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Supplemental information

Expression of gain-of-function CFTR in cystic fibrosis airway cells restores epithelial function better than wild-type or codon-optimized CFTR

Maximillian Woodall, Robert Tarran, Rhianna Lee, Hafssa Anfishi, Stella Prins, John Counsell, Paola Vergani, Stephen Hart, and Deborah Baines

Supplemental Material

Table S1. Demographics of human bronchial epithelial cell donors.

	Donor	Age at collection	Sex	Ethnicity	Genotype	Smoking history
Normal (Non-CF)	1	49	M	Caucasian	N/A	<1PY
	2	30	M	Caucasian	N/A	non-smoker
	3	43	M	Caucasian	N/A	non-smoker
	4	25	F	Caucasian	N/A	non-smoker
CF	1	26	M	Caucasian	F508del/F508del	non-smoker
	2	28	F	Caucasian	F508del/F508del	non-smoker
	3	28	F	Unknown	F508del/F508del	non-smoker
	4	36	F	Caucasian	F508del/F508del	non-smoker
	5	28	M	Unknown	F508del/F508del	non-smoker
	6	34	F	Unknown	W1282X/R1162X	non-smoker
	7	41	M	Unknown	W1282X/R1162X	non-smoker

<IPY, less than one pack per year smoking history.

Table S2. Demographics of induced sputum donors (pooled for CF sputum).

	Donor	Age at collection	Sex	Ethnicity	FEV_1	FVC_1	Genotype
CF sputum samples	1	49	F	Caucasian	1.21	2.28	F508del/F508del
	2	30	F	Caucasian	2.89	4.02	F508del/2184insA
	3	43	M	Caucasian	0.92	2.46	3849+10kbC>T/C3659del
	4	25	M	Caucasian	1.29	2.40	F508del/F508del
	5	26	M	Caucasian	2.38	4.46	F508del/F508del
	6	28	F	Caucasian	1.21	2.35	F508del/F508del
	7	28	M	Caucasian	2.27	3.30	F508del/G451V
	8	36	M	Caucasian	1.32	3.33	F508del/S945L
	9	28	M	Caucasian	1.00	2.00	F508del/F508del
	10	34	M	Caucasian	1.09	2.38	3849+10kbC>T/C3659del
Normal Lung sputum samples	1	22	F	Caucasian	3.29	2.28	N/A
	2	20	F	African American	3.03	4.02	N/A
	3	25	F	Caucasian	3.97	2.46	N/A
	4	28	M	Asian	4.78	2.40	N/A
	5	19	F	Caucasian	2.81	4.46	N/A
	6	25	F	Caucasian	3.28	2.35	N/A
	7	29	F	Asian	4.71	3.30	N/A
	8	23	F	African American	2.43	3.33	N/A
	9	34	M	Caucasian	5.97	2.00	N/A
	10	34	F	Caucasian	3.31	2.38	N/A

Table S3. Primary antisera.

Name	Species	Dilution	Catalogue #	Supplier
Anti-a-Tubulin, clone YL1/2	Rat	3 µg/mL	mab1864	Sigma-Aldrich
Anti-CFTR 596	Mouse	1:2000	A4	Cystic Fibrosis Foundation Therapeutics
Anti-GFP - ChIP Grade	Rabbit	1:5000	ab290	Abcam

Table S4. Secondary antisera and fluorophores

Name	Species	Fluorophore	Dilution	Catalogue #	Supplier
Anti-Mouse IgG	Donkey	Alexa Fluor 594	1:1000	A32744	Thermo Fisher Scientific
Anti-Goat IgG	Donkey	Alexa Fluor 555	1:1000	A32816	Thermo Fisher Scientific
Anti-Rabbit IgG	Donkey	Alexa Fluor 488	1:1000	711-545-152	Jackson ImmunoResearch
Phalloidin	-	Alexa Fluor 647	1:50	A22287	Thermo Fisher Scientific
DAPI	-	Ex/Em 340/ 488 nm	1:200	D9542	Sigma-Aldrich
IRDye® anti-Mouse IgG	Goat	800CW	1:20000	926-32232	LI-COR Biosciences

Table S5.Buffer composition.

Solution	Composition
NP-40 lysis buffer	25 mM Tris-HCL pH 7.4, 150mM NaCl, 1mM EDTA, 1% NP-40, 5% Glycerol. Add 1 tablet/10ml cOmplete™, Protease Inhibitor Cocktail at use
Ussing buffer	117 mM NaCl, 2.5 mM $CaCl_2$, 4.7 mM KCl, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 11 mM D-glucose, 5 mM Hepes (pH 7.4)
Cl^- free Ussing buffer	115 mM Na-isethionate, 25 mM $NaHCO_3$, 3mM Ca-gluconate, 2.4mM Mg-gluconate, 2.4mM K_2HPO_4, 1.1 mM KH_2PO, 11 mM D-glucose, 5 mM Hepes (pH 7.4)
Blocking buffer	1% BSA, 1% fish gelatin, 0.1% Triton X-100, 5% normal goat serum (this can be replaced with BSA) in 1x TBS.
Standard buffer	140 mM NaCl, 4.7 mM KCl, 1.2 mM MgCl2, 5 mM HEPES, 2.5 mM CaCl2, 11 mM glucose, pH 7.4.
DNA lysis buffer	10%SDS, 50 mM EDTA, 50 mM Tris-HCL, 100 mM NaCl, 5 mM DTT, 0.5 mM spermidine. Add Proteinase K 1:100 at use.

Table S6. Primers.

Name	Sequence 5'-3'	Supplier
K987C_F	GTGGGATTCTTAATAGATTCTCCTGCGATATAGCA ATTTTGGATGACCTTC	Sigma-Aldrich
K978C_R	GAAGGTCATCCAAAATTGCTATATCGCAGGAGAA TCTATTAAGAATCCCAC	Sigma-Aldrich
h^K987C_F	GCAGGAGGAATACTAAATAGATTTAGTTGCGATAT AGCAATACTAGATGATCTACTACC	Sigma-Aldrich
h^K978C_R	GGTAGTAGATCATCTAGTATTGCTATATCGCAACT AAATCTATTTAGTATTCCTCCTGC	Sigma-Aldrich
T2A_P16A_F	CGTGGAGGAGAATGCGGGCCCTATGCAGC	Sigma-Aldrich
T2A_P16A_R	GCTGCATAGGGCCCGCATTCTCCTCCACG	Sigma-Aldrich
AMEL_F	CCCTGGGCTCTGTAAAGAATAGTG	Amplification
AMEL_R	CAGGCTTGAGGCCAACCAT	Amplification
Amelogenin-X isoform (AMELX+FAM)	6-FAM-ATCCCAGATGTTTCTCAA-MGB-NFQ Designed by (George *et al.*, 2013)	ddPCR
Amelogenin-Y isoform (AMELY+VIC)	VIC-CATCCCAAATAAAGTGGTT-MGN-NFQ Designed by (George *et al.*, 2013)	ddPCR

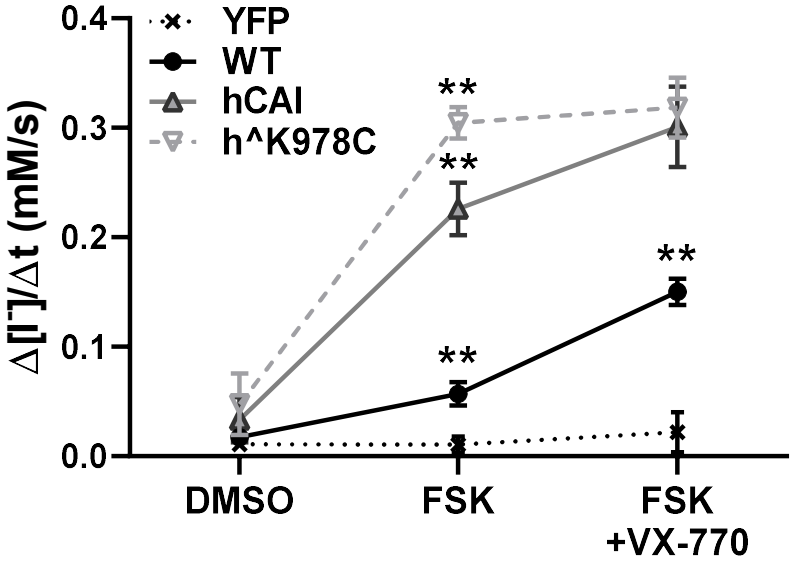


Figure S1. The maximal rate of I^- entry ($\Delta[I^-]/\Delta t$) summarised for HEK293T cells transduced with CFTR cDNA and subject to treatment with DMSO (vehicle control), forskolin (FSK) or FSK + VX-770. Data are shown as mean values points and means +/- SD. Treatments were compared by two-way ANOVA with Tukey's post hoc analyses; Significantly different as shown *: $p<0.05$; **: $p<0.01$; *** :$p<0.001$, n=3

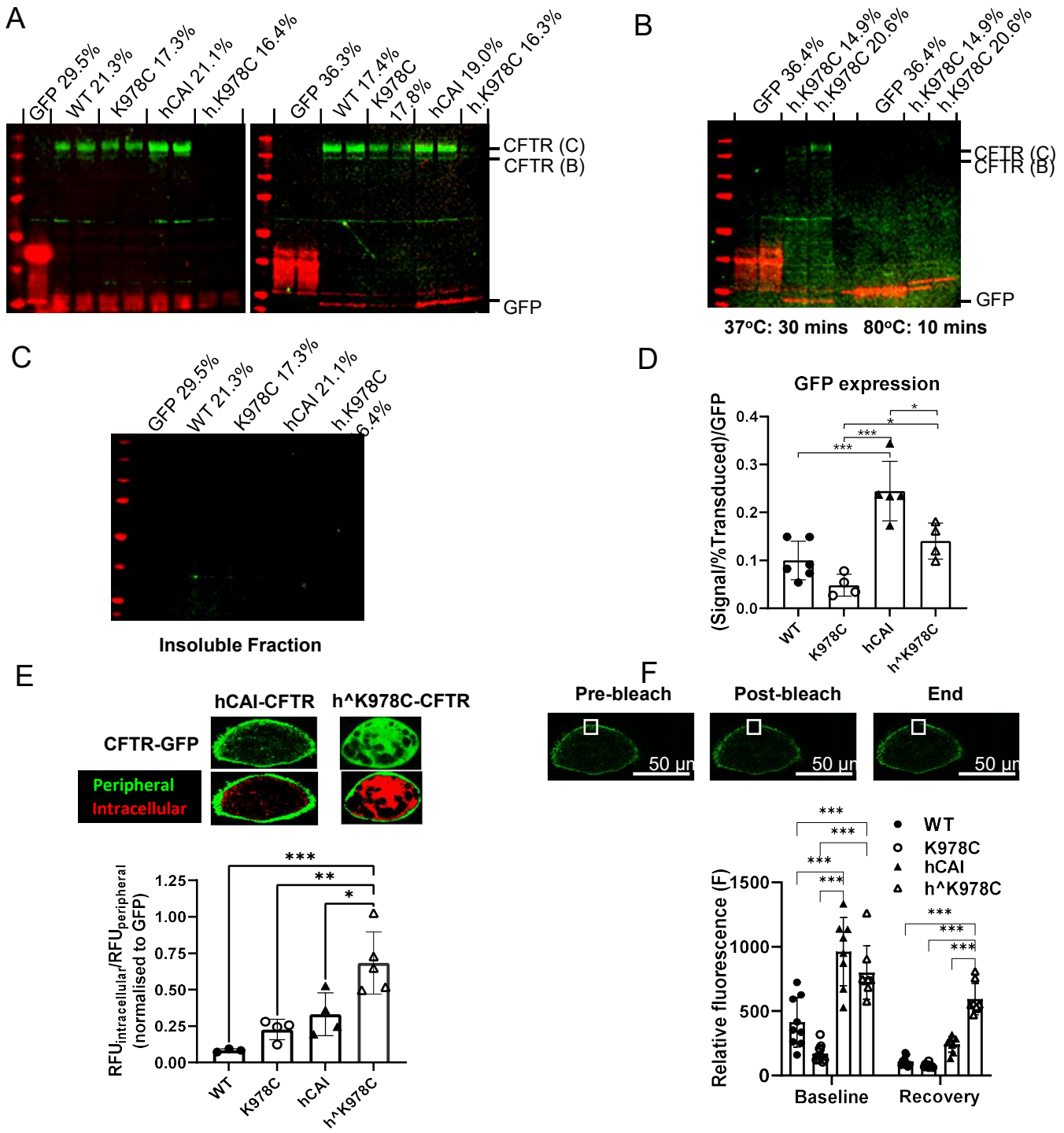
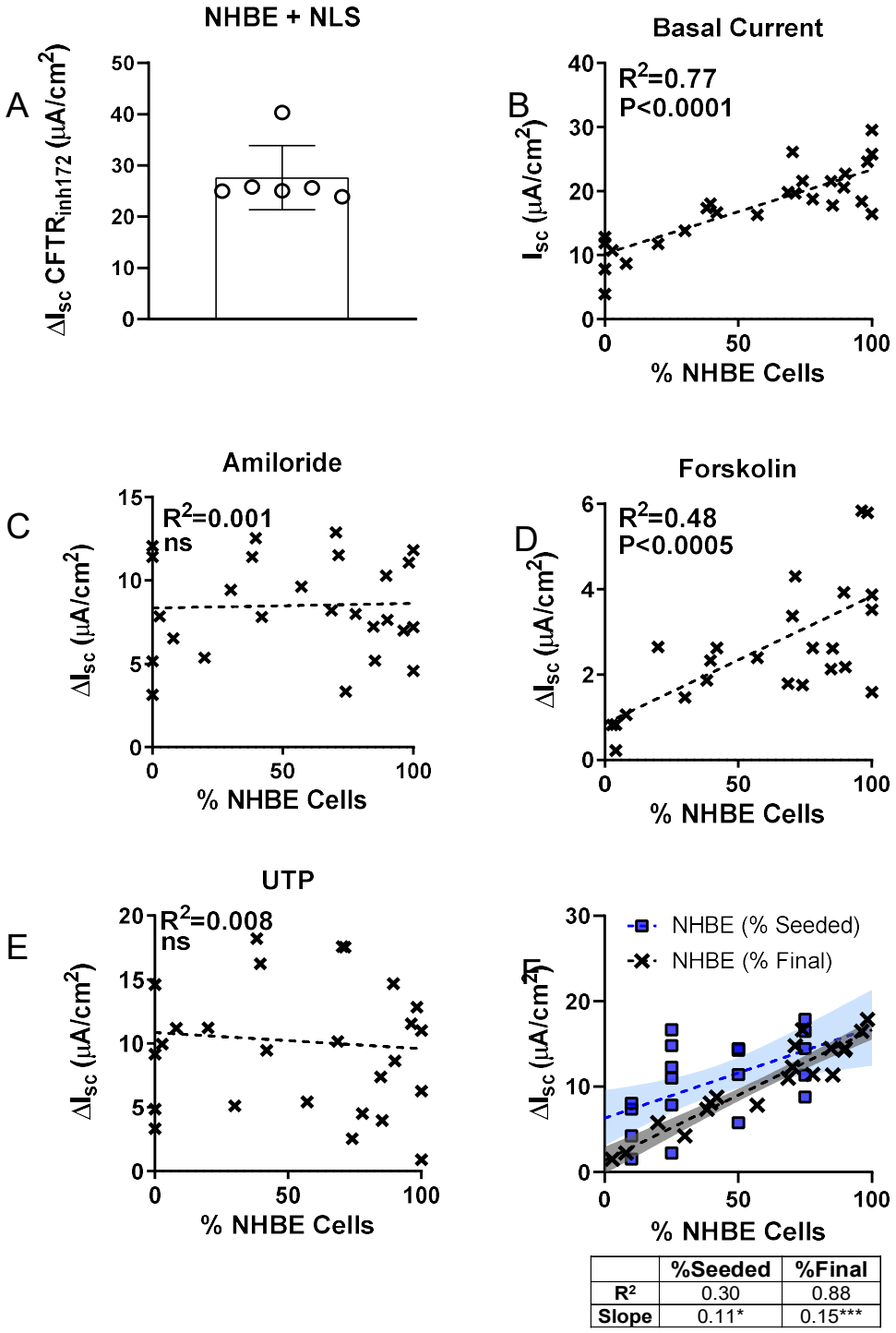
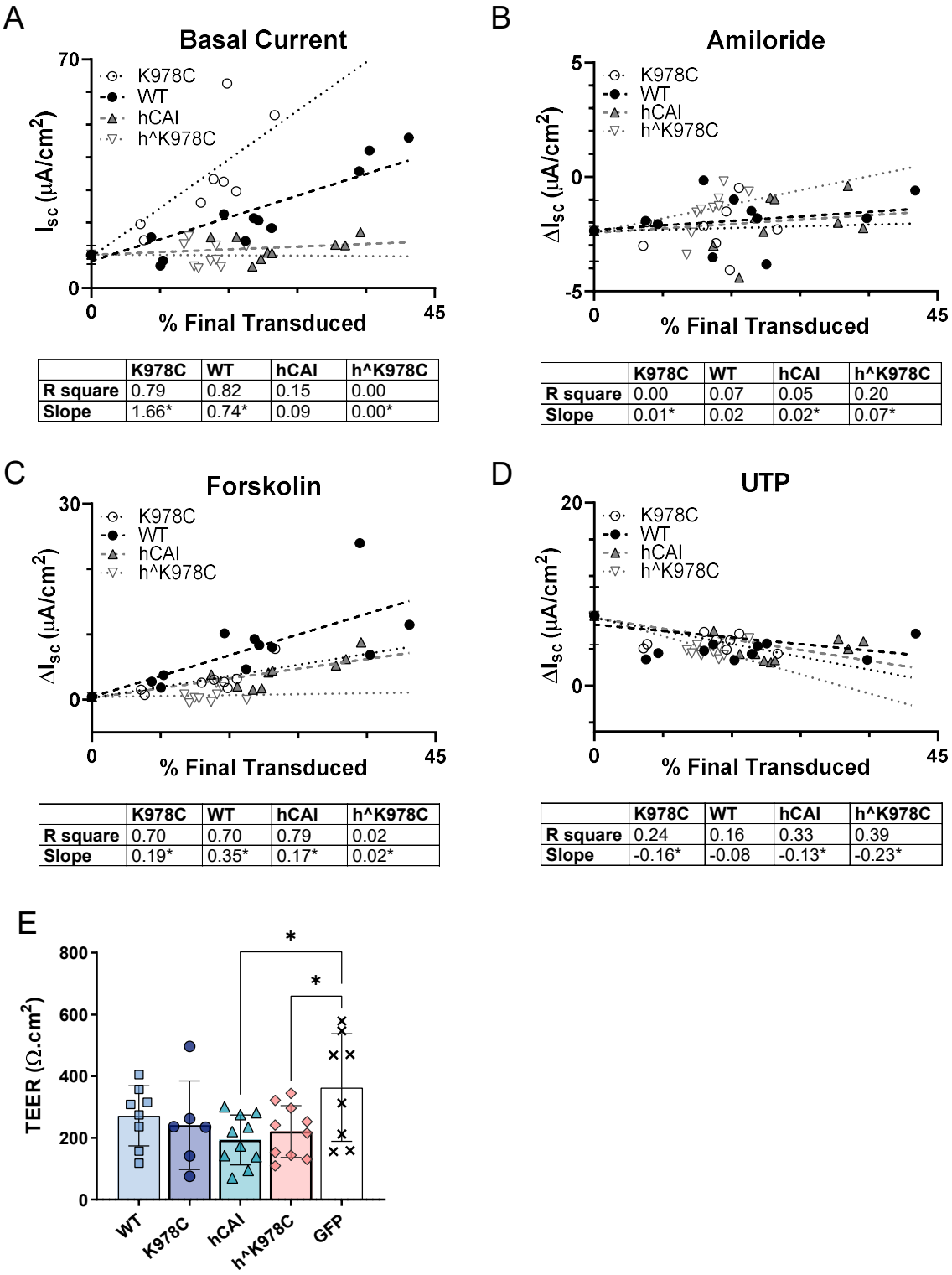


Figure S2. (*A*) Example western blot of lysate soluble fraction from CFBE cultures transduced with different CFTR cDNAs or GFP alone (as labelled above wells and run in duplicate). Blots were immunostained with anti-CFTR (CFTR 596) and anti-GFP. CFTR protein bands C and B (green), GFP (red) with molecular weight markers shown to the left of the blot (red). (*B*) Example western blot showing the difference in the CFTR and GFP proteins after different preparations of the same lysates (as labelled). (*C*) Example western blot of insoluble fractions from the same samples as shown in (a). (*D*) Summary of analysis of relative fluorescence units (RFU) for GFP bands relative to % transduced and normalised to GFP only transduced for each CFTR cDNA. Presented as mean ± SD (n=4-7 from 3 donors). Treatments were compared by one-way ANOVA with Tukey's post hoc analyses; Significantly different * $p<0.05$, *** $p<0.001$. (*E*) Quantification of the ratio of cytosolic GFP RFU (mask shows red) to peripheral GFP RFU (mask shows green) for each transduction, normalised to cytosolic GFP in cells transduced with GFP. Data are shown as mean +/- SD (n=2-5 from 1 CF donor). CFTR cDNAs were compared to WT CFTR by one-way ANOVA with Tukey's multiple comparisons test. Significantly different as shown; *: $p<0.05$, ** $p<0.01$, *** $p<0.001$. (*F*) Summary of fluorescence (F) from region of interest (ROI) of greatest fluorescence on individual cells pre-bleach (baseline) and 70 seconds after bleach (recovery).



	%Seeded	%Final
R^2	0.30	0.88
Slope	0.11*	0.15***

Figure S3. (A) ΔI_{sc} after addition of $CFTR_{inh}172$ (10 μM) for NHBE exposed to NLS (n=6 from 3 non-CF donors). I_{sc} plotted against % NHBE:CFBE (n=8-12 from 3 CF donors for (B) basal and ΔI_{sc} after addition with (C) amiloride, (D) forskolin or (E) UTP (10 μM). Forskolin was added bilaterally, amiloride and UTP was added apically. The R^2 values and P value for slope significantly different from zero, are shown on the graphs. ns., not significantly different. (F) Summary of ΔI_{sc} after addition of $CFTR_{inh}172$ (10 μM) for mixed cultures using % seeded values or % Final values, after exposure to CF sputum. Dotted lines show linear regression with 90% confidence intervals. R^2 values and significance values for variation of the slope from zero for each condition are shown in the table below *: $p<0.05$; ***: $p<0.001$ (n=8-12 from 3 CF donors).



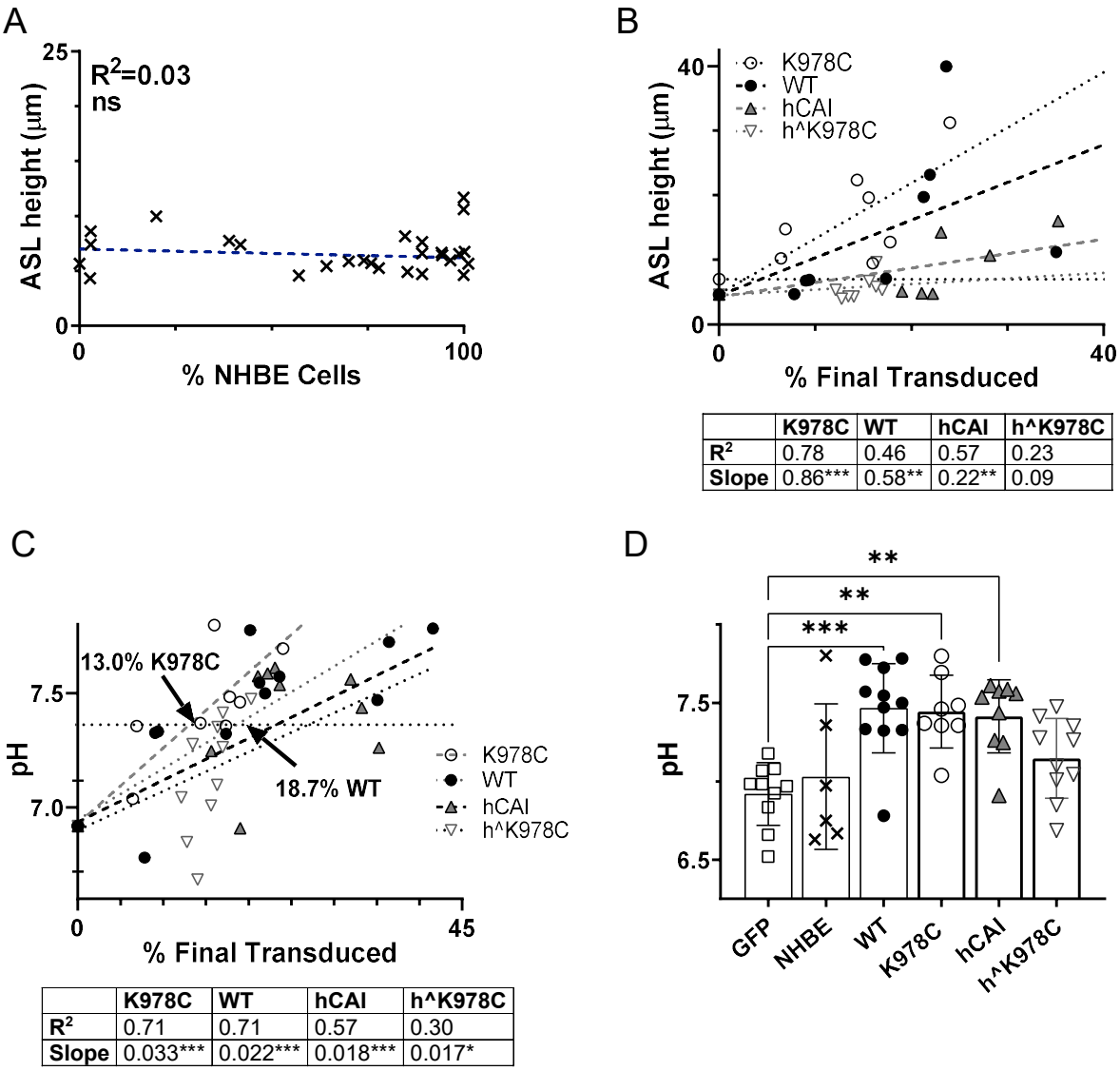
	K978C	WT	hCAI	h^K978C
R square	0.79	0.82	0.15	0.00
Slope	1.66*	0.74*	0.09	0.00*

	K978C	WT	hCAI	h^K978C
R square	0.00	0.07	0.05	0.20
Slope	0.01*	0.02	0.02*	0.07*

	K978C	WT	hCAI	h^K978C
R square	0.70	0.70	0.79	0.02
Slope	0.19*	0.35*	0.17*	0.02*

	K978C	WT	hCAI	h^K978C
R square	0.24	0.16	0.33	0.39
Slope	-0.16*	-0.08	-0.13*	-0.23*

Figure S4. I_{sc} plotted against plotted against % final transduced for each CFTR variant for (A) basal or (B) ΔI_{sc} after addition of amiloride (10 µM), (C) forskolin (10 µM) and (D) UTP (10 µM). All drugs were added apically except forskolin which was bilateral. Dotted lines show linear regression with R^2 values and significance values for variation of the slope from zero for each CFTR cDNA are shown in the table below *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$ (n=8-12 from 3 CF donors). (E) TEER measurements of transduced CFBE cultures after CFS exposure from. Data are presented as mean±SD; $p<0.05$ (n=8-12 from 3 CF donors: 1-W1282X/R1162X, 2-DF508/DF508, 3-W1282X/R1162X).



	K978C	WT	hCAI	h^K978C
R^2	0.78	0.46	0.57	0.23
Slope	0.86***	0.58**	0.22**	0.09

	K978C	WT	hCAI	h^K978C
R^2	0.71	0.71	0.57	0.30
Slope	0.033***	0.022***	0.018***	0.017*

Figure S5. (A) ASL height (um) in the presence of CFS before stimulation with vasoactive intestinal peptide (VIP) Plotted against % NHBE:CFBE (n=8-12 from 3 CF donors Data were subject to linear regression. (B) ASL height measured in CFBE transduced with CFTR cDNAs in the presence of CFS before stimulation with VIP. Dotted lines show linear regression with Slope, R square values and significance values for variation of the slope from zero. (C) ASL pH, after exposure to CF sputum and stimulation with vasoactive intestinal peptide (VIP), plotted against % final NHBE or % final transduced CFBE for CFTR variants. Dotted lines show linear regression for each CFTR variant. (D) ASL pH for all conditions, regardless of the rate of transduction. Data are shown as mean +/- SD with individual data points for n=6-11 from 3 CF donors and 3 non-CF donors. Treatments were compared by two-way ANOVA with Tukey's post hoc analyses; significantly increased as compared to CFBE are shown **: $p<0.01$; ***: $p<0.001$

Supplemental videos

Video S1: Video Abstract

Video S2: WT-CFTR transduced CFBE, immunostained with phalloidin (F-actin; red), anti-β-tubulin (cilia; yellow), GFP (transduced cells; green) and DAPI (blue).

Video S3: hCAI-CFTR transduced CFBE, immunostained with phalloidin (F-actin; red), anti-β-tubulin (cilia; yellow), GFP (transduced cells; green) and DAPI (blue).

Video S4: K978C-CFTR transduced CFBE, immunostained with phalloidin (F-actin; red), anti-β-tubulin (cilia; yellow), GFP (transduced cells; green) and DAPI (blue).

Video S5: h^K978C-CFTR transduced CFBE, immunostained with phalloidin (F-actin; red), anti-β-tubulin (cilia; yellow), GFP (transduced cells; green) and DAPI (blue).

Video S6: CFBE, immunostained with phalloidin (F-actin; red), anti-β-tubulin (cilia; yellow), GFP (transduced cells; green) and DAPI (blue).