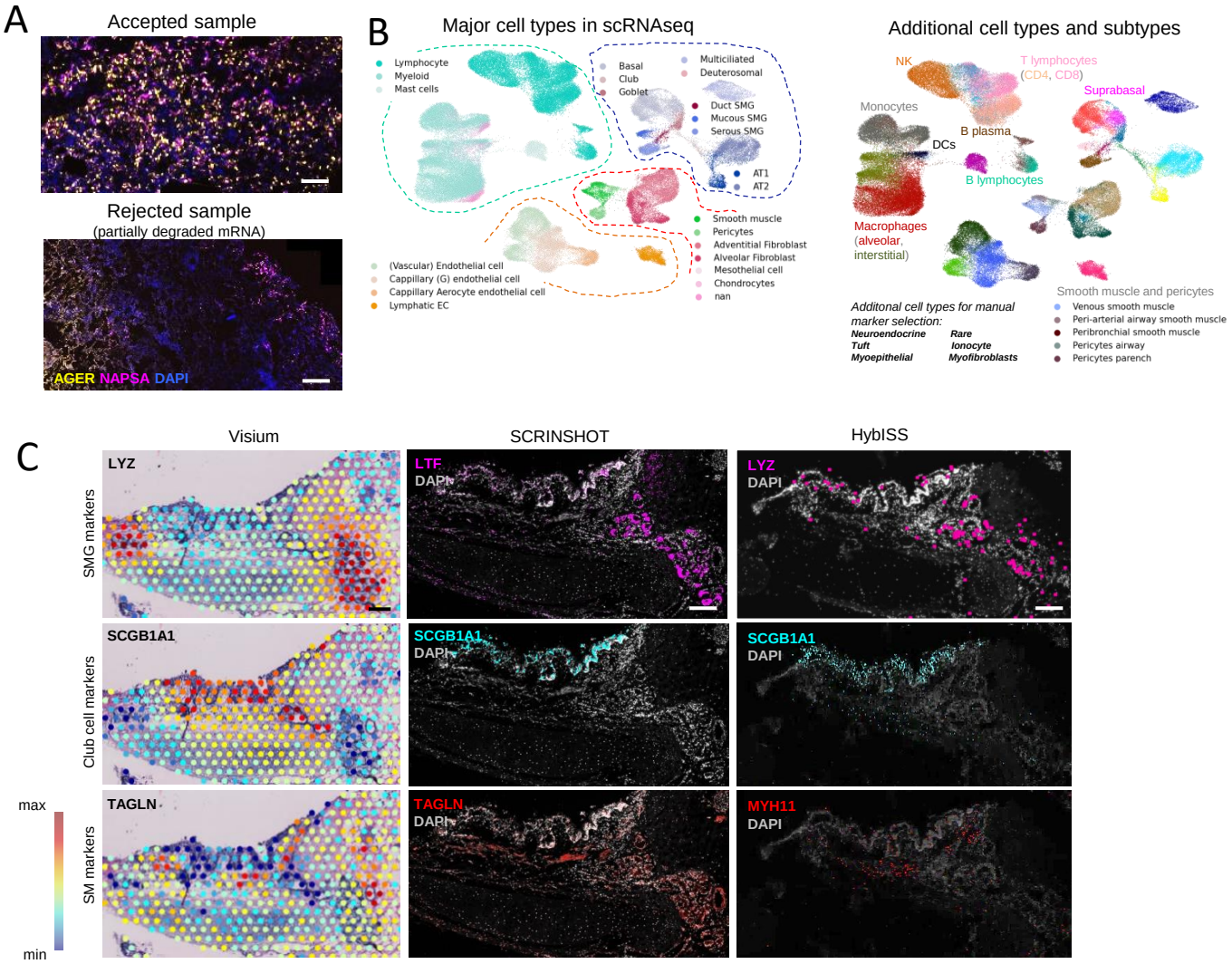
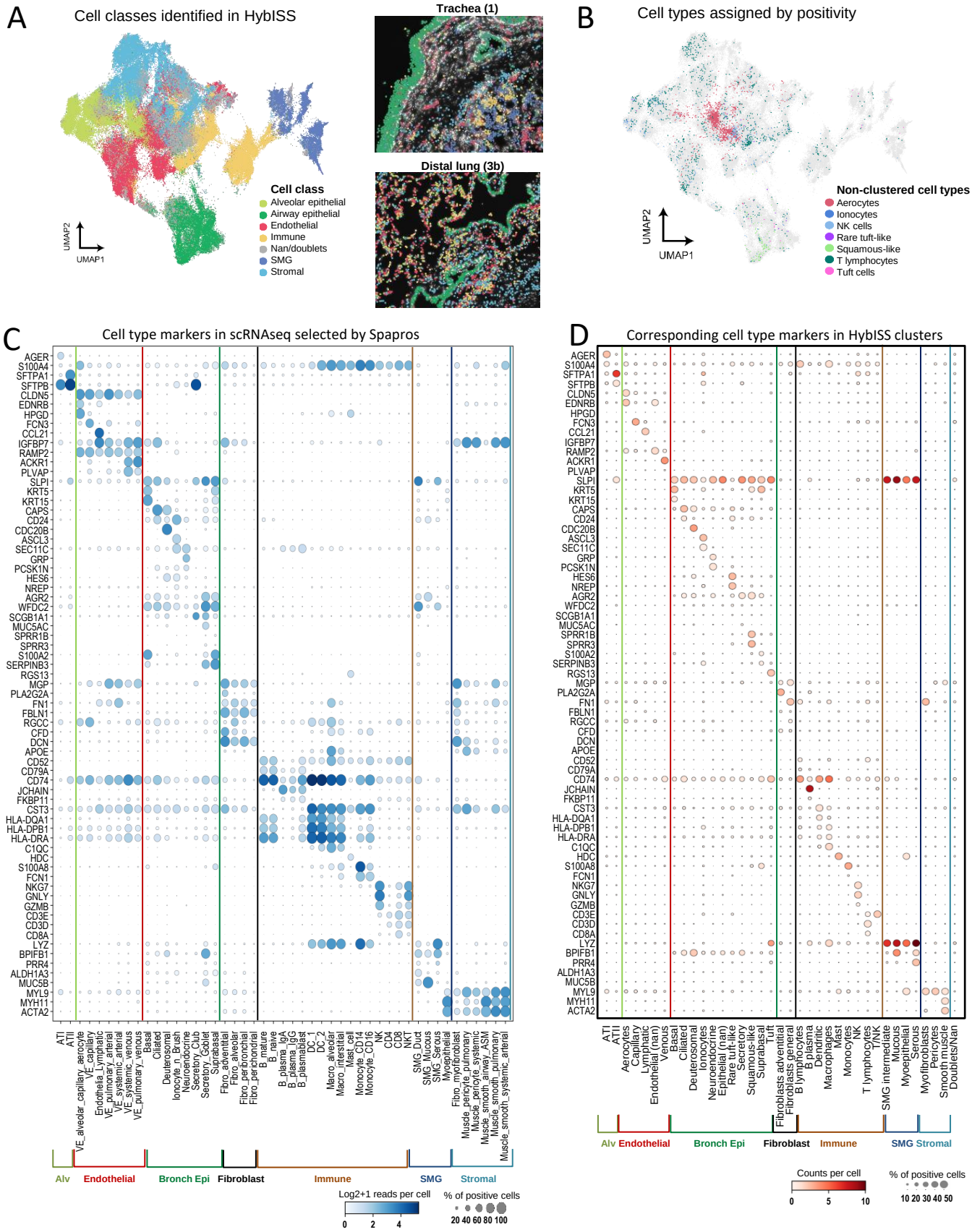
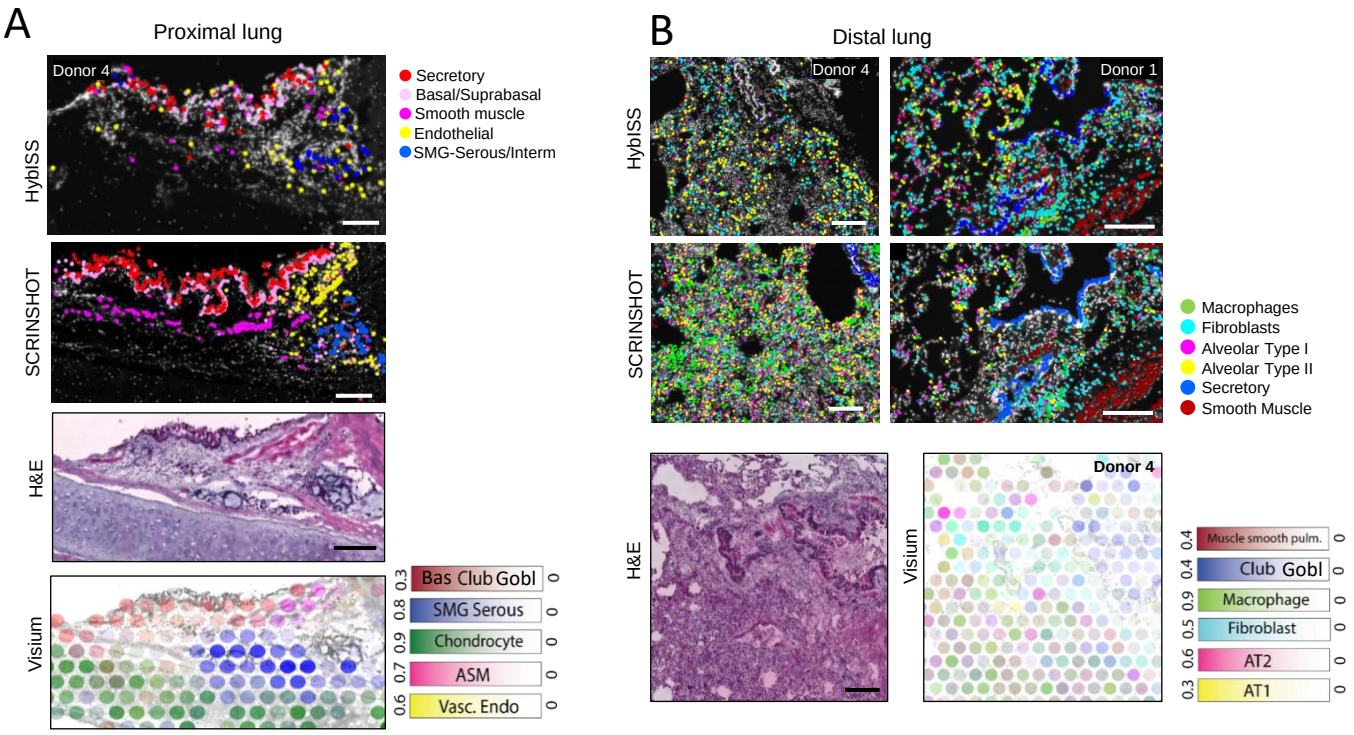


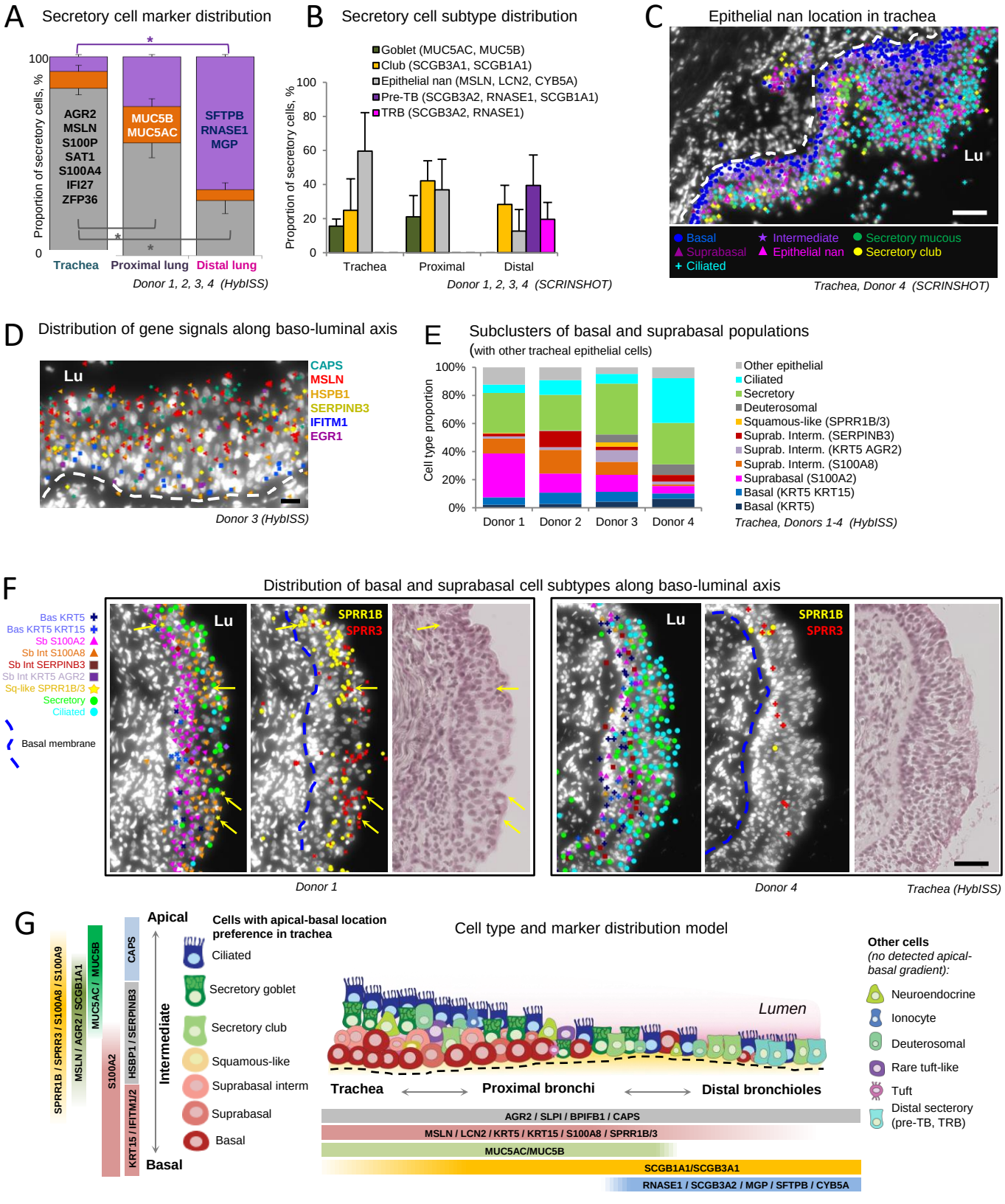
Supplementary figure 1. Quality control prior to cell type annotations. A. Representative accepted and rejected samples demonstrating mRNA signal for cell type marker genes for alveolar type I (AT1, AGER, yellow) and II (AT2, NAPSA, magenta) cells on top of nuclei (DAPI, blue) in alveolar region tissue sections. Scale bar: 200 µm. B. Clusters from scRNA-seq datasets [1] used for probe panel selection for targeted methods. Left – cell types targeted with SCRINSHOT marker panel, right – additional cell types targeted with HybISS panel. Additional cell types (not represented in scRNA-seq) based on other publications were targeted based on manual marker selection. C. Signal of characteristic markers of the indicated cell types plotted on the images of proximal lung consecutive sections, analyzed with Visium, SCRINSHOT and HybISS. Detected transcripts are shown on top of hematoxylin and eosin staining (Visium) of DAPI (gray, SCRINSHOT and HybISS). Scale bar: 200 µm. SMG – submucosal gland, SM – smooth muscle, EC – endothelial cells, G - general.



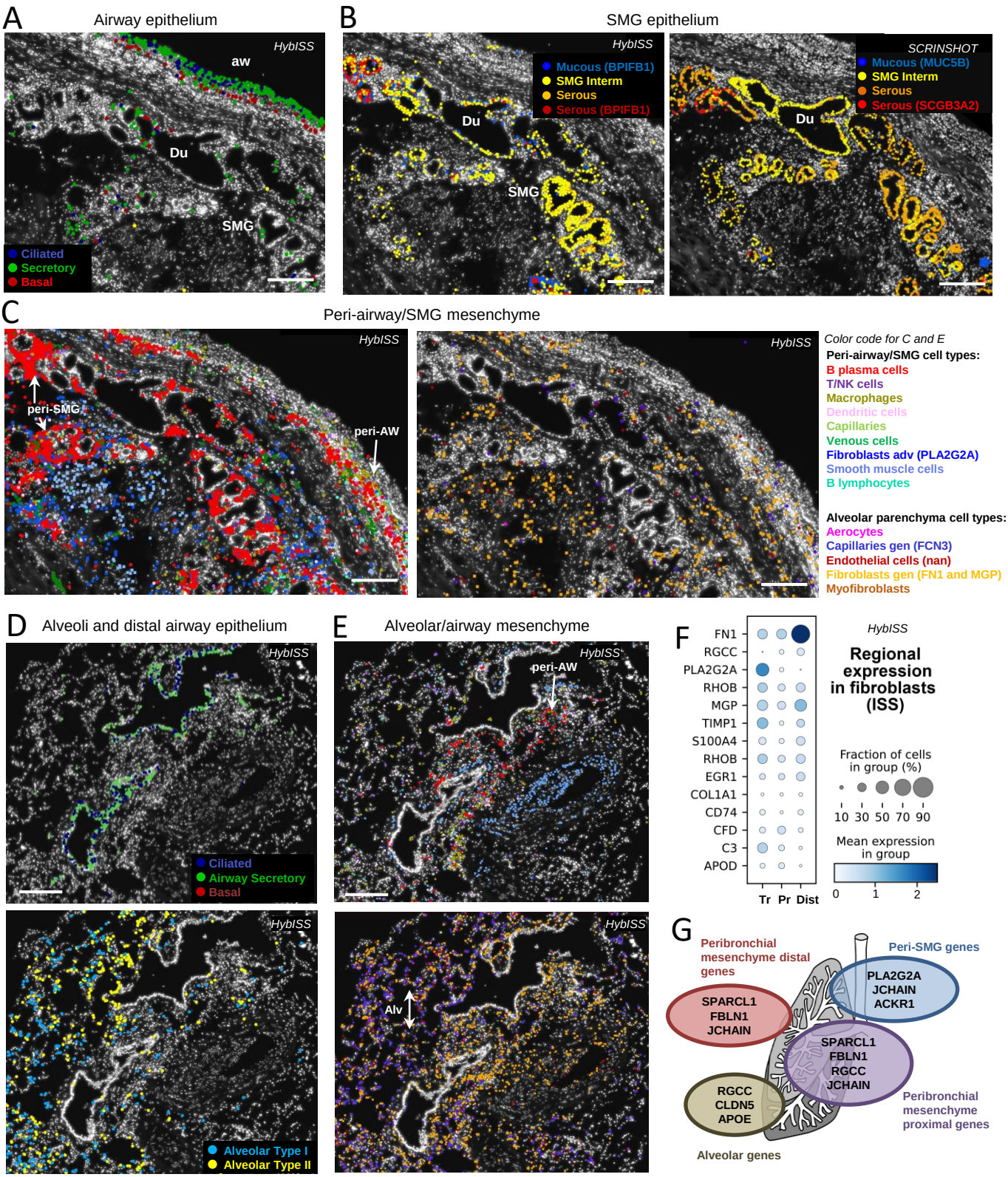
Supplementary figure 2. Characterization of cell types identified in HybISS analysis. A. (left) UMAP plot of cells profiled with HybISS, colored by assigned cell class. (right) Complementary maps of cell classes identified by HybISS in the trachea and distal lung sections. Colors as in the UMAP plot. Nuclei: gray. Scale bar: 200 µm. B. UMAP plot of the cells analyzed by HybISS, highlighting the indicated, additional cell types that were manually annotated using distinct marker positivity. C. Dotplot of the selected cell type panel markers in the reference scRNA-seq dataset [1]. Color intensity: $\log_2$(normalized UMI-counts+1) (library size was normalized to 10.000). Dot size: percentage of positive cells. D. Dotplot of the same markers as in (C) in the detected cell types of the HybISS dataset. Color intensity: mean counts per cell. Dot size: percentage of positive cells.



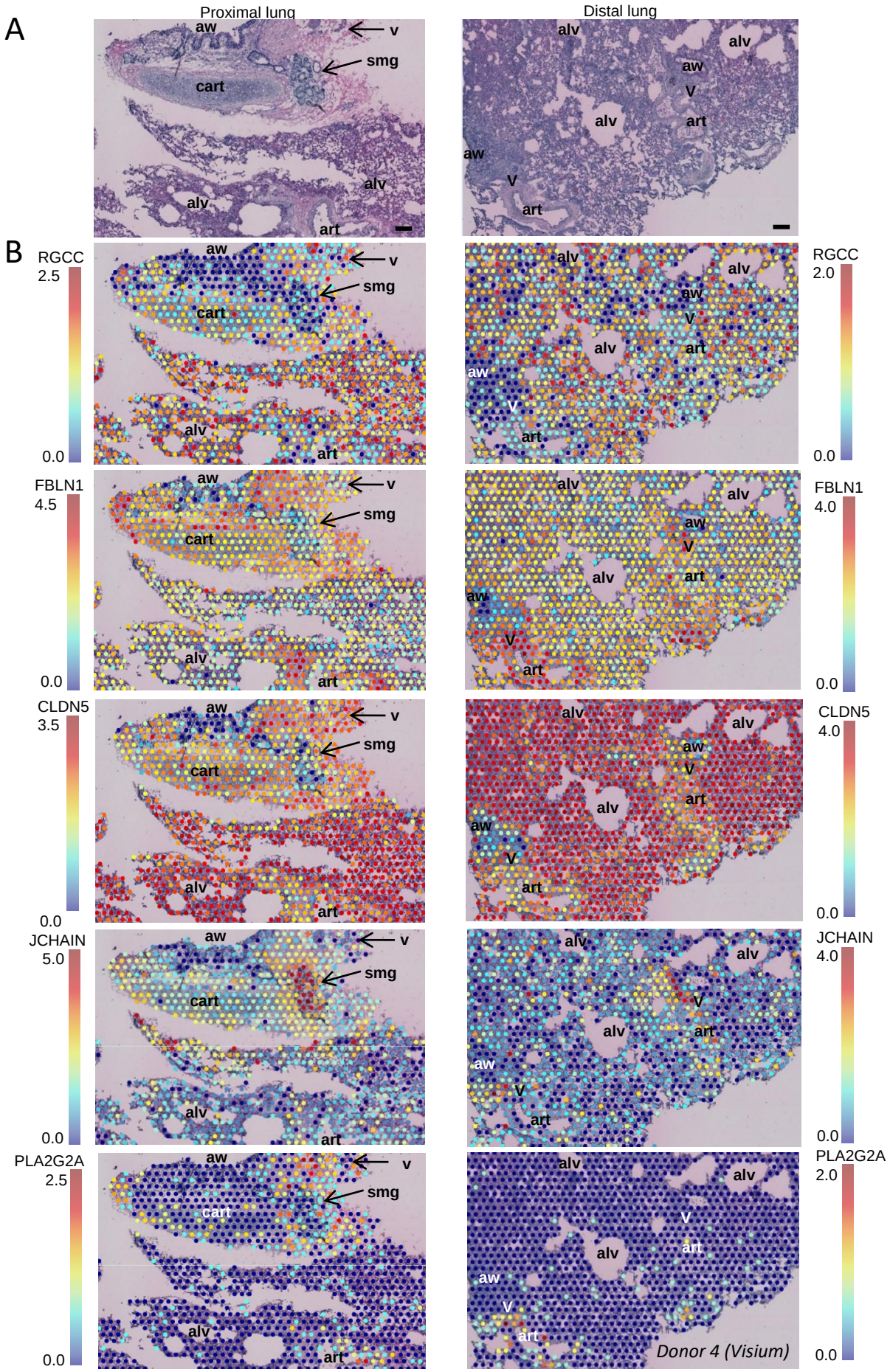
Supplementary figure 3. Cell type map validation by gene expression using Visium, SCRINSHOT and HybISS. Maps of the indicated cell types, detected by HybISS (top), SCRINSHOT (middle) and Visium (bottom) in proximal (A) and distal (B) lung tissue sections. Detected transcripts are shown on top of hematoxylin and eosin staining for Visium or DAPI (gray) for SCRINSHOT and HybISS. Cell types for Visium are detected using stereoscope method with 80 annotated cell subtypes [1]. Scale bar 200 µm.



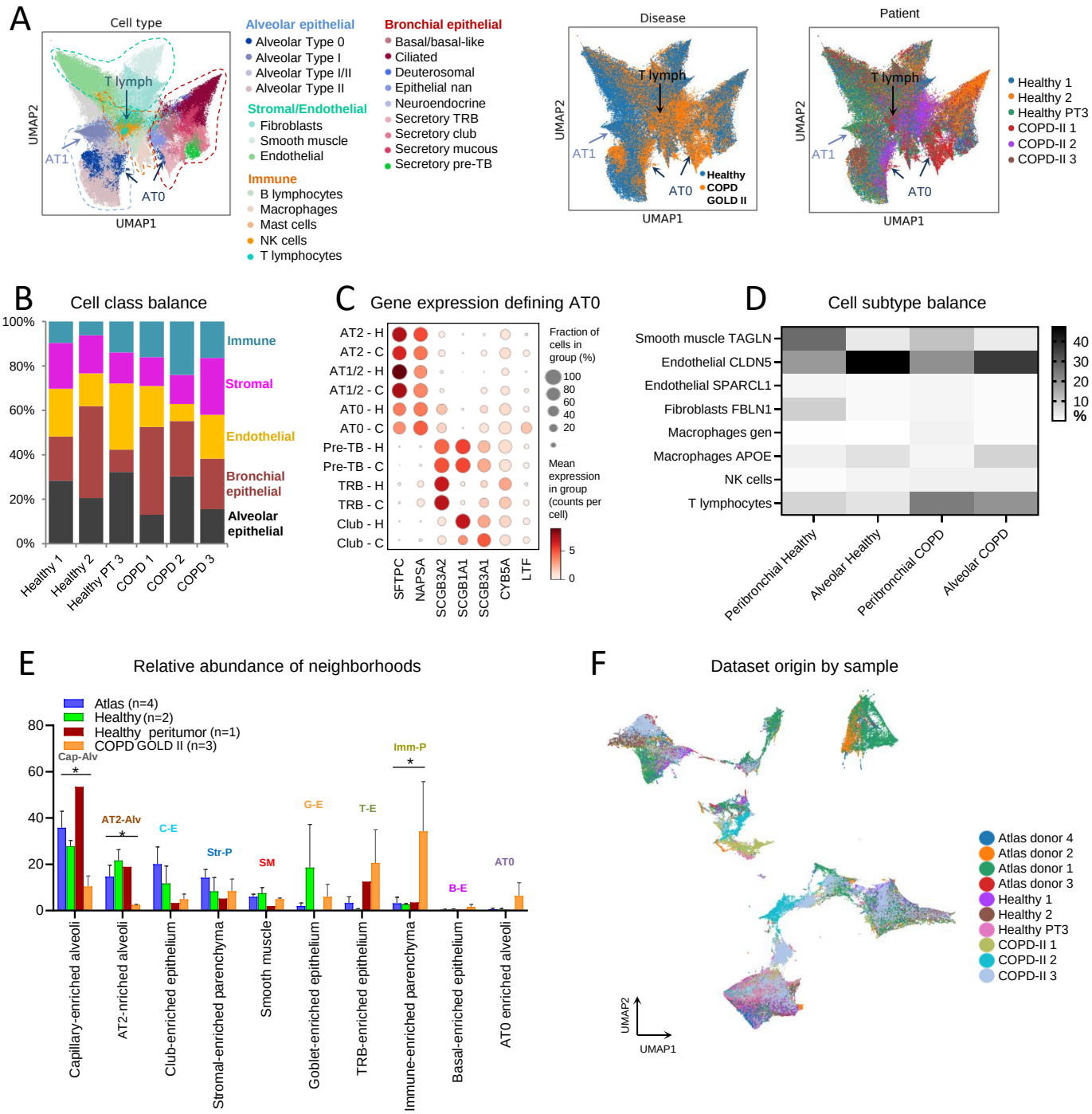
Supplementary figure 4. Defining epithelial cell states by gene expression and spatial distribution. A. Quantification of grouped subtypes of secretory cells in the bronchial epithelium from four donors (n=4), detected by HybISS. Error bars: standard deviation. Significant differences according to two-way ANOVA followed by Tukey's multiple comparisons test, are highlighted with asterisk, color-coded according to bars, * p<0.05, n=4. B. Quantification of goblet, club, pre-terminal bronchiole (pre-TB), terminal bronchiole (TRB) and other secretory cells in the epithelium of four donors (n=3 for trachea, n=4 for lung), detected by SCRINSHOT. Error bars: standard error. C. Representative map of epithelial cell types in tracheal epithelium of donor 4, based on SCRINSHOT. Scale bar 50 µm. D. Map of gene reads detected by HybISS on top of nuclei (gray) in a representative tracheal sample demonstrating variable gene expression along apical-basal axis. Scale bar: 20 µm. E. Barplot showing the percent of basal and suprabasal cell states in tracheal samples in four analysed donors, indicating the high variability in the abundance of suprabasal, squamous-like, and intermediate cell states. F. Representative images of tracheal epithelium from two donors, showing (left) the maps of the indicated cell types, (middle) the SPRR1B and SPRR3 squamous marker detected transcripts and (right) hematoxylin and eosin staining after HybISS. Arrows: squamous-like cells. Scale bar 50 µm. Lu – lumen, dashed line indicates approximate location of basal membrane. G. Schematic summary of cell type distribution and gene expression patterns along proximo-distal and apical-basal axes.



Supplementary figure 5. Spatial distribution of SMG, stromal, endothelial and immune cell subtypes A. Representative airway epithelial cell type maps in the trachea and submucosal gland (SMG) in HybISS dataset of donor 1 trachea. B. Representative maps of epithelial SMG cell types serial sections, analyzed with HybISS (left) and SCRINSHOT (right). C. Maps of (left) peribronchial/peri-SMG-dominating (arrows) and (right) alveolar-dominating cell types based on the HybISS dataset of donor 1 trachea. D. Representative airway epithelial (top) and alveolar epithelial (bottom) cell type maps in HybISS dataset from donor 1 distal lung. Scale bar 200 µm. E. Non-epithelial cell type maps of the peribronchial (arrow) and alveolar (double arrow) neighborhoods in the same regions as in (D). F. Dotplot showing the gene expression levels (color intensity) and the percent of positive cells for the indicated markers in the annotated fibroblasts across regions. Data from four donors, HybISS. G. Schematic summary of the gene expression in the parenchyma of each neighborhood. In all images nuclei: gray and scale bar 200 µm.



Supplementary figure 6. Gene expression maps by RRST in alveolar and peri-epithelial neighborhoods. A. Histological images of proximal (left) and distal (right) lung sections stained with hematoxylin and eosin from donor 4. Scale bar 200 µm. B. Maps of gene expression levels projected on top of proximal (left) and distal (right) lungs shown in A. Data from donor 4, RRST. Arrows and letters indicate histologic compartments around submucosal gland (SMG), airways (aw), arteries (art), veins (v), cartilage (cart) and alveoli (alv). Genes expression is shown in log-normalised values obtained in Seurat.



Supplementary figure 7. Quantification of COPD-related changes in cell types and neighborhoods. A. UMAP plots of cell type clusters (left), health status (middle) and donors/patient identities (right), from distal lung biopsies analyzed by SCRINSHOT. B. Proportions of the indicated cell classes in the analyzed donors. C. Dotplot showing the mean expression of the indicated genes in the AT2-related clusters compared to distal secretory cell types. Color intensity: number of counts per cell. Dot size: percentage of positive cells. D. Heatmap of the mean proportions (%) of the indicated cell type populations in the annotated peri-epithelial compartments. E. Barplot showing the relative abundance mean values of the indicated cell neighborhoods across all datasets (Atlas, COPD, healthy and healthy-peritumor). Error bars: standard error. Significant differences are highlighted with asterisk (*, p≤0.05), according to one-way ANOVA of logit-transformed data (0 values changed to the half of minimal detection limit values) followed by Dunnet's posthoc with Atlas group used as a control, and Healthy/Healthy peritumor treated as one group. TRB – terminal respiratory bronchiole. F. UMAP plot of the detected cellular neighborhood clusters (Figure 5C) colored by donor/patient. Metadata presented in supplementary table 1.