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Supplemental Material to:

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A balance between activating and repressive histone modifications regulates cystic fibrosis transmembrane conductance regulator (CFTR) expression in vivo

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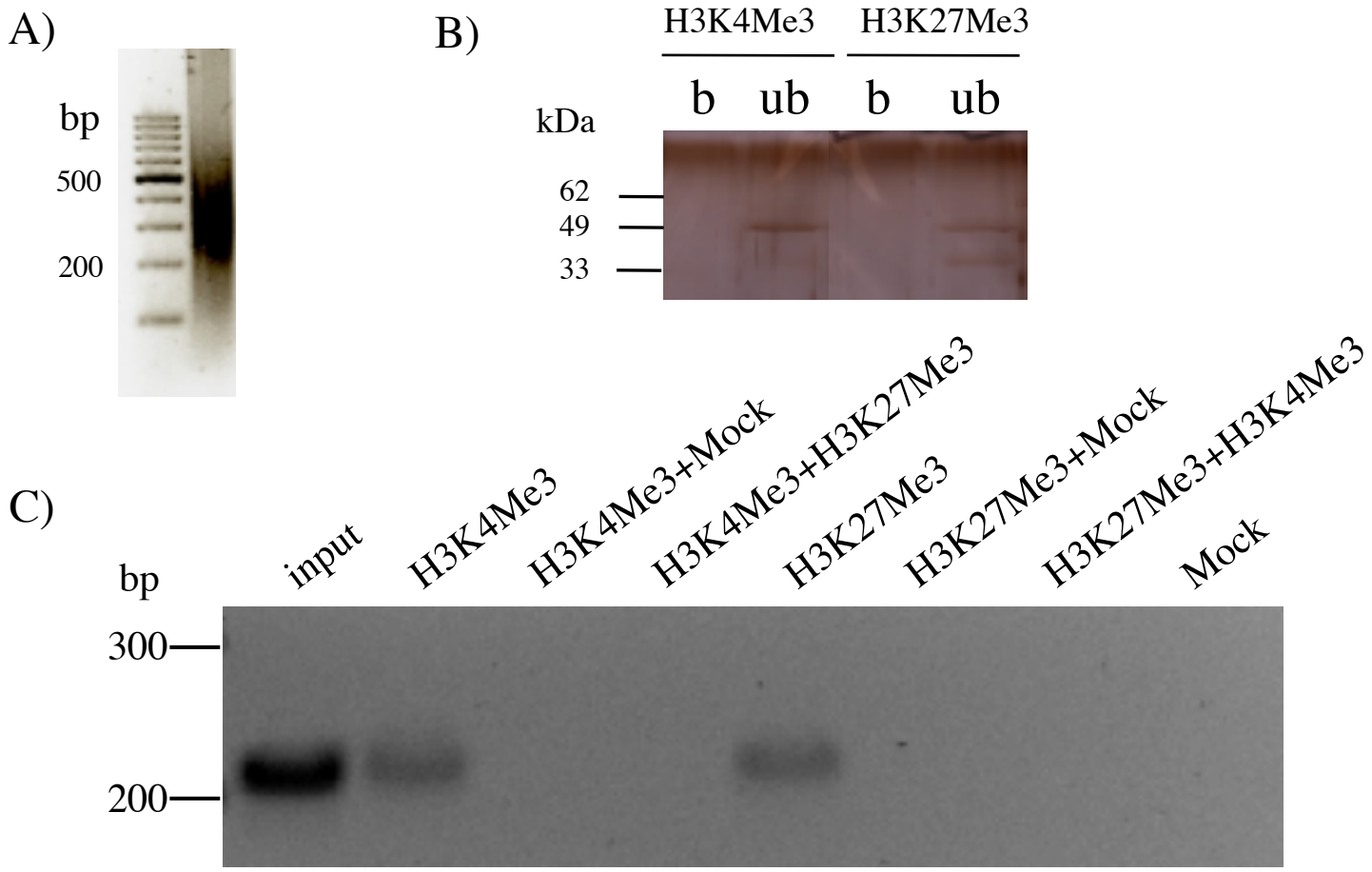
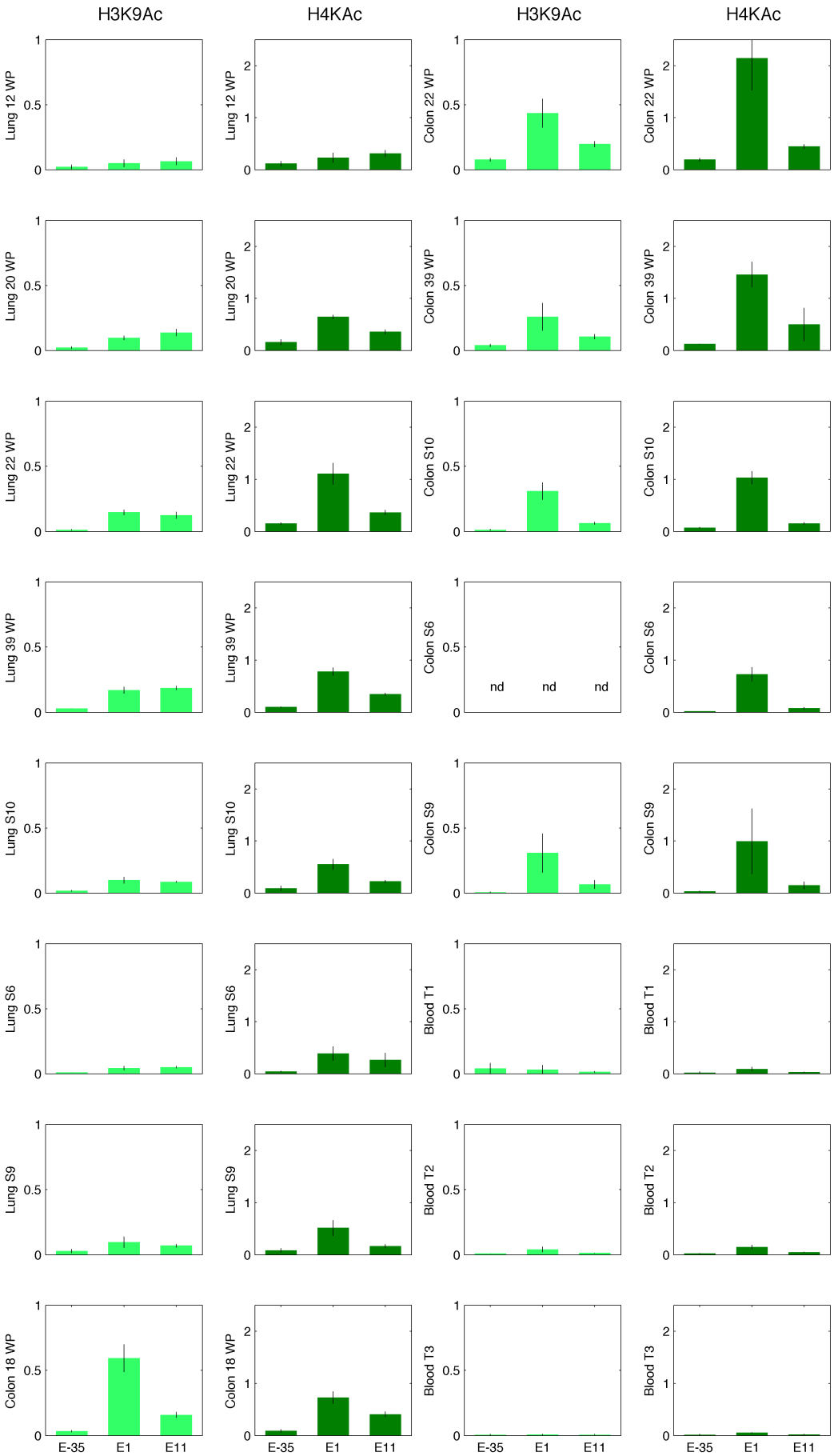
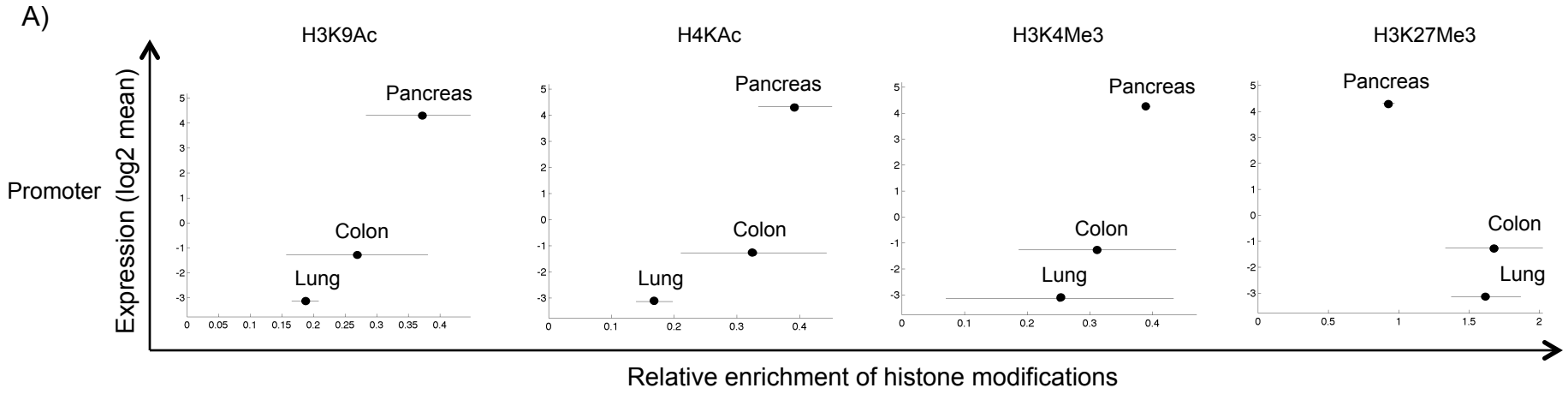
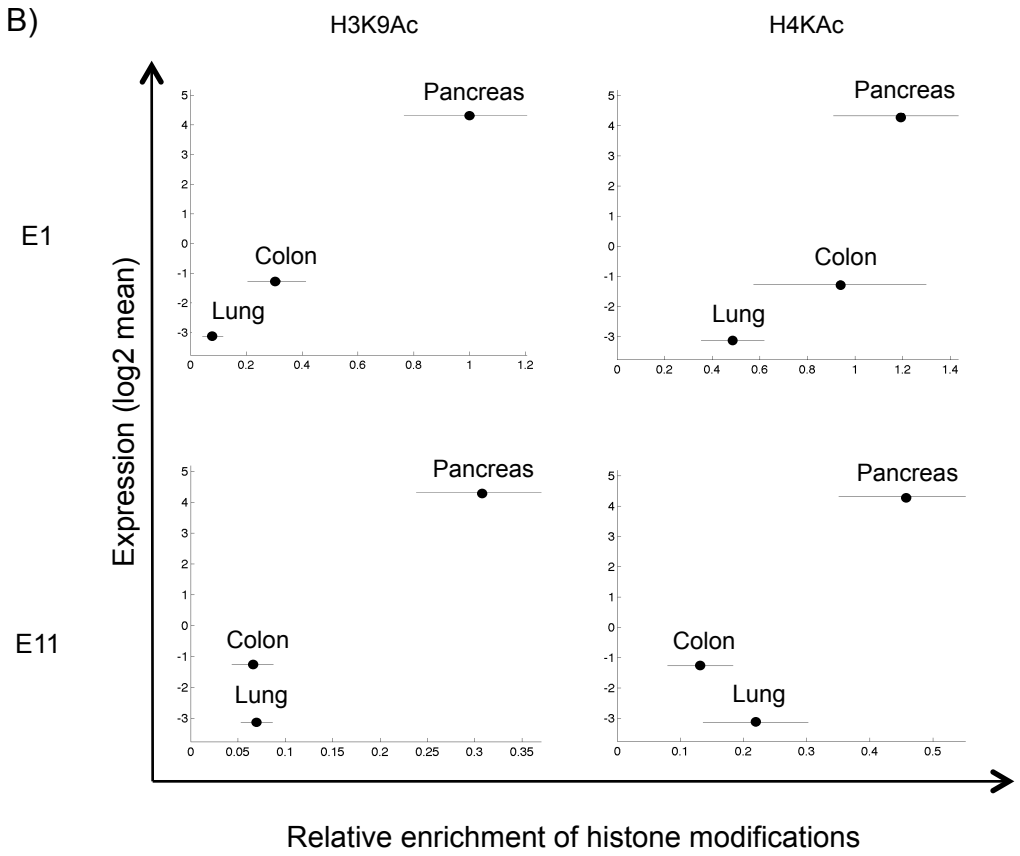


Supplementary Figure 1. Sequential ChIP on adult lung. A) Sonicated chromatin. B) Antibodies directed against either $H3K4Me_3$ (left side) or $H3K27Me_3$ (right side) were crosslinked to agarose beads. The crosslink stability was tested with 0.1M NaHCO3 and 1% SDS (silver-stained acrylamide gel): no antibody was released from the bound (b) fraction. The ub fraction was the exceeding unbound antibody. C) Adult lung chromatin was immunoprecipitated with a first antibody directed against $H3K4Me_3$ followed by a second antibody directed against $H3K27Me_3$. A reciprocal procedure was done in parallel. Primers specific to the promoter of *CFTR* were used to detect DNA in input chromatin, chromatin precipitated by one antibody, chromatin precipitated by one antibody followed by a mock antibody, and chromatin precipitated by two consecutive antibodies. No antibody was added in Mock control.



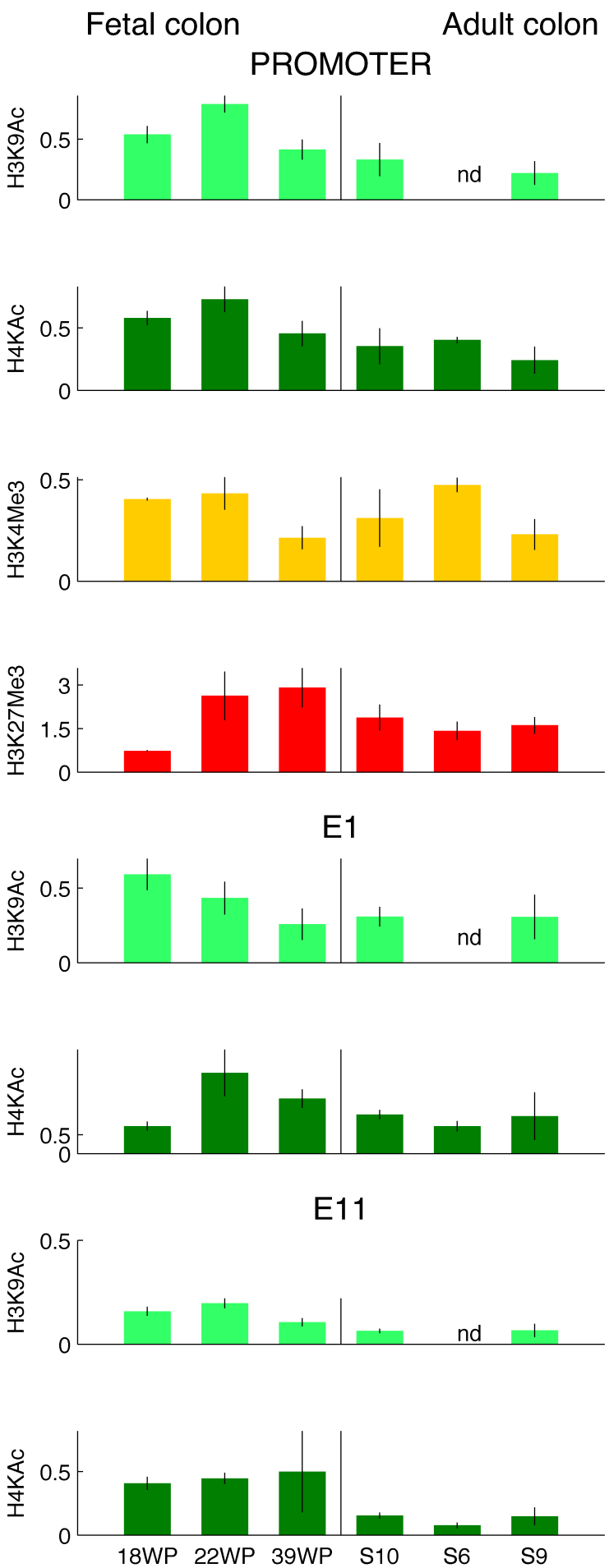
Supplementary Figure 2. Relative enrichment of histone acetylation in three enhancers (E-35, E1, and E11) of the *CFTR* gene. In fetal tissues, WP is weeks of pregnancy.





Supplementary Figure 3.

Correlation between levels of expression and relative enrichment of various histone modifications in the promoter (A) and in two intronic enhancers of *CFTR*, E1 and E11 (B). Expression was measured by real-time RT-PCR in a pool of mRNAs from several individuals. Relative enrichment of histone modifications was measured by ChIP. The figure displays the mean (filled circle) and standard deviation (horizontal line) of data obtained from three individuals.



Supplementary Figure 4.

Histone modifications in fetal and adult colon samples. The promoter (top lines) and two enhancers of *CFTR*, E1 (middle lines) and E11 (bottom lines), were analyzed. Bars represent relative DNA enrichment (means ± sd calculated on ≥3 independent replicates). The amount of immunoprecipitated DNA was expressed relative to the amount of histone modifications either in *GAPDH* (H3K9Ac, H4KAc, H3K4Me3) or in the STS JB10 (H3K27Me3) as previously described.[30,32] On the x-axis, adult tissues are indicated by the donor code, fetal tissues by weeks of pregnancy.

Figure Legends

Figure S1. Sequential ChIP on adult lung. A) Sonicated chromatin. B) Antibodies directed against either H3K4me3 (left side) or H3K27me3 (right side) were cross-linked to agarose beads. The crosslink stability was tested with 0.1 M NaHCO3 and 1% SDS (silver-stained acrylamide gel): no antibody was released from the bound (b) fractions. The ub fraction was the exceeding unbound antibody. C) Adult lung chromatin was immunoprecipitated with a first antibody directed against H3K4me3 followed by a second antibody directed against H3K27me3. A reciprocal procedure was done in parallel. Primers specific to the promoter of *CFTR* were used to detect DNA in input chromatin, chromatin precipitated by one antibody, chromatin precipitated by one antibody followed by a mock antibody, and chromatin precipitated by two consecutive antibodies. No antibody was added in Mock control.

Figure S2. Relative enrichment of histone acetylation in three enhancers (E-35, E1, and E11) of the *CFTR* gene. In fetal tissues, WP is weeks of pregnancy.

Figure S3. Correlation between levels of expression and relative enrichment of various histone modifications in the promoter and in two intronic enhancers, E1 and E11, of *CFTR*. Expression was measured by real-time RT-PCR in a pool of mRNAs from several individuals. Relative enrichment of histone modifications was measured by ChIP. The figure displays the mean (filled circle) and standard deviation (horizontal line) of data obtained from three individuals.

Figure S4. Histone modifications in fetal and adult colon samples. The promoter (top lines) and two enhancers of *CFTR,* E1 (middle lines) and E11 (bottom lines), were analyzed. Bars represent relative DNA enrichment (means $\pm$ sd calculated on $\geq$3 independent replicates). The amount of immunoprecipitated DNA was expressed relative to the amount of histone modifications either in *GAPDH* (H3K9Ac, H4KAc, H3K4me3) or in the STS JB10 (H3K27me3) as previously described.[30,32] On the x-axis, adult tissues are indicated by the donor code, fetal tissues by weeks of pregnancy.

Supplementary Table 1: Correlation between DNA methylation measured by pyrosequencing in *CFTR* promoter and donor age (Spearman's test).

Tissue	Nb of samples	Rs coefficient	P-value
Nasal cells	12	-0.41	0.19
Colon	16	-0.36	0.18
Blood	12	0.41	0.18

Supplementary Table 2: Clinical features of fetuses

Code	Weeks of pregnancy	Sex
F4	12	M
F2	18	F
F1	20	M
F5	22	M
F3	36	F
F6	39	M

Supplementary Table 3: List of Primers

Gene	Forward	Reverse	Tm (°C)	Amplicon size (bp)
Real-Time PCR on cDNAs				
CFTR	5'-GGAGAAGGAAGAGTTGGTATTAT-3'	5'-TATGGTTTGGTTGACTTGGTAG-3'	60	173
β-actin	5'-ACTGGAACGGTGAAGGTGAC-3'	5'-AGAGAAGTGGGGTGGCTTTT-3'	60	169
Chromatin Immunoprecipitation				
CFTR/Promoter	5'-CAGGCACCCAGAGTAGT-3'	5'-CCAAACCCAACCCATACACA-3'	60	212
CFTR/E1	5'-AATGATAACACTCTTTAGGAG-3'	5'-GGAAAGTCATTCTTCAGTCC-3'	60	240
CFTR/E11	5'-GGAGGAGGTCCCTTAGTAG-3'	5'-GAAACATTTGACATCAGAGTCAC-3'	60	156
CFTR/E-35 (2)	5'-ATCTACCTTACCCTGCTGTCCATT-3'	5'-TCTGAATTATCAGCCCACAGTCA-3'	60	64
JB10	5'-GTTTCCCACCAGCCAAATGAG-3'	5'GGGCAGCACGCAGATACC-3'	60	185
GAPDH	5'-CCATCTCAGTCGTTCCCAAAGTC-3'	5'-GCCAGTCCCAGCCCAAGG-3'	68	294
Pyrosequencing				
CFTR	5'-GTTGTTAATTGGATTTAAAGAGAGG-3'	5'-CTTTCCCCATTCTAACTCCCAACC-3'	57	133
		5'-TAACTCCCAACCTCC-3' (1)		
Bisulfite and Genomic Sequencing				
CFTR	5'-GAAGGAGGGTTTAGGAAGTTTTT-3'	5'-CTTATTCCTTTACCCCAAACCCAACC-3'	58	1000
CFTR-nested	5'-TTGTTAATTGGATTTAAAGAGAGGT-3'	5'- ATTAACCACCTTCTCACCTAAAAAA-3'	57	537
Bisulfite and Next-Generation Sequencing				
CFTR	5'-GTTGTTAATTGGATTTAAAGAGAGG-3'	5'-CTTTCCCCATTCTAACTCCCAACC-3'	56	133

(1) Reverse primer for sequencing
(2) Designed by Zhang *et al.* [12]