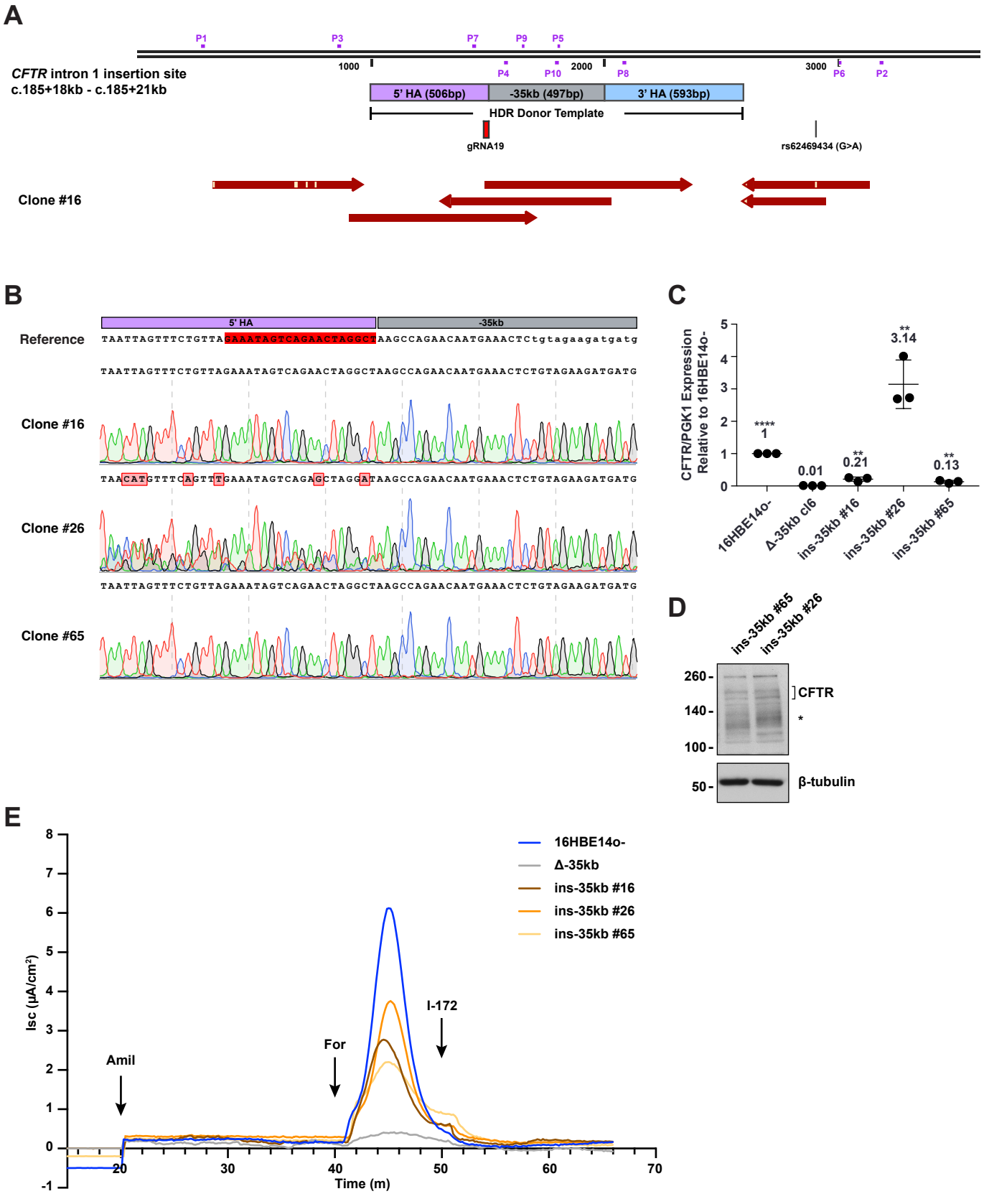
SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Sequence confirmation, CFTR mRNA, protein and function in 3 independent ins-35kb clones. A. Diagram showing the homology-directed repair (HDR) donor template used for insertion of -35kb enhancer into intron 1 of *CFTR*. Location of the CRISPR/Cas9 gRNA is noted. Sanger sequencing alignments are shown below for a single clone #16 (red arrows) indicating sequencing coverage across the modified region. Primers used for amplification and sequencing are shown in purple. Primers P1 and P2 were used to generate an amplicon that was used for sequencing with additional primers. B. Sanger sequencing chromatograms for the three 16HBE14o$^-$ ins-35kb clones around the region of the 5' junction between intron 1 and -35kb sequence. Clone #65 chromatogram indicates sequence variants introduced near the gRNA cut site. C. *CFTR* expression normalized to *PGK1*, shown relative to 16HBE14o$^-$ WT cells (n=3). Note: clones #16 and #65 were averaged for presentation in Figure 1C. **** denotes P< .0001 and ** denotes P<0.01 compared to 16HBE14o$^-$ Δ-35kb cells using an unpaired t-test. D. CFTR protein expression by western blot analysis, normalized to β-tubulin, highlighting additional CFTR isoforms in the multicopy insertion clone #26 and single copy clone #65. E. Representative traces of short-circuit current in 16HBE14o$^-$ -derived clones. Cells grown on permeable filters with apical and basolateral media. CFTR function was assessed in Ussing chambers. At the indicated times amiloride (Amil, 100 μM, apical), forskolin (For, 10 μM, basolateral), and Inh-172 (10 μM, apical) were added.

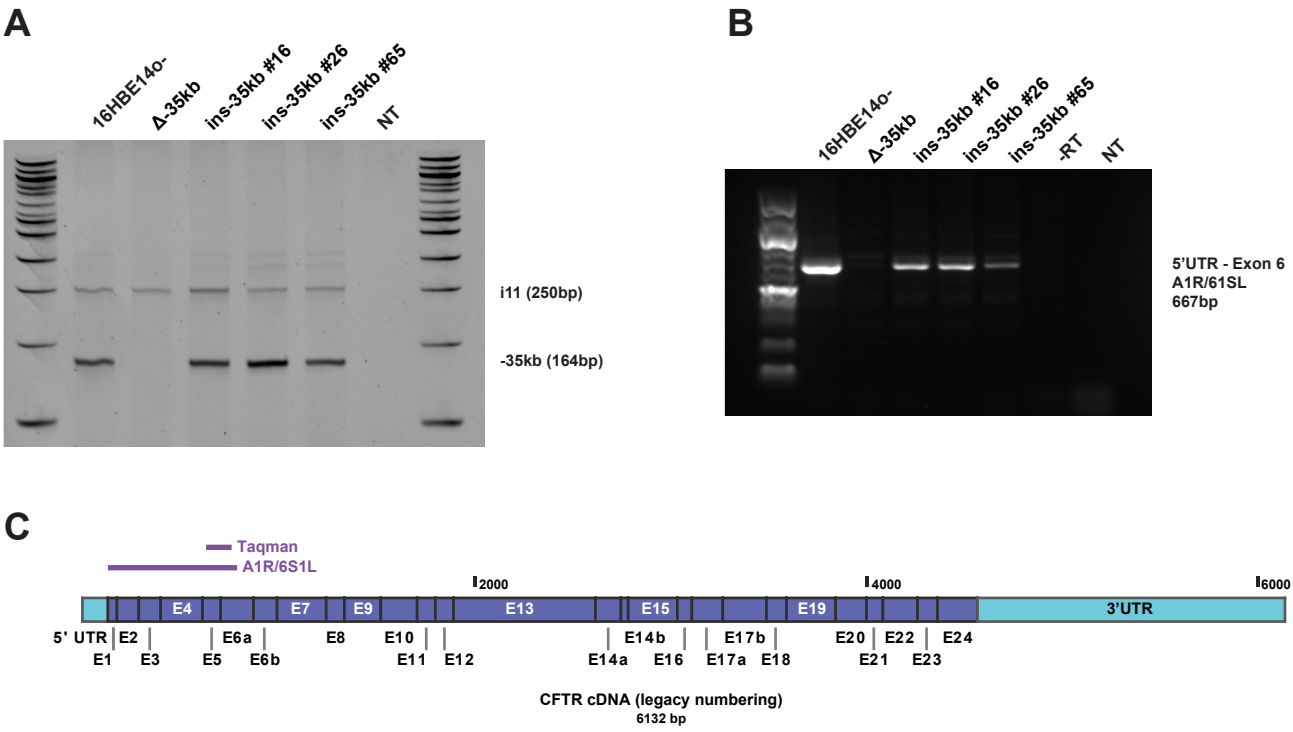
Figure S2: *CFTR* splicing and copy number assessment in 16HBE14o$^-$ ins-35kb clones. A. Multiplex PCR to examine copy number of -35kb in 16HBE14o$^-$ WT and modified cells. Products run on an 8% polyacrylamide gel, with an amplicon in *CFTR* intron 11 (250bp) serving as control to which the amplicon for -35kb (164bp) is compared. According to this ratio, clone #26 has more -35kb product than clones #16, #65 and WT, suggesting a higher allele number. NT: no template PCR control. B. Comparison of *CFTR* cDNA amplicons using RT-PCR, with a primer set encompassing the 5'UTR through exon 6. cDNA was synthesized with random hexamers and products separated on a 1.5% agarose gel. A WT *CFTR* product size of 667bp is detected for all samples with the exception of 16HBE14o$^-$ Δ-35kb, which expresses very low *CFTR* as expected ((19)) (Figure 1C, Figure S1C). -RT: no reverse transcriptase control cDNA, NT: no template PCR control. C. Diagram of *CFTR* cDNA with the locations of the Taqman primer/probe set (Figure 1C and Figure S1C) and A1R/6S1L primers used for splicing assessment (panel B).

Figure S3: Clonal variation in open chromatin and active histone mark occupancy in 16HBE14o$^-$ ins-35kb clones. A. Open chromatin mapping of 16HBE14o$^-$ Δ-35kb and ins-35kb clones, mapped to a modified hg19 genome accounting for the -35kb deletion (red dotted line) and insertion into intron 1 (blue line). Note the peak height at the intron 1 insertion compared to the int10c peak (orange arrows) in 16HBE14o$^-$ ins-35kb #26 versus that of clone #16 or #65, indicating more open chromatin at intron 1 in these cells. H3K27ac (B.) and RNAPII (C.) enrichment in 16HBE14o$^-$, 16HBE14o$^-$ Δ-35kb, and 16HBE14o$^-$ ins-35kb clones #16, #26 and #65 cells. Data normalized to percent input, with the chr11p13 region shown as control. B., n-3; C., n=2; *** denotes P<0.001 compared to

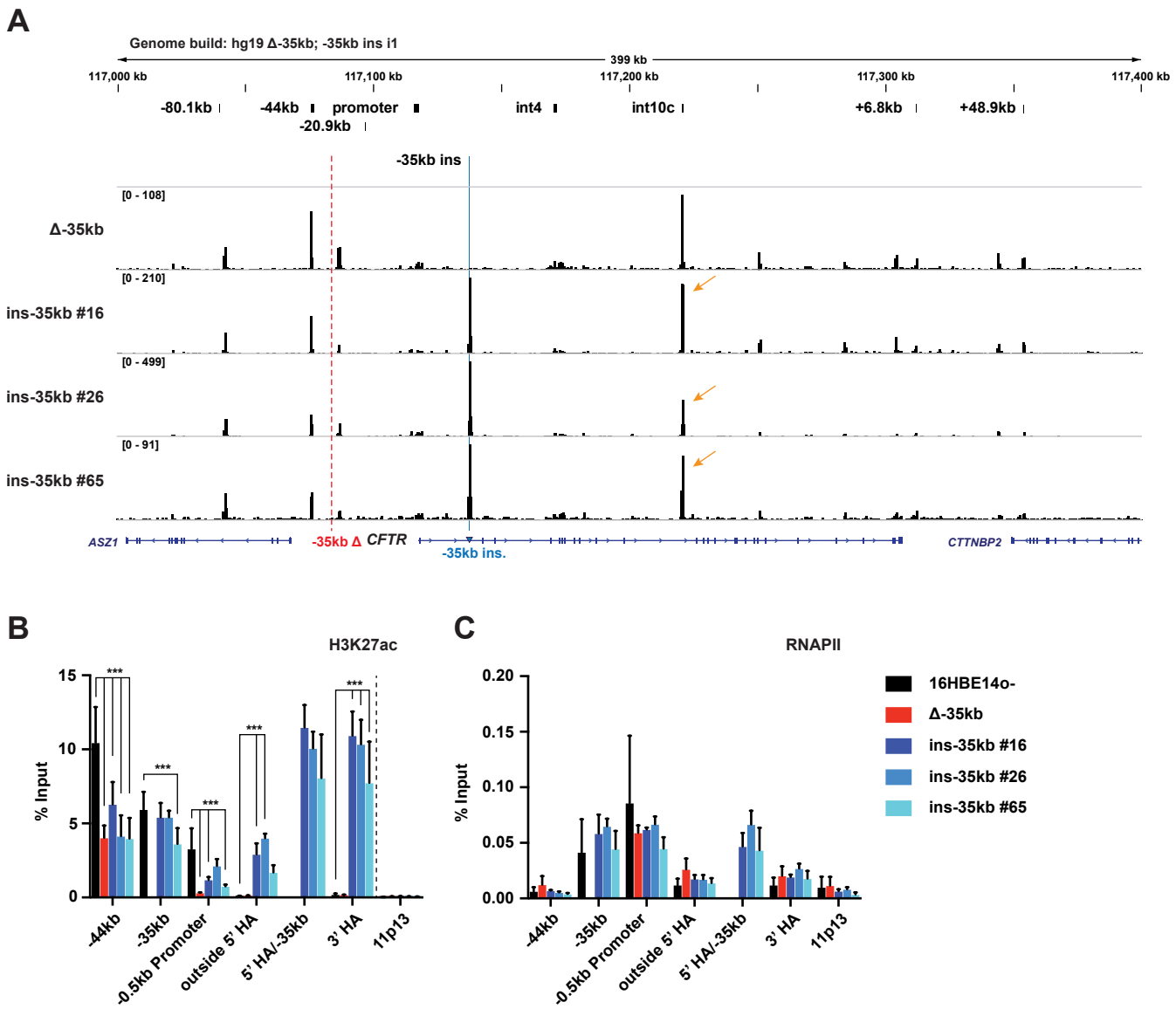
16HBE14o$^-$ enrichment levels at each site using the Holm-Sidak multiple t test (not significant not shown). Note the higher H3K27ac enrichment at the *CFTR* promoter in 16HBE14o$^-$ ins-35kb #26 cells compared to clones #16 and #26.



Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3

Table S1: Oligonucleotides

Gibson Assembly

Region	Forward Sequence (5'>3')	Reverse Sequence (5'>3')
5' Intron 1 HA	ATTTGGGAGAAGTGTCATGCAATTAG	atacaagtacaggtctagggAGCCTAGTTCTGACTATTTCTAAC
-35kb	gaaatagtcagaactaggctCCCTAGACCTGTACTTGTATCTTTAAC	catgagccaccaccacacccATGCTCTTTCGTGGAACCTAGTATTC
3' Intron 1 HA	taggttccacgaaagagcatGGGTGTGGTGGTGGCTCATGCCTG	AATATGTCCCATCAGGCCAGGCGC

Intron 1 Insertion

Name	Sequence (5'>3')
CFTR 185 + 17.2kb gBlock	TGTACAAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATAT ACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTG CAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG**GA** **AATAGTCAGAACTAGGCT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTCTAGAC CCAGCTTTCTTGTACAAAGTTGGCATTA
ins-35kb EF	GTGGCCTATTTCAAACTCATGGC
ins-35kb ER	AGCTTATGCTGCCCAACAAT
ins-35kb IF	GAGCCACATTCTTGCCTCTC
ins-35kb IR	GAGCTGGAGGATGAAATGGA

TaqMan Assay

Gene	Sequence (5'>3')
CFTR	AGCTGTCAAGCCGTGTTCTAGATA ATGAGGAGTGCCACTTGCAAA /56-FAM/CACACGAAA/ZEN/TGTGCCAATGCAAGTCCTT/3IABkFQ/ TAACAAGCTGACGCTGGAC GCAGCCTTAATCCTCTGGTT
PGK1	/56-JOEN/CGACTCTCA/ZEN/TAACGACCCGCTTCC/3IABkFQ/

ChIP-qPCR

Amplicon Site	Forward Sequence (5'>3')	Reverse Sequence (5'>3')
CFTR -44kb	AGTGAGATTAGTTGTCTCTTTTGGAGATAA	CCCTTGACTATTTTGTGCACATG
CFTR -35kb	ATCTACCTTACCCTGCTGTCCATT	TCTGAATTATCAGCCCACAGTCA
CFTR -0.5kb Promoter	GTTCTCCCGCCGGTGG	CAGTCGCGGCCTCTCTTTAG
outside 5' HA	CTGTGCTCTAGAAGTTTGGG	GCCACACAATCTGGATAGTC
5' HA::-35kb	GCCTTAGGGATCTCTGTTTGC	TGAAATGGACAGCAGGGTAA
3' HA	GTGAGAGGGGAAGACAGCAG	CAAAGCAGACACACCTGGAA
11p13	TCCTTCCAGGTTTTGGCTCC	GCCCCAGATCAGGAGAGAGA

4C-seq

Viewpoint (enzyme combo.)	Reading Primer (5'>3')	Non-reading Primer (5'>3')
DHS -20.9 kb (NlaIII/DpnII)	tacacgacgctcttccgatctTTAACAAAGTTTAGGTAAATGACCA	actggagttcagacgtgtgctcttccgatctCAAAGTGAGCTATTTTGTTTTCTC
int1+19.5kb (NlaIII/DpnII)	tacacgacgctcttccgatctAGGTGTGTCTGCTTTGTCTC	actggagttcagacgtgtgctcttccgatctGTCCTTTGTTTGTTTGTTTG

Semi-quantitative Copy Number Analysis

Primer location	Forward Primer (5'>3')	Reverse Primer (5'>3')
CFTR -35kb	TTCTTCCAATTCCTACCAGCA	GAATGTGGCTCAGTTAGAGAC
CFTR intron 11	CCAGCTTTAGGCTTCTTGGT	CGCCATATGCCACAAAGCTCTCTCCTTGA

***CFTR* Splicing Analysis**

Primer location	Forward Primer (5'>3')	Reverse Primer (5'>3')
A1R/6S1L	CGAGAGACCATGCAGAGGTC	GTAACAACTCCCAGATTAGC