SUPPLEMENTARY INFORMATION

For

Elevated miR-155 promotes inflammation in Cystic Fibrosis lung epithelial cells by driving hyper-expression of Interleukin-8

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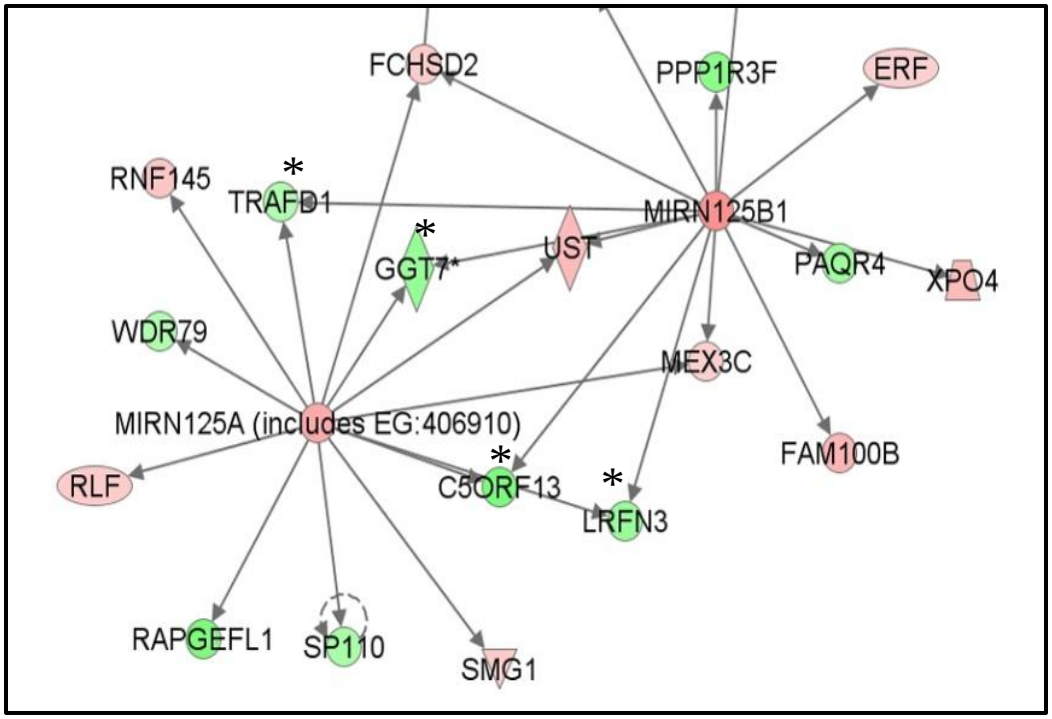
Figure S1**. Comprehensive analyses of CF-dependent gene expression known to be regulated by these CF-specific miRNAs:** The two major genes targeted by multiple miRNAs are ELL2 and Fam125b (Table 2). Relevantly, ELL2 is the elongation factor that can increase the catalytic rate of RNA polymerase II transcription by suppressing transient pausing by the polymerase at multiple sites along the DNA. The down-regulation of ELL2 expression by a miRNA-mediated mechanism emphasizes the functional role of miRNAs in the diseased state. Moreover, Fam125b is a component of the vesicular trafficking process, required for the sorting of endocytic ubiquitinated cargos into multivesicular bodies. TXNIP, a target gene of miR-301a, is a mediator of oxidative stress, by inhibiting thioredoxin activity or by limiting its bioavailability. SOX11, a target gene of miR-31 and miR-328, belongs to a family of transcription factors involved in the determination of cell fate. Moreover, TGF-β signaling is affected by the concerted mis-expression of miRNAs in IB3-1 CF cells. Consistently, pathway analyses of miR-125a and miR-125b identifies a set of four target genes: TRAFD, GGT7, C5ORF13 and LRFN3 (marked with asterisks, supplemental Fig. S1A), down-regulated in the IB3-1 cells, that coordinately affect Toll like Receptor (TLR) and TGFβ signaling pathways. Relevant to the CF disease status, TRAFD has been implicated as a negative regulator of TLR4 signaling, while C5ORF13 is known to regulate TGFβ-signaling pathway. . Additionally, GGT7 and LRFN3 are involved in glutathione metabolism, and protein degradation tagging activity, respectively, both of which are key pathways in CF.

Additionally as summarized in supplemental Fig.S1B miR-27b, which is predicted to target TTP (ZFP36) mRNA, is up-regulated in IB3-1 cells. We also noted that miR-660 is down-regulated in IB3-1 cells compared to IB3-1/S9 CFTR-repaired cells, and the corresponding target IL-8 mRNA is up-regulated. Thus, miR-27b elevates IL-8 mRNA, by potentially destabilizing TTP mRNA, which leads to stabilizing IL-8 mRNA as shown in our earlier work (12). Additionally, over-expression of miR-660 in IB3-1 CF cells causes translational repression of IL-8 expression but does not affect IL-8 mRNA stability (Biswas and Bhattacharyya, personal communication).

Figure S2. **Effect of anti-miR-155 on miR-155 and IL-8 expression.** (**A**) IB3-1 CF cells were transfected with anti-miR-155 (Ambion, Inc.) or with negative control anti-miR. A dose of 150 nM was most effective in down-regulating miR-155. (**B**)The mRNA expression was analyzed by ILLUMINA bead chip arrays. The data was analyzed by IPA. The depicted networks (down-regulated genes are in green symbols and up-regulated genes in red symbols) indicate the attenuation of inflammation. While the expression of the pro-inflammatory genes, IL-8 and IL-6, are down-regulated, expression of the anti-inflammatory gene IL-10 is up-regulated.

Figure S1

A


FCHSD2
PPP1R3F
ERF
RNF145
TRAFD1
*
MIRN125B1
GGT7*
*
UST
PAQR4
XPO4
WDR79
MEX3C
MIRN125A (includes EG:406910)
FAM100B
RLF
C5ORF13
*
*
LRFN3
RAPGEFL1
SP110
SMG1

B

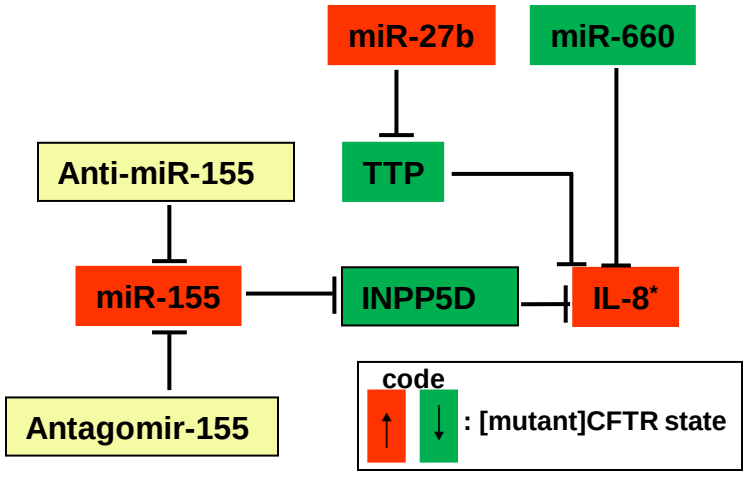
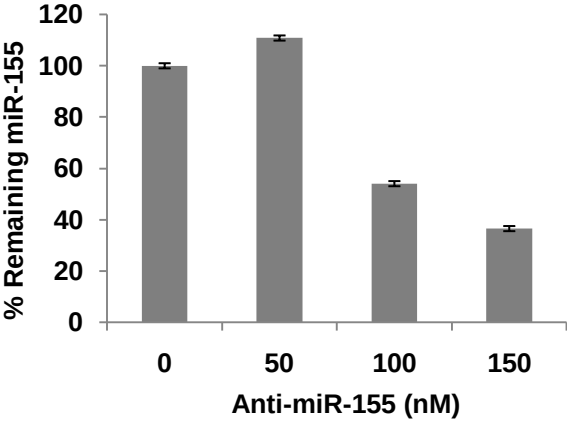
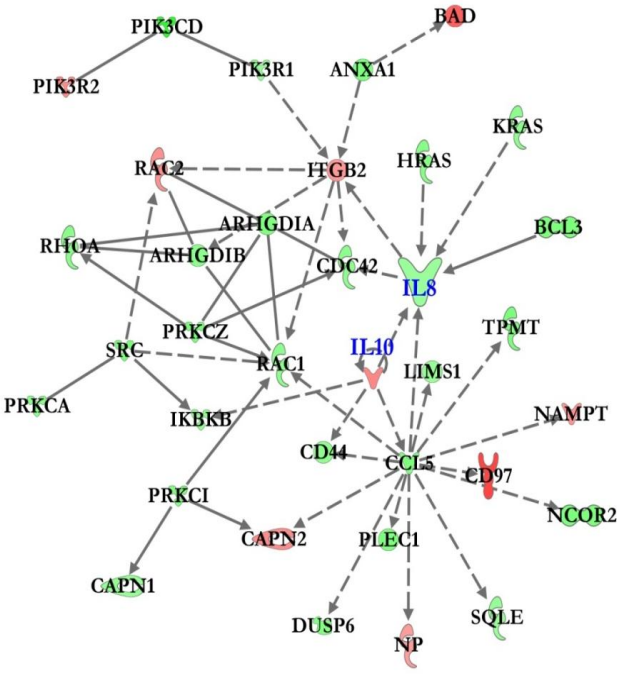

miR-27b
miR-660
Anti-miR-155
TTP
miR-155
INPP5D
IL-8*
Antagomir-155
code
: [mutant]CFTR state

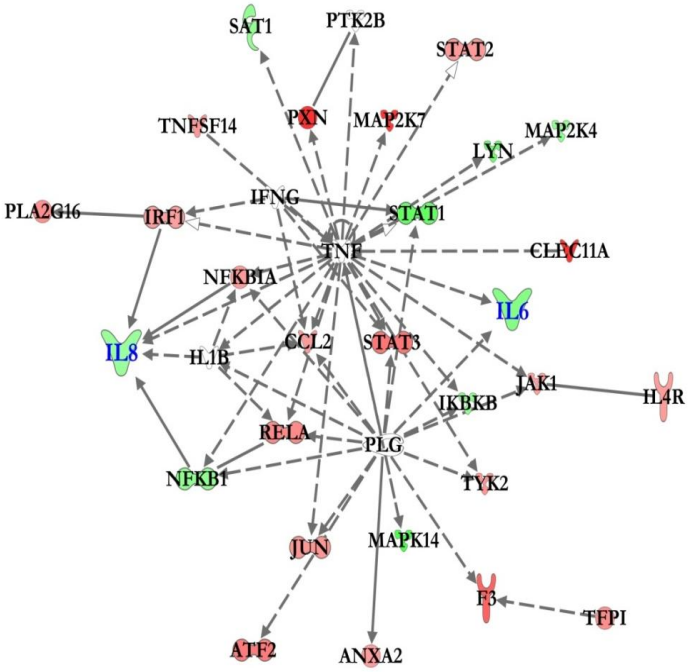
Figure S2

A


% Remaining miR-155
120
100
80
60
40
20
0
0
50
100
150
Anti-miR-155 (nM)

B


PIK3CD
BAD
PIK3R2
PIK3R1
ANXA1
KRAS
RAC2
ITGB2
HRAS
ARHGDIA
BCL3
RHOA
ARHGDIB
CDC42
IL8
PRKCZ
TPMT
SRC
IL10
RAC1
LIMS1
PRKCA
IKBKB
NAMPT
CD44
CCL5
CD97
PRKCI
NCOR2
CAPN2
PLEC1
CAPN1
DUSP6
SQLE
NP


SAT1
PTK2B
STAT2
TNFSF14
PXN
MAP2K7
MAP2K4
LYN
IFNG
PLA2G16
IRF1
STAT1
TNF
CLEC11A
NFKBIA
IL6
IL8
CCL2
STAT3
IL1B
JAK1
IL4R
IKBKB
RELA
PLG
NFKB1
TYK2
JUN
MAPK14
F3
TFPI
ATF2
ANXA2