METHODS

Single cell RNA-seq using 3' v3 and 5' v1 chemistries (10X Genomics) was performed on 11 explanted lung tissue samples, including both SSc-ILD and healthy control lungs (Table S1). In addition, we analyzed five of the lung samples by scATAC-seq. Several of the lung samples underwent negative selection to partially deplete overepresented cell populations (Table S1). ScRNA-seq and scATAC-seq was performed on lung tissue obtained from previously healthy organ donors whose lungs could not be utilized for transplant and SSc-ILD patients at the time of lung transplant. Protocols for research collection of control lungs was approved by the University of Pittsburgh, Committee for Oversight of Research and Clinical Training Involving Decendents and for research collection of explanted diseased tissue by the University of Pittsburgh, Institutional Review Board. Single Cell Reagents (10X Genomics, 3' v3 or 5' v1 chemistries) were used for the library preparation samples after digestion as described by Valenzi et al. (13). Briefly, lung cells in single-cell suspensions were mixed with reverse transcription reagents, loaded onto a single-cell chip along with gel beads containing unique barcoded primers enabling cell-specific transcript identification, followed by separation into oil micro-droplet partitions by the Chromium instrument. Following partitioning, cell mRNAs were reverse transcribed and cDNAs amplified by PCR. Library quantification and sequencing of the scRNA-seq cDNA libraries was carried out by the UPMC Genome Center, on an Illumina NextSeq-500 (14). The number of cells per sample partitioned on the 10X Chromium instrument and sequencing reads per sample are provided in Table S1.

Healthy control and SSc-ILD lung samples were also analyzed for scATAC-seq (10X Genomics, Single Cell ATAC Reagent Kits, v1 Chemistry). The lung samples were digested into single cells, nuclei prepared by detergent treatment, followed by transposition of open nuclear DNA through hyperactive Tn5 transposase. Nuclei were then encapsulated in droplets with barcoding and sequencing by the UPMC Genome Center (Illumina NextSeq-500), followed by alignment of resulting sequences (Cell Ranger) with identification of open chromatin peaks and clustering of cell types on the basis of open chromatin patterns. Data were analyzed using the R packages Seurat v 4.0 (17) and Signac v 1.0.9004 (18), Loupe Browser 3.1.1 (10X Genomics) and Single-cell regulatory network inference and clustering (SCENIC) (19).