

Assessing the effect of NBD1 stabilizers SION-451 and SION-719 in combination with other CFTR modulators on primary human bronchial epithelial cells from F508del/F508del and F508del/null donors

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Abstract

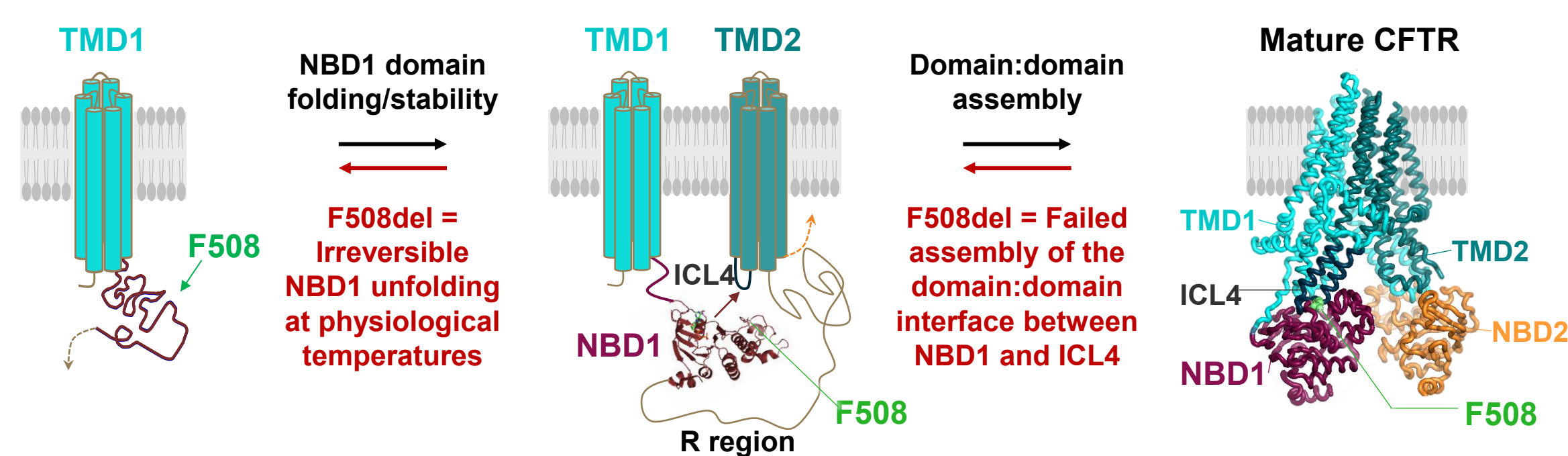
Background: The most prevalent CF-causing mutation, F508del, directly destabilizes NBD1 and leads to significant CFTR folding and functional defects. F508del also weakens CFTR domain-domain interactions, further impairing folding [1]. Thus, restoration of F508del CFTR function may require a combination of drugs that stabilize NBD1 and other domains that help improve CFTR global assembly, such as ICL4- or TMD1-directed correctors [2]. Approved CFTR modulators, which address domain assembly but do not directly impact NBD1, have led to substantial improvements in the lives of people with CF (pwCF) with at least one copy of F508del. However, approximately two-thirds of pwCF treated with approved modulators still have abnormal sweat chloride levels (≥ 30 mmol/L), indicating abnormal CFTR function [3]. Sionna is developing a novel class of small molecules designed to stabilize NBD1 with the potential to improve CFTR function up to wild-type levels when combined with other modulators that address CFTR global assembly. In these experiments, we assess the effect of Sionna's NBD1 stabilizers, alone and in combination with other complementary modulators, in primary CF human bronchial epithelial cells (CFHBE) from donors with either two copies of F508del (dF/dF) or one copy of F508del (dF/X, where X stands for a CFTR mutation resulting in no protein).

Methods: In functional and biochemical assays, we assessed the ability of Sionna's clinical-stage NBD1 stabilizers SION-451 and SION-719 to address F508del defects and improve channel function across a broad panel of CFHBEs from both dF/dF and dF/X donors, when used in combination with complementary modulators, including SION-2222 (TMD1 directed) and SION-109 (ICL4 directed).

Results: In primary CFHBEs from both dF/dF and dF/X donors, combinations of Sionna's NBD1 stabilizers with either ETI or other complementary CFTR modulators improved CFTR trafficking and chloride transport up to wild-type levels at E_{max} concentrations.

Introduction

F508del-CFTR Leads to NBD1 Instability and Defective CFTR Domain-Domain Assembly



- NBD1 instability is a key driver of defects in F508del-CFTR folding, trafficking, and ion channel function⁴⁻⁵
- F508del also impairs CFTR domain-domain assembly, adding to F508del-CFTR dysfunction⁴⁻⁵
- Suppressor mutations that stabilize NBD1 combined with those that improve CFTR domain-domain assembly (eg, NBD1-ICL4 interface stabilizers) can correct F508del-CFTR maturation up to wild-type CFTR levels⁴⁻⁵

Pharmacological F508del-CFTR Restoration May Require Addressing Both Domain Assembly and NBD1 Stability Defects⁴⁻⁶

- Sionna's NBD1 stabilizers are novel small molecules that directly bind to and stabilize NBD1 at physiological concentrations in non-clinical models
- Sionna is developing a proprietary dual combination of NBD1 stabilizer SION-451 and complementary TMD1-directed modulator SION-2222 (galicafitor)

A

Western Blots demonstrate robust trafficking rescue by NBD1 modulator combinations at E_{max} in both dF/dF and dF/X CFHBEs

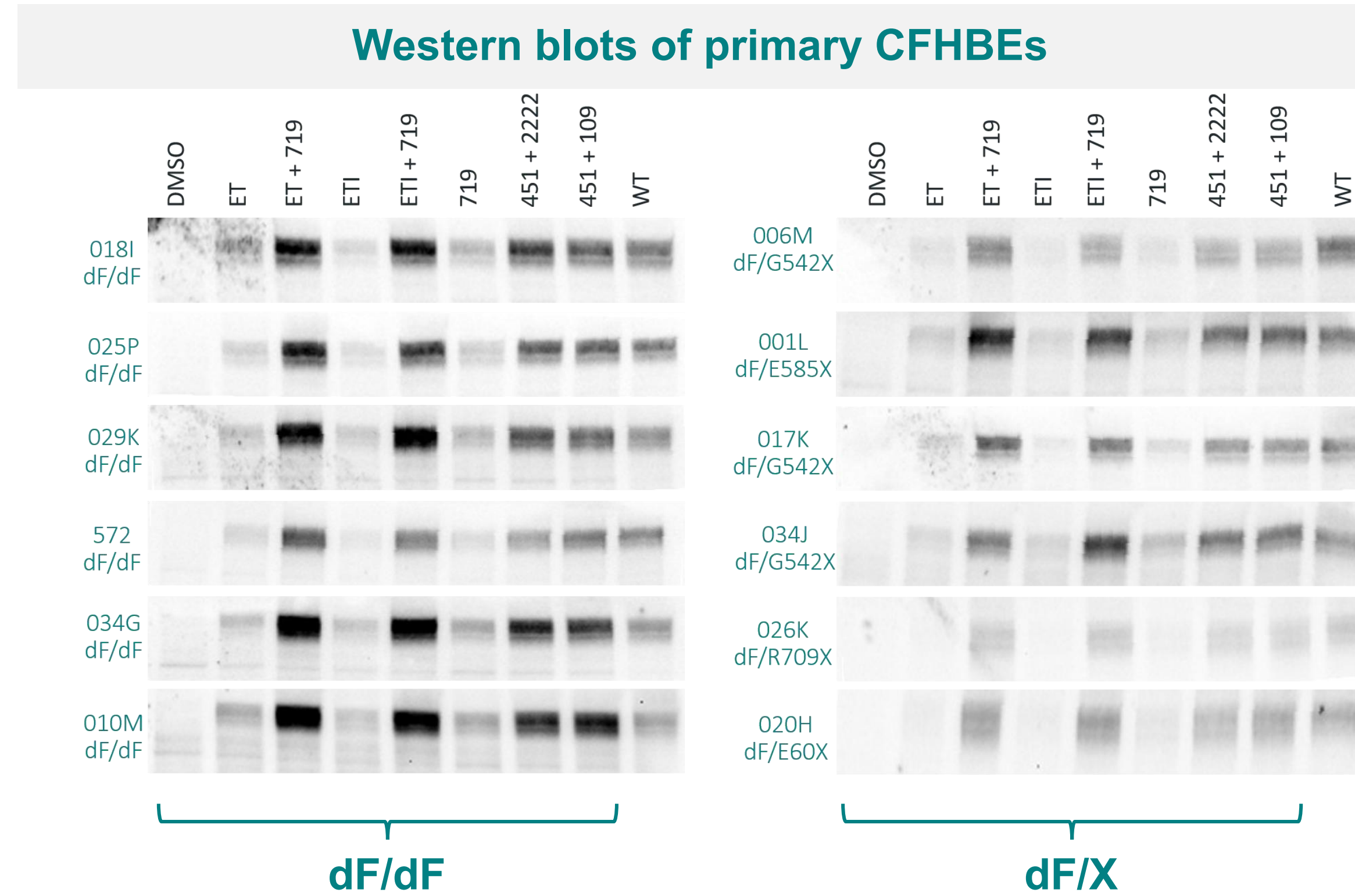


Fig. 1a. HBE cells from dF/dF and dF/X donors with CF treated with compound at their respective E_{max} . Western blots of each compound treatment represent a pooled sample from three replicate filters, and wild-type bands are a composite of 4 different wild-type donors with each donor being a pooled sample of 24 filters.

Western quantitation relative to wild-type

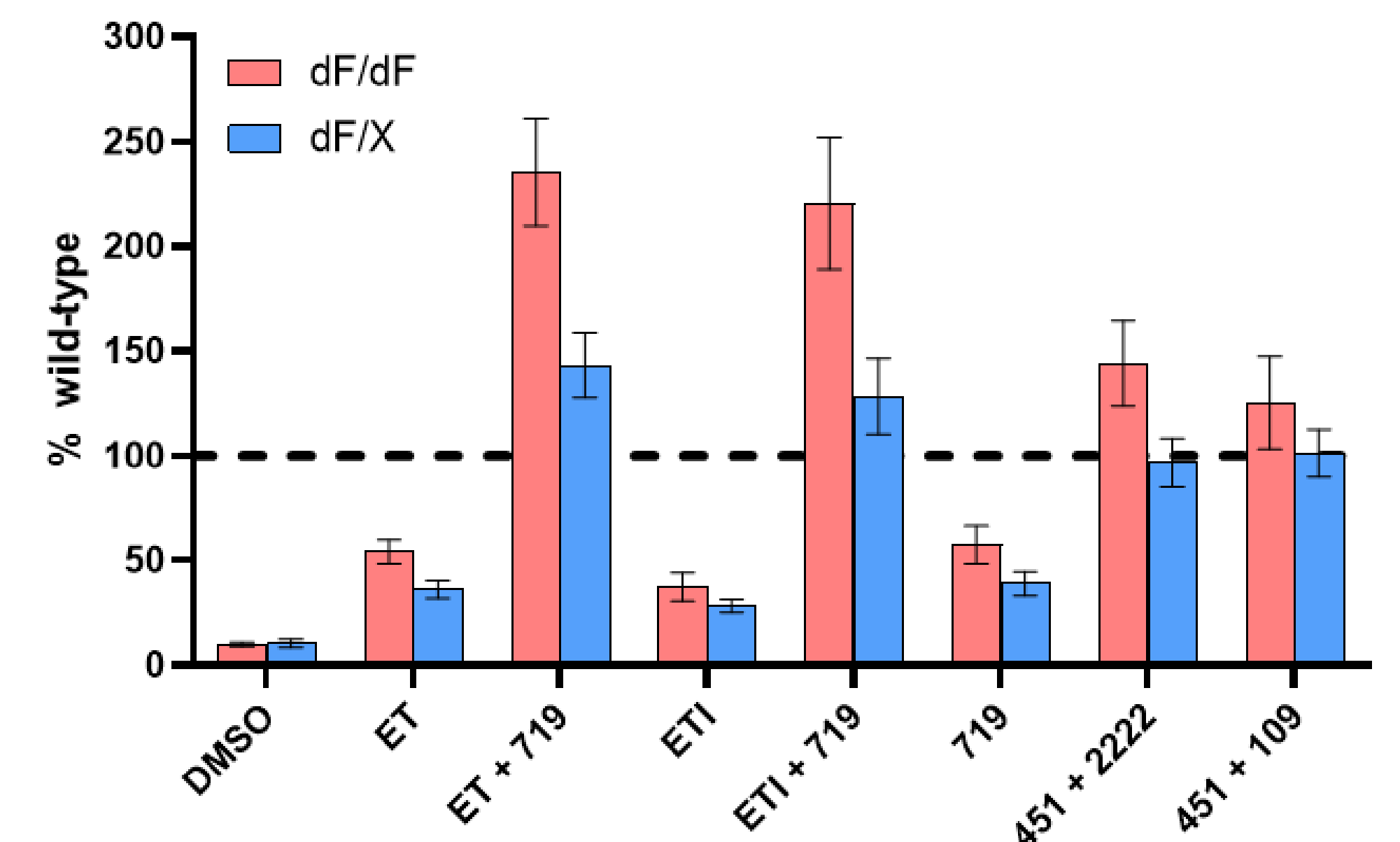


Fig. 1b. Data represent the average quantitation as a percent of wild-type for each compound(s) of all 6 dF/dF donors (red bars) or all 6 dF/X donors (blue bars); error bars display $\pm$ SEM. Mature C band of CFTR was normalized to wild-type across all samples.

B

NBD1 modulator combinations at E_{max} improve chloride secretion in both dF/dF and dF/X CFHBEs

CFTR peak Forskolin (FSK) to post-inhibitor current relative to wild-type

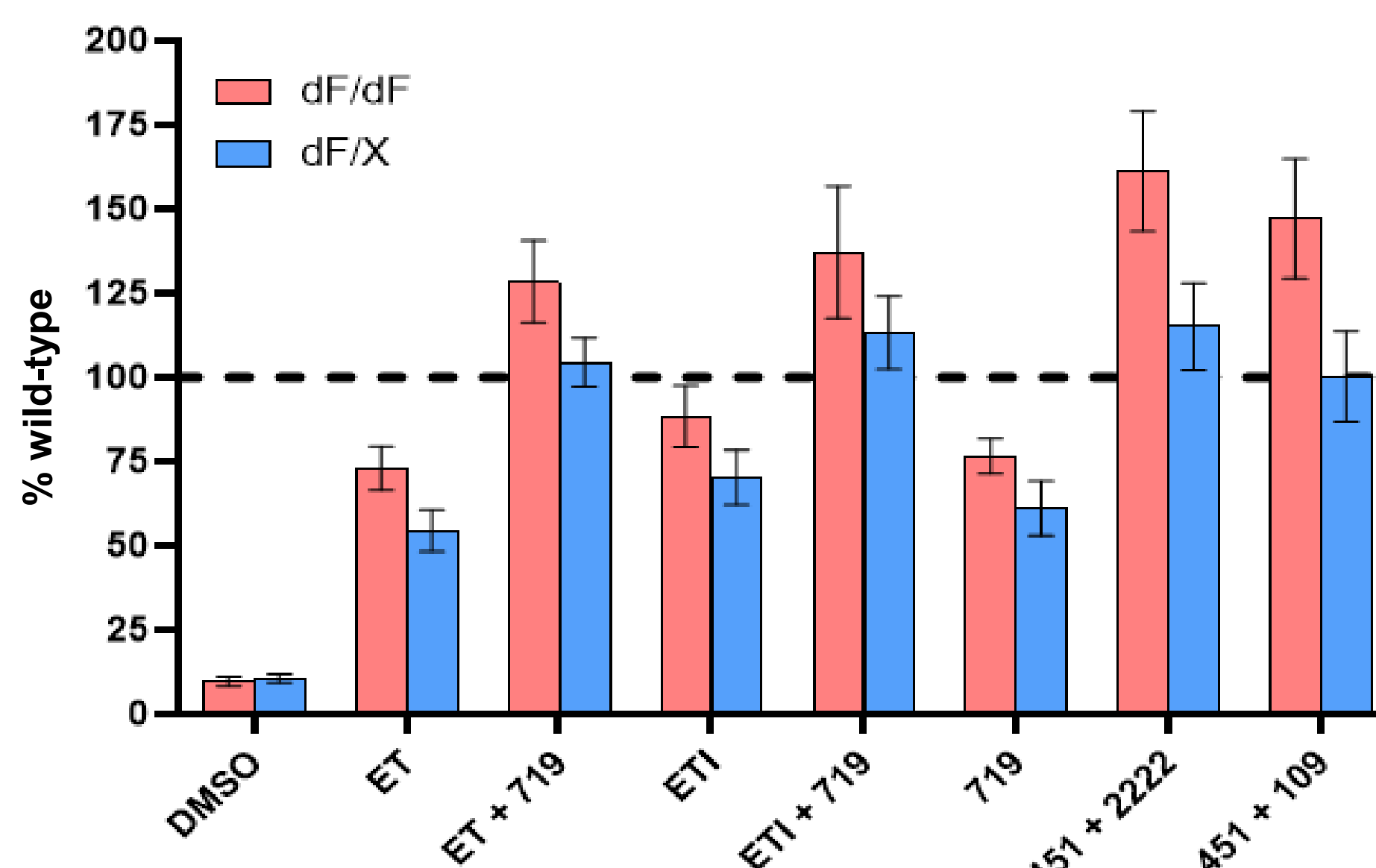


Fig. 2. Peak minus post-inhibitor-baseline current normalized to average current of six wild-type donors. Bar graphs represent average $\pm$ SEM of six dF/dF HBE donors (red bars) or six dF/X HBE donors (blue bars). All experiments were run on a TECC instrument under current clamp with symmetrical chloride.

Pre-stimulus baseline considerations in measuring CFTR activity using FSK current

- Historically, peak FSK current has correlated with clinical activity across approved CFTR modulators⁷, and the high pre-stimulation current observed when NBD1 stabilizer SION-719 was combined with ETI was interpreted as increased CFTR activity
- Following the PreciSION CF Phase 2a trial post-hoc analysis, we hypothesize that the combination with ivacaftor, the potentiator in ETI, may have blunted the driving force for chloride ion flow in this 4-drug combination trial
- Elevated pre-stimulation current is not observed in Sionna NBD1 anchored combinations that do not contain a potentiator

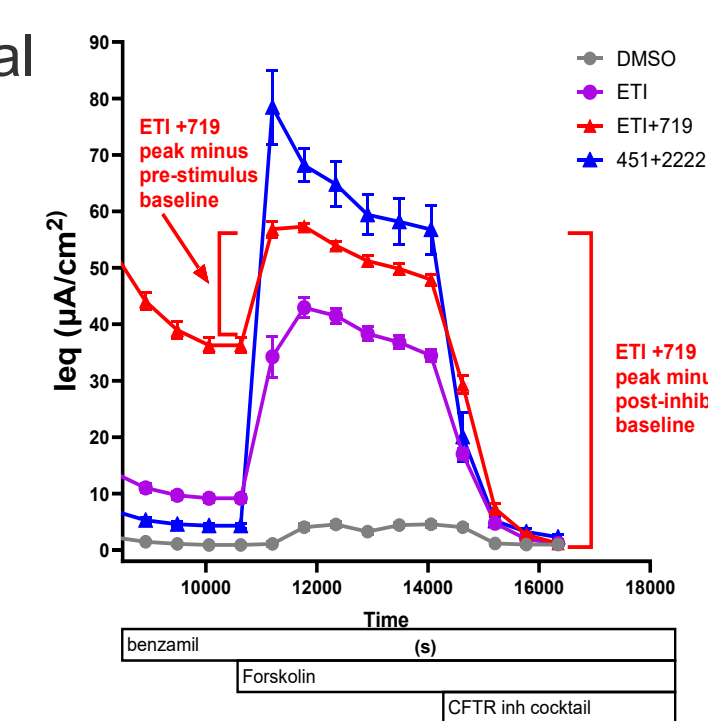


Fig. 3. Representative TECC traces demonstrate how CFTR activity can be measured at compound E_{max} .

C

Dual combination of NBD1 Stabilizer SION-451 with SION-2222 resembles wild-type HBE CFTR behavior

Wild-type HBEs demonstrate low pre-stimulation current, a strong response following stimulation, and a decline following CFTR inhibition

The activity profile of Sionna's NBD1 dual combination SION-451 + SION-2222, which does not contain a potentiator, closely mirrors that of wild-type in its pre-stimulation, stimulated, and inhibited phases

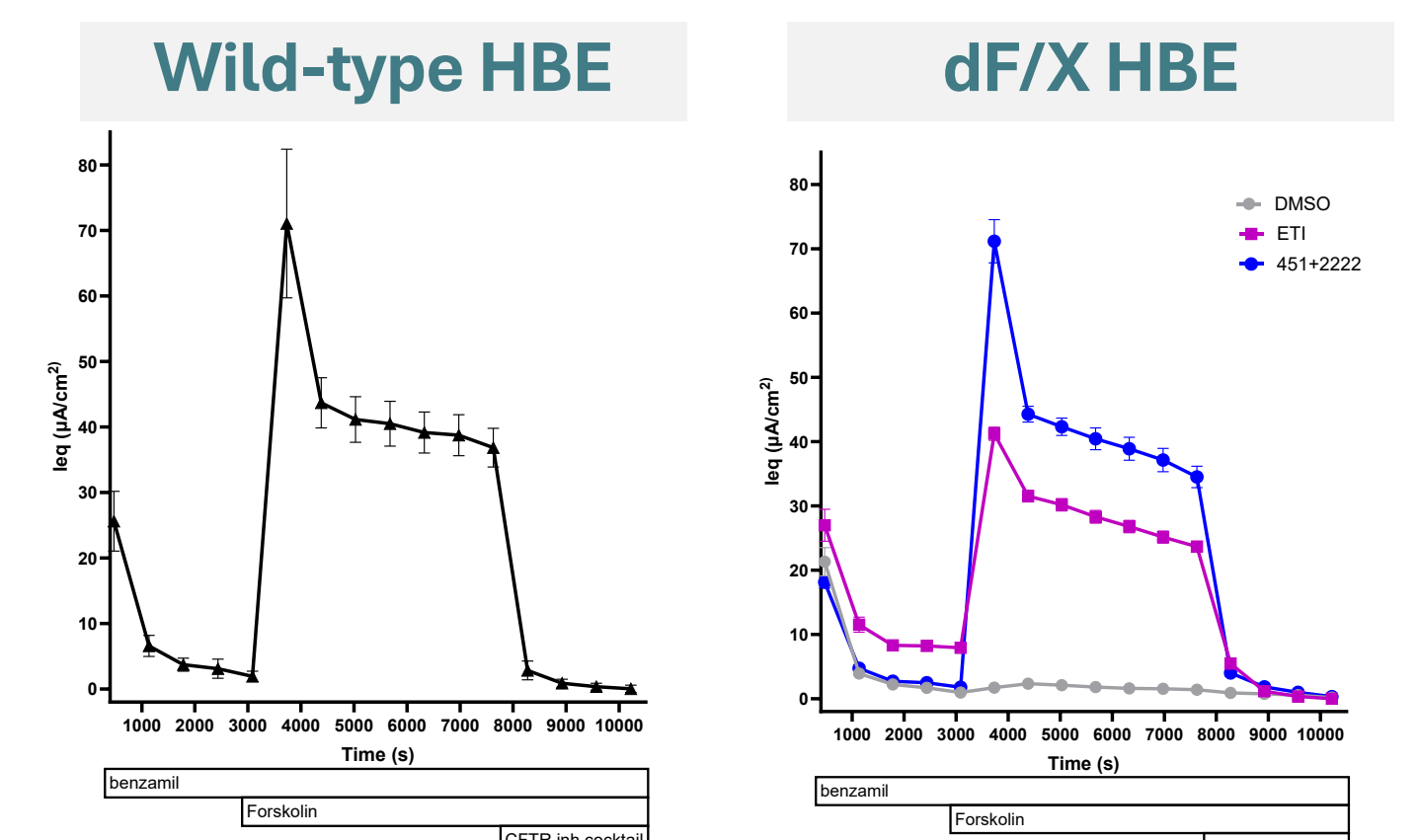


Fig. 4. Representative TECC traces extracted from either a wild-type or dF/X donor. Each trace is the average $\pm$ SEM of four replicate wells. Experiment was run under current clamp with symmetrical chloride.

Conclusions

SION-451 and SION-719 are novel, clinical-stage NBD1 stabilizers designed to address the CFTR defect caused by F508del. In primary CFHBEs from both dF/dF and dF/X donors, combinations of NBD1 stabilizers with other complementary modulators, including SION-2222 and SION-109, demonstrated improved CFTR trafficking and function up to wild-type levels. These findings support the potential of NBD1-stabilizer combinations to benefit a broad population of people with CF.

References

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