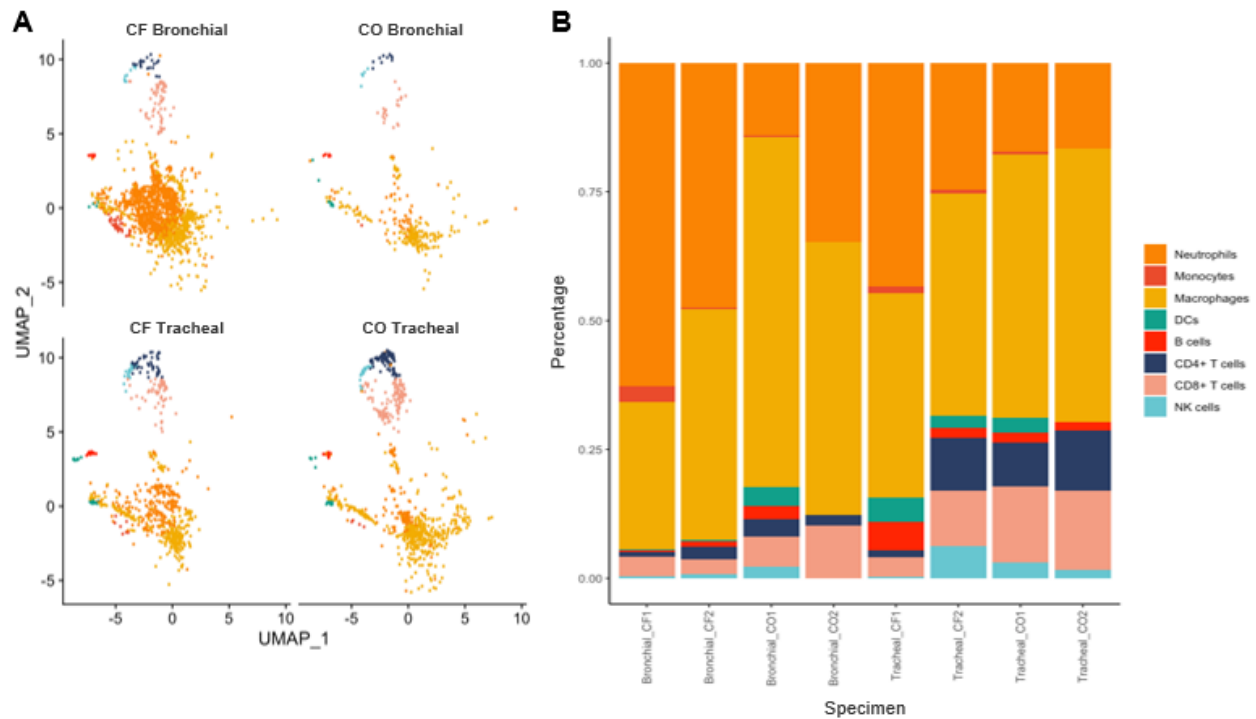
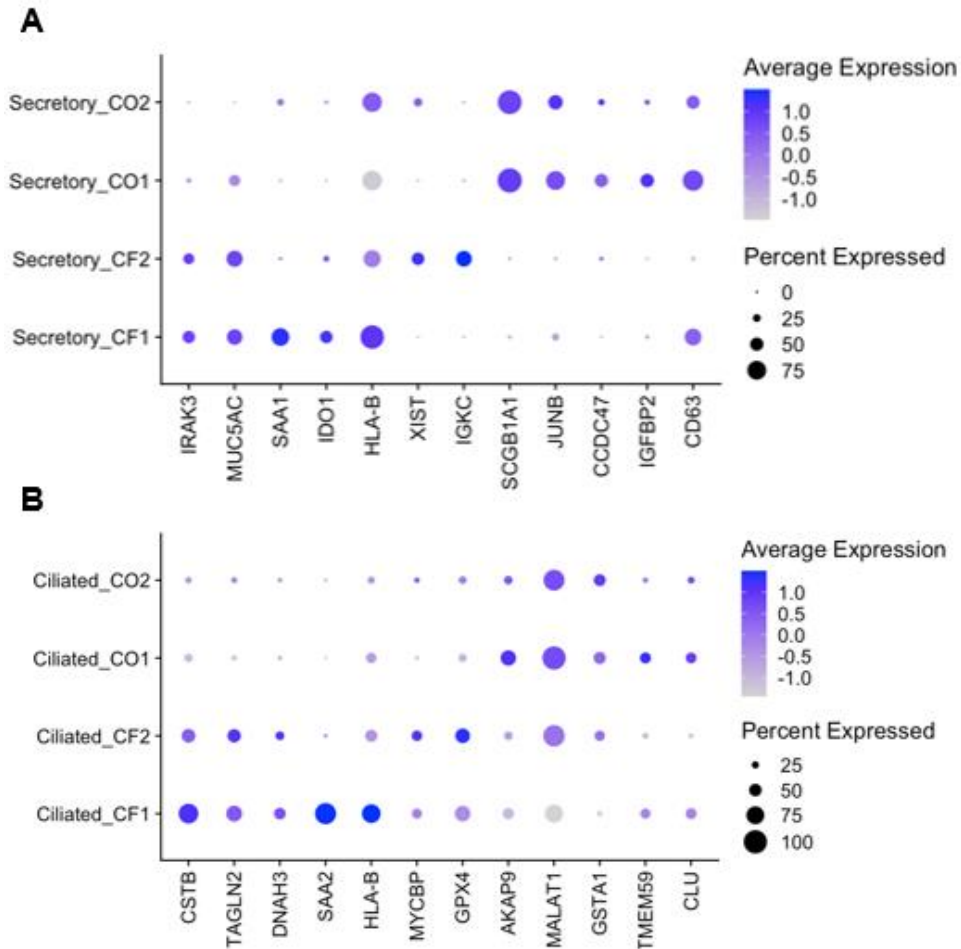
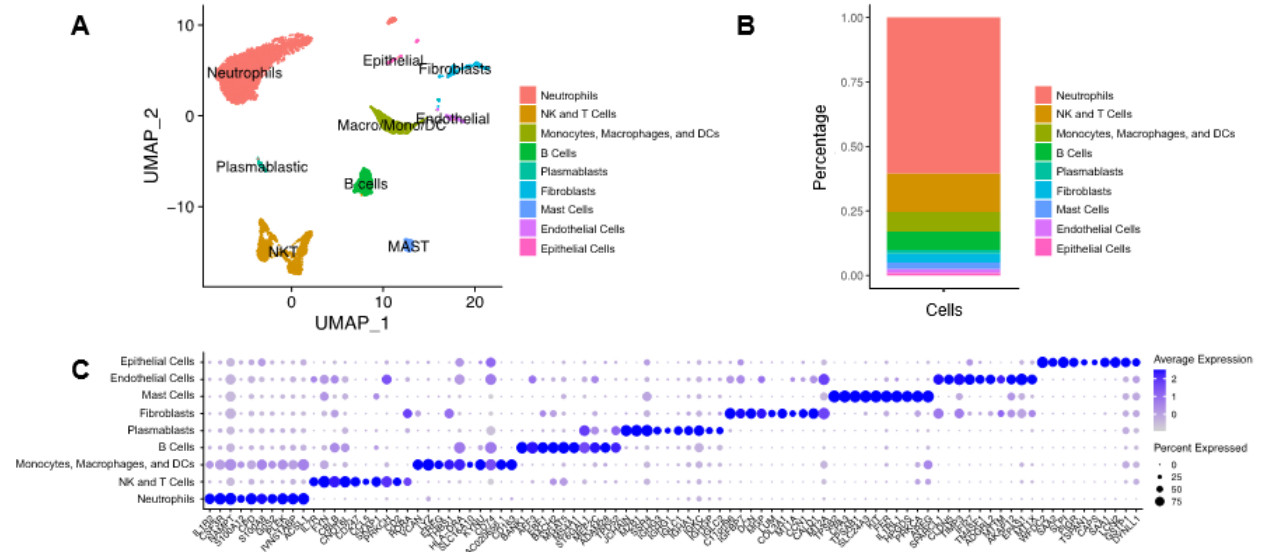




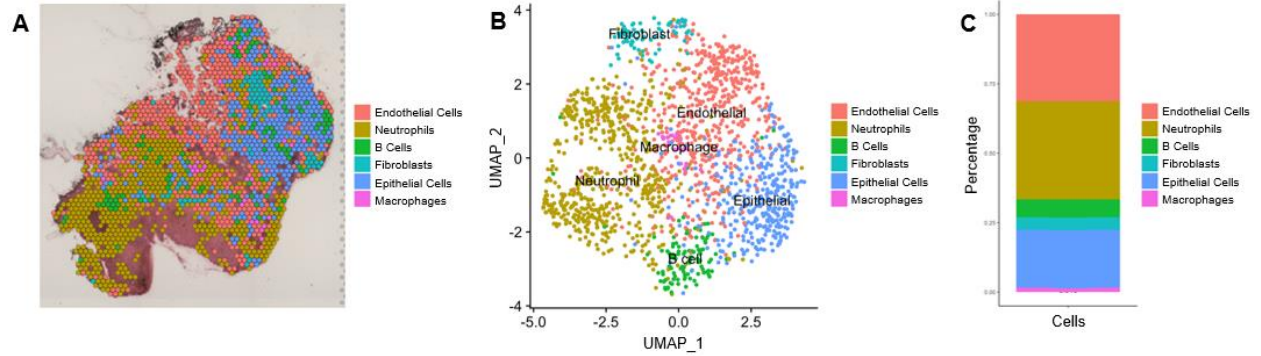
**Supplementary Figure S1.** Single-cell transcriptional profiling of the broad immune cell populations in the tracheobronchial brush biopsy specimens collected from pediatric patients with CF (n=2) and pediatric controls (CO) (n=2). **(A)** UMAP visualization of the broad immune cell populations divided by disease state and sampling site and colored by cell population. **(B)** Stacked column chart visualization of the distributions of the broad immune cell populations by disease state, pediatric patient, and sampling site. DC: Dendritic cell; NK: Natural killer cell.



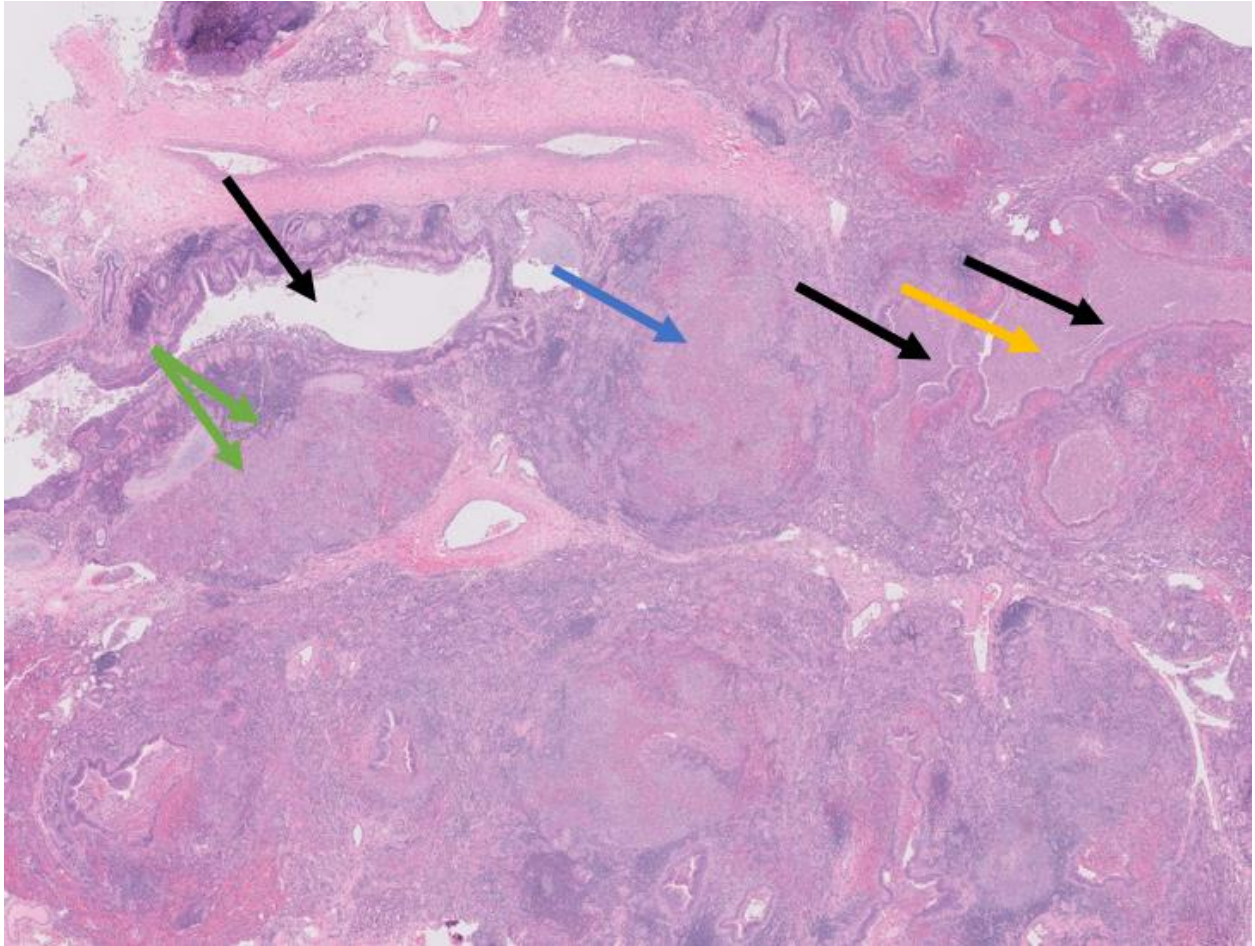
**Supplementary Figure S2.** Differential gene expression analysis in airway epithelial cell populations from pediatric patients with CF and pediatric controls (CO). **(A)** Dot plot visualization of select differentially expressed genes in secretory cells by expression level and frequency. **(B)** Dot plot visualization of select differentially expressed genes in ciliated cells by expression level and frequency.



**Supplementary Figure S3.** Single-cell transcriptional profiling of the broad immune and epithelial cell populations in resected lung tissue from a pediatric patient with CF. **(A)** UMAP visualization of the broad immune and epithelial cell populations colored by cell population. **(B)** Stacked column chart visualization of the distribution of the broad immune and epithelial cell populations. **(C)** Dot plot visualization of the top differentially expressed genes in each broad immune and epithelial cell population by expression level and frequency. DC: Dendritic cell; NK: Natural killer cell.



**Supplementary Figure S4.** Spatial transcriptional profiling of the pediatric CF lung. **(A)** Spatial transcriptome of resected lung tissue from a pediatric patient with CF. **(B)** UMAP visualization of the broad immune, epithelial, and stromal cell populations colored by cell population. **(C)** Stacked column chart visualization of the distribution of the broad immune, epithelial, and stromal cell populations.



**Supplementary Figure S5.** H&E staining (20x) of resected lung tissue from a pediatric patient with CF showing extensive bronchiectasis and bronchiolectasis (black arrows) as well as marked lymphocyte and histiocyte infiltration (green arrows). Neutrophils are present within the airway lumens (yellow arrow) and at the center of lymphohistiocytic nodules (blue arrow).

|                           | CF1  | CF2       | CO1       | CO2       | CO3       |
|---------------------------|------|-----------|-----------|-----------|-----------|
| Cell count                |      |           |           |           |           |
| Neutrophils (%)           | NP   | 94        | 0         | 5         | 2         |
| Monocytes (%)             | NP   | 1         | 0         | 3         | 3         |
| Macrophages (%)           | NP   | 2         | 86        | 63        | 14        |
| Eosinophils (%)           | NP   | 2         | 0         | 2         | 0         |
| Lymphocytes (%)           | NP   | 1         | 4         | 23        | 17        |
| Mesothelial cells (%)     | NP   | 0         | 10        | 4         | 64        |
| Microbial culture results |      |           |           |           |           |
| Bacterial culture         | MSSA | MRSA      | URF       | No growth | No growth |
| Fungal culture            | NP   | No growth | No growth | No growth | No growth |
| AFB culture               | NP   | No growth | NP        | No growth | No growth |

**Supplementary Table S1.** Clinical cell count and microbial culture results from the BALF specimens from the five included pediatric patients. AFB: Acid-fast bacilli, CF: Cystic fibrosis, CO: Control, MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-susceptible *Staphylococcus aureus*, NP: Not performed; URF: Usual respiratory flora.

|                          | <b>CF1</b>     | <b>CF2</b>     | <b>CO1</b>     | <b>CO2</b>     | <b>CO3</b>     |
|--------------------------|----------------|----------------|----------------|----------------|----------------|
| Sampling sites           |                |                |                |                |                |
| Tracheal brush biopsies  | Distal trachea | Distal trachea | Distal trachea | Distal trachea | Distal trachea |
| Bronchial brush biopsies | RLL            | LLL*           | LLL*           | RLL            | RLL            |
| Bronchoalveolar lavage   | RML            | RML            | LUL*           | RML            | RML            |

**Supplementary Table S5.** Sampling sites for each of the collected minimally invasive respiratory specimens from the five included pediatric patients. Sites representing a clinically indicated deviation from the methods are denoted with an (\*). CF: Cystic fibrosis, CO: Control, LLL: Left lower lobe, LUL: Left upper lobe, RLL: Right lower lobe, RML: Right middle lobe.