Supplementary Material (REVISED)

FOLDING AND RESCUE OF A CFTR TRAFFICKING MUTANT IDENTIFIED USING HUMAN-MURINE CHIMERIC PROTEINS

Ana Carina Da Paula[1,2], Marisa Sousa[1,2], Zhe Xu[3], Elizabeth S Dawson[4], A Christopher Boyd[4], David N Sheppard[3] and Margarida D Amaral[1,2§]

From University of Lisboa, Faculty of Sciences, BioFIG - Centre for Biodiversity, Functional and Integrative Genomics, Portugal[1], Department of Genetics, National Institute of Health, 1649-016 Lisboa, Portugal[2], Department of Physiology and Pharmacology, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, UK[3], Medical Genetics Section, Molecular Medicine Centre, Institute of Genetics and Molecular Medicine, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, UK[4]

Supplemental Figure Legends

Supplemental Fig. 1: Alignment of murine NBD1 and NBD2 amino acid sequences in the hmCFTR chimeras 12b-NBD1 and 114c-NBD2 with the corresponding sequences of hCFTR. (*A*) For clone 12b-NBD1 (aa 518-586) there are 12 residues divergent between the amino acid sequences of human and murine CFTR (shown in red). Among these, the 6 alterations with highest PCD values (indicated by black filled circles) were selected to be introduced (by site-directed mutagenesis) into the hCFTR cDNA. The 6 CFTR constructs generated were: E527Q, E528Q, S531T, K536Q, I539T and K584E. (*B*) For clone 114c-NBD2 (aa 1260-1412) there are 27 residues divergent between the amino acid sequences of human and murine CFTR (shown in red). Among these, the 12 alterations with highest PCD values (indicated by black filled circles) were selected to be introduced (by site-directed mutagenesis) into the hCFTR cDNA. The 12 CFTR constructs generated were: T1263I, P1290T, K1302Q, Y1307N, Q1309K, S1311K, R1325K, V1338T, C1344Y, L1367I, D1394G and E1409D. Numbers refer to amino acid co-ordinates in human CFTR. The alignment of the murine region of 114c-NBD2 was extended until residue 1419 to highlight differences between it and the hmCFTR chimera 323c-NBD2 (M1260-S1419) (see Discussion). The known (NBD1) and predicted (NBD2) secondary structures of the NBDs are shown above the sequence alignments with α-helices labeled as "H" and ß-sheets labeled as "S"; orange denote the F1-type ATP-binding core subdomain; blue the ABC α-subdomain and green the ABC ß-subdomain (11). For clarity, H6b and H6c are labeled 6b and 6c. Boxes denote the positions of the ABC signature and Walker B motifs.

Supplemental Fig. 2: Time courses of iodide efflux from BHK cells stably expressing CFTR variants in NBD1 and NBD2. (*A*) wt- (open squares long dashed line), F508del-CFTR (open circles dotted line) and murine-CFTR (open triangles medium dashed line); **(***B***)** E527Q (black circles), E528Q (black squares), S531T (black triangles); **(***C***)** K536Q (black inverted triangles), I539T (black diamonds) and K584E (black hexagons); **(***D***)** T1263I (open circles), P1290T (open squares), K1302Q (open triangles), Y1307N (open inverted triangles); **(***E***)** Q1309K (open diamonds), S1311K (open hexagons), R1325K (gray circles), V1338T (gray squares); **(***F***)** C1344Y (gray triangles), L1367I (gray inverted triangles), D1394G (gray diamonds) and E1409D (gray hexagons). During the periods indicated by the black bars, cells were stimulated with the cAMP agonist forskolin (10 μM) and the

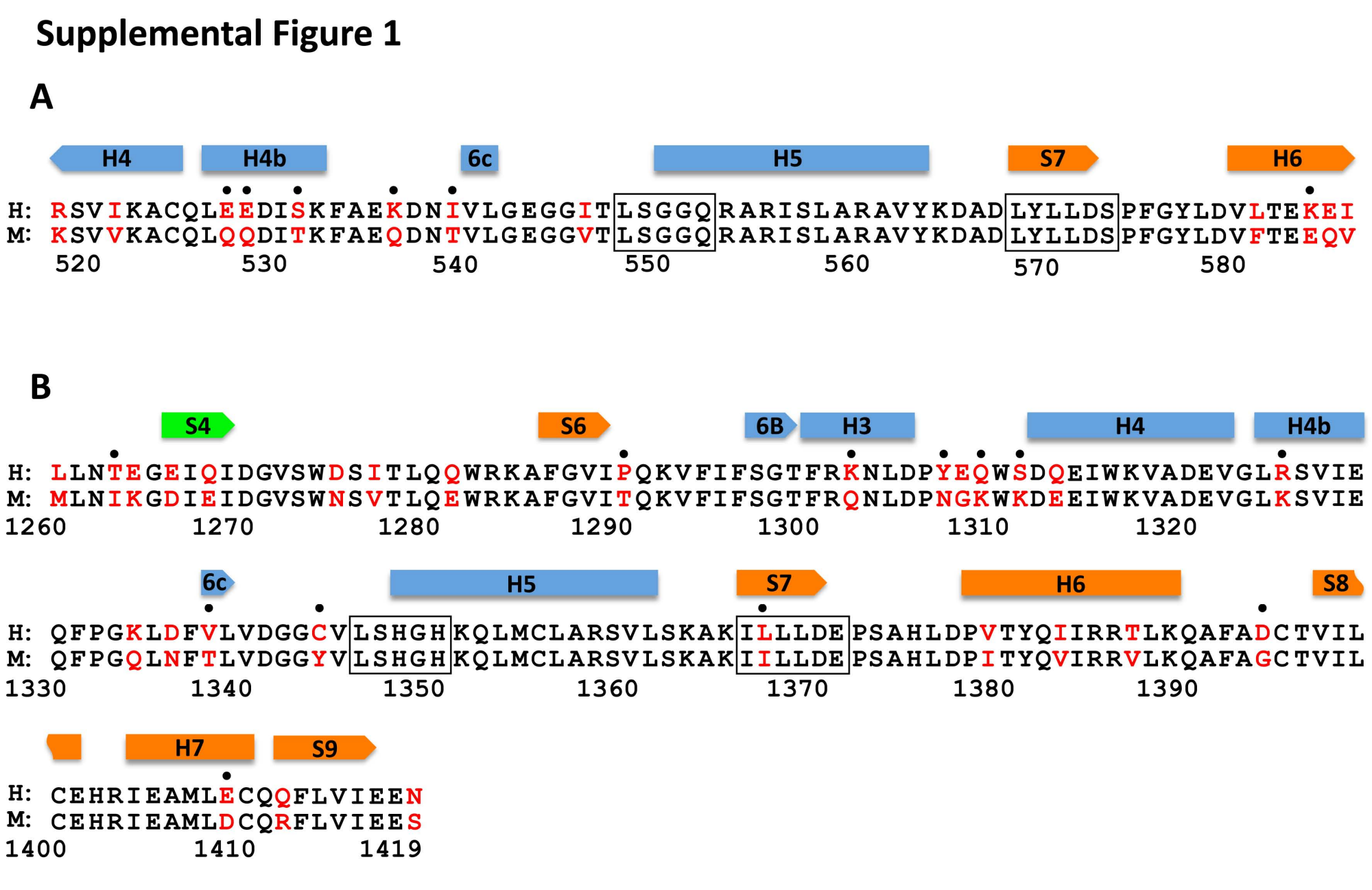
CFTR potentiator genistein (50 μM). All BHK cells were cultured at 37 ºC prior to assaying CFTR function using the iodide efflux technique. Data are means ± SEM (n = 6); where not shown error bars are smaller than symbol size.

Supplemental Fig. 3: Restoration of CFTR-mediated iodide efflux to K584E by the revertant mutation L581F. (*A*) Time courses of CFTR-mediated iodide efflux from BHK cells stably expressing wt- (long dashed line), F508del- (open circles), K584E- (black circles), L581F (black triangles) and L581F-K584E (black inverted triangles) cultured at 37 ºC and (*B*) wt- (long dashed line), F508del- (black squares) and K584E- (open diamonds) cultured at 26 ºC. During the periods indicated by the black bar, forskolin (10 μM) and genistein (50 μM) were added to the efflux buffer to stimulate CFTR-mediated iodide efflux. Data are means ± SEM (*n* = 6); where not shown, error bars are smaller than symbol size.

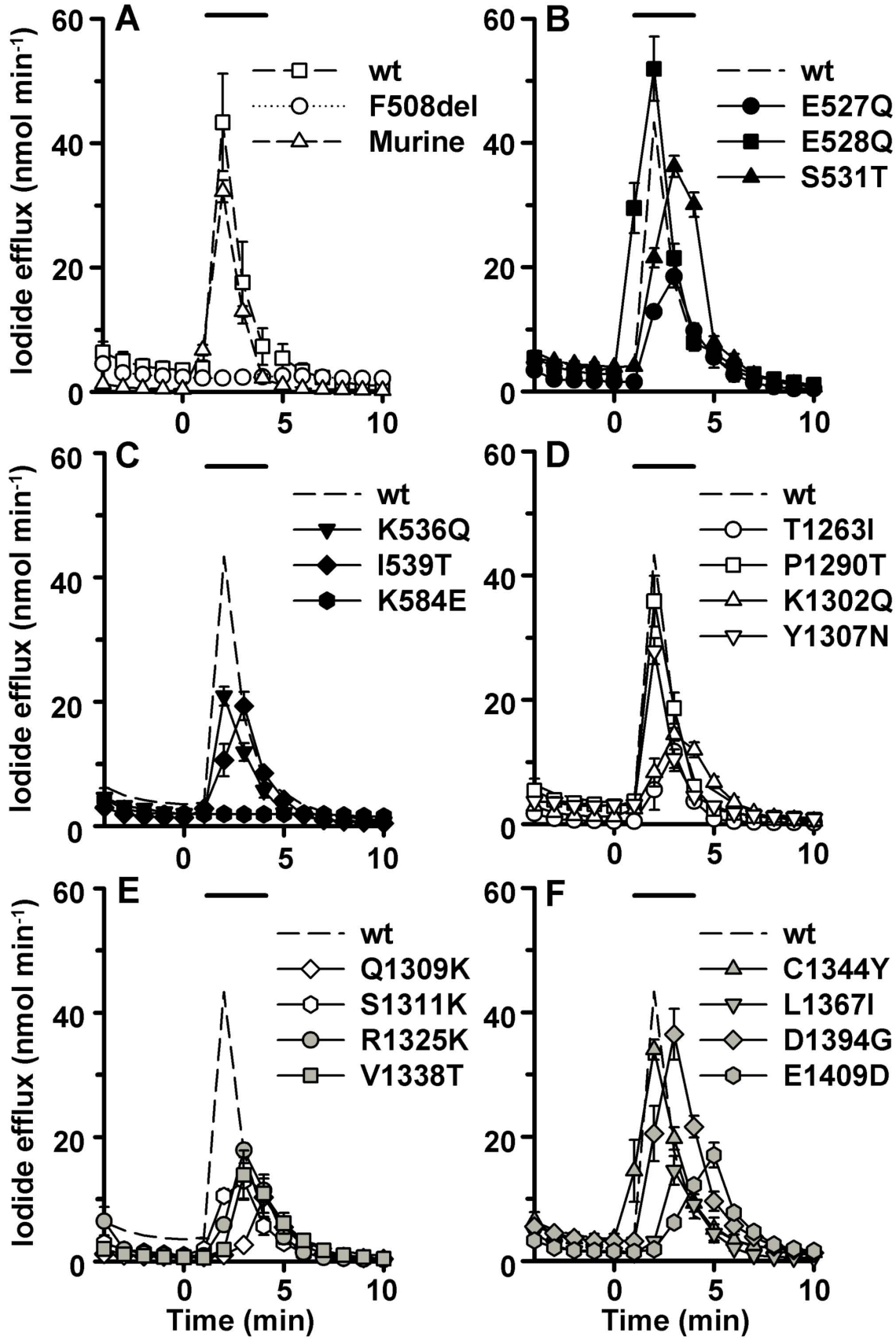
Supplemental Table 1: (*A*) Physico-chemical distances of murine/human divergent residues for 12b-NBD1. (*B*) Physico-chemical distances of murine/human divergent residues for 114c-NBD2. The human residue precedes the amino acid position, whereas the murine residue follows the position. Asterisks denote the residues that we selected for study. The criteria we adopted for selection of divergent residues to be generated by mutagenesis for further studies is provided on the right column.

Supplemental Table 2: Custom designed primers used to introduce various mutations into pNUT CFTR cDNA by site-directed mutagenesis.

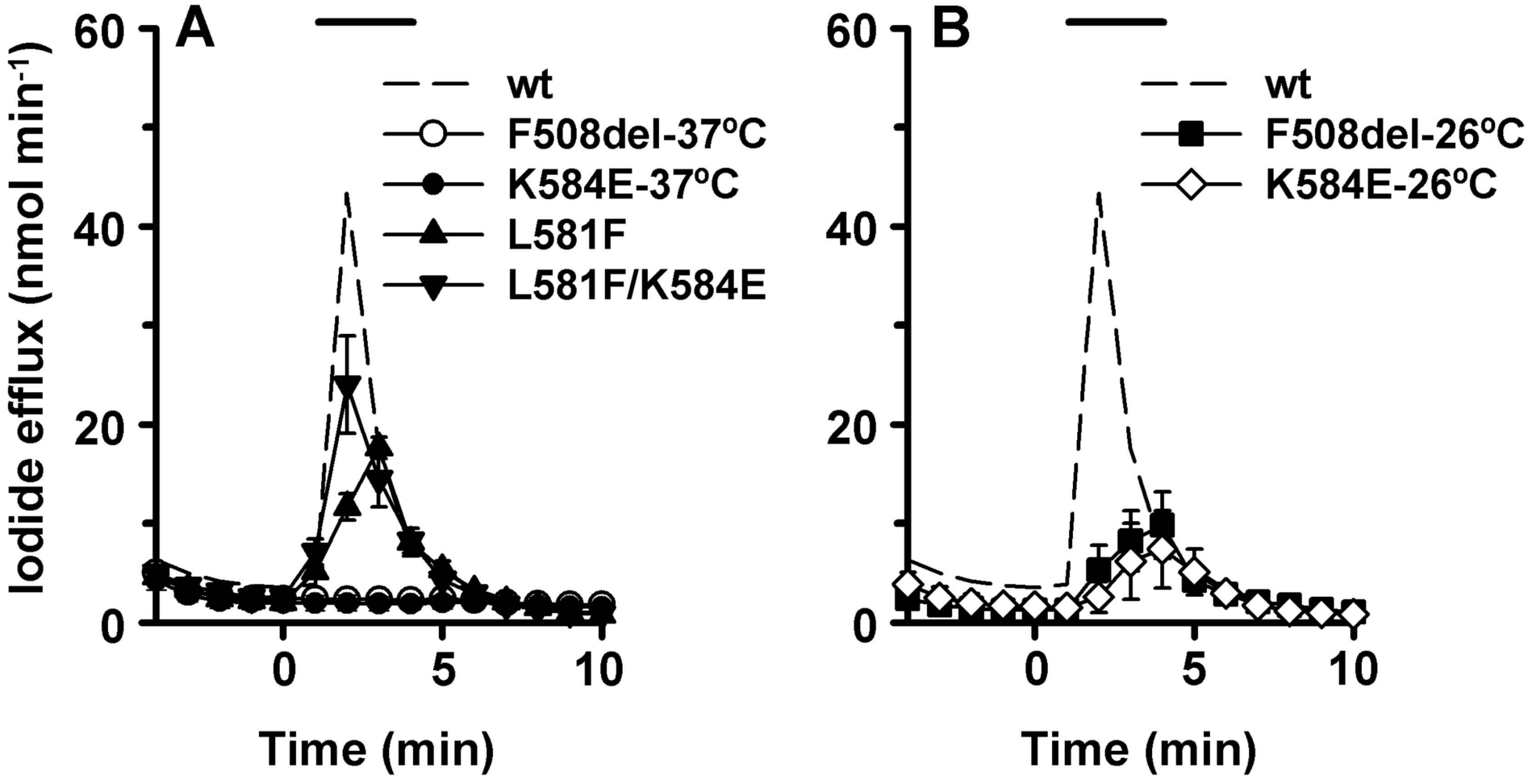
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Table 1

Table 1A: Physico-chemical distance (PCD) of murine/human divergent residues for 12b-NBD1. Asterisks denote the residues selected for this study.

Residues	PCD values	Selection criteria for mutagenesis
R518K	26	-
I521V	29	-
E527Q *	29	CF-causing mutation
E528Q *	29	Adjacent to CF-causing mutation
S531T *	58	High PCD
K536Q *	53	High PCD
I539T *	89	High PCD
I546V	29	-
L581F	22	-
K584E *	56	High PCD
E585Q	29	-
I586V	29	-

Table 1B: Physico-chemical distance (PCD) of murine/human divergent residues for 114c-NBD2. Asterisks denote the residues selected for this study.

Residues	PCD values	Selection criteria for mutagenesis
L1260M	15	-
T1263I *	89	High PCD + CF-causing mutation
E1264K	56	-
E1266D	45	-
Q1268E	29	-
D1275N	23	-
I1277V	29	-
Q1281E	29	-
P1290T *	38	CF-causing mutation
K1302Q *	53	-
Y1307N *	143	High PCD
E1308G	98	-
Q1309K *	53	Hotspot residue for CF-causing mutations
S1311K *	121	High PCD
Q1313E	29	-
R1325K *	26	Possible arginine-framed tripeptide
K1334Q	53	-
D1336N	23	-
V1338T *	69	High PCD
C1344Y *	194	High PCD
L1367I *	5	Hotspot residue for CF-causing mutations
V1379I	29	-
I1383V	29	-
T1387V	69	-
D1394G *	94	High PCD
E1409D *	45	Hotspot residue for CF-causing mutations
Q1412R	43	-

Supplemental Table 2

Table 2: Custom designed primers used to introduce various mutations into pNUT-CFTR cDNA by site-directed mutagenesis.

Name of Forward Primer	Sequence 5'→ 3'
E527Q	GCATGCCAACTACAGGAGGACATCTCC
E528Q	TGCCAACTAGAACAGGACATCTCCAAG
S531T	CAACTAGAAGAGGACATCACCAAGTTTGCAGAGAAAGAC
K536Q	GACATCTCCAAGTTTGCAGAGCAAGACAATATAGTTCTTGG
I539T	GCAGAGAAAGACAATACAGTTCTTGGAGAAGG
L581F	CCTTTTGGATACCTAGATGTTTTTACAGAAAAAGAAATATTTGAAAGC
L581F-K584E	CCTTTTGGATACCTAGATGTTTTTACAGAAGAAGAAATATTTGAAAGC
K584E	CCTTTTGGATACCTAGATGTTTTAACAGAAGAAGAAATATTTGAAAGC
T1263I	GAGACTACTGAACATTGAAGGAGAAATCC
P1290T	GGAGGAAAGCCTTTGGAGTGATAACACAGAAAGTATTTATTTTTTCT
K1302Q	CTGGAACATTTAGACAAAACTTGGATCCC
Y1307N	GAAAAAACTTGGATCCCAATGAACAGTGGAGTGATC
Q1309K	GGATCCCTATGAAAAATGGAGTGATCAAG
S1311K	GATCCCTATGAACAGTGGAAAGATCAAGAAATATGGAAAG
R1325K	GAGGTTGGGCTCAAGTCTGTGATAGAAC
V1338T	GGGAAGCTTGACTTTACCCTTGTGGATGGGGGC
C1344Y	GTGGATGGGGGCTATGTCCTAAGCCATG
L1367I	CTCAGTAAGGCGAAGATCATACTGCTTGATGAACCCAG
D1394G	CAAGCATTTGCTGGTTGCACAGTAATTC
E1409D	GGATAGAAGCAATGCTGGATTGCCAACAATTTTTGGTC