Material and Methods

Source of islets and tissues. All animal experimentation was approved by the Institutional Animal Care and Use Committee at the University of Iowa. Adult ferret islets were isolated as previous described (1). Non-diabetic human islets were obtained from the Integrated Islet Distribution Program.

Generation of recombinant adenoviruses to knockdown CFTR. To knockdown CFTR expression in WT ferret and human islets, two adenoviral vectors expressing a shRNA to human or ferret CFTR were generated to the same region of the *CFTR* cDNA (Exon 14; 2083-2111 bp of the transcript with the start codon ATG as +1). The coding sequence homologous to the 22 bp shRNA core (italics) was 5'-AAAAAA*GGAAGAATTCTATTCTCAATCC*-3' for human *CFTR* and 5'-AAAAAA*GGAAGAACTCT ATTCTTAATCC*-3' for ferret *CFTR*. For construction of the adenoviral vector expressing shRNA-CFTR, double stranded oligonucleotides for the following complete shRNA sequences were synthesized and cloned downstream of the murine U6 promoter within the paced shuttle vector (Vector core, University of Iowa): Human 5'-GAAGAATTCTATTCTCAATCTGTAAAGCCACAGATGGGATTGAGAATAG AATTCTTCCTTTTTT-3'; Ferret 5'-GAAGAACTCTATTCTTAATCTGTAAAGCCACAGATGGGA TTAAGAATAGAGTTCTTCCTTTTTT-3'. Similarly, a scrambled shRNA was also generated with a core sequence (italics) of 5'-TTTTTT*ATTGCGTTAGTTATACTAATCA*-3' using synthetic oligonucleotides of 5'-AACGCAATCAATGATTAGTCTGTAAAGCCACAGATGGGACTAAT CATTGATTGCGTTATTTTTT-3'. Recombinant adenoviral vectors were generated by the co-transfection of 293 cells with the linearized proviral plasmid containing the shRNA cassette in the E1 region and an adenoviral genome plasmid containing an RSV-driven eGFP cassette in the E3 region, as previously described (28). Viral stocks were amplified by CsCl banding and desalted prior to use. Viruses are designated: Ad.fCFTR-shRNA (ferret *CFTR* targeting vector), Ad.hCFTR-shRNA (human *CFTR* targeting vector), and Ad.Scr-shRNA (scrambled shRNA control vector).

CFTR knockdown in ferret and human islets using recombinant adenovirus. *CFTR* gene expression was inhibited in isolated ferret and human islets using recombinant adenovirus (Ad.fCFTR-shRNA and Ad.hCFTR-shRNA) encoding an shRNA against each *CFTR* species. Virus containing a scrambled shRNA

(Ad.Scr-shRNA) was used as a negative control. Approximately 100 adult human or ferret islets were cultured with recombinant adenoviruses ($1.0x10^9$ particles/ml) in RPMI1640 without serum for 4hrs and then supplemented with 10% fetal bovine serum thereafter. After 24hrs, the transduced islets were transferred into fresh culture medium for another 24hrs prior to using the islets for experiments.

CFTR RNA quantification in islets. CFTR RNA expression was measured by the QuantiGene Plex Assay Kit (Affymetrix). Bead-based oligonucleotide probe sets specific for ferret CFTR and peptidylprolyl isomerase B (PPIB) RNA were developed by Affymetrix, as detailed in the RNA probe map on the next page. Islet homogenates were prepared using the QuantiGene Sample Processing Kit (Affymetrix) and quantified as previously described (2), using PPIB as the internal control (housekeeping gene) by which to normalize expression for each sample.

Islet hormone secretion assays. Insulin secretion assays using isolated islets were performed in static culture as previously described (3). Briefly, five adult islets (150-200μm diameter) were equilibrated for 1hr in 1.67mM glucose Krebs-Ringers Bicarbonate Buffer (KRB: 120mM NaCl, 4.8mM KCl, 2.5mM $CaCl_2$, 1.2mM $MgCl_2$, 20mM $NaHCO_3$, 5mM HEPES, pH7.4) at 37°C (human islets) or 38.5°C (ferret islets). Each group of islets was then divided equally into either 1.67 or 16.7mM glucose KRB and cultured for 1hr. Insulin was measured using human insulin ELISA kit (Calbiotech, Cat#IS130D). Insulin release was calculated on a per islet basis as previously described (2).

1. Li DS, Yuan YH, Tu HJ, Liang QL, Dai LJ 2009 A protocol for islet isolation from mouse pancreas. *Nat Protoc* **4** 1649-1652.

2. Yi Y, Sun X, Gibson-Corley K, Xie W, Liang B, He N, Tyler SR, Uc A, Philipson LH, Wang K, Hara M, Ode KL, Norris AW, Engelhardt JF 2016 A Transient Metabolic Recovery from Early Life Glucose Intolerance in Cystic Fibrosis Ferrets Occurs During Pancreatic Remodeling. *Endocrinology* **157** 1852-1865.

3. Olivier AK, Yi Y, Sun X, Sui H, Liang B, Hu S, Xie W, Fisher JT, Keiser NW, Lei D, Zhou W, Yan Z, Li G, Evans TI, Meyerholz DK, Wang K, Stewart ZA, Norris AW, Engelhardt JF 2012 Abnormal endocrine pancreas function at birth in cystic fibrosis ferrets. *J Clin Invest* **122** 3755-3768

RNA Probe Map
Capture Extender Oligo (CE) is BLUE
Label Extender Oligo (LE) is RED
Blocking Probe Oligo (BL) is GREEN

>gi|859759950|ref|XM_004741906| PREDICTED: Mustela putorius furo cystic fibrosis transmembraneconductance regulator (ATP-binding cassette sub-family C, member 7)(CFTR), transcript variant X2, mRNA. [Mustela putorius furo] MAM 01-JUL-2015

gggcttggaaggaacgcgcgtgagggaggaaggctgggagtcagaatgggaaaagggaaacgcggagcagccaggagcga
aggagagaaggagcggtcagggtgcgcagagggtggagaaagccgctagggcaaactgggggctggaccaggcagcgccc
agcttttaacctgggcagtgggggcgggggaaggagctaaagggaaggggaggtgtgtgaagtggggtgggggggggttg
gaagcaaatgacatcacagcaggtcagagaaaaaggggcgagcagcctgtgccagaggagtaaggcgtttggcattagga
gctcgagtccagcctgcccgggcagggaccctgacgcccgcagagaccatgcagaggtcgcctctggagaaggccagcgt
cctctccaaacttttttcagctggaccagaccaattttgacgaaaggatatagacagcgcctggaattatcagacatat
atcaaatcccttctgctgattctgctgacaatctctctgaaaaattggaaagagaatgggaccgagaactggcatcaaag
aaaaaccccaaactcattaatgcccttcggcgatgctttttctggagatttatgttctatggaatcatattatatttagg
ggaagtcaccaaatcggtccagcctctcttactgggaagaatcatagcttcctatgatccagataacaaggaggaacgct
ctatcgcaatttacctaggcataggcttatgccttctctttgtcatgaggacgctgctcctgcacccggccatttttggc
cttcatcgtattggaatgcagatgaggatagctatgtttagcttgatttataagaagggacttgcactggcacattttgt
gtggattgctcctttacaagtgacgctcttgatgggcctgctctgggatttgttacaagcctctgccttctgtggtcttg
ctttcctcatagtcctcgccctttttcaagccgggttggggagaatgatgatgaagtacagagatcagagagctggaaag
atcaacgaaagactcgtgatcacctcagaaatgattgaaaatatccaatctgttaaggcatattgctgggaggaagcaat
ggaaaaaatgattgaaagcataagacaaacagaactgaaactgacccggaaggcagcctatgtgagatacttcaatagct
cagccttcttcttctcagggttctttgtggtgttttatctgtgcttccctatgcactgatcaaaacaatcgtccttcga
aaaatattcacaaccatctcattctgcatcgttttgcgcatggcagtcactcggcaattcccctgggctgtacaaacatg
gtatgactctcttggagcaataaacaaaatacaggatttcttacaaaagcaggagtataagacgttggaatataacttaa
caactacagaagtagtgatggagaatgtaacagccttctgggaggagggatttggggaattacttgagaaagcaaaacaa
aacagtaacgacagaaaaatttccaatgctgataacagccttttcttcagcaatttctcacttcttggtgctcctgtcct
gaaagatatcagtttcaagatagaaagaggacagttgttggcagttgctggatctactggagcaggcaagacttcacttc
tgatgatgattatgggagagttggagccttcagagggcaaaattaagcacagtggaagaatttcattctgctctcagttt
tcctggatcatgcctggcaccatcaaagaaaacatcatctttggtgtttcttatgatgagtatagatacaggagtgtcat
caaagcatgccagctagaggaggacatctccaagtttgcagagaaggacaatatcgtccttggagaaggtggaatcacac
tgagtggagggcagcgagcaagaatttccttagcaagagcagtatataaagatgctgatttatacctattagactctcct
tttggatacctggatgttttaacagaaaaagaaatatttgaaagctgtgtctgtaaattgatggctaacaaaactaggat
tttggtcacttctaaaatggaacatttaaagaaagctgacaaaatattaattttacatgaaggtagctgctatttttatg
ggacattttctgaattacaaaatctacggcccgacttcagctcaaagctcatgggatatgattcttttgaccagtttagt
gcagaaagaagaaattcaatcataactgagactttacggcgtttttcattagaaggagatgcgcctgtctcctggaatga
aacaaaaaagcaatcttttaaacagactggagagtttggtgaaaaaaggaagaactctattcttaatccagtgaattcta
tcagaaaattttcagttgtacagaagactccattacaaatgaatggcattgaagaagattccgatgagcccttagagaga
aggctgtccttggttcctgatggtgctgagcaaggagaggccatcctgcctcgaagcaacatgatcaacactggccccac
gttgcagagacaacgaaggcagtctgttctgaatctgatgacatgctccccagggaaccaaggtcaaagctttcatggaa
ggacagcctcatccacacgtaaaatgtcgctggctcctcaggcaaacttaactgaaatggatatatattcaagacggcta
tctcaagatagtggcttggaaatcagtgaggaaattaacgaagaagatttaaaggagtgctttattgacgatgttgaaag
cataccaccagtgacaacatggaacacatacctccgatatgttactatccacaagagcttagtttttgtgctaatttggt
gcttagttatttttctggctgaggtggctatttctttggttgtgttgtggctacttaaaaaaacagcttctcaagacaaa
gggaatagtactcagagcataaatagcagttacacagtgatcttcaccagtaccagcacatattatgtttttttacattta
tgtgggagtagcagacactttgcttgccctgggattcttcagaggtttaccactggtgcatactctaatcacagtgtcga
aaatttttacaccacaaaatgttacatgctgttcttcaagcacctatgtcaaccctcaacgcattgaaagcaggtgggatt
cttaatagattctccaaagacatagcaattttggatgatcttctgccccttaccatatttgacttcgtccagttgttact
aattgtgattggagctgtgacagtagtttcggctttgcaaccctacatcttcctagcaacagtgcctgtcatagcagctt
tcattatgctgagggcctacttcctccacacctcacagcaactcaagcaactggaatctgaaggcaggagtccaattttc
actcatcttgtcacaagcttgaaaggactatggacacttcgggcctttggacgtcagccttacttcgaaactctatttca
caaggctctgaacttacataccgccaactggttcttgtacctatccacactacgctggttccaaatgagaatggaaatta
tttttgtcatattcttcattgctattaccttcatttccattttaacaacaggtgaaggagtaggagcagttggtattatt

ctaacgttagccatgaatatcatgggtacattgcaatgggctgtaaactccagcatagatgtggacagcttgatgcgatc
tgtaagcagagtcttcaagtttattgacatgccagcagaagaaagtaaaccacccaccaagtcattcaaaccatccaagg
atgtccagctctcaaaagttttgattactgagaatcatcatgtgagagaagatgacatctggccctcaggggccaaatg
actgtcaaagacctcacagcaaagtatatagatggtgggaatgccatattagagaacatttccttctcaataagtcctgg
ccagagggtgggcctcttgggaagaactggatcagggaaaagtactttgttatctgctttttgagactgctgaacactg
agggagaaatccaaatagatggtgtgtcttgggattcgataactttgcaagagtggaggaaagcctttggagtgatccca
cagaaagtatttatattctctggaacatttagaaaaaacttggatccctatggacagtggaatgatcaagaaatatggaa
ggttgcagatgaggttggactcagatctgtgatagagcagtttcctgggaagcttgattttgtcctggtggatggaggtt
gcgtcctaagccatggccacaagcagttgatgtgcttggccagatctgttctcagcaaggcaaagatcttgctgcttgat
gaacccagtgctcatttggatccaataacatatcaaatcattcgaagaaccctaaaacaagcatttgcagattgcacagt
aatcctctctgaacacaggatagaagctatgttggaatgtcagcgatttttggtcatagaggaaaacaaggtgcggcagt
atgattccctccagaggctgctgagcgagaagagcctcttccggcaggccatcagcccctcggaccggctgaggttcttc
ccgcaccggaactcgagcaagcacaagtctcgctctcaaattgctgctctgaaggaggagacggaagaagaggtgcaaga
aacaaggctttagagaacactccagatattggcatgggctggccgctcatgaaattggaggagaacccagtttctgcccc
ttctttctaccacaaggatgagtaatgtttatttttaaaaggaaaaattttgagaagaggaattaaggacgctcagatgg
gtctggataagtagcttcctggcagtggtcgaattgtgtgaaaggtacttcaaagccttgaagatttaccacttgtgttt
gcaagtcagatcctcctgaaaacttttcctcttggtagtaattggaagggtagctatccatgtcagccagtgtaattgt
tcaacgtactgtttagtgagacttgctaatttgtatttttggaggagaactgtaatcatactacttcaagatatgattaa
gtaaagatgattggaaactttagtggtttatgtaagtttatatcactttgcctctactctccccagatacttaaaaatac
aactttgttgtctcttacgtatttggactgttctatttcaaagcaagtattagagtactgcaaaagtcacaagtcagctc
atcacagtatacaccgtgtaacatctacgtgtcaggagagagaacttcttgatctggaaaatccgtgttaataccactca
ctgctttaccaaaaatccaaataattcaggcaattagtatacatcccttacccaggaagagggctgttacagtcccatgt
gggggacaggataattgccttgatgcagaagttgacatgactttccccagtttccagaaagtaacaggctcataagacct
ttgaacagaaggatagctaggaaaaaatgctttaatgaaaattggcacaggaaatccttcttgcccctaaaaactctggg
aagaatatgtaagcatataggctgtgggcactgcaggtagatgtacatgaaattgaaagatgtagggtacgtatagggta
atgtcagtacagtttcaggctaactgtaggcctttcctcctagtgtagactgagtgggacagataatgggagacacaggg
aagagtcaatcaattcgttttatatgcttctgttttataagtttgtcatgcaagtaaaatttctctaggaaatatttat
tttaatatttcaaacttataaatggctgtattttacaatgattctattatgaattatatttgtattaaaagaattttata
tgtgaaatgttggtttttttatggcactagctctttttatgatatggtaaaactgaaggaaaaaaactgggcgggggcta
ttgaccagaagttacaaattaccttgatggtgtagagagaagcatgggggctcacaaagatttgcacctctgtacgattc
cctgccagcatacaggttccctcttggaaaatggtaccatgaaatctgacctctcctatcaaggacacacgccttcccaa
ctccaaactaactgttaatattacattctatttattactgtaagaaaatatcatgtgccaataaaatccctatatttgtg
tgaaa

>gi|859817605|ref|XM_004758725| PREDICTED: Mustela putorius furo peptidylprolyl
isomerase B(cyclophilin B) (PPIB), mRNA. [Mustela putorius furo] MAM 01-JUL-2015

ccagaggggaaaatagggacgacctcctgtcaggagactggcaggagccccggtttccccctcagtcgctctcggtgg
tggcgccggtgctctcgccaacgcgtccccaccctcttccggccctcggctgccggggccgcgctctctttgcccggtgg
atgctgcgcctctctgagcggaacatgaaggtcctctttgccgccgccctcatcgtgggtccgtcttcttcctgctgtt
gccaggtccctccacggccgacgagaagaagaagggcccaaagtcaccgtcaaggtgtactttgatctgcgaattggag
atgaagatataggccgggtagtcattggtctctttggaaagacagttccaaaaacagtggataatttttgtggccttagcc
acgggagagaaaggatttggctacaaaaacagcaaattccatcgtgtgatcaaggacttcatgatccagggtggagactt
tacccggggagatggcactggaggaaagagcatctatggtgaacgcttccctgatgagaacttcaagctgaaacactacg
ggcctggctgggtgagcatggccaatgcaggcaaagacaccaatggctcccagttctttatcacgacggtgaagacagcc
tggctagatggcaagcacgtggtattcggcaaagttctggaaggcatggaggtagtacggaaggtggagagcaccaagac
cgacagtcgggacaagcccctgaaggatgtgacgattgcagactgtggcaagatcgaggtggagaagcccttcgccatcg
ccaaggagtagggcccccgggacctcttccctttgagccaccatctgtgcagctctgttgccttcccctgggtggagacc
ggctgccgcagggttctgtgctgctctggccccagtgctggcctctgacagagtggacccctccccgcacattctgcagg
cctacctaccggcctggttttgtaacaaagtcctaccaatgctgaccaattaaaaaagatgtttttttttataa