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Supplemental information

Increased CFTR expression and function from an optimized lentiviral vector for cystic fibrosis gene therapy

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Supplemental Methods

Immunohistochemistry

Epithelia were fixed overnight in 4% paraformaldehyde at 4°C. SuperBlock (Thermo Fischer Scientific/Gibco, Waltham, MA) with 0.2% Triton X-100 was used to block. Epithelia were incubated with an acetylated α-tubulin antibody diluted 1:200 (Cell Signal D20G3 K40, Danvers, MA), before incubating with the secondary antibody Alexa Fluor 568 diluted 1:600 (Invitrogen A11036, Carlsbad, CA). A conjugated phalloidin Alexa Fluor 647 (Invitrogen A22287, Carlsbad, CA) was then used at 1:100 dilution. All incubation steps were done at room temperature for 1 hour, and 3 washes for 10 minutes with TBST were performed between each step. Finally, epithelia were mounted on microscope slides in VECTASHIELD with DAPI (Vector Laboratories, Burlingame, CA).

Supplemental Figures

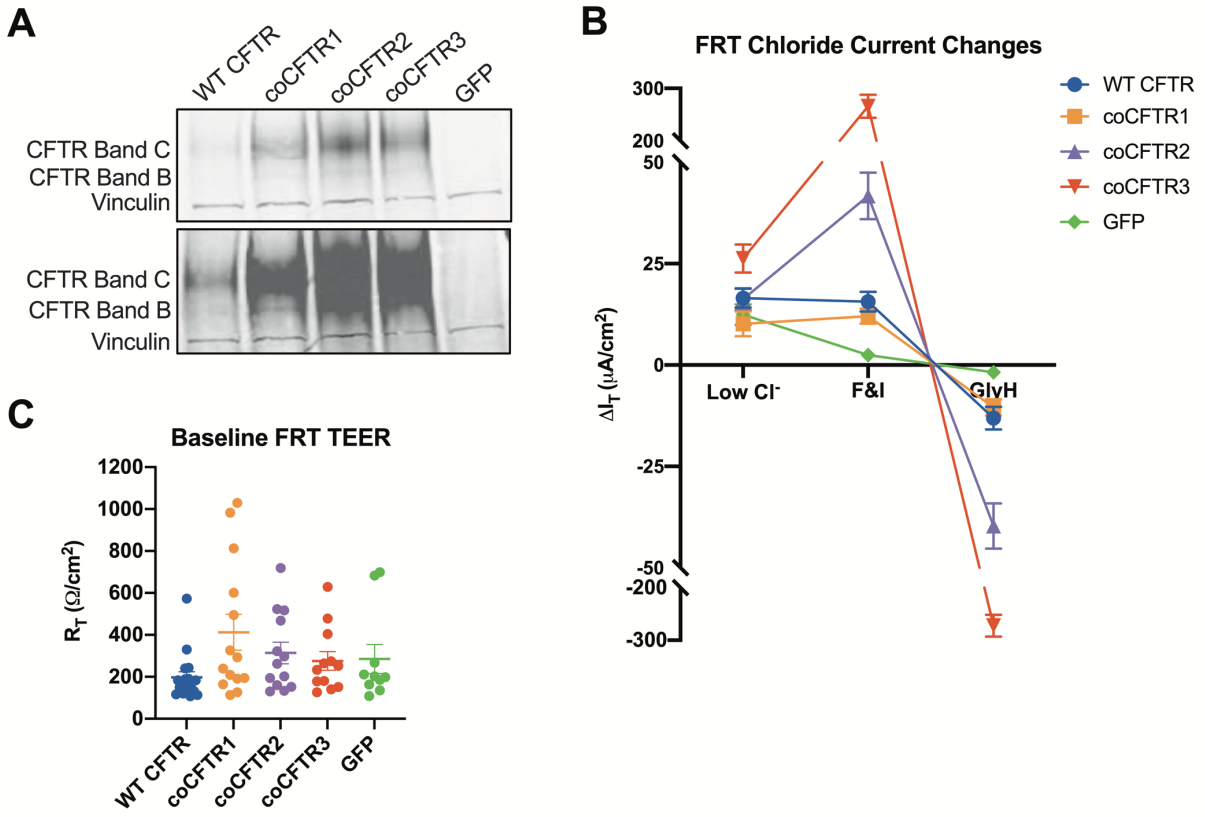

Figure S1. Codon optimized *CFTR* sequences increase protein production and generate unique changes in transepithelial chloride current in Fischer rat thyroid cells. Fischer rat thyroid (FRT) cells were transfected with pcDNA3.1(+) plasmids expressing wildtype (WT) *CFTR*, codon optimized (co) co*CFTR*1, co*CFTR*2, co*CFTR*3, or a GFP control. (A) Three days post transfection, cell lysate was collected and CFTR was quantified by western blot. The same representative blot is shown with normal exposure (top) and with overexposure of the CFTR channel (bottom) to visualize WT CFTR. Similarly, FRT cells were electroporated with the same plasmids, seeded on semipermeable membranes and allowed to form an epithelial layer under air-liquid interface culture conditions. (B) Epithelia were mounted in Ussing chambers and changes in transepithelial Cl^- current (ΔI_T) in response to an apical low Cl^- gradient, CFTR activation by forskolin and 3-isobutyl-1-methylxanthine (F&I), and CFTR inhibition by GlyH were calculated. (C) The baseline transepithelial electrical resistance (TEER) was quantified. No significant differences in TEER were observed between any of the treatment groups. Mean ± SE are shown.

Figure S2

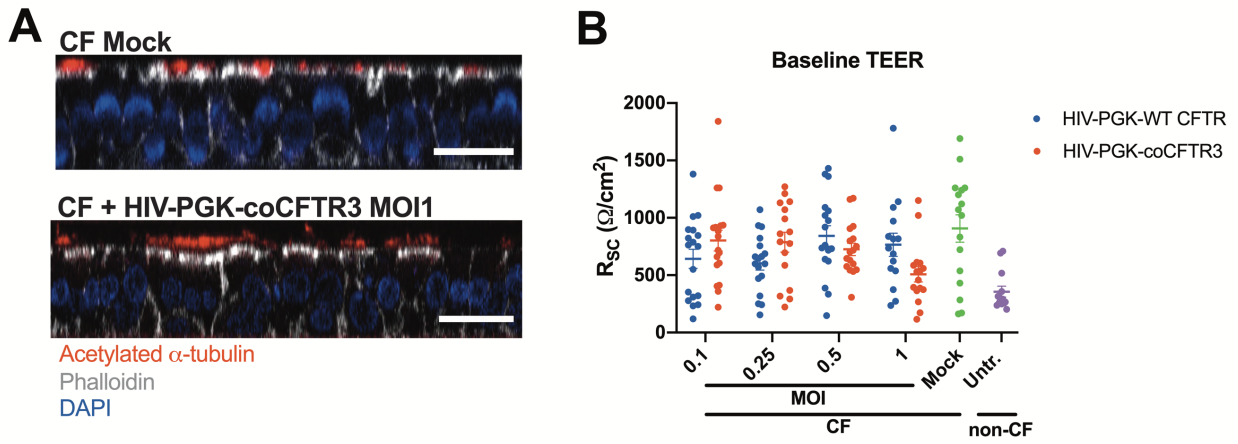

Figure S2. Formation of a differentiated epithelium is not affected by exogenous CFTR expression from a lentiviral vector. CF basal cells were transduced with HIV-PGK-WT CFTR or HIV-PGK-coCFTR3 at MOI 0.1, 0.25, 0.5 or 1 at the time of seeding on semipermeable membranes. (A) After four weeks of differentiation under air-liquid interface culture conditions, pseudostratified ciliated columnar epithelia were observed. Scale bars represent 20 μm. (B) The baseline transepithelial electrical resistance (TEER) of epithelia studied in Ussing chambers was quantified and no significant differences were observed between any of the treatment groups. Mean ± SE are shown.