Stem Cell Reports

Supplemental Information

Targeted Correction and Restored Function of the *CFTR* Gene in Cystic Fibrosis Induced Pluripotent Stem Cells

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Supplemental Information

Supplemental Data

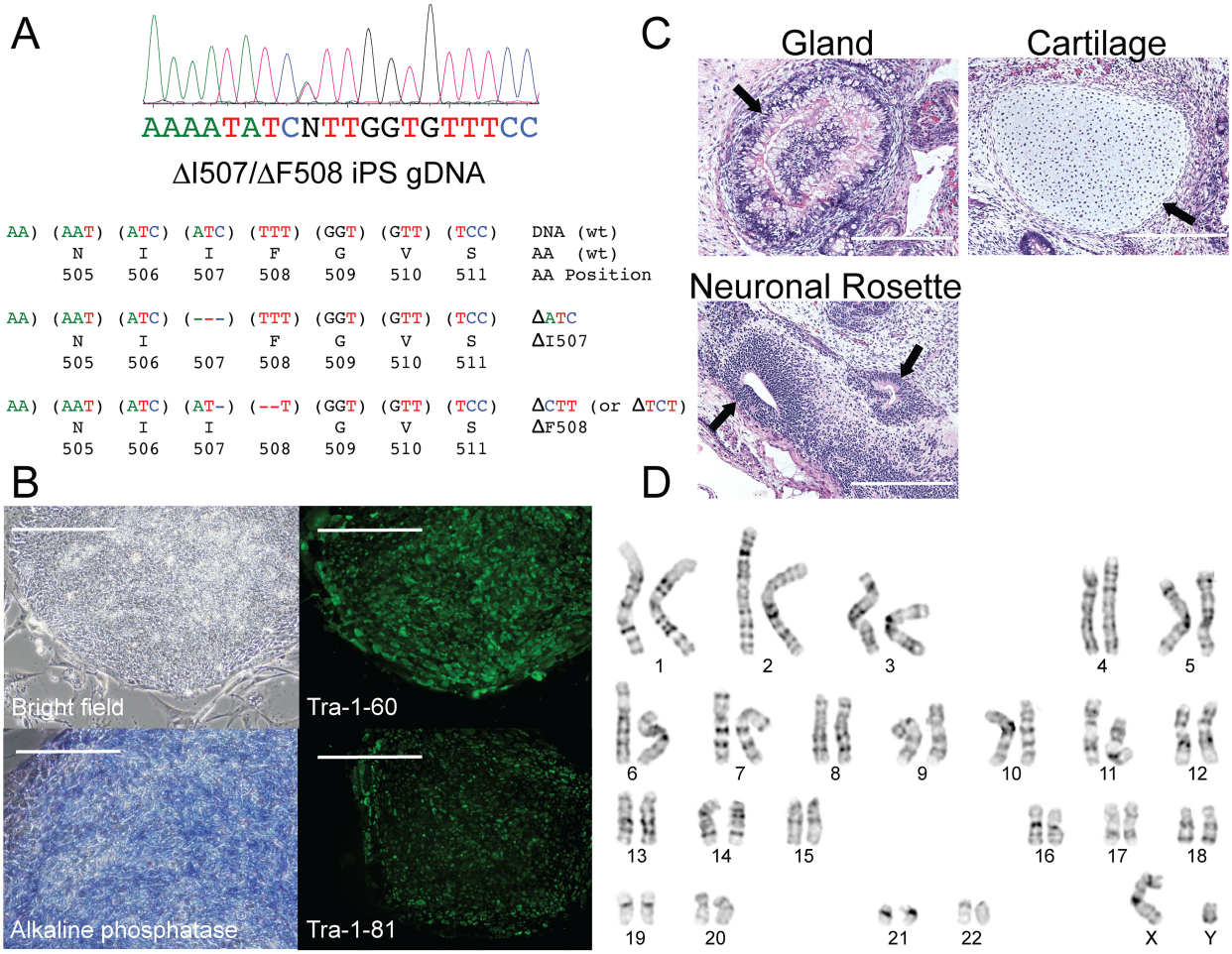
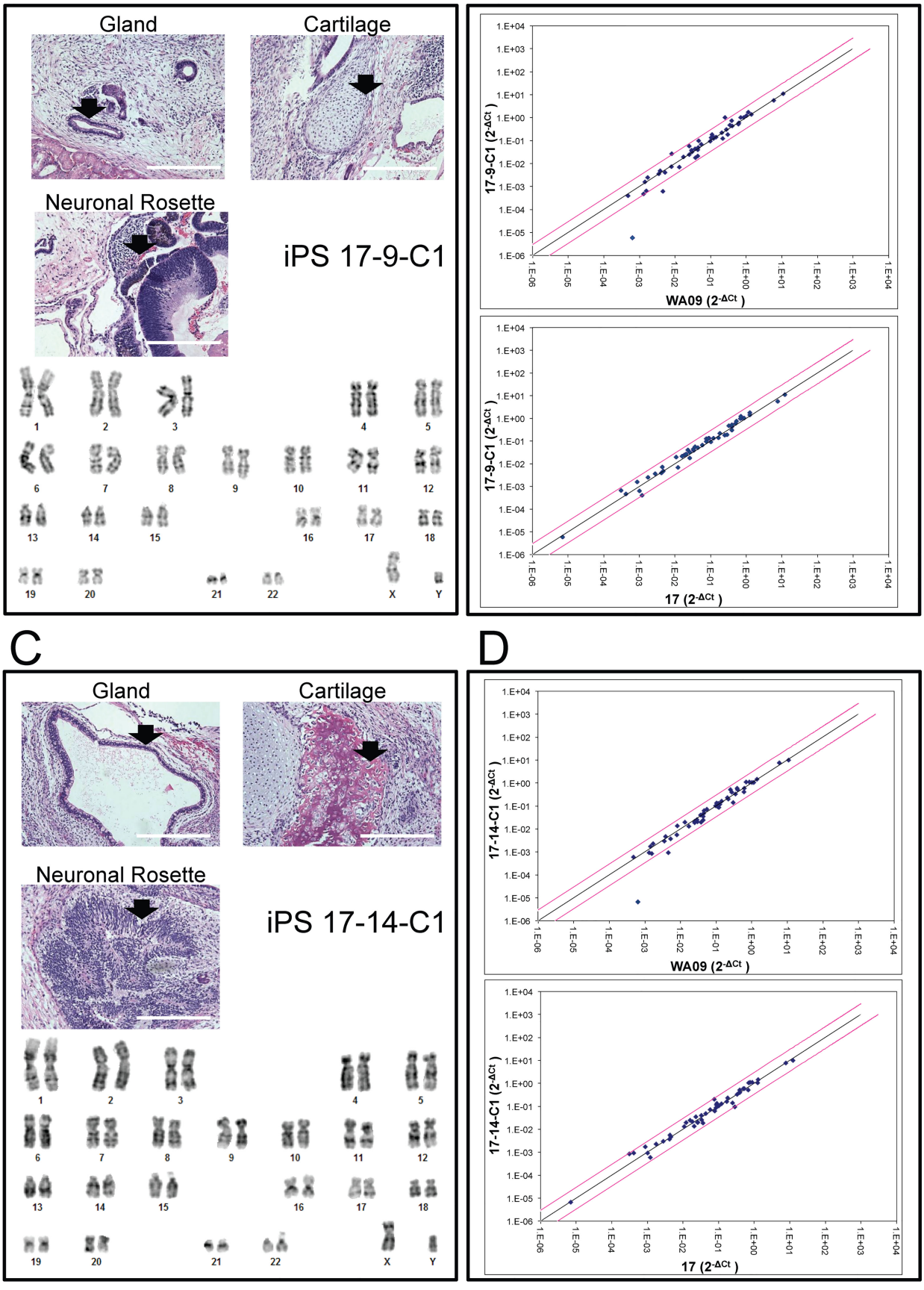


Figure S1. Characterization of mutant CF iPSCs. (related to Figure 1). **A.** DNA sequence of PCR amplicon spanning the *CFTR* exon 10 region from Clone 17 CF iPSCs containing ΔI507 and ΔF508 mutations. The mixture of bases (A and T; marked by the arrow) is consistent with amplification arising from two alleles: one ΔI507 and the other ΔF508. **B.** CF iPSCs express antigens characteristic of human ES cells. Expression of Tra-1-60, Tra-1-81, and non-specific alkaline phosphatase by CF iPSCs, Clone 17. Scale bars = 200 μm. **C.** Hematoxylin and Eosin staining of teratomas formed by CF iPSCs in immunodeficient mice; the three panels include examples of endoderm (gland), ectoderm (neuronal rosette), and mesoderm (cartilage). Scale bars = 200 μm. **D.** CF iPSCs exhibit normal male karyotype.


A
Gland
Cartilage
Neuronal Rosette
iPS 17-9-C1
1
2
3
4
5
6
7
8
9
10
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12
13
14
15
16
17
18
19
20
21
22
X
Y
B
17-9-C1 (2-ΔCt)
WA09 (2-ΔCt)
17 (2-ΔCt)
C
Gland
Cartilage
Neuronal Rosette
iPS 17-14-C1
D
17-14-C1 (2-ΔCt)
WA09 (2-ΔCt)
17 (2-ΔCt)

Figure S2. Characterization of corrected CF iPSCs. (related to Figure 2). Hematoxylin and Eosin staining of teratomas formed by corrected (wt/ΔF508) CF iPSCs (**A.** 17-9-C1, **C.** 17-14-C1) in immunodeficient mice; the three panels include examples of endoderm (gland), ectoderm (neuronal rosette), and mesoderm (cartilage). Corrected CF iPSCs exhibit normal male karyotype. Scale bars = 200 μm. Transcriptional RT^2 Profiler PCR array of corrected CF iPSCs (**B.** 17-9-C1 and **D.** 17-14-C1)**.** The data were analyzed by the ΔCt method and the black line represents fold changes ((2^(-ΔCt)) of 1. Pink lines indicate a three-fold change in gene expression. Top scatter plot: Comparison of corrected CF iPSCs vs. WA09 hES cells. *FGF-5* is the only significant outlier in the corrected 17-14-C1 and 17-9-C1 CF iPSCs with approximately 100-fold down-regulation in comparison to WA09 ES cells. Lower scatter plot: Comparison of corrected CF iPSCs vs. initial uncorrected CF iPSCs (clone 17). Data shown reflect single assays for samples 17-9-C1 and WA09 and average of duplicate assays for samples 17-14-C1 and Clone 17.

Table S1. Non-Synonymous Coding Variants (NSCVs) Identified in ZFN-Corrected iPSCs

CFTR chromosome 7:117199532 CCAGACTTCACTTCTAatggtgATTATGGGAGAACTG

Gene_ID	Chr	Position	Variant type	Ref	Mut	AA Change	Variant DNA Sequence	17-9-C1	17-14-C1
PRG4	1	186276439	snp	G	T	p.A396S	cacccaccaccaccaagtct**T**cacccaccactaccaaggag		
CYTIP	2	158272220	snp	C	T	p.R350H	tttcttcctcttccacagca**T**gatgaaggccagggataaat		
MTHFD1L	6	151243396	snp	G	A	p.R347K	acagcagcacaggcggtgga**A**acttcactgcttgaaacttc		
SPON1	11	14063077	snp	G	A	p.E119K	gttttccgtagatcatagac**A**aagaagaaactcagtttatg		
CLPB	11	72141383	snp	C	T	p.R143H	cttcttgcatattgttggca**T**gggcagcttccaacagggct		
APOBEC1	12	7802196	snp	G	T	p.P220T	tgtagctaaaaggatgtgtg**T**cggaatcgtttggtaatggc		
OR6C68	12	55886587	snp	G	C	p.M147I	aacaaagtgtgcaaaacaat**C**gttatttgttgttggatggc		
ITGBL1	13	102366883	snp	G	T	p.D459Y	acagagactgcgacaaacat**T**atggtctcatttgtacaggt		
SHPK	17	3527384	snp	G	A	p.T151M	tggttgcacagccgaagccc**A**tggccacactgagatgagac		
PHACTR3	20	58322854	snp	C	A	p.L108I	gcaggcaaggccgagaggag**A**tcatcaagaaggggctgctg		

NSCVs found in corrected clones 17-9-C1 or 17-14-C1 consist of single base pair substitutions (snp) with the reference (Ref) changed to Mut. Mut changes are in bold and capitalized shown with surrounding sequence under Variant DNA Sequence. Shown above are the CFTR ZFN recognition sequences with ZFN-L/ZFN-R sequences in capitals and the spacer sequences in lowercase.

Table S2. Bioinformatic analysis to predict the top 100 potential ZFN off-target sites in the human genome.

Rank	SELEX Score	Chrom.	Target Site	Mismatch	Gene
homo-dimer: ZFN pair 9940/ 9940 (recognizing: ATTATGGGAGAACTG/ ATTATGGGAGAACTG)					
1	1.37E-12	chr4	CCAGTTCaCCCGTgTACGTGCGTAgATGGatGAACacA	7	None
2	8.36E-13	chr2	CaGaTaCTCCCAgATACAGAGTATcTGGGAttACaGG	8	ARHGAP15
3	4.36E-14	chrX	CCGGTgCTCCgAgAgcCCCCGgATcacGGcGAACTGG	10	POU3F4
4	3.77E-14	chr15	CaGtTTCaCCCATgTAAGAAGTAgATGGGAGAACTtG	6	None
5	3.40E-14	chr8	ACAGTgCaCCCAgcTcCTAGTCTcTgTGGGtGAtCaGG	10	None
6	2.47E-14	chr11	CCtGTgCTCCCAgggcCCTGGgATggGGGtGAtCTGC	11	ST14
7	1.87E-14	chr5	CtGGTTCTtCCAgATAATTGGTAaATGGGAGtAgTtG	7	C5orf58
8	1.44E-14	chr11	CCtGTagTCCCAgATACTCAGgAcAgGGGtGAggTGG	10	LGR4
9	1.11E-14	chr12	CCAGcaCcCCCAccTcCTGCAGTATcTGGGAccACaGG	10	ATF1
10	9.72E-15	chr13	TCAGTaCaCCtGTATACGGCATtTcTGGGtGAAtCaG	8	None
11	9.62E-15	chrX	GCAGTgCTCCtGTAgcCCCAGCTATgCGGGAGgAtTGT	7	TBL1X
12	8.81E-15	chr3	CCtGTaCTCCCtgcTACTCAGgAggCGGGAGAAtTGT	9	None
13	8.79E-15	chrX	CCAGagCaCCtGTgTcCAGCAGacTcaGGGAGcACTGT	11	DHRSX
14	8.79E-15	chrX	CCAGagCaCCtGTgTcCAGCAGacTcaGGGAGcACTGT	11	DHRSX
15	8.79E-15	chrX	CCAGagCaCCtGTgTcCAGCAGacTcaGGGAGcACTGT	11	DHRSX
16	8.79E-15	chrY	CCAGagCaCCtGTgTcCAGCAGacTcaGGGAGcACTGT	11	DHRSX
17	8.79E-15	chrY	CCAGagCaCCtGTgTcCAGCAGacTcaGGGAGcACTGT	11	DHRSX
18	8.79E-15	chrY	CCAGagCaCCtGTgTcCAGCAGacTcaGGGAGcACTGT	11	DHRSX
19	8.21E-15	chr17	CCGaTaCTgCCATgTATCAAGgcTcgGGGAGAACTGT	8	TRIM37
20	6.05E-15	chr11	CCAGaaCTCCCAgggACTTAGgATgCaGGAGctCCtG	11	PRDM10
hetero-dimer: ZFN pair 9940/ 12897 (recognizing: ATTATGGGAGAACTG/ TAGAAG**t**GAAGTCTGG [Linker Collapse*])					
1	2.52E-13	chr1	CCAGTTCTCCCGgcTAAGTAACAAaaAGGAAtTCTGTC	5	MIER1
2	2.01E-13	chr4	ACAGaaCTCCCGgAaACCAGAGAcaaAGGAAGTtTGTA	8	PDLIM3
3	1.96E-13	chr3	CCtGgTCTCCCATATAACATACCAaaAGGAAGTtGGTG	5	None
4	1.15E-13	chr9	TAtAaACgTaCTGaTGCATACGTATATGGGAtcACTGG	7	None
5	9.26E-14	chr1	CCAGTTCTtCtGcAgACAAAAGCAtCAGGAAGTaCcCA	7	None
6	8.99E-14	chr3	CTCAcACaTaCTGtcTTTGGAAGgATATGGaAGAACTGT	7	PTPRG
7	6.21E-14	chr5	GCCAGcCTTCCTGGaTCTTGAGGTtTATGGGAatACTtG	6	None
8	4.90E-14	chr16	CCAGTTCTCtCATtTACTCCTCTgtCAGGAAcTCTGCC	5	LOC283867
9	4.18E-14	chr8	TCAGTTCTCCCcgATACCCCAGTTAaCAGGcAGcCAaCC	6	None
10	3.66E-14	chr2	CCAGTgCTtCCAgATcCTCATGTAgaaAGGctGTCCtGA	10	ARHGEF4
11	3.50E-14	chr10	AGaGGgCTTgCTGtcCACAGGAGgATtTGGGAGAACTGG	7	None
12	3.23E-14	chr11	CCAGTTtcCaCATtTACTTTGGCAACCAGGAAGTgTGGC	5	DAGLA
13	3.03E-14	chr16	CCAcTgCaCCCAgccACATTATCttCAGGAAGTCTGTG	8	TMCO7
14	2.90E-14	chrX	ACAGagCaCCCAgAgACTAGCAGCACCtGGAAGctCtTG	9	None
15	2.85E-14	chr17	CCAGTTCTCCCAgATACGCTAGGctCAaGcccTCAGCC	7	SPNS2
16	2.66E-14	chr17	ACCTGgCTTCCTGGaTCCAGGGGTgTcTGGGtGAAaTGA	6	AARSD1
17	2.62E-14	chr11	CCtAGgCTTCCTaGcACTGGGAGgcTATGGGAGAACCaG	7	TRIM68
18	2.59E-14	chr18	ATCCcACTTCCctGgGCCTAACTATgTGGGtGAACTGT	6	None
19	2.48E-14	chr12	AAgTGtCTTCaTGacCATTTTGTATATGGGgGAACTGG	6	PLXNC1
20	2.48E-14	chr15	CCtGTgCTCCCAcATcTCTCAGAAgCaAGGAAGTgGGTG	7	RASGRF1
homo-dimer: ZFN pair 12897/ 12897 (recognizing: TAGAAG**t**GAAGTCTGG/ TAGAAG**t**GAAGTCTGG [Linker Collapse*])					
1	1.20E-13	chr1	AGgTGACTTCCTGGgCCCATTAAAAaCAGGAAGTCAGGC	3	None
2	2.32E-14	chr14	CCCAGAggTCCTGGTTGCATGAATGACCAGGAAGgCCaGG	4	PPP1R13B
3	2.14E-14	chr18	CACAGACTTCCTGtaGGTCACTGAACaAGGAAGcCGtTT	5	None
4	1.96E-14	chr11	TGaAtACTTCCTGGTGTAAGTTAGAACCAGaAAtTCTGGA	4	None
5	1.84E-14	chr1	AACAacCTTCCTGtcTGAATCAAGAAgCAGGAAGTtTGGG	6	AXDND1
6	1.84E-14	chrX	GTCCaACTTCCTGtTTCCATCATGgCCAGGAAcTCAGTT	4	None
7	1.64E-14	chr1	TCaTGACaTCCTtGTAAGAACCTTAAtCAGGAAGTCCtAT	5	None
8	1.53E-14	chr1	TCaAGgCaTCCTGGTACTGTCAAGTACCAGGAAcTtGGGG	5	KCNH1
9	1.45E-14	chr5	TCaAtACTTCCTtGcTCTGCGTGTCtCCAGGAAGTtCtGT	7	None
10	1.41E-14	chr17	ACaGaACaTCCTGGgTCTAATTTGAACtgGGAAGTCTGGC	6	None
11	1.26E-14	chr3	AGgGGgCTTCCTGGTCCCAGAGAGCACagGGAtGcCTGGG	6	None
12	1.20E-14	chr7	CCaCaACTTCCTGcTTTCCTAAATGgaCAGGAAGTCCtTG	6	None
13	1.10E-14	chr5	GGCAGAtTTCCTGGTTCTCAAGAGAAaCtGGAAaTCTtGA	5	ADAMTS2
14	1.03E-14	chr12	CACAcACTTCCTGGTGACCAGAGGtCCcaGAAGTCTtCT	5	PRICKLE1
15	1.02E-14	chr17	AAaAGACcTtCTGtTTCTGCCAGAAtCAGGAAGTCTtTC	6	None
16	8.89E-15	chr17	AAaAGACTTgCTttTTACGCAGATAAaCAGGAAGTtTGAA	6	YPEL2
17	8.57E-15	chr14	AGaGaACTTtCTGGTCCTTGAGCTAcCCAGGAAGcCAGCA	5	TMEM63C
18	7.86E-15	chr21	CTCCacCTTCtTGtTCTTCTTGTGCACCAGGAAGTtCtTG	6	TIAM1
19	7.61E-15	chr20	CCaGGcCcTCCTGaTTTGCTCCCCACCAGGAAGTtTtTCT	6	None
20	7.25E-15	chr7	GTtTGACTTCCTtaTGAAGTAGAGACCAGGtAGTtTGGA	5	None
hetero-dimer: ZFN pair 9940/ 12897 (recognizing: ATTATGGGAGAACTG/ TAGAAG**T**GAAGTCTGG [Linker Gapped*])					
1	4.85E-12	chr5	TCAGTTCTCCCAgAgcCTTGGAAaCAGTGAAGTtAcCT	6	None
2	4.94E-13	chr4	CCAGTTCTCCCAggTcTCTGGCAtCAGTGAtGTtTtAG	7	None
3	4.56E-13	chr18	ATCCcACTTCCCTGGgCCTAACTATgTGGGtGAACTGT	4	None
4	2.88E-13	chr11	CCtGTaCTCCCAgATACTTGGGAAgCAGAGAtGggAGAA	7	SBF2
5	1.16E-13	chr4	ACAGaaCTCCCGgAaACCAGAGACaAaGGAAGTtTGTA	7	PDLIM3
6	9.14E-14	chr4	GAgTGACTTCTgTGGTTCAAAATATATGaGAGcACTGG	4	None
7	8.01E-14	chr22	CCAGTTCTCCtGgATcCATGGCtCCAGGGAtGgaGGAT	7	None
8	7.25E-14	chr8	CCCCacCTTCCCTGGTGAAGGGGgcTcTGGGAGgACaGA	7	MMP16
9	6.87E-14	chr7	GCGGTgCTCCCATATtGGGATAgCCAGAGAAGTgTGTA	4	CHN2
10	6.85E-14	chr9	CCAGTTCTCCCATtTcCTCCACCACagGAGAAtaCTGCT	6	TMOD1
11	6.36E-14	chr13	CCAGgaCTCCCATAacGATGTGGcCCAGGGAAGTCTtCC	6	None
12	5.72E-14	chr11	CCAGTTtTCCCAgATACCAGTGAaaAGTGcAGctAtGT	8	None
13	5.20E-14	chr8	ACAGTTCTtCCAcAgACCAGGAgCaAGGGAtGgtTtCA	9	RIMS2
14	5.13E-14	chr1	CCAGTTCaCCCAgAcAGCGAGTAgaCAGAGgAGTCTGCT	6	DAB1
15	4.00E-14	chr3	ACAGTTCTCCCATATACCACATtCCAGGGcAtcCTaTG	5	None
16	3.60E-14	chr9	CCAGTTCTCCCATATACTTGTGAgCaAtAaAAaTgCaTA	7	None
17	3.50E-14	chr6	CCAGTTCTCCCAgcacCATATAAAaCAGGGAAtTtTtTC	8	BCKDHB
18	3.48E-14	chr6	CTCCGAgTTCACTGtTGTCTATTcTcTGGGtGcACCGT	6	None
19	3.35E-14	chr5	TCAGTcCTCCCGgATcCCTGCTctCAGCtAAGgCTGGT	7	N4BP3
20	3.12E-14	chr1	CCAGTgCTCCCcTATATCTCCTCcCaAGGGAtcTCAGCC	6	SYT2
homo-dimer: ZFN pair 12897/ 12897 (recognizing: TAGAAG**T**GAAGTCTGG/ TAGAAG**T**GAAGTCTGG [Linker Gapped*])					
1	4.24E-13	chr21	TCCAGgCTTCCCTGtTGTCAGTGAACCAGGGAAGcCTGGT	3	None
2	1.15E-13	chr7	ACCCGACTTCTCTtGTGCTGGTCCACaAGAGAAGTtCaTT	4	DGKI
3	5.71E-14	chr9	TGaAGgCTTCCCTaGcTTCCTATGACCAGAGAAGTttTGTA	5	ENG
4	3.38E-14	chr4	ACCTcACTTCTCTGGTGGGGAGAACaAGTGAAcTgTGAA	4	WHSC1
5	3.34E-14	chr2	GCtGGACTTCACTGaTGCTCCAGGcCCAGAGAgGTCAGTG	4	None
6	1.66E-14	chr15	AGgGGACTTgGCTaGTTCTAGGAACCAGGGAAGTCTtTT	4	USP8
7	1.63E-14	chr9	TCCAaACTTCGCTGGTCTTTGGAACCAGTGAAaaCCcTT	4	None
8	1.56E-14	chr18	TCaAGACTTCACTGtgGTCTGTggtCAGAGAAGgCTGCA	6	KCNG2
9	1.27E-14	chr5	TCgGGcCaTCACTGaTCTTCACCCACCAGAGAAGTtTtTC	6	None
10	1.16E-14	chr4	TGCTGcCTTCACTGGgCACATGAAaCAGGGgAGTCAGCA	4	None
11	1.05E-14	chr1	CCaAaACTTCTCTGGaCCTCAATGtCCAGAGAAGcCAtGT	6	LGR6
12	1.05E-14	chr9	CCaAaACTTCCCTGGaCCTCAATGtCCAGAGAAGcCAtGT	6	C9orf44
13	7.52E-15	chr3	GAaGcACTTtGCTGaTCATAATTAAtCAGGGAAGTtTGCT	6	None
14	6.35E-15	chr15	AGaGGACTTCACTGaaTATGCTCAtCAGTGAtGTtGGCT	6	
15	6.34E-15	chr4	CAaCGACTTCACTGGaGGGACCActCAGAGAAGTgTtGA	6	None
16	5.53E-15	chr17	GGCTGACTTCCCTGaTGAAGAGGAtCaAGTGAttTCTGTG	5	None
17	5.42E-15	chr3	CACAGACTTCACTccaCCTTTGAAaaAGAGAAGTCTGCG	5	DNAH12
18	5.21E-15	chr4	GGgAGACTTCTtTGGaCCAGGTGgCCAGGGAAGTCAtCT	5	ODZ3
19	5.20E-15	chr1	TGCAGcCTTCCCTGaTTCCCAGCCAgCAGGGAtcTCTGAC	5	IL28RA
20	4.75E-15	chr9	TCaGagCcTCTCTGGTTCTTTCAAtCgGGGAtGTCTGCA	7	C9orf53

*ZFN 12897 was designed to skip a base (t) in the middle of the binding site (TAGAAGtGAAGTCTGG). Therefore, for the top 20 potential off-target searches we generated two sets for homo-/ heterodimers including ZFN 12897 with either the intended base pair position skipped (Linker Collapse) or where the intended base was not skipped (Linker Gapped).

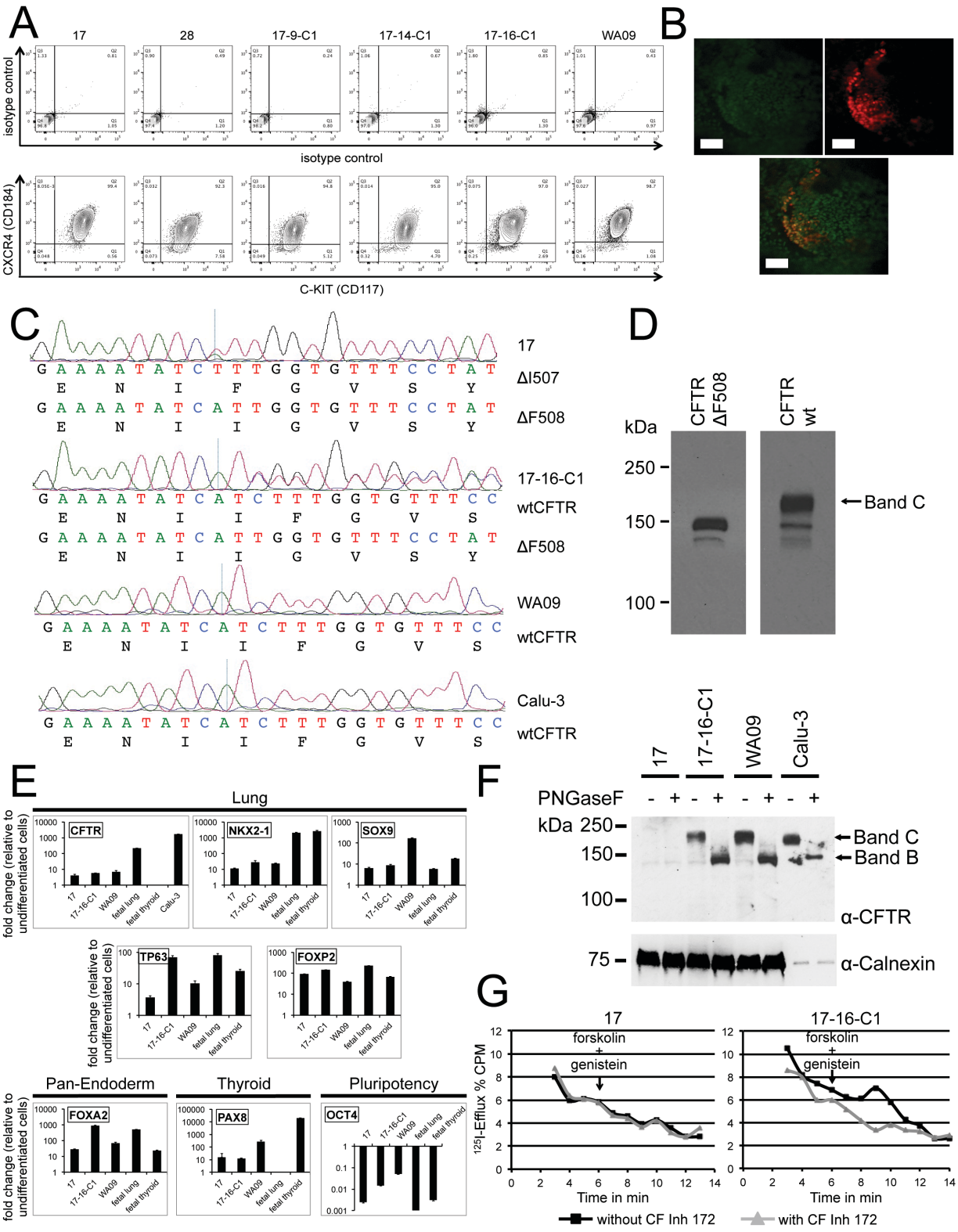
A
17
28
17-9-C1
17-14-C1
17-16-C1
WA09
isotype control
isotype control
CXCR4 (CD184)
C-KIT (CD117)
B
C
17
ΔI507
ΔF508
17-16-C1
wtCFTR
ΔF508
WA09
wtCFTR
Calu-3
wtCFTR
D
CFTR ΔF508
CFTR wt
kDa
250
150
100
Band C
E
Lung
fold change (relative to undifferentiated cells)
CFTR
NKX2-1
SOX9
TP63
FOXP2
Pan-Endoderm
Thyroid
Pluripotency
FOXA2
PAX8
OCT4
17
17-16-C1
WA09
fetal lung
fetal thyroid
Calu-3
F
17
17-16-C1
WA09
Calu-3
PNGaseF
kDa 250
150
100
75
Band C
Band B
α-CFTR
α-Calnexin
G
17
17-16-C1
forskolin
+
genistein
125I-Efflux % CPM
Time in min
without CF Inh 172
with CF Inh 172

Figure S3. *In vitro* differentiation of corrected CF wt/ΔF508 iPSCs. (related to Figure 3). **A.** Representative flow cytometry analysis showing CXCR4 vs. CKIT co expression after 4 days of differentiation to definitive endoderm of all mutant and corrected CF iPSC clones studied. Isotype control (top panels) were used to set gate for experimental clones (bottom panels) **B.** Corrected CF wt/ΔF508 iPSCs (RC 202) were differentiated towards anterior foregut endoderm/NKX2-1 according to the directed differentiation protocol shown in Fig. 3A. Immunostaining for FOXA2 (green) and NKX2-1 (red) was performed on day 14 of the differentiation and demonstrates that the majority of cells are endodermal (FOXA2+) and a subpopulation is FOXA2+/NKX2-1+. Scale bar = 100 μm. **C.** Sequencing of *CFTR* mRNA from 19 day differentiated mutant Clone 17 CF (ΔI507 and ΔF508) and corrected 17-16-C1 (corrected wt and mutant ΔF508) iPSCs confirm the expected expression pattern. Calu-3 and differentiated WA09 cells express wt *CFTR* mRNA. **D.** HEK 293 cells transiently transfected with ΔF508 or wt CFTR expression constructs produce immature or mature glycoforms, respectively. **E.** Gene expression analysis of day 19 differentiated mutant (17), corrected CF iPSCs (17-16-C1), and WA09 hESCs. Data (mean +/- standard deviation, 2-3 well replicates) from a representative experiment (this experiment corresponds to the Western blot in Fig. S3F). **F.** Western blot analysis of 50 μg protein lysates from day 19 differentiation cultures treated with (+) or without (-) PNGaseF and probed with a CFTR-specific antibody. Whereas the mature, fully glycosylated CFTR protein (the 170 kDa protein band C) is present in Calu-3 cells and differentiated WA09 cells, Clone 17 iPSCs are deficient in expression of this glycoform. Differentiation of the corrected Clone 17-16-C1 iPSCs demonstrates restored expression of the mature CFTR glycoform. PNGaseF treated samples showed a shift of the mature 170 kDa band C to the core glycosylated, immature CFTR band B (~ 130 kDa). Detection of Calnexin demonstrated equal sample loading for differentiated iPSC/hESC samples, with less sample (5μg) loaded for the positive control Calu-3 cells. See also Fig. 3C.

G. cAMP-dependent ^{125}I-efflux from confluent monolayers of differentiated mutant Clone 17 and corrected 17-16-C1 CF iPSCs results in the release of radioactive ^{125}I exclusively in corrected CF cells after addition of forskolin and genestein at 6 minutes. Treatment with CF Inhibitor 172 (grey graph) from minute one completely blocks CFTR channels and iodide release from corrected 17-16-C1 CF cells. Results were expressed as ^{125}I-efflux % CPM released per minute into to the media; the average of in duplicate measurements is shown with and without CF inhibitor 172.

Supplemental Experimental Procedures

Cystic fibrosis primary fibroblasts:

CF primary fibroblast line GM04320 was obtained (Coriell Repository, Camden, NJ) from a patient (17 year old male) reported homozygous for the ΔF508 mutation. Clinical symptoms for this patient were reported as advanced pulmonary disease and pancreatic insufficiency; in addition, defective cAMP stimulated chloride channel activity was demonstrated in fibroblasts from this patient (Lin and Gruenstein 1987). Our sequencing of the *CFTR* alleles in genomic DNA isolated from the GM04320 fibroblasts demonstrated that the patient was, in fact, a compound heterozygote with one allele being ΔF508 and the other ΔI507. ΔF508/ΔI507 compound heterozygosity has previously been reported in CF patients (Kerem et al., 1990).

CF iPSC generation and characterization:

pMXs retroviral vectors encoding human reprogramming factors (OCT4 [17964], SOX2 [17965], KLF4 [17967], C-MYC [17966], NANOG [18115]) were graciously provided by K. Plath through Addgene (Lowry et al., 2008). VSV-G enveloped viral stocks were prepared by transfection of Plat-GP cells (Cell Biolabs) with vector DNA and VSV-G expression plasmids (pCMV-VSV-G [8454], kindly provided by B. Weinberg through Addgene) and concentrated 100 fold by ultracentrifugation. Parallel production of pMXs-GFP vector stocks was performed; titration of the pMXs-GFP virus was performed by infection of primary human fibroblasts and subsequent

FACS analysis for GFP-expressing cells. CF fibroblasts, plated at 10^5 cells per well of a 6-well plate on day 0, were transduced on days 1 and 2 by spin-fection (200 g for 30 minutes) at a multiplicity of infection of 21.5, in the presence of 10 μg/ml protamine sulfate. On day 4, fibroblasts were transferred onto irradiated mouse embryo fibroblasts (MEFs; CF-1 mouse strain, Charles River), and one day later media was switched to human embryonic stem (ES) cell media (per National Stem Cell Bank protocol SOP-CC-001C; http://nationalstemcellbank.org) containing 40 ng/ml basic Fibroblast growth factor (bFGF) and re-fed daily. Starting on day 12, cells were re-fed daily with human ES cell media pre-conditioned on irradiated MEFs. Beginning at 16 days post transduction, iPS-like colonies were first identified based on morphological criteria. Live-cell staining with either Alexa 488-conjugated anti-Tra-1-60 monoclonal antibody (Stemgent, 09-0068), or anti-Tra-1-81 monoclonal antibody (Millipore, MAB4381) followed by Alexa 488 goat anti-mouse IgM (Life Technologies, A-21042), was then used to identify reprogrammed colonies for subsequent expansion and characterization. Of 32 colonies originally picked (all of which stained positive for Tra-1-60 and/or Tra-1-81), 9 colonies were subsequently expanded and cryopreserved and two iPS clones (17 and 28) were selected for more extensive characterization.

Teratoma assay: 2-3 million uncorrected (clone 17) or corrected (17-9-C1, 17-14-C1) CF iPSCs in 30% matrigel were injected into the kidney capsule or testis of six week old Fox Chase SCID beige mice (3 mice per cell line; Charles River) and monitored weekly for the appearance of tumor growth. Six to eight weeks post injection, tumors were removed, paraffin embedded, prepared for histological examination by hematoxylin and eosin, and analyzed (Applied Stem Cell Inc.).

ZFN-mediated targeting:

Potential ZFN target sequences in the vicinity of *CFTR* exon 10 were first examined against a database of ZF DNA binding specificities. ZFN pair 12897 (recognizing TAGAAGtGAAGTCTGG) /9940 (recognizing ATTATGGGAGAACTG) was constructed by fusing

the desired DNA binding motifs to the cleavage domain of the Fok I endonuclease. ZFNs were delivered to cells either in the form of a DNA expression plasmid or *in vitro* generated RNA (MessageMAX T7 ARCA-Capped Message Transcription Kit, A-PlusPolyA Polymerase Tailing from Cell Script Inc. and MegaClear kit from Ambion). A 1.6 kb donor plasmid construct to facilitate HDR containing wt exon 10 sequences (approximately 860 bp and 290 bp of homology sequences upstream and downstream of exon 10, respectively) was originally constructed by PCR amplification of genomic DNA sequences from BAC clone RP11-1152A23. Two silent single base pair substitutions were introduced into the right ZFN binding site with the goal of interfering the ability of the introduced ZFNs to cleave the donor either prior or subsequent to homology-directed repair; an additional silent single base pair substitution was introduced into the wt exon 10 donor sequences in order to create a novel Cla I restriction enzyme site to facilitate identification of target integrated clones. The 1.6 kb amplicon was cloned into pSC-B as per StrataClone Blunt PCR Cloning Kit (Stratagene). Three additional single base pair changes were introduced into intron 10 donor sequences 125 bp downstream of exon 10 to create a unique Avr II restriction enzyme site to introduce a selection marker conferring phosphoglycerate kinase promoter-driven puromycin- resistance and thymidine kinase sensitivity (pgk-puro-TK-bpA). All changes in the wt donor were introduced via Quikchange Lightning Site-Directed Mutagenesis (Agilent). PCR amplification of pgk-puroTK-bpA sequences from plasmid pPthC-Oct3/4 (Masui et al., 2007)(kindly provided by N. Nakayama) with primers including loxP recognition sequences and Avr II sites generated material was cloned into the introduced Avr II site of *CFTR* donor by Phusion Hot Start II high fidelity DNA polymerase (New England Biolabs) in antisense orientation to CFTR gene. Donor plasmid was confirmed by sequencing.

ZFNs, either in the form of DNA expression plasmids (1 or 2 μg) or *in vitro* transcribed RNA (1.5 or 3 μg were delivered together with donor DNA (4 or 8 μg) to CF iPSCs (2 million single cells in suspension obtained via Accutase treatment; clone 17) via nucleofection (Lonza

Amaxa hStemCell kit1 program A23) and cells were plated in the presence of 10 μM Rock-inhibitor (Alexis Biochemicals, Y27632) onto irradiated puromycin-resistant MEFs (Stem Cell Technology). Puromycin selection (0.5 μg/ml) was initiated 4 days post transfection, and puromycin-resistant colonies were picked starting 5-9 days later and expanded, in the presence of puromycin, to establish clonal cell lines.

The donors utilized included either "A" or "G" in intron 9, 76 bp upstream of the ZFN cleavage site. We did not observe a significant difference in the ratio of CFTR-targeted vs. non-targeted puro^R colonies as a function of donor. For example, in a total of two experiments with the "A" donor, we analyzed a total of 63 puro^R colonies, of which 21 exhibited targeting within the *CFTR* locus as evidenced by PCR amplification at the upstream end of the donor. For the "G" donor, we analyzed a total of 57 puro^R colonies, of which 15 gave evidence for targeting within the *CFTR* locus (one of the 15 colonies did not subsequently pass one of our further criteria for targeting and was thus not included in the total in Fig. 4).

Molecular analysis of targeted iPS clones:

Genomic DNA was isolated from puromycin-resistant clones beginning at passage 2 by QIAprep Spin Miniprep Kit (Qiagen) or ArchivePure DNA Cell Tissue Kit (5 Prime). PCR amplification utilizing various primers (e.g. Fig. 2) was performed according to manufacturer protocols. Sequencing was performed on an ABI 3730XL sequencer. Sequences of oligonucleotide primers utilized for PCR amplification and sequencing are available upon request.

Southern blotting:

In order to generate a radio-labeled DNA for probing Southern blotted genomic DNAs, the donor plasmid was digested with Nde I + Spe I, separated on 0.8% agarose gel, and then the 2.3 kb fragment was cut out and gel-purified (Qiagen). The 2.3 kb fragment was labeled with [alpha-^{32}P]dCTP using Prime-It II Random Primer Labeling kit (Agilent Technologies) following manufacturer's instruction. 25 μg of genomic DNAs (gDNAs) were digested with Spe I overnight

and purified by phenol/chloroform extraction. The gDNAs were then resolved on 1% agarose gell and transferred to a Nytran Super Charge membrane (Schleicher and Schuell) and hybridized with ^{32}P-labeled probe. The membrane was exposed and image scanned using a phosphorimager system (Molecular Dynamics).

Cre-mediated excision of selectable marker:

Cre-expression plasmids (pBS513 EF1alpha-cre [B. Sauer, 11918](Le et al., 1999) , pCAG-Cre [C. Cepko, 13775])(Matsuda and Cepko 2007) were delivered to Accutase-treated single cells via Amaxa nucleofection (same conditions as ZFN mediated targeting) and plated onto irradiated puromycin-resistant MEFs. Individual colonies were picked and expanded, and then plated in replicate to identify those clones that had become sensitive to puromycin. Alternatively, some clones were first identified based on their resistance to FIAU (1 μM, Moravek Biochemicals), expanded, and then plated in replicate to identify puromycin sensitive clones.

In vitro differentiation:

CF iPSCs, either mutant or corrected, and WA09 ESCs were differentiated based on (Green et al., 2011) and (Longmire et al., 2012) with minor modifications. Single cell adapted and mTeSR1 (StemCell Technologies) cultured clones were lifted from the plate by using 1mg/ml Dispase (StemCell Technologies). To form embryoid bodies (EBs), cells were transferred into serum-free differentiation medium (SFDM, previously described in (Longmire et al., 2012)) supplemented with 10 μM Y-27632 (Reagent Direct), 3 ng/ml BMP4 and 10 ng/ml Wnt3a onto low attachment six well plates (Corning). All differentiation factors were purchased from R&D Systems if not otherwise indicated. Next day the medium was changed to SFDM containing 10 μM Y-27632, 0.5 ng/ml BMP4, 2.5 ng/ml b-FGF and 100 ng/ml Activin A to induce definitive endoderm over 72 hours. Depending on EB number the medium was refreshed on the second day of Activin A treatment. Alternatively, we derived definitive endoderm from human iPSCs/ESCs cultured as a monolayer, using the StemDiff kit (Stem Cell Technology) according to manufacturer's protocol. On day four or five of differentiation cells were analyzed by flow

cytometry for expression of endodermal markers using CXCR4- (BioLegend, 306505) and C-KIT-antibodies (Life Technologies, CD11705). Subsequently 50,000- 100,000 single cells/cm^2 were plated either into each well of a gelatin (Millipore) or fibronectin (3ug/cm^2,Sigma) coated 24 well plate or a 12.5 cm^2 flask (Falcon) and cultured for 2 days in SFDM supplemented with 10 μM Y-27632, 200 ng/ml NOGGIN and 250 nM SB-431542 (Sigma-Aldrich) to generate anterior foregut endoderm. On day 7 of differentiation the medium was switched to ventralization/ NKX2-1 induction medium containing 100 ng/ml WNT3a, 10 ng/ml KGF, 10 ng/ml FGF10, 10 ng/ml BMP4, 20 ng/ml EGF and 100 nM Retinoic Acid (RA) and refreshed every day until day 19.

Analysis of mRNA:

RNA isolation from iPS and iPS-derived cells with the RNeasy kit (Qiagen), cDNA synthesis was performed with Improm-II Reverse Transcriptase oligo dT kit (Promega), and RT-PCR was performed with Gotaq Hot Start polymerase (Promega) or Universal TaqMan PCR (Applied Biosystems). For detection of *CFTR* expression, two sets of PCR primers were utilized: one set with primers in exons 9 and 13 (yielding a 0.77 kb product; data not shown), and an alternative set with one primer spanning exons 8 and 9 and the other primer in exon 11 (yielding a 0.5 kb product; Fig. 2C).

Quantitative transcriptional profiling: The RT^2 profiler human ES cell PCR array (Qiagen PAHS-081Z), designed to assay the expression of 44 genes involved in maintenance of pluripotency and self-renewal of human ES cells, was used to profile corrected (17-9-C1, 17-14-C1), uncorrected (Clone 17) CF iPSCs, and WA09 hES cells.

In profiling the differentiated cells, gene expression levels were determined in 2-3 well replicates, normalized against housekeeping gene 18S, and compared to undifferentiated samples. All TaqMan gene expression assays were obtained from Life Technologies.

CFTR constructs and transfection:

CFTR wt and ΔF508 over-expression plasmids were previously described in (Hoelen et al., 2010) and a kind gift from Jeffrey Beekman. HEK293 cells grown in six well plates were

transfected with 2 µg of pcDNA3-CFTRwt and pcDNA-F508del plasmids using X-treme Gene HP DNA Transfection Reagent (Roche). 48 hours post transfection cells were lysed in 500ul lysis buffer (1% Triton X-100, 50 mM Tris HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, supplemented with protease inhibitor mixture [Roche]). CFTR protein analysis was performed after removal of cell debris.

Western blot analysis:

Day 19 differentiated cells and Calu-3 controls were lysed using 1% Triton X-100 containing lysis buffer in the presence of protease inhibitor. Cell debris was removed by centrifugation and protein amount quantified by BCA protein assay (Thermo Fisher). 100 µg of total protein from differentiated samples and 10 µg from Calu-3 cells were treated with 10X glycoprotein denaturation buffer, 10X G7 reaction buffer, 10% NP-40 and finally supplemented with and without 3,000 units of PNGaseF (New England Biolabs). Protein samples were incubated for 1 h at 37 deg C until deglycosylation reaction was stopped by adding 4X Laemmli sample buffer (Bio-Rad). 50 µg of PNGaseF-treated and untreated protein samples (or 5 µg for Calu-3 samples) were separated on a 7% NuPAGE Tris-Acetate gel (Life Technologies) and transferred onto a nitrocellulose membrane. Membranes were blocked for 1 hour in 5% nonfat dry milk (Bio-Rad) in PBS and probed with a monoclonal 596 anti-CFTR antibody (1:1000, Cystic Fibrosis Foundation Therapeutics, A4 596). A secondary HRP conjugated anti-mouse IgG (1:5000, Cell Signaling Technology, 7076S) and enhanced chemiluminescence reagent (Amersham) was used to visualize the bands. As loading control an anti-Calnexin polyclonal antibody (1:300, abcam, ab22595) was used together with a secondary anti-rabbit IgG IRDye 800CW (LI-COR, 925-32211). An Odyssey Quantitative Infrared Imaging System measured the fluorescence intensities of the protein bands.

Immunostaining:

Cultured cells were fixed with fresh 4% paraformaldehyde for 30 minutes, rinsed with PBS, permeabilized with 0.2% Triton X-100 for 15 minutes, blocked in 4% goat serum for 30 minutes

and then incubated in primary antibody overnight at 4 degrees. After 16 hours the cells were washed twice with PBS and incubated in secondary antibody (1:100) for 2 hours at room temperature. The antibodies used were FOXA2 (1:100, Santa Cruz Biotechnology, sc-101060) and NKX2-1 (1:100, abcam, ab76013). Parallel wells stained with secondary antibodies alone were included as negative controls. Goat anti-mouse Alexa 488 (Life Technologies, A-11001) and goat anti-rabbit Alexa 546 (Life Technologies, A-11010) were used as secondaries.

Functional CFTR chloride channel assays:

Short circuit current: Inserts were mounted in Ussing chambers and short circuit currents (Isc) were tested under voltage clamp conditions as previously described (Bates et al., 2007; McClure et al., 2014). Briefly, iPS cells differentiated in flasks for 19-20 days under the conditions described above were seeded on permeable supports (3×10^5 cells/cm2 filter (Costar 3470),) coated with fibronectin (3ug/cm^2,Sigma) and grown until confluent monolayers established. At time of seeding, differentiated cells expressed, by Western Blot, either corrected (e.g. Clones 17-9-C1, 17-14-C1, 17-16-C1) or mutant (e.g. Clones 17, 28) CFTR proteins, respectively. Prior to short circuit measurements, cells were treated for 24 hours with 0.03% DMSO or 3 uM VX-809 (Selleckchem). Filters in modified Ussing chambers (Jim's Instruments,Iowa City, IA) were bathed on both sides with identical Ringers buffer containing 120 mM NaCl, 25 mM, $NaHCO_3$, 2.4 mM KH_2PO_4, 1.24 mM K_2HPO_4, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM D-glucose (pH 7.4). Bath solutions were vigorously stirred and gassed with 95% O_2: 5% CO_2 and maintained at 37°C. Isc expressed as μA/cm^2, was monitored using an epithelial voltage clamp (University of Iowa Bioengineering). A 3-mV pulse of 1 second duration was imposed every 100 seconds to track resistance by Ohm's law. To measure stimulated Isc, the mucosal bathing solution was changed to a low Cl^- buffer containing 1.2 mM NaCl, 115 mM Na gluconate, and other components as above plus 100 μM amiloride to block residual Na^+ channel activity. Agonists (20 μM forskolin and 50 μM genistein) were included in the bath solutions (minimum 5 min observation at each concentration). CFTRInh-172 (10 μM) was added

to the mucosal bathing solution at the conclusion of each experiment to block CFTR-dependent Isc. Statistical Analysis: Study subjects were clustered by clonal cell line and experiment number. To conduct statistical analysis, while accounting for correlated replicates, we applied a linear mixed-effect model (LMM) by restricted maximum likelihood (REML) (Laird and Ware 1982; Pinheiro and Bates 2000) and the corresponding ANOVA (Analysis of Variance) that incorporate random-effects to explain within-subject variations. For independent observations, we use a t-test or Mann-Whitney U test for normally-distributed and non-normally distributed variables, respectively. All statistical analyses were performed using the computing environment R (R Development Core Team, 2014; www.r-project.org).

cAMP-dependent ^{125}I-efflux: Corrected (17-16-C1) and mutant (Clone 17) CF iPSCs were differentiated for 19 to 23 days to carry a sub-population of lung epithelial cell types. CF-iPSC-derived epithelial monolayer was loaded with 0.5 μCi/cm^2 Na^{125}I in HEPES-phosphate buffered Ringer solution (HPBR) and incubated at 37°C in 5 % CO_2 for 1-2 hours (Venglarik et al., 1990). Briefly, cells were washed 4 times with 1 ml aliquots of isotope-free HPBR solution to establish an iodide efflux baseline. From minute one, isotope-free HPBR solution with or without CF inhibitor 172 (10 μM) was added by first removing all the solution from duplicate wells and replacing it with fresh solution at one minute intervals indicated times. At minute six, we added 1 ml cAMP buffer (HPBR with forskolin (10 μM) and genistein (50 μM) in the presence or absence of CF inhibitor 172 (10 μM)). Samples were collected until approximate 14 minutes. Buffer changes at each time point were collected and analyzed for radioactive ^{125}I. Remaining counts were obtained by cell lysis with 1% SDS, 0.1 N NaOH solution. ^{125}I-efflux are expressed in % counts per minute (CPM) as determined by the ratio of counts at a specific time point versus total of measured radioactivity (i.e., released iodide in supernatants versus remaining iodide

from lysed cells). We also assayed Calu-3 cells to establish robustness of the assay (data not shown, (Cantin et al., 2006)).

Genome sequencing and analysis:

Human iPSCs were first depleted of MEFs by passage on matrigel in MEF-conditioned media. Genomic DNA was extracted with the QIAprep Spin Miniprep Kit. DNA sequences were obtained as described previously using DNA nanoball amplification and combinatorial probe-anchor ligation sequencing (Drmanac et al., 2010; Lee et al., 2010). Possible de novo non-synonymous coding variants (NSCV) were analyzed and filtered based on two independent criteria (somaticScore and refScore) and then combined to generate the list in Table S1. Since each cell line was compared to the reference genome (GRCh37/hg19) we used the *calldiff* function with somatic option in CGA Tools to generate pairwise comparisons of our four cell lines. All NSCV with SomaticScore greater than -10 from the 8 pairwise comparisons were combined with NSCV which had a refScore greater than 60 (indicating the likelihood of being homozygous reference) in the mutant CF fibroblast cell line. The compendium of putative NSCV was examined across all four cell lines by loading evidence file based BAM files for each cell line into the Integrative Genomics Viewer (Robinson et al., 2011). We considered false NSCV to consist of variations in areas with poor coverage (<9 reads), seen in the CF mutant fibroblast(Gore et al., 2011), variations present at <10% frequency, and those duplicating nearby repetitive sequences. The 10% frequency is based on observations of loci with variant reads present at 10% of total reads that were not called variant. Since the BAM files were generated from evidence files after *de novo* alignment, coverage was assessed indirectly based on calls in the masterVarbeta files; regions with “no call” or “complex” in any of the cell lines. In other instances where there was no sequence data in the BAM files and a call of “hom ref”, we assumed homozygous reference sequence when coverage scores were positive.

Exome Capture and Sequencing:

Two μg of genomic DNA was submitted to Axeq Technologies for human exome capture

sequencing using TrueSeq 62 Mb target enrichment (http://www.illumina.com/documents/products/datasheets/datasheet_truseq_exome_enrichment_kit.pdf). Axeq Technologies performed sample validation, library preparation, exon enrichment, clustering and sequencing using illumina HiSeq 2000 Sequencer. Approximately 63,000,000 reads of an average size of 107 bp per sample was returned to us as two fastq files (one file per orientation).

Read Mapping:

Each pair of fastq files were aligned to human genome (hg19) using Novoalign (http://www.novocraft.com). All parameters were kept at the default settings, as recommended by Novocraft. SAMtools (http://samtools.sourceforge.net/) (Li et al., 2009) was used to sort the SAM files, create BAM files and generate their index files. Picard (http://picard.sourceforge.net/) was used to remove all the PCR duplicates from the BAM files. The Genome Analysis Toolkit (GATK) (McKenna et al., 2010)was used for local realignments, base quality recalibration, and variant calling. Parameters were set as described in GATK's Best Practices v3. GATK generated standard variant call format files (VCF http://www.1000genomes.org/wiki/Analysis/Variant%20Call%20Format/vcf-variant-call-format-version-41). The VCF files were annotated using snpEff (snpeff.sourceforge.net) (Cingolani et al., 2012) and ANNOVAR(Wang et al., 2010). From this point on we focused our analysis only on the putative coding non-synonymous variants.

NSCV described were independently found in both the whole genome (WGS) and the exome sequencing, which incorporates data from two different sequencing platforms and analysis methods (Lam et al., 2012). Both WGS and exome sequencing identified 4 NSCV in the CF iPS, but only 1 NSCV was present in both data sets. For the 17-9-C1 cells, 1 additional NSCV was identified by exome sequencing while for the 17-14-C1 cells, 1 additional NSCV was found by WGS. Whole genome sequencing was also employed to identify the integration sites of the reprogramming vectors in Clone 17 CF iPSCs; as expected for retroviral vectors, the majority of

integrants mapped to introns but none of the 25 vectors integrated directly into protein coding sequences (data not shown).

SELEX was used to identify the binding site preference for the left and right zinc finger proteins comprising the CFTR ZFNs. Base preferences for the CFTR ZFNS as determined by SELEX were used to guide a genome wide bioinformatics prediction of the top 100 potential off-target sites in the human genome, taking into account either homo-dimer or hetero-dimer binding (Perez et al., 2008). Whole genome sequences for two corrected CF iPSC lines (17-9-C1 and 17-14-C1) were then interrogated at these potential off-target ZFN binding sites for any evidence of mutation.

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