Type-2 inflammation reduces SARS-CoV-2 replication in the airway epithelium in allergic asthma through the functional alteration of ciliated epithelial cells

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ONLINE DATA SUPPLEMENT

Table E1: List of DEGs comparing samples with SARS-CoV-2 virus and with or without IL-13 stimulation in allergic asthma and healthy children (excel file).

Table E2: List of genes whose expression is positively correlated with viral load and down-regulated by IL-13 stimulation or genes whose expression is negatively correlated with viral load and up-regulated by IL-13 stimulation identified from bulk and 10X data in allergic asthma children (excel file).

Table E3: List of genes whose expression is positively correlated with viral load and down-regulated by IL-13 stimulation or genes whose expression is negatively correlated with viral load and up-regulated by IL-13 stimulation identified from bulk data in healthy children (excel file).

Table E4: Number of cell counts for cell clusters (cell types) for allergic asthma and healthy children from 10X data (excel file).

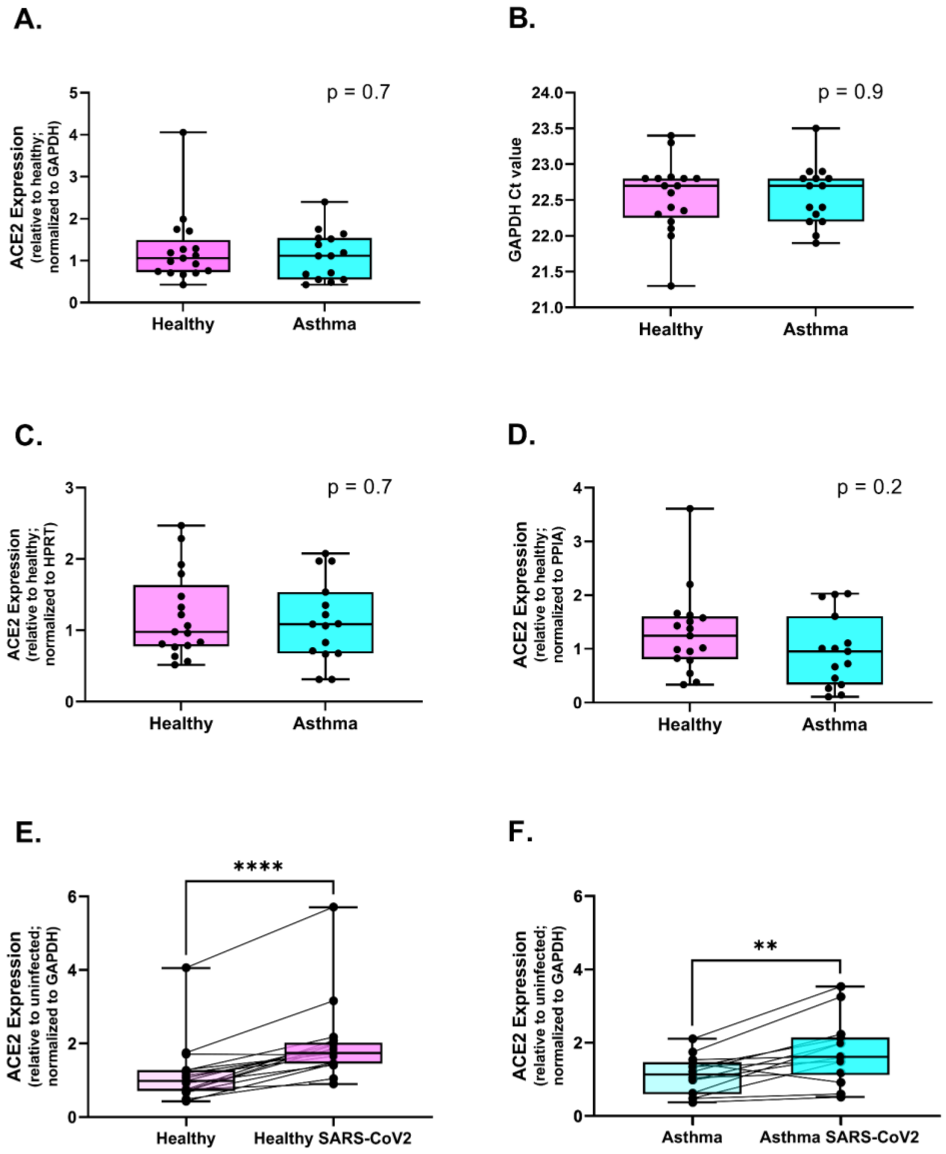

Figure E1: (A) *ACE2* expression relative to healthy AEC cultures, normalized to *GAPDH* expression. (B) Therehold cycle (Ct) values for *GAPDH* expression by AEC cultures from healthy and asthma donors. (D) *ACE2* expression relative to healthy AEC cultures, normalized to *HPRT* expression. (D) *ACE2* expression relative to healthy AEC cultures, normalized to *PPIA* expression. (E) *ACE2* expression in uninfected and SARS-CoV-2 infected healthy AEC cultures. (F) *ACE2* expression in uninfected and SARS-CoV-2 infected asthma AEC cultures. **p<0.001 (paired t-test); ****p<0.0001 (Wilcoxon matched-pairs signed rank test).

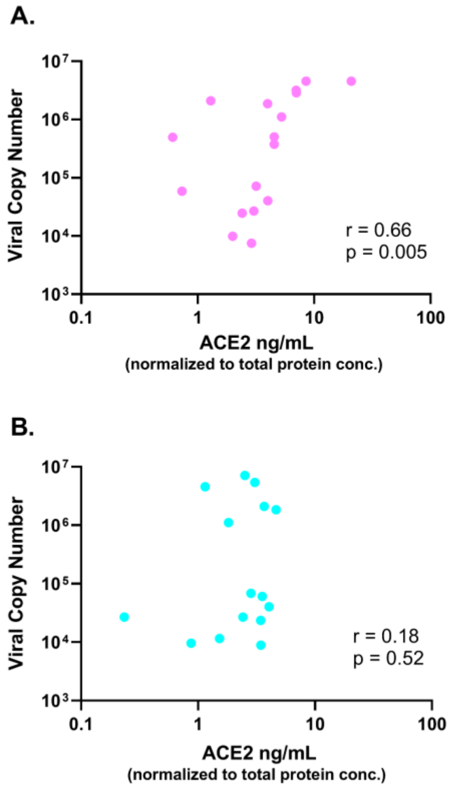

Figure E2: Plot showing correlation between SARS-CoV-2 viral copy number in infected cultures and ACE2 protein abundance in parallel uninfected cultures from healthy controls (A) and allergic asthma (B). Spearman r and p values are shown on the plots.

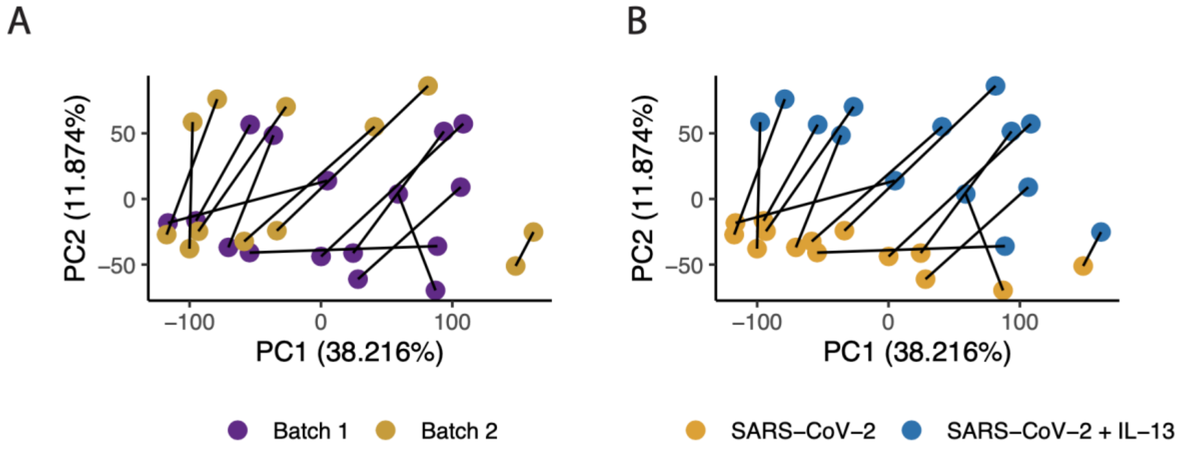

Figure E3: Distribution of samples on PCA by batch (A**)** and condition (B). Samples from the same donor are connected by lines.

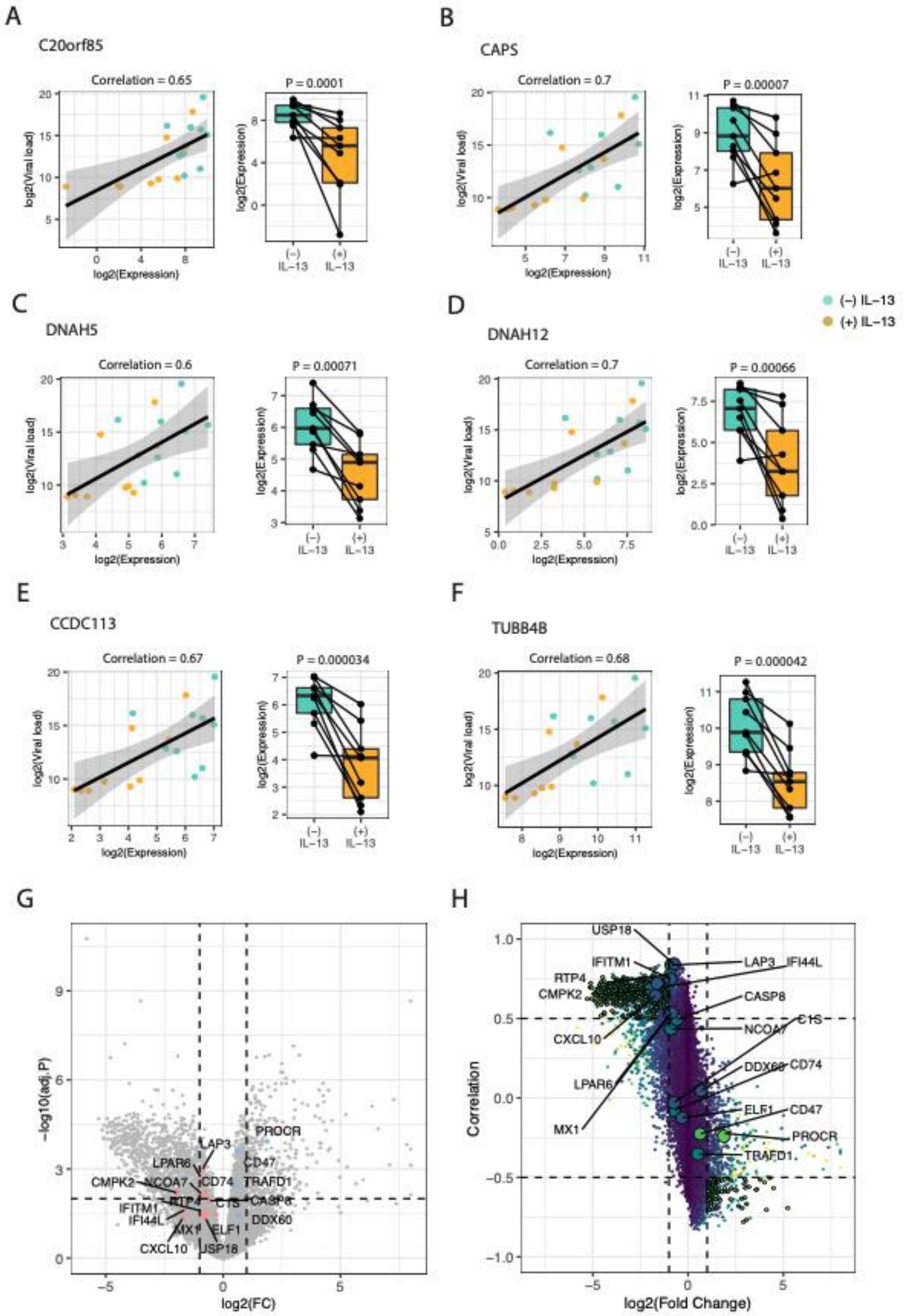
A
C20orf85
Correlation = 0.65
log2(Viral load)
log2(Expression)
P = 0.0001
log2(Expression)
(−) IL−13
(+) IL−13
B
CAPS
Correlation = 0.7
P = 0.00007
C
DNAH5
Correlation = 0.6
P = 0.00071
D
DNAH12
Correlation = 0.7
P = 0.00066
(−) IL−13
(+) IL−13
E
CCDC113
Correlation = 0.67
P = 0.000034
F
TUBB4B
Correlation = 0.68
P = 0.000042
G
−log10(adj.P)
log2(FC)
PROCR
LAP3
LPAR6
CD47
CMPK2
NCOA7
CD74
TRAFD1
RTP4
C1S
CASP8
IFITM1
IFI44L
MX1
ELF1
DDX60
CXCL10
USP18
H
Correlation
log2(Fold Change)
USP18
IFITM1
RTP4
CMPK2
LAP3
IFI44L
CASP8
NCOA7
C1S
CXCL10
DDX60
CD74
LPAR6
ELF1
CD47
MX1
PROCR
TRAFD1

Figure E4: (A-F) Examples of ciliated marker genes showing positive association between viral load and expression (scatter plots) and significant differential expression with IL-13 stimulation (box plots). (G) Volcano plot showing the differentially up- and down-regulated genes by IL-13 stimulation. Select interferon type-I genes are highlighted and up-regulated and down-regulated genes are denoted by blue and red color points, respectively. (H) Scatter plot showing the association between viral load and IL-13 induced differential gene expression. The X-axis represents the change in expression due to IL-13 stimulation, and the Y-axis the correlation to viral load. The color of the point shows the statistical significance of differential expression [-log10(adj.P)]. Select interferon type-I genes are highlighted by larger point size.

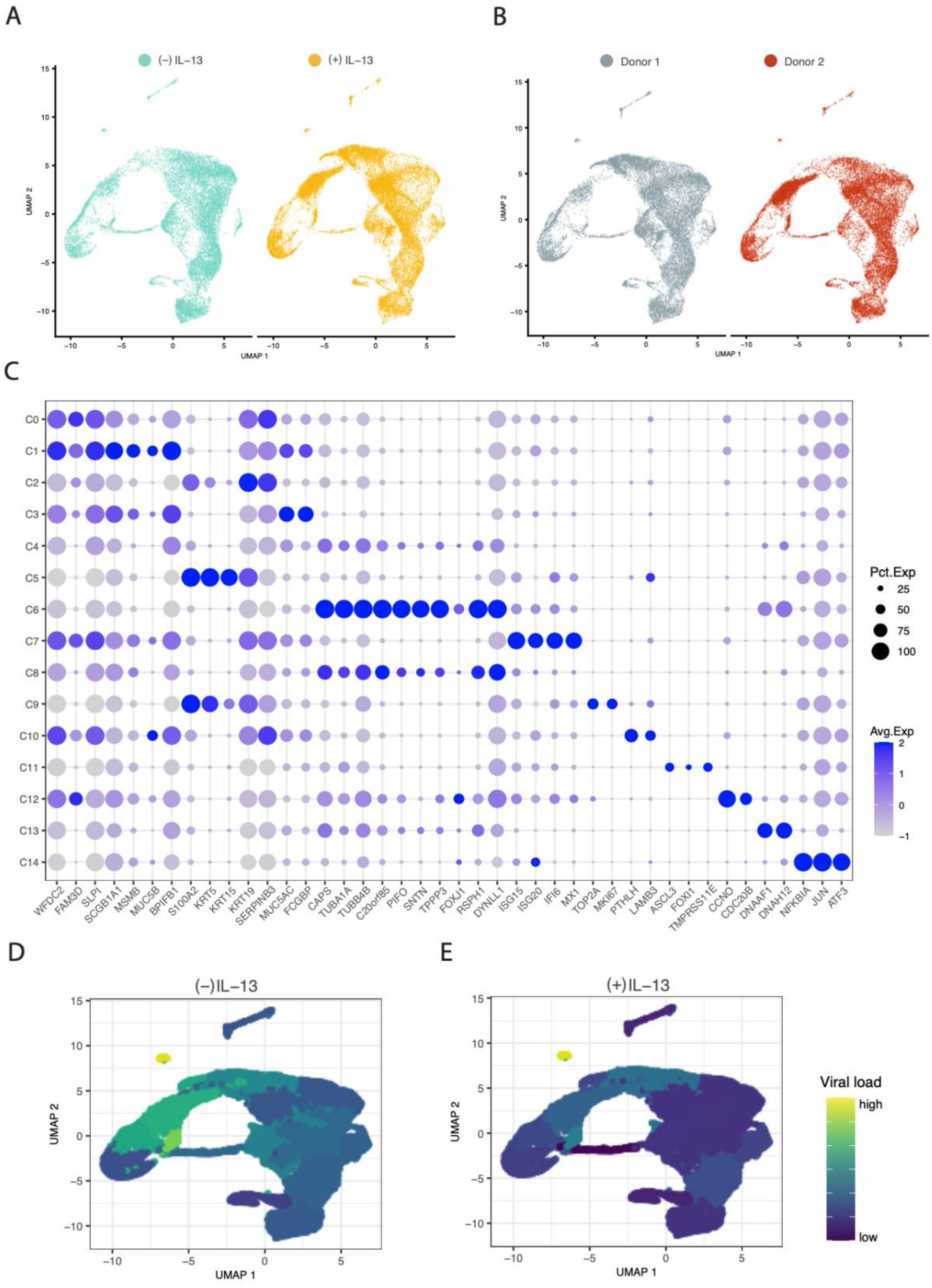
A
(–) IL–13
(+) IL–13
UMAP 2
UMAP 1
B
Donor 1
Donor 2
UMAP 2
UMAP 1
C
C0
C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
C11
C12
C13
C14
WFDC2
FAM3D
SLPI
SCGB1A1
MSMB
MUC5B
BPIFB1
S100A2
KRT5
KRT15
KRT19
SERPINB3
MUC5AC
FCGBP
CAPS
TUBA1A
TUBB4B
C20orf85
PIFO
SNTN
TPPP3
FOXJ1
RSPH1
DYNLL1
ISG15
ISG20
IFI6
MX1
TOP2A
MKI67
PTHLH
LAMB3
ASCL3
FOXI1
TMPRSS11E
CCNO
CDC20B
DNAAF1
DNAH12
NFKBIA
JUN
ATF3
Pct.Exp
25
50
75
100
Avg.Exp
2
1
0
−1
D
(–)IL–13
UMAP 2
UMAP 1
E
(+)IL–13
UMAP 2
UMAP 1
Viral load
high
low

Figure E5: UMAP clustering and visualization of scRNA-seq data from AECs from two children with allergic asthma separated by stimulated condition (SARS-CoV-2 infection with or without IL-13) (A) and by donor id (B). (C) Dot plot showing expression of key marker genes used for annotation of UMAP clusters to known cell types. Color denotes magnitude of marker genes expression and size of the circle denotes percent of cells expressing the markers. (D-E) Cell type specific viral loads in allergic asthma samples infected with SARS-CoV-2 without and with IL-13 stimulated shown in each cell cluster on the UMAP.

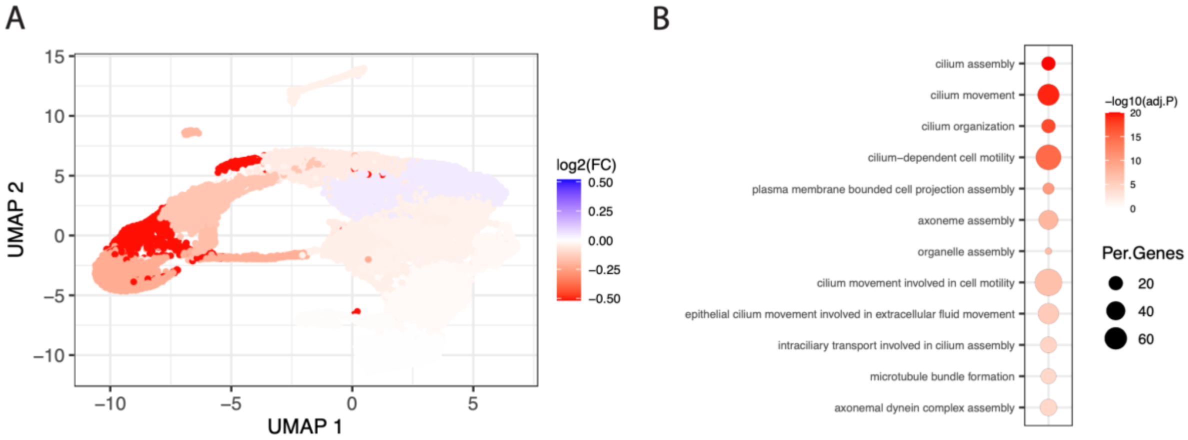

Figure E6: (A) Cell type specific expression of genes showing positive association between viral load and expression and down-regulated by IL-13 stimulation from scRNA-seq data. Expression values are presented as the ratio of expression in the IL-13 stimulated compared to unstimulated condition on a log2 scale shown in each cell cluster on the UMAP. (B) Enriched GO terms for genes showing positive association between viral load and expression and down-regulated by IL-13 stimulation from scRNA-seq data in allergic asthma. The size of each dot denotes the percent of genes and the intensity of color denotes statistical significance as -log10(adj.P).

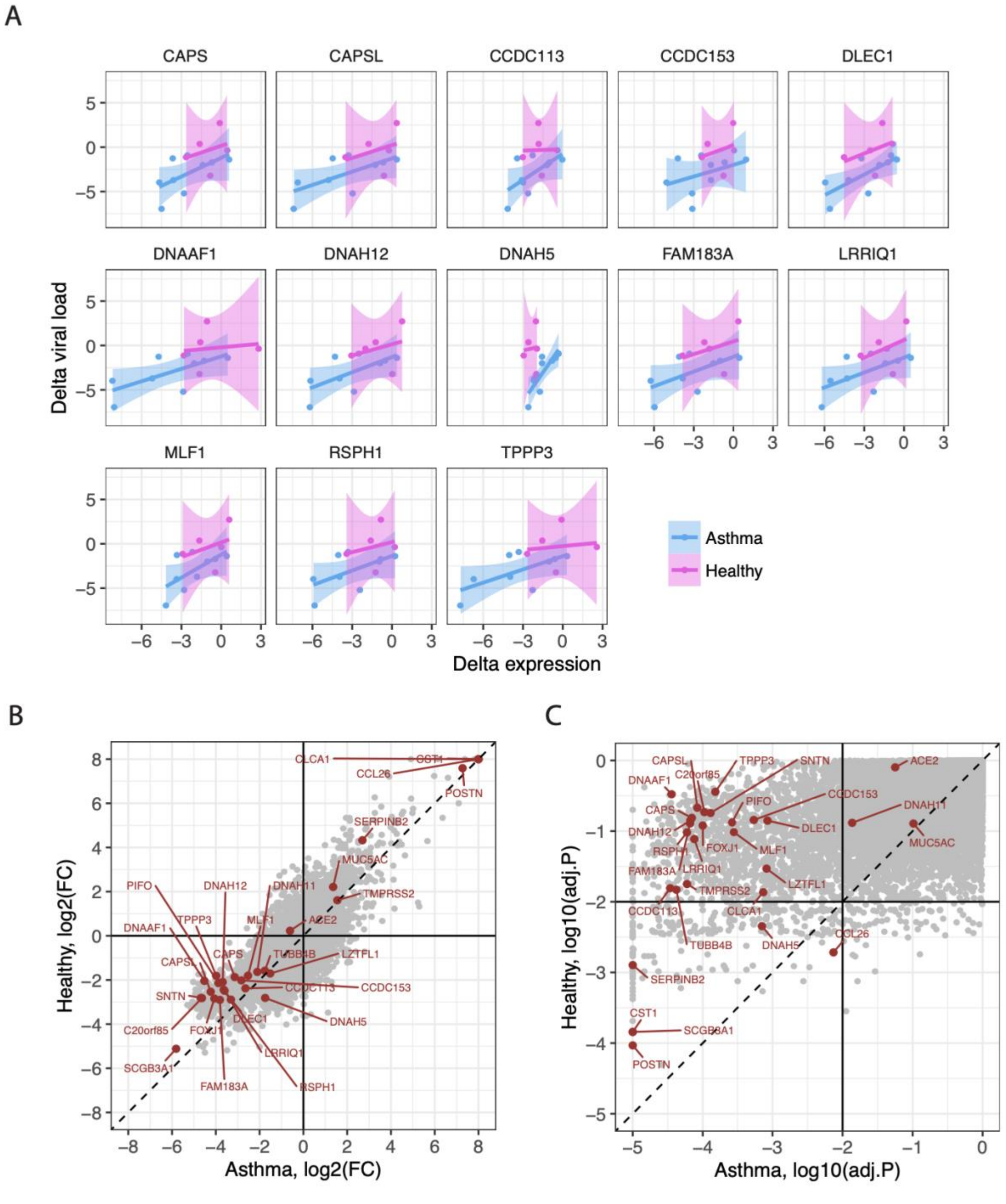
A
CAPS
CAPSL
CCDC113
CCDC153
DLEC1
DNAAF1
DNAH12
DNAH5
FAM183A
LRRIQ1
MLF1
RSPH1
TPPP3
Delta viral load
Delta expression
5
0
−5
−6
−3
0
3
Asthma
Healthy
B
Healthy, log2(FC)
Asthma, log2(FC)
8
6
4
2
0
−2
−4
−6
−8
CLCA1
CST1
CCL26
POSTN
SERPINB2
MUC5AC
TMPRSS2
PIFO
DNAH12
DNAH11
MLF1
ACE2
DNAAF1
TPPP3
TUBB4B
LZTFL1
CAPSL
CAPS
CCDC113
CCDC153
SNTN
DNAH5
C20orf85
FOXJ1
DLEC1
LRRIQ1
SCGB3A1
FAM183A
RSPH1
C
Healthy, log10(adj.P)
Asthma, log10(adj.P)
0
−1
−2
−3
−4
−5
CAPSL
TPPP3
SNTN
ACE2
DNAAF1
C20orf85
CCDC153
CAPS
PIFO
DNAH11
DNAH12
DLEC1
MUC5AC
RSPH1
FOXJ1
MLF1
FAM183A
LRRIQ1
LZTFL1
CCDC113
TMPRSS2
CLCA1
TUBB4B
DNAH5
CCL26
SERPINB2
CST1
SCGB3A1
POSTN

Figure E7: (A) Examples of select ciliated marker genes association between delta viral load (difference in viral load between IL-13 stimulated and unstimulated) and delta expression (difference in expression between IL-13 stimulated and unstimulated) in allergic asthma and healthy non-sensitized donors. Scatter plots comparing (B) effect sizes (log2FC) and (C) statistical significance (adjusted P.values) due to IL-13 stimulation in allergic asthma and healthy non-sensitized children. Select ciliary and type 2 specific genes are highlighted the same as in Figure 2c and Figure 4c.

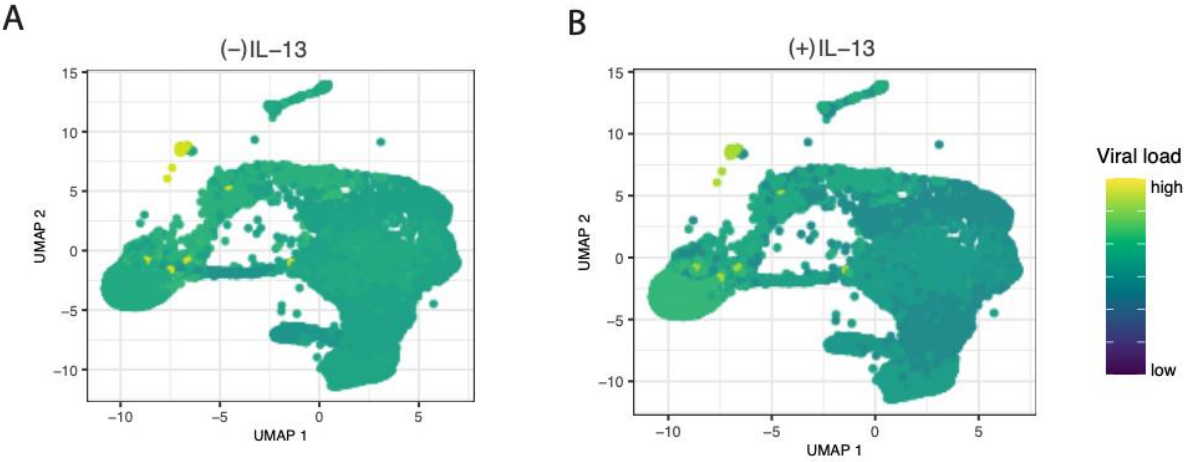

Figure E8: (A-B) Cell type specific viral loads in healthy non-sensitized AEC samples infected with SARS-CoV-2 without and with IL-13 stimulated shown in each cell cluster on the UMAP.