Clinical-stage NBD1 stabilizers can increase F508del-CFTR protein half-life to wild-type levels when used alone or in combination with other CFTR modulators

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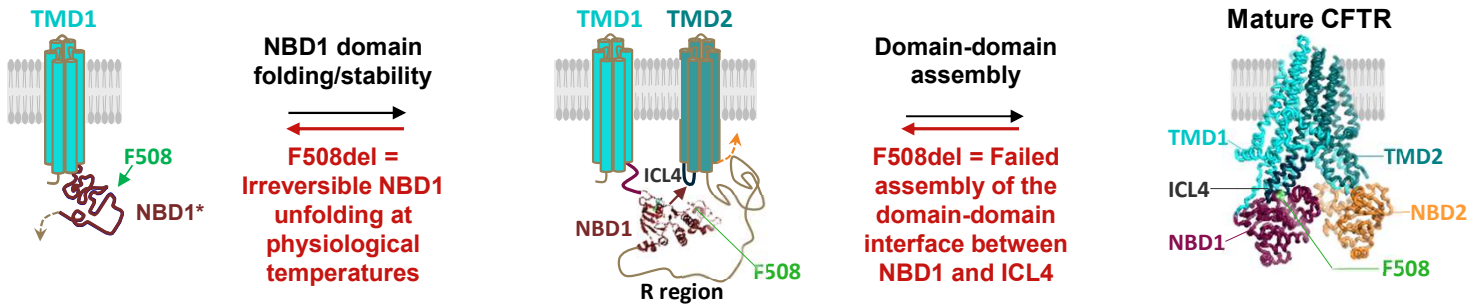
Abstract # ECFS-2026-00214

NBD1 Instability and Defective CFTR Domain-Domain Assembly are Central Drivers of F508del-CFTR Dysfunction

F508del-CFTR's critical NBD1 instability and domain assembly errors must be addressed for full correction



- F508del-CFTR leads to NBD1 instability causing the domain to unfold at physiological temperatures
- NBD1 instability is a key driver of F508del-CFTR's folding, trafficking, and channel function defects
- F508del also impairs CFTR domain-domain assembly, adding to F508del-CFTR dysfunction

**NBD1 = Nucleotide Binding Domain 1; TMD = Transmembrane Domain; ICL4 = Intracellular Loop 4*

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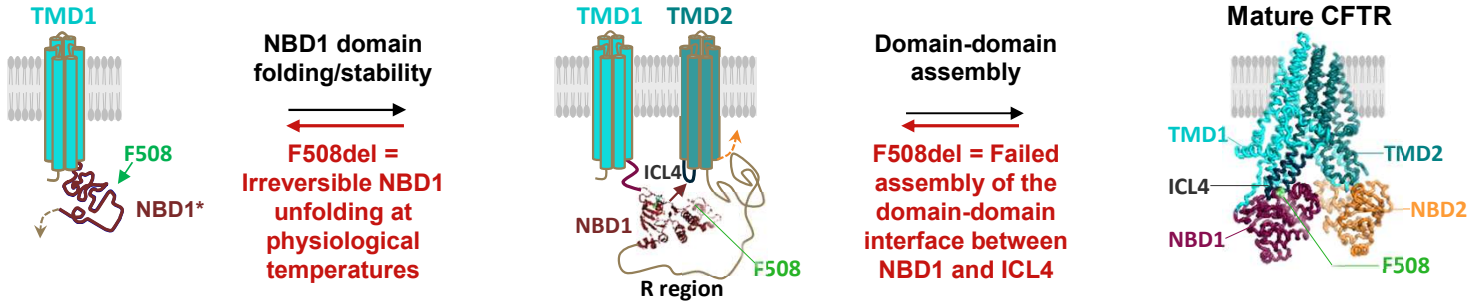
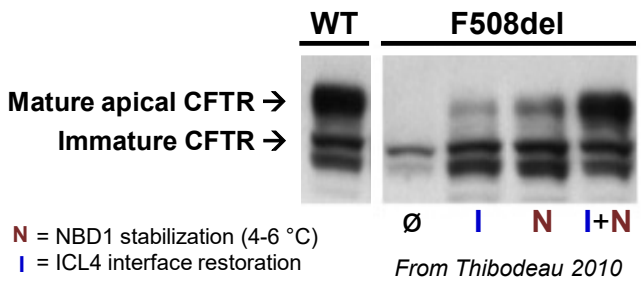


Proof of Hypothesis: Stabilizing NBD1 with Second-Site Mutations



N = NBD1 stabilization (4-6 °C)
I = ICL4 interface restoration

From Thibodeau 2010

F508del-CFTR suppressor mutations that stabilize NBD1 and the NBD1-ICL4 interface restore F508del-CFTR maturation and function to near wild type (WT) levels, providing a roadmap to more effective therapies

Thibodeau *et al*. J Biol Chem. 2010 Nov 12;285(46):35825-35.

Hypothesis:* *Stabilize NBD1* + *Improve Domain Assembly* = *Fully Restorative Therapy

**NBD1 = Nucleotide Binding Domain 1; TMD = Transmembrane Domain; ICL4 = Intracellular Loop 4*

Sionna is Developing NBD1 Stabilizers and Complementary Modulators that Correct Domain Assembly

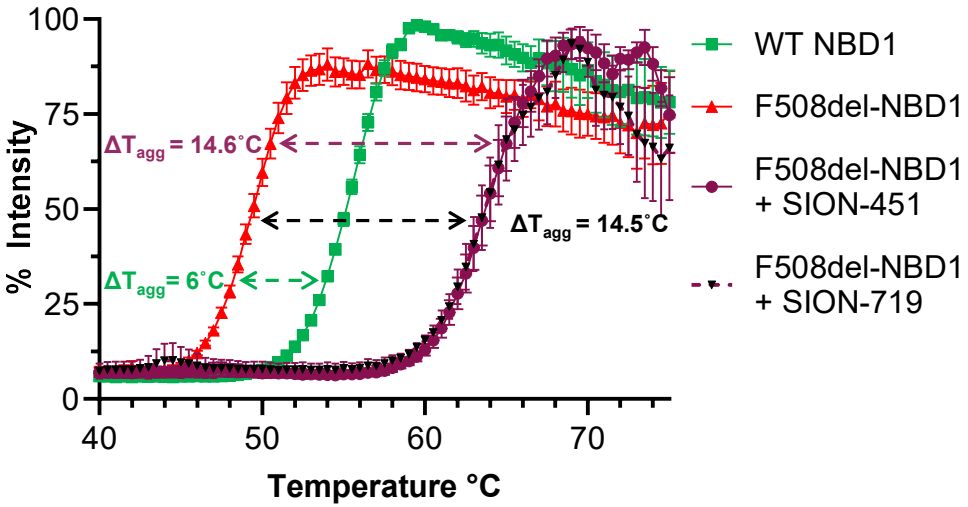
- **Marketed modulators have significantly improved outcomes for people with CF, but do not fully restore normal CFTR function in at least two-thirds of patients[1,2]**
- **Leveraging over 15 years of investment by Sionna, CFF, Genzyme and Sanofi, we've had unique success identifying potent small molecules that directly target NBD1, which was previously considered undruggable**
  - >10 screening campaigns (biophysical, cell-based and virtual) covering >2 million compounds
  - ~150 X-ray co-structures were solved to guide structure-based optimization of NBD1 stabilizers
  - >5,000 compounds across different NBD1 ligand series were designed, synthesized and assessed
  - >250 *in vivo* PK studies, >20 multiple-dose tox studies conducted, 4 INDs to date
- **Sionna NBD1 stabilizers and complementary CFTR modulators have progressed to clinical studies**
  - Our goal is to deliver differentiated medicines, anchored by novel NBD1 stabilizers, to enable more people with CF to achieve normal CFTR function
- **Here we utilized metabolic pulse-chase labeling to demonstrate Sionna NBD1 stabilizers increase the half-life of F508del-CFTR**
  - SION-451 in the presence and absence of TMD1-directed corrector SION-2222 (galicaftor), or ICL4-directed corrector SION-109
  - SION-719 in the presence and absence of ETI

1. *Konstan M, Mayer-Hamblett N, Odem-Davis K, et al. CFTR modulator-induced sweat chloride changes across the CF population from the CHEC-SC Study: 2022 Update. J Cyst Fibros. 2022;21:S26-S27. Poster presented at the NACFC, Nov 3-5, 2022.*
2. *Zemanick ET, Emerman I, McCreary M, et al. Heterogeneity of CFTR modulator-induced sweat chloride concentrations in people with CF. J Cyst Fibros. 2024;23:676-684.*

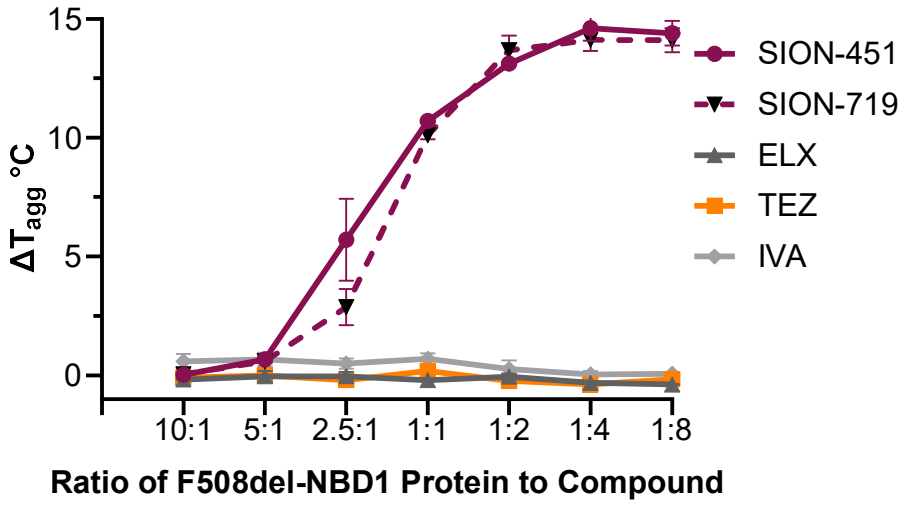
Sionna Clinical-stage NBD1 Stabilizers Correct the Thermal Stability Defect of F508del-NBD1

SION-451 and SION-719 are high affinity NBD1 ligands that can stabilize F508del-NBD1 to levels exceeding WT

NBD1 Differential Static Light Scattering



N=4, error bars indicate standard error

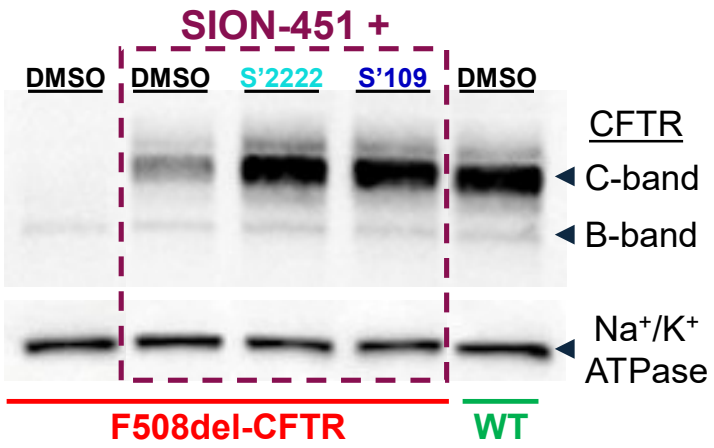


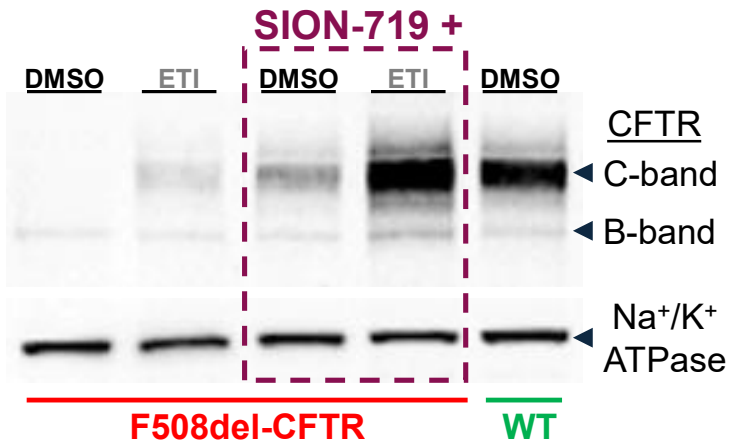
N=4, error bars indicate standard deviation

WT: wild type

NBD1 Stabilizers Increase Steady-State Levels of Mature F508del-CFTR both Alone and in Combination with Complementary Modulators

F508del-CFTR C-band similar to WT levels at steady state





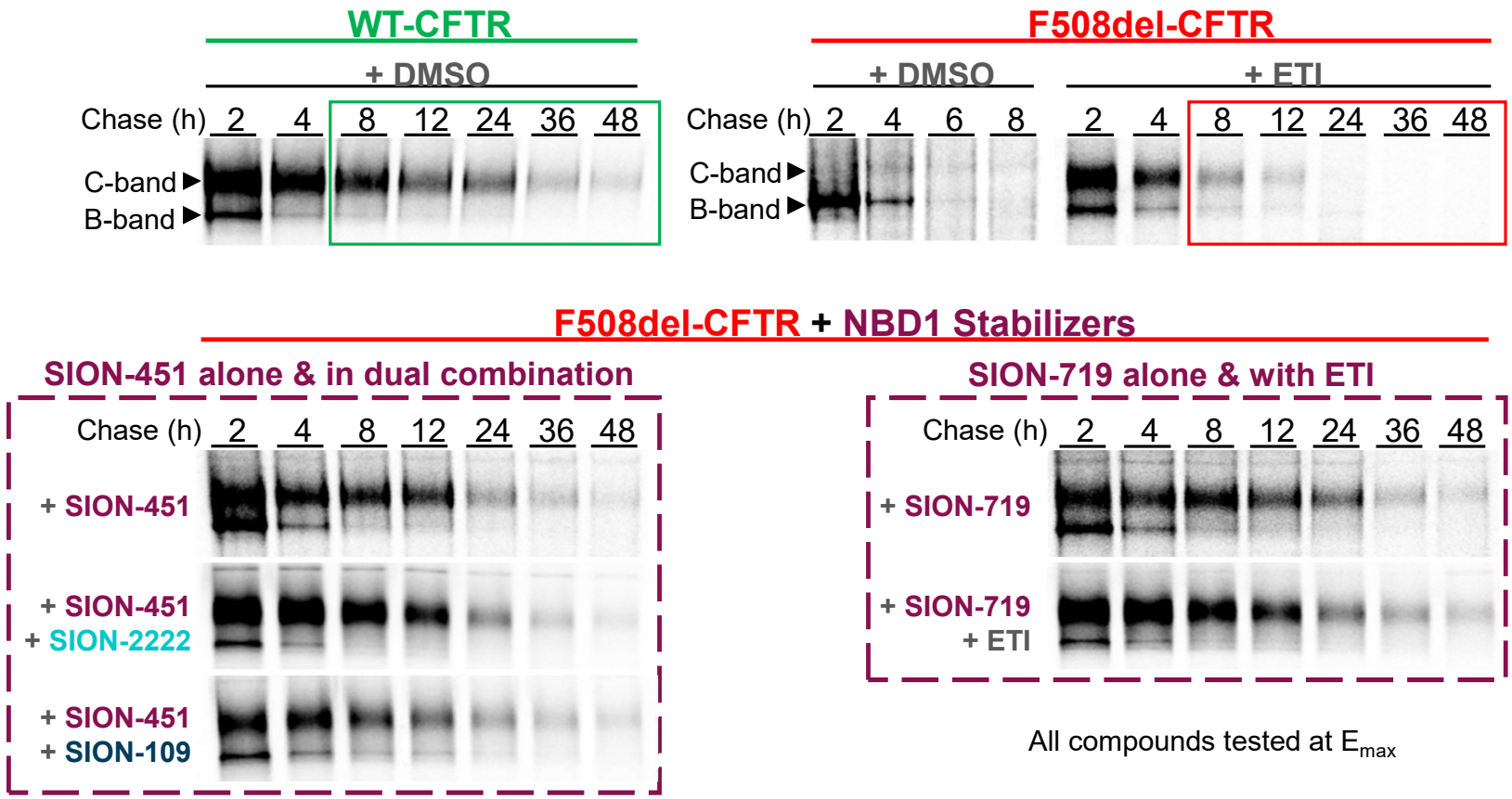
SION-451: 1.5μM – Sionna NBD1 Stabilizer
SION-2222: 5μM – Sionna TMD1 Corrector
SION-109: 3μM – Sionna ICL4 Corrector

SION-719: 1.5μM – Sionna NBD1 Stabilizer
ETI at E_{max}
- **ELX: 10μM – approved ICL4 Corrector**
- **TEZ: 5μM – approved TMD1 Corrector**
- **IVA: 0.1μM – approved Potentiator**

Western blots of CFTR-expressing CFSMEo- cells 48 hours of indicated treatment with indicated compounds at their respective E_{max}

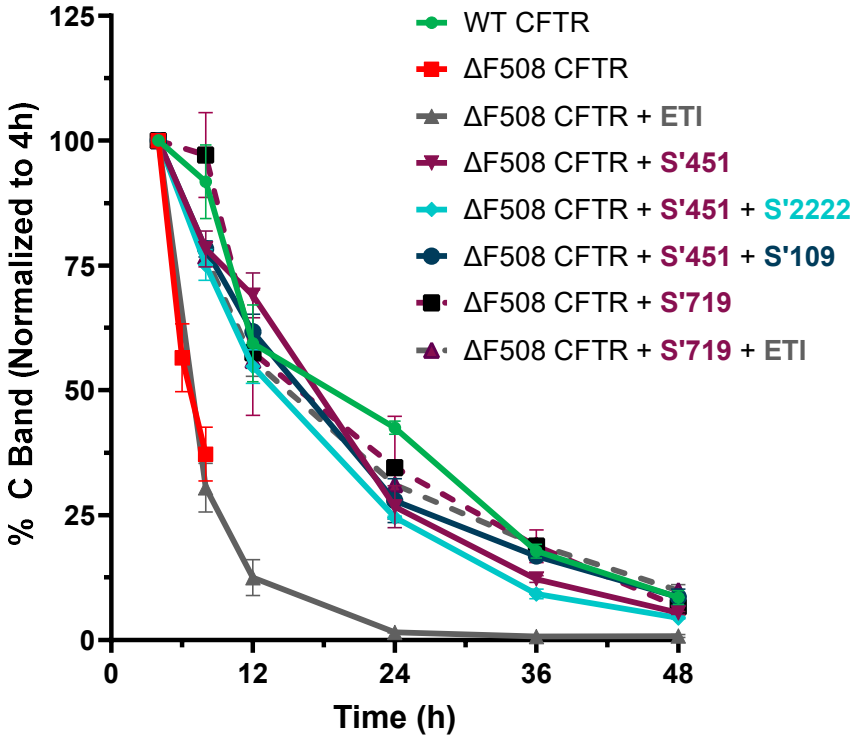
Hypothesis: Stabilize NBD1 + Improve Domain Assembly = Fully Restorative Therapy

NBD1 Stabilizers Improve F508del-CFTR Protein Maturation and Function, And Pulse Chase Analysis Demonstrates They Increase F508del-CFTR Half-life to WT-CFTR Levels

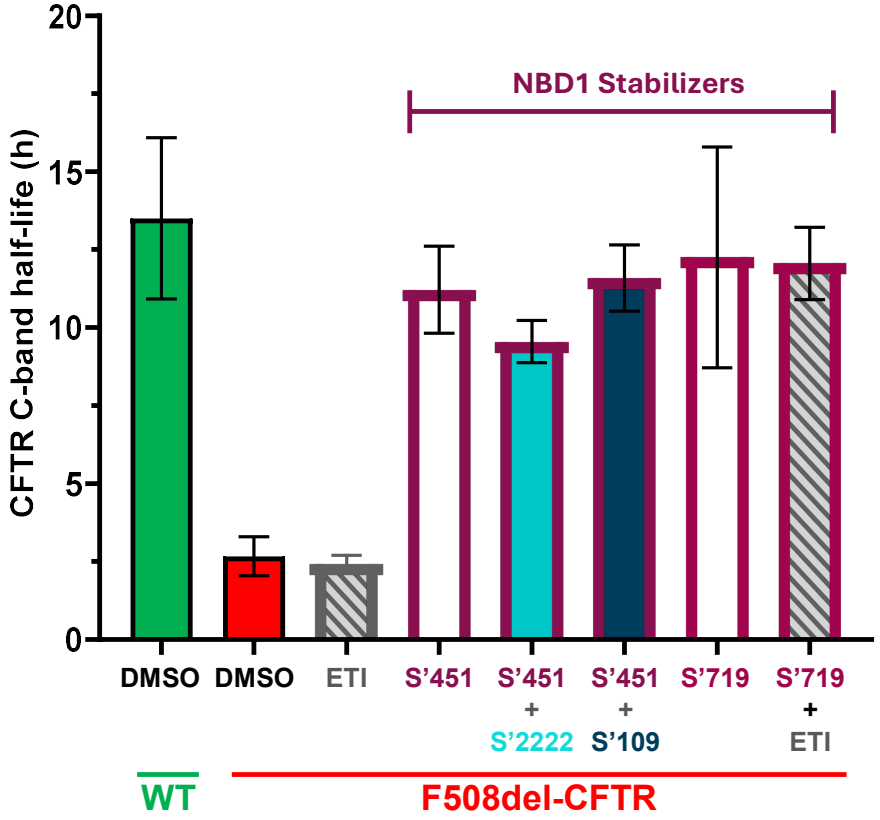


All compounds tested at E_{max}

Metabolic Pulse Chase Analysis Demonstrates NBD1 Stabilizers Improve the Half-life of F508del-CFTR to WT-CFTR Levels

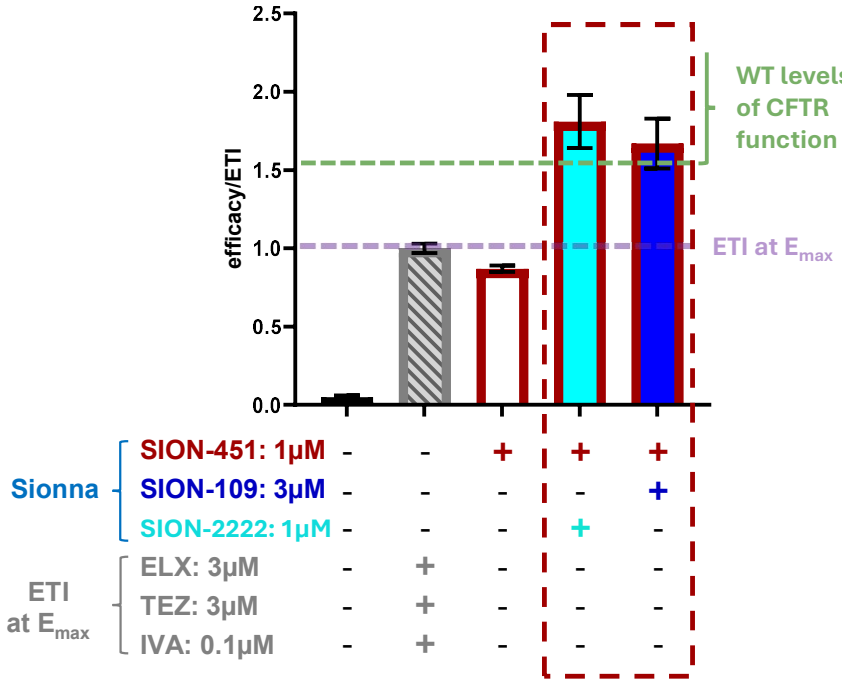


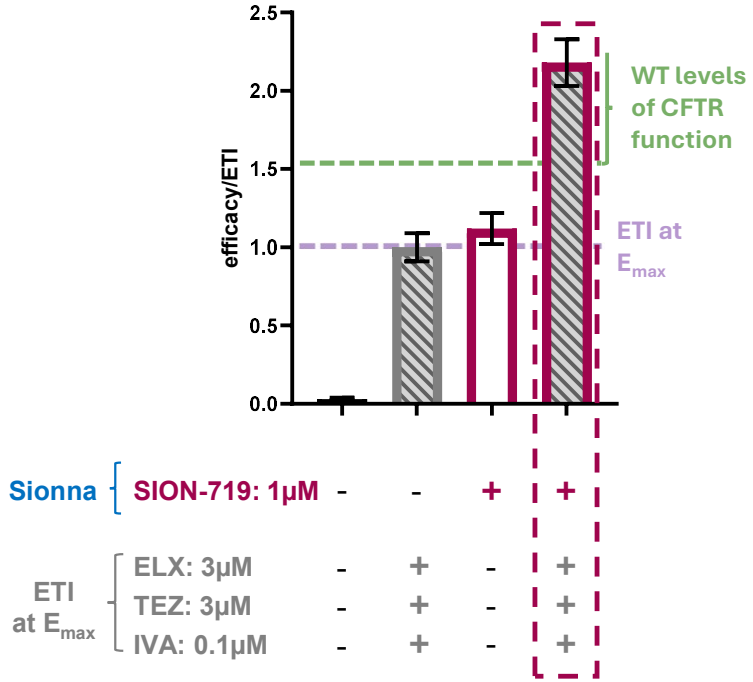
Treatment with ETI alone had a minimal effect on F508del-CFTR half-life



Alone and in Combination, NBD1 Stabilizers Improve F508del-CFTR Function in the CFHBE Translation Model

Combinations of SION-451 with SION-2222 or SION-109, and SION-719 with ETI were Highly Active in the F508del/F508del CFHBE* (VALI) Model at E_{max}





*CFHBE: CF Human Bronchial Epithelial cell model

***Hypothesis:** Stabilize NBD1 + Improve Domain Assembly = Fully Restorative Therapy*

To Fully Normalize F508del-CFTR, Sionna is Developing Novel Modulators to Address Key Drivers of Dysfunction

- **NBD1 stabilizers SION-451 and SION-719, in combination with complementary modulator(s), enable full restoration of WT-like CFTR function in preclinical CF models**
- **Here, we demonstrate that NBD1 stabilizers, even when used as single agents, can increase the half-life of F508del-CFTR to WT levels, an effect not observed with ETI**
- **These findings suggest NBD1 instability is a central driver of F508del-CFTR's greatly increased degradation rate, further highlighting the potential of NBD1 stabilizers in the treatment of CF**
- **Sionna NBD1 stabilizers and complementary CFTR modulators have progressed to clinical studies. Our goal is to deliver differentiated medicines, anchored by novel NBD1 stabilizers, that enable more people with CF to achieve normal CFTR function**
  - SION-719, is being studied as an add-on to SOC in a Phase 2 study in people with CF
  - SION-451, is being studied in combination with complementary modulators in Phase 1 studies in healthy volunteers

Hypothesis:* *Stabilize NBD1* + *Improve Domain Assembly* = *Fully Restorative Therapy

Thank You