Supplementary Figure Legends

Supplementary Figure 1. Hi-C data at the *CFTR* locus from human fibroblasts cells generated by the Ren Lab (data source: http://www.3dgenome.org). Dotted red lines indicate the TAD boundaries identified by 4C-seq, which are consistent with the Hi-C data. CTCF ChIP-seq data from ENCODE are also shown.

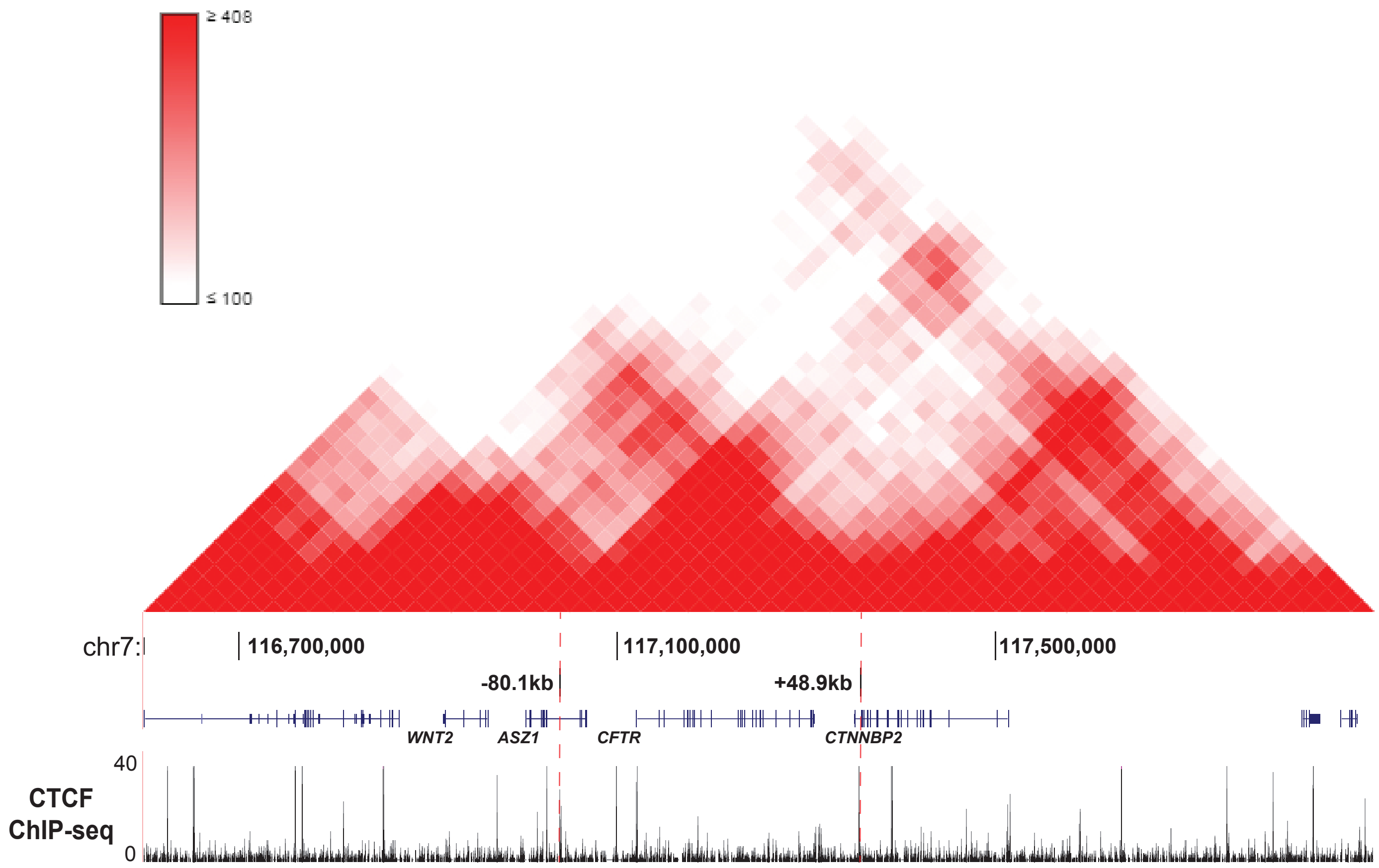
Supplementary Figure 2. Validation of 4C interactions by FISH. (A) Screen shot from UCSC genome browser showing the locations of BACs used for the FISH experiment. (B) Representative FISH images showing nuclear staining with DAPI, individual fluorescent BAC probes, and all signals merged. (C) Box and whisker plot showing quantification of physical distances between BAC probes. The box represent 25% to 75% percentile and bars represent 5% to 95% percentile. Images from 94 nuclei (282 pairs) were used for the quantification.

Supplementary Figure 3. Design and validation of CRISPR/Cas9 mediated deletion of *cis*-regulatory elements. (A, B) -20.9 kb site deletion. (A) UCSC genome browser graphic showing schematic for the design of the CRISPR/Cas9 target deletion and its location relative to the -20.9kb DHS. Below, the location of the deletion breakpoints is shown following direct sequencing of gel-purified PCR products generated using flanking primers (Suppl. Table 3). (B) Agarose gel image showing PCR product with

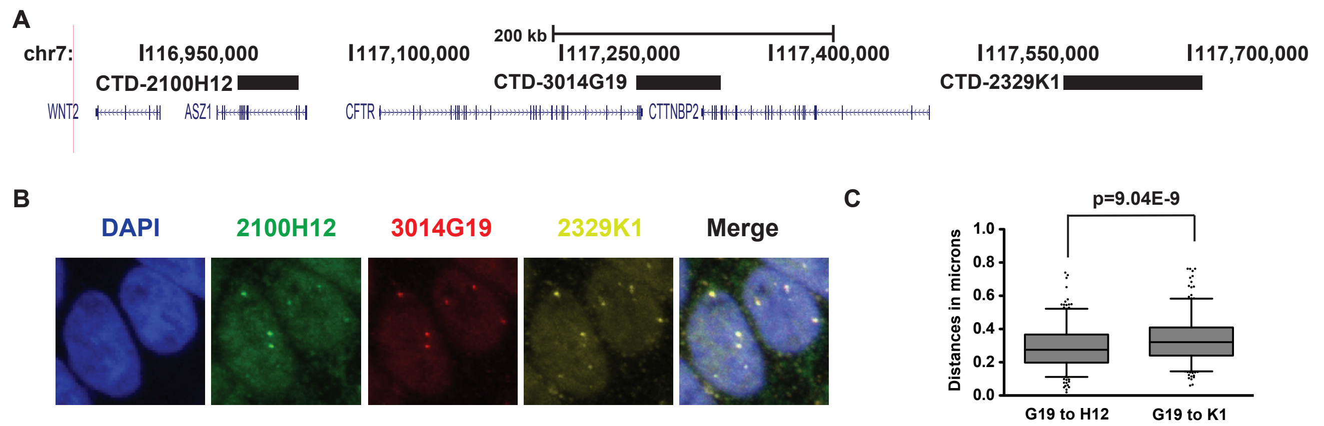
no template (1), 2 alleles deleted (2), and all three alleles deleted (3) (note Caco2 is 3N for CFTR). (C, D) intron 11 DHS deletion. (C) UCSC genome browser graphic showing schematics for the design of CRISPR/Cas9 deletion and its location relative to the intron 11 DHS. Below, the location of the deletion breakpoints is shown (as for A) (Suppl. Table 3). (D) Agarose gel image showing PCR product using template from WT (1), one allele deleted (2), two alleles deleted (3), and all three alleles deleted clones (4).

Supplementary Figure 4. Test splicing variants in WT and del11 clones by RT-PCR. cDNA from 2 WT and 4 del11 clones were used for RT-PCR. Primers used are illustrated by the schematic and described in Chalkley et al. J. Med. Genet. 1991. All WT and del11 clones show same splicing variants: 1kb product being the full length product; and 0.8kb product that lacks exon 12 (exon 12 (-)), which is common in normal tissues.

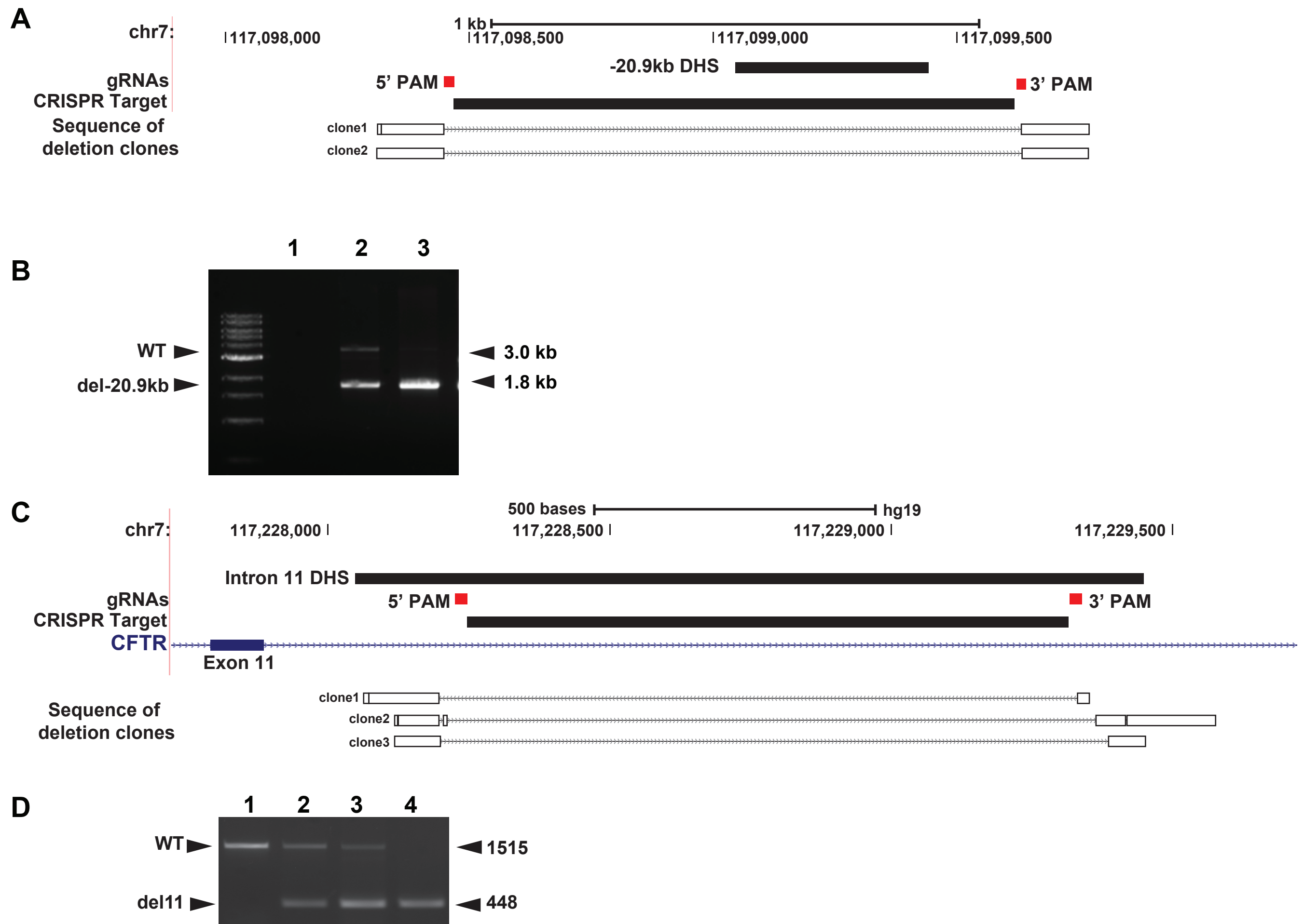
Supplementary Figure 5. Measurement of CTCF enrichment at the gel-forming mucin gene cluster on chromosome 11, in del-20.9 clones. (A) Schematic showing the position of CTCF binding sites (numbered I to XIII) relative to the mucin genes. (B) CTCF enrichment was assayed by ChIP-qPCR at site IV and IX. Results are mean ± S.E.M., n=4, not significant.



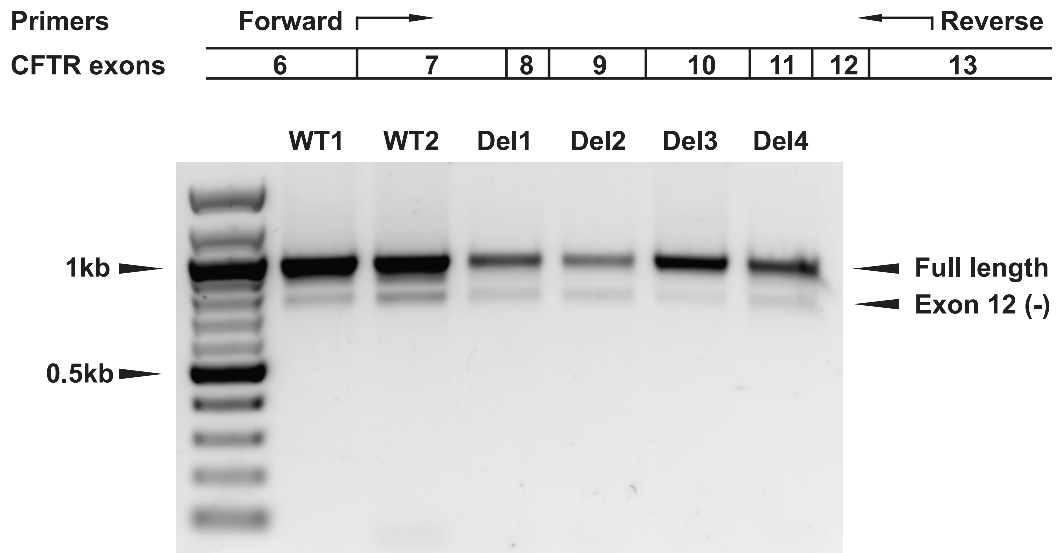
Supplementary Figure 1



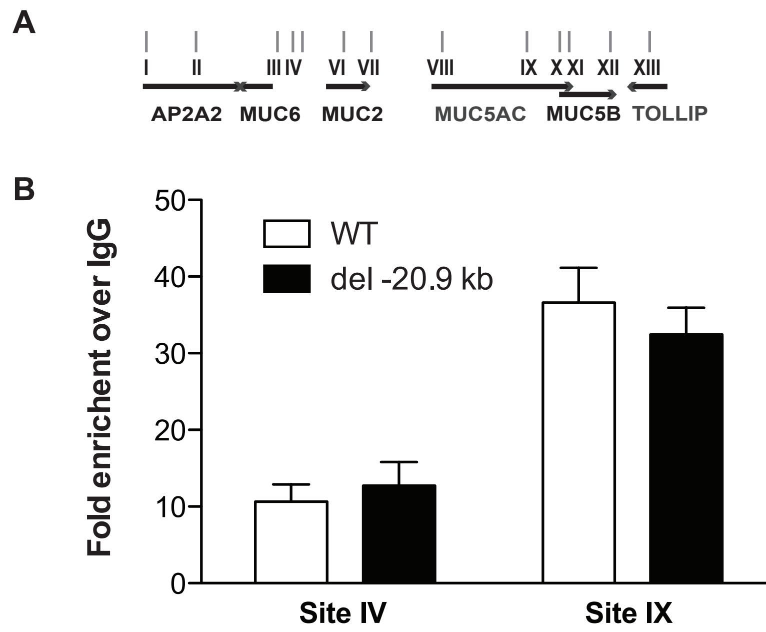
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5

Supplementary Table 1. Primer sequences for ChIP-qPCR and RT-PCR

ChIP-qPCR Primer Sets	Sequences
-80.1kb F	GGGCATTCAAAGAAAAGCAGAAAGC
-80.1kb R	ACCCCAGTACAGAGACGTGACA
Promoter F	GTTCTCCCGCCGGTGG
Promoter R	CAGTCGCGGCCTCTCTTTAG
Intron 1 CTCF F (185+1kb)	GCAGTTAATCCTGGAACTCCGGTGC
Intron 1 CTCF R (185+1kb)	AAGTCCTTCTCTCTCATCCACAGGG
Intron 1 enhancer F (185+10kb)	TCATTGTCAACTGTCAGGTAGCAA
Intron 1 enhancer R (185+10kb)	CAGAGTTAGGATTCCAGCCAGG
Intron 10 F	TGCTTTATTGAATGGCATTACCTCTA
Intron 10 R	AGATGCTTGTGGTAAGGGAGGAG
Intron 11 F	TCCAAAAGCTGAGACAGGAAACT
Intron 11 R	ATTACATACACACAAAAGTACACACATGACT
+48.9kb F	GGCATCAGCCAGTCAAGGTT
+48.9kb R	AGCAGAGGGCAAAGTGGTACTT
+83.7kb F	CCGGGTCGAACATGCAAAGC
+83.7kb R	AGCCCTCTGCTGGAAGCGTG

RT-PCR Primer Sets	Sequences
B1R	GACAAACAGAACTGAAACTG
B1L	CAGCTTTCTTTAAATGTTCC

Supplementary Table 2. Restriction enzyme pairs and primer sequences used for 4C library generation.

Viewpoint	Primary enzyme	Secondary enzyme	Reading primer	Non-reading primer
Promoter	NlaIII	Csp6I	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTGCACTTACTATATGCAGGCATG	CAAGCAGAAGACGGCATACGATGAAGTGTTCTTTGGATATTGC
Promoter CG	NlaIII	Csp6I	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTCGGCACTTACTATATGCAGGCATG	CAAGCAGAAGACGGCATACGATGAAGTGTTCTTTGGATATTGC
Promoter AT	NlaIII	Csp6I	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTATGCACTTACTATATGCAGGCATG	CAAGCAGAAGACGGCATACGATGAAGTGTTCTTTGGATATTGC
Promoter GA	NlaIII	Csp6I	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTGAGCACTTACTATATGCAGGCATG	CAAGCAGAAGACGGCATACGATGAAGTGTTCTTTGGATATTGC
Promoter TA	NlaIII	Csp6I	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGCACTTACTATATGCAGGCATG	CAAGCAGAAGACGGCATACGATGAAGTGTTCTTTGGATATTGC
Promoter AG	NlaIII	Csp6I	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGCACTTACTATATGCAGGCATG	CAAGCAGAAGACGGCATACGATGAAGTGTTCTTTGGATATTGC
Promoter GC	NlaIII	Csp6I	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTGCGCACTTACTATATGCAGGCATG	CAAGCAGAAGACGGCATACGATGAAGTGTTCTTTGGATATTGC
-20.9kb	NlaIII	DpnII	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTTTAACAAAGTTTAGGTAAATGACCA	CAAGCAGAAGACGGCATACGACAAAGTGAGCTATTTTGTTTTCTC
-20.9kb CG	NlaIII	DpnII	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTCGTTAACAAAGTTTAGGTAAATGACCA	CAAGCAGAAGACGGCATACGACAAAGTGAGCTATTTTGTTTTCTC
-20.9kb AT	NlaIII	DpnII	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTATTTAACAAAGTTTAGGTAAATGACCA	CAAGCAGAAGACGGCATACGACAAAGTGAGCTATTTTGTTTTCTC
-20.9kb GA	NlaIII	DpnII	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTGATTAACAAAGTTTAGGTAAATGACCA	CAAGCAGAAGACGGCATACGACAAAGTGAGCTATTTTGTTTTCTC

+48.9kb	NlaIII	DpnII	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTGAGTGAGCTTGAAAGCCATG	CAAGCAGAAGACGGCATACGATGGAACATCGTCAGTGGAAG
+48.9kb AT	NlaIII	DpnII	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTATGAGTGAGCTTGAAAGCCATG	CAAGCAGAAGACGGCATACGATGGAACATCGTCAGTGGAAG
+48.9kb CG	NlaIII	DpnII	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTCGGAGTGAGCTTGAAAGCCATG	CAAGCAGAAGACGGCATACGATGGAACATCGTCAGTGGAAG
+48.9kb GA	NlaIII	DpnII	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTGAGAGTGAGCTTGAAAGCCATG	CAAGCAGAAGACGGCATACGATGGAACATCGTCAGTGGAAG
-80.1kb	NlaIII	Csp6I	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTACTGAGAACTTACAGGGCAGTC	CAAGCAGAAGACGGCATACGACTGGTAGCTTTTGGTTGAATG
-80.1kb AT	NlaIII	Csp6I	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTATACTGAGAACTTACAGGGCAGTC	CAAGCAGAAGACGGCATACGACTGGTAGCTTTTGGTTGAATG
-80.1kb CG	NlaIII	Csp6I	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTCGACTGAGAACTTACAGGGCAGTC	CAAGCAGAAGACGGCATACGACTGGTAGCTTTTGGTTGAATG
-80.1kb GA	NlaIII	Csp6I	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTGAACTGAGAACTTACAGGGCAGTC	CAAGCAGAAGACGGCATACGACTGGTAGCTTTTGGTTGAATG
+15.6kb	NlaIII	Csp6I	AATGATACGGCGACCACCGAACACTCTTTCCCTACACGACGCTCTTCCGATCTTGAAACCCTATTTGAATAGCATG	CAAGCAGAAGACGGCATACGAATTTGTGTGTTTGGCTTTGG

Supplementary Table 3. List of gRNA sequences and PCR primers used for generation and validation of -20.9kb and intron 11 CRISPR/Cas9 clones.

Name	Sequences
-20.9kb 5' gRNA	CTGTAGACCATCCTTATTAACT
-20.9kb 3' gRNA	ACATCTTAATATAGCCATT
Intron 11 5' gRNA	CTGCCACTCCAATGTACACACC
Intron 11 3' gRNA	CATTATGTAGCTCTTGCATACT
-20.9kb validation PCR F	AACCTCATGCTTGTGCAAAT
-20.9kb validation PCR R	TCCATCACATCCCATTTGAG
Intron 11 validation PCR F	GCCCGTATCTTGGTGTCAGT
Intron 11 validation PCR R	AAGATGAAGACACAGTTCC