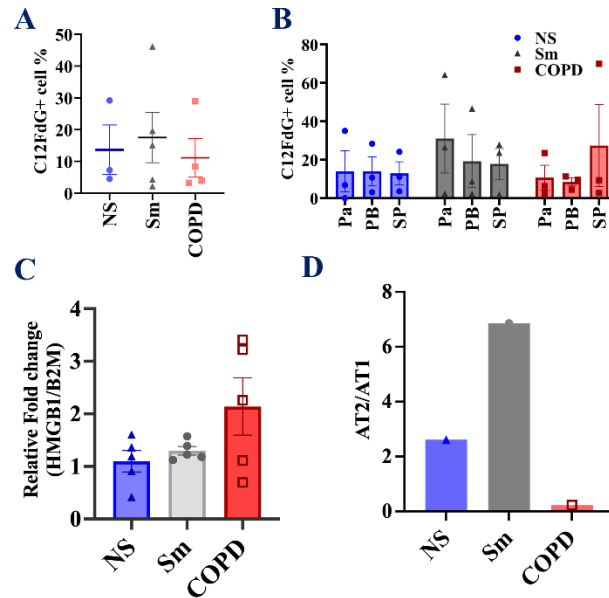


SUPPLEMENTARY FIGURE FILE

Characterizing the B-cell senescence signature in driving the disease phenotype in COPD/Emphysema

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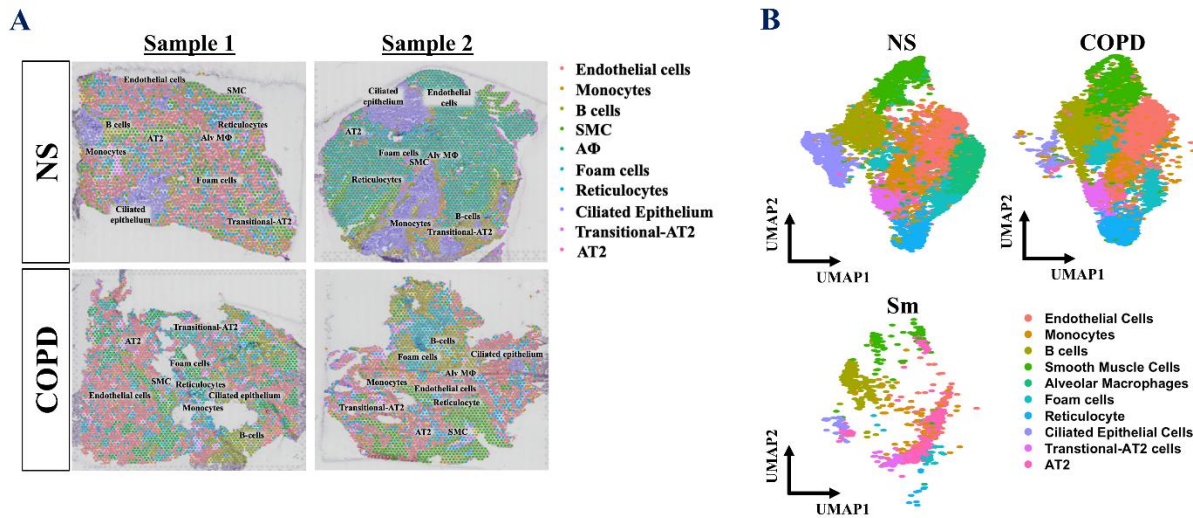


Supplementary Figure 1: Characterization of senescence in whole tissue and at single cell level in healthy non-smokers, smokers and COPD donors

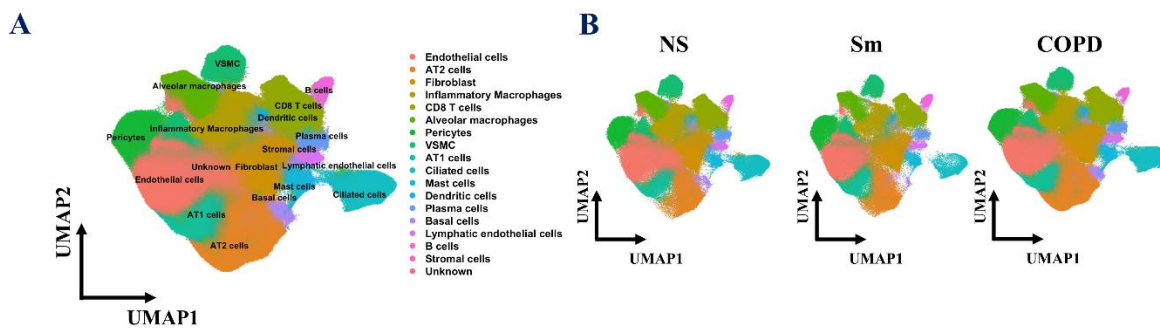
The total cellular senescence in the whole lung tissue was determined by performing fluorescence-based SA- β -Gal assay (C₁₂FdG) on three regions from the donor lungs – parenchymal (Pa), peri-bronchial (PB) and sub-pleural (SP). **(A)** Groupwise and **(B)** tissue region-specific changes in the level of C₁₂FdG+ cells amongst healthy non-smokers, smokers and COPD donors were plotted.

(C) Changes in the gene expression of *HMGB1*, known as a marker of senescence in lung tissues, was determined using qPCR.

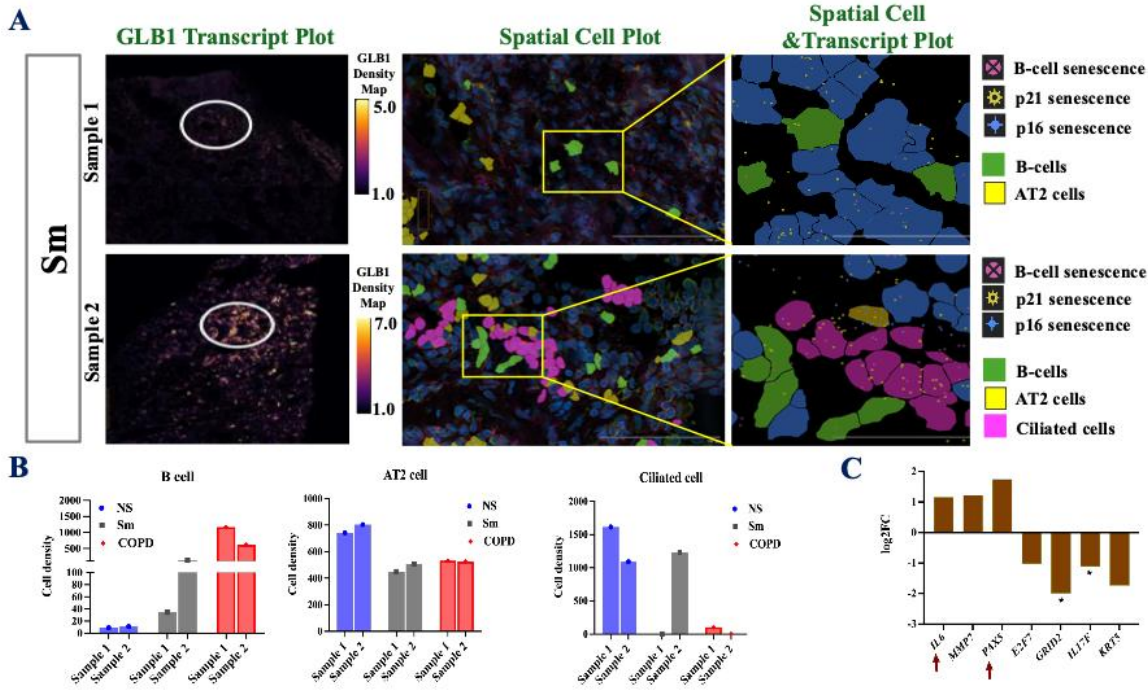
(D) The AT2/AT1 ratio in healthy non-smokers, smokers and COPD donors represented as cell frequencies determined after dimensionality reduction and clustering of scRNA seq data. Here: NS: non-smoker, Sm: smoker and COPD: COPD patient with emphysema.



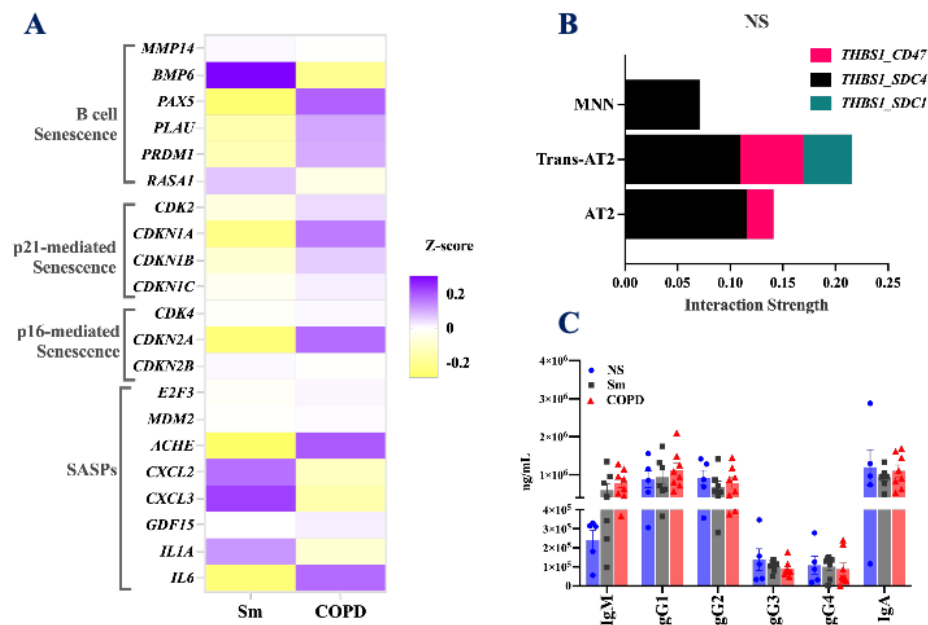
Supplementary Figure 2: (A) Spatial plots showing the cellular distributions of identified cell types in tissue regions for NS and COPD lungs. Representative plots for two donors per group are displayed here. **(B)** UMAP showing the groupwise comparisons of the identified cell clusters from the analyses of spatial transcriptomics data from NS, Sm and COPD donors.



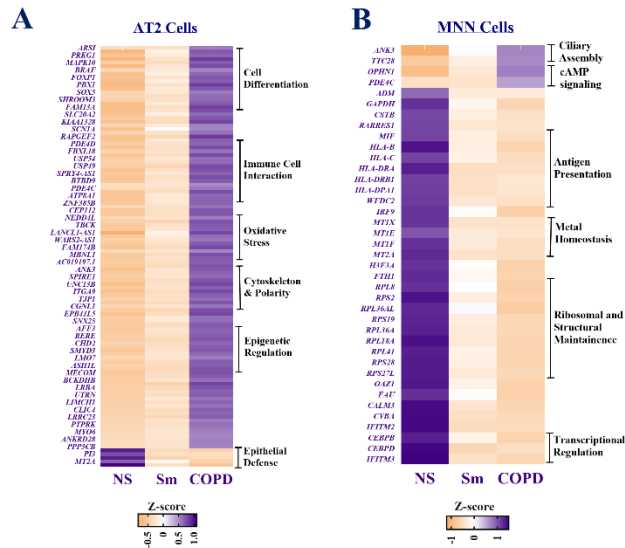
Supplementary Figure 3: (A) UMAP showing the cell clusters identified upon analyses of the transcriptomic profile of tissue sections from non-diseased (NS/Sm) and diseased donors using Xenium Spatial profiling. n = 6-8 tissue sections per group. **(B)** UMAP showing the groupwise comparisons of the distribution of identified cell clusters from the analyses of targeted spatial transcriptomics (Xenium) data from NS, Sm and COPD donors.



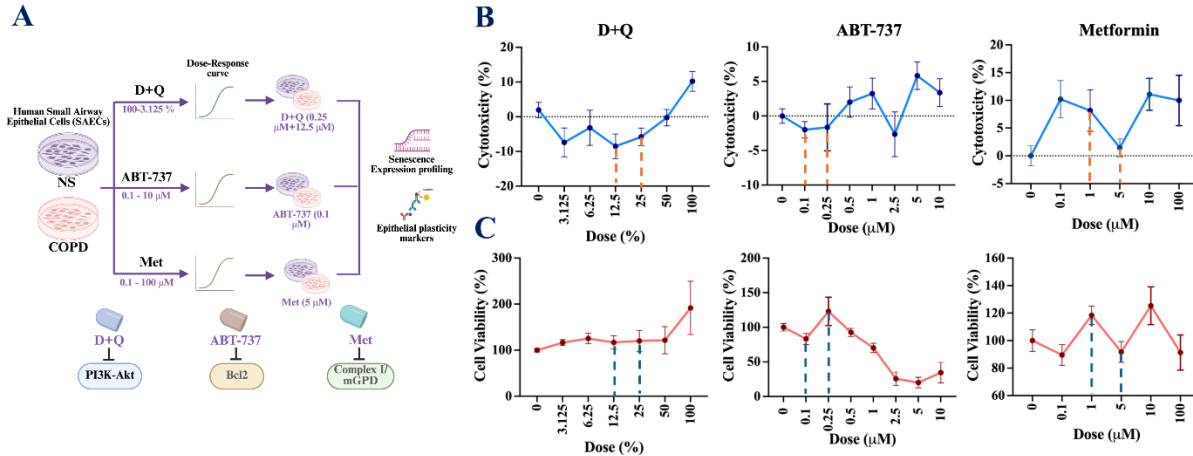
Supplementary Figure 4: (A) Representative spatial plots (n = 2/group) showing the strategy of ‘senescent’ niche identification (using GLB1+ density plot) in lung tissues with the representative spatial cell plot and senescence-relevant transcript (p16 and p21) distribution in Smokers (Sm). **(B)** Cell density plots (density per mm²) showing distribution of B-, AT2, and ciliated cells in the ‘senescent niches’ from NS, Sm and COPD donor lung sections. Each bar shows the cell density for the representative images shown in Figure 5B and S4A. **(C)** Bar plot showing the log₂FC for the differentially regulated genes in ‘senescent niche’ from COPD donor tissues as compared to Sm. Data is a normalized mean of results from two randomly selected regions per tissue section from 8 tissue section/group. * indicates significantly (p<0.05) dysregulated genes per unpaired two-sample t-test (Welch's t-test).



Supplementary Figure 5: (A) Heatmap showing the changes in the transcript expression for mediators of proliferation, SASPs, p16 and p21-mediated signaling and B cell senescence (as identified using scRNA seq analyses) in ‘senescent niches’ of COPD and Sm donor lungs using targeted spatial analyses using Xenium technology. Data are normalized means of results from two randomly selected regions per tissue section from 8 tissue section/group. **(B)** Bar plot showing the top ligand-receptor interactions with interaction strength identified for each cell types, considering B cells as sender for NS lung donors following CellChat analyses in scRNA seq data set. **(C)** Bar plot showing the changes in the level of various immunoglobulin isotypes (IgG1, IgG2, IgG3, IgG4, IgA, IgM) in the plasma of COPD, Sm and NS donors as determined using immunoglobulin isotyping. Data is shown as mean $\pm$ SEM in n = 7-8 / group.

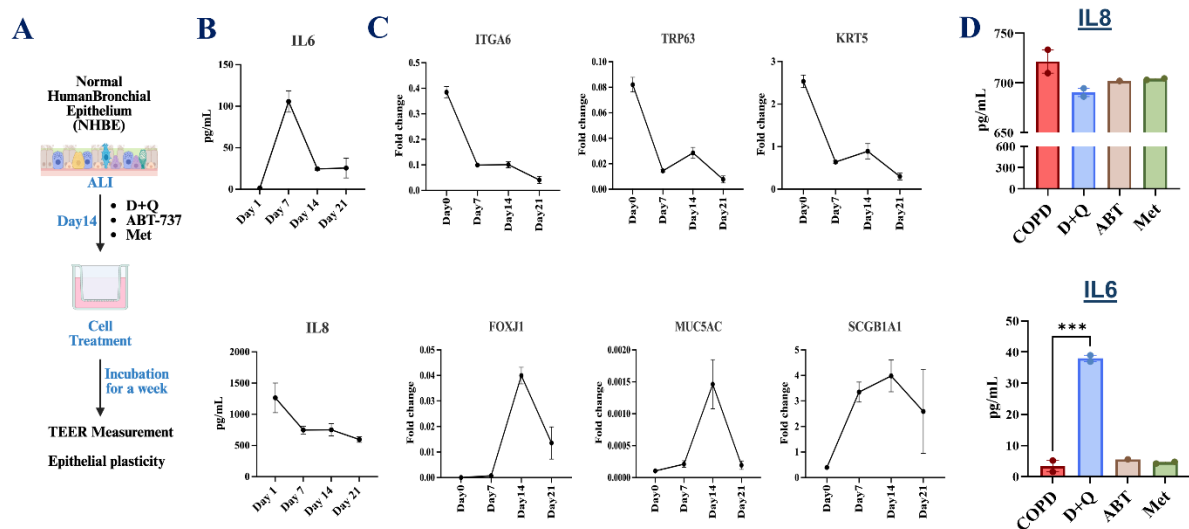


Supplementary Figure 6: Heatmap showing the significant ($p < 0.05$) DEGs from **(A)** AT2 and **(B)** MNN cell clusters in NS, Sm and COPD donor lungs as determined by DESeq2 analyses of scRNA seq data. Data shows the normalized mean of the counts data for $n = 4-5$ /group.



Supplementary Figure 7: (A) Schematics showing the cell intervention experiment using treatment with Dasatanib + Quercetin (D+Q), ABT-737 (ABT) or Metformin (Met) in small airway epithelial cells (SAEC; grown as submerged culture) from age- and sex-matched NS and COPD donors.

Dose-response Curve showing the **(B)** cytotoxicity and **(C)** Cell Viability of small airway epithelial cells (SAEC) from age- and sex-matched healthy and diseased (COPD) donors upon treatment with varying doses of dasatanib + quercetin (D+Q), ABT-737, and Metformin. The chosen drug doses have been indicated with the dotted arrows on the graphs.



Supplementary Figure 8: (A) Schematics showing cell intervention studies performed using diseased-human bronchial epithelial (D-HBE) cells from COPD donor cultured in air-liquid interface (ALI).

Characterization of the differentiation of ALI culture using D-HBE cells by quantifying the release of **(B)** epithelial plasticity markers (IL6 and IL8) in cell media and **(C)** gene expression of epithelial differentiation markers for basal (KRT5, TRP63, ITGA6), ciliated (FOXJ1) and club/goblet (MUC5AC, SCGB1A1) cells at Days 0, 7, 14 and 21 of ALI culture.

(D) The changes in the release of IL6 and IL8 upon 7-day treatment of D-HBE with D+Q, ABT or Met. Data is shown as mean $\pm$ SEM from 2 replicates per treatment from one independent experiment. *** $p < 0.0009$ vs untreated COPD per one-way ANOVA for multiple comparisons.