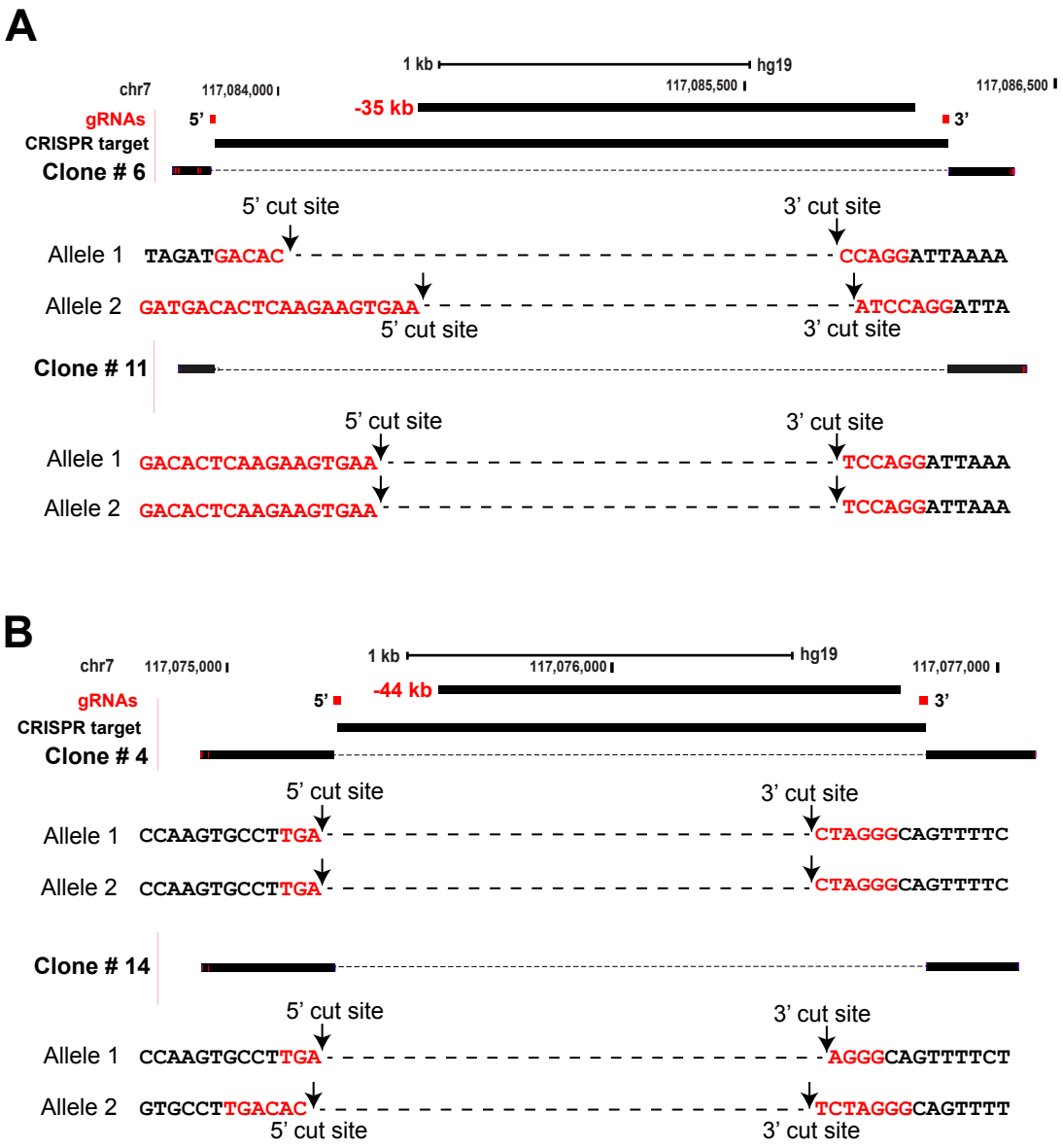
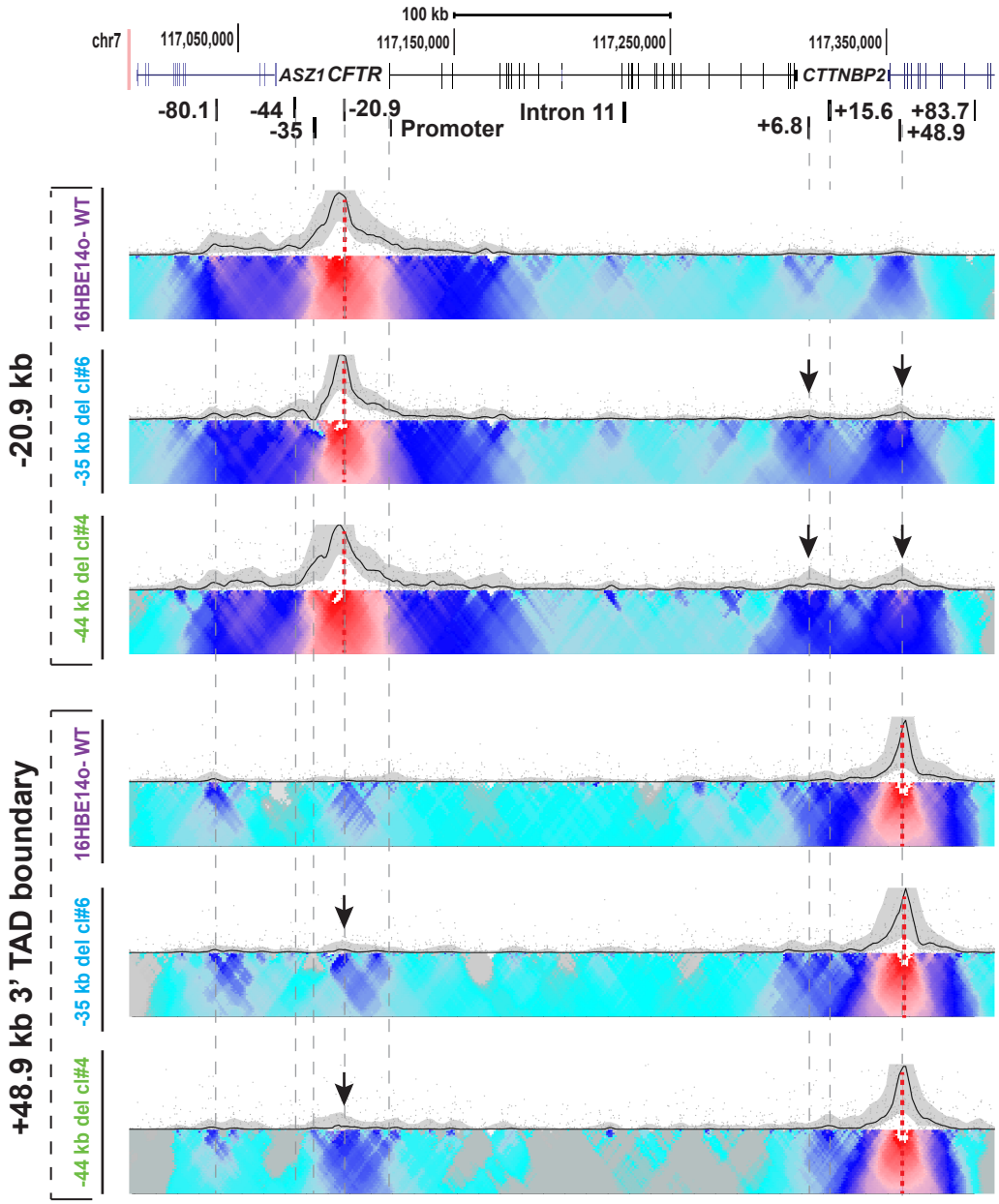
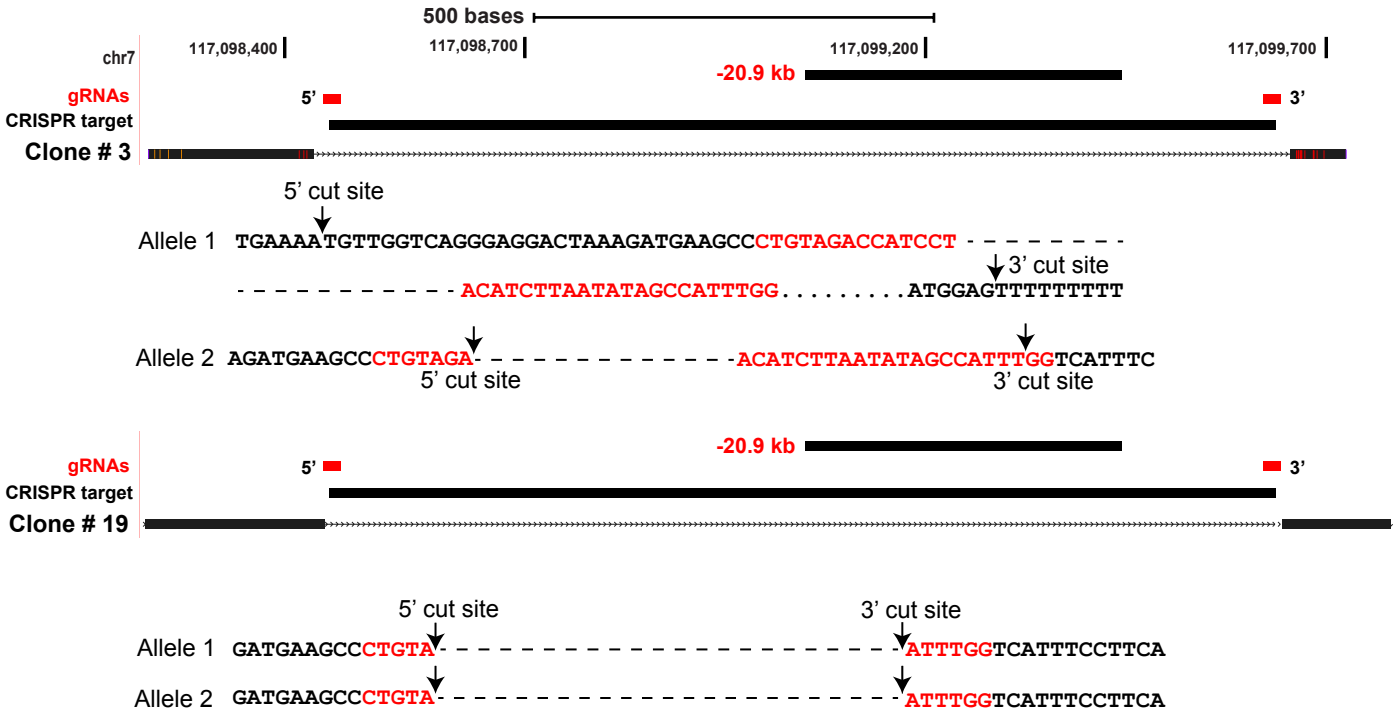


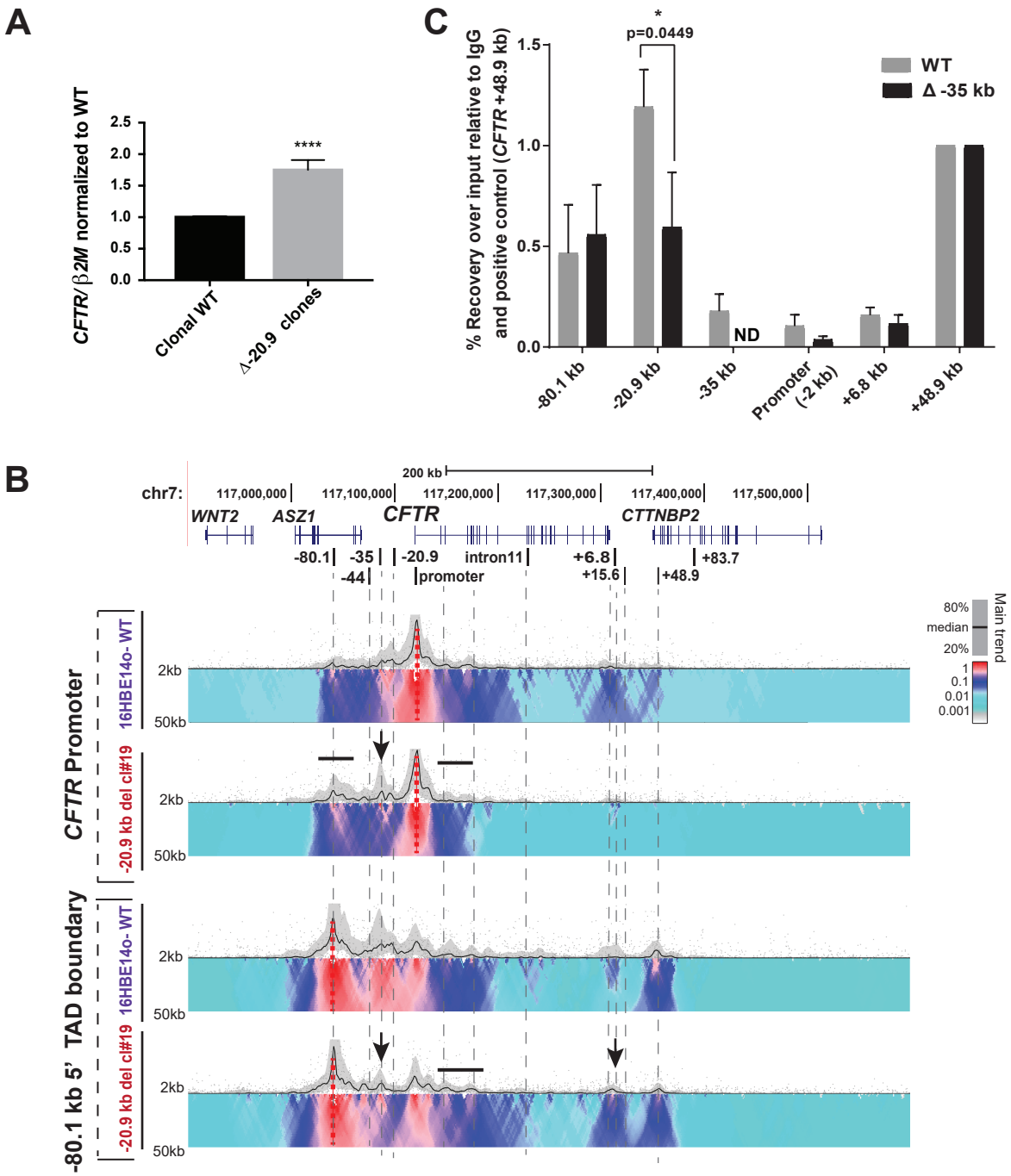
Supplementary Figure S1: Design and validation of CRISPR/Cas9 mediated deletions of -35 kb and -44 kb regulatory elements. UCSC genome browser graphic showing the schematic for the design of the CRISPR/Cas9 deletion of -35 kb CRE (A) and -44 kb CRE (B). The gRNAs used (red) and the target regions are shown above the sequences of two deletion clones for each CRE. The exact positions of the cuts in the 2 alleles are indicated by black arrows and were validated by sequencing from the external primers (Suppl. Table S1).



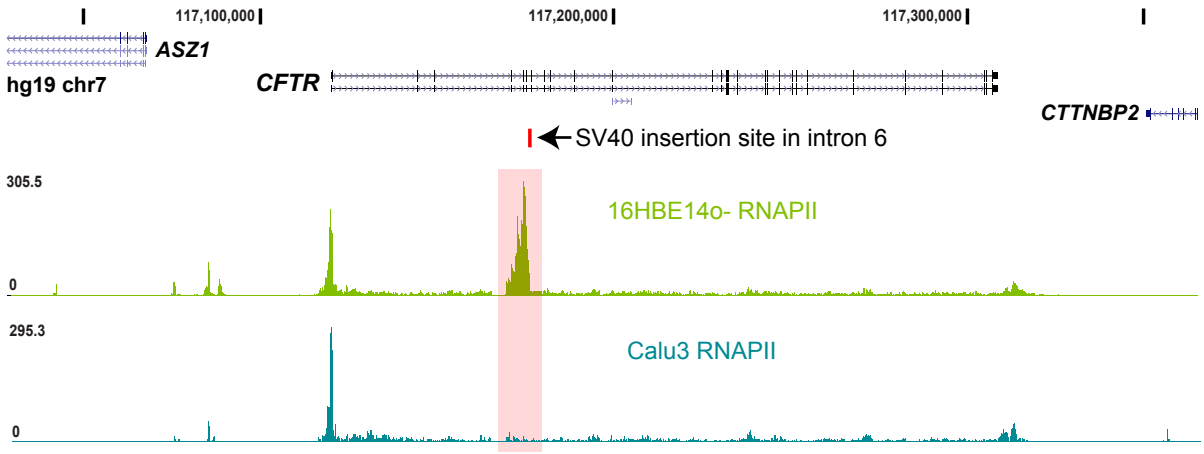
Supplementary Figure S2: Deletion of the -35 kb or the -44 kb CRE from the *CFTR* locus has a major impact on locus architecture. 4C-seq interaction profiles of the same WT, Δ-35 kb (Clone #6 and Δ–44 kb (Clone #4) 16HBE14o- clones shown in Fig.4, using viewpoints at the -20.9 kb and the +48.9 kb 3' TAD boundary. Data from a single representative clone are shown, and were consistent with an independent replica clone for both deletions.



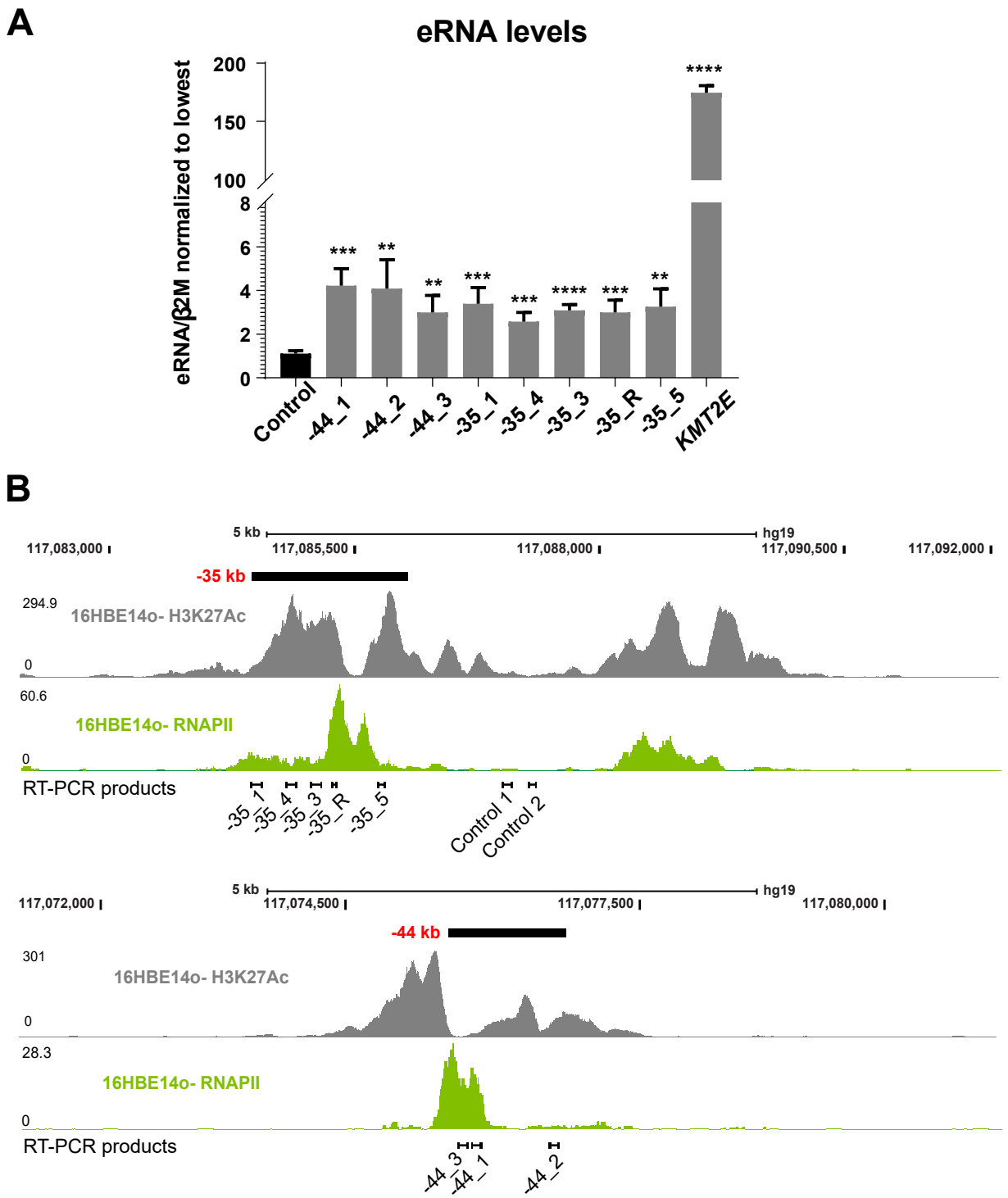
Supplementary Figure S3: Design and validation of CRISPR/Cas9 mediated -20.9 kb CRE deletion in 16HBE14o-. UCSC genome browser graphic showing the schematic for the design of the CRISPR/Cas9 deletion of -20.9 CRE. The gRNAs used (red) and the target regions are shown above the sequences of two deletion clones. The exact positions of the cuts in the 2 alleles are indicated by black arrows and were validated by sequencing using the external primers shown in Suppl. Table S1.



Supplementary Figure S4: Removal of the -20.9 kb CRE alters *CFTR* gene expression and chromatin conformation in 16HBE14o- cells. A) *CFTR* gene expression measured by RT-qPCR in two Δ-20.9 kb clones compared to 2 non-targeted WT clones from the same experiment. Results are the average of 3 independent experiments. Error bars represent S.E.M. B) 4C-seq interaction profiles of Δ -20.9 kb clone #19 with viewpoints at *CFTR* promoter (top) and the -80.1 kb 5' TAD boundary (below). Data from a single representative clone are shown, and were consistent in a replica clone. C) Deletion of the -35kb CRE decreases CTCF occupancy at the adjacent -20.9 kb insulator element. CTCF ChIP-qPCR analysis in a non-targeted WT (grey) and -35 kb deletion clone (# 6, black) in 16HBE14o- cells. CTCF occupancy was assayed at the -80.1 kb, -35 kb, -20.9 kb, +6.8 kb and +48.9 kb binding sites and the promoter (-2 kb). The graph shows % input values normalized to IgG and relative to CTCF occupancy at the +48.9 kb invariant site. Error bars represent S.E.M., n=3.



Supplementary Figure S5: The impact of SV40 integration on RNAPII recruitment. The UCSC genome browser image defines the insertion site of the SV40 genome in intron 6 of *CFTR* gene [35]. ChIP-seq shows RNAPII occupancy adjacent to this insertion in 16HBE14o- cells (olive green), but not in Calu3 cells (teal). Both RNAPII ChIP-seq experiments were performed in 2 replicates and data are shown as IDR plots.



Supplementary Figure S6: The -35 and -44 kb CREs may produce low abundance eRNAs. A) qRT-PCR of eRNAs generated from the -44 and -35 kb CREs normalized to the lowest values (an average of control 1 and control 2, which showed almost negligible eRNA levels), set to 1. The ncRNA upstream of *KMT2E* gene was used as the positive control. The error bars indicate the S.E.M and n=4. B) UCSC genome browser images showing the location of the qRT-PCR products below the 16HBE14o- H3K27Ac and RNAPII ChIP-seq data for -35 kb (above) and -44 kb (below).

Supplementary Tables

Supplementary Table S1: List of gRNA sequences and PCR primers used for the generation and validation of -35 kb, -44 kb and -20.9 kb CRISPR/Cas9 deletion clones in 16HBE14o-.

Name	Sequence (5'-3')
-35 kb 5' gRNA	GACACTCAAGAAGTGAACAG
-35 kb 3' gRNA	AACAAATTACATGTACATCC
-44 kb 5' gRNA	AAGTGTTTAGAAAAGTGTCA
-44 kb 3' gRNA	GATATCAGACAACAAGTCTA
-35 kb validation PCR Fwd External	AGGTGAAAAGGCGAAGAAGAAA
-35 kb validation PCR Rev External	CTGTAACCAACAAGGACCTC
-35 kb validation PCR Fwd Internal	CAACTGCTCACGTAAATGGGTA
-44 kb validation PCR Fwd External	CTATCCAGGAGGGCAGGAAAC
-44 kb validation PCR Rev External	GTCTCCTACATCATCCTTTCAC
-44 kb validation PCR Rev Internal	ACTCAGGAACTAGGAAGAAGAGC
-20.9 kb 5'gRNA	Yang *et al*, 2016
-20.9 kb 3' gRNA	Yang *et al*, 2016
-20.9 kb validation PCR Fwd External	GCTCAACGTAGGTTTGGC
-20.9 kb validation PCR Rev External	ACAGGCAAAAATCCAGGTTG
-20.9 kb validation PCR Fwd Internal	CCGGGATGTTGTTTGAAGCTT

Supplementary Table S2: Primers for ChIP-qPCR and RT-qPCR

Taqman RT-qPCR primer sets	Sequences
CFTR exon 5 F	AGCTGTCAAGCCGTGTTCTAGATA
CFTR exon 6 R	ATGAGGAGTGCCACTTGCAAA
CFTR exon 5/6 probe	CACACGAAATGTGCCAATGCAAGTCCTT
B2M F	AAGTGGGATCGAGACATGTAAG
B2M R	GCAAGCAAGCAGAATTTGGA
B2M probe	5- /56-JOEN/TCA TGG AGG /ZEN/TTT GAA GAT GCC GCA /3IABkFQ/ -3

SYBR RT-qPCR primer sets	Sequences
-44 kb_1 F	TGCTTGGTTCGCTGATGACA
-44 kb_1 R	TCAGGAACTAGGAAGAAGAGCC
-44 kb_2 F	GGGAGCAAGTGTAGAATCAGGAA
-44 kb_2 R	TCAACTGGGGCTGGCTAAAA
-44 kb_3 F	AGTCACAAGCTTTATTCCTGGC
-44 kb_3 R	GGCATTCTGACCCACTCCAG
-35 kb_1 F	AGATGTCACCCCTAGACCTGT
-35 kb_1 R	AAGAGGGTTGATGGTGTGCT
-35 kb_3 F	CACATTTATCAGCACCAGCC
-35 kb_3 R	AGTTCTCTGCTCTTGGGTTC
-35 kb_4 F	CTTCCAGCAGAGCATGACACA

-35 kb_4 R	CCACCACTTGGGCTTTTTCAC
-35 kb_5 F	GAAGTGCCTCAACTGCTCAC
-35 kb_5 R	TGTAAGAACAACCGAACCCGA
-35 kb RNAPII F	ATCTACCTTACCCTGCTGTCCATT
-35 kb RNAPII R	TCTGAATTATCAGCCCACAGTCA
KMT2E F	CCAGCTATGACCAGGGTTCAC
KMT2E R	TCTGCATGGTGGGTTCAGTAT
-35_6 F (Control 1)	AGAGAGTTGAACTTTGGTATGGC
-35_6 R (Control 1)	GTAGGGAAAGCCTACACTCTCA
-35_7 F (Control 2)	GCCATCAGTATCATCCGTTTC
-35_7 R (Control 2)	GTAAAGGGGAATGGGGAGTT

ChIP-qPCR primer sets	**Sequences**
-80.1 kb F	GGGCATTCAAAGAAAAGCAGAAAGC
-80.1 kb R	ACCCCAGTACAGAGACGTGACA
-44 kb F	AGTGAGATTAGTTGTCTCTTTTGGAGATAA
-44 kb R	CCCTTGACTATTTTGTGCACATG
-35 kb F	ATCTACCTTACCCTGCTGTCCATT
-35 kb R	TCTGAATTATCAGCCCACAGTCA
-20.9 kb F	CCGGGATGTTGTTTGAAGCTT
-20.9 kb R	TTTAAATAGTTGAATAGAGGACGAGATACTTT
Promoter -4 kb F	TTCCACAGTACAAGGGCAACC
Promoter -4 kb R	CAGATTAAGTTAGGGTCTCTCTACCTCAG
Promoter -3.4 kb F	AGAAGCACCCAGCACATT
Promoter -3.4 kb R	AAGAGCGAGGATGAGTAGGA
Promoter -2 kb F	TTGAACAATTTTCTGGTGGATAAGTC
Promoter -2 kb R	ATGCACTAATTGCGACATGATATTC
Intron 1 F	TCATTGTCAACTGTCAGGTAGCAA
Intron 1 R	CAGAGTTAGGATTCCAGCCAGG
Intron 10 F	TGCTTTATTGAATGGCATTACCTCTA
Intron 10 R	AGATGCTTGTGGTAAGGGAGGAG
Intron 23 F	CCCTATGGTTTAGTCACAAGGAAGTT
Intron 23 R	GGCTCAAAAGCCTGAACAGAA
+6.8 kb F	TCTTCTTTCCCATTCACCTTTGTC
+6.8 kb R	TTTTGGTTTCATTTATCAGCACATC
11p13 F	TCCTTCCAGGTTTTGGCTCC
11p13 R	GCCCCAGATCAGGAGAGAGA
M6PR F	CGCCTTTTCTGGTTGCCTTT
M6PR R	ACCCTCTTCACTCGAACCCT
+48.9 F	GGCATCAGCCAGTCAAGGTT
+48.9 R	AGCAGAGGGCAAAGTGGTACTT

Supplementary Table S3

Luciferase mutagenesis primer	Sequence
pZL350 – CEBPβ mutant	cccacaggaatgctctggagcacaaatTCTCGAGcagaattagccctaccttgaagcaagtgg

Supplementary Table S4: Restriction enzyme pairs and primer sequences used for 4C library generation.

Viewpoint	Primary enzyme	Secondary enzyme	Reading primer	Non-reading primer
CFTR promoter No barcode	NlaIII	Csp6I	AATGATACGGCGACCACCGAA CACTCTTTCCCTACACGACGCT CTTCCGATCTGCACTTACTAT ATGCAGGCATG	CAAGCAGAAGACGG CATACGATGAAGTG TTCTTTGGATATTG C
CFTR promoter AT	NlaIII	Csp6I	AATGATACGGCGACCACCGAA CACTCTTTCCCTACACGACGCT CTTCCGATCTATGCACTTACT ATATGCAGGCATG	CAAGCAGAAGACGG CATACGATGAAGTG TTCTTTGGATATTG C
CFTR promoter GA	NlaIII	Csp6I	AATGATACGGCGACCACCGAA CACTCTTTCCCTACACGACGCT CTTCCGATCTGAGCACTTACT ATATGCAGGCATG	CAAGCAGAAGACGG CATACGATGAAGTG TTCTTTGGATATTG C
CFTR promoter GC	NlaIII	Csp6I	AATGATACGGCGACCACCGAA CACTCTTTCCCTACACGACGCT CTTCCGATCTGCGCACTTACT ATATGCAGGCATG	CAAGCAGAAGACGG CATACGATGAAGTG TTCTTTGGATATTG C
-80.1 kb No barcode	NlaIII	Csp6I	AATGATACGGCGACCACCGAA CACTCTTTCCCTACACGACGCT CTTCCGATCTACTGAGAACTT ACAGGGCAGTC	CAAGCAGAAGACGG CATACGACTGGTAG CTTTTGGTTGAATG
-80.1 kb GA	NlaIII	Csp6I	AATGATACGGCGACCACCGAA CACTCTTTCCCTACACGACGCT CTTCCGATCTGAACTGAGAAC TTACAGGGCAGTC	CAAGCAGAAGACGG CATACGACTGGTAG CTTTTGGTTGAATG
-80.1 kb AT	NlaIII	Csp6I	AATGATACGGCGACCACCGAA CACTCTTTCCCTACACGACGCT CTTCCGATCTATACTGAGAAC TTACAGGGCAGTC	CAAGCAGAAGACGG CATACGACTGGTAG CTTTTGGTTGAATG
-80.1 kb AG	NlaIII	Csp6I	AATGATACGGCGACCACCGAA CACTCTTTCCCTACACGACGCT CTTCCGATCTAGACTGAGAAC TTACAGGGCAGTC	CAAGCAGAAGACGG CATACGACTGGTAG CTTTTGGTTGAATG
-20.9 No barcode	NlaIII	DpnII	AATGATACGGCGACCACCGAA CACTCTTTCCCTACACGACGCT CTTCCGATCTTTAACAAAGTT	CAAGCAGAAGACGG CATACGACAAAGTG AGCTATTTTGTTTT

			TAGGTAAATGACCA	CTC
-20.9 kb AT	NlaIII	DpnII	AATGATACGGCGACCACCGAA CACTCTTTCCCTACACGACGCT CTTCCGATCTATTTAACAAAG TTTAGGTAAATGACCA	CAAGCAGAAGACGG CATACGACAAAGTG AGCTATTTTGTTTT CTC
+48.9 kb No barcode	NlaIII	DpnII	AATGATACGGCGACCACCGAA CACTCTTTCCCTACACGACGCT CTTCCGATCTGAGTGAGCTTG AAAGCCATG	CAAGCAGAAGACGG CATACGATGGAACA TCGTCAGTGGAAG
+48.9 kb AT	NlaIII	DpnII	AATGATACGGCGACCACCGAA CACTCTTTCCCTACACGACGCT CTTCCGATCTATGAGTGAGCT TGAAAGCCATG	CAAGCAGAAGACGG CATACGATGGAACA TCGTCAGTGGAAG