## Table S1.

Primer sequences for RT-qPCR, ChIP-qPCR, or mutagensis of the luciferase plasmids

| Taqman RT-qPCR | Sequence |
|---|---|
| CFTR exon 5 F | AGCTGTCAAGCCGTGTTCTAGATA |
| CFTR exon 6 R | ATGAGGAGTGCCACTTGCAAA |
| CFTR exon 5/6 probe | CACACGAAATGTGCCAATGCAAGTCCTT |
| β2M F | AAGTGGGATCGAGACATGTAAG |
| β2M R | GCAAGCAAGCAGAATTTGGA |
| β2M probe | 5- /56-JOEN/TCA TGG AGG /ZEN/TTT GAA GAT GCC GCA /3IABkFQ/ -3 |

| SYBR RT-qPCR | Sequence |
|---|---|
| β2M F | CTCTCTCTTTCTGGCCTGGAG |
| β2M R | TCTGCTGGATGACGTGAGTA |
| KLF5 F | ACCTCCATCCTATGCTGCTAC |
| KLF5 R | TTTGTGCAACCAGGGTAATC |
| CTCF F | GGAGAGCATACCAGCAGTGACT |
| CTCF R | TTCCAAGGAGCCACAGCACAAC |
| RAD21 F | GTGGAAAGAGACAGGAGGAGTAG |
| RAD21 R | AGGTCTTCTGGTACAAGCGGTG |

| ChIP-qPCR | Sequence |
|---|---|
| 11p13 F | TCCTTCCAGGTTTTGGCTCC |
| 11p13 R | GCCCCAGATCAGGAGAGAGA |
| -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 -2 kb F | TTGAACAATTTTCTGGTGGATAAGTC |
| Promoter -2 kb R | ATGCACTAATTGCGACATGATATTC |
| Intron 10ab F | TGCTTTATTGAATGGCATTACCTCTA |
| Intron 10ab R | AGATGCTTGTGGTAAGGGAGGAG |
| +6.8 kb F | TCTTCTTTCCCATTCACCTTTGTC |
| +6.8 kb R | TTTTGGTTTCATTTATCAGCACATC |
| +48.9 F | GGCATCAGCCAGTCAAGGTT |
| +48.9 R | AGCAGAGGGCAAAGTGGTACTT |

| Luciferase Construct mutagenesis | Sequence |
|---|---|
| 5' Mutagenesis | AGTTACAAAGGGCTAGCCACTTGCTTCAA taagcttCTAATTCTGTGGCGCAATTTGTGCTCCAG |
| 3' Mutagenesis | CCACATTCTTGCCTCTCCTGACCTAT gagctcaagTTCACTTTTCTTCTTTTGAGAGT |

## Table S2.

Primer sequences for the 4C-seq libraries, CRISPR HDR guide sequence, template, and clone screening primers.

| 4C Viewpoints | Primary Enzyme | Secondary Enzyme | | Sequence |
|---|---|---|---|---|
| CFTR Promoter | NlaIII | Csp6I | Reading | TACACGACGCTCTTCCGATCT GCACTTACTATATGCAGGCATG |
| | | | Nonreading | ACTGGAGTTCAGACGTGTGCT CTTCCGATCTTGAAGTGTTCTT TGGATATTGC |
| -80.1kb | NlaIII | Csp6I | Reading | TACACGACGCTCTTCCGATCT ACTGAGAACTTACAGGGCAGTC |
| | | | Nonreading | ACTGGAGTTCAGACGTGTGCT CTTCCGATCTCTGGTAGCTTTT GGTTGAATG |
| -20.9kb | NlaIII | DpnII | Reading | TACACGACGCTCTTCCGATCT TTAACAAAGTTTAGGTAAATG ACCA |
| | | | Nonreading | ACTGGAGTTCAGACGTGTGCT CTTCCGATCTCAAAGTGAGCT ATTTTGTTTTCTC |
| +48.9kb | NlaIII | DpnII | Reading | TACACGACGCTCTTCCGATCT GAGTGAGCTTGAAAGCCATG |
| | | | Nonreading | ACTGGAGTTCAGACGTGTGCT CTTCCGATCTTGGAACATCGT CAGTGGAAG |
| Chr11.2516 CRE | NlaIII | DpnII | Reading | TACACGACGCTCTTCCGATCT CTCCCCAAATTAGCACCATG |
| | | | Nonreading | ACTGGAGTTCAGACGTGTGCT CTTCCGATCTAGGCAGCCTTC TTGCTTTCT |
| APIP Intron 4 | NlaIII | DpnII | Reading | TACACGACGCTCTTCCGATCT CCCTCTATAAATAGCCTGAAG ACATG |
| | | | Nonreading | ACTGGAGTTCAGACGTGTGCT CTTCCGATCTAACCCTTGAGA AATTTAGATGGT |

| CRISPR Sequences | Sequence |
|---|---|
| HDR Guide | GTACTCTCAAAAGAAGAAAAGTGA |
| HDR Template | TAATTCTGTGGCGCAATTTGTGCTCCAGAGCATTCCTGTGGGATCA AACTGAAACTAGTCTCTAACTGAGCCACATTCTTGCCTCTCCTGAC CTATgagctcaagTTCACTTTTCTTCTTTTGAGAGTACAGCCCAGTAAAT CACTTGGACAAGAAAACTCATCTCATGCTTTCCCAGGAAACCTGAC TTAAACCAGACC |
| HDR Screening F | CTGTATTGTACTACCCATTTACGTGAG |
| HDR Screening R | GTAGGGAAAGCCTACACTCTCA |

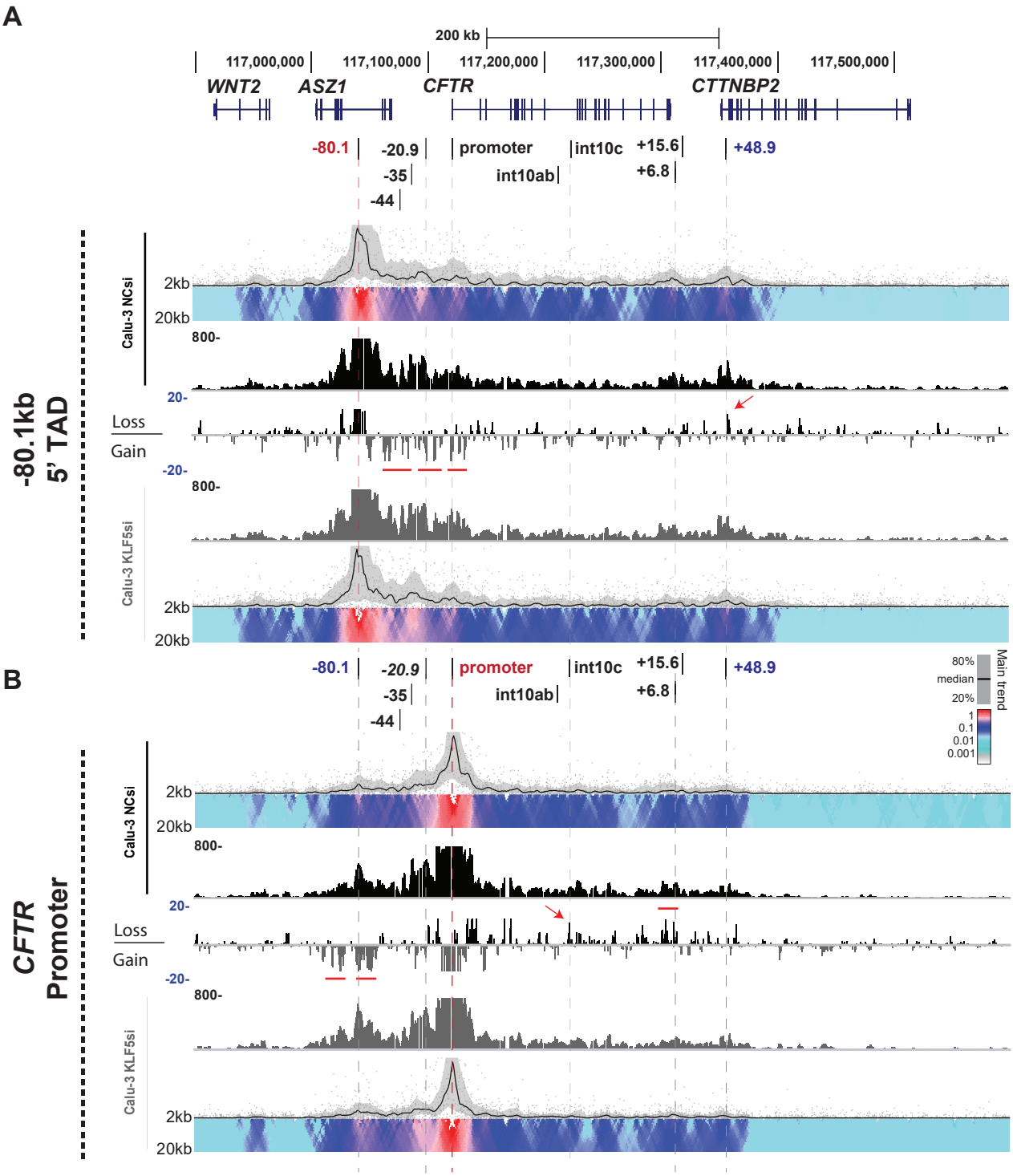


**Figure S1. KLF5 depletion alters the 3D structure of the CFTR locus in Calu-3 cells**

As described in Fig.1, but here showing viewpoints at A) the -80.1kb 5' TAD boundary, and B) the *CFTR* promoter. 4C-seq data for Calu-3 treated with either negative control siRNA or siRNA targeting KLF5. Domainograms for relative read enrichment of each condition are shown together with the read quantification track. Between each condition, the comparison shows the subtraction of KLF5si results from NCsi, calculating loss or gain of looping interaction.

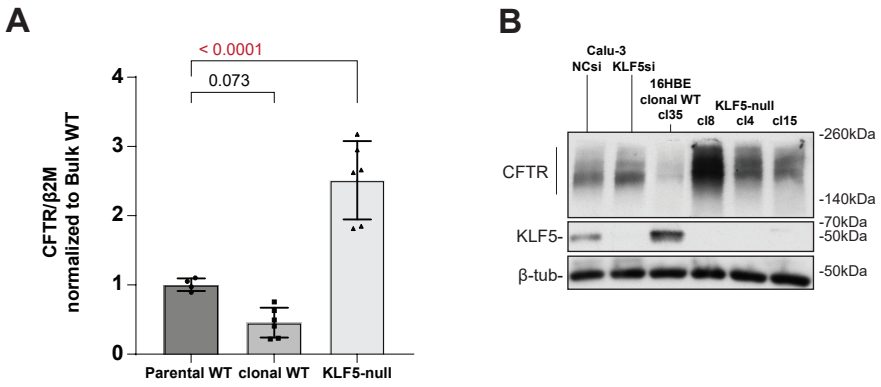


**Figure S2. CFTR expression in KLF5-null 16HBE14o$^-$ cells**

A. RT-qPCR for CFTR in parental WT 16HBE14o$^-$, clonal WT cells generated in the CRISPR experiment (n=2), and KLF5-null lines (n=3). cDNA was prepared from four passages of RNA for the bulk WT, three passages of each of the clonal WT, and two passages of KLF5-null cell lines. B. Western blot probed with antibodies specific for CFTR (CFF-596), KLF5, or β-tubulin as a loading control. Protein samples are from Calu-3 cells treated with negative control siRNA or siRNA targeting KLF5, 16HBE14o$^-$ clonal WT cells, or KLF5-null 16HBE14o$^-$ lines (n=3, as shown).

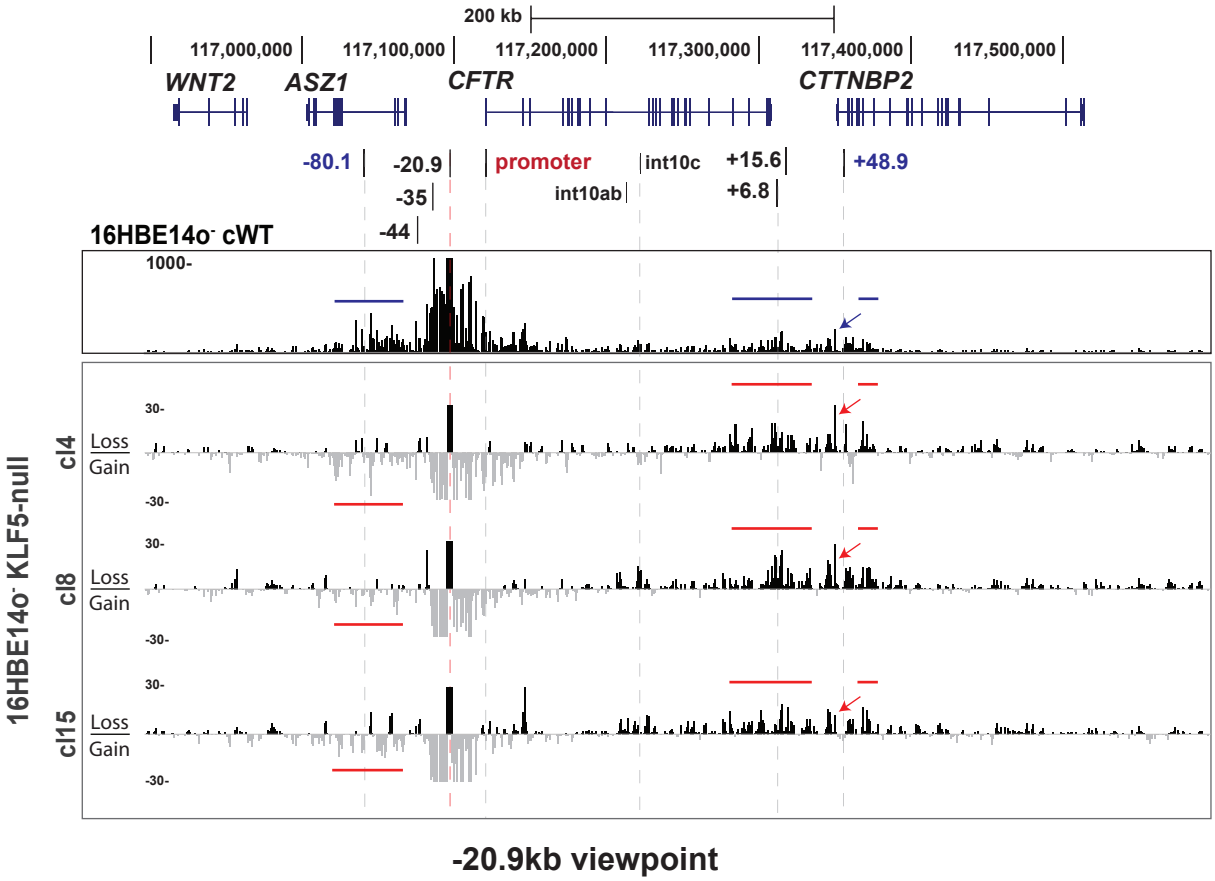


**Figure S3. KLF5-null 16HBE14o$^-$ cells exhibit altered looping interactions with the -20.9kb CRE**

As described in Fig. 2, but here showing a viewpoint at the -20.9kb CRE. 4C-seq data are shown for clonal WT (n=2) or KLF5-null (n=3) 16HBE14o$^-$ clones. The subtraction of the read quantification tracks of each KLF5-null clone 4C-seq from the WT cells in $\log_2$ scale is shown. Regions of specific interest are marked by bars and arrows in blue for the clonal WT and red for the KLF5-null clones.

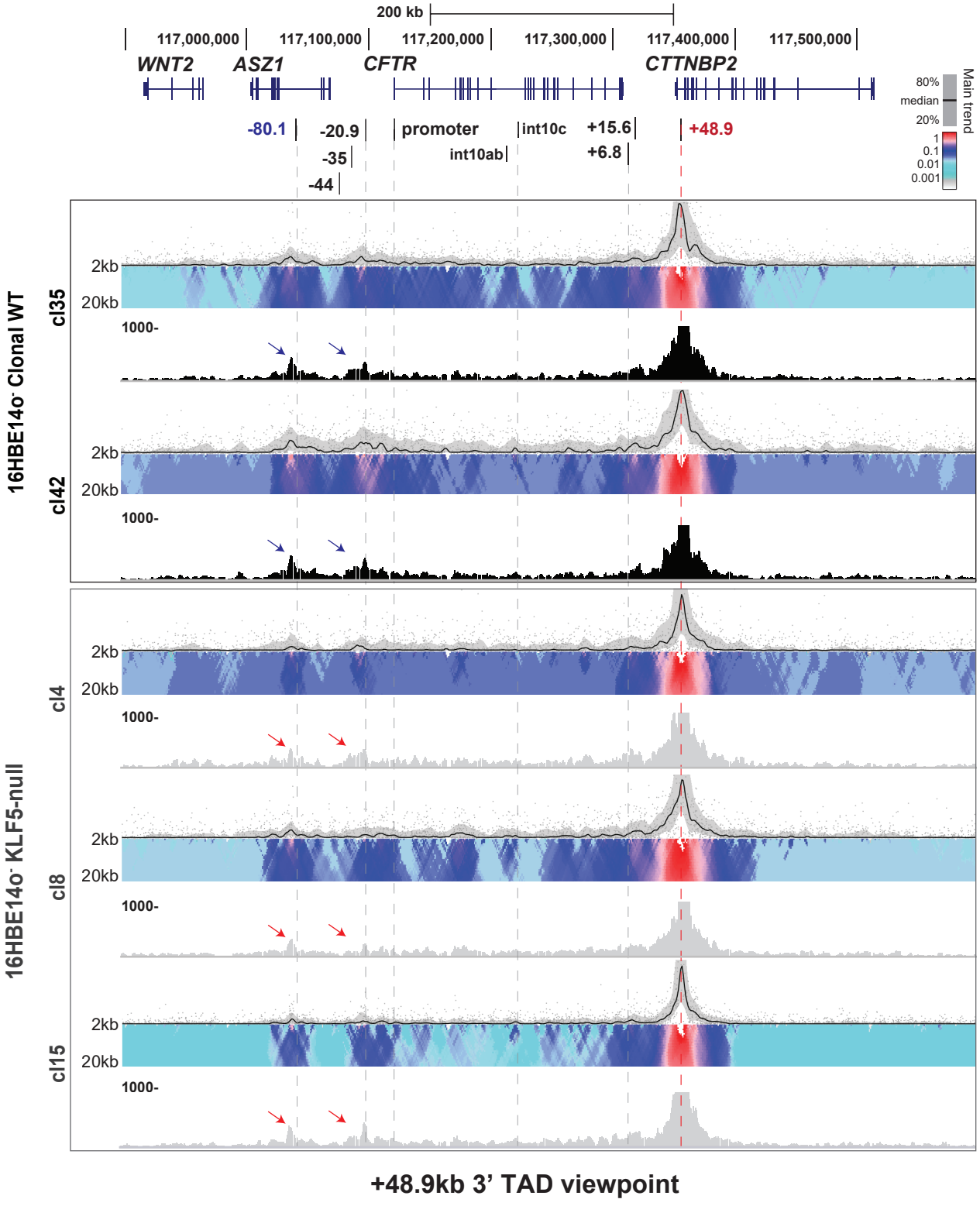


**Figure S4. Changes in 3D structure around the +48.9kb 3' TAD boundary in KLF5-null 16HBE14o- cells**

As described in Fig.2, but here showing viewpoint at the +48.9kb 3' TAD boundary. 4C-seq data are shown for clonal WT (n=2) or KLF5-null (n=3) 16HBE14o- clones. Domainograms for relative read enrichment of each condition are shown together with the read quantification track. Regions of specific interest are marked by bars and arrows in blue for the clonal WT and red for the KLF5-null clones.

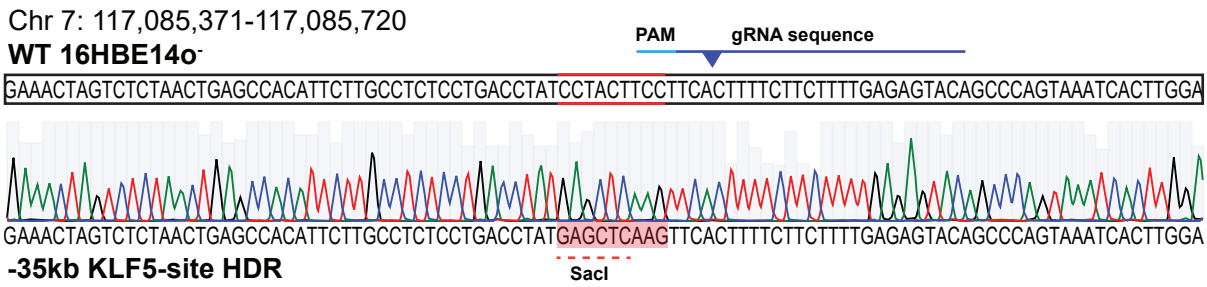


**Figure S5. CRISPR homology directed repair mutagenesis of the KLF5 binding motif at the -35kb CRE**

Sequence within the -35kb CRE upstream of *CFTR* containing the 3' KLF5 binding site underlined in red (RSAT motif similarity *p*-value = 0.01). Mutated region is shown in red, with the introduced SacI site, gRNA sequence, and PAM sequence detailed.

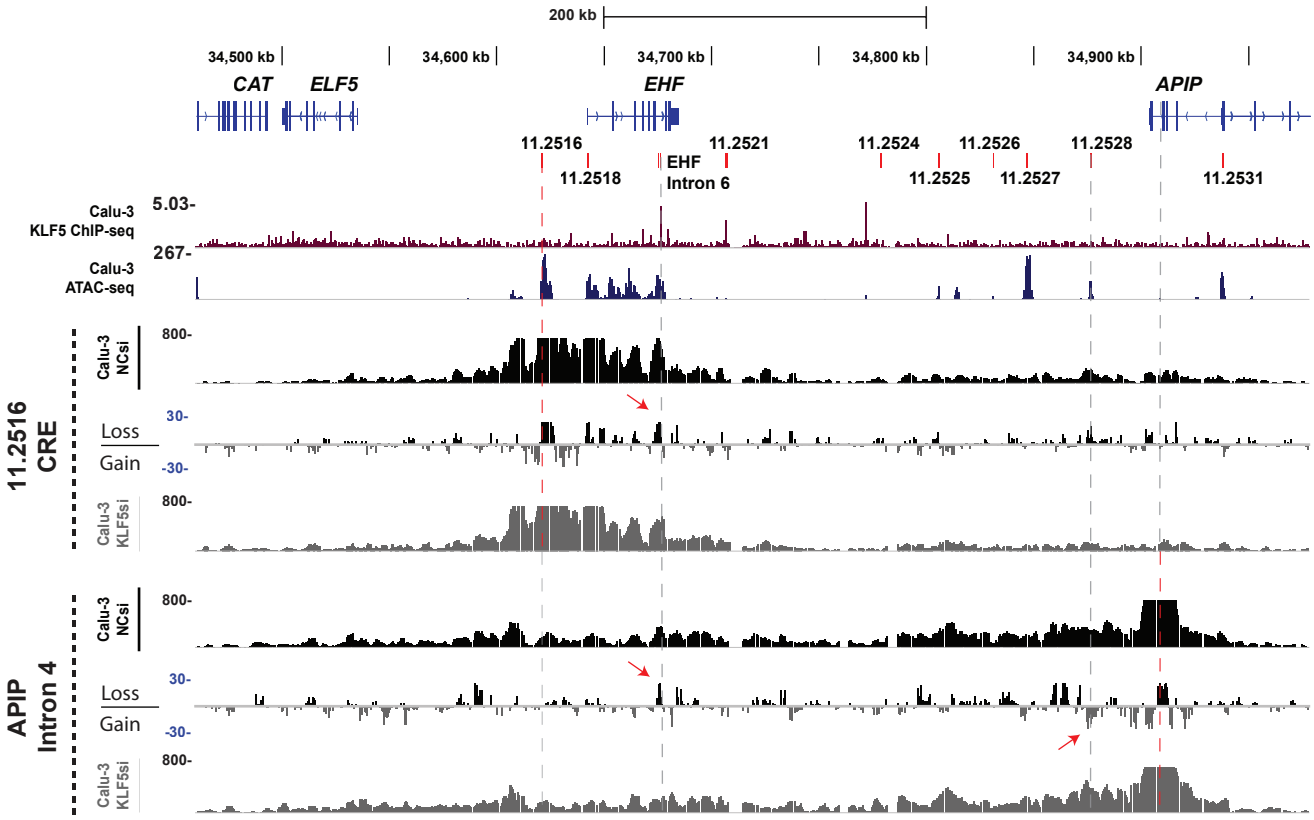


**Figure S6. KLF5 depletion alters the 3D structure of the Chr11p13 locus**

4C-seq data are shown for Calu-3 cells treated with negative control siRNA (NCsi) or siRNA targeting KLF5 (KLF5si). The chr11p13 region, genes contained within the locus, select DNaseI hypersensitive sites (DHS), KLF5 ChIP-seq IDR, and ATAC-seq in Calu-3 cells are shown in order above. 4C-seq data generated using the 11.2516 DHS (A) and APIP Intron 4 (B) viewpoints are shown. Read quantification tracks in the IGV genome browser are shown for NCsi or above for KLF5si. Between the conditions are read tracks of the subtraction of the KLF5si 4C-seq from the NCsi cells in $\log_2$ scale. Sites of interest and labelled with red arrows.