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Canadian Intellectual Property Office

CA 3137015 A1 2020/10/22

(21) 3 137 015

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2020/04/15
(87) Date publication PCT/PCT Publication Date: 2020/10/22
(85) Entrée phase nationale/National Entry: 2021/10/14
(86) N° demande PCT/PCT Application No.: US 2020/028264
(87) N° publication PCT/PCT Publication No.: 2020/214668
(30) Priorités/Priorities: 2019/04/15 (US62/833,972); 2019/10/25 (US62/926,308); 2020/01/29 (US62/967,214)

(51) Cl.Int./Int.Cl. *A61K 48/00* (2006.01), *C12N 15/86* (2006.01)

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(54) Titre : COMPOSITIONS ET METHODES DE TRAITEMENT DE LA FIBROSE KYSTIQUE
(54) Title: COMPOSITIONS AND METHODS FOR TREATMENT OF CYSTIC FIBROSIS

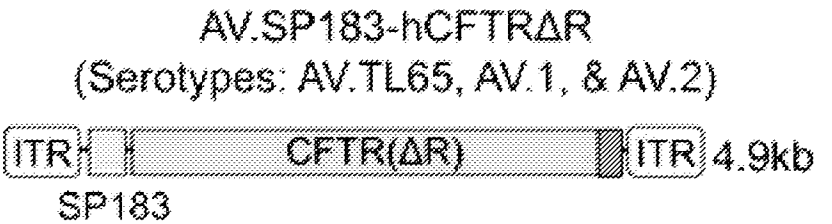

FIG 1A

(57) Abrégé/Abstract:
Provided herein are polynucleotides, rAAV vectors, pharmaceutical compositions, and methods of making and using the same, e.g., for treatment of cystic fibrosis (CF). For example, the disclosure provides a recombinant adeno-associated virus (rAAV) that includes, in one embodiment, an AV.TL65 capsid protein and a polynucleotide that includes an F5 enhancer and a tg83 promoter operably linked to a CFTRΔR minigene, pharmaceutical compositions thereof, and methods of use thereof, e.g., for treatment of CF.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 October 2020 (22.10.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/214668 A1

(51) International Patent Classification:
A61K 48/00 (2006.01) *C12N 15/86* (2006.01)

(21) International Application Number:
PCT/US2020/028264

(22) International Filing Date:
15 April 2020 (15.04.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/833,972	15 April 2019 (15.04.2019)	US
62/926,308	25 October 2019 (25.10.2019)	US
62/967,214	29 January 2020 (29.01.2020)	US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

(54) **Title:** COMPOSITIONS AND METHODS FOR TREATMENT OF CYSTIC FIBROSIS

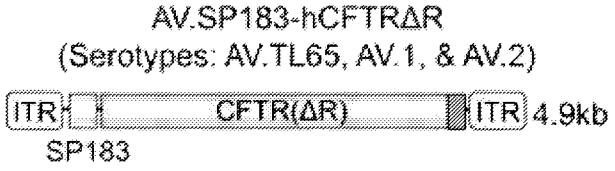

FIG 1A

(57) **Abstract:** Provided herein are polynucleotides, rAAV vectors, pharmaceutical compositions, and methods of making and using the same, e.g., for treatment of cystic fibrosis (CF). For example, the disclosure provides a recombinant adeno-associated virus (rAAV) that includes, in one embodiment, an AV.TL65 capsid protein and a polynucleotide that includes an F5 enhancer and a tg83 promoter operably linked to a CFTRΔR minigene, pharmaceutical compositions thereof, and methods of use thereof, e.g., for treatment of CF.

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COMPOSITIONS AND METHODS FOR TREATMENT OF CYSTIC FIBROSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing date of U.S. application No. 62/833,972, filed on April 15, 2019, U.S. application No. 62/926,308, filed on October 25, 2019, and U.S. application No. 62/967,214, filed on January 29, 2020, the disclosures of which are incorporated by reference herein.

BACKGROUND

Gene therapy using adeno-associated virus (AAV) is an emerging treatment modality, including for treatment of single-gene defects. Cystic fibrosis (CF) is a lethal, autosomal-recessive disorder that affects at least 30,000 people in the U.S. alone, and at least 70,000 people worldwide. The average survival age for CF patients is about 40 years. CF is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a channel that conducts chloride and bicarbonate ions across epithelial cell membranes. Impaired CFTR function leads to inflammation of the airways and progressive bronchiectasis. Because of the single-gene etiology of CF and the various CFTR mutations in the patient population, gene therapy potentially provides a universal cure for CF.

Adeno-associated virus (AAV), a member of the human parvovirus family, is a non-pathogenic virus that depends on helper viruses for its replication. For this reason, recombinant AAV (rAAV) vectors are among the most frequently used in gene therapy pre-clinical studies and clinical trials. Indeed, CF lung disease clinical trials with rAAV2 demonstrated both a good safety profile and long persistence of the viral genome in airway tissue (as assessed by biopsy) relative to other gene transfer agents (such as recombinant adenovirus). Nevertheless, gene transfer failed to improve lung function in CF patients because transcription of the rAAV vector-derived CFTR mRNA was not detected.

Therefore, there remains a need in the art for improved compositions and methods for treatment of CF.

SUMMARY

The disclosure provides, *inter alia*, rAAVs, pharmaceutical compositions, isolated polynucleotides, and methods of making and using the same, e.g., for treatment of CF.

In one aspect, the disclosure features a recombinant adeno-associated virus (rAAV) including (i) an AV.TL65 capsid protein; and (ii) a polynucleotide including an F5 enhancer and a tg83 promoter operably linked to a CFTRΔR minigene.

In some embodiments, the AV.TL65 capsid protein includes the amino acid sequence of SEQ ID NO:13 or a variant thereof with at least 80% amino acid sequence identity to SEQ ID NO:13.

In some embodiments, the F5 enhancer includes the polynucleotide sequence of SEQ ID NO:1 or SEQ ID NO:14, or a variant thereof with at least 80% nucleic acid sequence identity to SEQ ID NO:1 or SEQ ID NO:14. In some embodiments, the F5 includes the polynucleotide sequence of SEQ ID NO:1. In other embodiments, the F5 enhancer includes the polynucleotide sequence of SEQ ID NO:14.

In some embodiments, the tg83 promoter includes the polynucleotide sequence of SEQ ID NO:2.

In some embodiments, the CFTRΔR minigene is a human CFTRΔR minigene.

In some embodiments, the human CFTRΔR minigene is encoded by a polynucleotide including the sequence of SEQ ID NO:4.

In some embodiments, the polynucleotide includes, in a 5'-to-3' direction, the F5 enhancer, the tg83 promoter, and the CFTRΔR minigene.

In another aspect, the disclosure features a pharmaceutical composition including any one of the rAAVs described herein and a pharmaceutically acceptable carrier.

In another aspect, the disclosure features a method of treating cystic fibrosis, the method including administering to a subject in need thereof a therapeutically effective amount of the any one of the rAAVs described herein or any one of the pharmaceutical compositions described herein. In some embodiments, the method further includes administering one or more additional therapeutic agents to the subject.

In another aspect, the mammal is a human. In one aspect, the human is a neonate. In one aspect, the human is a juvenile.

In another aspect, the disclosure features an rAAV for use in treating cystic fibrosis in a subject in need thereof, the rAAV including (i) an AV.TL65 capsid protein; and (ii) a polynucleotide including an F5 enhancer and a tg83 promoter operably linked to a CFTRΔR minigene. In some embodiments, the rAAV is for use in combination with one or more additional therapeutic agents.

In some embodiments, the one or more additional therapeutic agents includes an augmenter (e.g., a proteasome modulating agent such as an anthracycline (e.g., doxorubicin, idarubicin, aclarubicin, daunorubicin, epirubicin, valrubicin, or mitoxantrone), a proteasome inhibitor (e.g., bortezomib, carfilzomib, and ixazomib), a tripeptidyl aldehyde (e.g., *N*-acetyl-l-leucyl-l-leucyl-l-norleucine (LLnL)), or a combination thereof), an antibiotic, a mucus thinner, a CFTR modulator, a mucolytic, an immunosuppressive agent, normal saline, hypertonic saline, or a combination thereof. In some embodiments, the augmenter is doxorubicin. In other embodiments, the augmenter is idarubicin. In some embodiments, the one or more additional therapeutic agents includes an immunosuppressive agent (e.g., a corticosteroid (e.g., an inhaled corticosteroid)).

In some embodiments, the administering is by inhalation, nebulization, aerosolization, intranasally, intratracheally, intrabronchially, orally, intravenously, subcutaneously, or intramuscularly.

In some embodiments, the administering is by inhalation, nebulization, aerosolization, intranasally, intratracheally, and/or intrabronchially.

In another aspect, the disclosure features an isolated polynucleotide including the sequence of SEQ ID NO:7.

In some embodiments, the polynucleotide further includes, in the 3' direction, a 3' untranslated region (3'-UTR) including the sequence of SEQ ID NO:5.

In some embodiments, the polynucleotide further includes, in the 3' direction, a synthetic polyadenylation site including the sequence of SEQ ID NO:6.

In some embodiments, the polynucleotide further includes a 5' adeno-associated virus (AAV) inverted terminal repeat (ITR) at the 5' terminus of the polynucleotide and a 3' AAV ITR at the 3' terminus of the polynucleotide. In some embodiments, the 5' AAV ITR comprises the sequence of SEQ ID NO:15 or a variant thereof with at least 80% nucleic acid sequence identity to SEQ ID NO:15. In some embodiments, the 3' AAV ITR comprises the sequence of SEQ ID NO:16 or a variant thereof with at least 80% nucleic acid sequence identity to SEQ ID NO:16.

In some embodiments, the polynucleotide includes the sequence of SEQ ID NO:11 or SEQ ID NO:17, or a variant thereof with at least 80% nucleic acid sequence identity to SEQ ID NO:11 or SEQ ID NO:17. In some embodiments, the polynucleotide includes the sequence of SEQ ID NO:11. In other embodiments, the polynucleotide includes the sequence of SEQ ID NO:17.

In another aspect, the disclosure features an isolated polynucleotide including the sequence of SEQ ID NO:18. In another aspect, the disclosure features a recombinant adeno-associated virus (rAAV) including any one of the polynucleotides described herein (e.g., a polynucleotide including the sequence of SEQ ID NO:7, SEQ ID NO:11, or SEQ ID NO:17).

In some embodiments, the rAAV has a tropism for airway cells.

In some embodiments, the rAAV has a tropism for airway epithelial cells.

In some embodiments, the rAAV has a tropism for lung epithelial cells.

In some embodiments, the rAAV includes an AV.TL65 capsid protein, an AAV1 capsid protein, an AAV2 capsid protein, an AAV5 capsid protein, an AAV6 capsid protein, or an AAV9 capsid protein.

In some embodiments, the rAAV includes an AV.TL65 capsid protein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1C show functional complementation of CFTR-mediated chloride transport in polarized human CF airway epithelium. Fig. 1A shows the rAAV2 viral genome AV.TL65-SP183-hCFTRΔR was packaged into three capsid serotypes

(AV.TL65, AV.1, and AV.2) and used to apically infect polarized human CF ALI cultures from the apical (AV.TL65 and AV.1) or basolateral surface (AV.2). Basolateral infection with AAV2 was used as a positive control since it efficiently infects from the basolateral surface. 2.5 μM doxorubucin and 20μM LLnL were added to the viral inoculum and ALI cultures were infected for 16 h. Virus was then removed and cultures were re-fed in the absence of proteasome inhibitors. Fig. 1B shows Isc tracing of two cultures for each conditions. Arrows mark the addition of IBMX/forskolin (I&F) and CFTR inhibitor (GlyH101). Fig. 1C shows the mean +/-SEM ΔIsc at 12 days post-infection.

FIGS. 2A-2D are a series of graphs showing gene transfer efficiency of AV.TL65-SP183-hCFTRΔR to the ferret trachea and lung. Figs. 2A and 2B show the number of copies of hCFTR and fCFTR mRNA per 500 ng RNA in the trachea (Fig. 2A) and lung (Fig. 2B). Copy number was determined using a standard curve generated from serial dilutions of plasmid CFTR cDNA for each species. Figs. 2C and 2D show the ratio of transgene-derived hCFTR to endogenous fCFTR mRNA in the trachea (Fig. 2C) and lung (Fig. 2D). C1-C3 represent animals in the mock-infected group and A1-A3 represent animals in the AAV-infected group. The average is also shown for the three AAV-infected animals. The dashed line represents endogenous levels of CFTR (ratio = 1). Data depict the mean +/-SEM for N=3 animals in each group.

FIGS. 3A-3D are a series of graphs showing that AV.TL65 effectively transduces the mature ferret airways. Fig. 3A shows the results of TaqMan® RNA-specific PCR (RS-PCR) for human CFTR mRNA and endogenous ferret GAPDH mRNA for vector and mock treated animals. Results show the ratio of hCFTR/fGAPDH mRNA. Fig. 3B shows TaqMan® RS-PCR for endogenous ferret CFTR mRNA and endogenous ferret GAPDH mRNA for vector and mock treated animals. Results show the ratio of fCFTR/fGAPDH mRNA. Fig. 3C shows TaqMan® Q-PCR for the number vector genomes in each sample per 100 ng DNA. Fig. 3D shows the ratio of mRNA copies for hCFTR/fCFTR for each sample. 1 is equal to endogenous levels of CFTR (red dashed line). Lung samples contained on average 3.0+/-0.5 copies of transgene derived hCFTR mRNA per copy of fCFTR mRNA. Trachea and nasal tissue transduction was more variable, but averaged one copy of transgene derived hCFTR/fCFTR mRNA. Results depict the mean +/-SEM for the vector treated animals.

FIG. 4 is a graph showing representative CF traces for the experiment described in Example 5.

FIG. 5 is a series of graphs showing ΔIsc ($\mu A/cm^2$) under the indicated conditions for CF donors or non-CF donors for the experiment described in Example 5. The error bars indicate standard error of the mean (SEM).

FIG. 6 is a series of graphs showing representative I_{eq} traces (37°C) from individual wells of a 24-well Transwell filter plate for the experiment described in Example 6.

FIG. 7 is a series of graphs showing mean CFTR-mediated chloride secretion after forskolin/IBMX stimulation for each condition for the experiment described in Example 6, n=4. The error bars indicate SEM.

FIGS. 8A-8D. *In vitro and in vivo* comparison of rAAV vector performance. (A) CF (F508del/F508del) human polarized ALI airway cultures were infected apically with AV1-SP183-hCFTRΔR or the AV.TL65-SP183-hCFTRΔR (MOI=100,000 DRP/cell) in the presence of augmenter. Short circuit current (Isc) measurements were then performed in Ussing chambers at 12-days post-infection. Shown is the ΔIsc response to forskolin/IBMX and GlyH101 (CFTR inhibitor). Data show the mean ± SD for n=4 transwells from two donors. Non-infected ALI cultures served as baseline controls (n=4 from two donors). (B) After Isc measurements, two transwell inserts from each group were pooled and lysed to quantify the vector-derived hCFTRΔR mRNA copies by reverse transcriptase quantitative-PCR (RT-qPCR), and normalized to human GAPDH mRNA copies. Values were then expressed as a ratio of hCFTRΔR/GAPDH. Data shows mean ± range for n=2. (C) Human and ferret polarized tracheobronchial epithelia at ALI were infected apically with AV.TL65-SP183gLuc at a multiplicity of infection (MOI) of 100,000. DNase-resistant particles (DRP)/cell in the presence of augmenter. Gaussia luciferase activity was measured at 5-days post-infection as relative luminescence units (RLU). Data show the mean ± SD for n=6 transwells from two donors of each species. (D) Three-days-old ferrets or one-month-old ferrets were intratracheally infected with AV.TL65-SP183-hCFTRΔR mixed with augmenter (4×10^{10} DRP per gram body weight). The mock-infected group was inoculated with PBS with augmenter. The tracheae and lungs were then harvested at 11-days post-infection for quantification of vector-derived hCFTRΔR and endogenous fCFTR mRNA copies by RT-qPCR with GAPDH mRNA copy number normalization. The data represents the ratio (hCFTRΔR /fCFTR) of mRNA copies of hCFTRΔR and fCFTRΔR. Data show the mean+/-SD for n=3 animals in each group. ns, not significantly different.

FIGS. 9A-9C. Repeat dosing of AV.TL65 in neonatal ferrets. (A) Study design involving three groups of neonatal ferrets receiving 0-, 1-, or 2-doses of virus at 1×10^{13} DRP/kg via intra-tracheal administration. The ferrets receiving one dose were administered the reporter vector AV.TL65-SP183-gLuc at 4 wks of age, whereas the ferrets receiving two doses were administered AV.TL65-SP183-fCFTRΔR at 1 wk of age and AV.TL65-SP183-gLuc at 4 wks of age. Plasma and BALF samples were collected at the indicated ages. (B) Gaussia luciferase activity in the plasma at the indicated time points post-delivery of AV.TL65-SP183-gLuc. (C) Gaussia luciferase activity in BALF at 14-days post-delivery of AV.TL65-SP183-gLuc. Results show the mean ± SD for n=6 animals per group. The statistical significance was analyzed with one-way ANOVA followed by Tukey's post-test. ns, non-significant. RLU, relative luminescence units.

FIGS. 10A-10C. Repeat dosing of AV.TL65 in juvenile ferrets. (A) Study design involving three groups of juvenile ferrets receiving 0-, 1-, or 2-doses of virus at 1×10^{13} DRP/kg via intra-tracheal administration. The ferrets receiving one dose were

administered the reporter vector AV.TL65-SP183-gLuc at 8 wks of age, whereas the ferrets receiving two doses were administered AV.TL65-SP183-fCFTRΔR at 4 wk of age and AV.TL65-SP183-gLuc at 8 wks of age. Plasma and BALF samples were collected at the indicated ages. (B) Gaussia luciferase activity in the plasma at the indicated time points post-delivery of AV.TL65-SP183-gLuc. (C) Gaussia luciferase activity in BALF at 14-days post-delivery of AV.TL65-SP183-gLuc. Results show the mean ± SD for n=9-10 animals per group. The statistical significance was analyzed with one-way ANOVA followed by Tukey's post-test: $^{**}P<0.01$, $^{****}P<0.0001$. RLU, relative luminescence units.

FIGA. 11A-11D. Titers of AV.TL65 neutralizing antibodies in the BALF and plasma of infected ferrets. (A, B) Neonatal ferrets samples as collected in Figure 9A were evaluated for NAbs in the (A) BALF and (B) plasma using transduction inhibition assay. Serial dilutions of BALF or plasma were incubated with AV.TL65-fLuc prior to infection of A549 cells. The titer of NAbs were calculated the concentration of BALF or plasma (dilution ratio) that resulted 50% inhibition (IC50) of transduction as assessed by firefly luciferase activity. AV.TL65-fLuc only infected cells served as the baseline control and mock-infected cells served as blank. (C, D) Juvenile ferret samples as collected in Figure 10A were evaluated for NAbs in the (C) BALF and (D) plasma using the above described transduction inhibition assays. Results show the mean ± SD for n=6 neonatal animals per group and n=9-10 juvenile animals per group. The statistical significance was analyzed with one-way ANOVA followed by Tukey's post-test: $^{**}P<0.01$, $^{****}P<0.0001$. ns, non-significant.

FIGS. 12A-12B. Development of an ELISA-based assay for quantifying anti-capsid antibody isotypes. Immune plasma was generated from a ferret infected with AV-TL65 to the lung four times at 1-2 months intervals starting at 1 month of age. The naive plasma was derived from a ferret of similar age. ELISA plates were coated with (A) AAV5 or (B) AAV2 and then evaluated for binding of immune and naive ferret plasma. Secondary detections antibodies were against IgG. Results show the mean ± range for two technical replicates on each sample.

FIGS. 13A-13F. Quantification of IgG, IgM, and IgA capsid binding antibodies in the plasma of AV.TL65 infected ferrets. (A-F) Quantification of capsid binding antibodies in the plasma of (A-C) neonatal and (D-F) juvenile ferrets for (A,D) IgG, (B,E) IgM, and (C,F) IgA. Results show the mean+/-SD for n=6 neonatal animals per group and n=9-10 juvenile animals per group. The statistical significance was analyzed with one-way ANOVA followed by Tukey's post test: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$. Unlabeled comparisons between single- and repeat-dose groups were not significantly different.

FIGS. 14A-14F. Quantification of IgG, IgM, and IgA capsid binding antibodies in the BALF of AV.TL65 infected ferrets. (A-F) Quantification of capsid binding antibodies in the BALF of (A-C) neonatal and (D-F) juvenile ferrets for (A,D) IgG, (B,E) IgM, and (C,F) IgA. Results show the mean+/-SD for n=6 neonatal animals per group and n=9-10

juvenile animals per group. The statistical significance was analyzed with one-way ANOVA followed by Tukey's post test: *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Unlabeled comparisons between single- and repeat-dose groups were not significantly different.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE DISCLOSURE

Gene therapy is the only mutation-agnostic approach to treat cystic fibrosis (CF). The present disclosure is based, at least in part, on the discovery that the rAAV vectors described herein (e.g., AV.TL65-SP183-hCFTRΔR) are unexpectedly effective in complementing CFTR-mediated chloride transport in polarized human CF airway epithelium. The rAAV vectors described herein utilize a combination of components to achieve improved functional payload capacity, more effective cell delivery, and more efficient transgene expression relative to existing CF gene therapy approaches. In particular, the rAAVs include a highly functional CFTR minigene (CFTRΔR), a short but highly active 183 bp synthetic promoter (SP183, which includes an F5 enhancer and a tg83 promoter), and an evolved chimeric rAAV vector, AV.TL65, that is highly tropic for the human airway. In one embodiment, the vector is administered to a human. In one aspect, the human is a neonate. In one aspect, the human is a juvenile.

Definitions

The term "AAV" refers to adeno-associated virus, and may be used to refer to the naturally occurring wild-type virus itself or derivatives thereof. The term covers all subtypes, serotypes and pseudotypes, and both naturally occurring and recombinant forms, except where required otherwise. The AAV genome is built of single stranded DNA, and comprises inverted terminal repeats (ITRs) at both ends of the DNA strand, and two open reading frames: *rep* and *cap*, encoding replication and capsid proteins, respectively. A foreign polynucleotide can replace the native *rep* and *cap* genes. AAVs can be made with a variety of different serotype capsids which have varying transduction profiles or, as used herein, "tropism" for different tissue types. As used herein, the term "serotype" refers to an AAV which is identified by and distinguished from other AAVs based on capsid protein reactivity with defined antisera, e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, and AAVrh10. For example, serotype AAV2 is used to refer to an AAV which contains capsid proteins encoded from the cap gene of AAV2 and a genome containing 5' and 3' ITR sequences from the same AAV2 serotype. Pseudotyped AAV as refers to an AAV that contains capsid proteins from one serotype and a viral genome including 5'-3' ITRs of a second serotype. Pseudotyped rAAV would be expected to have cell surface binding properties of the capsid serotype and genetic properties consistent with the ITR serotype. Pseudotyped rAAV are produced using standard techniques described in the art.

The term “about” is used herein to mean a value that is ±10% of the recited value.

As used herein, by “administering” is meant a method of giving a dosage of a composition described herein (e.g., an rAAV or a pharmaceutical composition thereof) to a subject. The compositions utilized in the methods described herein can be administered by any suitable route, including, for example, by inhalation, nebulization, aerosolization, intranasally, intratracheally, intrabronchially, orally, parenterally (e.g., intravenously, subcutaneously, or intramuscularly), orally, nasally, rectally, topically, or buccally. In some embodiments, a composition described herein is administered in aerosolized particles intratracheally and/or intrabronchially using an atomizer sprayer (e.g., with a MADgic® laryngo-tracheal mucosal atomization device). The compositions utilized in the methods described herein can also be administered locally or systemically. The method of administration can vary depending on various factors (e.g., the components of the composition being administered and the severity of the condition being treated).

The term “AV.TL65” refers to an evolved chimeric AAV capsid protein that is highly tropic for the human airway. AV.TL65 is described in Excoffon et al. *Proc. Natl. Acad. Sci. USA* 106(10):3865-3870, 2009, which is incorporated by reference herein in its entirety, and is also known in the art as AAV2.5T. AV.TL65 is a chimera between AAV2 (a.a. 1–128) and AAV5 (a.a. 129–725) with a substitution based on one point mutation (A581T). The amino acid sequence of the AV.TL65 capsid is shown below:

MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFN
GLD
KGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRA
VFQ
AKKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQ
QLQ
IPAQPASSLGADTMSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRT
WVL
PSYNNHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWG
FRP
RSLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDYQLPYVVGNGTEGCLPAF
PPQ
VFTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSS
FAP
SQNLFKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPMGRTQGWN
LGS
GVNRASVSAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPANP
GTT
ATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAPTTGTYNLQEIVPGSVW
MER
DVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTPVPGNITSFSDVPVS
SFI
TQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIG
TRY
LTRPL (SEQ ID NO:13).

A “control element” or “control sequence” is a nucleotide sequence involved in an interaction of molecules that contributes to the functional regulation of a polynucleotide, including replication, duplication, transcription, splicing, translation, or

degradation of the polynucleotide. The regulation may affect the frequency, speed, or specificity of the process, and may be enhancing or inhibitory in nature. Control elements known in the art include, for example, transcriptional regulatory sequences such as promoters and enhancers. A promoter is a DNA region capable under certain conditions of binding RNA polymerase and initiating transcription of a coding region usually located downstream (in the 3' direction) from the promoter. Promoters include AAV promoters, e.g., P5, P19, P40 and AAV ITR promoters, as well as heterologous promoters.

An “expression vector” is a vector comprising a region which encodes a polypeptide of interest, and is used for effecting the expression of the protein in an intended target cell. An expression vector also comprises control elements operatively linked to the encoding region to facilitate expression of the protein in the target. The combination of control elements and a gene or genes to which they are operably linked for expression is sometimes referred to as an “expression cassette,” a large number of which are known and available in the art or can be readily constructed from components that are available in the art.

A “gene” refers to a polynucleotide containing at least one open reading frame that is capable of encoding a particular protein after being transcribed and translated.

The term “gene delivery” refers to the introduction of an exogenous polynucleotide into a cell for gene transfer, and may encompass targeting, binding, uptake, transport, localization, replicon integration and expression.

The term “gene transfer” refers to the introduction of an exogenous polynucleotide into a cell which may encompass targeting, binding, uptake, transport, localization and replicon integration, but is distinct from and does not imply subsequent expression of the gene.

The term “gene expression” or “expression” refers to the process of gene transcription, translation, and post-translational modification.

A “helper virus” for AAV refers to a virus that allows AAV (e.g., wild-type AAV) to be replicated and packaged by a mammalian cell. A variety of such helper viruses for AAV are known in the art, including adenoviruses, herpes viruses and poxviruses such as vaccinia. The adenoviruses encompass a number of different subgroups, although Adenovirus type 5 of subgroup C is most commonly used. Numerous adenoviruses of human, non-human mammalian and avian origin are known and available from depositories such as the ATCC. Viruses of the herpes family include, for example, herpes simplex viruses (HSV) and Epstein-Barr viruses (EBV), as well as cytomegaloviruses (CMV) and pseudorabies viruses (PRV); which are also available from depositories such as ATCC.

A “detectable marker gene” is a gene that allows cells carrying the gene to be specifically detected (e.g., distinguished from cells which do not carry the marker gene). A large variety of such marker genes are known in the art.

A "selectable marker gene" is a gene that allows cells carrying the gene to be specifically selected for or against, in the presence of a corresponding selective agent. By way of illustration, an antibiotic resistance gene can be used as a positive selectable marker gene that allows a host cell to be positively selected for in the presence of the corresponding antibiotic. A variety of positive and negative selectable markers are known in the art, some of which are described below.

"Heterologous" means derived from a genotypically distinct entity from that of the rest of the entity to which it is compared. For example, a polynucleotide introduced by genetic engineering techniques into a different cell type is a heterologous polynucleotide (and, when expressed, can encode a heterologous polypeptide).

"Host cells," "cell lines," "cell cultures," "packaging cell line" and other such terms denote eukaryotic cells, e.g., mammalian cells, such as human cells, useful in the present disclosure. These cells can be used as recipients for recombinant vectors, viruses or other transfer polynucleotides, and include the progeny of the original cell that was transduced. It is understood that the progeny of a single cell may not necessarily be completely identical (in morphology or in genomic complement) to the original parent cell.

An "isolated" plasmid, virus, or other substance refers to a preparation of the substance devoid of at least some of the other components that may also be present where the substance or a similar substance naturally occurs or is initially prepared from. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Enrichment can be measured on an absolute basis, such as weight per volume of solution, or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Increasing enrichments of the embodiments of this disclosure are increasingly more some. Thus, for example, a 2-fold enrichment is some, 10-fold enrichment is more some, 100-fold enrichment is more some, 1000-fold enrichment is even more some.

As used herein, the term "operable linkage" or "operably linked" refers to a physical or functional juxtaposition of the components so described as to permit them to function in their intended manner. More specifically, for example, two DNA sequences operably linked means that the two DNAs are arranged (*cis* or *trans*) in such a relationship that at least one of the DNA sequences is able to exert a physiological effect upon the other sequence. For example, an enhancer and/or a promoter can be operably linked with a transgene (e.g., a therapeutic transgene, such as a CFTRΔR minigene).

"Packaging" as used herein refers to a series of subcellular events that results in the assembly and encapsidation of a viral vector, particularly an AAV vector. Thus, when a suitable vector is introduced into a packaging cell line under appropriate conditions, it can be assembled into a viral particle. Functions associated with packaging of viral vectors, particularly AAV vectors, are described herein and in the art.

The term "polynucleotide" refers to a polymeric form of nucleotides of any length, including deoxyribonucleotides or ribonucleotides, or analogs thereof. A polynucleotide may comprise modified nucleotides, such as methylated or capped nucleotides and nucleotide analogs, and may be interrupted by non-nucleotide components. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The term polynucleotide, as used herein, refers interchangeably to double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of the disclosure described herein that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

The terms "polypeptide" and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, acetylation, phosphorylation, lipidation, or conjugation with a labeling component. Polypeptides such as "CFTR" and the like, when discussed in the context of gene therapy and compositions therefor, refer to the respective intact polypeptide, or any fragment or genetically engineered derivative thereof that retains the desired biochemical function of the intact protein. Similarly, references to CFTR, and other such genes for use in gene therapy (typically referred to as "transgenes" to be delivered to a recipient cell), include polynucleotides encoding the intact polypeptide or any fragment or genetically engineered derivative possessing the desired biochemical function.

By "pharmaceutical composition" is meant any composition that contains a therapeutically or biologically active agent (e.g., a polynucleotide comprising a transgene (e.g., a CFTRΔR minigene; see, e.g., Ostedgaard et al. *Proc. Natl. Acad. Sci. USA* 108(7):2921-6, 2011)), either incorporated into a viral vector (e.g., an rAAV vector) or independent of a viral vector (e.g., incorporated into a liposome, microparticle, or nanoparticle)) that is suitable for administration to a subject. Any of these formulations can be prepared by well-known and accepted methods of art. See, for example, Remington: The Science and Practice of Pharmacy (21st ed.), ed. A.R. Gennaro, Lippincott Williams & Wilkins, 2005, and Encyclopedia of Pharmaceutical Technology, ed. J. Swarbrick, Informa Healthcare, 2006, each of which is hereby incorporated by reference.

By "pharmaceutically acceptable diluent, excipient, carrier, or adjuvant" is meant a diluent, excipient, carrier, or adjuvant which is physiologically acceptable to the subject while retaining the therapeutic properties of the pharmaceutical composition with which it is administered.

"Recombinant," as applied to a polynucleotide means that the polynucleotide is the product of various combinations of cloning, restriction and/or ligation steps, and other procedures that result in a construct that is distinct from a polynucleotide found in nature. A recombinant virus is a viral particle comprising a recombinant polynucleotide.

The terms respectively include replicates of the original polynucleotide construct and progeny of the original virus construct.

By "recombinant adeno-associated virus (AAV)" or "rAAV vector" is meant a recombinantly-produced AAV or AAV particle that comprises a polynucleotide sequence not of AAV origin (e.g., a polynucleotide comprising a transgene, which may be operably linked to one or more enhancer and/or promoters) to be delivered into a cell, either *in vivo*, *ex vivo,* or *in vitro*. The rAAV may use naturally occurring capsid proteins from any AAV serotype. In some embodiments, non-naturally occurring (e.g., chimeric) capsids may be used in the rAAVs described herein, e.g., AV.TL65.

By "reference" is meant any sample, standard, or level that is used for comparison purposes. A "normal reference sample" or a "wild-type reference sample" can be, for example, a sample from a subject not having the disorder (e.g., cystic fibrosis). A "positive reference" sample, standard, or value is a sample, standard, value, or number derived from a subject that is known to have a disorder (e.g., cystic fibrosis), which may be matched to a sample of a subject by at least one of the following criteria: age, weight, disease stage, and overall health.

The terms "subject" and "patient" are used interchangeably herein to refer to any mammal (e.g., a human, a primate, a cat, a dog, a ferret, a cow, a horse, a pig, a goat, a rat, or a mouse). For example, the subject is a human.

A "terminator" refers to a polynucleotide sequence that tends to diminish or prevent read-through transcription (i.e., it diminishes or prevent transcription originating on one side of the terminator from continuing through to the other side of the terminator). The degree to which transcription is disrupted is typically a function of the base sequence and/or the length of the terminator sequence. In particular, as is well known in numerous molecular biological systems, particular DNA sequences, generally referred to as "transcriptional termination sequences" are specific sequences that tend to disrupt read-through transcription by RNA polymerase, presumably by causing the RNA polymerase molecule to stop and/or disengage from the DNA being transcribed. Typical example of such sequence-specific terminators include polyadenylation ("polyA") sequences, e.g., SV40 polyA. In addition to or in place of such sequence-specific terminators, insertions of relatively long DNA sequences between a promoter and a coding region also tend to disrupt transcription of the coding region, generally in proportion to the length of the intervening sequence. This effect presumably arises because there is always some tendency for an RNA polymerase molecule to become disengaged from the DNA being transcribed, and increasing the length of the sequence to be traversed before reaching the coding region would generally increase the likelihood that disengagement would occur before transcription of the coding region was completed or possibly even initiated. Terminators may thus prevent transcription from only one direction ("uni-directional" terminators) or from both directions ("bi-directional" terminators), and may be comprised of sequence-specific termination sequences or sequence-non-specific terminators or both. A variety of such terminator sequences are

known in the art; and illustrative uses of such sequences within the context of the present disclosure are provided below.

A "therapeutic gene," "prophylactic gene," "target polynucleotide," "transgene," "gene of interest" and the like generally refer to a gene or genes to be transferred using a vector. Typically, in the context of the present disclosure, such genes are located within the rAAV vector (which vector is flanked by inverted terminal repeat (ITR) regions and thus can be replicated and encapsidated into rAAV particles). Target polynucleotides can be used in this disclosure to generate rAAV vectors for a number of different applications. Such polynucleotides include, but are not limited to: (i) polynucleotides encoding proteins useful in other forms of gene therapy to relieve deficiencies caused by missing, defective or sub-optimal levels of a structural protein or enzyme; (ii) polynucleotides that are transcribed into anti-sense molecules; (iii) polynucleotides that are transcribed into decoys that bind transcription or translation factors; (iv) polynucleotides that encode cellular modulators such as cytokines; (v) polynucleotides that can make recipient cells susceptible to specific drugs, such as the herpes virus thymidine kinase gene; (vi) polynucleotides for cancer therapy, such as E1A tumor suppressor genes or p53 tumor suppressor genes for the treatment of various cancers; and (vii) polynucleotides for gene editing (e.g., CRISPR). To effect expression of the transgene in a recipient host cell, it is in one embodiment operably linked to a promoter, either its own or a heterologous promoter. A large number of suitable promoters are known in the art, the choice of which depends on the desired level of expression of the target polynucleotide; whether one desires constitutive expression, inducible expression, cell-specific or tissue-specific expression, etc. The rAAV vector may also contain a selectable marker. Exemplary transgenes include, without limitation, cystic fibrosis transmembrane conductance regulator (CFTR) or derivatives thereof (e.g., a CFTRΔR minigene; see, e.g., Ostedgaard et al. *Proc. Natl. Acad. Sci. USA* 108(7):2921-6, 2011, which is incorporated by reference herein in its entirety), α-antitrypsin, β-globin, γ-globin, tyrosine hydroxylase, glucocerebrosidase, aryl sulfatase A, factor VIII, dystrophin, erythropoietin, alpha 1-antitrypsin, surfactant protein SP-D, SP-A or SP-C, erythropoietin, or a cytokine, e.g., IFN-alpha, IFNγ, TNF, IL-1, IL-17, or IL-6, or a prophylactic protein that is an antigen such as viral, bacterial, tumor or fungal antigen, or a neutralizing antibody or a fragment thereof that targets an epitope of an antigen such as one from a human respiratory virus, e.g., influenza virus or RSV including but not limited to HBoV protein, influenza virus protein, RSV protein, or SARS protein.

By "therapeutically effective amount" is meant the amount of a composition administered to improve, inhibit, or ameliorate a condition of a subject, or a symptom of a disorder or disease, e.g., cystic fibrosis, in a clinically relevant manner. Any improvement in the subject is considered sufficient to achieve treatment. In one embodiment, an amount sufficient to treat is an amount that reduces, inhibits, or prevents the occurrence or one or more symptoms of cystic fibrosis or is an amount that

reduces the severity of, or the length of time during which a subject suffers from, one or more symptoms of cystic fibrosis (e.g., by at least about 10%, about 20%, or about 30%, or by at least about 50%, about 60%, or about 70%, or by at least about 80%, about 90%, about 95%, about 99%, or more, relative to a control subject that is not treated with a composition described herein). An effective amount of the pharmaceutical composition used to practice the methods described herein (e.g., the treatment of cystic fibrosis) varies depending upon the manner of administration and the age, body weight, and general health of the subject being treated. A physician or researcher can decide the appropriate amount and dosage regimen.

"Transduction" or "transducing" as used herein, are terms referring to a process for the introduction of an exogenous polynucleotide, e.g., a transgene in rAAV, into a host cell leading to expression of the polynucleotide, e.g., the transgene in the cell. The process generally includes 1) endocytosis of the AAV after it has bound to a cell surface receptor, 2) escape from endosomes or other intracellular compartments in the cytosol of a cell, 3) trafficking of the viral particle or viral genome to the nucleus, 4) uncoating of the virus particles, and generation of expressible double stranded AAV genome forms, including circular intermediates. The rAAV expressible double stranded form may persist as a nuclear episome or optionally may integrate into the host genome. The alteration of any or a combination of endocytosis of the AAV after it has bound to a cell surface receptor, escape from endosomes or other intracellular compartments to the cytosol of a cell, trafficking of the viral particle or viral genome to the nucleus, or uncoating of the virus particles, and generation of expressive double stranded AAV genome forms, including circular intermediates, may result in altered expression levels or persistence of expression, or altered trafficking to the nucleus, or altered types or relative numbers of host cells or a population of cells expressing the introduced polynucleotide. Altered expression or persistence of a polynucleotide introduced via rAAV can be determined by methods well known to the art including, but not limited to, protein expression, e.g., by ELISA, flow cytometry and Western blot, measurement of DNA and RNA production by hybridization assays, e.g., Northern blots, Southern blots and gel shift mobility assays, or quantitative or non-quantitative reverse transcription, polymerase chain reaction (PCR), or digital droplet PCR assays.

"Treatment" of an individual or a cell is any type of intervention in an attempt to alter the natural course of the individual or cell at the time the treatment is initiated, e.g., eliciting a prophylactic, curative or other beneficial effect in the individual. For example, treatment of an individual may be undertaken to decrease or limit the pathology caused by any pathological condition, including (but not limited to) an inherited or induced genetic deficiency (e.g., cystic fibrosis), infection by a viral, bacterial, or parasitic organism, a neoplastic or aplastic condition, or an immune system dysfunction such as autoimmunity or immunosuppression. Treatment includes (but is not limited to) administration of a composition, such as a pharmaceutical composition, and administration of compatible cells that have been treated with a composition. Treatment

may be performed either prophylactically or therapeutically; that is, either prior or subsequent to the initiation of a pathologic event or contact with an etiologic agent. Treatment may reduce one or more symptoms of a pathological condition. For example, symptoms of cystic fibrosis are known in the art and include, e.g., persistent cough, wheezing, breathlessness, exercise intolerance, repeated lung infections, inflamed nasal passages or stuffy nose, foul-smelling or greasy stools, poor weight gain and growth, intestinal blockage, constipation, elevated salt concentrations in sweat, pancreatitis, and pneumonia. Detecting an improvement in, or the absence of, one or more symptoms of a disorder (e.g., cystic fibrosis), indicates successful treatment.

A “variant” refers to a polynucleotide or a polypeptide that is substantially homologous to a native or reference polynucleotide or polypeptide. For example, a variant polynucleotide may be substantially homologous to a native or reference polynucleotide, but which has a polynucleotide sequence different from that of the native or reference polynucleotide because of one or a plurality of deletions, insertions, and/or substitutions. In another example, a variant polypeptide may be substantially homologous to a native or reference polypeptide, but which has an amino acid sequence different from that of the native or reference polypeptide because of one or a plurality of deletions, insertions, and/or substitutions. Variant polypeptide-encoding polynucleotide sequences encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to a native or reference polynucleotide sequence, but that encode a variant protein or fragment thereof that retains activity. A wide variety of mutagenesis approaches are known in the art and can be applied by a person of ordinary skill in the art.

A variant polynucleotide or polypeptide sequence can be at least 80%, at least 85%, at least at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more, identical to a native or reference sequence. The degree of homology (percent identity) between a native and a variant sequence can be determined, for example, by comparing the two sequences using freely available computer programs commonly employed for this purpose on the world wide web (e.g., BLASTp or BLASTn with default settings).

A “vector” as used herein refers to a macromolecule or association of macromolecules that comprises or associates with a polynucleotide and which can be used to mediate delivery of the polynucleotide to a cell, either *in vitro* or *in vivo*. Illustrative vectors include, for example, plasmids, viral vectors, liposomes and other gene delivery vehicles. The polynucleotide to be delivered, sometimes referred to as a transgene, may comprise a coding sequence of interest in gene therapy (such as a gene encoding a protein of therapeutic or interest), a coding sequence of interest in vaccine development (such as a polynucleotide expressing a protein, polypeptide or peptide suitable for eliciting an immune response in a mammal), and/or a selectable or detectable marker.

Polynucleotides

The disclosure provides polynucleotides which may be incorporated into rAAV vectors, or used in the preparation of rAAV vectors. The polynucleotide may include any suitable elements or components, including one or more elements selected from a 5' AAV ITR (e.g., an AAV2 5' ITR), an F5 enhancer, a tg83 promoter, a 5' untranslated region (UTR), a CFTRΔR minigene, a '3 UTR, a polyadenylation site, and/or a 3' AAV ITR (e.g., an AAV2 3' ITR). Although the polynucleotides are generally incorporated into rAAV vectors, it is to be understood that they could be delivered or administered in the context of other types of vectors that are known in the art.

In one aspect, the disclosure provides an isolated polynucleotide that includes the sequence of SEQ ID NO:7, or a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:7. In some embodiments, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:1, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4. In another some embodiment, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:14, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4.

In some embodiments, the polynucleotide further comprises, in the 3' direction, a 3' untranslated region (3'-UTR) comprising the sequence of SEQ ID NO:5, or a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:5.

In some embodiments, the polynucleotide further comprises, in the 3' direction (e.g., 3' relative to the 3'-UTR), a synthetic polyadenylation site comprising the sequence of SEQ ID NO:6.

In some embodiments, the polynucleotide further comprises a 5' adeno-associated virus (AAV) inverted terminal repeat (ITR) at the 5' terminus of the polynucleotide and/or a 3' AAV ITR at the 3' terminus of the polynucleotide. In some embodiments, the polynucleotide comprises the sequence of SEQ ID NO:11, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:11. In some embodiments, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:1, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4. In another some embodiment, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:14, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4.

In other embodiments, the polynucleotide comprises the sequence of SEQ ID NO:17, or a variant thereof, e.g.,sequence having at least at least 80%, 85%, 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:17. In some embodiments, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:1, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4. In another some embodiment, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:14, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4.

Any of the polynucleotides may contain a 5' AAV ITR. Any suitable 5' AAV ITR may be used, including a 5' AAV ITR from any AAV serotype (e.g., AAV2). In some embodiments, the 5' AAV ITR comprises the sequence of SEQ ID NO:9, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:9. In another example, in some embodiments, the polynucleotide includes a 5' AAV ITR comprising the sequence of SEQ ID NO:15, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:15. Any of the polynucleotides may contain a 3' AAV ITR. Any suitable 3' AAV ITR may be used, including a 3' AAV ITR from any AAV serotype (e.g., AAV2). In some embodiments, the 3' AAV ITR comprises the sequence of SEQ ID NO:10, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:10. In another example, in some embodiments, the polynucleotide includes a 3' AAV ITR comprising the sequence of SEQ ID NO:16, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:16. The ITR sequences may be palindromic, e.g., as in SEQ ID NO:15 and SEQ ID NO:16, where the ITR sequence on the 5' end is located on the reverse strand, and the ITR sequence on the 3' end is located on the forward strand.

Any of the polynucleotides may contain an F5 enhancer. See, e.g., U.S. Patent Application No. 16/082,767, which is incorporated herein by reference in its entirety. In some embodiments, the F5 enhancer comprises the sequence of SEQ ID NO:1 or SEQ ID NO:14, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:1 or SEQ ID NO:14. In some embodiments, the F5 includes the polynucleotide sequence of SEQ ID NO:1. In other embodiments, the F5 enhancer includes the polynucleotide sequence of SEQ ID NO:14.

Any of the polynucleotides may contain a tg83 promoter. See, e.g., U.S. Patent Application No. 16/082,767. In some embodiments, the tg83 promoter comprises the sequence of SEQ ID NO:2, or a variant thereof, e.g., a sequence having at least at least

80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:2.

Any of the polynucleotides may contain a 5'-UTR. Any suitable 5'-UTR may be used. In some embodiments, the 5'-UTR comprises the sequence of SEQ ID NO:3, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:3.

Any of the polynucleotides may contain a sequence encoding a CFTRΔR minigene. Any suitable CFTRΔR minigene may be used, including human CFTRΔR (hCFTRΔR) or ferret CFTRΔR. In some embodiments, the sequence encoding an hCFTRΔR minigene comprises the sequence of SEQ ID NO:4, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:4.

Any of the polynucleotides may contain a 3'-UTR. Any suitable 3'-UTR may be used. In some embodiments, the 3'-UTR comprises the sequence of SEQ ID NO:3, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:5.

Any of the polynucleotides may contain a polyadenylation site. Any suitable polyadenylation site may be used. In some embodiments, the polyadenylation site comprises the sequence of SEQ ID NO:6, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:6.

In one aspect, the disclosure provides an isolated polynucleotide that includes the sequence of SEQ ID NO:8, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:8. In some embodiments, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:1, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4. In another some embodiment, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:14, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4.

In one aspect, the disclosure provides an isolated polynucleotide that includes the sequence of SEQ ID NO:11, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:11. In some embodiments, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:1, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4. In another some embodiment, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:14, a

tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4.

In one aspect, the disclosure provides an isolated polynucleotide that includes the sequence of SEQ ID NO:12, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:12. In some embodiments, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:1, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4. In another some embodiment, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:14, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4.

In another aspect, the disclosure provides an isolated polynucleotide that includes the sequence of SEQ ID NO:18, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:18. In some embodiments, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:1, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4. In another some embodiment, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:14, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4.

The polynucleotide may also contain one or more detectable markers. A variety of such markers are known, including, by way of illustration, the bacterial beta-galactosidase (lacZ) gene; the human placental alkaline phosphatase (AP) gene and genes encoding various cellular surface markers which have been used as reporter molecules both *in vitro* and *in vivo*. The polynucleotide may also contain one or more selectable markers.

Recombinant AAV Vectors

Recombinant AAV vectors are potentially powerful tools for human gene therapy, particularly for diseases such as cystic fibrosis. A major advantage of rAAV vectors over other approaches to gene therapy is that they generally do not require ongoing replication of the target cell in order to exist episomally or become stably integrated into the host cell. In general, the disclosure provides an rAAV that includes an AV.TL65 capsid protein and a polynucleotide that includes an F5 enhancer and a tg83 promoter operably linked to a transgene.

For example, in one aspect, the disclosure provides an rAAV that includes (i) an AV.TL65 capsid protein; and (ii) a polynucleotide including an F5 enhancer and a tg83 promoter operably linked to a CFTRΔR minigene.

In another aspect, the disclosure provides an rAAV for use in treating cystic fibrosis in a subject in need thereof, the rAAV including (i) an AV.TL65 capsid protein; and (ii) a polynucleotide including an F5 enhancer and a tg83 promoter operably linked to a CFTRΔR minigene.

In some embodiments, the AV.TL65 capsid protein includes the amino acid sequence of SEQ ID NO:13, or a variant thereof, e.g., an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the amino acid sequence of SEQ ID NO:13.

In some embodiments, the F5 enhancer includes the polynucleotide sequence of SEQ ID NO:1 or SEQ ID NO:14, or a variant thereof, e.g., a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:1 or SEQ ID NO:14. In some embodiments, the F5 includes the polynucleotide sequence of SEQ ID NO:1. In other embodiments, the F5 enhancer includes the polynucleotide sequence of SEQ ID NO:14.

In some embodiments, the tg83 promoter includes the polynucleotide sequence of SEQ ID NO:2, or a variant thereof, e.g., a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:2.

Any suitable CFTRΔR minigene or a derivative thereof may be used. In some embodiments, the CFTRΔR minigene is a human CFTRΔR minigene. In other embodiments, the CFTRΔR minigene is a ferret CFTRΔR minigene. In some embodiments, the human CFTRΔR minigene is encoded by a polynucleotide including the sequence of SEQ ID NO:4, or a variant thereof, e.g., a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:4.

In some embodiments, the polynucleotide includes, in a 5'-to-3' direction, the F5 enhancer, the tg83 promoter, and the CFTRΔR minigene. In some particular embodiments, the polynucleotide comprises, in a 5'-to-3' direction, a 5' AAV ITR (e.g., an AAV2 5' ITR), the F5 enhancer, the tg83 promoter, a 5' untranslated region (UTR), the CFTRΔR minigene, a '3-UTR, a polyadenylation site, and a 3' AAV ITR (e.g., an AAV2 3' ITR).

In another aspect, the disclosure provides an rAAV comprising any of the polynucleotides described herein, e.g., a polynucleotide comprising the sequence of SEQ ID NO:7, SEQ ID NO:11, or SEQ ID NO:17, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:7, SEQ ID NO:11, or SEQ ID NO:17. For example, the disclosure provides an rAAV comprising a polynucleotide comprising the sequence of SEQ ID NO:17, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:17. In some embodiments, the rAAV has a tropism for airway epithelial cells (e.g., lung

epithelial cells). In some embodiments, the rAAV comprises an AV.TL65 capsid protein, an AAV1 capsid protein, an AAV2 capsid protein, an AAV5 capsid protein, an AAV6 capsid protein, or an AAV9 capsid protein. In some embodiments, the rAAV comprises an AV.TL65 capsid protein. In some embodiments, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:1, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4. In another some embodiment, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:14, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4.

The heterologous polynucleotide is integrated by recombinant techniques into or in place of the AAV genomic coding region (i.e., in place of the AAV rep and cap genes), but is generally flanked on either side by AAV inverted terminal repeat (ITR) regions. This means that an ITR appears both upstream and downstream from the coding sequence, either in direct juxtaposition, e.g., (although not necessarily) without any intervening sequence of AAV origin in order to reduce the likelihood of recombination that might regenerate a replication-competent AAV genome. However, a single ITR may be sufficient to carry out the functions normally associated with configurations comprising two ITRs (see, for example, WO 94/13788), and vector constructs with only one ITR can thus be employed in conjunction with the packaging and production methods of the present disclosure.

The native promoters for rep are self-regulating, and can limit the amount of AAV particles produced. The rep gene can also be operably linked to a heterologous promoter, whether rep is provided as part of the vector construct, or separately. Any heterologous promoter that is not strongly down-regulated by rep gene expression is suitable; but inducible promoters are some because constitutive expression of the rep gene can have a negative impact on the host cell. A large variety of inducible promoters are known in the art; including, by way of illustration, heavy metal ion inducible promoters (such as metallothionein promoters); steroid hormone inducible promoters (such as the MMTV promoter or growth hormone promoters); and promoters such as those from T7 phage which are active in the presence of T7 RNA polymerase. One sub-class of inducible promoters are those that are induced by the helper virus that is used to complement the replication and packaging of the rAAV vector. A number of helper-virus-inducible promoters have also been described, including the adenovirus early gene promoter which is inducible by adenovirus E1A protein; the adenovirus major late promoter; the herpesvirus promoter which is inducible by herpesvirus proteins such as VP16 or 1CP4; as well as vaccinia or poxvirus inducible promoters.

Given the relative encapsidation size limits of various AAV genomes, insertion of a large heterologous polynucleotide into the genome necessitates removal of a portion of the AAV sequence. Removal of one or more AAV genes is in any case desirable, to reduce the likelihood of generating replication-competent AAV (“RCA”).

Accordingly, encoding or promoter sequences for rep, cap, or both, are in one embodiment removed, since the functions provided by these genes can be provided in *trans*.

The resultant vector is referred to as being "defective" in these functions. In order to replicate and package the vector, the missing functions are complemented with a packaging gene, or a plurality thereof, which together encode the necessary functions for the various missing rep and/or cap gene products. The packaging genes or gene cassettes are in one embodiment not flanked by AAV ITRs and in one embodiment do not share any substantial homology with the rAAV genome. Thus, in order to minimize homologous recombination during replication between the vector sequence and separately provided packaging genes, it is desirable to avoid overlap of the two polynucleotide sequences. The level of homology and corresponding frequency of recombination increase with increasing length of homologous sequences and with their level of shared identity. The level of homology that will pose a concern in a given system can be determined theoretically and confirmed experimentally, as is known in the art. Typically, however, recombination can be substantially reduced or eliminated if the overlapping sequence is less than about a 25 nucleotide sequence if it is at least 80% identical over its entire length, or less than about a 50 nucleotide sequence if it is at least 70% identical over its entire length. Of course, even lower levels of homology further reduce the likelihood of recombination. It appears that, even without any overlapping homology, there is some residual frequency of generating RCA. Even further reductions in the frequency of generating RCA (e.g., by nonhomologous recombination) can be obtained by "splitting" the replication and encapsidation functions of AAV, as described by Allen et al., WO 98/27204).

The rAAV vector construct, and the complementary packaging gene constructs can be implemented in this disclosure in a number of different forms. Viral particles, plasmids, and stably transformed host cells can all be used to introduce such constructs into the packaging cell, either transiently or stably.

In certain embodiments of this disclosure, the AAV vector and complementary packaging gene(s), if any, are provided in the form of bacterial plasmids, AAV particles, or any combination thereof. In other embodiments, either the AAV vector sequence, the packaging gene(s), or both, are provided in the form of genetically altered (e.g., inheritably altered) eukaryotic cells. The development of host cells inheritably altered to express the AAV vector sequence, AAV packaging genes, or both, provides an established source of the material that is expressed at a reliable level.

A variety of different genetically altered cells can thus be used in the context of this disclosure. By way of illustration, a mammalian host cell may be used with at least one intact copy of a stably integrated rAAV vector. An AAV packaging plasmid comprising at least an AAV rep gene operably linked to a promoter can be used to supply replication functions (as described in U.S. Pat. No. 5,658,776). Alternatively, a stable mammalian cell line with an AAV rep gene operably linked to a promoter can be

used to supply replication functions (see, e.g., Trempe et al., (WO 95/13392); Burstein et al. (WO 98/23018); and Johnson et al. (U.S. Pat. No. 5,656,785)). The AAV cap gene, providing the encapsidation proteins as described above, can be provided together with an AAV rep gene or separately (see, e.g., the above-referenced applications and patents as well as Allen et al. (WO 98/27204). Other combinations are possible and included within the scope of this disclosure.

Approaches for producing rAAVs, e.g., rAAVs that contain AV.TL65 capsid proteins are known in the art. See, e.g., Excoffon et al. *Proc. Natl. Acad. Sci. USA* 106(10):3865-3870, 2009 and U.S. Patent No. 10,046,016, each of which is incorporated herein by reference in its entirety.

Augmenters

The rAAVs described herein can be used in combination with augmenters of AAV transduction to achieve significant increases in transduction and/or expression of transgenes. Any suitable augmenter can be used. For example, U.S. Patent No. 7,749,491, which is incorporated by reference herein in its entirety, describes suitable augmenters. The augmenter may be a proteasome modulating agent. The proteasome modulating agent may be an anthracycline (e.g., doxorubicin, idarubicin, aclarubicin, daunorubicin, epirubicin, valrubicin, or mitoxantrone), a proteasome inhibitor (e.g., bortezomib, carfilzomib, and ixazomib), a tripeptidyl aldehyde (e.g., *N*-acetyl-l-leucyl-l-leucyl-l-norleucine (LLnL)), or a combination thereof. In some embodiments, the augmenter is doxorubicin. In other embodiments, the augmenter is idarubicin.

The rAAV and the augmenter(s) may be contacted with a cell, or administered to a subject, in the same composition or in different compositions (e.g., pharmaceutical compositions). The contacting or the administration of the rAAV and the augmenter(s) may be sequential (e.g., rAAV followed by the augmenter(s), or vice versa) or simultaneous.

Pharmaceutical Compositions

The disclosure provides pharmaceutical compositions, including pharmaceutical compositions that include any of the rAAVs described herein. The pharmaceutical carrier may include one or more pharmaceutically acceptable carriers, excipients, diluents, buffers, and the like.

For example, in one aspect, the disclosure provides a pharmaceutical composition that includes an rAAV, the rAAV including (i) an AV.TL65 capsid protein; and (ii) a polynucleotide including an F5 enhancer and a tg83 promoter operably linked to a CFTRΔR minigene.

In another aspect, the disclosure provides a pharmaceutical composition comprising an rAAV for use in treating cystic fibrosis in a subject in need thereof, the rAAV including (i) an AV.TL65 capsid protein; and (ii) a polynucleotide including an F5 enhancer and a tg83 promoter operably linked to a CFTRΔR minigene.

In some embodiments, the AV.TL65 capsid protein includes the amino acid sequence of SEQ ID NO:13, or a variant thereof, e.g., an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the amino acid sequence of SEQ ID NO:13.

In some embodiments, the F5 enhancer includes the polynucleotide sequence of SEQ ID NO:1 or SEQ ID NO:14, or a variant thereof, e.g., a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:1 or SEQ ID NO:14. In some embodiments, the F5 includes the polynucleotide sequence of SEQ ID NO:1. In other embodiments, the F5 enhancer includes the polynucleotide sequence of SEQ ID NO:14.

In some embodiments, the tg83 promoter includes the polynucleotide sequence of SEQ ID NO:2, or a variant thereof, e.g., a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:2.

Any suitable CFTRΔR minigene or a derivative thereof may be used. In some embodiments, the CFTRΔR minigene is a human CFTRΔR minigene. In other embodiments, the CFTRΔR minigene is a ferret CFTRΔR minigene. In some embodiments, the human CFTRΔR minigene is encoded by a polynucleotide including the sequence of SEQ ID NO:4, or a variant thereof, e.g., a sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:4.

In some embodiments, the polynucleotide includes, in a 5'-to-3' direction, the F5 enhancer, the tg83 promoter, and the CFTRΔR minigene. In some particular embodiments, the polynucleotide comprises, in a 5'-to-3' direction, a 5' AAV ITR (e.g., an AAV2 5' ITR), the F5 enhancer, the tg83 promoter, a 5' untranslated region (UTR), the CFTRΔR minigene, a 3'-UTR, a polyadenylation site, and a 3' AAV ITR (e.g., an AAV2 3' ITR).

In another aspect, the disclosure provides a pharmaceutical composition comprising an rAAV, the rAAV comprising any of the polynucleotides described herein, e.g., a polynucleotide comprising the sequence of SEQ ID NO:7, 11, or 17, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:7, 11, or 17). For example, provided herein is a pharmaceutical composition comprising an rAAV, the rAAV comprising a polynucleotide comprising the sequence of SEQ ID NO:17, or a variant thereof, e.g., a sequence having at least at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with the polynucleotide sequence of SEQ ID NO:17. In some embodiments, the rAAV has a tropism for airway epithelial cells (e.g., lung epithelial cells). In some embodiments, the rAAV comprises an AV.TL65 capsid protein, an AAV1 capsid protein, an AAV2 capsid protein, an AAV5 capsid protein, an AAV6 capsid protein, or an AAV9 capsid protein. In some embodiments, the rAAV comprises an AV.TL65 capsid protein.

In some embodiments, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:1, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4. In another some embodiment, the polynucleotide includes an F5 enhancer comprising the sequence of SEQ ID NO:14, a tg83 promoter comprising the sequence of SEQ ID NO:2, and/or a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4.

The pharmaceutical compositions described herein may include an rAAV alone, or an rAAV in combination with one or more additional therapeutic agents. Exemplary additional therapeutic agents include, without limitation, an augmenter (e.g., any augmenter described herein, e.g., doxorubicin or idarubicin), an antibiotic (e.g., azithromycin (ZITHROMAX®), amoxicillin and clavulanic acid (AUGMENTIN®), cloxacillin and diclocacillin, ticarcillin and clavulanic acid (TIMENTIN®), cephalexin, cefdinir, cefprozil, cefaclor; sulfamethoxazole and trimethoprim (BACTRIM®), erythromycin/sulfisoxazole, erythromycin, clarithromycin, tetracycline, doxycycline, minocycline, tigecycline, vancomycin, imipenem, meripenem, Colistimethate/COLISTIN®, linezolid, ciprofloxacin, levofloxacin, or a combination thereof), a mucus thinner (e.g., hypertonic saline or dornase alfa (PULMOZYME®)), a CFTR modulator (e.g., ivacaftor (KALYDECO®), lumacaftor, lumacaftor/ivacaftor (ORKAMBI®), tezacaftor/ivacaftor (SYMDEKO®), or TRIKAFTA® (elexacaftor/ivacaftor/tezacaftor)), a mucolytic (e.g., acetylcysteine, ambroxol, bromhexine, carbocisteine, erdosteine, mecysteine, and dornase alfa), an immunosuppressive agent, normal saline, hypertonic saline, or a combination thereof.

For example, pharmaceutical compositions described herein may include an one or more immunosuppressive agents. Any suitable immunosuppressive agent may be used. For example, non-limiting examples of immunosuppressive agents include corticosteroids (e.g., an inhaled corticosteroid (e.g., beclomethasone (QVAR®), budesonide (PULMICORT®), budesonide/formoterol (SYMBICORT®), ciclesonide (ALVESCO®), fluticasone (FLOVENT HFA®), fluticasone propionate (FLOVENT DISKUS®), fluticasone furoate (ARNUITY ELLIPTA®), fluticasone propionate/salmeterol (ADVAIR®), fluticasone furoate/umeclidinium/vilanterol (TRELEGY ELLIPTA®), mometasone furoate (ASMANEX®), or mometasone/formoterol (DULERA®), predisone, or methylprednisone), polyclonal anti-lymphocyte antibodies (e.g., anti-lymphocyte globulin (ALG) and anti-thymocyte globulin (ATG) antibodies, which may be, for example, horse- or rabbit-derived), monoclonal anti-lymphocyte antibodies (e.g., anti-CD3 antibodies (e.g., murmomab and alemtuzumab) or anti-CD20 antibodies (e.g., rituximab)), interleukin-2 (IL-2) receptor antagonists (e.g., daclizumab and basiliximab), calcineurin inhibitors (e.g., cyclosporin A and tacrolimus), cell cycle inhibitors (e.g., azathioprine, mycophenolate mofetil (MMF), and mycophenolic acid (MPA)), mammalian target of rapamycin (mTOR) inhibitors (e.g., sirolimus (rapamycin) and everolimus), methotrexate, cyclophosphamide, an anthracycline (e.g., doxorubicin, idarubicin, aclarubicin, daunorubicin, epirubicin, valrubicin, mitoxantrone, or a

combination thereof), a taxane (e.g., TAXOL® (paclitaxel)), and a combination thereof (e.g., a combination of a calcineurin inhibitor, a cell cycle inhibitor, and a corticosteroid).

In particular embodiments, pharmaceutical compositions described herein may include an one or more corticosteroids (e.g., an inhaled corticosteroid (e.g., beclomethasone (QVAR®), budesonide (PULMICORT®), budesonide/formoterol (SYMBICORT®), ciclesonide (ALVESCO®), fluticasone (FLOVENT HFA®), fluticasone propionate (FLOVENT DISKUS®), fluticasone furoate (ARNUITY ELLIPTA®), fluticasone propionate/salmeterol (ADVAIR®), fluticasone furoate/umeclidinium/vilanterol (TRELEGY ELLIPTA®), mometasone furoate (ASMANEX®), or mometasone/formoterol (DULERA®), predisone, or methylprednisone). In some embodiments, the corticosteroid is an inhaled corticosteroid.

An immunosuppressive agent (e.g., any immunosuppressive agent described herein) may be administered by inhalation or administered systemically (e.g., intravenously or subcutaneously).

Typically, the viral vectors are in a pharmaceutically suitable pyrogen-free buffer such as Ringer's balanced salt solution (pH 7.4). Although not required, pharmaceutical compositions may optionally be supplied in unit dosage form suitable for administration of a precise amount. Pharmaceutical compositions are generally sterile.

Methods of Treating CF

The disclosure provides methods of treating and/or preventing CF.

For example, in one aspect, the disclosure provides a method of treating CF, the method comprising administering to a subject in need thereof a therapeutically effective amount of an rAAV comprising (i) an AV.TL65 capsid protein; and (ii) a polynucleotide comprising an F5 enhancer and a tg83 promoter operably linked to a CFTRΔR minigene. The rAAV may include any of the polynucleotides described herein.

In another aspect, the disclosure features an rAAV for use in treating cystic fibrosis in a subject in need thereof, the rAAV including (i) an AV.TL65 capsid protein; and (ii) a polynucleotide including an F5 enhancer and a tg83 promoter operably linked to a CFTRΔR minigene. In some embodiments, the rAAV is for use in combination with one or more additional therapeutic agents (e.g., any augmenter described herein). The rAAV may include any of the polynucleotides described herein.

Compositions described herein (e.g., rAAVs or pharmaceutical compositions) may be used *in vivo* as well as *ex vivo*. *In vivo* gene therapy comprises administering the vectors of this disclosure directly to a subject. Pharmaceutical compositions can be supplied as liquid solutions or suspensions, as emulsions, or as solid forms suitable for dissolution or suspension in liquid prior to use. For administration into the respiratory tract, one exemplary mode of administration is by aerosol, using a composition that provides either a solid or liquid aerosol when used with an appropriate aerosolubilizer

device. Another some mode of administration into the respiratory tract is using a flexible fiberoptic bronchoscope to instill the vectors.

A composition described herein (e.g., rAAVs or pharmaceutical compositions) can be administered by any suitable route, e.g., by inhalation, nebulization, aerosolization, intranasally, intratracheally, intrabronchially, orally, parenterally (e.g., intravenously, subcutaneously, or intramuscularly), orally, nasally, rectally, topically, or buccally. They can also be administered locally or systemically. In some embodiments, a composition described herein is administered in aerosolized particles intratracheally and/or intrabronchially using an atomizer sprayer (e.g., with a MADgic® laryngo-tracheal mucosal atomization device). In some embodiments, the composition is administered parentally. In other some embodiments, the composition is administered systemically. Vectors can also be introduced by way of bioprostheses, including, by way of illustration, vascular grafts (PTFE and dacron), heart valves, intravascular stents, intravascular paving as well as other non-vascular prostheses. General techniques regarding delivery, frequency, composition and dosage ranges of vector solutions are within the skill of the art.

For administration to the upper (nasal) or lower respiratory tract by inhalation, the compositions described herein (e.g., rAAVs or pharmaceutical compositions) are conveniently delivered from an insufflator, nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the composition may take the form of a dry powder, for example, a powder mix of the agent and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges, or, e.g., gelatine or blister packs from which the powder may be administered with the aid of an inhalator, insufflator or a metered-dose inhaler.

For intra-nasal administration, the agent may be administered via nose drops, a liquid spray, such as via a plastic bottle atomizer or metered-dose inhaler. Typical of atomizers are the Mistometer (Wintrop) and the Medihaler (Riker).

Administration of the compositions described herein (e.g., rAAVs or pharmaceutical compositions) may be continuous or intermittent, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The compositions described herein can be administered once, or multiple times (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, or more times), at the same or at different sites. The administration of the agents of the disclosure may be essentially continuous over a preselected period of time or may be in a series of spaced doses.

The compositions described herein (e.g., rAAVs or pharmaceutical compositions) may be administered as a monotherapy. The compositions described herein (e.g., rAAVs or pharmaceutical compositions) can also be administered in combination with one or more additional therapeutic agent. Any suitable additional therapeutic agent(s) may be used, including standard of care therapies for CF. In some embodiments, the one or more additional therapeutic agents includes an antibiotic (e.g., azithromycin (ZITHROMAX®), amoxicillin and clavulanic acid (AUGMENTIN®), cloxacillin and diclocacillin, ticarcillin and clavulanic acid (TIMENTIN®), cephalexin, cefdinir, cefprozil, cefaclor; sulfamethoxazole and trimethoprim (BACTRIM®), erythromycin/sulfisoxazole, erythromycin, clarithromycin, tetracycline, doxycycline, minocycline, tigecycline, vancomycin, imipenem, meripenem, Colistimethate/COLISTIN®, linezolid, ciprofloxacin, levofloxacin, or a combination thereof), a mucus thinner (e.g., hypertonic saline or dornase alfa (PULMOZYME®)), a CFTR modulator (e.g., ivacaftor (KALYDECO®), lumacaftor, lumacaftor/ivacaftor (ORKAMBI®), tezacaftor/ivacaftor (SYMDEKO®), or TRIKAFTA® (elexacaftor/ivacaftor/tezacaftor)), a mucolytic (e.g., acetylcysteine, ambroxol, bromhexine, carbocisteine, erdosteine, mecysteine, and dornase alfa), an immunosuppressive agent, normal saline, hypertonic saline, or a combination thereof.

For example, any one the compositions described herein (e.g., rAAVs or pharmaceutical compositions) may be administered in combination with one or more immunosuppressive agents. Any suitable immunosuppressive agent may be used. For example, non-limiting examples of immunosuppressive agents include corticosteroids (e.g., an inhaled corticosteroid (e.g., beclomethasone (QVAR®), budesonide (PULMICORT®), budesonide/formoterol (SYMBICORT®), ciclesonide (ALVESCO®), fluticasone (FLOVENT HFA®), fluticasone propionate (FLOVENT DISKUS®), fluticasone furoate (ARNUITY ELLIPTA®), fluticasone propionate/salmeterol (ADVAIR®), fluticasone furoate/umeclidinium/vilanterol (TRELEGY ELLIPTA®), mometasone furoate (ASMANEX®), or mometasone/formoterol (DULERA®), predisone, or methylprednisone), polyclonal anti-lymphocyte antibodies (e.g., anti-lymphocyte globulin (ALG) and anti-thymocyte globulin (ATG) antibodies, which may be, for example, horse- or rabbit-derived), monoclonal anti-lymphocyte antibodies (e.g., anti-CD3 antibodies (e.g., murmomab and alemtuzumab) or anti-CD20 antibodies (e.g., rituximab)), interleukin-2 (IL-2) receptor antagonists (e.g., daclizumab and basiliximab), calcineurin inhibitors (e.g., cyclosporin A and tacrolimus), cell cycle inhibitors (e.g., azathioprine, mycophenolate mofetil (MMF), and mycophenolic acid (MPA)), mammalian target of rapamycin (mTOR) inhibitors (e.g., sirolimus (rapamycin) and everolimus), methotrexate, cyclophosphamide, an anthracycline (e.g., doxorubicin, idarubicin, aclarubicin, daunorubicin, epirubicin, valrubicin, mitoxantrone, or a combination thereof), a taxane (e.g., TAXOL® (paclitaxel)), and a combination thereof (e.g., a combination of a calcineurin inhibitor, a cell cycle inhibitor, and a corticosteroid).

In particular embodiments, any one the compositions described herein (e.g., rAAVs, pharmaceutical compositions, and/or augmenters) may be administered in combination with one or more corticosteroids (e.g., an inhaled corticosteroid (e.g., beclomethasone (QVAR®), budesonide (PULMICORT®), budesonide/formoterol (SYMBICORT®), ciclesonide (ALVESCO®), fluticasone (FLOVENT HFA®), fluticasone propionate (FLOVENT DISKUS®), fluticasone furoate (ARNUITY ELLIPTA®), fluticasone propionate/salmeterol (ADVAIR®), fluticasone furoate/umeclidinium/vilanterol (TRELEGY ELLIPTA®), mometasone furoate (ASMANEX®), or mometasone/formoterol (DULERA®), predisone, or methylprednisone). In some embodiments, the corticosteroid is an inhaled corticosteroid.

An immunosuppressive agent (e.g., any immunosuppressive agent described herein) may be administered by inhalation or administered systemically (e.g., intravenously or subcutaneously).

The compositions described herein (e.g., rAAVs or pharmaceutical compositions) may be administered to a mammal alone or in combination with pharmaceutically acceptable carriers. As noted above, the relative proportions of active ingredient and carrier are determined by the solubility and chemical nature of the compound, chosen route of administration and standard pharmaceutical practice.

The dosage of the present compositions will vary with the form of administration, the particular compound chosen and the physiological characteristics of the particular patient under treatment. It is desirable that the lowest effective concentration of virus be utilized in order to reduce the risk of undesirable effects, such as toxicity.

EXAMPLES

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1: Development of AV.TL65-SP183-CFTRΔR and Functional Complementation of CFTR-mediated Chloride Transport in Polarized Human CF Airway Epithelium

rAAV vectors have a limited packaging capacity, which has hindered the development of this virus for gene therapy of cystic fibrosis (CF). For example, viral genomes >4.9 kb in total size incur small deletions at the ends of the genome. In the

case of CFTR vectors, for which the transgene cassette is joined directly to the ITRs, this can lead to compromised CFTR function.

This study describes development of an rAAV vector referred to as AV.TL65-SP183-hCFTRΔR. This vector utilizes a combination of elements that are expected to overcome many of the obstacles that have been holding back CF lung gene therapy efforts: an evolved chimeric AAV capsid protein, AV.TL65, that is highly tropic for the human airway; a short but highly active 183 base pair synthetic enhancer and promoter (SP183, which includes an F5 enhancer and a tg83 promoter), and a highly functional CFTR minigene (human CFTRΔR (referred to as hCFTRΔR)). The examples described herein utilized an rAAV vector that included a polynucleotide comprising: a 5' AAV ITR comprising the sequence of SEQ ID NO:15, an F5 enhancer comprising the sequence of SEQ ID NO:14 (which may include a 5' EcoRI site and a 3' XhoI site, as in SEQ ID NO:1), a tg83 promoter comprising the sequence of SEQ ID NO:2, a 5' UTR comprising the sequence of SEQ ID NO:3, a hCFTRΔR minigene comprising the sequence of SEQ ID NO:4, a 3' UTR comprising the sequence of SEQ ID NO:5, a s-pA comprising the sequence of SEQ ID NO:6, and a 3' AAV ITR comprising the sequence of SEQ ID NO:16. For example, the packaged polynucleotide may include the sequence of SEQ ID NO:17. AV.TL65-SP183-hCFTRΔR may be used alone or in combination with one or more augmenters of rAAV transduction (e.g., small molecule augmenters). Interestingly, as described herein, we have determined that AV.TL65 can also infect the airway of ferrets, enabling use of ferret CF models. A ferret version of the CFTRΔR minigene (fCFTRΔR) can also be used in such models.

Surprisingly, AV.TL65-SP183-hCFTRΔR outperformed AV.1-SP183-hCFTRΔR in a head-to-head comparison on CF air-liquid interface (ALI) cultures evaluating CFTR-mediated chloride transport (**FIGS. 1A-1C**). In these experiments, the rAAV2 viral genome AV.TL65-SP183-hCFTRΔR was packaged into three capsid serotypes (AV.TL65, AV.1, and AV.2) and used to apically infect polarized human CF ALI cultures from the apical (AV.TL65 and AV.1) or basolateral surface (AV.2). Basolateral infection with AAV2 was used as a positive control since it efficiently infects from the basolateral surface. 2.5 μM doxorubucin and 20μM LLnL were added to the viral inoculum and ALI cultures were infected for 16 h. Virus was then removed and cultures were re-fed in the absence of proteasome inhibitors. Prior to developing AV.TL65, rAAV serotype 1 (AV.1) was the best performing vector tested in human ALI cultures and lungs of chimpanzees. AV.TL65-SP183-hCFTRΔR outperformed AV.1-SP183-hCFTRΔR by ~2-fold (**FIGS. 1B and 1C**). Thus, these data demonstrate functional complementation of CFTR-mediated chloride transport in polarized human CF airway epithelium.

Example 2: In Vivo Expression of AV.TL65-SP183-hCFTRΔR

This study describes testing of the clinical candidate vector AV.TL65-SP183-hCFTRΔR for hCFTR expression in the newborn and mature ferret airway. An endpoint of these analyses was the ratio of transgene-derived human CFTR (hCFTR) to that of

endogenous ferret CFTR (fCFTR) mRNA. Three day old newborn ferrets were infected with a 100 μl volume of 6 x 10^{11} DRP of AV.TL65-SP183-hCFTRΔR in 500 μM doxorubicin. Non-infected animals were given an equal volume of vehicle with doxorubicin. At 10 days post-infection, the entire lung and trachea were harvested and snap frozen in liquid nitrogen. Tissue was pulverized and mRNA and cDNA generated for Q-PCR of human and ferret CFTR. As shown in **FIGS. 2A-2D**, AV.TL65-SP183-CFTRΔR led to 240% greater expression of human CFTR compared with endogenous (ferret) CFTR following gene delivery to the lung. Unexpectedly, treated ferrets also showed ~90-fold increase in endogenous CFTR in lungs (but not trachea) compared with controls, implying that receptor binding and/or the infectious process of AV.TL65-SP183-CFTRΔR may induce endogenous CFTR expression. Without wishing to be bound by theory, this could provide additional therapeutic effect for partial function CFTR mutants or in patients who are taking CFTR modulators.

Newborn ferrets are born with an immature airway that lacks submucosal glands and contains few ciliated cells. By the end of the first 3 weeks of life, ciliogenesis and submucosal gland formation is complete throughout the cartilaginous airways of ferrets. Given that the phenotype of ferret airway epithelia and the secretions in the airway will change during this maturation phase, we evaluated whether AV.TL65 transduces the mature ferret airway. To this end, we evaluated the ability of AV.TL65 to transduce the lung of 1 month old ferrets. The lungs of 1 month old ferrets (N=3) were transduced with 7.5 x 10^{12} DRP of AV.TL65 harboring the SP183-hCFTRΔR cDNA in a 500 μl volume of PBS in the presence of 250 μM doxorubicin. A mock-infected control animal (N=1) received 500 μl PBS with no vector in the presence of 250 μM doxorubicin. Vector was delivered to the lung with a PennCentury microsprayer through tracheal intubation. Nasal delivery in the same animals was also performed using 100 μl containing 1.5 x 10^{12} DRP with 250 μM doxorubicin by instillation of fluid. Mock-infected nasal delivery received PBS with 250 μM doxorubicin. At 12 days following infection, the lung lobes were harvested separately along with the trachea, carina, and nasal turbinates with surrounding adventitia. The tissues were snap frozen and pulverized samples were processed separately for mRNA and DNA. In 1 month old mature ferrets (**Figs. 3A-3D**), AV.TL65-SP183-hCFTRΔR led to 3-fold greater expression of human CFTR compared with endogenous (ferret) CFTR following gene delivery to the lung. While AV.TL65 was developed to effectively transduce the apical surface of differentiated human airway epithelial, these findings suggest that the receptor and co-receptor that determines efficacy of AV.TL65 is conserved in ferret. Of note, in these experiments, the induction of the endogenous ferret CFTR transcript observed in 3 day old ferrets following AV.TL65 infection (**Fig. 2B**) was not observed in the mature ferret lung (**Fig. 3B**), suggesting that this biology may be specific to the neonatal airway. These findings from newborn and mature ferrets indicate that the present approach to gene therapy for CF translates robustly *in vivo*.

Example 3: Large safety margin indicates clinical feasibility of inhaled doxorubicin to augment gene therapy

The clinical feasibility of an inhaled proteasome inhibitor has been demonstrated with doxorubicin, which was tested in two clinical trials for patients with lung cancer or metastases administered as an inhaled, aerosolized formulation. The maximum tolerated doses in these studies were 6.0 mg/m^2 and 7.5 mg/m^2 once every 3 weeks for up to 8 cycles. The dose of doxorubicin that achieved efficacy in the mature ferret lung (Figs. 3A-3D) was 100 µl of 250 µM doxorubicin, which is equivalent to 0.34 mg/m^2, assuming a ferret body surface area of 0.043 m^2. Thus, there is anticipated to be an 18 to 22-fold safety margin between an effective ferret dose and the maximum tolerated human dose using mg/m^2 allometric scaling some by the FDA. This large safety margin with doxorubicin is supports the concept of utilizing an inhaled augmenter to improve transduction efficiency with rAAVs.

Example 4: CFTR functional complementation by nasal potential difference (PD) measurements and bacterial clearance in juvenile and adult CF ferrets infected with AV.TL65-SP183-fCFTRΔR

This Example describes a model clinical trial in CF ferrets to demonstrating functional complementation of nasal PD measurements and enhanced bacterial clearance following AV.TL65-SP183-fCFTRΔR infection. These studies utilize a gut-corrected CFTR-KO ferret model, which will prevent an immune response to ferret CFTR. It is expected that delivery of AV.TL65-SP183-fCFTRΔR to the nasal epithelium of CF animals will lead to CFTR-dependent changes in Vt.

Experimental Design and Methods

Gene therapy to the nasal epithelium in CF ferrets. AV.TL65-SP183-fCFTRΔR is delivered to the nasal epithelium of 5 month old adult CF ferrets at a dose of 1 x 10^{12} DRP/kg alone or with augmenter. Age matched non-CF controls are also be evaluated in the absence of vector and/or augmenter to determine baseline values. Nasal transepithelial voltage (Vt) measurement are taken at baseline, and 10, 20, and 30 days post-infection using previously described protocols. Transepithelial voltage measurements are assessed using the sequential addition of the following agents/solutions sequentially added to the epithelial perfusate after baseline measurements: amiloride (100 µM), Cl-free solution, isoproterenol (10 µM), ATP (100 µM), and GlyH-101 (100 µM). The change in transepithelial voltage in the presence of isoproterenol reflects CFTR-mediated Cl permeability and the addition of GlyH-101 should block this change in voltage if due to CFTR. In one example, 8 CF animals are evaluated (4 males and 4 females) and 8 non-CF controls (4 males and 4 females).

Gene therapy to the lung of CF ferrets. AV.TL65-SP183-fCFTRΔR is delivered to the lung epithelium of 1 month old CF ferrets at a dose of 1 x 10^{13} DRP/kg alone or

with augmenter using a Penn-Century microsprayer (similar to the experiment described in **Figs. 3A-3D**). Control CF and non-CF ferrets will receive controls (e.g., vehicle or augmenter alone). At the time of gene transfer, both CF and non-CF animals are removed from antibiotics used during rearing to prevent bacterial colonization of the lung. At 12 days post-infection or control delivery of augmenter alone, animals are challenged with an equal mixture of ampicillin-resistant *P. aeruginosa* (PA01) (1 x 10^6 CFU/100 grams body weight) and erythromycin-resistant *Staphylococcus pseudintermedius* (1 x 10^6 CFU/100 grams body weight) using a Penn-Century microsprayer using procedures similar to those previously described in newborn CF and non-CF ferrets demonstrating defective CF bacterial clearance. In one example, 16 CF animals are evaluated with and without vector administration (4 males and 4 females for each condition) and 8 non-CF controls (4 males and 4 females). At 24 h post-bacterial challenge, whole lung homogenates are generated for quantification of the following endpoints: 1) total bacterial CFU on blood agar, 2) ampicillin-resistant bacterial CFU on blood agar, 3) erythromycin-resistant bacterial CFU on blood agar, 4) transgene and endogenous CFTR mRNA, and 5) vector-derived genomes.

Example 5: CFTR functional complementation in polarized human CF airway epithelium

In this Example, short circuit current was measured to assess rescue of functional CFTR using AV.TL65-SP183-fCFTRΔR. This assay evaluates cAMP-regulated chloride channel activity in the apical membrane of human bronchial epithelia (HBE's) in a Ussing chamber. Amiloride was used to block epithelial Na^+ channel activity, ensuring that changes in short-circuit current (ΔISC) during subsequent manipulation were secondary to effects on Cl^- transport. The anion transport inhibitor 4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS) is more effective at blockade of the Cl^-/HCO_3^- exchanger putative anion transporter-1 when applied in buffers containing low Cl^- concentrations (~5 mM) compared with physiological Ringers solutions with ~120 mM Cl^- concentration. The cAMP agonists Forskolin and IBMX activate CFTR via a cyclic adenosine monophosphate (cAMP)-dependent mechanism. GlyH101 is a specific inhibitor of CFTR that allowed the relative contribution of CFTR (versus other anion transport pathways) to be determined in Ussing chamber systems. In this study, CF HBE (dF508/dF508) (N=6) cells were compared to non-CF HBE cells(N=6), cells were grown in USG media; and the assays were performed 7 days post AAV & PI treatment (AAV, 10K MOI). In this experiment, the ferret CFTRΔR minigene was used, but in other examples, the human CFTRΔR can be used.

Fig. 4 shows representative CF traces. The forskolin-stimulated, CFTR-mediated chloride transport (Isc) in CF HBE treated with either AV.TL65+doxorubicin or AV.TL65+idarubicin exceeded 50% of the forskolin-stimulated, CFTR-mediated chloride transport in non-CF HBE (Fig. 5). Trans-epithelial resistance (TEER) measurements

before and after addition of AAV, PI, or both showed no changes and was not affected (and if anything, there was subtle increase in TEER), thus demonstrating no significant toxicity to or cell death of the HBE in response to AV.TL65+doxorubicin or AV.TL65+idarubicin treatment.

Example 6: AV.TL65-SP183-hCFTRΔR can significantly increase CFTR activity relative to standard of care therapies

The complementation of CFTR activity by AV.TL65-SP183-hCFTRΔR was compared to a current standard of care therapy, the combination of VX-809 (LUMACAFTOR) and VX-770 (IVACAFTOR) - ORKAMBI®. The effect of AV.TL65-SP183-hCFTRΔR + Dox or Ida was compared to VX-770/VX-809 ("VX") in two separate CF HBE P3 cell lines: dF508/dF508 and dF508/R553X. Cells were treated with proteasome inhibitor (PI) or vehicle and with AV.TL65 (MOI = 10K, 25K, 80K). Cells were grown in BronchiaLife/Vertex air-liquid interface (ALI) medium and assayed 7 days post-AAV & PI treatment. A trans-epithelial cell current clamp amplifier for 24-well plate assay (TECC-24) was performed. CFTR activation by Forskolin/VX caused an increase in Cl conductance leading to membrane depolarization. Addition of DMSO only caused minor deviation.

Fig. 6 shows representative I_{eq} traces (37°C) from individual wells of a 24-well Transwell filter plate. A MOI-dependent increase in CFTR activity in AV.TL65-SP183-hCFTRΔR-treated cells was observed in the presence of PI. No change in CFTR activity was observed in cells treated with AV.TL65-SP183-hCFTRΔR without PI. VX-809/VX-770 significantly increased CFTR activity.

Fig. 7 shows area under the curve (AUC) graphs showing mean CFTR mediated chloride secretion after Forskolin stimulation for each condition, n=4. Trans-epithelial resistance measurements before and after addition of AAV or of PI or both showed no changes and was not affected.

These data demonstrate that CFTR activity is significantly increased by AV.TL65-SP183-hCFTRΔR relative to current standard of care, e.g., VX-770/VX-809 combination treatment.

SEQUENCE LISTING

SEQ ID NO	Name	Sequence
1	F5 Enhancer with 5' EcoRI and 3' XhoI sites	GAATTCGTGGTGAGCGTCTGGGCATGTCTGGGCATGTCTGGGCATGTCTGGGCATGTCGGGCATTCTGGGCGTCTGGGCATGTCTGGGCATGTCTGGGCATCTCGAG
2	tg83 Promoter	AACGGTGACGTGCACGCGTGGGCGGAGCCATCACGCAGGTTGCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGA

3	5'-UTR	GTCGAGCCCGAGAGACC
4	hCFTRΔR	ATGCAGAGGTCGCCTCTGGAAAAGGCCAGCGTTGTCTCCAA ACTTTTTTTCAGCTGGACCAGACCAATTTTGAGGAAAGGATA CAGACAGCGCCTGGAATTGTCAGACATATACCAAATCCCTTC TGTTGATTCTGCTGACAATCTATCTGAAAAATTGGAAAGAGAA TGGGATAGAGAGCTGGCTTCAAAGAAAAATCCTAAACTCATT AATGCCCTTCGGCGATGTTTTTTCTGGAGATTTATGTTCTATG GAATCTTTTTATATTTAGGGGAAGTCACCAAAGCAGTACAGC CTCTCTTACTGGGAAGAATCATAGCTTCCTATGACCCGGATA ACAAGGAGGAACGCTCTATCGCGATTTATCTAGGCATAGGCT TATGCCTTCTCTTTATTGTGAGGACACTGCTCCTACACCCAG CCATTTTTGGCCTTCATCACATTGGAATGCAGATGAGAATAG CTATGTTTAGTTTGATTTATAAGAAGACTTTAAAGCTGTCAAG CCGTGTTCTAGATAAAATAAGTATTGGACAACTTGTTAGTCTC CTTTCCAACAACCTGAACAAATTTGATGAAGGACTTGCATTG GCACATTTCGTGTGGATCGCTCCTTTGCAAGTGGCACTCCTC ATGGGGCTAATCTGGGAGTTGTTACAGGCGTCTGCCTTCTGT GGACTTGGTTTCCTGATAGTCCTTGCCCTTTTTCAGGCTGGG CTAGGGAGAATGATGATGAAGTACAGAGATCAGAGAGCTGG GAAGATCAGTGAAAGACTTGTGATTACCTCAGAAATGATCGA GAACATCCAATCTGTTAAGGCATACTGCTGGGAAGAAGCAAT GGAAAAAATGATTGAAAACTTAAGACAAACAGAACTGAAACT GACTCGGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGC CTTCTTCTTCTCAGGGTTCTTTGTGGTGTTTTTATCTGTGCTT CCCTATGCACTAATCAAAGGAATCATCCTCCGGAAAATATTC ACCACCATCTCATTCTGCATTGTTCTGCGCATGGCGGTCACT CGGCAATTTCCCTGGGCTGTACAAACATGGTATGACTCTCTT GGAGCAATAAACAAAATACAGGATTTCTTACAAAAGCAAGAA TATAAGACATTGGAATATAACTTAACGACTACAGAAGTAGTGA TGGAGAATGTAACAGCCTTCTGGGAGGAGGGATTTGGGGAA TTATTTGAGAAAGCAAAACAAAACAATAACAATAGAAAAACTT CTAATGGTGATGACAGCCTCTTCTTCAGTAATTTCTCACTTCT TGGTACTCCTGTCCTGAAAGATATTAATTTCAAGATAGAAAGA GGACAGTTGTTGGCGGTTGCTGGATCCACTGGAGCAGGCAA GACTTCACTTCTAATGATGATTATGGGAGAACTGGAGCCTTC AGAGGGTAAAATTAAGCACAGTGGAAGAATTTCATTCTGTTC TCAGTTTTCCTGGATTATGCCTGGCACCATTAAAGAAAATATC ATCTTTGGTGTTTCCTATGATGAATATAGATACAGAAGCGTCA TCAAAGCATGCCAACTAGAAGAGGACATCTCCAAGTTTGCAG AGAAAGACAATATAGTTCTTGGAGAAGGTGGAATCACACTGA GTGGAGGTCAACGAGCAAGAATTTCTTTAGCAAGAGCAGTAT ACAAAGATGCTGATTTGTATTTATTAGACTCTCCTTTTGGATA CCTAGATGTTTTAACAGAAAAAGAAATATTTGAAAGCTGTGTC TGTAAACTGATGGCTAACAAAACTAGGATTTTGGTCACTTCTA AAATGGAACATTTAAAGAAAGCTGACAAAATATTAATTTTGCA TGAAGGTAGCAGCTATTTTTATGGGACATTTTCAGAACTCCAA AATCTACAGCCAGACTTTAGCTCAAAACTCATGGGATGTGAT TCTTTCGACCAATTTAGTGCAGAAAGAAGAAATTCAATCCTAA CTGAGACCTTACACCGTTTCTCATTAGAAGGAGATGCTCCTG TCTCCTGGACAGAAACAAAAAAACAATCTTTTAAACAGACTG GAGAGTTTGGGGAAAAAAGGAAGAATTCTATTCTCAATCCAA TCAACTCTACGCTTCAGGCACGAAGGAGGCAGTCTGTCCTG AACCTGATGACACACTCAGTTAACCAAGGTCAGAACATTCAC CGAAAGACAACAGCATCCACACGAAAAGTGTCACTGGCCCC TCAGGCAAACTTGACTGAACTGGATATATATTCAAGAAGGTT ATCTCAAGAAACTGGCTTGGAAATAAGTGAAGAAATTAACGA AGAAGACTTAAAGGAGTGCCTTTTTGATGATATGGAGAGCAT ACCAGCAGTGACTACATGGAACACATACCTTCGATATATTAC TGTCCACAAGAGCTTAATTTTTGTGCTAATTTGGTGCTTAGTA ATTTTTCTGGCAGAGGTGGCTGCTTCTTTGGTTGTGCTGTGG

		CTCCTTGGAAACACTCCTCTTCAAGACAAAGGGAATAGTACT CATAGTAGAAATAACAGCTATGCAGTGATTATCACCAGCACC AGTTCGTATTATGTGTTTTACATTTACGTGGGAGTAGCCGAC ACTTTGCTTGCTATGGGATTCTTCAGAGGTCTACCACTGGTG CATACTCTAATCACAGTGTCGAAAATTTTACACCACAAAATGT TACATTCTGTTCTTCAAGCACCTATGTCAACCCTCAACACGTT GAAAGCAGGTGGGATTCTTAATAGATTCTCCAAAGATATAGC AATTTTGGATGACCTTCTGCCTCTTACCATATTTGACTTCATC CAGTTGTTATTAATTGTGATTGGAGCTATAGCAGTTGTCGCA GTTTTACAACCCTACATCTTTGTTGCAACAGTGCCAGTGATA GTGGCTTTTATTATGTTGAGAGCATATTTCCTCCAAACCTCAC AGCAACTCAAACAACTGGAATCTGAAGGCAGGAGTCCAATTT TCACTCATCTTGTTACAAGCTTAAAAGGACTATGGACACTTCG TGCCTTCGGACGGCAGCCTTACTTTGAAACTCTGTTCCACAA AGCTCTGAATTTACATACTGCCAACTGGTTCTTGTACCTGTCA ACACTGCGCTGGTTCCAAATGAGAATAGAAATGATTTTTGTC ATCTTCTTCATTGCTGTTACCTTCATTTCCATTTTAACAACAG GAGAAGGAGAAGGAAGAGTTGGTATTATCCTGACTTTAGCCA TGAATATCATGAGTACATTGCAGTGGGCTGTAAACTCCAGCA TAGATGTGGATAGCTTGATGCGATCTGTGAGCCGAGTCTTTA AGTTCATTGACATGCCAACAGAAGGTAAACCTACCAAGTCAA CCAAACCATACAAGAATGGCCAACTCTCGAAAGTTATGATTA TTGAGAATTCACACGTGAAGAAAGATGACATCTGGCCCTCAG GGGGCCAAATGACTGTCAAAGATCTCACAGCAAAATACACAG AAGGTGGAAATGCCATATTAGAGAACATTTCCTTCTCAATAAG TCCTGGCCAGAGGGTGGGCCTCTTGGGAAGAACTGGATCAG GGAAGAGTACTTTGTTATCAGCTTTTTTGAGACTACTGAACAC TGAAGGAGAAATCCAGATCGATGGTGTGTCTTGGGATTCAAT AACTTTGCAACAGTGGAGGAAAGCCTTTGGAGTGATACCACA GAAAGTATTTATTTTTTCTGGAACATTTAGAAAAAACTTGGAT CCCTATGAACAGTGGAGTGATCAAGAAATATGGAAAGTTGCA GATGAGGTTGGGCTCAGATCTGTGATAGAACAGTTTCCTGG GAAGCTTGACTTTGTCCTTGTGGATGGGGGCTGTGTCCTAAG CCATGGCCACAAGCAGTTGATGTGCTTGGCTAGATCTGTTCT CAGTAAGGCGAAGATCTTGCTGCTTGATGAACCCAGTGCTCA TTTGGATCCAGTAACATACCAAATAATTAGAAGAACTCTAAAA CAAGCATTTGCTGATTGCACAGTAATTCTCTGTGAACACAGG ATAGAAGCAATGCTGGAATGCCAACAATTTTTGGTCATAGAA GAGAACAAAGTGCGGCAGTACGATTCCATCCAGAAACTGCT GAACGAGAGGAGCCTCTTCCGGCAAGCCATCAGCCCCTCCG ACAGGGTGAAGCTCTTTCCCCACCGGAACTCAAGCAAGTGC AAGTCTAAGCCCCAGATTGCTGCTCTGAAAGAGGAGACAGA AGAAGAGGTGCAAGATACAAGGCTTTAG
5	3’-UTR	AGAGCAGCATAAATGTTGACATGGGACATTTGCTCATGGAAT TGG
6	s-pA	AATAAAGAGCTCAGATGCATCGATCAGAGTGTGTTGGTTTTT TGTGTGTA
7	F5 Enhancer, Tg83 Promoter, 5’-UTR, hCFTRΔR	GAATTCGTGGTGAGCGTCTGGGCATGTCTGGGCATGTCTGG GCATGTCTGGGCATGTCGGGCATTCTGGGCGTCTGGGCATG TCTGGGCATGTCTGGGCATCTCGAGAACGGTGACGTGCACG CGTGGGCGGAGCCATCACGCAGGTTGCTATATAAGCAGAGC TCGTTTAGTGAACCGTCAGAGTCGAGCCCGAGAGACCATGC AGAGGTCGCCTCTGGAAAAGGCCAGCGTTGTCTCCAAACTTT TTTTCAGCTGGACCAGACCAATTTTGAGGAAAGGATACAGAC AGCGCCTGGAATTGTCAGACATATACCAAATCCCTTCTGTTG ATTCTGCTGACAATCTATCTGAAAAATTGGAAAGAGAATGGG ATAGAGAGCTGGCTTCAAAGAAAAATCCTAAACTCATTAATG CCCTTCGGCGATGTTTTTTCTGGAGATTTATGTTCTATGGAAT CTTTTTATATTTAGGGGAAGTCACCAAAGCAGTACAGCCTCT CTTACTGGGAAGAATCATAGCTTCCTATGACCCGGATAACAA GGAGGAACGCTCTATCGCGATTTATCTAGGCATAGGCTTATG

		CCTTCTCTTTATTGTGAGGACACTGCTCCTACACCCAGCCAT TTTTGGCCTTCATCACATTGGAATGCAGATGAGAATAGCTAT GTTTAGTTTGATTTATAAGAAGACTTTAAAGCTGTCAAGCCGT GTTCTAGATAAAATAAGTATTGGACAACTTGTTAGTCTCCTTT CCAACAACCTGAACAAATTTGATGAAGGACTTGCATTGGCAC ATTTCGTGTGGATCGCTCCTTTGCAAGTGGCACTCCTCATGG GGCTAATCTGGGAGTTGTTACAGGCGTCTGCCTTCTGTGGA CTTGGTTTCCTGATAGTCCTTGCCCTTTTTCAGGCTGGGCTA GGGAGAATGATGATGAAGTACAGAGATCAGAGAGCTGGGAA GATCAGTGAAAGACTTGTGATTACCTCAGAAATGATCGAGAA CATCCAATCTGTTAAGGCATACTGCTGGGAAGAAGCAATGGA AAAAATGATTGAAAACTTAAGACAAACAGAACTGAAACTGACT CGGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGCCTTC TTCTTCTCAGGGTTCTTTGTGGTGTTTTTATCTGTGCTTCCCT ATGCACTAATCAAAGGAATCATCCTCCGGAAAATATTCACCA CCATCTCATTCTGCATTGTTCTGCGCATGGCGGTCACTCGGC AATTTCCCTGGGCTGTACAAACATGGTATGACTCTCTTGGAG CAATAAACAAAATACAGGATTTCTTACAAAAGCAAGAATATAA GACATTGGAATATAACTTAACGACTACAGAAGTAGTGATGGA GAATGTAACAGCCTTCTGGGAGGAGGGATTTGGGGAATTATT TGAGAAAGCAAAACAAAACAATAACAATAGAAAAACTTCTAAT GGTGATGACAGCCTCTTCTTCAGTAATTTCTCACTTCTTGGTA CTCCTGTCCTGAAAGATATTAATTTCAAGATAGAAAGAGGAC AGTTGTTGGCGGTTGCTGGATCCACTGGAGCAGGCAAGACT TCACTTCTAATGATGATTATGGGAGAACTGGAGCCTTCAGAG GGTAAAATTAAGCACAGTGGAAGAATTTCATTCTGTTCTCAGT TTTCCTGGATTATGCCTGGCACCATTAAAGAAAATATCATCTT TGGTGTTTCCTATGATGAATATAGATACAGAAGCGTCATCAAA GCATGCCAACTAGAAGAGGACATCTCCAAGTTTGCAGAGAAA GACAATATAGTTCTTGGAGAAGGTGGAATCACACTGAGTGGA GGTCAACGAGCAAGAATTTCTTTAGCAAGAGCAGTATACAAA GATGCTGATTTGTATTTATTAGACTCTCCTTTTGGATACCTAG ATGTTTTAACAGAAAAAGAAATATTTGAAAGCTGTGTCTGTAA ACTGATGGCTAACAAAACTAGGATTTTGGTCACTTCTAAAATG GAACATTTAAAGAAAGCTGACAAAATATTAATTTTGCATGAAG GTAGCAGCTATTTTTATGGGACATTTTCAGAACTCCAAAATCT ACAGCCAGACTTTAGCTCAAAACTCATGGGATGTGATTCTTT CGACCAATTTAGTGCAGAAAGAAGAAATTCAATCCTAACTGA GACCTTACACCGTTTCTCATTAGAAGGAGATGCTCCTGTCTC CTGGACAGAAACAAAAAAACAATCTTTTAAACAGACTGGAGA GTTTGGGGAAAAAAGGAAGAATTCTATTCTCAATCCAATCAA CTCTACGCTTCAGGCACGAAGGAGGCAGTCTGTCCTGAACC TGATGACACACTCAGTTAACCAAGGTCAGAACATTCACCGAA AGACAACAGCATCCACACGAAAAGTGTCACTGGCCCCTCAG GCAAACTTGACTGAACTGGATATATATTCAAGAAGGTTATCTC AAGAAACTGGCTTGGAAATAAGTGAAGAAATTAACGAAGAAG ACTTAAAGGAGTGCCTTTTTGATGATATGGAGAGCATACCAG CAGTGACTACATGGAACACATACCTTCGATATATTACTGTCCA CAAGAGCTTAATTTTTGTGCTAATTTGGTGCTTAGTAATTTTT CTGGCAGAGGTGGCTGCTTCTTTGGTTGTGCTGTGGCTCCTT GGAAACACTCCTCTTCAAGACAAAGGGAATAGTACTCATAGT AGAAATAACAGCTATGCAGTGATTATCACCAGCACCAGTTCG TATTATGTGTTTTACATTTACGTGGGAGTAGCCGACACTTTGC TTGCTATGGGATTCTTCAGAGGTCTACCACTGGTGCATACTC TAATCACAGTGTCGAAAATTTTACACCACAAAATGTTACATTC TGTTCTTCAAGCACCTATGTCAACCCTCAACACGTTGAAAGC AGGTGGGATTCTTAATAGATTCTCCAAAGATATAGCAATTTTG GATGACCTTCTGCCTCTTACCATATTTGACTTCATCCAGTTGT TATTAATTGTGATTGGAGCTATAGCAGTTGTCGCAGTTTTACA ACCCTACATCTTTGTTGCAACAGTGCCAGTGATAGTGGCTTT TATTATGTTGAGAGCATATTTCCTCCAAACCTCACAGCAACTC

		AAACAACTGGAATCTGAAGGCAGGAGTCCAATTTTCACTCAT CTTGTTACAAGCTTAAAAGGACTATGGACACTTCGTGCCTTC GGACGGCAGCCTTACTTTGAAACTCTGTTCCACAAAGCTCTG AATTTACATACTGCCAACTGGTTCTTGTACCTGTCAACACTGC GCTGGTTCCAAATGAGAATAGAAATGATTTTTGTCATCTTCTT CATTGCTGTTACCTTCATTTCCATTTTAACAACAGGAGAAGGA GAAGGAAGAGTTGGTATTATCCTGACTTTAGCCATGAATATC ATGAGTACATTGCAGTGGGCTGTAAACTCCAGCATAGATGTG GATAGCTTGATGCGATCTGTGAGCCGAGTCTTTAAGTTCATT GACATGCCAACAGAAGGTAAACCTACCAAGTCAACCAAACCA TACAAGAATGGCCAACTCTCGAAAGTTATGATTATTGAGAATT CACACGTGAAGAAAGATGACATCTGGCCCTCAGGGGGCCAA ATGACTGTCAAAGATCTCACAGCAAAATACACAGAAGGTGGA AATGCCATATTAGAGAACATTTCCTTCTCAATAAGTCCTGGCC AGAGGGTGGGCCTCTTGGGAAGAACTGGATCAGGGAAGAGT ACTTTGTTATCAGCTTTTTTGAGACTACTGAACACTGAAGGAG AAATCCAGATCGATGGTGTGTCTTGGGATTCAATAACTTTGC AACAGTGGAGGAAAGCCTTTGGAGTGATACCACAGAAAGTAT TTATTTTTTCTGGAACATTTAGAAAAAACTTGGATCCCTATGA ACAGTGGAGTGATCAAGAAATATGGAAAGTTGCAGATGAGGT TGGGCTCAGATCTGTGATAGAACAGTTTCCTGGGAAGCTTGA CTTTGTCCTTGTGGATGGGGGCTGTGTCCTAAGCCATGGCC ACAAGCAGTTGATGTGCTTGGCTAGATCTGTTCTCAGTAAGG CGAAGATCTTGCTGCTTGATGAACCCAGTGCTCATTTGGATC CAGTAACATACCAAATAATTAGAAGAACTCTAAAACAAGCATT TGCTGATTGCACAGTAATTCTCTGTGAACACAGGATAGAAGC AATGCTGGAATGCCAACAATTTTTGGTCATAGAAGAGAACAA AGTGCGGCAGTACGATTCCATCCAGAAACTGCTGAACGAGA GGAGCCTCTTCCGGCAAGCCATCAGCCCCTCCGACAGGGTG AAGCTCTTTCCCCACCGGAACTCAAGCAAGTGCAAGTCTAAG CCCCAGATTGCTGCTCTGAAAGAGGAGACAGAAGAAGAGGT GCAAGATACAAGGCTTTAG
8	F5 Enhancer, Tg83 Promoter, 5'-UTR, hCFTRΔR, 3'-UTR	GAATTCGTGGTGAGCGTCTGGGCATGTCTGGGCATGTCTGG GCATGTCTGGGCATGTCGGGCATTCTGGGCGTCTGGGCATG TCTGGGCATGTCTGGGCATCTCGAGAACGGTGACGTGCACG CGTGGGCGGAGCCATCACGCAGGTTGCTATATAAGCAGAGC TCGTTTAGTGAACCGTCAGAGTCGAGCCCGAGAGACCATGC AGAGGTCGCCTCTGGAAAAGGCCAGCGTTGTCTCCAAACTTT TTTTCAGCTGGACCAGACCAATTTTGAGGAAAGGATACAGAC AGCGCCTGGAATTGTCAGACATATACCAAATCCCTTCTGTTG ATTCTGCTGACAATCTATCTGAAAAATTGGAAAGAGAATGGG ATAGAGAGCTGGCTTCAAAGAAAAATCCTAAACTCATTAATG CCCTTCGGCGATGTTTTTTCTGGAGATTTATGTTCTATGGAAT CTTTTTATATTTAGGGGAAGTCACCAAAGCAGTACAGCCTCT CTTACTGGGAAGAATCATAGCTTCCTATGACCCGGATAACAA GGAGGAACGCTCTATCGCGATTTATCTAGGCATAGGCTTATG CCTTCTCTTTATTGTGAGGACACTGCTCCTACACCCAGCCAT TTTTGGCCTTCATCACATTGGAATGCAGATGAGAATAGCTAT GTTTAGTTTGATTTATAAGAAGACTTTAAAGCTGTCAAGCCGT GTTCTAGATAAAATAAGTATTGGACAACTTGTTAGTCTCCTTT CCAACAACCTGAACAAATTTGATGAAGGACTTGCATTGGCAC ATTTCGTGTGGATCGCTCCTTTGCAAGTGGCACTCCTCATGG GGCTAATCTGGGAGTTGTTACAGGCGTCTGCCTTCTGTGGA CTTGGTTTCCTGATAGTCCTTGCCCTTTTTCAGGCTGGGCTA GGGAGAATGATGATGAAGTACAGAGATCAGAGAGCTGGGAA GATCAGTGAAAGACTTGTGATTACCTCAGAAATGATCGAGAA CATCCAATCTGTTAAGGCATACTGCTGGGAAGAAGCAATGGA AAAAATGATTGAAAACTTAAGACAAACAGAACTGAAACTGACT CGGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGCCTTC TTCTTCTCAGGGTTCTTTGTGGTGTTTTTATCTGTGCTTCCCT ATGCACTAATCAAAGGAATCATCCTCCGGAAAATATTCACCA

		CCATCTCATTCTGCATTGTTCTGCGCATGGCGGTCACTCGGC AATTTCCCTGGGCTGTACAAACATGGTATGACTCTCTTGGAG CAATAAACAAAATACAGGATTTCTTACAAAAGCAAGAATATAA GACATTGGAATATAACTTAACGACTACAGAAGTAGTGATGGA GAATGTAACAGCCTTCTGGGAGGAGGGATTTGGGGAATTATT TGAGAAAGCAAAACAAAACAATAACAATAGAAAAACTTCTAAT GGTGATGACAGCCTCTTCTTCAGTAATTTCTCACTTCTTGGTA CTCCTGTCCTGAAAGATATTAATTTCAAGATAGAAAGAGGAC AGTTGTTGGCGGTTGCTGGATCCACTGGAGCAGGCAAGACT TCACTTCTAATGATGATTATGGGAGAACTGGAGCCTTCAGAG GGTAAAATTAAGCACAGTGGAAGAATTTCATTCTGTTCTCAGT TTTCCTGGATTATGCCTGGCACCATTAAAGAAAATATCATCTT TGGTGTTTCCTATGATGAATATAGATACAGAAGCGTCATCAAA GCATGCCAACTAGAAGAGGACATCTCCAAGTTTGCAGAGAAA GACAATATAGTTCTTGGAGAAGGTGGAATCACACTGAGTGGA GGTCAACGAGCAAGAATTTCTTTAGCAAGAGCAGTATACAAA GATGCTGATTTGTATTTATTAGACTCTCCTTTTGGATACCTAG ATGTTTTAACAGAAAAAGAAATATTTGAAAGCTGTGTCTGTAA ACTGATGGCTAACAAAACTAGGATTTTGGTCACTTCTAAAATG GAACATTTAAAGAAAGCTGACAAAATATTAATTTTGCATGAAG GTAGCAGCTATTTTTATGGGACATTTTCAGAACTCCAAAATCT ACAGCCAGACTTTAGCTCAAAACTCATGGGATGTGATTCTTT CGACCAATTTAGTGCAGAAAGAAGAAATTCAATCCTAACTGA GACCTTACACCGTTTCTCATTAGAAGGAGATGCTCCTGTCTC CTGGACAGAAACAAAAAAACAATCTTTTAAACAGACTGGAGA GTTTGGGGAAAAAAGGAAGAATTCTATTCTCAATCCAATCAA CTCTACGCTTCAGGCACGAAGGAGGCAGTCTGTCCTGAACC TGATGACACACTCAGTTAACCAAGGTCAGAACATTCACCGAA AGACAACAGCATCCACACGAAAAGTGTCACTGGCCCCTCAG GCAAACTTGACTGAACTGGATATATATTCAAGAAGGTTATCTC AAGAAACTGGCTTGGAAATAAGTGAAGAAATTAACGAAGAAG ACTTAAAGGAGTGCCTTTTTGATGATATGGAGAGCATACCAG CAGTGACTACATGGAACACATACCTTCGATATATTACTGTCCA CAAGAGCTTAATTTTTGTGCTAATTTGGTGCTTAGTAATTTTT CTGGCAGAGGTGGCTGCTTCTTTGGTTGTGCTGTGGCTCCTT GGAAACACTCCTCTTCAAGACAAAGGGAATAGTACTCATAGT AGAAATAACAGCTATGCAGTGATTATCACCAGCACCAGTTCG TATTATGTGTTTTACATTTACGTGGGAGTAGCCGACACTTTGC TTGCTATGGGATTCTTCAGAGGTCTACCACTGGTGCATACTC TAATCACAGTGTCGAAAATTTTACACCACAAAATGTTACATTC TGTTCTTCAAGCACCTATGTCAACCCTCAACACGTTGAAAGC AGGTGGGATTCTTAATAGATTCTCCAAAGATATAGCAATTTTG GATGACCTTCTGCCTCTTACCATATTTGACTTCATCCAGTTGT TATTAATTGTGATTGGAGCTATAGCAGTTGTCGCAGTTTTACA ACCCTACATCTTTGTTGCAACAGTGCCAGTGATAGTGGCTTT TATTATGTTGAGAGCATATTTCCTCCAAACCTCACAGCAACTC AAACAACTGGAATCTGAAGGCAGGAGTCCAATTTTCACTCAT CTTGTTACAAGCTTAAAAGGACTATGGACACTTCGTGCCTTC GGACGGCAGCCTTACTTTGAAACTCTGTTCCACAAAGCTCTG AATTTACATACTGCCAACTGGTTCTTGTACCTGTCAACACTGC GCTGGTTCCAAATGAGAATAGAAATGATTTTTGTCATCTTCTT CATTGCTGTTACCTTCATTTCCATTTTAACAACAGGAGAAGGA GAAGGAAGAGTTGGTATTATCCTGACTTTAGCCATGAATATC ATGAGTACATTGCAGTGGGCTGTAAACTCCAGCATAGATGTG GATAGCTTGATGCGATCTGTGAGCCGAGTCTTTAAGTTCATT GACATGCCAACAGAAGGTAAACCTACCAAGTCAACCAAACCA TACAAGAATGGCCAACTCTCGAAAGTTATGATTATTGAGAATT CACACGTGAAGAAAGATGACATCTGGCCCTCAGGGGGCCAA ATGACTGTCAAAGATCTCACAGCAAAATACACAGAAGGTGGA AATGCCATATTAGAGAACATTTCCTTCTCAATAAGTCCTGGCC AGAGGGTGGGCCTCTTGGGAAGAACTGGATCAGGGAAGAGT

		ACTTTGTTATCAGCTTTTTTGAGACTACTGAACACTGAAGGAG AAATCCAGATCGATGGTGTGTCTTGGGATTCAATAACTTTGC AACAGTGGAGGAAAGCCTTTGGAGTGATACCACAGAAAGTAT TTATTTTTTCTGGAACATTTAGAAAAAACTTGGATCCCTATGA ACAGTGGAGTGATCAAGAAATATGGAAAGTTGCAGATGAGGT TGGGCTCAGATCTGTGATAGAACAGTTTCCTGGGAAGCTTGA CTTTGTCCTTGTGGATGGGGGCTGTGTCCTAAGCCATGGCC ACAAGCAGTTGATGTGCTTGGCTAGATCTGTTCTCAGTAAGG CGAAGATCTTGCTGCTTGATGAACCCAGTGCTCATTTGGATC CAGTAACATACCAAATAATTAGAAGAACTCTAAAACAAGCATT TGCTGATTGCACAGTAATTCTCTGTGAACACAGGATAGAAGC AATGCTGGAATGCCAACAATTTTTGGTCATAGAAGAGAACAA AGTGCGGCAGTACGATTCCATCCAGAAACTGCTGAACGAGA GGAGCCTCTTCCGGCAAGCCATCAGCCCCTCCGACAGGGTG AAGCTCTTTCCCCACCGGAACTCAAGCAAGTGCAAGTCTAAG CCCCAGATTGCTGCTCTGAAAGAGGAGACAGAAGAAGAGGT GCAAGATACAAGGCTTTAGAGAGCAGCATAAATGTTGACATG GGACATTTGCTCATGGAATTGG
9	5’ AAV ITR	TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGC CGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGG GCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGG CCAACTCCATCACTAGGGGTTCCT
10	3’ AAV ITR	AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGC GCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGC GTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCG AGCGCGCAGAGAGGGAGTGGCCAA
11	5’ AAV ITR through 3’ ITR	TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGC CGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGG GCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGG CCAACTCCATCACTAGGGGTTCCTCAGATCTGAATTCGTGGT GAGCGTCTGGGCATGTCTGGGCATGTCTGGGCATGTCTGGG CATGTCGGGCATTCTGGGCGTCTGGGCATGTCTGGGCATGT CTGGGCATCTCGAGAACGGTGACGTGCACGCGTGGGCGGA GCCATCACGCAGGTTGCTATATAAGCAGAGCTCGTTTAGTGA ACCGTCAGAGTCGAGCCCGAGAGACCATGCAGAGGTCGCCT CTGGAAAAGGCCAGCGTTGTCTCCAAACTTTTTTTCAGCTGG ACCAGACCAATTTTGAGGAAAGGATACAGACAGCGCCTGGA ATTGTCAGACATATACCAAATCCCTTCTGTTGATTCTGCTGAC AATCTATCTGAAAAATTGGAAAGAGAATGGGATAGAGAGCTG GCTTCAAAGAAAAATCCTAAACTCATTAATGCCCTTCGGCGA TGTTTTTTCTGGAGATTTATGTTCTATGGAATCTTTTTATATTT AGGGGAAGTCACCAAAGCAGTACAGCCTCTCTTACTGGGAA GAATCATAGCTTCCTATGACCCGGATAACAAGGAGGAACGCT CTATCGCGATTTATCTAGGCATAGGCTTATGCCTTCTCTTTAT TGTGAGGACACTGCTCCTACACCCAGCCATTTTTGGCCTTCA TCACATTGGAATGCAGATGAGAATAGCTATGTTTAGTTTGATT TATAAGAAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAA TAAGTATTGGACAACTTGTTAGTCTCCTTTCCAACAACCTGAA CAAATTTGATGAAGGACTTGCATTGGCACATTTCGTGTGGAT CGCTCCTTTGCAAGTGGCACTCCTCATGGGGCTAATCTGGG AGTTGTTACAGGCGTCTGCCTTCTGTGGACTTGGTTTCCTGA TAGTCCTTGCCCTTTTTCAGGCTGGGCTAGGGAGAATGATGA TGAAGTACAGAGATCAGAGAGCTGGGAAGATCAGTGAAAGA CTTGTGATTACCTCAGAAATGATCGAGAACATCCAATCTGTTA AGGCATACTGCTGGGAAGAAGCAATGGAAAAAATGATTGAAA ACTTAAGACAAACAGAACTGAAACTGACTCGGAAGGCAGCCT ATGTGAGATACTTCAATAGCTCAGCCTTCTTCTTCTCAGGGTT CTTTGTGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAA GGAATCATCCTCCGGAAAATATTCACCACCATCTCATTCTGC ATTGTTCTGCGCATGGCGGTCACTCGGCAATTTCCCTGGGCT GTACAAACATGGTATGACTCTCTTGGAGCAATAAACAAAATA

		CAGGATTTCTTACAAAAGCAAGAATATAAGACATTGGAATATA ACTTAACGACTACAGAAGTAGTGATGGAGAATGTAACAGCCT TCTGGGAGGAGGGATTTGGGGAATTATTTGAGAAAGCAAAA CAAAACAATAACAATAGAAAAACTTCTAATGGTGATGACAGC CTCTTCTTCAGTAATTTCTCACTTCTTGGTACTCCTGTCCTGA AAGATATTAATTTCAAGATAGAAAGAGGACAGTTGTTGGCGG TTGCTGGATCCACTGGAGCAGGCAAGACTTCACTTCTAATGA TGATTATGGGAGAACTGGAGCCTTCAGAGGGTAAAATTAAGC ACAGTGGAAGAATTTCATTCTGTTCTCAGTTTTCCTGGATTAT GCCTGGCACCATTAAAGAAAATATCATCTTTGGTGTTTCCTAT GATGAATATAGATACAGAAGCGTCATCAAAGCATGCCAACTA GAAGAGGACATCTCCAAGTTTGCAGAGAAAGACAATATAGTT CTTGGAGAAGGTGGAATCACACTGAGTGGAGGTCAACGAGC AAGAATTTCTTTAGCAAGAGCAGTATACAAAGATGCTGATTTG TATTTATTAGACTCTCCTTTTGGATACCTAGATGTTTTAACAG AAAAAGAAATATTTGAAAGCTGTGTCTGTAAACTGATGGCTAA CAAAACTAGGATTTTGGTCACTTCTAAAATGGAACATTTAAAG AAAGCTGACAAAATATTAATTTTGCATGAAGGTAGCAGCTATT TTTATGGGACATTTTCAGAACTCCAAAATCTACAGCCAGACTT TAGCTCAAAACTCATGGGATGTGATTCTTTCGACCAATTTAGT GCAGAAAGAAGAAATTCAATCCTAACTGAGACCTTACACCGT TTCTCATTAGAAGGAGATGCTCCTGTCTCCTGGACAGAAACA AAAAAACAATCTTTTAAACAGACTGGAGAGTTTGGGGAAAAA AGGAAGAATTCTATTCTCAATCCAATCAACTCTACGCTTCAGG CACGAAGGAGGCAGTCTGTCCTGAACCTGATGACACACTCA GTTAACCAAGGTCAGAACATTCACCGAAAGACAACAGCATCC ACACGAAAAGTGTCACTGGCCCCTCAGGCAAACTTGACTGA ACTGGATATATATTCAAGAAGGTTATCTCAAGAAACTGGCTTG GAAATAAGTGAAGAAATTAACGAAGAAGACTTAAAGGAGTGC CTTTTTGATGATATGGAGAGCATACCAGCAGTGACTACATGG AACACATACCTTCGATATATTACTGTCCACAAGAGCTTAATTT TTGTGCTAATTTGGTGCTTAGTAATTTTTCTGGCAGAGGTGG CTGCTTCTTTGGTTGTGCTGTGGCTCCTTGGAAACACTCCTC TTCAAGACAAAGGGAATAGTACTCATAGTAGAAATAACAGCT ATGCAGTGATTATCACCAGCACCAGTTCGTATTATGTGTTTTA CATTTACGTGGGAGTAGCCGACACTTTGCTTGCTATGGGATT CTTCAGAGGTCTACCACTGGTGCATACTCTAATCACAGTGTC GAAAATTTTACACCACAAAATGTTACATTCTGTTCTTCAAGCA CCTATGTCAACCCTCAACACGTTGAAAGCAGGTGGGATTCTT AATAGATTCTCCAAAGATATAGCAATTTTGGATGACCTTCTGC CTCTTACCATATTTGACTTCATCCAGTTGTTATTAATTGTGATT GGAGCTATAGCAGTTGTCGCAGTTTTACAACCCTACATCTTT GTTGCAACAGTGCCAGTGATAGTGGCTTTTATTATGTTGAGA GCATATTTCCTCCAAACCTCACAGCAACTCAAACAACTGGAA TCTGAAGGCAGGAGTCCAATTTTCACTCATCTTGTTACAAGC TTAAAAGGACTATGGACACTTCGTGCCTTCGGACGGCAGCCT TACTTTGAAACTCTGTTCCACAAAGCTCTGAATTTACATACTG CCAACTGGTTCTTGTACCTGTCAACACTGCGCTGGTTCCAAA TGAGAATAGAAATGATTTTTGTCATCTTCTTCATTGCTGTTAC CTTCATTTCCATTTTAACAACAGGAGAAGGAGAAGGAAGAGT TGGTATTATCCTGACTTTAGCCATGAATATCATGAGTACATTG CAGTGGGCTGTAAACTCCAGCATAGATGTGGATAGCTTGATG CGATCTGTGAGCCGAGTCTTTAAGTTCATTGACATGCCAACA GAAGGTAAACCTACCAAGTCAACCAAACCATACAAGAATGGC CAACTCTCGAAAGTTATGATTATTGAGAATTCACACGTGAAGA AAGATGACATCTGGCCCTCAGGGGGCCAAATGACTGTCAAA GATCTCACAGCAAAATACACAGAAGGTGGAAATGCCATATTA GAGAACATTTCCTTCTCAATAAGTCCTGGCCAGAGGGTGGG CCTCTTGGGAAGAACTGGATCAGGGAAGAGTACTTTGTTATC AGCTTTTTTGAGACTACTGAACACTGAAGGAGAAATCCAGAT CGATGGTGTGTCTTGGGATTCAATAACTTTGCAACAGTGGAG

		GAAAGCCTTTGGAGTGATACCACAGAAAGTATTTATTTTTTCT GGAACATTTAGAAAAAACTTGGATCCCTATGAACAGTGGAGT GATCAAGAAATATGGAAAGTTGCAGATGAGGTTGGGCTCAG ATCTGTGATAGAACAGTTTCCTGGGAAGCTTGACTTTGTCCT TGTGGATGGGGGCTGTGTCCTAAGCCATGGCCACAAGCAGT TGATGTGCTTGGCTAGATCTGTTCTCAGTAAGGCGAAGATCT TGCTGCTTGATGAACCCAGTGCTCATTTGGATCCAGTAACAT ACCAAATAATTAGAAGAACTCTAAAACAAGCATTTGCTGATTG CACAGTAATTCTCTGTGAACACAGGATAGAAGCAATGCTGGA ATGCCAACAATTTTTGGTCATAGAAGAGAACAAAGTGCGGCA GTACGATTCCATCCAGAAACTGCTGAACGAGAGGAGCCTCTT CCGGCAAGCCATCAGCCCCTCCGACAGGGTGAAGCTCTTTC CCCACCGGAACTCAAGCAAGTGCAAGTCTAAGCCCCAGATT GCTGCTCTGAAAGAGGAGACAGAAGAAGAGGTGCAAGATAC AAGGCTTTAGAGAGCAGCATAAATGTTGACATGGGACATTTG CTCATGGAATTGGCAGGCCTAATAAAGAGCTCAGATGCATCG ATCAGAGTGTGTTGGTTTTTTGTGTGTACTGAGGAACCCCTA GTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCT CACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACC TTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAG AGGGAGTGGCCAA
12	pAV-F5tg83-hCFTR-dR vector	TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGC CGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGG GCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGG CCAACTCCATCACTAGGGGTTCCTCAGATCTGAATTCGTGGT GAGCGTCTGGGCATGTCTGGGCATGTCTGGGCATGTCTGGG CATGTCGGGCATTCTGGGCGTCTGGGCATGTCTGGGCATGT CTGGGCATCTCGAGAACGGTGACGTGCACGCGTGGGCGGA GCCATCACGCAGGTTGCTATATAAGCAGAGCTCGTTTAGTGA ACCGTCAGAGTCGAGCCCGAGAGACCATGCAGAGGTCGCCT CTGGAAAAGGCCAGCGTTGTCTCCAAACTTTTTTTCAGCTGG ACCAGACCAATTTTGAGGAAAGGATACAGACAGCGCCTGGA ATTGTCAGACATATACCAAATCCCTTCTGTTGATTCTGCTGAC AATCTATCTGAAAAATTGGAAAGAGAATGGGATAGAGAGCTG GCTTCAAAGAAAAATCCTAAACTCATTAATGCCCTTCGGCGA TGTTTTTTCTGGAGATTTATGTTCTATGGAATCTTTTTATATTT AGGGGAAGTCACCAAAGCAGTACAGCCTCTCTTACTGGGAA GAATCATAGCTTCCTATGACCCGGATAACAAGGAGGAACGCT CTATCGCGATTTATCTAGGCATAGGCTTATGCCTTCTCTTTAT TGTGAGGACACTGCTCCTACACCCAGCCATTTTTGGCCTTCA TCACATTGGAATGCAGATGAGAATAGCTATGTTTAGTTTGATT TATAAGAAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAA TAAGTATTGGACAACTTGTTAGTCTCCTTTCCAACAACCTGAA CAAATTTGATGAAGGACTTGCATTGGCACATTTCGTGTGGAT CGCTCCTTTGCAAGTGGCACTCCTCATGGGGCTAATCTGGG AGTTGTTACAGGCGTCTGCCTTCTGTGGACTTGGTTTCCTGA TAGTCCTTGCCCTTTTTCAGGCTGGGCTAGGGAGAATGATGA TGAAGTACAGAGATCAGAGAGCTGGGAAGATCAGTGAAAGA CTTGTGATTACCTCAGAAATGATCGAGAACATCCAATCTGTTA AGGCATACTGCTGGGAAGAAGCAATGGAAAAAATGATTGAAA ACTTAAGACAAACAGAACTGAAACTGACTCGGAAGGCAGCCT ATGTGAGATACTTCAATAGCTCAGCCTTCTTCTTCTCAGGGTT CTTTGTGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAA GGAATCATCCTCCGGAAAATATTCACCACCATCTCATTCTGC ATTGTTCTGCGCATGGCGGTCACTCGGCAATTTCCCTGGGCT GTACAAACATGGTATGACTCTCTTGGAGCAATAAACAAAATA CAGGATTTCTTACAAAAGCAAGAATATAAGACATTGGAATATA ACTTAACGACTACAGAAGTAGTGATGGAGAATGTAACAGCCT TCTGGGAGGAGGGATTTGGGGAATTATTTGAGAAAGCAAAA CAAAACAATAACAATAGAAAAACTTCTAATGGTGATGACAGC CTCTTCTTCAGTAATTTCTCACTTCTTGGTACTCCTGTCCTGA

		AAGATATTAATTTCAAGATAGAAAGAGGACAGTTGTTGGCGG TTGCTGGATCCACTGGAGCAGGCAAGACTTCACTTCTAATGA TGATTATGGGAGAACTGGAGCCTTCAGAGGGTAAAATTAAGC ACAGTGGAAGAATTTCATTCTGTTCTCAGTTTTCCTGGATTAT GCCTGGCACCATTAAAGAAAATATCATCTTTGGTGTTTCCTAT GATGAATATAGATACAGAAGCGTCATCAAAGCATGCCAACTA GAAGAGGACATCTCCAAGTTTGCAGAGAAAGACAATATAGTT CTTGGAGAAGGTGGAATCACACTGAGTGGAGGTCAACGAGC AAGAATTTCTTTAGCAAGAGCAGTATACAAAGATGCTGATTTG TATTTATTAGACTCTCCTTTTGGATACCTAGATGTTTTAACAG AAAAAGAAATATTTGAAAGCTGTGTCTGTAAACTGATGGCTAA CAAAACTAGGATTTTGGTCACTTCTAAAATGGAACATTTAAAG AAAGCTGACAAAATATTAATTTTGCATGAAGGTAGCAGCTATT TTTATGGGACATTTTCAGAACTCCAAAATCTACAGCCAGACTT TAGCTCAAAACTCATGGGATGTGATTCTTTCGACCAATTTAGT GCAGAAAGAAGAAATTCAATCCTAACTGAGACCTTACACCGT TTCTCATTAGAAGGAGATGCTCCTGTCTCCTGGACAGAAACA AAAAAACAATCTTTTAAACAGACTGGAGAGTTTGGGGAAAAA AGGAAGAATTCTATTCTCAATCCAATCAACTCTACGCTTCAGG CACGAAGGAGGCAGTCTGTCCTGAACCTGATGACACACTCA GTTAACCAAGGTCAGAACATTCACCGAAAGACAACAGCATCC ACACGAAAAGTGTCACTGGCCCCTCAGGCAAACTTGACTGA ACTGGATATATATTCAAGAAGGTTATCTCAAGAAACTGGCTTG GAAATAAGTGAAGAAATTAACGAAGAAGACTTAAAGGAGTGC CTTTTTGATGATATGGAGAGCATACCAGCAGTGACTACATGG AACACATACCTTCGATATATTACTGTCCACAAGAGCTTAATTT TTGTGCTAATTTGGTGCTTAGTAATTTTTCTGGCAGAGGTGG CTGCTTCTTTGGTTGTGCTGTGGCTCCTTGGAAACACTCCTC TTCAAGACAAAGGGAATAGTACTCATAGTAGAAATAACAGCT ATGCAGTGATTATCACCAGCACCAGTTCGTATTATGTGTTTTA CATTTACGTGGGAGTAGCCGACACTTTGCTTGCTATGGGATT CTTCAGAGGTCTACCACTGGTGCATACTCTAATCACAGTGTC GAAAATTTTACACCACAAAATGTTACATTCTGTTCTTCAAGCA CCTATGTCAACCCTCAACACGTTGAAAGCAGGTGGGATTCTT AATAGATTCTCCAAAGATATAGCAATTTTGGATGACCTTCTGC CTCTTACCATATTTGACTTCATCCAGTTGTTATTAATTGTGATT GGAGCTATAGCAGTTGTCGCAGTTTTACAACCCTACATCTTT GTTGCAACAGTGCCAGTGATAGTGGCTTTTATTATGTTGAGA GCATATTTCCTCCAAACCTCACAGCAACTCAAACAACTGGAA TCTGAAGGCAGGAGTCCAATTTTCACTCATCTTGTTACAAGC TTAAAAGGACTATGGACACTTCGTGCCTTCGGACGGCAGCCT TACTTTGAAACTCTGTTCCACAAAGCTCTGAATTTACATACTG CCAACTGGTTCTTGTACCTGTCAACACTGCGCTGGTTCCAAA TGAGAATAGAAATGATTTTTGTCATCTTCTTCATTGCTGTTAC CTTCATTTCCATTTTAACAACAGGAGAAGGAGAAGGAAGAGT TGGTATTATCCTGACTTTAGCCATGAATATCATGAGTACATTG CAGTGGGCTGTAAACTCCAGCATAGATGTGGATAGCTTGATG CGATCTGTGAGCCGAGTCTTTAAGTTCATTGACATGCCAACA GAAGGTAAACCTACCAAGTCAACCAAACCATACAAGAATGGC CAACTCTCGAAAGTTATGATTATTGAGAATTCACACGTGAAGA AAGATGACATCTGGCCCTCAGGGGGCCAAATGACTGTCAAA GATCTCACAGCAAAATACACAGAAGGTGGAAATGCCATATTA GAGAACATTTCCTTCTCAATAAGTCCTGGCCAGAGGGTGGG CCTCTTGGGAAGAACTGGATCAGGGAAGAGTACTTTGTTATC AGCTTTTTTGAGACTACTGAACACTGAAGGAGAAATCCAGAT CGATGGTGTGTCTTGGGATTCAATAACTTTGCAACAGTGGAG GAAAGCCTTTGGAGTGATACCACAGAAAGTATTTATTTTTTCT GGAACATTTAGAAAAAACTTGGATCCCTATGAACAGTGGAGT GATCAAGAAATATGGAAAGTTGCAGATGAGGTTGGGCTCAG ATCTGTGATAGAACAGTTTCCTGGGAAGCTTGACTTTGTCCT TGTGGATGGGGGCTGTGTCCTAAGCCATGGCCACAAGCAGT

		TGATGTGCTTGGCTAGATCTGTTCTCAGTAAGGCGAAGATCT TGCTGCTTGATGAACCCAGTGCTCATTTGGATCCAGTAACAT ACCAAATAATTAGAAGAACTCTAAAACAAGCATTTGCTGATTG CACAGTAATTCTCTGTGAACACAGGATAGAAGCAATGCTGGA ATGCCAACAATTTTTGGTCATAGAAGAGAACAAAGTGCGGCA GTACGATTCCATCCAGAAACTGCTGAACGAGAGGAGCCTCTT CCGGCAAGCCATCAGCCCCTCCGACAGGGTGAAGCTCTTTC CCCACCGGAACTCAAGCAAGTGCAAGTCTAAGCCCCAGATT GCTGCTCTGAAAGAGGAGACAGAAGAAGAGGTGCAAGATAC AAGGCTTTAGAGAGCAGCATAAATGTTGACATGGGACATTTG CTCATGGAATTGGCAGGCCTAATAAAGAGCTCAGATGCATCG ATCAGAGTGTGTTGGTTTTTTGTGTGTACTGAGGAACCCCTA GTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCT CACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACC TTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAG AGGGAGTGGCCAACCCCCCCCCCCCCCCCCTGCAGCCAG CTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCC AACAGTTGCGTAGCCTGAATGGCGAATGGCGCGACGCGCCC TGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGC GCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCT CCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGC TTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTC CGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGAT TAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGAC GGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGT GGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCG GTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCT ATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAA TTTTAACAAAATATTAACGTTTACAATTTCCTGATGCGGTATTT TCTCCTTACGCATCTGTGCGGTATTTCACACCGCATATGGTG CACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCA GCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGG GCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGAC CGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCAT CACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTA TTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGT CAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATT TGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAG ACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAG AGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTT TTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGC TGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGA GTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTT GAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACT TTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGAC GCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCA GAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCT TACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCAT AACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAAC GATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACAT GGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGC TGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATG CCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGC GAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGG ATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGC CCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGG TGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAG ATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGA GTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAG ATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAA GTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTA

		ATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATG ACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCA GACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTT TTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCG CTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACT CTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCA AATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTC AAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATC CTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTT ACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCA GCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGC TTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCG TGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGG CGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGA GCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTT ATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGAT TTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAAC GCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTG GCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCT GTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCT CGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCG AGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCC GCGCGTTGGCCGATTCATTAATGCAGCTGGGCTGCAGGGGG GGGGGGGGGGGGGG
13	AV.TL65 capsid protein	MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDS RGLVLPGYKYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDS GDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKKRVLEP FGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEA GPSGSQQLQIPAQPASSLGADTMSAGGGGPLGDNNQGADGV GNASGDWHCDSTWMGDRVVTKSTRTWVLPSYNNHQYREIKS GSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINN YWGFRPRSLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDD DYQLPYVVGNGTEGCLPAFPPQVFTLPQYGYATLNRDNTENPT ERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFHSSFAPSQNLF KLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFP GPMGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQ PNGMTNNLQGSNTYALENTMIFNSQPANPGTTATYLEGNMLIT SESETQPVNRVAYNVGGQMATNNQSSTTAPTTGTYNLQEIVPG SVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPM MLIKNTPVPGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKEN SKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTTRPIGTRYLTR PL
14	F5 enhancer	GTGGTGAGCGTCTGGGCATGTCTGGGCATGTCTGGGCATGT CTGGGCATGTCGGGCATTCTGGGCGTCTGGGCATGTCTGGG CATGTCTGGGCAT
15	5’ AAV ITR (flop)	CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCC CGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGG CCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA CTCCATCACTAGGGGTTCCT
16	3’ AAV ITR (flop)	AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGC GCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCC GACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCG AGCGCGCAGAGAGGGAGTGGCC
17	5’ AAV ITR (flop) through 3’ AAV ITR (flop)	CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCC CGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGG CCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA CTCCATCACTAGGGGTTCCTCAGATCTGAATTCGTGGTGAGC GTCTGGGCATGTCTGGGCATGTCTGGGCATGTCTGGGCATG TCGGGCATTCTGGGCGTCTGGGCATGTCTGGGCATGTCTGG GCATCTCGAGAACGGTGACGTGCACGCGTGGGCGGAGCCA

		TCACGCAGGTTGCTATATAAGCAGAGCTCGTTTAGTGAACCG TCAGAGTCGAGCCCGAGAGACCATGCAGAGGTCGCCTCTGG AAAAGGCCAGCGTTGTCTCCAAACTTTTTTTCAGCTGGACCA GACCAATTTTGAGGAAAGGATACAGACAGCGCCTGGAATTGT CAGACATATACCAAATCCCTTCTGTTGATTCTGCTGACAATCT ATCTGAAAAATTGGAAAGAGAATGGGATAGAGAGCTGGCTTC AAAGAAAAATCCTAAACTCATTAATGCCCTTCGGCGATGTTTT TTCTGGAGATTTATGTTCTATGGAATCTTTTTATATTTAGGGG AAGTCACCAAAGCAGTACAGCCTCTCTTACTGGGAAGAATCA TAGCTTCCTATGACCCGGATAACAAGGAGGAACGCTCTATCG CGATTTATCTAGGCATAGGCTTATGCCTTCTCTTTATTGTGAG GACACTGCTCCTACACCCAGCCATTTTTGGCCTTCATCACAT TGGAATGCAGATGAGAATAGCTATGTTTAGTTTGATTTATAAG AAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAATAAGTA TTGGACAACTTGTTAGTCTCCTTTCCAACAACCTGAACAAATT TGATGAAGGACTTGCATTGGCACATTTCGTGTGGATCGCTCC TTTGCAAGTGGCACTCCTCATGGGGCTAATCTGGGAGTTGTT ACAGGCGTCTGCCTTCTGTGGACTTGGTTTCCTGATAGTCCT TGCCCTTTTTCAGGCTGGGCTAGGGAGAATGATGATGAAGTA CAGAGATCAGAGAGCTGGGAAGATCAGTGAAAGACTTGTGA TTACCTCAGAAATGATCGAGAACATCCAATCTGTTAAGGCAT ACTGCTGGGAAGAAGCAATGGAAAAAATGATTGAAAACTTAA GACAAACAGAACTGAAACTGACTCGGAAGGCAGCCTATGTG AGATACTTCAATAGCTCAGCCTTCTTCTTCTCAGGGTTCTTTG TGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAAGGAAT CATCCTCCGGAAAATATTCACCACCATCTCATTCTGCATTGTT CTGCGCATGGCGGTCACTCGGCAATTTCCCTGGGCTGTACA AACATGGTATGACTCTCTTGGAGCAATAAACAAAATACAGGA TTTCTTACAAAAGCAAGAATATAAGACATTGGAATATAACTTA ACGACTACAGAAGTAGTGATGGAGAATGTAACAGCCTTCTGG GAGGAGGGATTTGGGGAATTATTTGAGAAAGCAAAACAAAAC AATAACAATAGAAAAACTTCTAATGGTGATGACAGCCTCTTCT TCAGTAATTTCTCACTTCTTGGTACTCCTGTCCTGAAAGATAT TAATTTCAAGATAGAAAGAGGACAGTTGTTGGCGGTTGCTGG ATCCACTGGAGCAGGCAAGACTTCACTTCTAATGATGATTAT GGGAGAACTGGAGCCTTCAGAGGGTAAAATTAAGCACAGTG GAAGAATTTCATTCTGTTCTCAGTTTTCCTGGATTATGCCTGG CACCATTAAAGAAAATATCATCTTTGGTGTTTCCTATGATGAA TATAGATACAGAAGCGTCATCAAAGCATGCCAACTAGAAGAG GACATCTCCAAGTTTGCAGAGAAAGACAATATAGTTCTTGGA GAAGGTGGAATCACACTGAGTGGAGGTCAACGAGCAAGAAT TTCTTTAGCAAGAGCAGTATACAAAGATGCTGATTTGTATTTA TTAGACTCTCCTTTTGGATACCTAGATGTTTTAACAGAAAAAG AAATATTTGAAAGCTGTGTCTGTAAACTGATGGCTAACAAAAC TAGGATTTTGGTCACTTCTAAAATGGAACATTTAAAGAAAGCT GACAAAATATTAATTTTGCATGAAGGTAGCAGCTATTTTTATG GGACATTTTCAGAACTCCAAAATCTACAGCCAGACTTTAGCT CAAAACTCATGGGATGTGATTCTTTCGACCAATTTAGTGCAG AAAGAAGAAATTCAATCCTAACTGAGACCTTACACCGTTTCTC ATTAGAAGGAGATGCTCCTGTCTCCTGGACAGAAACAAAAAA ACAATCTTTTAAACAGACTGGAGAGTTTGGGGAAAAAAGGAA GAATTCTATTCTCAATCCAATCAACTCTACGCTTCAGGCACGA AGGAGGCAGTCTGTCCTGAACCTGATGACACACTCAGTTAAC CAAGGTCAGAACATTCACCGAAAGACAACAGCATCCACACG AAAAGTGTCACTGGCCCCTCAGGCAAACTTGACTGAACTGGA TATATATTCAAGAAGGTTATCTCAAGAAACTGGCTTGGAAATA AGTGAAGAAATTAACGAAGAAGACTTAAAGGAGTGCCTTTTT GATGATATGGAGAGCATACCAGCAGTGACTACATGGAACAC ATACCTTCGATATATTACTGTCCACAAGAGCTTAATTTTTGTG CTAATTTGGTGCTTAGTAATTTTTCTGGCAGAGGTGGCTGCT TCTTTGGTTGTGCTGTGGCTCCTTGGAAACACTCCTCTTCAA

		GACAAAGGGAATAGTACTCATAGTAGAAATAACAGCTATGCA GTGATTATCACCAGCACCAGTTCGTATTATGTGTTTTACATTT ACGTGGGAGTAGCCGACACTTTGCTTGCTATGGGATTCTTCA GAGGTCTACCACTGGTGCATACTCTAATCACAGTGTCGAAAA TTTTACACCACAAAATGTTACATTCTGTTCTTCAAGCACCTAT GTCAACCCTCAACACGTTGAAAGCAGGTGGGATTCTTAATAG ATTCTCCAAAGATATAGCAATTTTGGATGACCTTCTGCCTCTT ACCATATTTGACTTCATCCAGTTGTTATTAATTGTGATTGGAG CTATAGCAGTTGTCGCAGTTTTACAACCCTACATCTTTGTTGC AACAGTGCCAGTGATAGTGGCTTTTATTATGTTGAGAGCATA TTTCCTCCAAACCTCACAGCAACTCAAACAACTGGAATCTGA AGGCAGGAGTCCAATTTTCACTCATCTTGTTACAAGCTTAAAA GGACTATGGACACTTCGTGCCTTCGGACGGCAGCCTTACTTT GAAACTCTGTTCCACAAAGCTCTGAATTTACATACTGCCAACT GGTTCTTGTACCTGTCAACACTGCGCTGGTTCCAAATGAGAA TAGAAATGATTTTTGTCATCTTCTTCATTGCTGTTACCTTCATT TCCATTTTAACAACAGGAGAAGGAGAAGGAAGAGTTGGTATT ATCCTGACTTTAGCCATGAATATCATGAGTACATTGCAGTGG GCTGTAAACTCCAGCATAGATGTGGATAGCTTGATGCGATCT GTGAGCCGAGTCTTTAAGTTCATTGACATGCCAACAGAAGGT AAACCTACCAAGTCAACCAAACCATACAAGAATGGCCAACTC TCGAAAGTTATGATTATTGAGAATTCACACGTGAAGAAAGAT GACATCTGGCCCTCAGGGGGCCAAATGACTGTCAAAGATCT CACAGCAAAATACACAGAAGGTGGAAATGCCATATTAGAGAA CATTTCCTTCTCAATAAGTCCTGGCCAGAGGGTGGGCCTCTT GGGAAGAACTGGATCAGGGAAGAGTACTTTGTTATCAGCTTT TTTGAGACTACTGAACACTGAAGGAGAAATCCAGATCGATGG TGTGTCTTGGGATTCAATAACTTTGCAACAGTGGAGGAAAGC CTTTGGAGTGATACCACAGAAAGTATTTATTTTTTCTGGAACA TTTAGAAAAAACTTGGATCCCTATGAACAGTGGAGTGATCAA GAAATATGGAAAGTTGCAGATGAGGTTGGGCTCAGATCTGTG ATAGAACAGTTTCCTGGGAAGCTTGACTTTGTCCTTGTGGAT GGGGGCTGTGTCCTAAGCCATGGCCACAAGCAGTTGATGTG CTTGGCTAGATCTGTTCTCAGTAAGGCGAAGATCTTGCTGCT TGATGAACCCAGTGCTCATTTGGATCCAGTAACATACCAAAT AATTAGAAGAACTCTAAAACAAGCATTTGCTGATTGCACAGTA ATTCTCTGTGAACACAGGATAGAAGCAATGCTGGAATGCCAA CAATTTTTGGTCATAGAAGAGAACAAAGTGCGGCAGTACGAT TCCATCCAGAAACTGCTGAACGAGAGGAGCCTCTTCCGGCA AGCCATCAGCCCCTCCGACAGGGTGAAGCTCTTTCCCCACC GGAACTCAAGCAAGTGCAAGTCTAAGCCCCAGATTGCTGCT CTGAAAGAGGAGACAGAAGAAGAGGTGCAAGATACAAGGCT TTAGAGAGCAGCATAAATGTTGACATGGGACATTTGCTCATG GAATTGGCAGGCCTAATAAAGAGCTCAGATGCATCGATCAGA GTGTGTTGGTTTTTTGTGTGTACTGAGGAACCCCTAGTGATG GAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGA GGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCC CGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGA GTGGCC
18	pAV-F5tg83-hCFTR-dR (flop ITR) vector	CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCC CGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGG CCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA CTCCATCACTAGGGGTTCCTCAGATCTGAATTCGTGGTGAGC GTCTGGGCATGTCTGGGCATGTCTGGGCATGTCTGGGCATG TCGGGCATTCTGGGCGTCTGGGCATGTCTGGGCATGTCTGG GCATCTCGAGAACGGTGACGTGCACGCGTGGGCGGAGCCA TCACGCAGGTTGCTATATAAGCAGAGCTCGTTTAGTGAACCG TCAGAGTCGAGCCCGAGAGACCATGCAGAGGTCGCCTCTGG AAAAGGCCAGCGTTGTCTCCAAACTTTTTTTCAGCTGGACCA GACCAATTTTGAGGAAAGGATACAGACAGCGCCTGGAATTGT CAGACATATACCAAATCCCTTCTGTTGATTCTGCTGACAATCT

		ATCTGAAAAATTGGAAAGAGAATGGGATAGAGAGCTGGCTTC AAAGAAAAATCCTAAACTCATTAATGCCCTTCGGCGATGTTTT TTCTGGAGATTTATGTTCTATGGAATCTTTTTATATTTAGGGG AAGTCACCAAAGCAGTACAGCCTCTCTTACTGGGAAGAATCA TAGCTTCCTATGACCCGGATAACAAGGAGGAACGCTCTATCG CGATTTATCTAGGCATAGGCTTATGCCTTCTCTTTATTGTGAG GACACTGCTCCTACACCCAGCCATTTTTGGCCTTCATCACAT TGGAATGCAGATGAGAATAGCTATGTTTAGTTTGATTTATAAG AAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAATAAGTA TTGGACAACTTGTTAGTCTCCTTTCCAACAACCTGAACAAATT TGATGAAGGACTTGCATTGGCACATTTCGTGTGGATCGCTCC TTTGCAAGTGGCACTCCTCATGGGGCTAATCTGGGAGTTGTT ACAGGCGTCTGCCTTCTGTGGACTTGGTTTCCTGATAGTCCT TGCCCTTTTTCAGGCTGGGCTAGGGAGAATGATGATGAAGTA CAGAGATCAGAGAGCTGGGAAGATCAGTGAAAGACTTGTGA TTACCTCAGAAATGATCGAGAACATCCAATCTGTTAAGGCAT ACTGCTGGGAAGAAGCAATGGAAAAAATGATTGAAAACTTAA GACAAACAGAACTGAAACTGACTCGGAAGGCAGCCTATGTG AGATACTTCAATAGCTCAGCCTTCTTCTTCTCAGGGTTCTTTG TGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAAGGAAT CATCCTCCGGAAAATATTCACCACCATCTCATTCTGCATTGTT CTGCGCATGGCGGTCACTCGGCAATTTCCCTGGGCTGTACA AACATGGTATGACTCTCTTGGAGCAATAAACAAAATACAGGA TTTCTTACAAAAGCAAGAATATAAGACATTGGAATATAACTTA ACGACTACAGAAGTAGTGATGGAGAATGTAACAGCCTTCTGG GAGGAGGGATTTGGGGAATTATTTGAGAAAGCAAAACAAAAC AATAACAATAGAAAAACTTCTAATGGTGATGACAGCCTCTTCT TCAGTAATTTCTCACTTCTTGGTACTCCTGTCCTGAAAGATAT TAATTTCAAGATAGAAAGAGGACAGTTGTTGGCGGTTGCTGG ATCCACTGGAGCAGGCAAGACTTCACTTCTAATGATGATTAT GGGAGAACTGGAGCCTTCAGAGGGTAAAATTAAGCACAGTG GAAGAATTTCATTCTGTTCTCAGTTTTCCTGGATTATGCCTGG CACCATTAAAGAAAATATCATCTTTGGTGTTTCCTATGATGAA TATAGATACAGAAGCGTCATCAAAGCATGCCAACTAGAAGAG GACATCTCCAAGTTTGCAGAGAAAGACAATATAGTTCTTGGA GAAGGTGGAATCACACTGAGTGGAGGTCAACGAGCAAGAAT TTCTTTAGCAAGAGCAGTATACAAAGATGCTGATTTGTATTTA TTAGACTCTCCTTTTGGATACCTAGATGTTTTAACAGAAAAAG AAATATTTGAAAGCTGTGTCTGTAAACTGATGGCTAACAAAAC TAGGATTTTGGTCACTTCTAAAATGGAACATTTAAAGAAAGCT GACAAAATATTAATTTTGCATGAAGGTAGCAGCTATTTTTATG GGACATTTTCAGAACTCCAAAATCTACAGCCAGACTTTAGCT CAAAACTCATGGGATGTGATTCTTTCGACCAATTTAGTGCAG AAAGAAGAAATTCAATCCTAACTGAGACCTTACACCGTTTCTC ATTAGAAGGAGATGCTCCTGTCTCCTGGACAGAAACAAAAAA ACAATCTTTTAAACAGACTGGAGAGTTTGGGGAAAAAAGGAA GAATTCTATTCTCAATCCAATCAACTCTACGCTTCAGGCACGA AGGAGGCAGTCTGTCCTGAACCTGATGACACACTCAGTTAAC CAAGGTCAGAACATTCACCGAAAGACAACAGCATCCACACG AAAAGTGTCACTGGCCCCTCAGGCAAACTTGACTGAACTGGA TATATATTCAAGAAGGTTATCTCAAGAAACTGGCTTGGAAATA AGTGAAGAAATTAACGAAGAAGACTTAAAGGAGTGCCTTTTT GATGATATGGAGAGCATACCAGCAGTGACTACATGGAACAC ATACCTTCGATATATTACTGTCCACAAGAGCTTAATTTTTGTG CTAATTTGGTGCTTAGTAATTTTTCTGGCAGAGGTGGCTGCT TCTTTGGTTGTGCTGTGGCTCCTTGGAAACACTCCTCTTCAA GACAAAGGGAATAGTACTCATAGTAGAAATAACAGCTATGCA GTGATTATCACCAGCACCAGTTCGTATTATGTGTTTTACATTT ACGTGGGAGTAGCCGACACTTTGCTTGCTATGGGATTCTTCA GAGGTCTACCACTGGTGCATACTCTAATCACAGTGTCGAAAA TTTTACACCACAAAATGTTACATTCTGTTCTTCAAGCACCTAT

		GTCAACCCTCAACACGTTGAAAGCAGGTGGGATTCTTAATAG ATTCTCCAAAGATATAGCAATTTTGGATGACCTTCTGCCTCTT ACCATATTTGACTTCATCCAGTTGTTATTAATTGTGATTGGAG CTATAGCAGTTGTCGCAGTTTTACAACCCTACATCTTTGTTGC AACAGTGCCAGTGATAGTGGCTTTTATTATGTTGAGAGCATA TTTCCTCCAAACCTCACAGCAACTCAAACAACTGGAATCTGA AGGCAGGAGTCCAATTTTCACTCATCTTGTTACAAGCTTAAAA GGACTATGGACACTTCGTGCCTTCGGACGGCAGCCTTACTTT GAAACTCTGTTCCACAAAGCTCTGAATTTACATACTGCCAACT GGTTCTTGTACCTGTCAACACTGCGCTGGTTCCAAATGAGAA TAGAAATGATTTTTGTCATCTTCTTCATTGCTGTTACCTTCATT TCCATTTTAACAACAGGAGAAGGAGAAGGAAGAGTTGGTATT ATCCTGACTTTAGCCATGAATATCATGAGTACATTGCAGTGG GCTGTAAACTCCAGCATAGATGTGGATAGCTTGATGCGATCT GTGAGCCGAGTCTTTAAGTTCATTGACATGCCAACAGAAGGT AAACCTACCAAGTCAACCAAACCATACAAGAATGGCCAACTC TCGAAAGTTATGATTATTGAGAATTCACACGTGAAGAAAGAT GACATCTGGCCCTCAGGGGGCCAAATGACTGTCAAAGATCT CACAGCAAAATACACAGAAGGTGGAAATGCCATATTAGAGAA CATTTCCTTCTCAATAAGTCCTGGCCAGAGGGTGGGCCTCTT GGGAAGAACTGGATCAGGGAAGAGTACTTTGTTATCAGCTTT TTTGAGACTACTGAACACTGAAGGAGAAATCCAGATCGATGG TGTGTCTTGGGATTCAATAACTTTGCAACAGTGGAGGAAAGC CTTTGGAGTGATACCACAGAAAGTATTTATTTTTTCTGGAACA TTTAGAAAAAACTTGGATCCCTATGAACAGTGGAGTGATCAA GAAATATGGAAAGTTGCAGATGAGGTTGGGCTCAGATCTGTG ATAGAACAGTTTCCTGGGAAGCTTGACTTTGTCCTTGTGGAT GGGGGCTGTGTCCTAAGCCATGGCCACAAGCAGTTGATGTG CTTGGCTAGATCTGTTCTCAGTAAGGCGAAGATCTTGCTGCT TGATGAACCCAGTGCTCATTTGGATCCAGTAACATACCAAAT AATTAGAAGAACTCTAAAACAAGCATTTGCTGATTGCACAGTA ATTCTCTGTGAACACAGGATAGAAGCAATGCTGGAATGCCAA CAATTTTTGGTCATAGAAGAGAACAAAGTGCGGCAGTACGAT TCCATCCAGAAACTGCTGAACGAGAGGAGCCTCTTCCGGCA AGCCATCAGCCCCTCCGACAGGGTGAAGCTCTTTCCCCACC GGAACTCAAGCAAGTGCAAGTCTAAGCCCCAGATTGCTGCT CTGAAAGAGGAGACAGAAGAAGAGGTGCAAGATACAAGGCT TTAGAGAGCAGCATAAATGTTGACATGGGACATTTGCTCATG GAATTGGCAGGCCTAATAAAGAGCTCAGATGCATCGATCAGA GTGTGTTGGTTTTTTGTGTGTACTGAGGAACCCCTAGTGATG GAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGA GGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCC CGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGA GTGGCCCCCCCCCCCCCCCCCCCTGCAGCCTGGCGTAATA GCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGC AGCCTGAATGGCGAATGGACGCGCCCTGTAGCGGCGCATTA AGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTA CACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCC CTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTC TAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTAC GGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCAC GTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGA CGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAA CTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTT ATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGA GCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTA ACGCTTACAATTTCCTGATGCGGTATTTTCTCCTTACGCATCT GTGCGGTATTTCACACCGCATATGGTGCACTCTCAGTACAAT CTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGC CAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCG GCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTG

		CATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGA GACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATG TCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCG GGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATA CATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAA TGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACA TTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTT CCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGAT GCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACT GGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGA AGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGT GGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACT CGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTA CTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGT AAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACAC TGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGG AGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTC GCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCA AACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAAC AACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGC TTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGT TGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGT TTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGC GGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCG TATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGA TGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGAT TAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTT TAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGT GAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGT GAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATC AAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCT GCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTT TGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTG GCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGT AGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCG CCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCT GCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAG ACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGG GGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTAC ACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGC CACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAA GCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCC AGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCG CCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGG GGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTT TACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTT TCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGC CTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCG AGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCC AATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTA ATGCAGGCTGCAGGGGGGGGGGGGGGGGG

Example 7: Repeat Dosing of AV.TL65 to Ferret Lungs Elicits an Antibody Response That Diminishes Transduction in an Age-dependent Manner

Repeat-dosing of recombinant adeno-associated virus (rAAV) may be necessary to treat cystic fibrosis (CF) lung disease using gene therapy. However, little is known about rAAV-mediated immune responses in the lung. Here we demonstrate that

the ferret is a suitable species for the preclinical testing of AV.TL65 for CFTR delivery to the lung and characterization of neutralizing antibody (NAb) responses. AV.TL65-hCFTRΔR efficiently transduced both human and ferret airway epithelial cultures, and complemented CFTR Cl^- currents in CF airway cultures. Delivery of AV.TL65-hCFTRΔR to neonatal and juvenile ferret lungs produced h*CFTR* mRNA at 200-300% greater levels than endogenous f*CFTR*. Single-dose (AV.TL65-gLuc) or repeat-dosing (AV.TL65-fCFTRΔR followed by AV.TL65-gLuc) of AV.TL65 was performed in neonatal and juvenile ferrets. Repeat-dosing significantly reduced transgene expression (11-fold) and increased bronchioalveolar lavage fluid (BALF) NAbs in juvenile but not neonatal ferrets, despite near equivalent plasma NAbs responses in both age groups. Notably, both age groups demonstrated a reduction in BALF anti-capsid binding IgG, IgM, and IgA antibodies following repeat-dosing. Unique to juvenile ferrets was a suppression of plasma anti-capsid binding IgM following the second vector administration. Thus, age-dependent immune system maturation and isotype switching may impact the development of high-affinity lung NAbs following repeat-dosing of AV.TL65 and may provide a path to blunt AAV neutralizing responses in the lung.

The above results were carried out as follows in greater detail below.

Results

The ferret is a suitable preclinical species for evaluation of AV.TL65 gene therapy to the lung

To evaluate whether the AV.TL65 capsid variant was capable of complementing CFTR function in the airway, we tested the ability of AV.TL65-SP183-hCFTRΔR virus to correct CFTR-mediated Cl^- current in human CF ALI cultures following apical infection. Because rAAV1 had been previously shown to be one of the best performing serotypes for apically transduction of human ALI cultures, we also pseudopackaged the same AV2-F5tg83-hCFTRΔR viral genome into the AAV1 capsid and performed a comparative analysis with AV.TL65. This comparison demonstrated that apical infection with AV.TL65-SP183-hCFTRΔR virus gave rise to higher levels of CFTR-mediated Cl^- current (Fig. 8A) and *CFTR* mRNA (Fig. 8B) than that following infection with the rAAV1 virus harboring the same genome (AV1.SP183-hCFTRΔR).

To evaluate whether AV.TL65 was also capable to transducing ferret airway epithelium, we first performed *in vitro* transduction assays in well-differentiated tracheobronchial ALI cultures derived from humans and ferrets using a secreted gaussia luciferase (gLuc) reporter vector, AV.TL65-SP183gLuc (Fig. 8C). Apical infection of these cultures with AV.TL65-SP183gLuc demonstrated no significant difference in the levels of gLuc transgene expression between the two species. To confirm the tropism of AV.TL65 for ferret lungs *in vivo*, we evaluated the transduction efficiency of AV.TL65-SP183-hCFTRΔR in neonatal and juvenile ferret following intratracheal delivery. In these studies, expression of the transgene-derived h*CFTR*ΔR

mRNA was referenced to endogenous f*CFTR* mRNA as an index (i.e., the ratio of h*CFTR*ΔR / f*CFTR* mRNA copies) for the efficiency of transduction. Using this metric, h*CFTR*ΔR mRNA expression in the lungs was 2- to 3-fold greater than endogenous f*CFTR* mRNA in both neonates and juvenile ferrets (Fig. 8D). By contrast, tracheal expression of h*CFTR*ΔR mRNA was lower than endogenous f*CFTR* mRNA in neonates and near equivalent in juvenile animals. The low neonatal and highly variable juvenile transduction of the trachea with AV.TL65 was potentially due to the delivery method, which used surgery to instill the virus into the middle of the trachea. Overall, these *in vitro* and *in vivo* studies indicate that the ferret is a suitable species to study immunologic responses in the lung to AV.TL65 infection.

Previous exposure of AV.TL65 to lungs of juvenile, but not neonatal, ferrets impairs transduction by a second administration

We utilized two rAAV vectors (AV.TL65-SP183-fCFTRΔR and AV.TL65-SP183-gLuc) to evaluate the feasibility of repeat-dosing of AV.TL65 to the ferret lung. AV.TL65-SP183-fCFTRΔR was chosen for the first viral infection, since this vector should not mount an immune response to the transgene (i.e., ferret *CFTR* or f*CFTR*). For the second viral infection, we wanted a robust reporter that would allow for temporal and quantitative analysis of transgene expression and thus chose a secreted gLuc reporter vector, AV.TL65-SP183-gLuc. The ferrets in the single-dose groups were infected with only the AV.TL65-SP183-gLuc vector and those of the repeat-dose group were infected first with AV.TL65-SP183-fCFTRΔR and second with AV.TL65-SP183-gLuc. We first evaluated the repeated dosing in younger animals (Fig. 9). We initiated these studies in neonatal ferrets, infecting the repeat-dose group at 1 week of age with AV.TL65-SP183-fCFTRΔR and then three weeks later infecting both the repeat-dose and single-dose (naive) groups with AV.TL65-SP183-gLuc virus (Fig. 9A). Luciferase activity was monitored in blood samples during the 14 days post-infection with AV.TL65-SP183-gLuc and in BALF at the termination of the experiment. Finding from this study demonstrated that gLuc activity in plasma peaked by 5-days post-infection and remained stable to 14 days in both dosing groups (Fig. 9B). There was also no significant difference in the level of plasma gLuc activity between the two dosing groups. Similarly, gLuc activity in the BALF at 14 days post-infection was also not significantly different between the two dosing groups (Fig. 9C). In both the plasma and BALF, gLuc activity was well above background levels in naive (uninfected) controls (Figs. 9B and 9C).

This study in neonatal ferrets demonstrated it was feasible to re-administer AV.TL65 without a significant decline in transduction to the lung; however, the possibility remained that an underdeveloped immune system in neonatal ferrets could produce a tolerized immunologic state against the AAV capsid. For these reasons, we repeated experiments in juvenile ferrets by initiating the first infection with AV.TL65-SP183-fCFTRΔR for the repeat-dose group at 1 month of age, which approximately represents

a 1-2 years old toddler, followed the delivery of the gLuc reporter vector (AV.TL65-SP183-gLuc) to both the single-dose and repeat-dose groups 4 weeks later (Fig. 10A). Findings from this second study demonstrated maximal plasma gLuc activity at 5-days post-infection in both groups, however, the repeat-dose group had lower (15- to 34-fold) plasma gLuc activity at all time points tested. In contrast to the stable plasma gLuc expression in single- and repeated-dose neonatal groups (Fig. 9B), we observed a gradually declined in plasma gLuc activity in both juvenile groups with steeper trend in the repeat-dose animals. (Fig. 10B). Similarly, BALF gLuc activity was also significantly lower (11-fold) in the repeat-dose juvenile group (Fig. 10C). Cumulatively, these studies suggested the potential for NAb responses against the AAV capsid in juvenile but not neonatal ferrets.

Repeat-dosing of AV.TL65 elicits a higher NAb response in the BALF and plasma

Given the reduced efficiency of AV.TL65 transduction in the lungs of juvenile ferrets previously exposed to this virus, we sought to evaluate the NAbs in the BALF and plasma of test animals. The titers of anti-AV.TL65 NAbs were determined as the IC_{50} for inhibition of AV.TL65-SP183-fLuc transduction in A594 cells, an human airway cell line. Consistent with similar levels of transgene expression in single- and repeat-dosed neonatal ferret, NAb titers in BALF were not significantly different between the two dosing conditions (Fig. 11A). By contrast, NAb titers in the BALF of juvenile ferrets were significantly higher in the repeat-dose as compared to the single-dose group (Fig. 11B). Furthermore, the absolute titers of NAbs in experiments with older animals of both single and repeat dose groups were higher (3- to 5-fold) than the neonatal test groups, suggestive of a more fully developed immune response in the older ferrets.

Similar analyses on the plasma samples demonstrated no pre-existing NAbs in the control naive group (Figs. 11C and 11D) and the test groups prior to AV.TL65 infection. In both age groups, single- and repeat-dose animals demonstrated gradual time-dependent increases in plasma NAb titers following infection and repeat-dose juvenile ferrets produced slightly higher plasma NAb titers (2-2.8 fold) than did neonatal ferrets. Juvenile ferrets also produced NAbs more rapidly in the plasma following single-dose infection with an appearance at 5-days post-infection as compared to 10-days for neonatal ferrets. The level of plasma NAbs in the repeat-dose group was also significantly higher than that of single-dose groups for both ages, with the exception of the 14-days post-infection time point in the juvenile ferrets.

Development of an ELISA-based assay for quantifying anti-AV.TL65 capsid antibody isotypes

Evolved from an AAV2/AAV5 capsid-shuffling library, VP2 and the most abundant VP3 capsid proteins of AV.TL65 are derived from AAV5 with a single A581T mutation in VP1. VP1 of AV.TL65 is a hybrid of AAV2 and AAV5 capsids with the N-

terminal unique sequence (VP1u) from the 1-131 aa of the AAV2 VP1 following by 128-724 aa of AAV5 capsid harboring the A581T mutation. The VP1u of AAV harbors a phospholipase A2 (PLA2) catalytic domain that is thought to be crucial to virion escape from the endosome. To evaluate AV.TL65 capsid-specific immunoglobins in the plasma and BALF (IgG, IgM, and IgA) of AV.TL65-infected ferrets, an ELISA assay using AAV viral particles as the coating antigen was developed. To validate the method, we used plasma collected from a 1-month-old ferret for which AV.TL65 virus was delivered to the lung four times at 1-2 months intervals. Using AAV5 particles as the coating antigen, differential IgG binding between naive and AV.TL65-immune plasma was seen starting at a 1:50 dilution, and by a 1:1250 dilution binding of naive plasma was absent while AV.TL65-immune plasma antibody binding remained high (Fig. 12A). By contrast, when AAV2 was used as the coating antigens, there was no difference in plasma IgG binding between the immune plasma and the naive plasma at all dilutions and the sensitivity of detecting IgG was much lower than AAV5 (Fig. 12B). These findings suggest the surface antigen epitopes of AV.TL65 displays similar immunogenicity to the AAV5 capsid and for these reasons we chose to use AAV5 as the coating antigen for classification of anti-capsid antibody isotypes in the BALF and plasma of test animals.

We next used this ELISA method for classification of anti-capsid antibody isotypes (IgG, IgM, and IgA) in the BALF and plasma of test animals (Figs. 12 and 13). In general, neonatal and juvenile ferrets elicited similar AAV5-reactive IgG responses in the plasma of both single- and repeat-dosing groups, but titers were higher following repeat-infection (Figs. 13A and 13D). By contrast, plasma AAV5-reactive IgM (Figs. 13B and 13E) and IgA (Figs. 13C and 13F) responses demonstrated differences from that of IgG with respect to age of the animal and dosing regimen. For example, capsid-binding plasma IgM levels were suppressed only in juvenile animals of the repeat-dose group (Figs. 13B and 13E), while capsid-binding plasma IgA levels were suppressed in both age groups following repeat dosing. Furthermore, neonatal animals initially mounted a large anti-capsid IgA response initially following second viral expose which subsided with time, while juvenile animals lacked this response (Figs. 13C and 13F). These findings suggest that age-dependent differences in antibody isotype switching may be impacted by prior expose to AV.TL65. Contrary to expectations, AAV5-reactive IgG, IgM and IgA in the BALF was significantly higher in the single-dose group, as compared to the repeat-dose group, for both neonatal and juvenile animals (Fig. 14). Furthermore, the absolute level of capsid-binding IgG, IgM and IgA were generally similar between both age groups and dosing conditions, despite higher levels of NAbs in the BALF of juvenile animals that were exposed twice to virus (Figs. 11A and 11B).

Materials and Methods

Production of recombinant AV.TL65 viral vectors

pAV.TL65repcap (Excoffon et al., 2009, *supra*) was the AAV helper plasmid used to generate AV.TL65 capsid for the production of AV1-SP183-hCFTRΔR, and

AV.TL65-SP183-hCFTRΔR, AV.TL65-SP183-fCFTRΔR, AV.TL65-SP183-fLuc, AV.TL65-SP183-gLuc. rAAV proviral plasmids used for packaging were pAV2.F5tg83-hCFTRΔR and pAV2.F5tg83-fCFTRΔR, as well as the pAV2-F5tg83fLuc (firefly luciferase reporter) and pAV2-F5tg83gLuc (gaussia luciferase reporter). AV.TL65 vectors were produced in the Vector Core of Children's Hospital of Philadelphia (CHOP) using a triple-plasmid transfection method. In brief, AAV helper pAV.TL65repcap and Adenovirus helper pAd were transfected into HEK293 cells together with one of the AAV proviral vector. rAAV vector produced from the transfected HEK293 cells were purified on CsCl-density gradients. The titers were determined by quantitative real-time polymerase chain reaction (qPCR) using primers and probes specific to the transgenes, and the purity of the vector stocks were evaluated by SDS-PAGE following silver-staining.

In vitro evaluation of AV.TL65 vector in human and ferret airway epithelium

In order to evaluate whether the ferret would be a suitable species for analysis of AV.TL65, we initially performed *in vitro* transduction experiments in well-differentiated tracheobronchial ALI cultures derived from humans and ferrets. The reporter vector, AV.TL65-SP183gLuc, was inoculated apically onto the airway epithelial ALI cultures of human (n=6 transwells from two donors) and ferret (n=6 transwells from two donors) at an MOI (multiplicity of infection) of 10,000 DRP (DNase-resistant particle)/cell. During the infection period, the culture medium was supplemented with doxorubicin at the final concentration of 4 μM, and the relative luminescence units (RLU) of gaussia luciferase activity was measured after 5-days infection according to the manufacturer's instructions for the Renilla Luciferase activity assay kit (Promega), which was designed for the measurement of Gaussia luciferase and Renilla luciferase. Two non-infected transwells were set as control.

In vitro comparison of CFTR-mediated currents following infection of human CF airway epithelium with AV1-SP183-hCFTRΔR and AV.TL65-SP183-hCFTRΔR viruses

The effectiveness of AV.TL65-SP183-hCFTRΔR and AV1-SP183-hCFTRΔR for expressing hCFTRΔR and complementation of CFTR function was evaluated in polarized human ALI cultures derived from the proximal airway of CF patients (F508del/F508del). Each vector was apically applied to the ALI cultures (n=4 transwells from two donors) at an MOI of 100,000 DRP/cell in the presence of doxorubicin (2.5μM) and LLnL (20μM). These two proteasome modulating agents have been shown to augment transduction by several AAV serotypes. At 12-day post-infection, CFTR-mediated Cl^- currents were measured in Ussing chambers as described previously to determine the change in short-circuit current (ΔIsc) following cAMP stimulation (IBMX/Forskolin) and CFTR inhibition (GlyH101). Non-infected ALI cultures (n=4 transwells from two donors) were used as baseline controls. After measure of the ΔIsc, two inserts from each virus infection group were pooled and lysed for total RNA using

the RNeasy® Plus Mini kit (Qiagene). After conversion of mRNA to cDNA, the vector-derived hCFTRΔR mRNA was quantitated by TaqMan® PCR and normalized to human GAPDH mRNA.

Analysis of AV.TL65 transduction in neonatal and juvenile ferret lungs

Three-day-old neonatal ferrets (n=3) or one-month-old juvenile ferrets (n=3) intratracheally received 4×10^{10} DRP per gram body weight of the AV.TL65-SP183-hCFTRΔR virus mixing with doxorubicin (final concentration 250μM). The ferrets in the mocked infection group (n=3) were only inoculated with Dox in PBS (250μM). The animals were euthanized at 11-days post-infection, the trachea and lung tissues were separately harvested, snap frozen, and pulverized for total RNA extraction. The vector-derived mRNA of the transgene hCFTRΔR and endogenous fCFTR were quantified by TaqMan®, and the copy numbers of hCFTRΔR and fCFTRΔR were normalized to GAPDH and then expressed as the ratio of hCFTRΔR / fCFTR.

Administration of AV.TL65-SP183-fCFTRΔR and/or AV.TL65-SP183-gLuc to ferrets for humoral response studies

We evaluated repeat dosing of AV.TL65 vectors to neonatal and juvenile ferrets using the following experimental design. Neonatal ferrets: AV.TL65-SP183-gLuc reporter vector was intratracheally administered to 4-week-old ferrets that were either naive to AV.TL65 capsid or previously infected with AV.TL65-SP183-fCFTΔR at 1-week of age. Juvenile ferrets: AV.TL65-SP183-gLuc reporter vector was intratracheally administered to 8-week-old ferrets that were either naive to AV.TL65 capsid or previously infected with AV.TL65-SP183-fCFTRΔR at 4-weeks of age. For each dose, the animal received an inoculum containing AV.TL65-SP183gLuc or AV.TL65-SP183-fCFTRΔR vector ($1x10^{13}$ DRP/kg) and doxorubicin (200 μM final concentration). Surgical intratracheal injection was performed in the 1-week-old neonatal ferrets with a 150 μl inoculum administered to kits under anesthesia with a mixture of isofluorane and oxygen. For other ages, virus was administered intratracheally with a MicroSprayer® aerosolizer under anesthesia via subcutaneous injection with a mixture of ketamine and xylazine. The volume of the vector/doxorubicin inoculum for aerosolization was normalized to ferret body weight (5 ml/kg).

Bleeding and bronchoalveolar lavage fluid collection for measurement of Gaussia Luciferase activity

Plasma was collected into heparinized tubes from anesthetized ferrets at the 0-, 5-, 10- and 14-days post-delivery of the AV.TL65-SP183-gLuc report vector. Animals were euthanized with EUTHASOL® (Virbac AH Inc) and bronchoalveolar lavage fluid (BALF) was collected from the tracheal/lung cassette by instillation of 5 ml

of PBS per 300-gram body weight. The gLuc activity in plasma and BALF were immediately measured after sample collection.

Antibody neutralization assays using plasma and BALF

Micro-neutralization assays were performed using modifications to a previously reported method (Wu et al. *Front Immunol.* 8:1649, 2017). The titer of NAb in the plasma and BALF was quantified as the reduction in reporter gene expression following infection of A549 cells with AV.TL65-SP183-fLuc virus incubated with serially diluted plasma or BALF prior to infection. Briefly, all plasma samples from ferrets were heat-inactivated (56°C, 30 min). Five-fold serial dilutions of plasma (started at 1:50 and ended at 1:156,250) were incubated with AV.TL65-SP183-fLuc in a total volume of 100 µl. For BALF, the same condition was applied, but the serial dilution started at 1:5 and ended at 1:3125. These mixtures were incubated at 37°C for 1 hr to facilitate antibody binding and neutralization, and then applied to a monolayer of A549 cells in 48-well plates (1×10^5/well, MOI=5000 DRP/cell) in duplicate for each dilution. After incubating cells for 1 hr at 37°C / 5% CO_2 with the virus mixture, the wells supplemented with DMEM containing of 2% fetal bovine serum and incubated for an additional 24 hrs. Firefly Luciferase activity in cell lysates were then measured with a Firefly Luciferase Assay Kit (Promega) according to manufacturer's instruction. Each time this assay was performed, A549 cells infected only with AV.TL65-SP183-fLuc served as the reference control for 100% transduction. The neutralization titer of each plasma or BALF sample was calculated as the half maximal inhibitory concentration (IC50).

ELISA measurements of capsid-binding IgG, IgM, and IgA in plasma and BALF

An ELISA procedure was used to capture and quantify the total capsid-binding IgG, IgM, and IgA in the plasma and BALF. In brief, rAAV5 in carbonate buffer was bound to 96 wells ELISA plates overnight at 4°C (1×10^9 DRP/well). The tested plasma samples (diluted to 1:2000 for IgG and IgM and 1:20 for IgA) and undiluted BALF samples were applied to each well, and incubated for 1 hr at room temperature. After washing three times in PBS-T (0.05% Tween-20), diluted HRP-conjugated second antibodies were added and incubated for 1 hr at room temperature. The HRP-conjugated second antibodies included chicken anti-ferret IgG (Gallus Immunotech or Abcam) and goat anti-ferret IgM or IgA (Life-Bio Inc). The HRP reaction product was then quantified by absorbance in a plate reader.

Statistical analysis

Experimental data are presented as mean ± SD and Prism 7 (GraphPad Software, Inc., San Diego, CA, USA) was used for data analysis. The statistical significance was analyzed with one-way analysis of variance (ANOVA) followed by Tukey test (*P<0.05; **P<0.01; ***P<0.001, ****P<0.0001).

Ethics Statement in Animal Care

All animal experimentation was performed according to protocols approved by the Institutional Animal Care and Use Committees of the University of Iowa.

All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification, this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details herein may be varied considerably without departing from the basic principles of the invention.

WHAT IS CLAIMED IS:

1. A recombinant adeno-associated virus (rAAV) comprising (i) an AV.TL65 capsid protein or a variant thereof; and (ii) a polynucleotide comprising an F5 enhancer, or a variant thereof, and a tg83 promoter, or a variant thereof, operably linked to a CFTRΔR minigene or a variant thereof.

2. The rAAV of claim 1, wherein the AV.TL65 capsid protein comprises the amino acid sequence of SEQ ID NO:13 or the variant comprises at least 80% amino acid sequence identity to SEQ ID NO:13.

3. The rAAV of claim 1 or 2, wherein the F5 enhancer comprises the polynucleotide sequence of SEQ ID NO:1 or the variant thereof comprises at least 80% nucleic acid sequence identity to SEQ ID NO:1.

4. The rAAV of claim 1 or 2, wherein the F5 enhancer comprises the polynucleotide sequence of SEQ ID NO:14 or the variant thereof comprises at least 80% nucleic acid sequence identity to SEQ ID NO:14.

5. The rAAV of any one of claims 1 to 4, wherein the tg83 promoter comprises the polynucleotide sequence of SEQ ID NO:2 or the variant thereof comprises at least 80% nucleic acid sequence identity to SEQ ID NO:2.

6. The rAAV of any one of claims 1 to 5, wherein the CFTRΔR minigene is a human CFTRΔR minigene.

7. The rAAV of claim 6, wherein the human CFTRΔR minigene is encoded by a polynucleotide comprising the sequence of SEQ ID NO:4 or the variant thereof comprising at least 80% nucleic acid sequence identity to SEQ ID NO:4.

8. The rAAV of any one of claims 1 to 7, wherein the polynucleotide comprises, in a 5'-to-3' direction, the F5 enhancer, the tg83 promoter, and the CFTRΔR minigene.

9. A pharmaceutical composition comprising the rAAV of any one of claims 1 to 8 and a pharmaceutically acceptable carrier.

10. A method of treating cystic fibrosis, comprising: administering to a subject in need thereof a therapeutically effective amount of the rAAV of any one of claims 1 to 8, or the pharmaceutical composition of claim 9.

11. The method of claim 10, further comprising administering one or more additional therapeutic agents to the subject.

12. The method of claim 11, wherein the one or more additional therapeutic agents includes an antibiotic, a mucus thinner, a CFTR modulator, a mucolytic, normal saline, hypertonic saline, an immunosuppressive agent, or a combination thereof.

13. The method of any one of claims 10 to 12, wherein the administering is by inhalation, by nebulization, or by aerosolization, or is intranasal, intratracheal, intrabronchial, oral, intravenous, subcutaneous, or intramuscular administration.

14. The method of claim 13, wherein the administering is by inhalation, by nebulization, or by aerosolization, or is intranasal, intratracheal, and/or intrabronchial administration.

15. An isolated polynucleotide comprising the sequence of SEQ ID NO:7 or a variant thereof with at least 80% nucleic acid sequence identity to SEQ ID NO:7.

16. The polynucleotide of claim 15, further comprising, in the 3' direction, a 3' untranslated region (3'-UTR) comprising the sequence of SEQ ID NO:5 or a variant thereof with at least 80% nucleic acid sequence identity to SEQ ID NO:5.

17. The polynucleotide of claim 15 or 16 further comprising, in the 3' direction, a synthetic polyadenylation site comprising the sequence of SEQ ID NO:6 or a variant thereof with at least 80% nucleic acid sequence identity to SEQ ID NO:6.

18. The polynucleotide of any one of claims 15 to 17, further comprising a 5' adeno-associated virus (AAV) inverted terminal repeat at the 5' terminus of the polynucleotide and a 3' AAV ITR at the 3' terminus of the polynucleotide.

19. The polynucleotide of claim 18, wherein the polynucleotide comprises the sequence of SEQ ID NO:17 or a variant thereof with at least 80% nucleic acid sequence identity to SEQ ID NO:17.

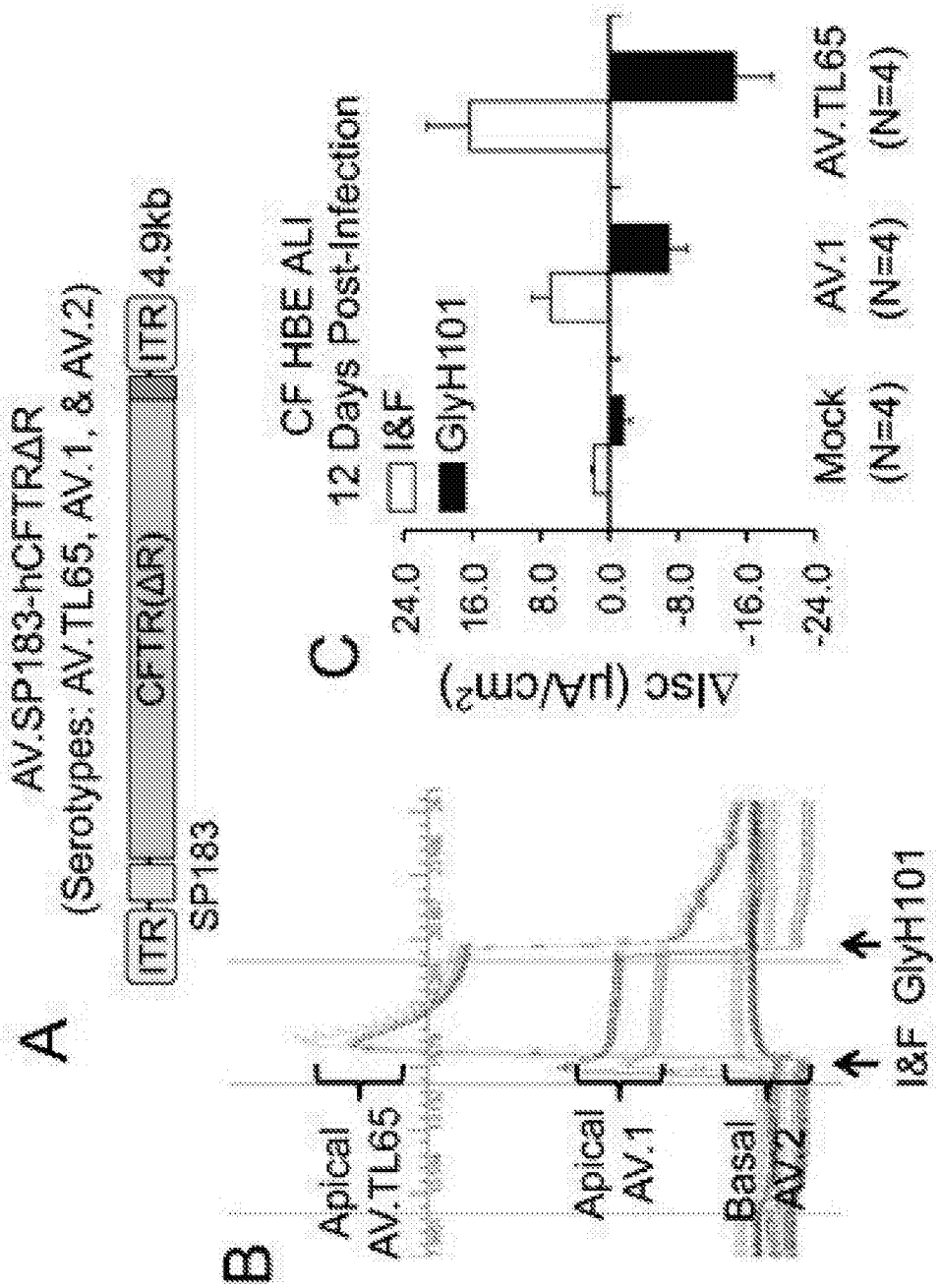
20. A recombinant adeno-associated virus (rAAV) comprising the polynucleotide of any one of claims 15 to 19.

21. The rAAV of claim 20, wherein the rAAV has a tropism for airway epithelial cells.

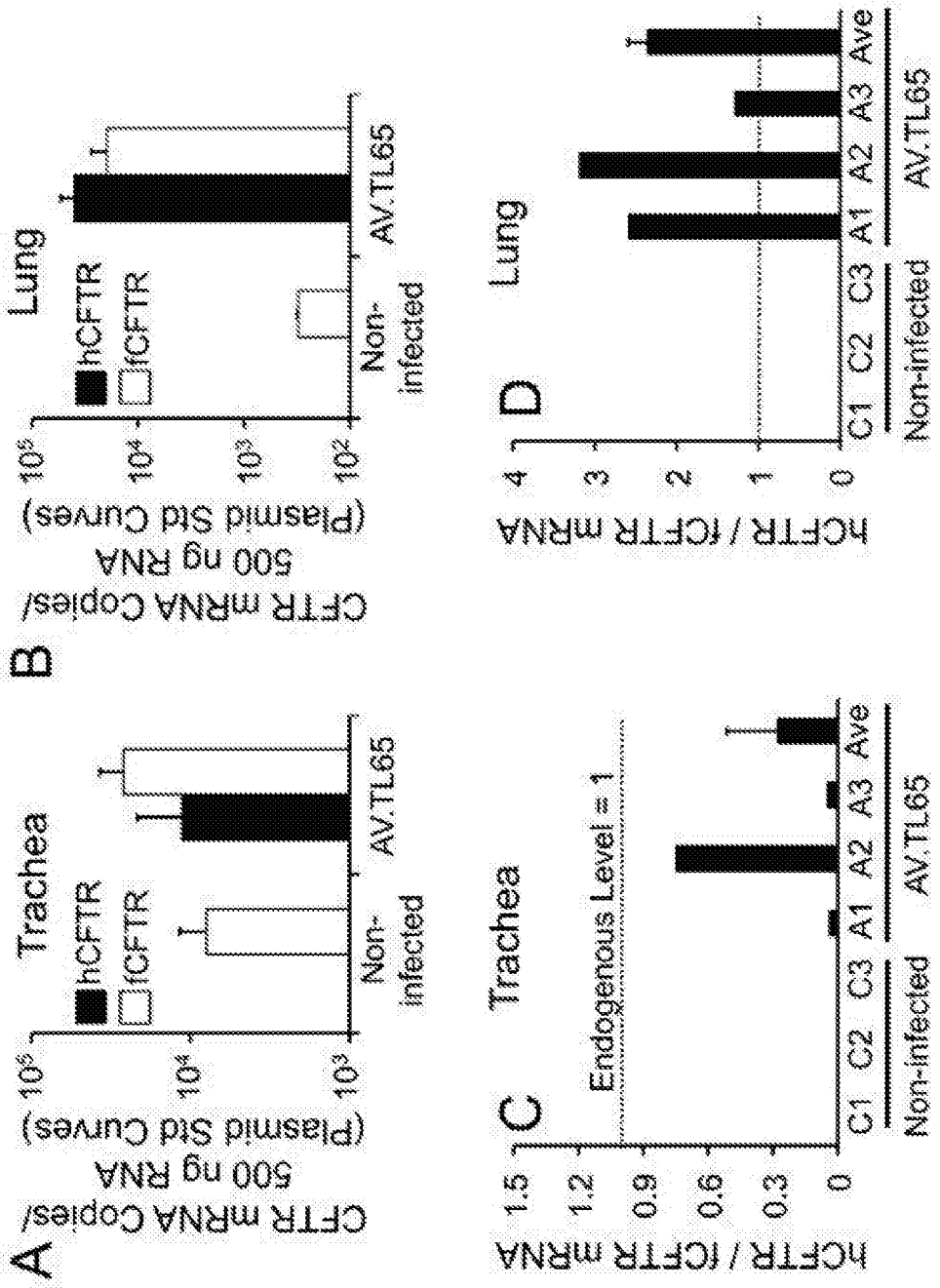
22. The rAAV of claim 21, wherein the rAAV has a tropism for lung epithelial cells.

23. The rAAV of any one of claims 20 to 22, wherein the rAAV comprises an AV.TL65 capsid protein, an AAV1 capsid protein, an AAV2 capsid protein, an AAV5 capsid protein, an AAV6 capsid protein, or an AAV9 capsid protein.

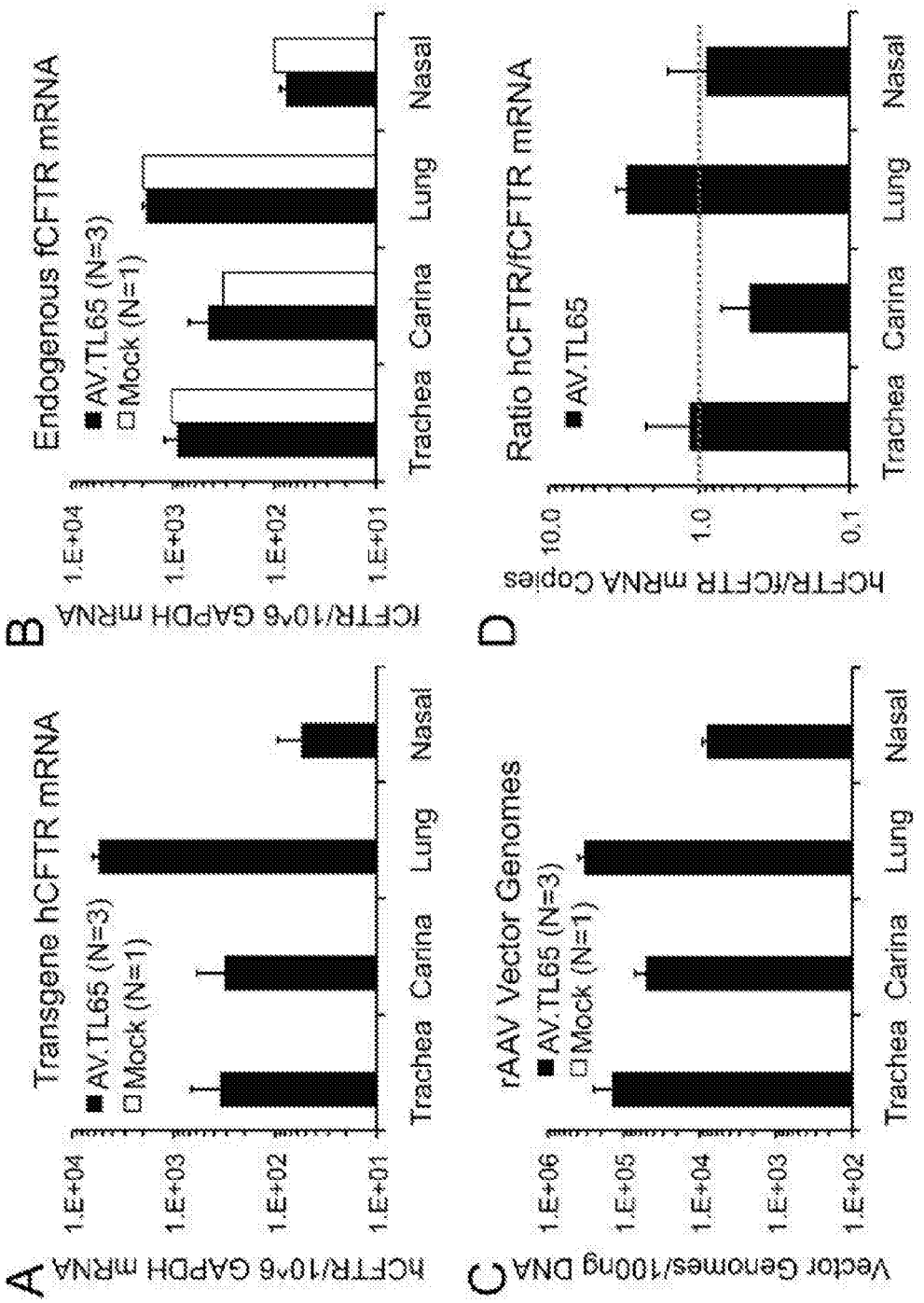
24. The rAAV of claim 23, wherein the rAAV comprises an AV.TL65 capsid protein.


A
AV.SP183-hCFTRΔR
(Serotypes: AV.TL65, AV.1, & AV.2)
ITR
CFTR(ΔR)
ITR
4.9kb
SP183
B
Apical
AV.TL65
Apical
AV.1
Basal
AV.2
I&F GlyH101
C
CF HBE ALI
12 Days Post-Infection
I&F
GlyH101
Isc (μA/cm²)
24.0
16.0
8.0
0.0
-8.0
-16.0
-24.0
Mock
(N=4)
AV.1
(N=4)
AV.TL65
(N=4)

FIGS. 1A-1C


A
Trachea
hCFTR
fCFTR
CFTR mRNA Copies/
500 ng RNA
(Plasmid Std Curves)
Non-
infected
AV.TL65
B
Lung
C
Trachea
Endogenous Level = 1
hCFTR / fCFTR mRNA
C1 C2 C3 A1 A2 A3 Ave
Non-infected
AV.TL65
D
Lung

FIGS. 2A-2D


A
hCFTR/10^6 GAPDH mRNA
Transgene hCFTR mRNA
AV.TL65 (N=3)
Mock (N=1)
1.E+04
1.E+03
1.E+02
1.E+01
Trachea
Carina
Lung
Nasal
B
hCFTR/10^6 GAPDH mRNA
Endogenous fCFTR mRNA
AV.TL65 (N=3)
Mock (N=1)
1.E+04
1.E+03
1.E+02
1.E+01
Trachea
Carina
Lung
Nasal
C
Vector Genomes/100ng DNA
rAAV Vector Genomes
AV.TL65 (N=3)
Mock (N=1)
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
Trachea
Carina
Lung
Nasal
D
hCFTR/fCFTR mRNA Copies
Ratio hCFTR/fCFTR mRNA
AV.TL65
10.0
1.0
0.1
Trachea
Carina
Lung
Nasal

FIGS. 3A-3D

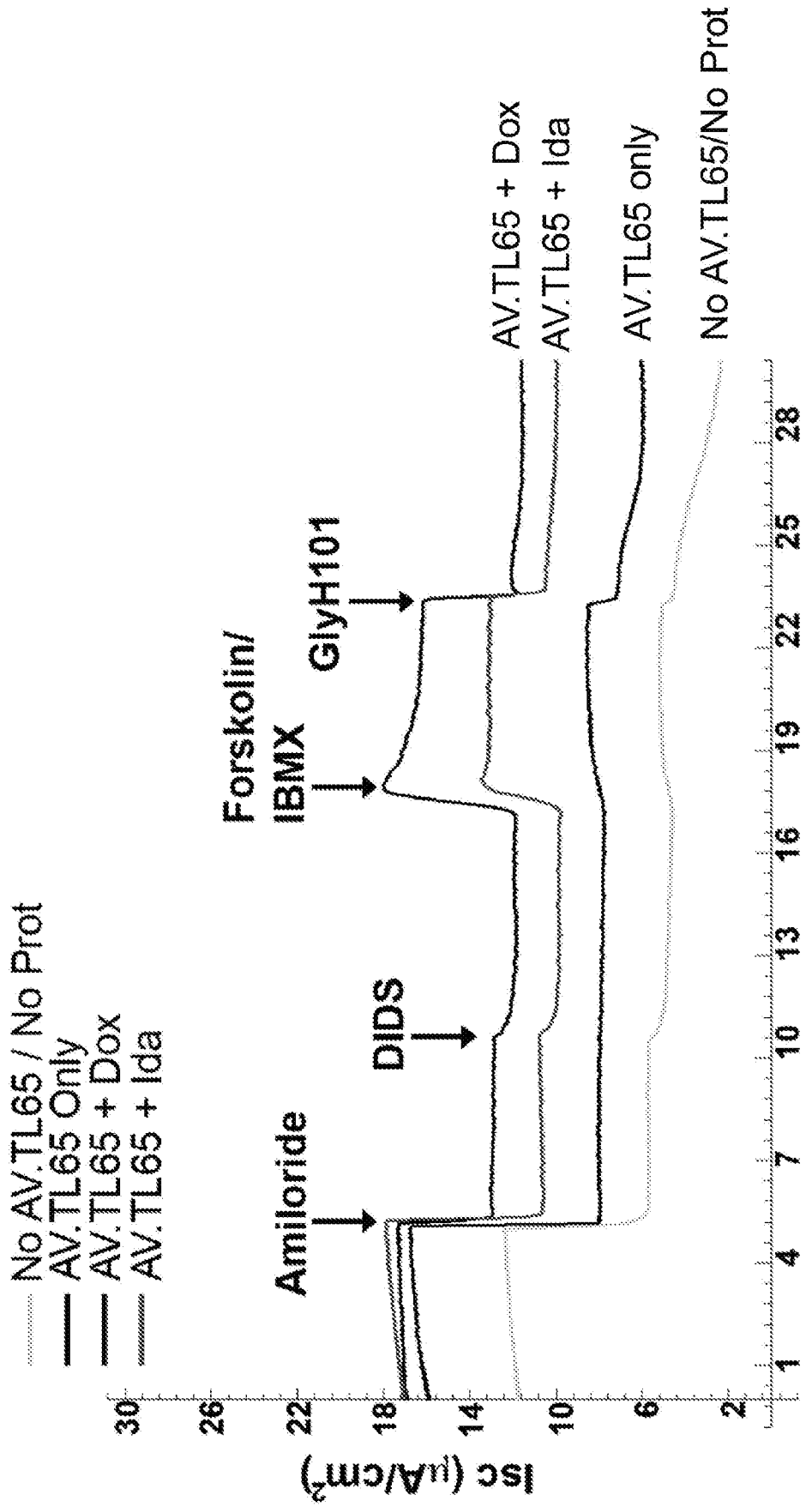

No AV.TL65 / No Prot
AV.TL65 Only
AV.TL65 + Dox
AV.TL65 + Ida
Amiloride
DIDS
Forskolin/
IBMX
GlyH101
AV.TL65 + Dox
AV.TL65 + Ida
AV.TL65 only
No AV.TL65/No Prot
Isc (μA/cm²)
30
26
22
18
14
10
6
2
1
4
7
10
13
16
19
22
25
28
Time (min)

FIG. 4

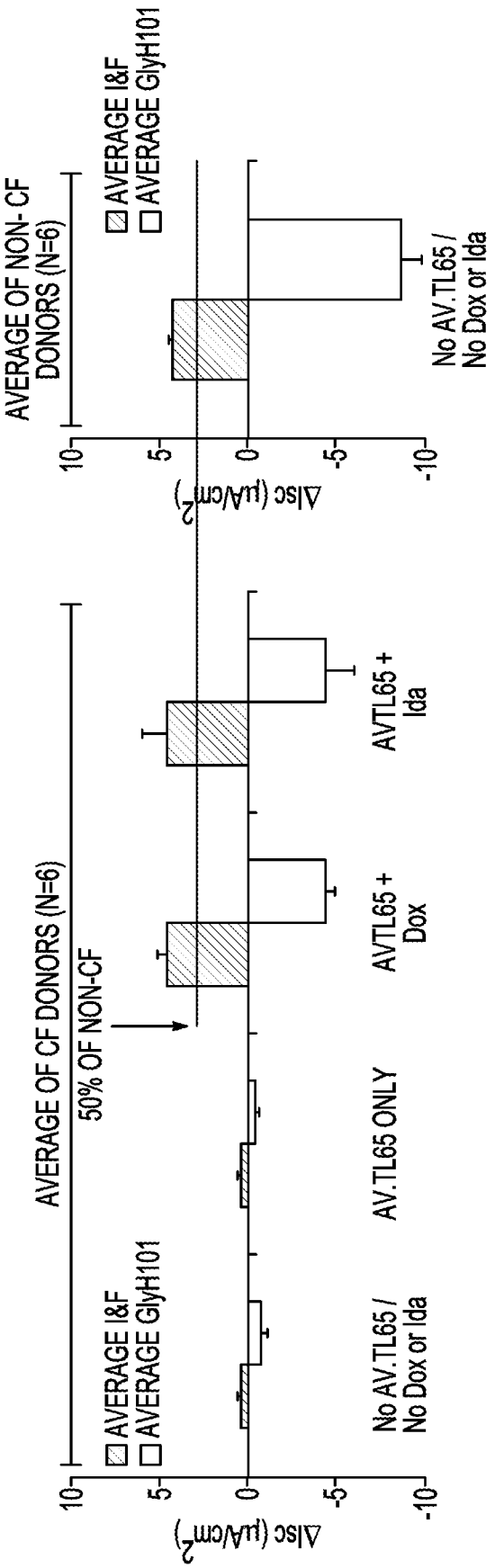

AVERAGE OF NON- CF DONORS (N=6)
AVERAGE I&F
AVERAGE GlyH101
No AV.TL65 / No Dox or Ida
ΔIsc (μA/cm²)
10
5
0
-5
-10
AVERAGE OF CF DONORS (N=6)
50% OF NON-CF
AVTL65 + Ida
AVTL65 + Dox
AV.TL65 ONLY
No AV.TL65 / No Dox or Ida

FIG. 5

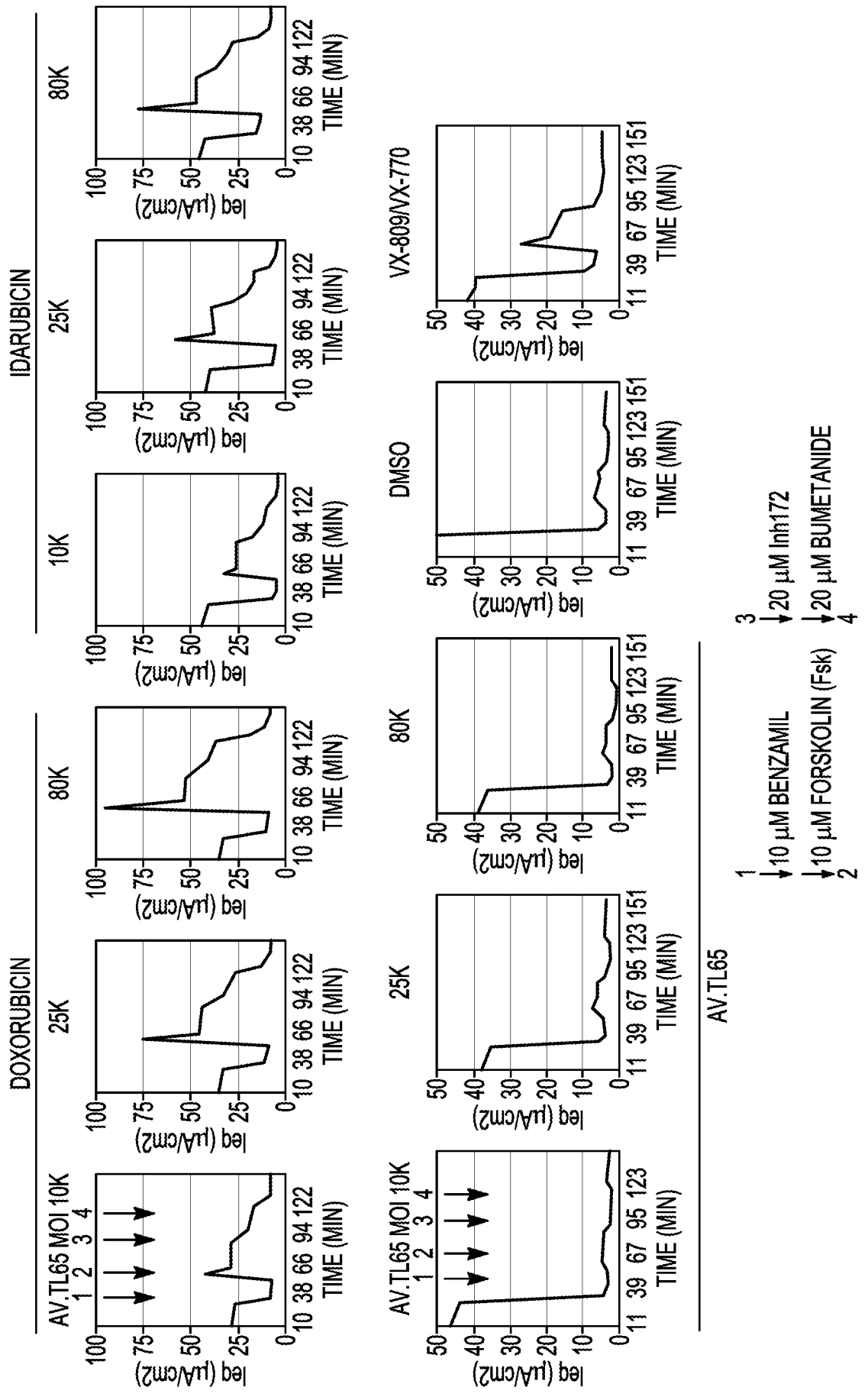

DOXORUBICIN
IDARUBICIN
AV.TL65 MOI 10K 25K 80K
1 2 3 4
Ieq (μA/cm2)
TIME (MIN)
AV.TL65
DMSO
VX-809/VX-770
1 10 μM BENZAMIL
2 10 μM FORSKOLIN (Fsk)
3 20 μM Inh172
4 20 μM BUMETANIDE

FIG. 6

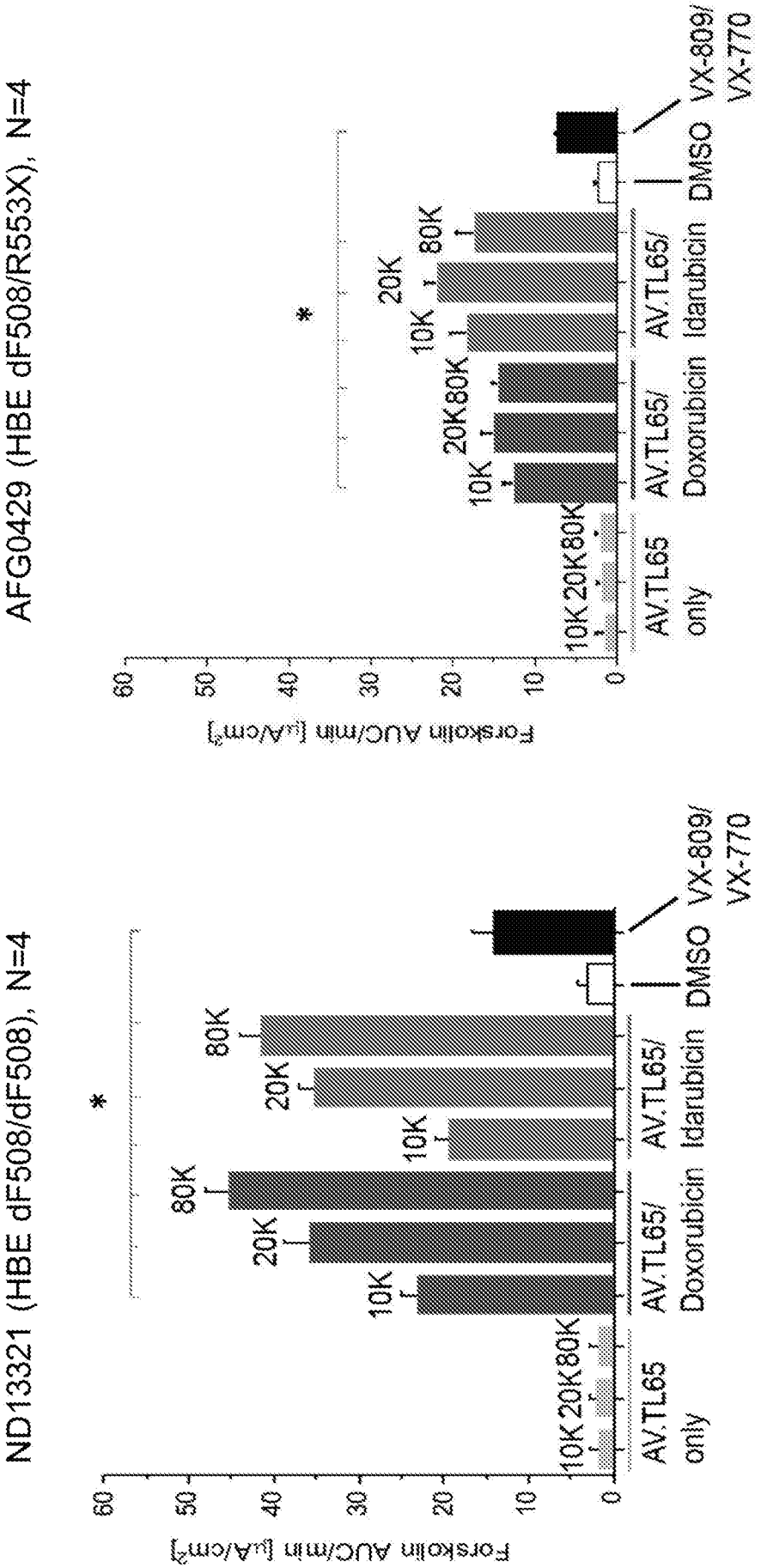

AFG0429 (HBE dF508/R553X), N=4
Forskolin AUC/min [μA/cm²]
0
10
20
30
40
50
60
*
AV.TL65 only
10K
20K
80K
AV.TL65/ Doxorubicin
10K
20K
80K
AV.TL65/ Idarubicin
10K
20K
80K
DMSO
VX-809/ VX-770
ND13321 (HBE dF508/dF508), N=4
Forskolin AUC/min [μA/cm²]
0
10
20
30
40
50
60
*
AV.TL65 only
10K
20K
80K
AV.TL65/ Doxorubicin
10K
20K
80K
AV.TL65/ Idarubicin
10K
20K
80K
DMSO
VX-809/ VX-770

FIG. 7

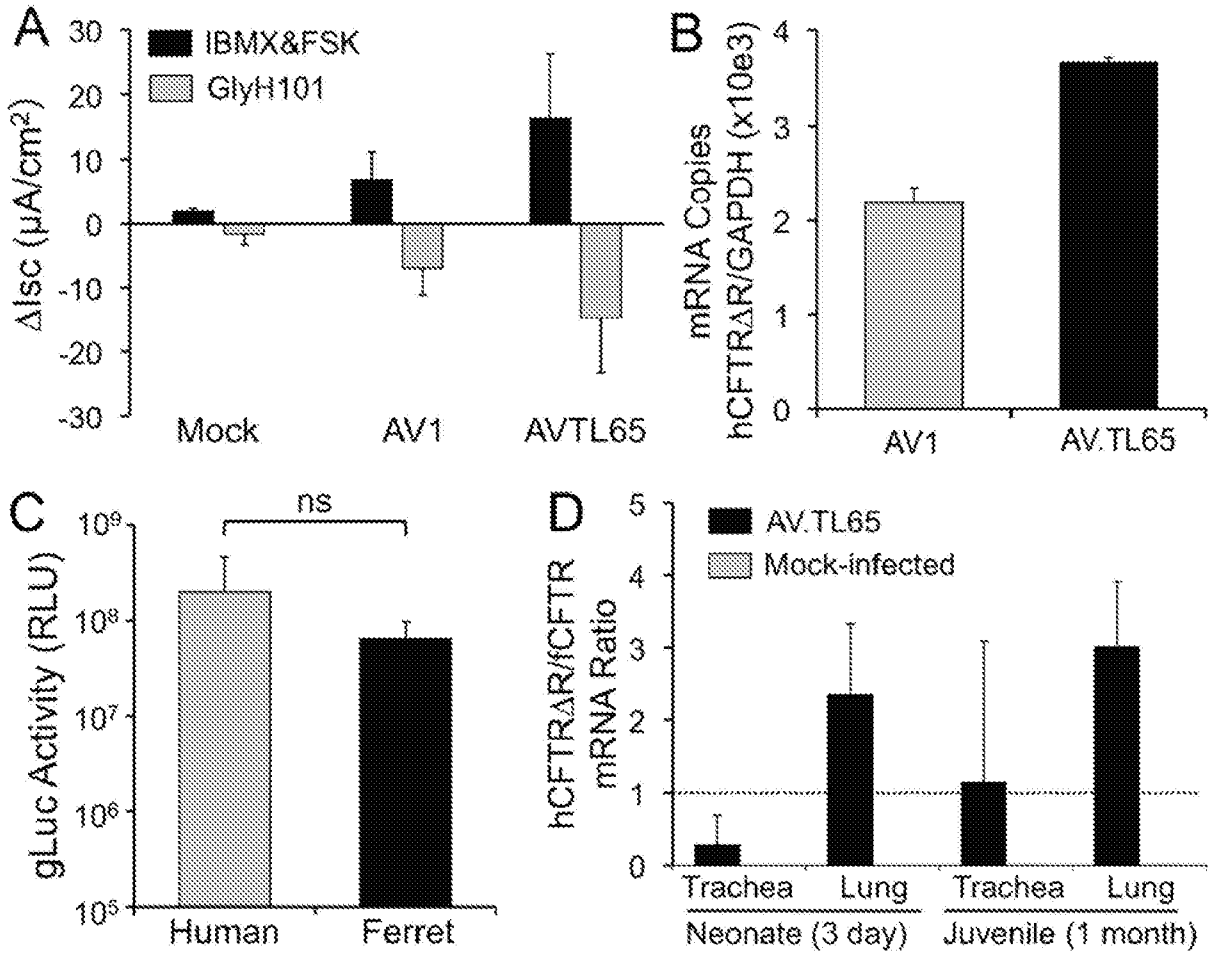

A
IBMX&FSK
GlyH101
ΔIsc (μA/cm²)
30
20
10
0
-10
-20
-30
Mock
AV1
AVTL65
B
mRNA Copies hCFTRΔR/GAPDH (x10e3)
4
3
2
1
0
AV1
AV.TL65
C
gLuc Activity (RLU)
ns
Human
Ferret
D
AV.TL65
Mock-infected
hCFTRΔR/fCFTR mRNA Ratio
Trachea
Lung
Trachea
Lung
Neonate (3 day)
Juvenile (1 month)

FIG. 8

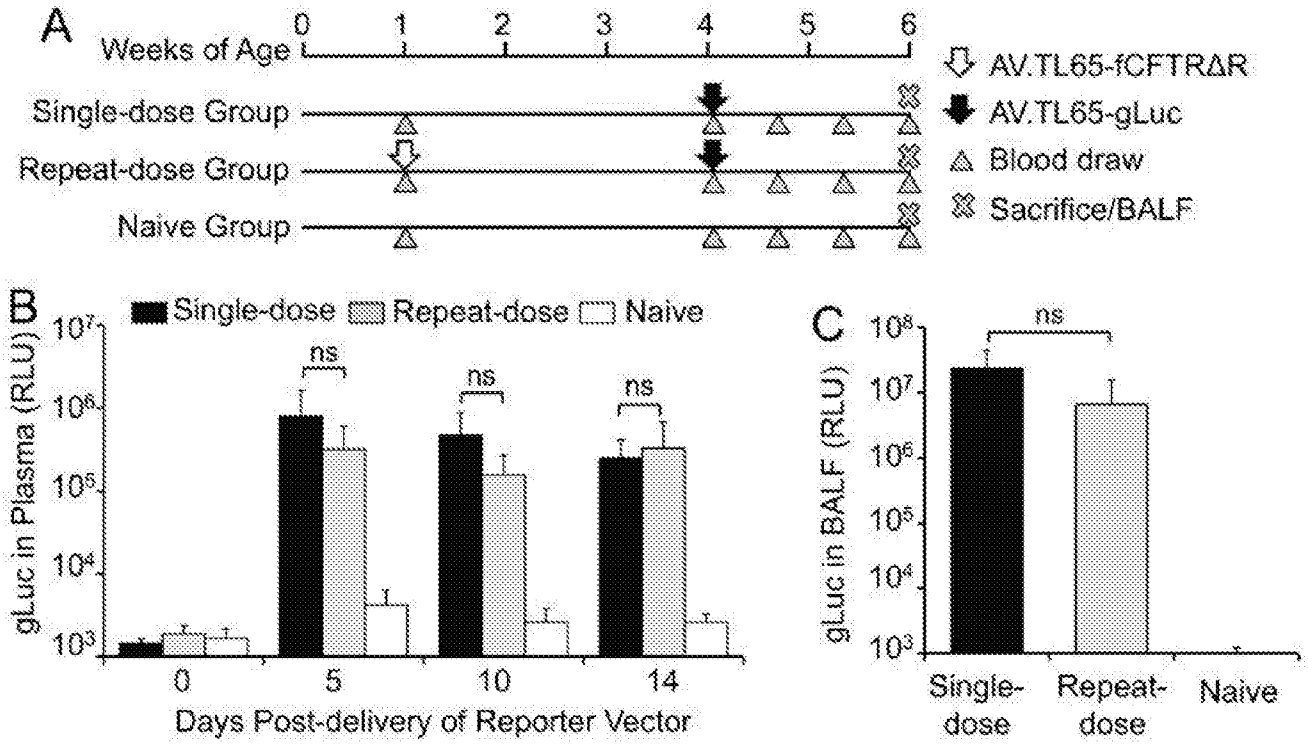

A
Weeks of Age
0
1
2
3
4
5
6
Single-dose Group
Repeat-dose Group
Naive Group
AV.TL65-fCFTRΔR
AV.TL65-gLuc
Blood draw
Sacrifice/BALF
B
Single-dose
Repeat-dose
Naive
gLuc in Plasma (RLU)
ns
Days Post-delivery of Reporter Vector
C
gLuc in BALF (RLU)
Single-dose
Repeat-dose
Naive

FIG. 9

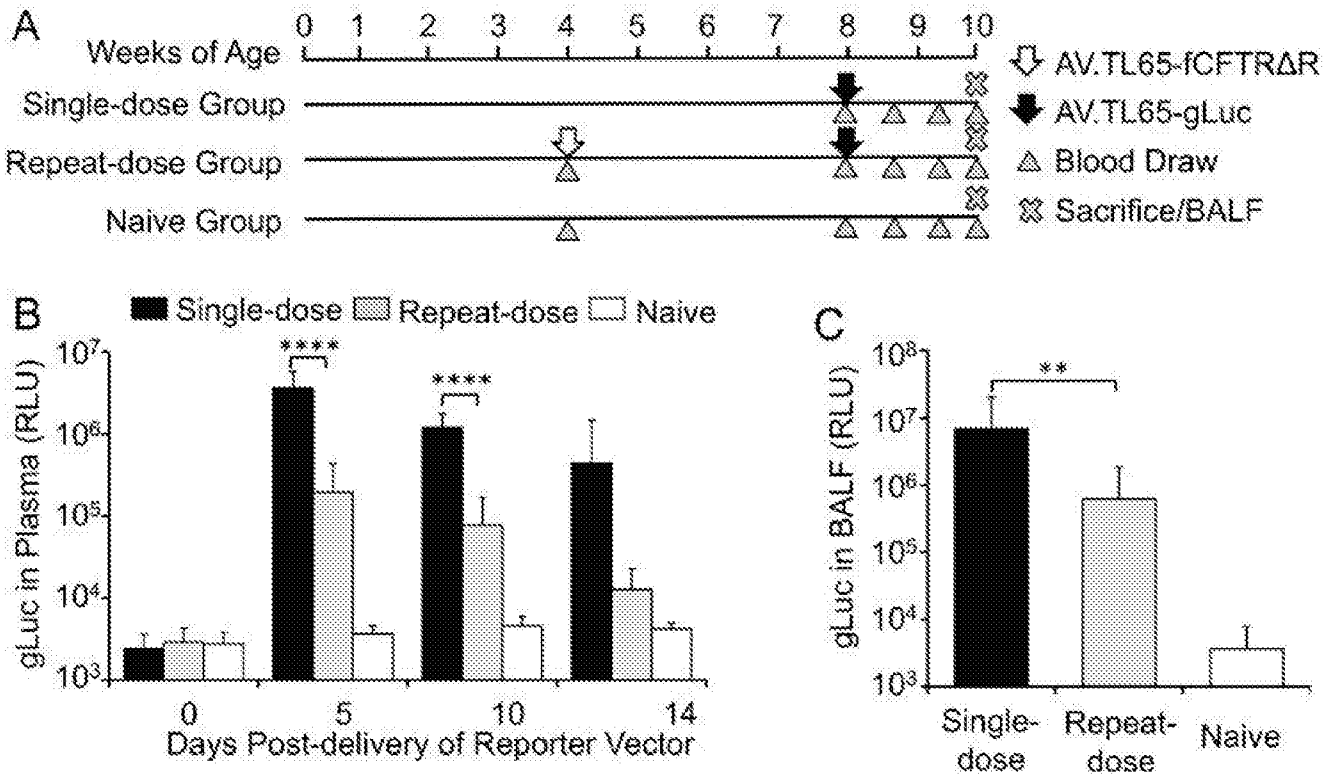

A
Weeks of Age
0 1 2 3 4 5 6 7 8 9 10
Single-dose Group
Repeat-dose Group
Naive Group
AV.TL65-fCFTRΔR
AV.TL65-gLuc
Blood Draw
Sacrifice/BALF
B
Single-dose
Repeat-dose
Naive
gLuc in Plasma (RLU)
****
****
Days Post-delivery of Reporter Vector
C
gLuc in BALF (RLU)
**
Single-dose
Repeat-dose
Naive

FIG. 10

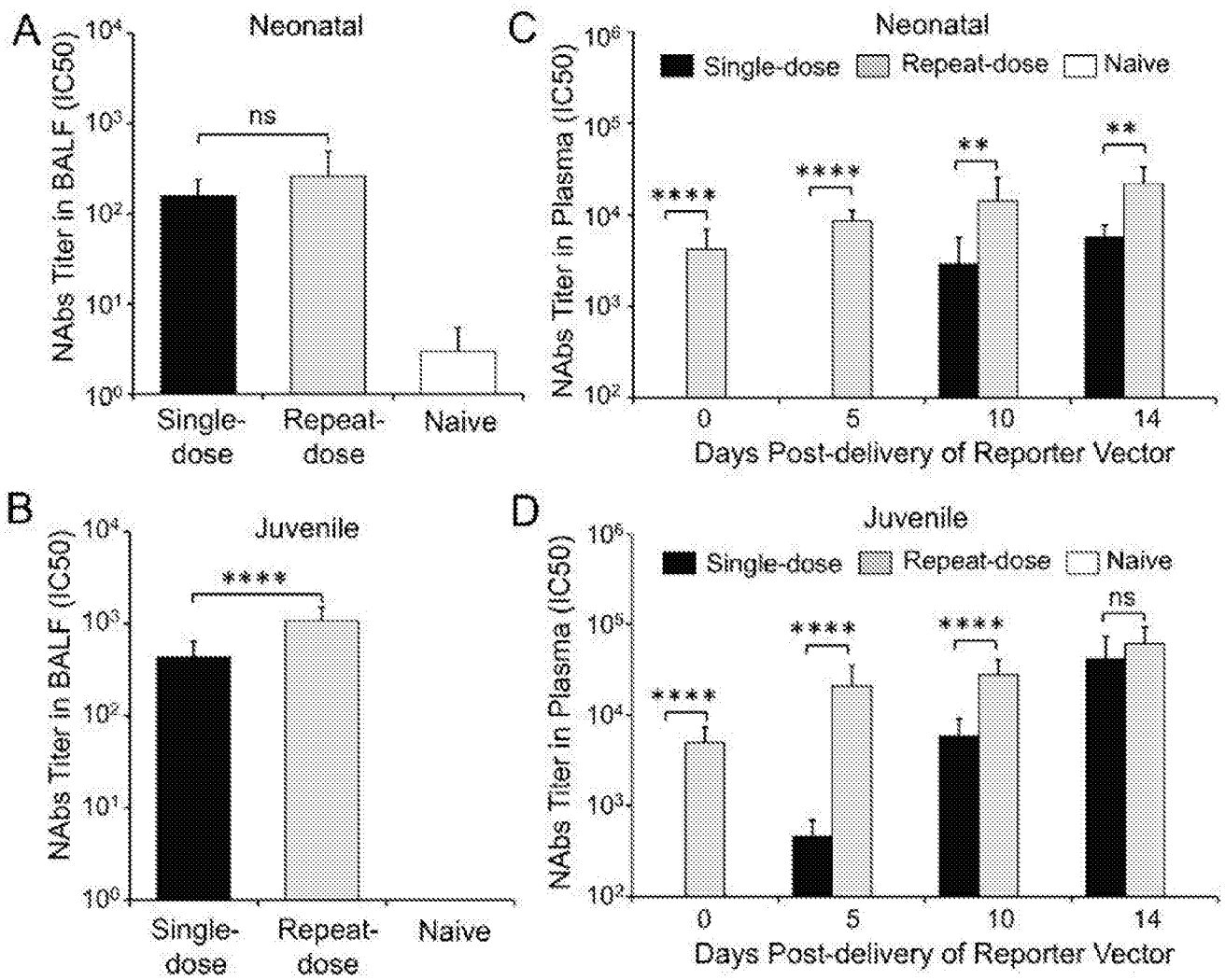

A
Neonatal
NAbs Titer in BALF (IC50)
ns
Single-dose
Repeat-dose
Naive
B
Juvenile
****
C
Neonatal
Single-dose
Repeat-dose
Naive
NAbs Titer in Plasma (IC50)
Days Post-delivery of Reporter Vector
D
Juvenile

FIG. 11

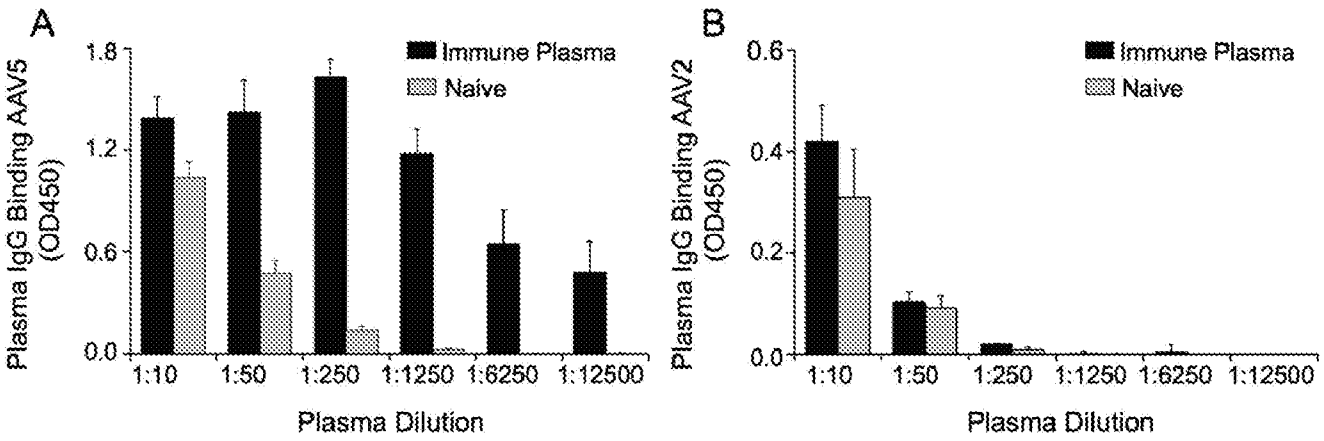

A
Plasma IgG Binding AAV5 (OD450)
1.8
1.2
0.6
0.0
Immune Plasma
Naive
1:10
1:50
1:250
1:1250
1:6250
1:12500
Plasma Dilution
B
Plasma IgG Binding AAV2 (OD450)
0.6
0.4
0.2
0.0
Immune Plasma
Naive
1:10
1:50
1:250
1:1250
1:6250
1:12500
Plasma Dilution

FIG. 12

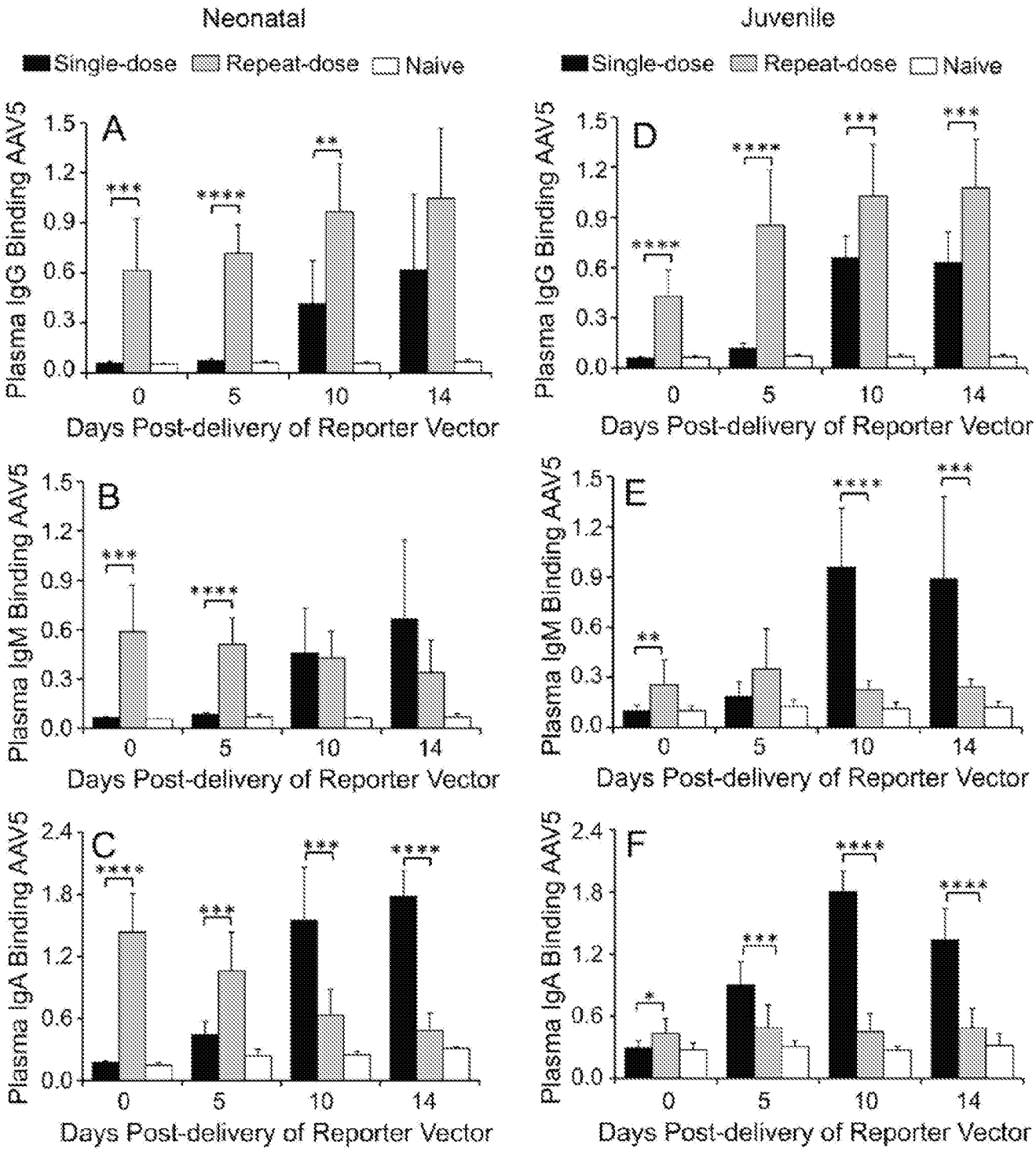

Neonatal
Juvenile
Single-dose
Repeat-dose
Naive
A
B
C
D
E
F
Plasma IgG Binding AAV5
Plasma IgM Binding AAV5
Plasma IgA Binding AAV5
Days Post-delivery of Reporter Vector
0
5
10
14

FIG. 13

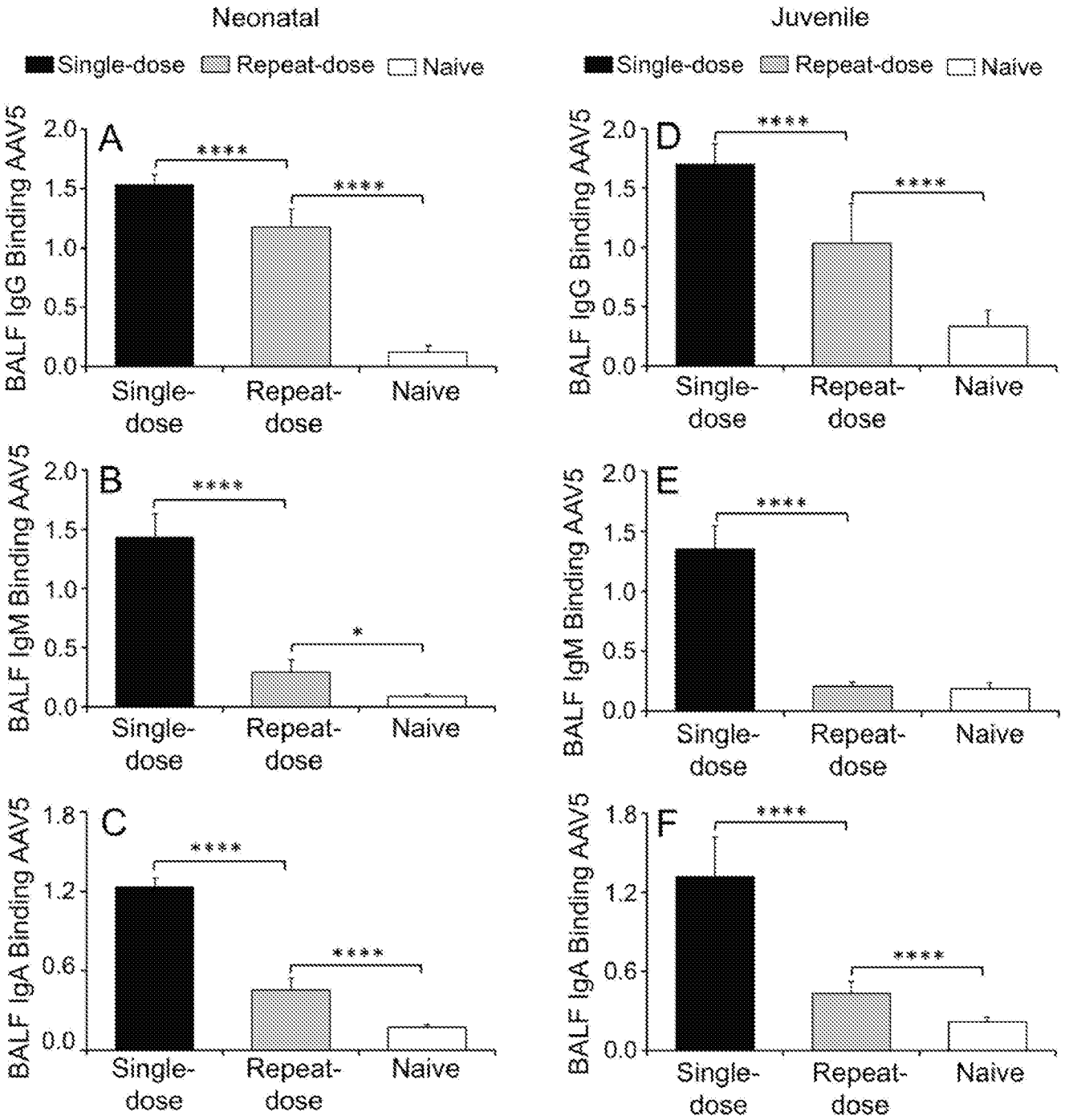

Neonatal
Juvenile
Single-dose
Repeat-dose
Naive
A
B
C
D
E
F
BALF IgG Binding AAV5
BALF IgM Binding AAV5
BALF IgA Binding AAV5
****
*
Single-dose
Repeat-dose
Naive

FIG. 14

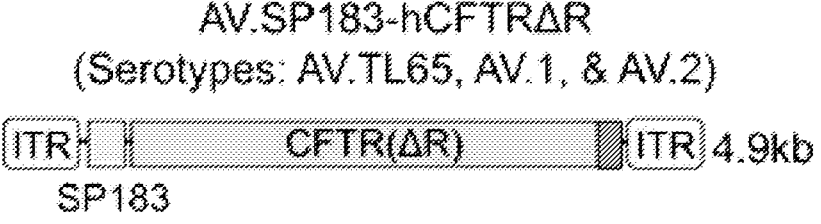

AV.SP183-hCFTRΔR
(Serotypes: AV.TL65, AV.1, & AV.2)
ITR
CFTR(ΔR)
ITR
4.9kb
SP183

FIG 1A