**Supporting materials and methods**

**ALI culture**

Human bronchial epithelial (HBE) cells were seeded on 6.5 mm Transwell permeable supports (Corning, Amsterdam, the Netherlands). These supports were coated with 300 µL phosphate-buffered saline (PBS) containing 30 µg/mL PureCol (CellSystems Biotechnologie Vertrieb GmbH, Troisdorf, Germany), 10 µg/mL fibronectin (Sigma Aldrich), and 10 µg/mL bovine serum albumin (BSA) for 2 hours at 37°C and 5% CO2. Next, the HBE cells were seeded at a density of 4.5 × 105 cells/cm2 in differentiation medium consisting of 1:1 bronchial epithelial cell growth medium (BEGM; Lonza, Castleford, UK) and Dulbecco’s modified Eagle’s medium (DMEM; Life Technologies), BEGM SingleQuot Kit Supplements & Growth Factors (Lonza, Breda, the Netherlands), 1% penicillin/streptomycin, 1.5 µg/mL BSA, and 15 ng/mL retinoic acid (Sigma Aldrich). The medium was refreshed three times per week, and once the cells had formed a fully confluent monolayer, the medium was removed from the apical side to establish an air–liquid interface (ALI). The cultures were maintained for at least 3 weeks in ALI before the start of treatment.

**Transfection of CFPAC-1 cells with eluforsen**

Eluforsen oligonucleotide and Lipofectamine 2000 were mixed at a 1:5 v/v ratio in Opti-MEM medium (Life Technologies), incubated at room temperature for 5 minutes, then added to the culture medium. After 6 hours, the transfection medium was replaced by culture medium and the cells were cultured for an additional 24–72 hours before being used for the *N*-(6-methoxyquinolyl) acetoethyl ester (MQAE) assay.

**MQAE assay**

CF pancreatic adenocarcinoma (CFPAC-1) cells were loaded for 6 hours with 10 mM MQAE in culture medium. Next, the cells were washed twice with chloride buffer (130 mM NaCl, 5 mM KCl, 1.2 mM CaCl2, 1 mM MgCl2, 10 mM glucose, and 20 mM [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HEPES]) and incubated for 15 minutes with chloride buffer. The buffer was then removed completely and replaced by low chloride buffers (130 mM NaNO3, 5 mM KCl, 1.2 mM CaCl2, 1 mM MgCl2, 10 mM glucose, and 20 mM HEPES) containing 10 µM forskolin and 10 µM VX-770 (ivacaftor [CFTR potentiator]). Fluorescence signal at 460 nm was measured every 30 seconds using a FluoDia T70 plate reader (Photon Technology International, Longjumeau, France) and the rate of increase in fluorescence signal over the first 2 minutes was used as a measure of eluforsen-mediated restoration of CFTR-mediated chloride efflux.

**Ussing chamber**

HBE cells grown on Transwell permeable supports were transferred to Ussing chambers (P2300, Physiological Instruments, San Diego, CA, USA) mounted in a heat block kept at 37°C, and chloride buffer (135 mM NaCl, 2.4 mM K2HPO4, 0.6 mM KH2PO4, 1.2 mM CaCl2, 1.2 mM MgCl2, 10 mM glucose, and 10 mM HEPES) was added to the basolateral and apical side of the cells. The cultures were left to equilibrate for 10 minutes prior to measuring the Isc. Transepithelial voltage (VTE) was clamped to 0 mV, and Isc was measured using a VCC MC6 Voltage/Current Clamp (Physiologic Instruments, San Diego, CA, USA) and digitally recorded using Acquire and Analyze software (version 2.3; Physiologic Instruments). The CFTR-specific current was measured by sequential addition of:

1. 30 µM amiloride (Sigma Aldrich) to the apical side (block the predominant sodium channel ENaC);

2. 5 µM VX-770 or 30 μM Genistein (CFTR potentiation);

3. 100 µM isoproterenol (CFTR activation) (Sigma Aldrich); and

4. 30 µM CFTRinh-172.

The difference between the maximum Isc after CFTR stimulation and the Isc after specific CFTR inhibition was calculated as a measure of CFTR activity.