TRANSCRIPTIONAL TARGETING IN THE AIRWAY USING NOVEL GENE REGULATORY ELEMENTS

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ONLINE DATA SUPPLEMENT

SUPPLEMENTARY MATERIALS AND METHODS

Promoter plasmid constructs.

Promoter sequences were cloned with 5' NotI or XhoI and 3' HindIII restriction endonuclease recognition sequences. The 5' LTR from Jaagsiekte Sheep Respiratory Virus (JSRV) was PCR amplified from the pJS21-luc plasmid (1) using the forward primer AGCGGCCGCCAGTGTGATG and the reverse primer TCGCCCTTCCAAGCTTACTTAGATCGCAG. The murine *CC10* minimal promoter was a kind gift from Dr. Barry Stripp and was amplified using the forward primer AATGCGGCCGCTTGTGTGAGTG and the reverse primer AGGAAGCTTATGTGGGGTGATGTTG. Genomic regions 5' of the *BPIFA1* genes from *Canis familiaris, Equus ferus caballus, Mus musculus, Rattus norvegicus,* and *Homo sapiens* were aligned using the VISTA genome browser (2). One kilobase of sequence 5' and 300 bases 3' of the transcription start site (TSS) were amplified from human genomic DNA based on homology via semi-nested PCR using the 1° forward primer AAGCGGCCGCAGGTCAGGAGTTCG and reverse primer AGAAGCTTGTTAAGGGTAGCAGAGGGGCC and the 2° forward and reverse primers AAGCGGCCGCAGGTCAGGAGTTCG and TCAAGCTTGGATGGCTCTTACCTG, respectively. The genomic regions 5' of *WDR65* genes from *Canis familiaris, Equus ferus caballus, Mus musculus, Rattus norvegicus,* and *Homo sapiens* were aligned using the VISTA genome

browser. 400 bases upstream and 100 bases downstream of the TSS were amplified from human genomic DNA based on homology using the forward and reverse primers AATGCGGCCGCTCACTCCCAGGG and AATAAGCTTTGCTACCAGCTCC. Amplicons were subcloned into pCR2.1 (Invitrogen Life Technologies, Carlsbad, CA, USA). 2150 bases upstream of human *CFTR* were PCR-amplified from human leukocyte DNA and cloned in the NheI/BglII sites of the luciferase expression vector pGL3-Basic (Promega). Primers used to PCR amplify the human *CFTR* upstream sequence were based upon the human *CFTR* 5' end (NCBI accession # M58478); phCFTR-F CGAGCTAGCGGCATTTACTTAACTTTTCTTGTTTGCTTAAC and phCFTR-R TTAAGATCTGGTCTCTCGGGCGCTGGGGTCCCTGCTA.

To clone the dual expression constructs for *in vitro* expression analysis, XhoI/HindIII fragments from each promoter subclone were ligated to the XhoI/HindIII linearized pGL3dual vector 5' of the firefly luciferase reporter gene. To clone the FIV transgene cassette constructs, the NotI/HindIII fragments from each promoter subclone were ligated to the NotI/HindIII linearized FIV3.3MCSLuc vector (3). To clone the adenoviral-promoter-ntLacZ plasmids, the *hBPIFA1*, *hWDR65*, and *hCFTR* upstream genetic elements were first subcloned with MluI and BglII restriction sites 5' and 3', respectively, into pCR2.1 as previously described. A BglII site in the SV40 polyadenylation signal of the final Ad5ntLacZ plasmid vector was removed via site-directed mutagenesis using the QuikChange II XL Mutagenesis Kit (Agilent Technologies, Santa Clara, CA,

USA) using the primers CTAGTCCCGCGGTGGCAAATCTGGAAGGTGCTGAGG and CCTCAGCACCTTCCAGATTTGCCACCGCGGGGACTAG according to the manufacturer's instructions. The MluI/BglII subcloned promoter fragments were then ligated into the Ad5ntLacZ plasmid.

To clone the Ad-CFTR shuttle plasmids, the porcine cDNA was PCR amplified using the oligos AAAACTAGTAATTCAAGTCGACCTAAAGTC and AAACTCGAGCCGGTGCCACCATGCAGAGG and subcloned into pCR2.1 (Invitrogen Life Technologies). The oligos contained 5' XhoI and 3' SpeI sites. The XhoI/SpeI fragment was cloned into an XhoI/XbaI-digested Ad5RSVShuttle vector. The *hBPIFA1*, *hWDR65*, and *hCFTR* upstream genetic elements were subcloned with MluI and XhoI sites 5' and 3', respectively into pCR2.1. The MluI/XhoI subcloned promoter fragments were then ligated into the Ad5pigCFTR plasmid.

Transient transfections in A549 cells.

A549 cells (ATCC #CCL-185) were cultured in DMEM (Gibco, Carslbad, CA, USA) supplemented with ten percent FBS and one percent penicillin/streptomycin. pGL3dual promoter constructs were transiently transfected (0.4 µg) into 24-well plates of A549 cells (150,000 cells/well) using Lipofectamine 2000 (Invitrogen Life Technologies) according to the

manufacturer's instructions. Transiently-transfected cells were harvested in 1X Passive Lysis Buffer (Promega, Madison, WI, USA) after 48 hours. Twenty µl lysates were assayed using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions.

Viral vector production.

Lentiviral and Adenoviral vectors were produced in the University of Iowa Gene Transfer Vector Core (4, 5). FIV packaging, transgene cassette, and envelope plasmids were co-transfected into TSA201 cells using Ca_2PO_3 precipitation as previously described (4). Collected supernatants were concentrated 250:1 via overnight centrifugation and reconstituted in alpha-lactose buffer (40 mg/ml in 1X PBS). Concentrated vector was titered on HT1080s and FIV genomes were quantified using Taqman qPCR.

Bromodeoxyuridine (BrdU) staining

Three mice received 7 consecutive intraperitoneal doses of BrdU (50 mg/kg/dose, 1 dose/day). Mice received 50 µl intra-nasal doses of 2% polidocanol on days 1 and 4. Mice were euthanized on day 8 (4 days following the second dose of polidocanol and 1 day following the final dose of BRDU) and the tissues were fixed in 10% formalin. BrdU staining was conducted using a ZYMED BrdU staining kit (cat# 93-3943) and the manufacturer's instructions. A

parallel cohort of mice (n = 3) received DMSO (vehicle control) instead of polidocanol.

Statistics.

All numerical data are represented as mean ± standard error. Standard one and two sample t tests were performed using the statistical computer program R (www.r-project.org) with the lme4 package. Analysis of variance was performed using Prism software.

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