## Supplementary Figure Legends

### SUPPLEMENTARY FIGURE 1

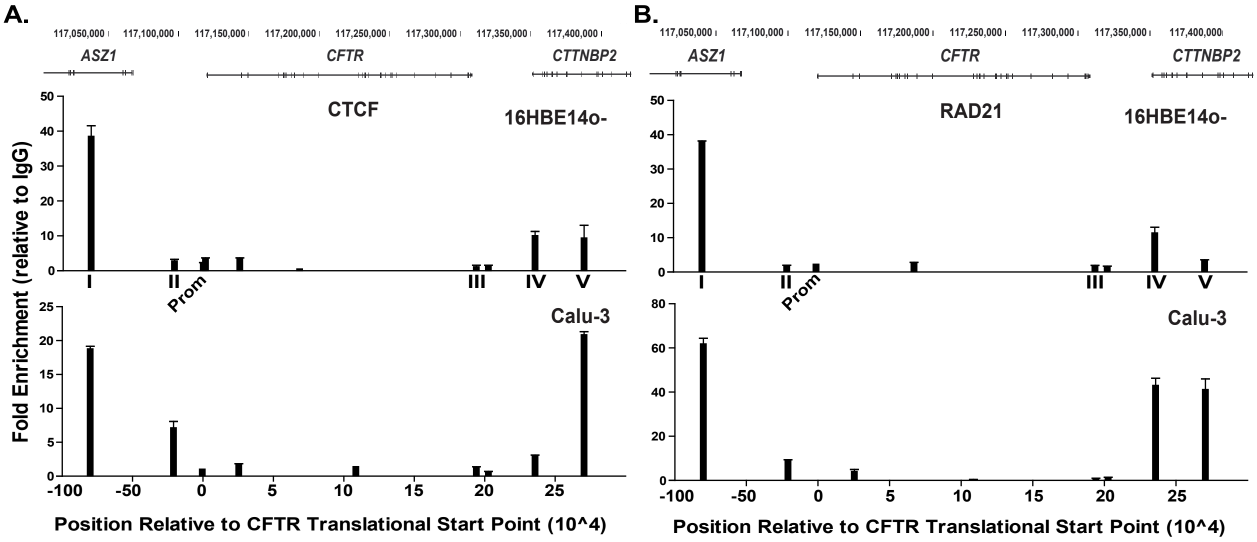
***CTCF and RAD21 occupancy across CFTR in airway cell lines.*** A, B. ChIP for CTCF (A) or RAD21 (B) was performed in each cell type indicated. Data are presented as fold enrichment over IgG. n≥2 for each cell line and representative data are shown.

### SUPPLEMENTARY FIGURE 2

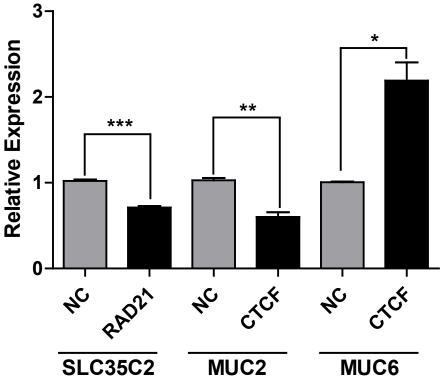
***Altered expression of other genes after CTCF or RAD21 loss.*** Changes in gene expression of *SLC35C2*, *MUC2*, *MUC6* measured by RT-qPCR after treatment with NC siRNA (grey) and either RAD21 or CTCF targeting siRNAs (black). *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ as determined by an unpaired, two-tailed Student's t-test.

### SUPPLEMENTARY FIGURE 3

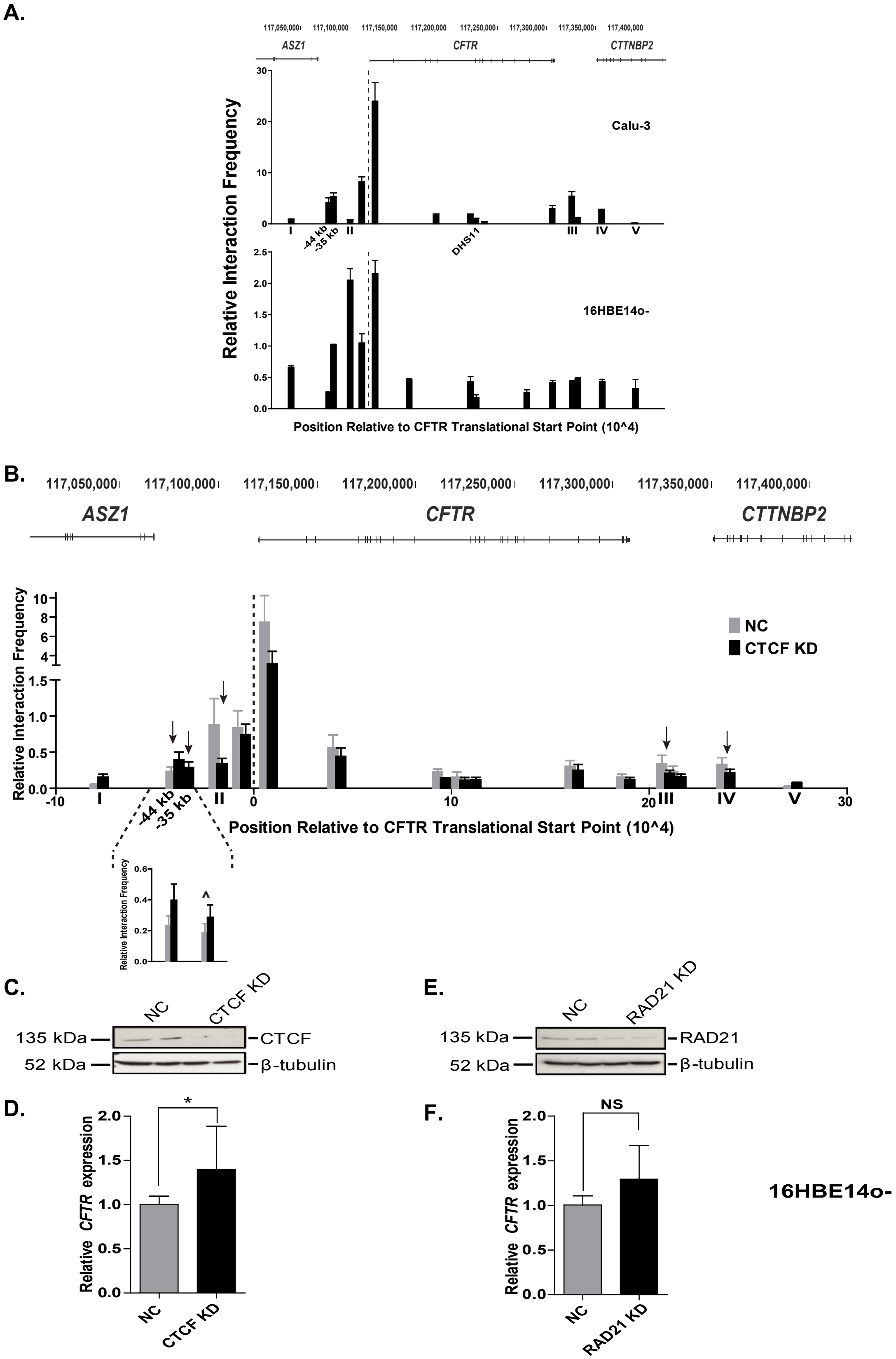
***Role of architectural proteins in airway cell lines.*** A. q3C interactions in Calu-3 and 16HBE14o- cell lines. Details as described in Fig. 2 legend. B. q3C interactions in 16HBE14o- treated with a NC siRNA (grey) or with a CTCF targeting siRNA (black). Arrows mark sites of interest. A graph below expands interactions at the airway-selective DHS-35 and -44 kb sites that show altered interactions with the promoter after CTCF depletion. Data shown have an n=3, ^ marks statistically significant change. Experimental details as in Fig. 2 legend. C, E. Western blots show siRNA mediated depletion of CTCF (C) or RAD21 (E) in 16HBE14o- cells with β-tubulin as the loading control. D, F. RT-qPCR for *CFTR* expression after knockdown of CTCF (D) or RAD21 (F) (black) compared to NC siRNA treated cells (grey). * $p<0.05$ as determined by an unpaired, two-tailed Student's t-test.



SUPPLEMENTARY FIGURE 1



SUPPLEMENTARY FIGURE 2



SUPPLEMENTARY FIGURE 3

## Supplementary Table 1

| **RT-qPCR Primers** | **Sequences** |
|---|---|
| 18s rRNA F | CGGCTACCACATCCAAGGAA |
| 18s rRNA R | GCTGGAATTACCGCGGCT |
| 18s rRNA probe | FAM-TGCTGGCACCAGACTTGCCCTC-TAMRA |
| CFTR exon 5/6a F | AGCTGTCAAGCCGTGTTCTAGATA |
| CFTR exon 5/6a R | ATGAGGAGTGCCACTTGCAAA! |
| CFTR exon 5/6a probe | FAM-CACACGAAATGTGCCAATGCAAGTCCTT-TAMRA |
| **ChIP-qPCR Primer Sets** | **Sequences** |
| -80.1 kb F | GGGCATTCAAAGAAAAGCAGAAAGC |
| -80.1 kb R | ACCCCAGTACAGAGACGTGACA |
| -20.9 kb F | CCGGGATGTTGTTTGAAGCTT |
| -20.9 kb R | TTTAAATAGTTGAATAGAGGACGAGATACTTT |
| Promoter F | GTTCTCCCGCCGGTGG |
| Promoter R | CAGTCGCGGCCTCTCTTTAG |
| Intron 1 CTCF F | GCAGTTAATCCTGGAACTCCGGTGC |
| Intron 1 CTCF R | AAGTCCTTCTCTCTCATCCACAGGG |
| Intron 1 DHS F | TCATTGTCAACTGTCAGGTAGCAA |
| Intron 1 DHS R | CAGAGTTAGGATTCCAGCCAGG |
| Intron 2 CTCF F | TGGTGGCATTTGCCTTATGCTGGT |
| Intron 2 CTCF R | AGGGCTCCTTCTTTCGTGGTCCT |
| Intron 8 F (NC)[1] | GCTTCCTCCTGATCAATCTTTAGG |
| Intron 8 R (NC)[1] | AAAAATTCCTTGCCTCACTATTGC |
| Intron 10a,b DHS F | TGCTTTATTGAATGGCATTACCTCTA |
| Intron 10a,b DHS R | AGATGCTTGTGGTAAGGGAGGAG |
| Intron 10c DHS F | GGAGAGTACTGTCTCTTATCAGCCATCT |
| Intron 10c DHS R | CTGGTCTTTTCAACACTTTGAGTCA |
| Intron 11 DHS F | TCCAAAAGCTGAGACAGGAAACT |
| Intron 11 DHS R | ATTACATACACACAAAAGTACACACATGACT |
| Intron 14a F | TGAGCCAGAAGTGGCAACCT |
| Intron 14a R | GCTGCAGACCTTCCCAGTGA |
| +6.8 kb DHS F | TCTTCTTTCCCATTCACCTTTGTC |
| +6.8 kb DHS R | TTTTGGTTTCATTTATCAGCACATC |
| +15.6 kb DHS F | ATCCATTTTCTTCAAGTCTCTCTCCAT |
| +15.6 kb DHS R | GGAATGAGGATTGTTTATGATTTGG |
| +48.9 kb F | GGCATCAGCCAGTCAAGGTT |
| +48.9 kb R | AGCAGAGGGCAAAGTGGTACTT |
| +83.7 kb F | CCGGGTCGAACATGCAAAGC |
| +83.7 kb R | AGCCCTCTGCTGGAAGCGTG |
| **q3C Primer Sets** | |
| Ercc3 F | CCCTGAGGTGAGTTTGTGGAAT |
| Ercc3 R | AGGATCTCTGTTTAATGGAAAAGCTT |
| Ercc3 Probe | FAM-CAGAGTCGGATCACGCCTTCATCCTT-TAMRA |
| Promoter Probe | FAM-CTCCGACACGCAAAGGAAGCGCT-TAMRA |
| Promoter R | GCAGTGTGGGTCTGATGCAT |
| -20.9kb Probe | FAM-CTTGTATTCATTAGGTGACCTGA-NFQ MGB |
| -20.9 kb R | CCAAATACTGGACAAAAATGAACTTC |

| | |
|---|---|
| -80.1 kb | GAGACTAACAAAAGGTTCTGTATCA |
| -41.7 kb | TGGACCTGGGTACATCTGTC |
| -36.8 kb | TGTTATGTGCTGGTCGAAGA |
| -20.9 kb | CCAAATACTGGACAAAAATGAACTTC |
| -3 kb | TGCCTGGCACAGAGATGGA |
| Promoter | GCAGTGTGGGTCTGATGCAT |
| S2 | CATTGTGTCAGCAAAGAGCTTAGG |
| S3 | GGTTTAAATATGTGCAAGGAGGATGTC |
| Int3 | TTTTCCCTAGCCTGTCAATTGC |
| Int8 | GCTTCCTCCTGATCAATCTTTAGG |
| Int10a,b | ACCCTATATCATTGCCCTTTGTATG |
| Int10c | AAGCATTTTCTCCTTTTCCTCAAC |
| Int11 | TGACTCTGATGTCAAATGTTTCTCAA |
| Int14a | TGAGCCAGAAGTGGCAACCT |
| Int18 | GCTTGCTGACCCTTCTTCAG |
| Int19 | GGGAGGAGGTGCATTGAAGTTA |
| Int20(2) | GCGGTTCATAAAGGGTCATA |
| Int21 | TGTTGACTTTTCTCTAATGAAGATG |
| Ex24 | GTGTTGGAGAAGAACTGAAATCATACTT |
| 3'Ins | CTGTGGTTTGAATGTGTTCCCTAA |
| 3'+20kb | CTGCTACAAGCCCACTATACAATATTG |
| +48.9 kb | AGTGCCGCTAGGTGATCAGATAA |
| +83.7 kb | GCTGGAAGCGTGGAGTA |

[1] NC – Negative control ChIP site

## Supplementary Table 2

| **q3C Statistics RAD21 Knockdown (Fig. 2C,D)** | **p-values** |
|---|---|
| -80.1 kb (I) | p=0.0195 |
| -20.9 kb (II) | p=0.0206 |
| DHS 10c | p=0.0059 |
| DHS 11 | p=0.0137 |
| 3'Insulator (+6.8 kb and +15.6 kb, III) | p=0.0208 |
| +48.9 kb (IV) | p=0.0207 |
| **q3C Statistics CTCF Knockdown -20.9 kb Bait (Fig. 2B)** | |
| +48.9 kb (IV) | p=0.0495 |
| **q3C Statistics FOXA1/A2 Knockdown (Fig. 2F)** | |
| -36.8 kb | p=0.0448 |
| DHS 10a,b | p=0.0089 |
| DHS 11 | p=0.002 |
| **q3C Statistics CTCF/RAD21 Knockdown Prom Bait (Fig. 3A)** | **p-values** |
| -80.1 kb | p=0.0082 |
| -20.9 kb | p=0.0125 |
| DHS 10a,b | p=0.0370 |
| DHS 10c | p=0.0388 |
| Int14a | p=0.0408 |
| Int19 | p=0.0448 |
| +48.9 kb (IV) | p=0.0315 |
| +83.7 kb (V) | p=0.0151 |
| **q3C Statistics CTCF/RAD21 Knockdown -20.9 kb Bait (Fig. 3B)** | **p-values** |
| Int14a | p=0.0224 |
| Int19 | p=0.0191 |
| +48.9 kb (IV) | p=0.0417 |
| **q3C Statistics CTCF Knockdown 16HBE14o- (Fig. S4B)** | |
| -35 kb | p=0.0132 |

| **H3K27ac Statistics (Fig. 5C)** | **p-values** |
|---|---|
| -80.1 kb (I) | 0.0381 |
| -2 kb promoter | 0.025 |
| DHS 1 | 0.004 |
| DHS 10a,b | 0.0058 |
| DHS 11 | 0.0451 |