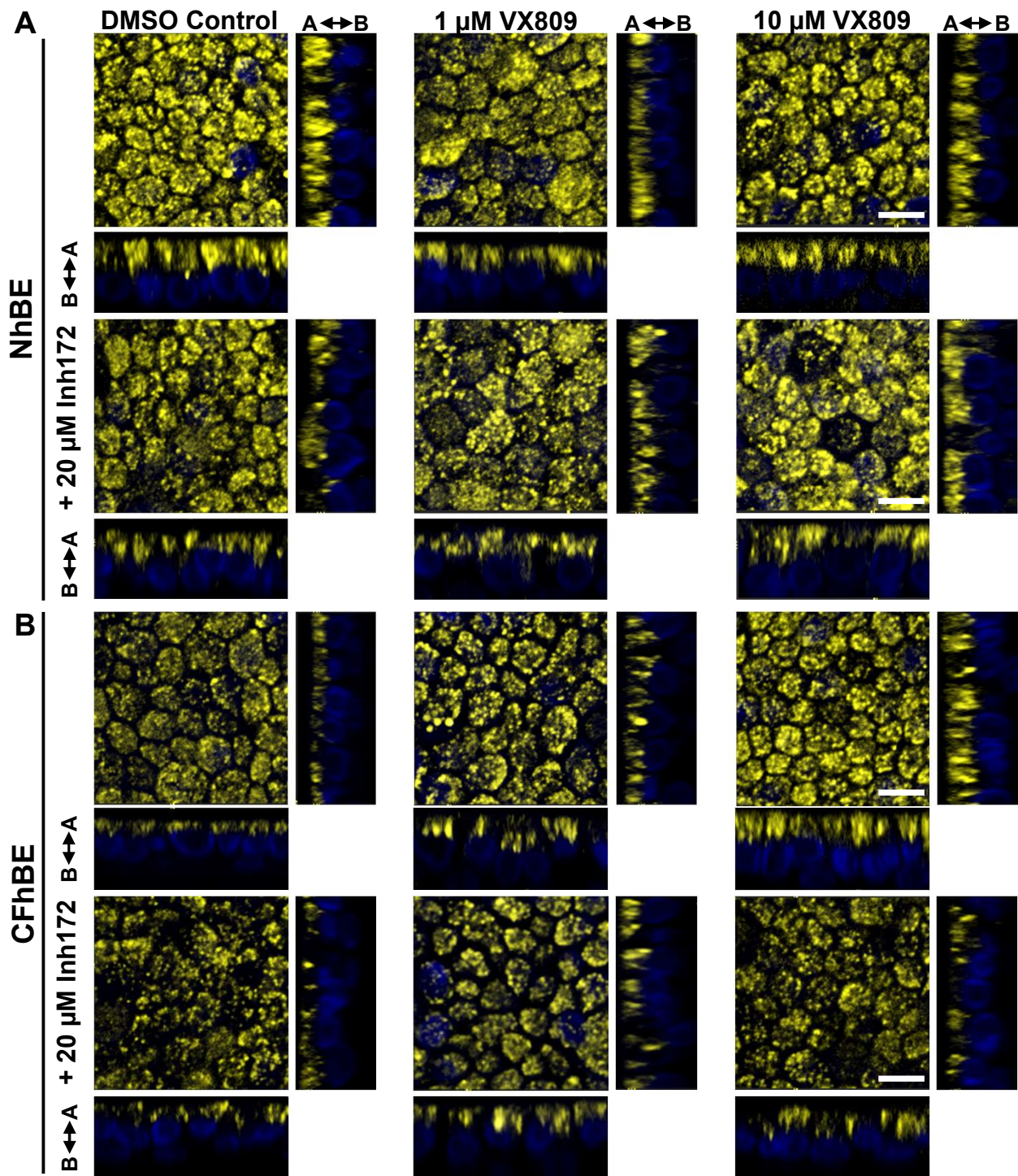


**Supplemental Figure S8. Analysis of association of CFTR and Nrf2 by immunofluorescence and immunoprecipitation.** (A) Separate channels for representative immunofluorescence images of NhBE and CFhBE cells with basolateral Na<sup>+</sup>/K<sup>+</sup>-ATPase staining. Cells were paraffin embedded and XY side sections are shown. Immunofluorescence for Nrf2 (green), CFTR (red), DAPI (blue), and Na<sup>+</sup>/K<sup>+</sup>-ATPase (purple), at 60X magnification. Scale Bar: 5 $\mu$ m (B) Input controls for the immunoprecipitation of CFTR and immunoblot against Nrf2 shown in **Figure 2F**. Whole cell lysate input controls (not incubated with anti-CFTR antibody) were subjected to SDS-PAGE, transferred to PVDF membrane, and probed with anti-Nrf2 antibody. (C) Reverse pulldown CFTR association with Nrf2 was analyzed by immunoprecipitation. NhBE and CFhBE cells were treated with the indicated doses of DMSO control, VX809 or VX661 for 48 hr. Whole cell lysates were incubated with anti-Nrf2 antibody, immunoprecipitated, subjected to SDS-PAGE, transferred to PVDF membrane, and probed with anti-CFTR antibody (lanes were run on the same gel but were noncontiguous). Whole cell lysate input controls (not incubated with anti-Nrf2 antibody) were probed with anti-Nrf2 antibody as control.





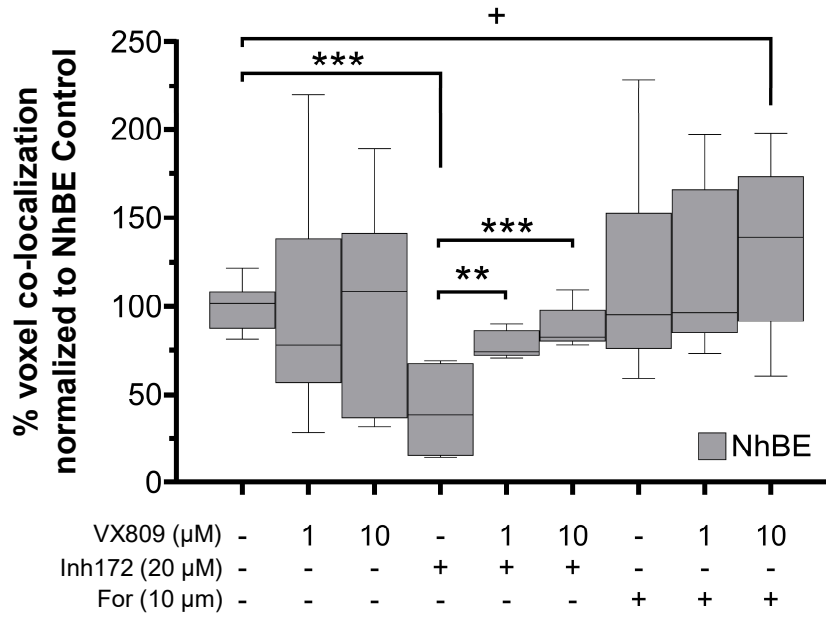
**Supplemental Figure S9. Nrf2-CFTR colocalization is decreased in CF mice.** Nrf2 and CFTR colocalization is decreased in the lung airway cells of DF508 CF mice compared to wild-type mice. Representative micrographs of separate channels, with immunofluorescence for Nrf2 (green), CFTR (red), or DAPI (blue), with Nrf2-CFTR colocalization in yellow, at 60X magnification, for at least n=4 mice per group. Scale Bar: 10 $\mu$ m.



**Supplemental Figure S10. Colocalization of Nrf2 and CFTR increases in CFhBE cells after VX809 treatment, and is blocked by co-incubation with CFTR<sub>inh</sub>172.** Representative micrograph at 60X magnification of primary (A) NrhBE and (B) CFhBE cells showing the colocalization channel (yellow) from the Nrf2 and CFTR immunofluorescence shown in **Figure 4**, with counterstaining of the nuclei (DAPI, blue), as analyzed in Imaris software. Cells were treated with the indicated doses of VX809 and/or CFTR<sub>inh</sub>-172 (Inh172) for 72 hr. Arrows indicate apical, A and basolateral, B sides. Cells were permeabilized as in **Figure 2**. Representative images of 5 independent experiments from 3 patient donors per cell type, with 3 technical replicates per treatment and experiment. Scale Bar: 10μm.

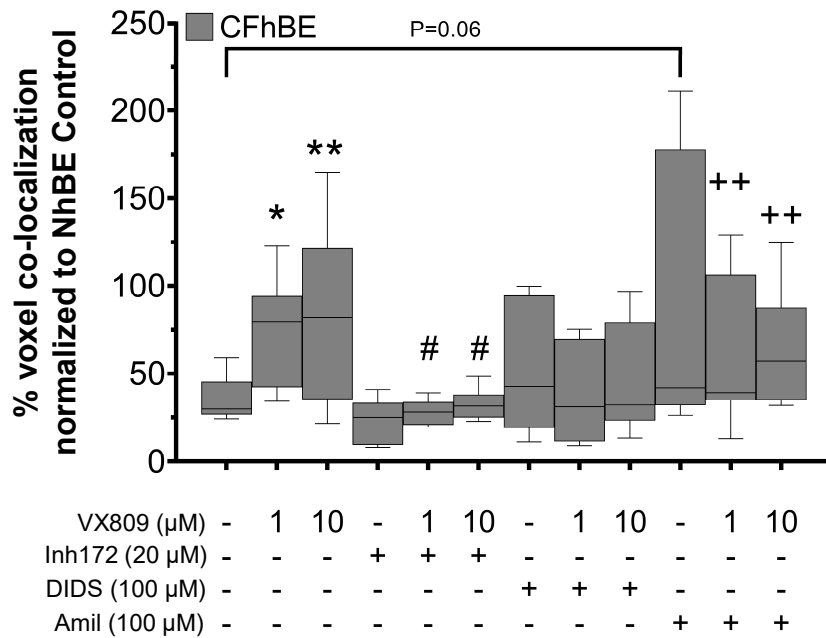
**A**

NhBE cells: Nrf2/CFTR Colocalization

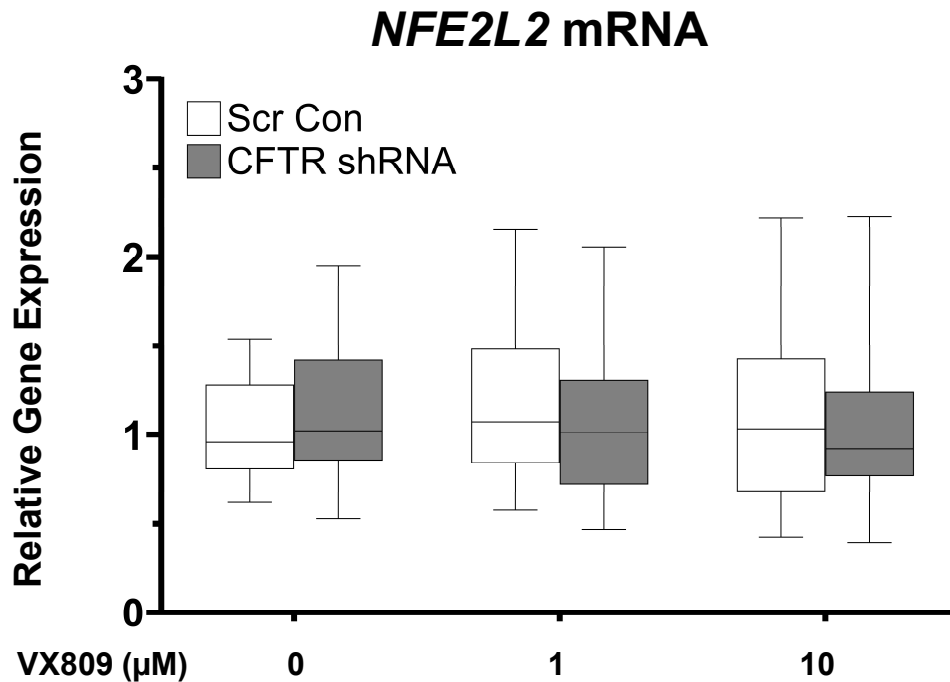


CFhBE cells: Nrf2/CFTR Colocalization

**B**



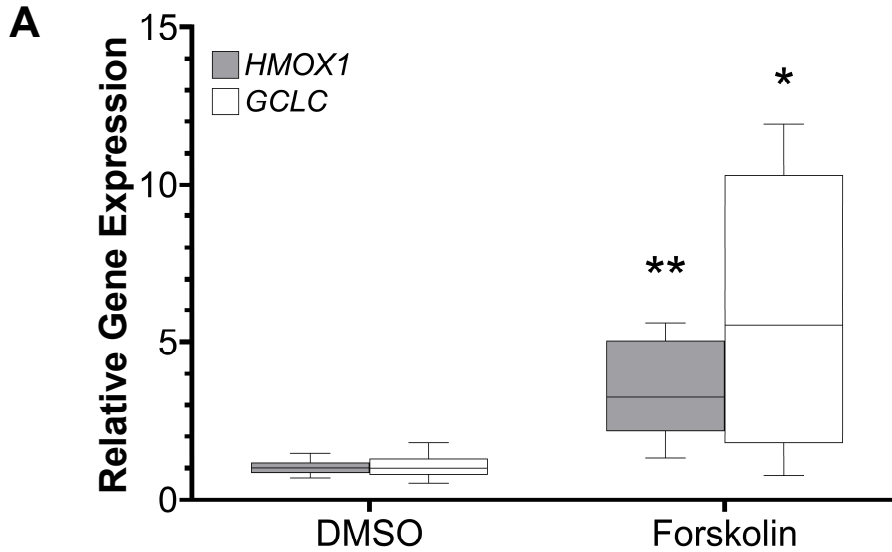
**Supplemental Figure S11. Colocalization of Nrf2 and CFTR increases in CFhBE cells after VX809 treatment, and is blocked by acute co-incubation with CFTR<sub>inh</sub>172.** Quantification of Nrf2-CFTR colocalization in NhBE (A) and CFhBE (B) cells treated with vehicle control or 1-10 μM VX809 for 46 hours, and then co-incubated for 2 hours with the indicated doses of CFTR<sub>inh</sub>172 (Inh172), forskolin (For), DIDS, or amiloride (Amil). Data for n=3 independent experiments, with 3 technical replicates per treatment and experiment are expressed as box-and-whisker plots. Horizontal bars indicate the median, box borders indicate 25<sup>th</sup> and 75<sup>th</sup> percentiles, and whiskers indicate 5<sup>th</sup> and 95<sup>th</sup> percentiles. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, compared to vehicle control; # p<0.05, vs. same dose of VX809 by 1-way ANOVA with Dunnett's multiple comparison test. +p<0.05, ++p<0.01 compared to vehicle control by 2-way ANOVA with Dunnett's multiple comparisons test.



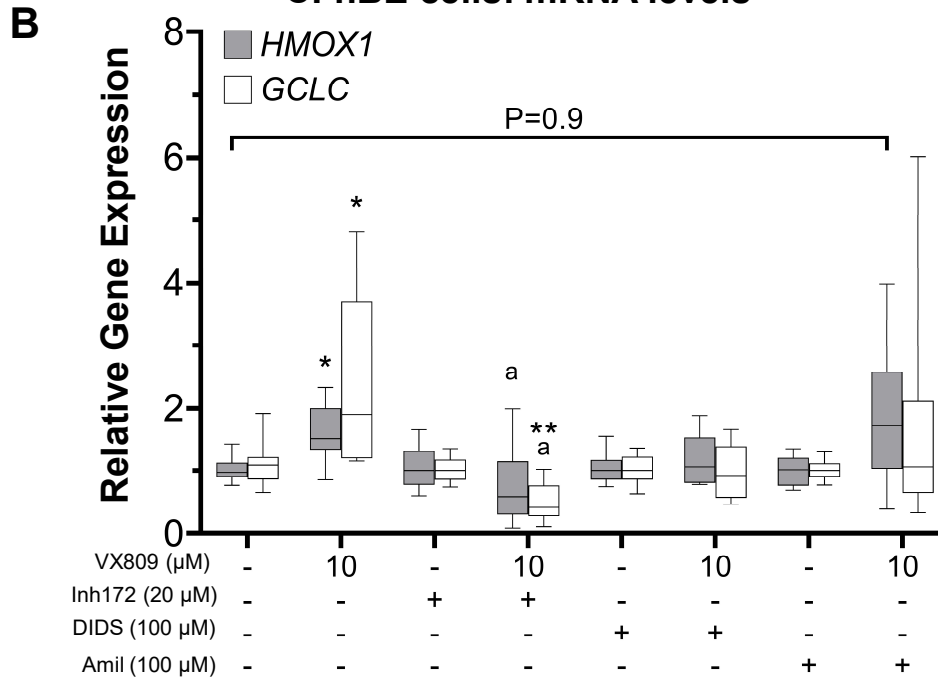
**Supplemental Figure S12. VX809 treatment and *CFTR* knockdown do not affect *NFE2L2* gene expression in CFhBE cells.** CFhBE cells were infected with lentivirus for *CFTR* shRNA or scrambled control (Scr Con) for 4 days, then incubated with DMSO control or 1-10 µM VX809 for 48 hours. Gene expression was determined by real-time qPCR, with mRNA levels shown as fold changes vs. scrambled control cells incubated with vehicle control (DMSO). Data for n=4 independent experiments from 3 patient donors are expressed as box-and-whisker plots. Horizontal bars indicate the median, box borders indicate 25<sup>th</sup> and 75<sup>th</sup> percentiles, and whiskers indicate 5<sup>th</sup> and 95<sup>th</sup> percentiles. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 by 1-way ANOVA with Dunnett's multiple comparison test.



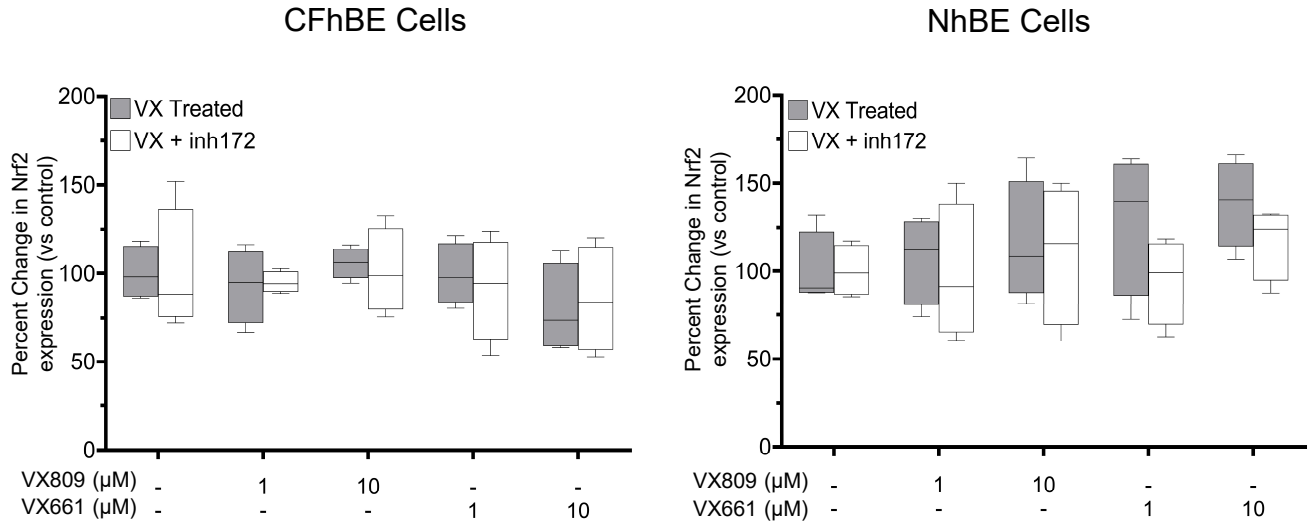
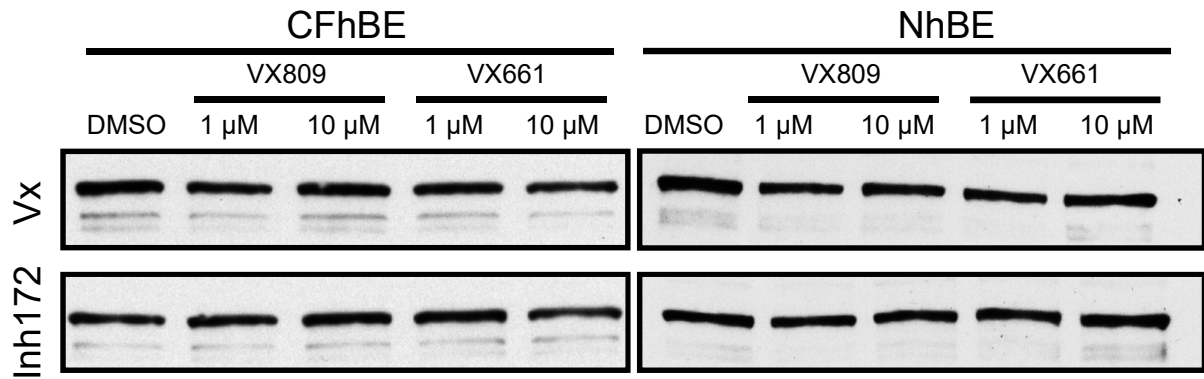
## NhBE cells: mRNA levels



## CFhBE cells: mRNA levels



**Supplemental Figure S13. Nrf2 transcriptional activity increases in CFhBE cells after VX809 treatment, and is blocked by acute co-incubation with CFTR<sub>inh</sub>172.** Gene expression of Nrf2-regulated genes *HMOX1* and *GCLC* in primary (A) NhBE, and (B) CFhBE cells, after incubation with DMSO control or 10 μM VX809 for 48 hours, with co-incubation for the last 6 hours with 20 μM CFTR<sub>inh</sub>172 (Inh172), 10 μM forskolin (For), 100 μM DIDS, and/or 100 μM amiloride (Amil), as determined by qPCR. Data presented as fold changes vs. untreated cells or CFTR<sub>inh</sub>172, forskolin, DIDS, and/or amiloride alone; calculated from cycle threshold measurements and normalized to control gene, 18S rRNA. Data for n=3 independent experiments from 3 donors per cell type are expressed as box-and-whisker plots. Horizontal bars indicate the median, box borders indicate 25<sup>th</sup> and 75<sup>th</sup> percentiles, and whiskers indicate 5<sup>th</sup> and 95<sup>th</sup> percentiles. For (A): \*p<0.05, \*\*p<0.01, vs vehicle control by Student's t-test. For (B): \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, compared to vehicle control or cells treated with CFTR<sub>inh</sub>172, DIDS, and/or amiloride alone; or <sup>a</sup> P<0.05, vs. same cells with same dose of VX809 by mixed-effects ANOVA with Dunnett's multiple comparison test.



**Supplemental Figure S14. Input Western blots for CBP immunoprecipitation.** Equally loaded aliquots of immunoprecipitations of CBP from CFhBE and NhBE cells following treatment for 48 hr with DMSO, VX809, VX661, or treatment + CFTR-Inh<sub>172</sub>. A) Example blot for Nrf2 levels in input lysate used for CBP immunoprecipitation. B) Levels of Nrf2 in inputs used in CBP immunoprecipitations in CFhBE cells following treatment. C) Levels of Nrf2 in inputs used in CBP immunoprecipitations in NhBE cells following treatment. Data for n=4 independent experiments from 4 CF donors and 3 non-CF donors are expressed as box-and-whisker plots. Horizontal bars indicate the median, box borders indicate 25<sup>th</sup> and 75<sup>th</sup> percentiles, and whiskers indicate 5<sup>th</sup> and 95<sup>th</sup> percentiles. Not significant vs vehicle control by 1-way ANOVA with Dunnett's multiple comparison test.