

Figure S1. Club cells, expressed the marker *SCGB1A1* (cluster 12), alveolar type I (AT1) cells expressed *AGER* (cluster 8), alveolar type II cells (AT2) expressed *SFTPC* (cluster 6), and ciliated cells expressed *FOXJ1* (cluster 9). *KRT5* expressing basal airway cells, and *MUC5B* expressing goblet cells were not found in discrete clusters. The rare cells expressing *KRT5* (basal cells) and *MUC5B* (goblet cells) are seen clearly upon reclustering of our combined IPF/control data, Figure 2D and Figure S15. Fibroblasts expressing *COL1A1* and smooth muscle cells expressing *DES* (cluster 7). Endothelial cells, expressing *VWF*, and lymphatic endothelial cells, expressing *LYVE1*, clustered in two distinct adjacent groups (clusters 4 and 11, respectively). Cells with pericyte markers *RGS5* were also distributed in cluster 4. Macrophages, identified by *AIF1* and *CD163* expression, were found in six different clusters, (clusters 0, 1, 3, 14 and 18). A small subset of *CD1C*-expressing dendritic cells were found in cluster 10. *TPSAB1*, a marker of mast cells, was expressed by cells in cluster 13. T lymphocytes, including *CD3* and *CD8A* positive cells, clustered together in cluster 2, while B lymphocyte lineage were marked with *MS4A1/CD20* cluster 15, including high expression of immunoglobulin genes (*IGKC*), and *MZB1*, a plasma cell marker. NK cells, expressing *GNLY*, were found in cluster 5.

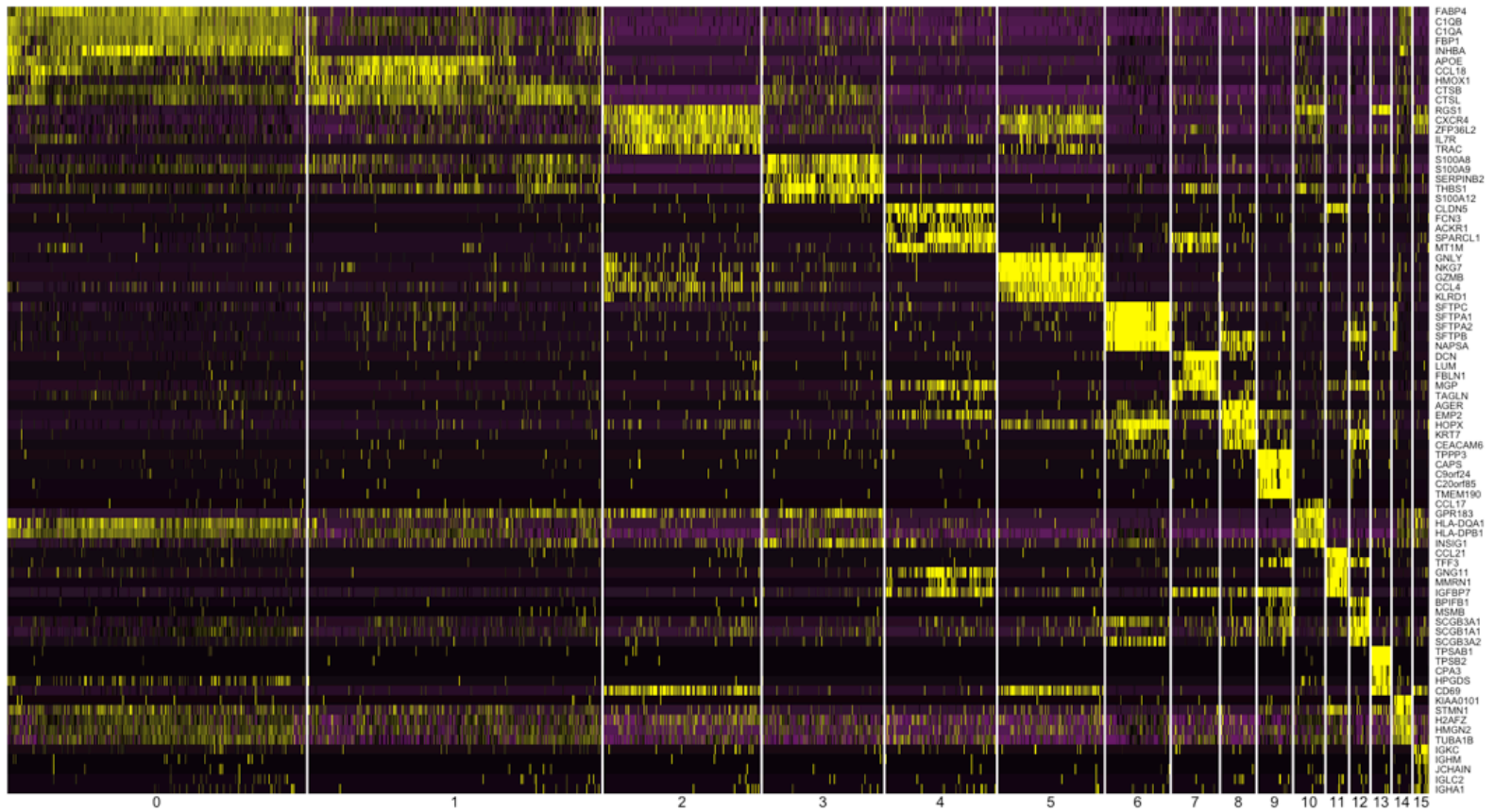
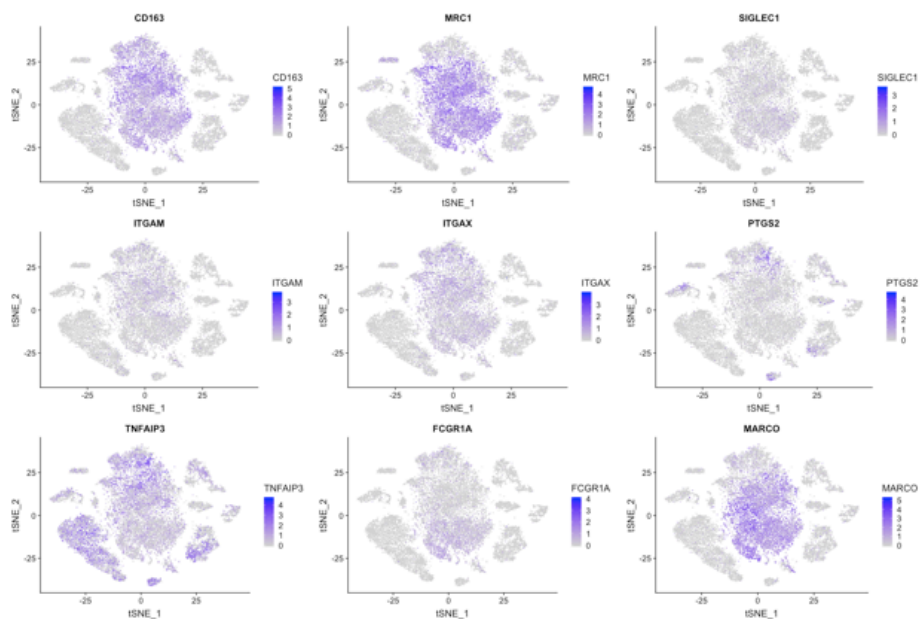
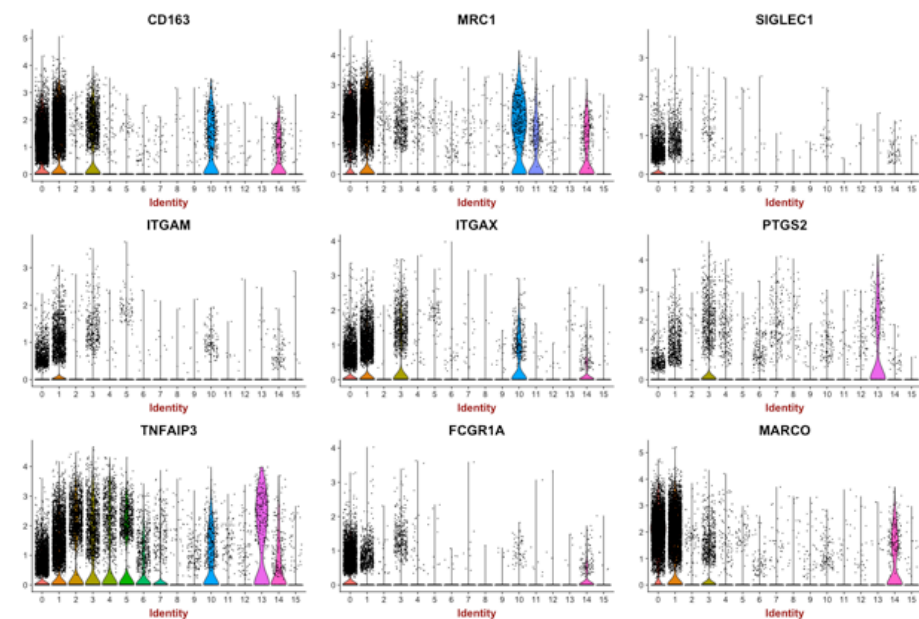


Figure S2. Clustering of top differentially expressed genes from scRNA-seq of normal lungs



A



B

Figure S3. Macrophage marker expression in scRNA-seq clusters from normal lungs. Feature plots (panel A) and Violin plots (panel B) show macrophage markers expressed by all macrophage/monocyte/DC populations (clusters 0, 1, 3, 10 and 14; CD163, ITGAX/CD11c) or restricted mainly to macrophage populations (clusters 0, 1 and 14; MRC1/CD206, SIGLEC1/CD169, ITGAM/CD11b, FCGR1A/CD64, MARCO)



Figure S4 Reclustering of macrophages from healthy lungs, cells colored by cluster (panel A) and by subject (panel B).

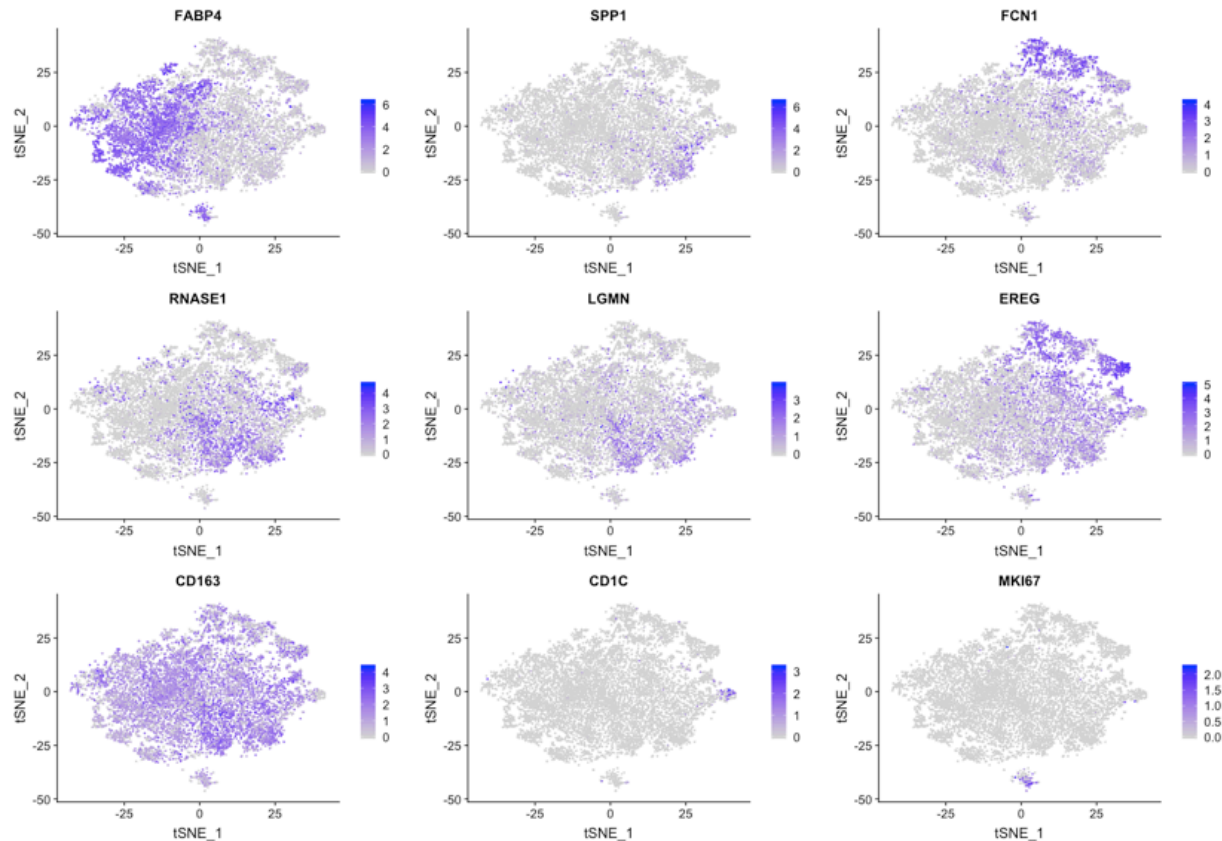


Figure S5 Reclustering of macrophages from healthy lungs, cells colored by gene expression (purple = high expression grey = low), showing FABP4 highly expressed by FABP4^{hi} macrophages; SPP1, LGMN and RNASE1 more highly expressed by SPP1^{hi} macrophages; CD163 and CD1C showing reciprocal expression in all macrophages and dendritic cells, respectively and FCN1 and EREG expressed more highly by FCN1^{hi} macrophages).

Figure S6 Breakout panels showing individual fluorescent channels and merged images staining from Figure 1E

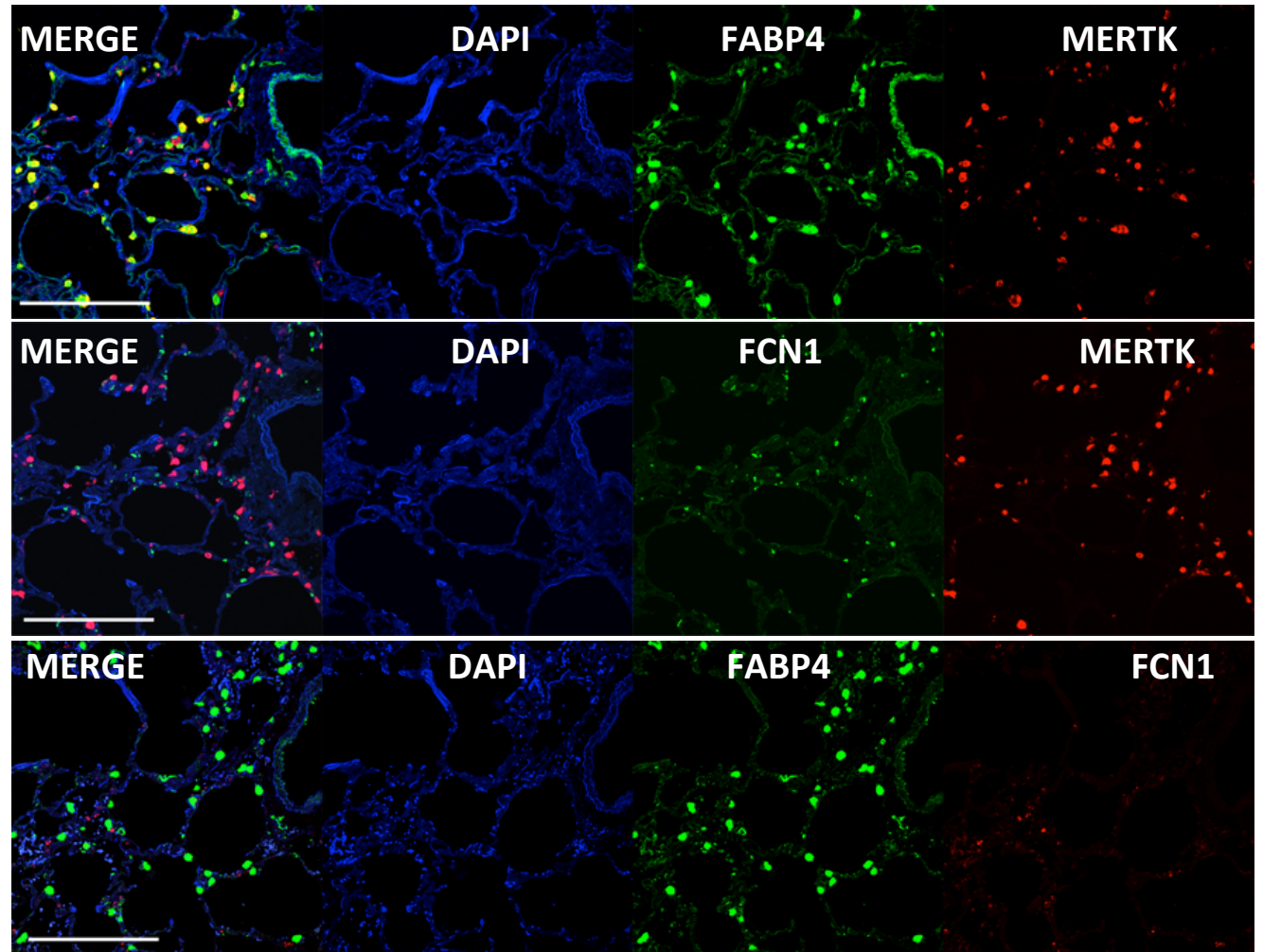
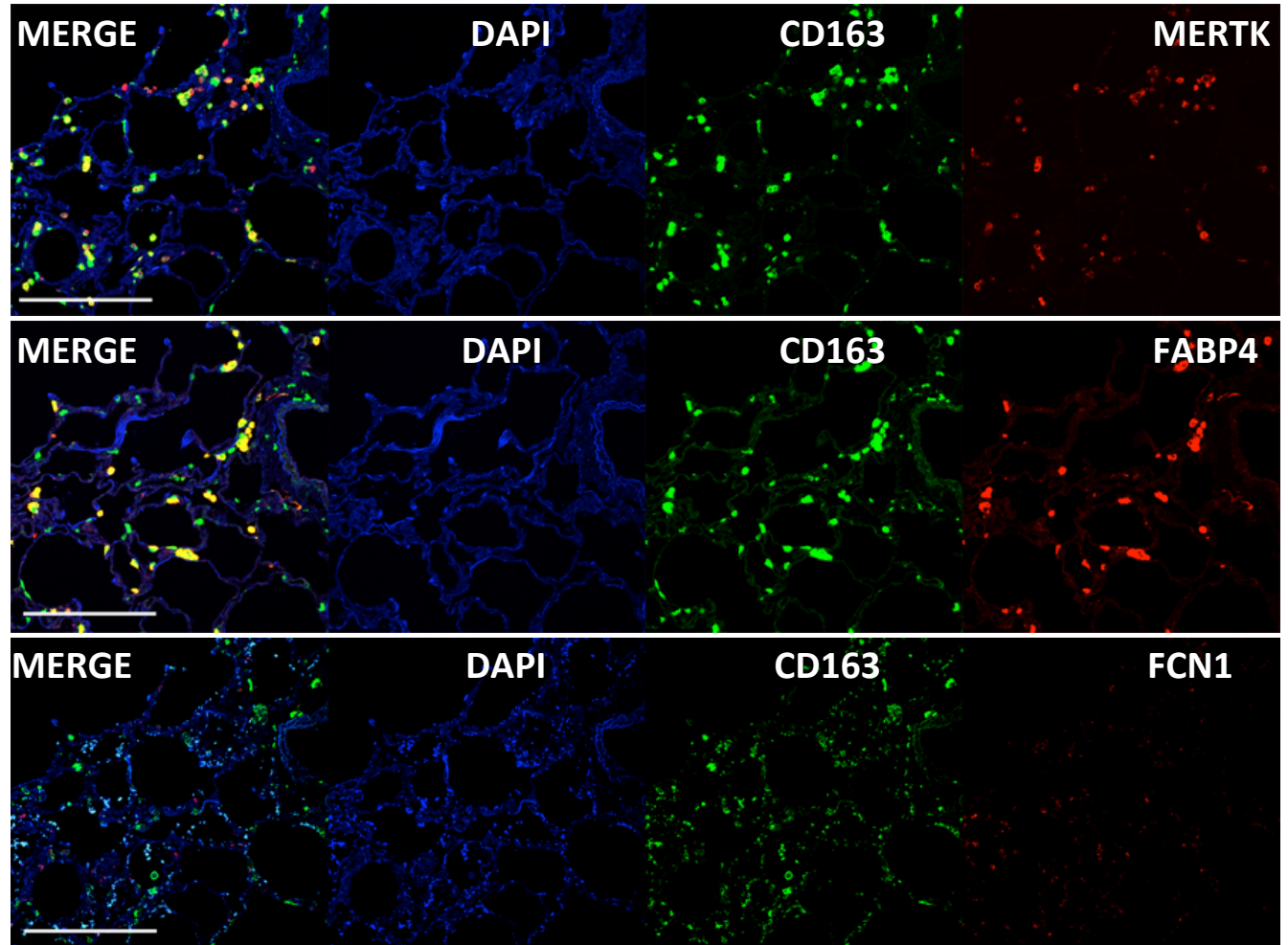
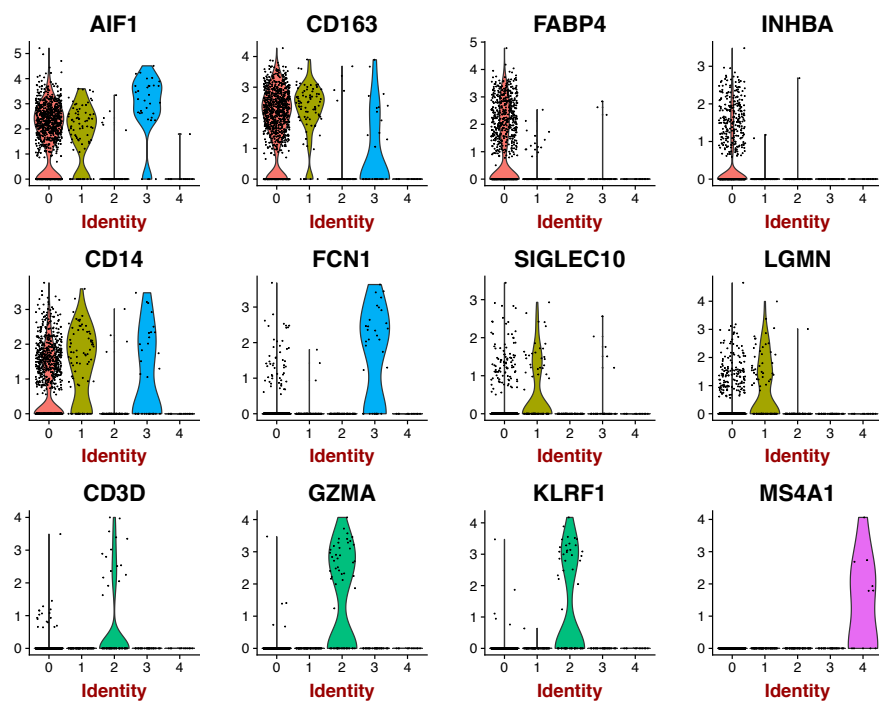
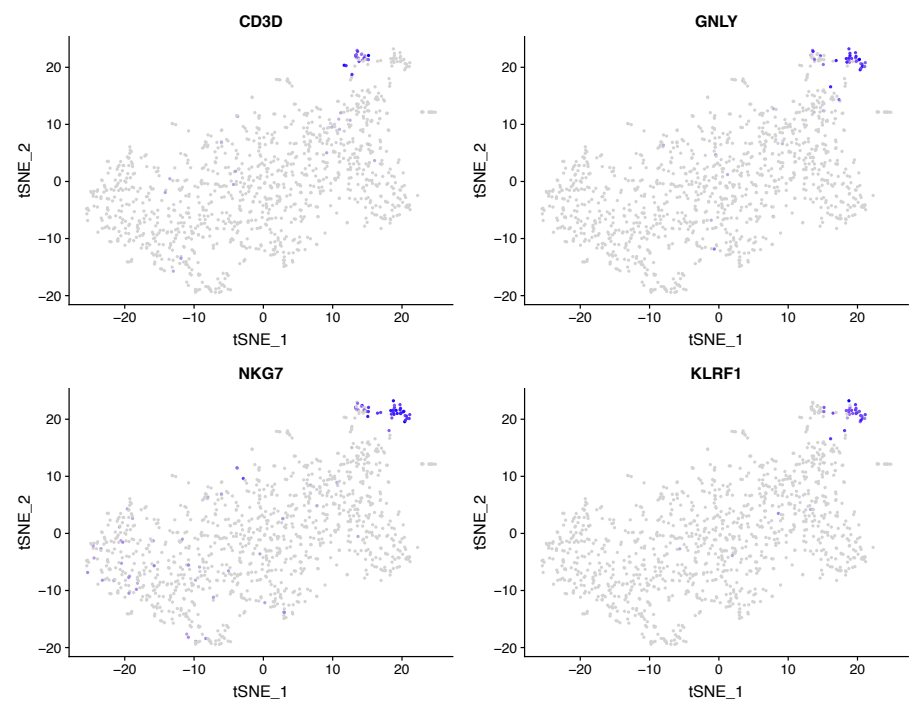


Figure S6 Breakout panels showing individual fluorescent channels and merged images staining from Figure 1E





A



B

Figure S7. Marker gene expression by cell clusters in healthy bronchoalveolar lavage (panel A) Cluster 2 includes subclusters of T cells (*CD3D*) and NK cells (*KLRF1*, *GNLY* and *NKG7*; panel B).

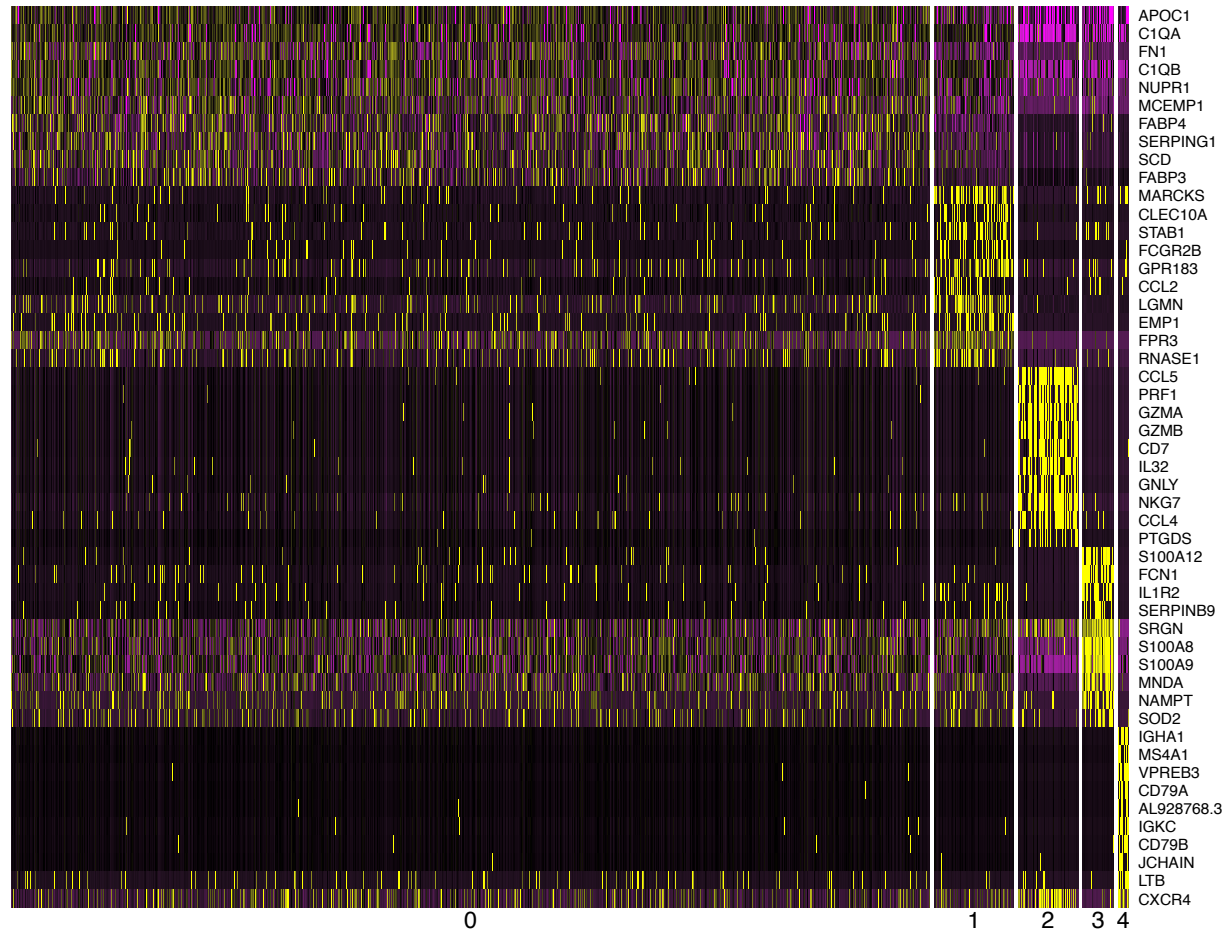


Figure S8. 10 top differentially expressed genes from scRNA-seq data of healthy bronchoalveolar lavage fluid

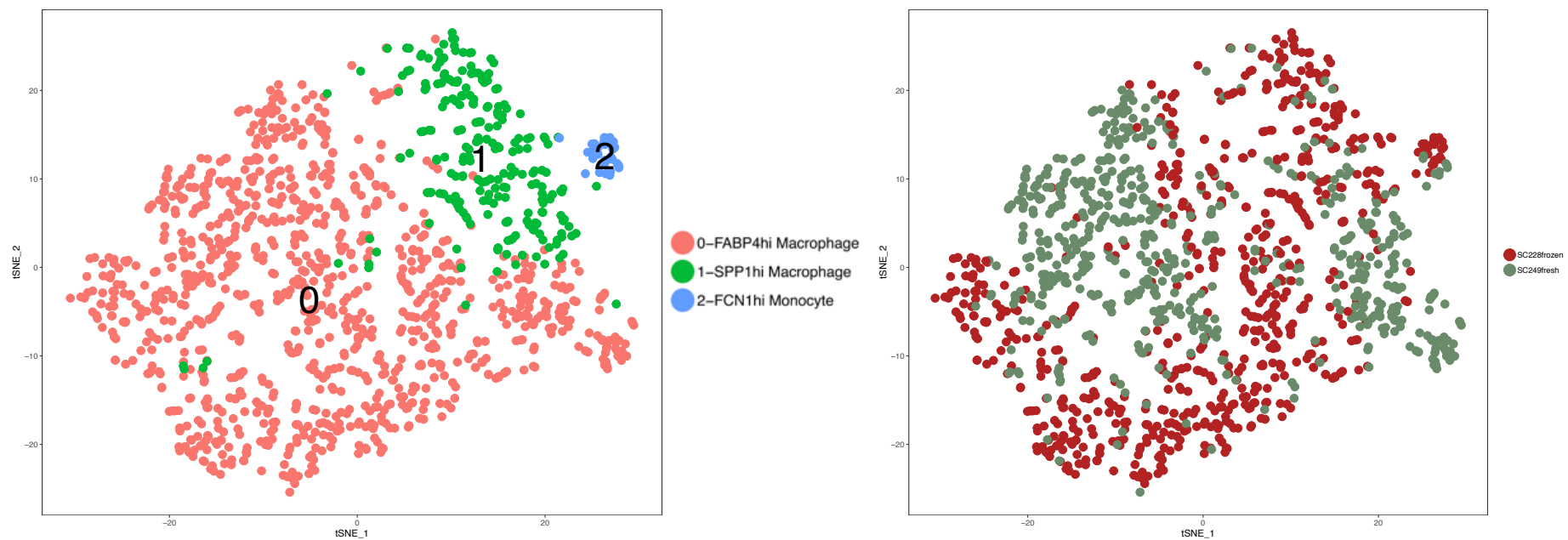


Figure S9. T-SNE plot recluster of macrophages from combined healthy lung BAL (SC228NORbal) and BAL with brushings (SC249NORbal), showing cells colored by cluster assignment (panel A) or by subject (panel B)

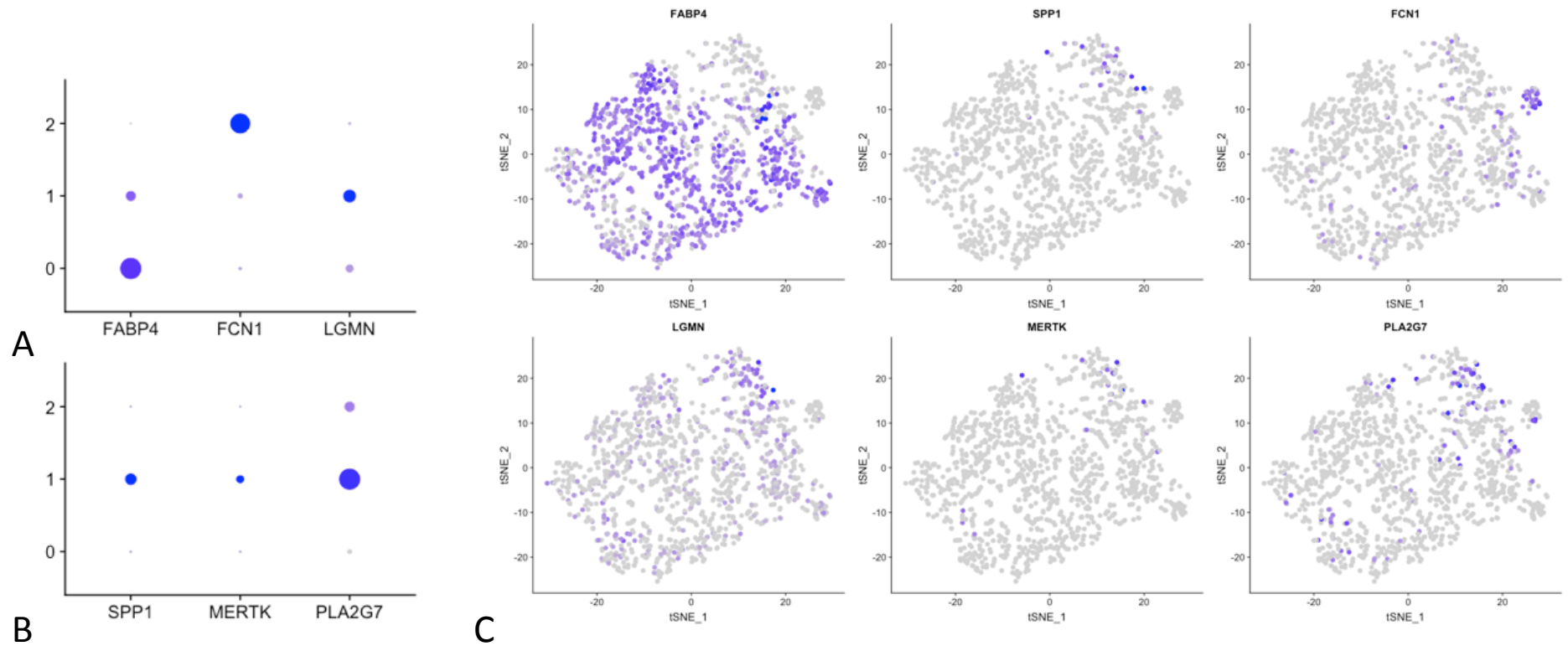
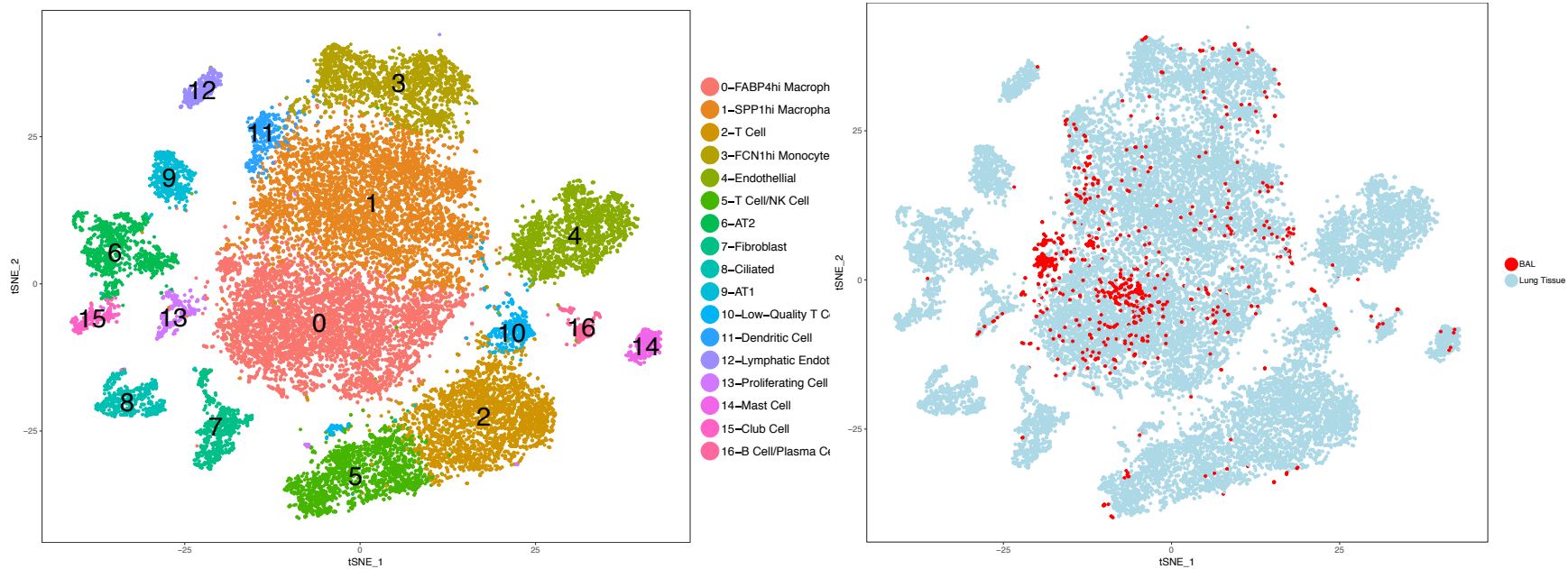


Figure S10. Dot plots (panels A and B) and feature plots (panel C) showing expression of marker genes for FABP4^{hi} (FABP4), SPP1^{hi} (SPP1, MERTK and PLA2G7) and FCN1^{hi} (FCN1) macrophages in BAL from two healthy control lungs



A

B

Figure S11. T-SNE plot of combined healthy lung tissues (SC14NOR, SC155NOR, SC156NOR, SC31NOR, SC31DNOR, SC45NOR, SC56NOR, SC59NOR), and healthy bronchoalveolar lavage fluid (SC228NORbal), showing cells colored by cluster assignment (panel A) or by subject (panel B).

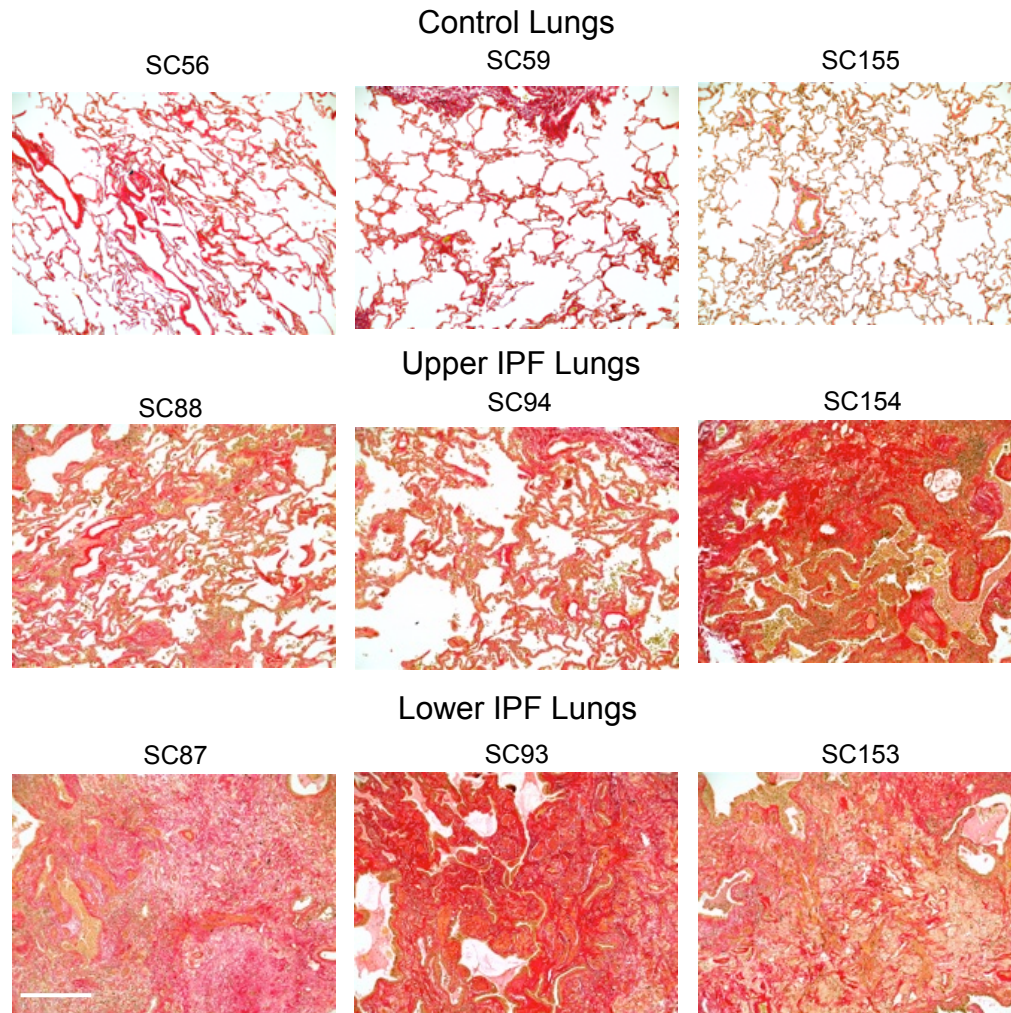
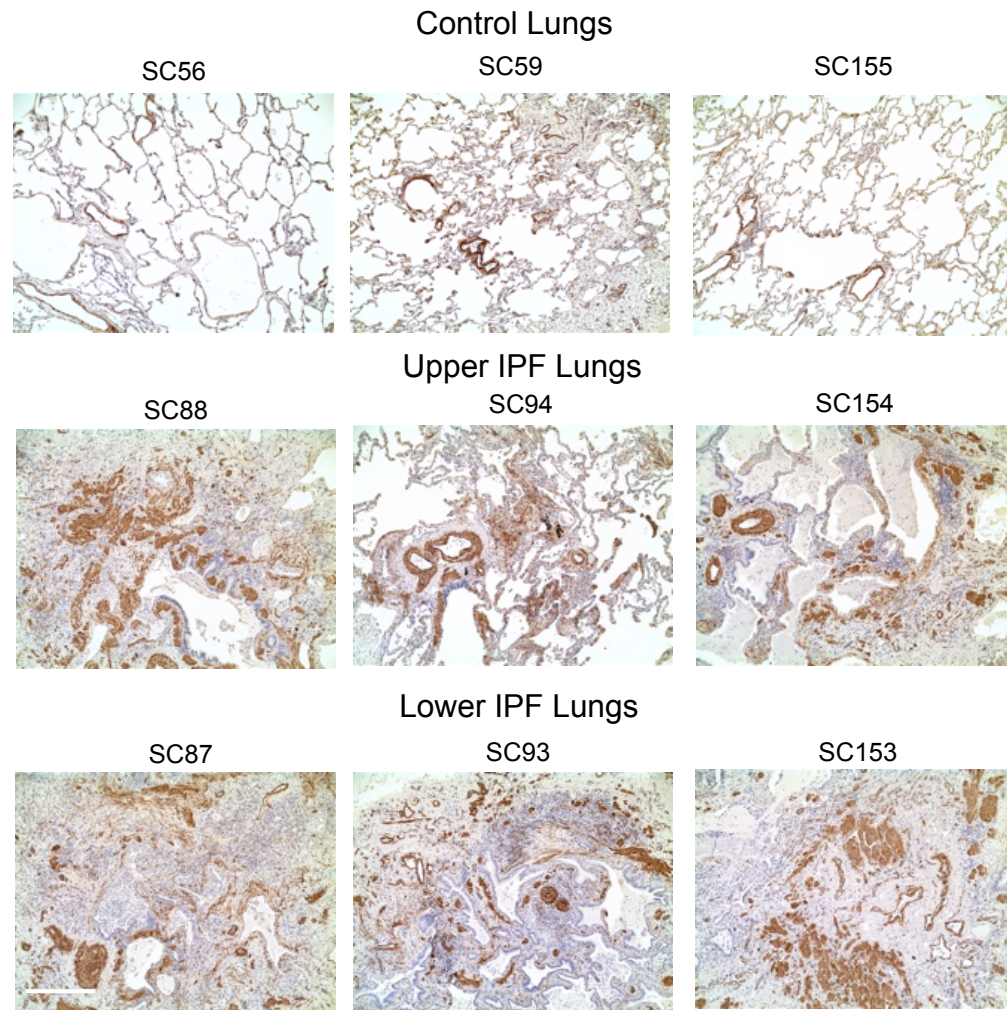


Figure S12A. Picro Sirius Red staining of control lung, and upper and lower lobe IPF lung tissues. Collagen staining (red) is seen in alveolar septa of control lungs, and densely and most prominently in lower lobes of fibrotic IPF tissues. (Scale bar = 400 μ m)

Figure S12B. Smooth muscle actin staining by immunohistochemistry of control lung, and upper and lower lobe IPF lung tissues. Perivascular smooth muscle/pericyte staining is seen in control lungs, and in addition staining of myofibroblasts is seen most prominently in lower lobes of IPF tissues (brown stain; scale bar = 400 μ m)



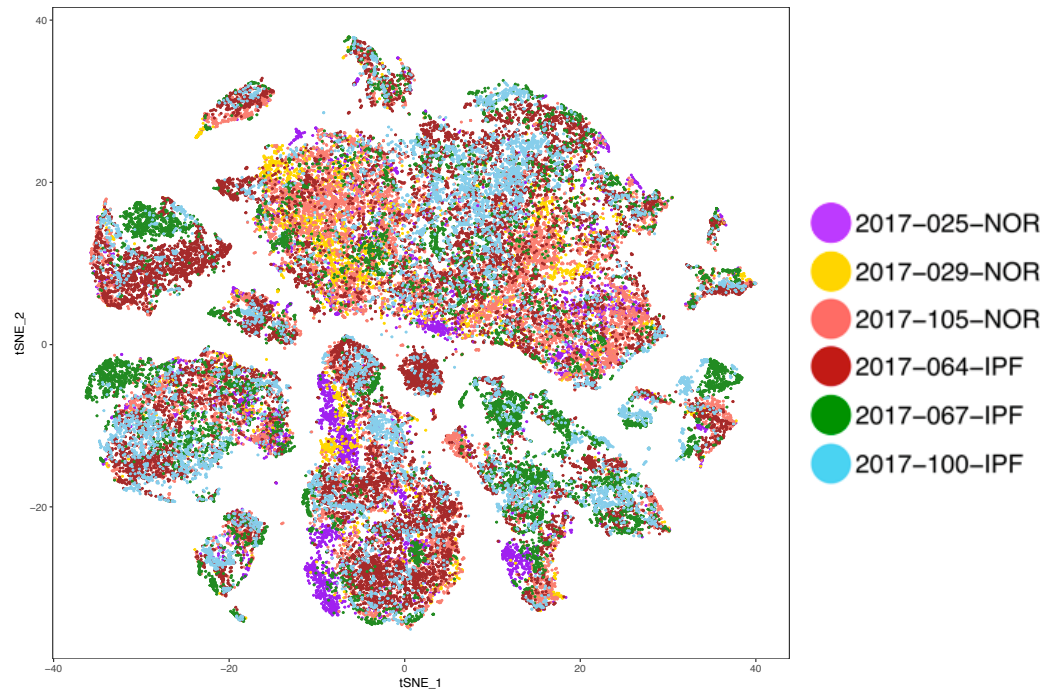


Figure S13. Combined analysis of healthy and IPF lungs, cells colored by subject

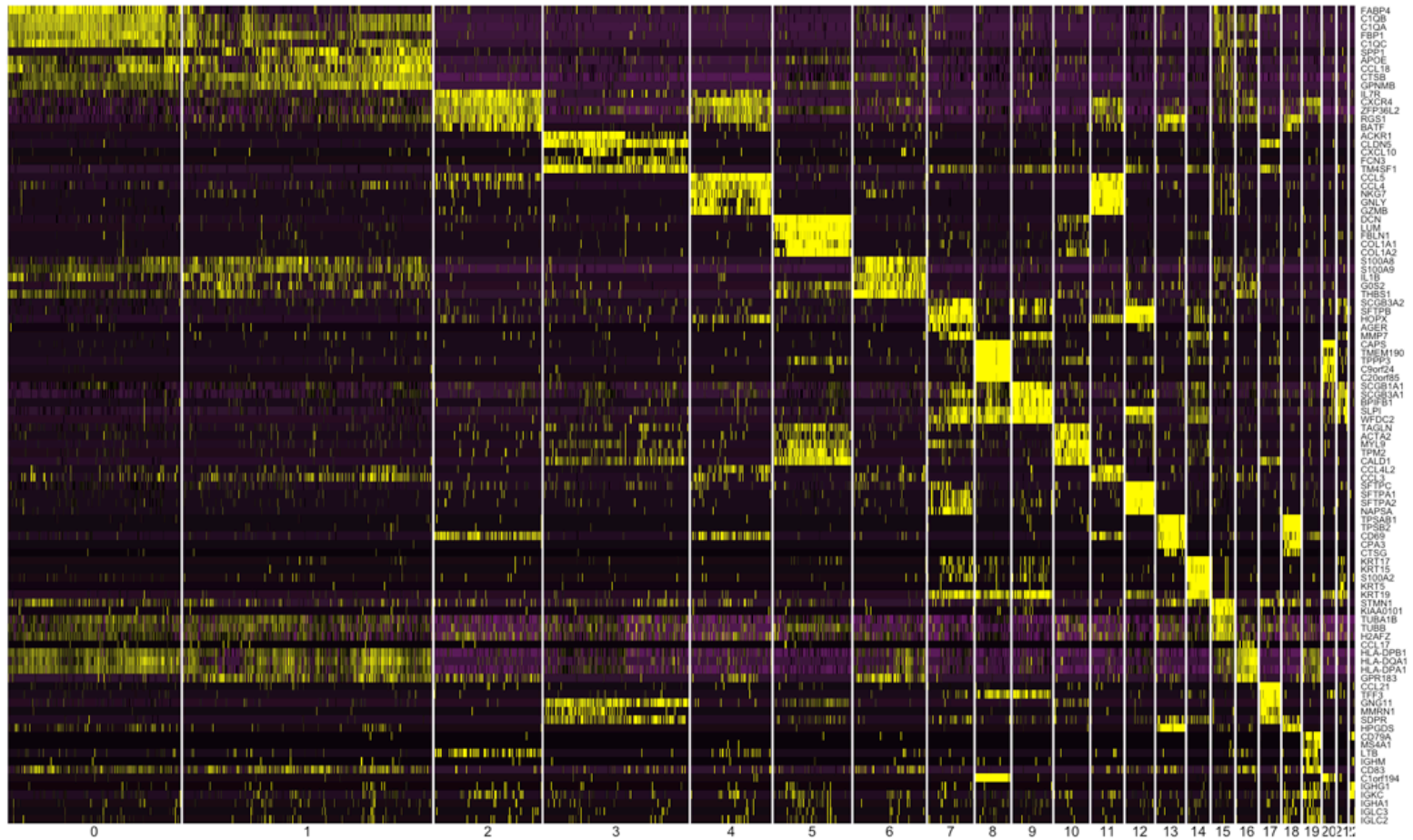


Figure S14. Clustering of top five differentially expressed genes from scRNA-seq of IPF and normal lungs

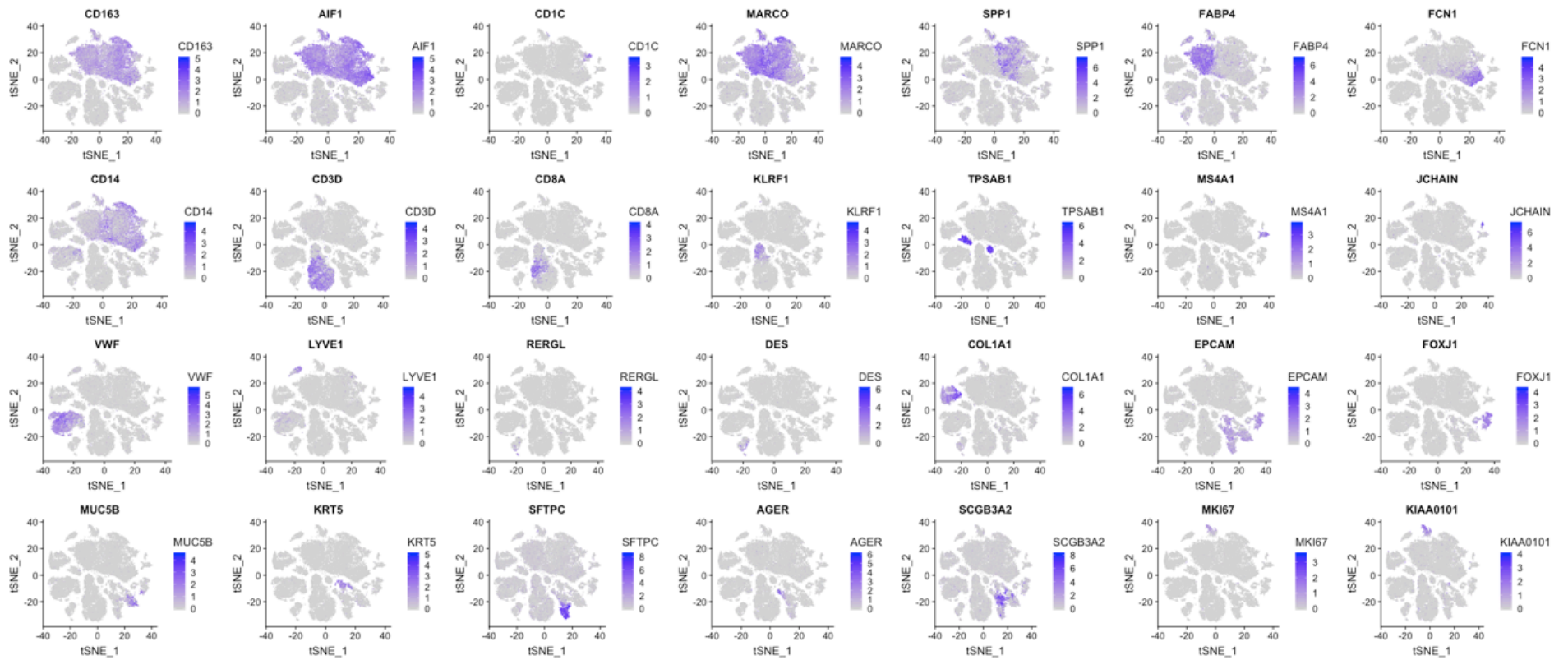


Figure S15. Feature plots of marker genes expressed from scRNA-seq of IPF and normal lungs

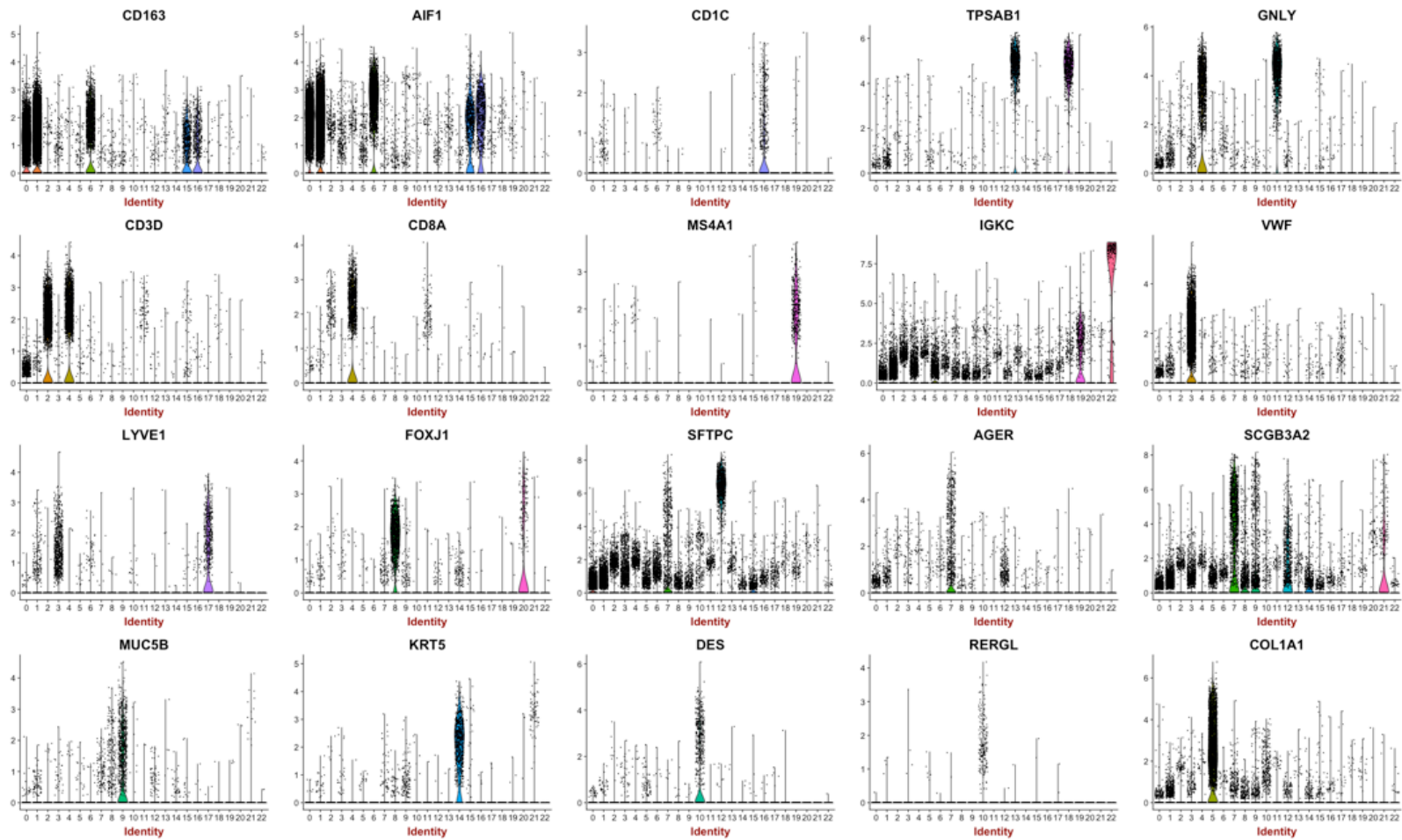


Figure S16. Violin plots of marker genes expressed from scRNA-seq of IPF and normal lungs

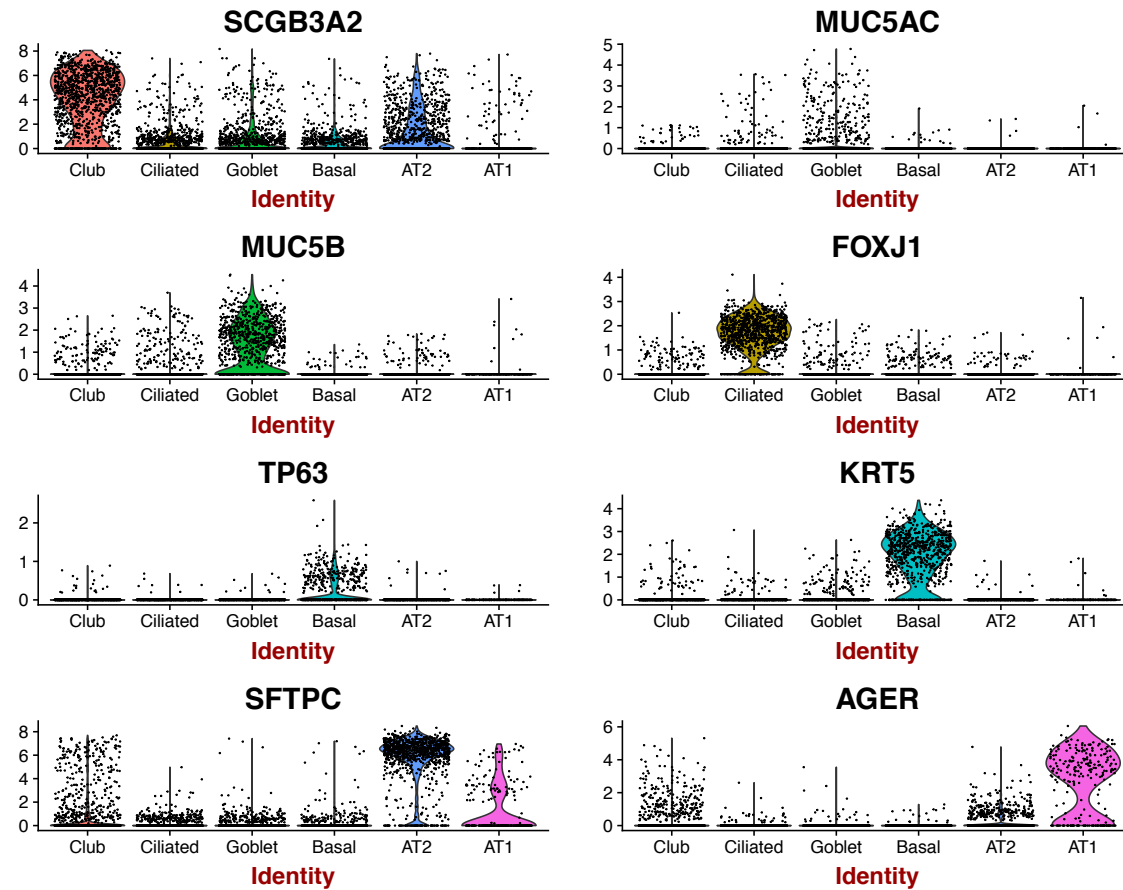


Figure S17. Violin plots of marker genes expressed from scRNA-seq of combined IPF and normal lungs in epithelial cell subclusters

Figure S18. Markers of AT1 and AT2 cells show distinct expression. Expression of high levels of AT1 marker AGER and AT2 marker SFTPC are larger confined to specific cell subsets with only very few cells expressing high levels of both. These cells may represent doublets or rare bipolar cells.

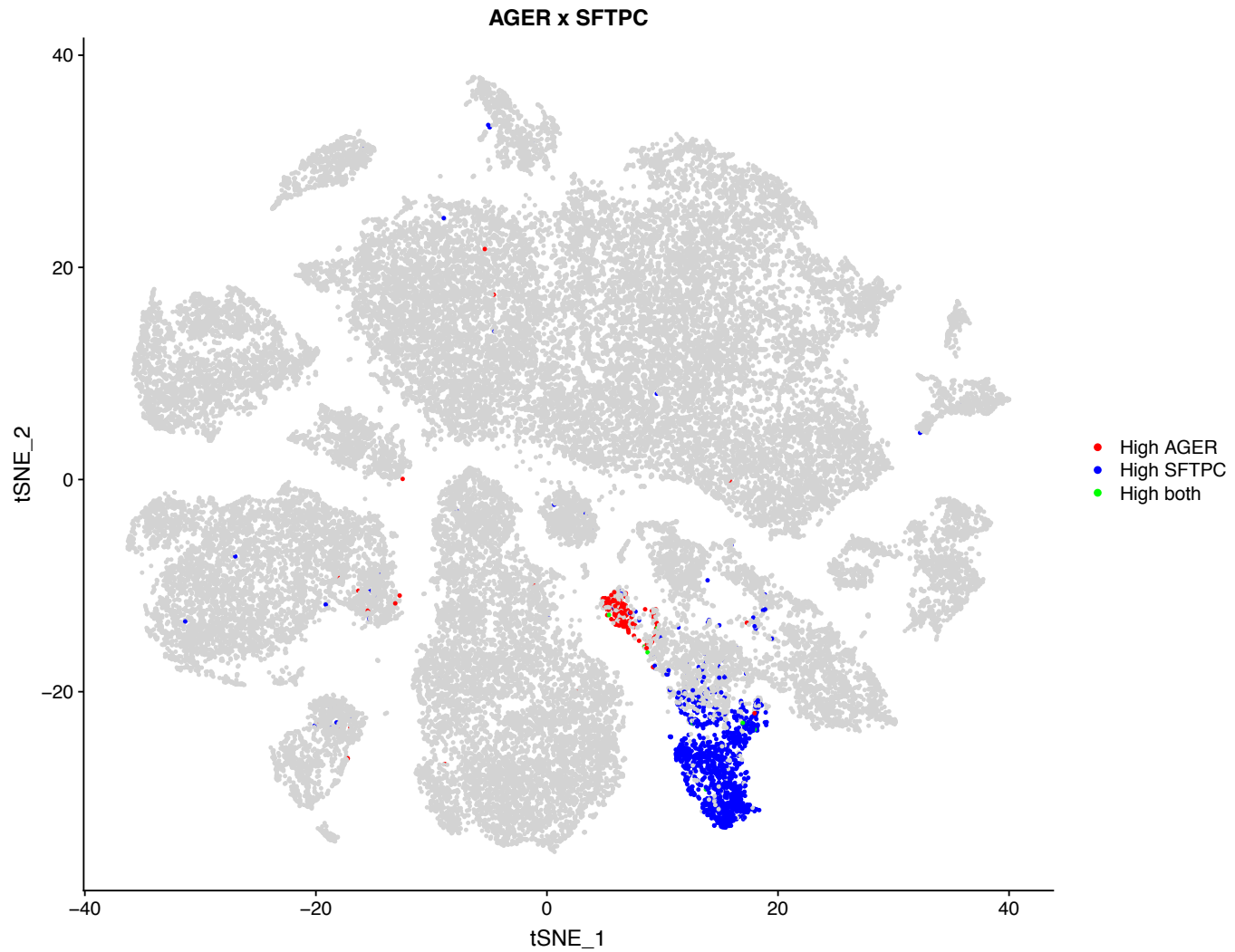
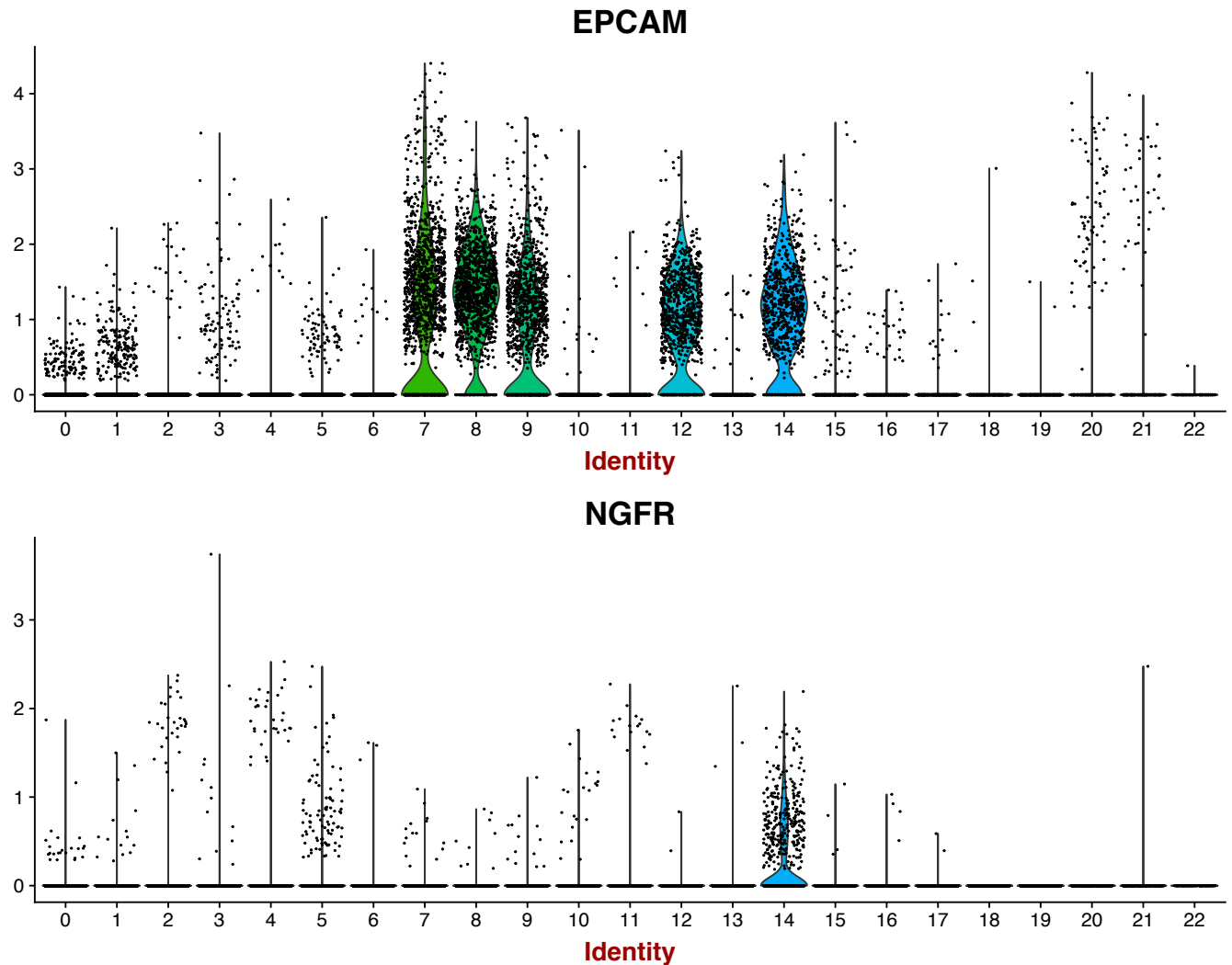
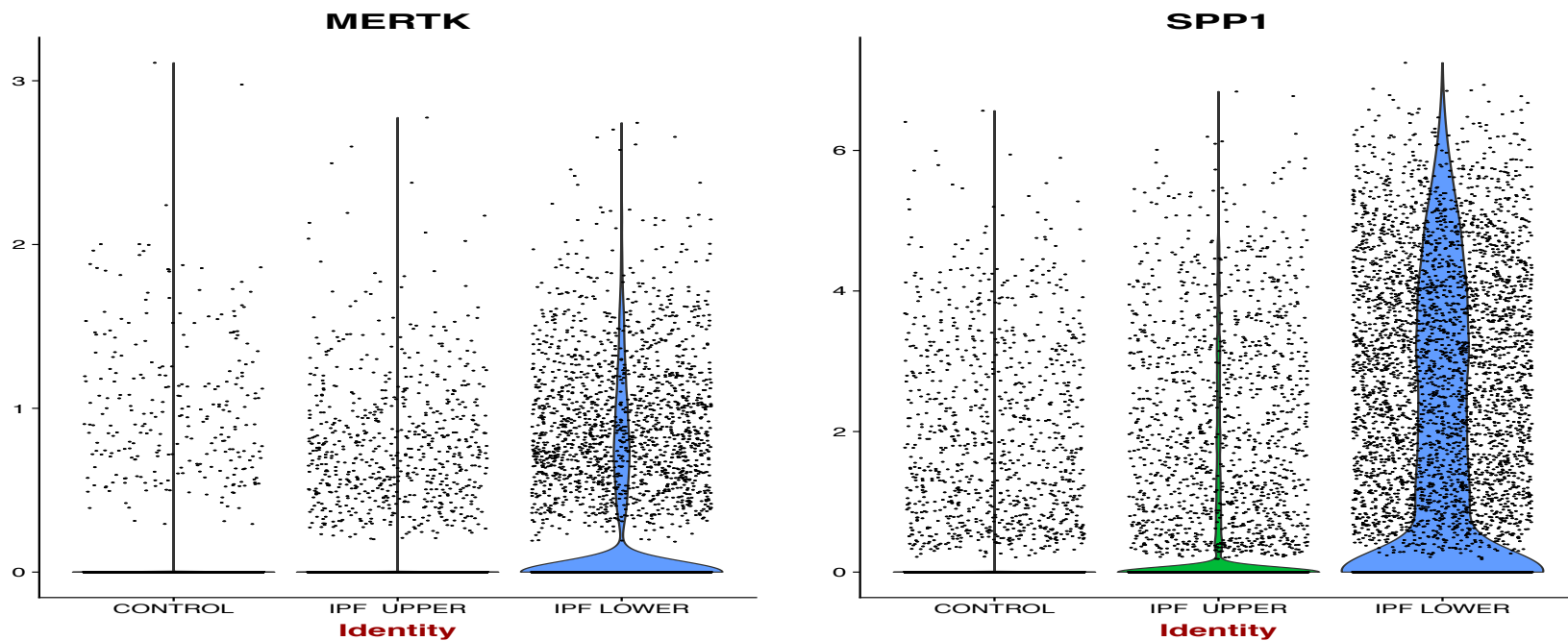


Figure S19. EPCAM and NGFR as markers of epithelial cells. EPCAM is expressed among all epithelial cell types, including, goblet (cluster 9), club and AT1 (cluster 7), ciliated (cluster 8), basal (cluster 14), and AT2 (cluster 12) while NGFR expression is elevated in basal cells (cluster 14).





MERTK gene expression				SPP1 gene expression			
Comparison	Z score	P-value	adjusted P-value	Comparison	Z score	P-value	adjusted P-value
CONTROL - IPF UPPER	-18.37804898	9.85E-76	9.85E-76	CONTROL - IPF UPPER	-19.91341385	1.56E-88	1.56E-88
CONTROL - IPF LOWER	-45.57615762	0	0	CONTROL - IPF LOWER	-67.75375612	0	0
IPF UPPER - IPF LOWER	-21.81872933	7.70E-106	1.16E-105	IPF UPPER - IPF LOWER	-39.18994918	0	0

Figure S20: MERTK and SPP1 gene expression is highly statistically significantly upregulated in IPF, comparing either upper or lower lobes with healthy controls or comparing IPF lower lobes with upper lobes

Figure S21: IF staining of idiopathic pulmonary fibrosis in upper and lower lung tissues. (a) Double IF staining of IPF upper lobe major lung macrophage population SPP1 (green) co-stained with FABP4 (red). (b) Double IF staining of IPF lower lobe major lung macrophage population SPP1 (green) co-stained with FABP4 (red). (c) Double IF staining of IPF upper lobe major lung macrophage population MERTK (green) co-stained with FABP4 (red). (d) Double IF staining in IPF lower lobe of major lung macrophage population MERTK (green) co-stained with FABP4 (red) MERTK is notably expanded in lower lobe as compared to upper lobe. For all panels, nuclei are counterstained with DAPI (blue). Scale bar = 100 μ m. IF, immunofluorescence. IPF, idiopathic pulmonary fibrosis.

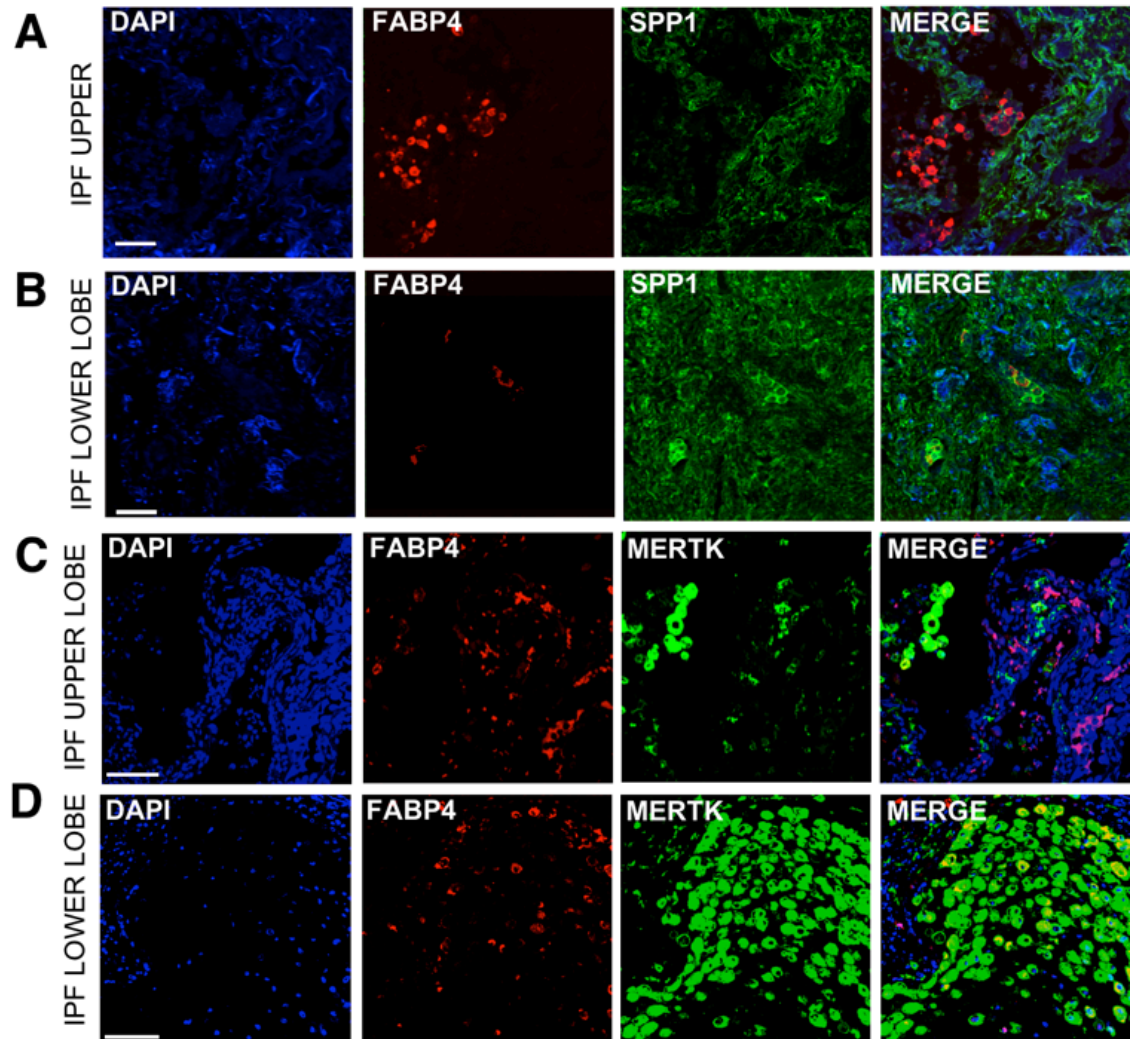


Figure S22: IF staining of serial sections of idiopathic pulmonary fibrosis lower lobe lung tissues, staining with combinations of MERTK, FABP4, FCN1 and CD163 as indicated. For all panels, nuclei are counterstained with DAPI (blue). Scale bar = 500 μ m. IF, Immunofluorescence.

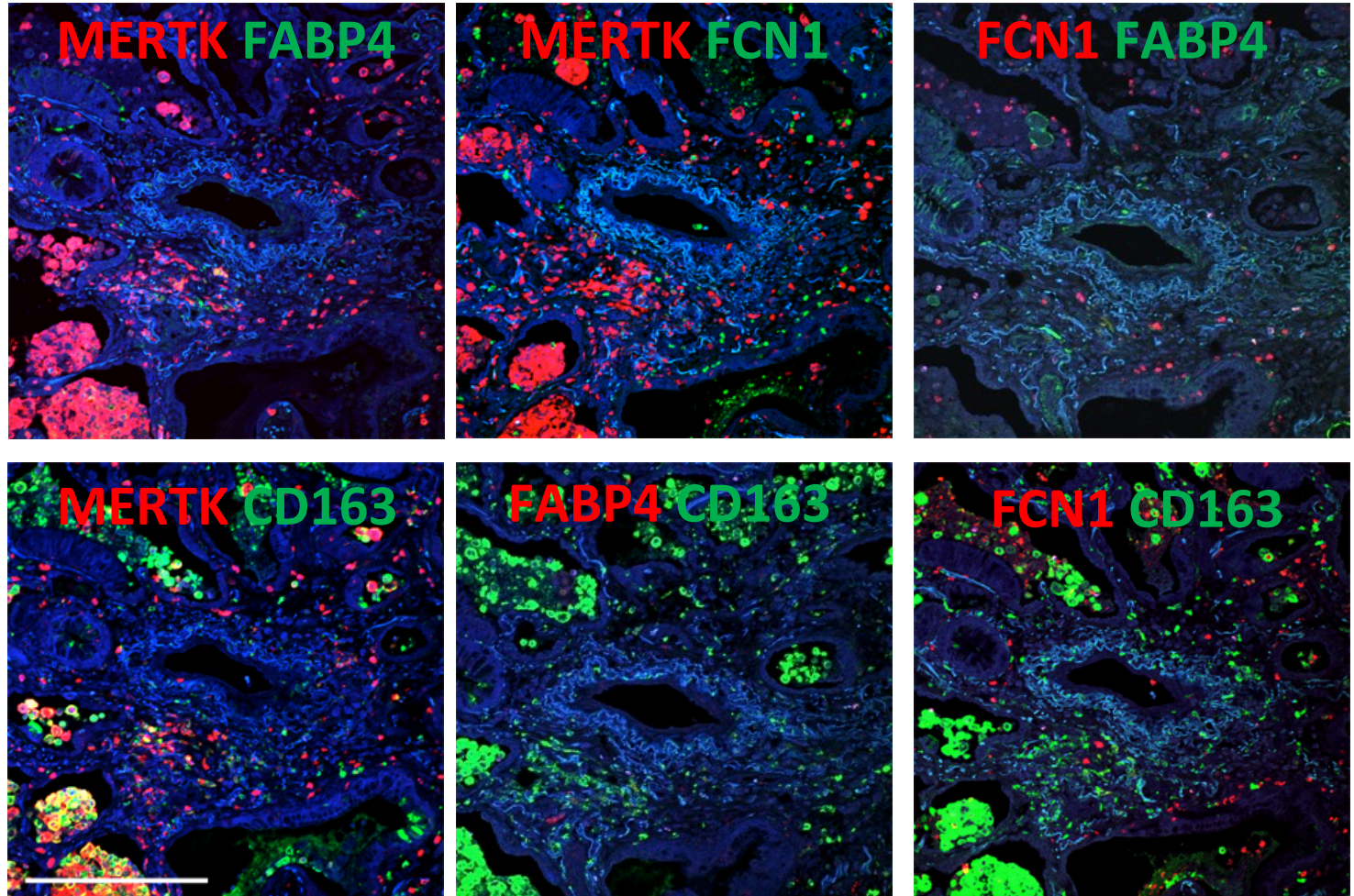


Figure S22: Merged and breakout micrographs of IF staining of serial sections of idiopathic pulmonary fibrosis lower lobe lung tissues, staining with combinations of FABP4 x MERTK; FCN1 x MERTK; and FABP4 x FCN1, as indicated. For all panels, nuclei are counterstained with DAPI (blue). Scale bar = 500 μ m.

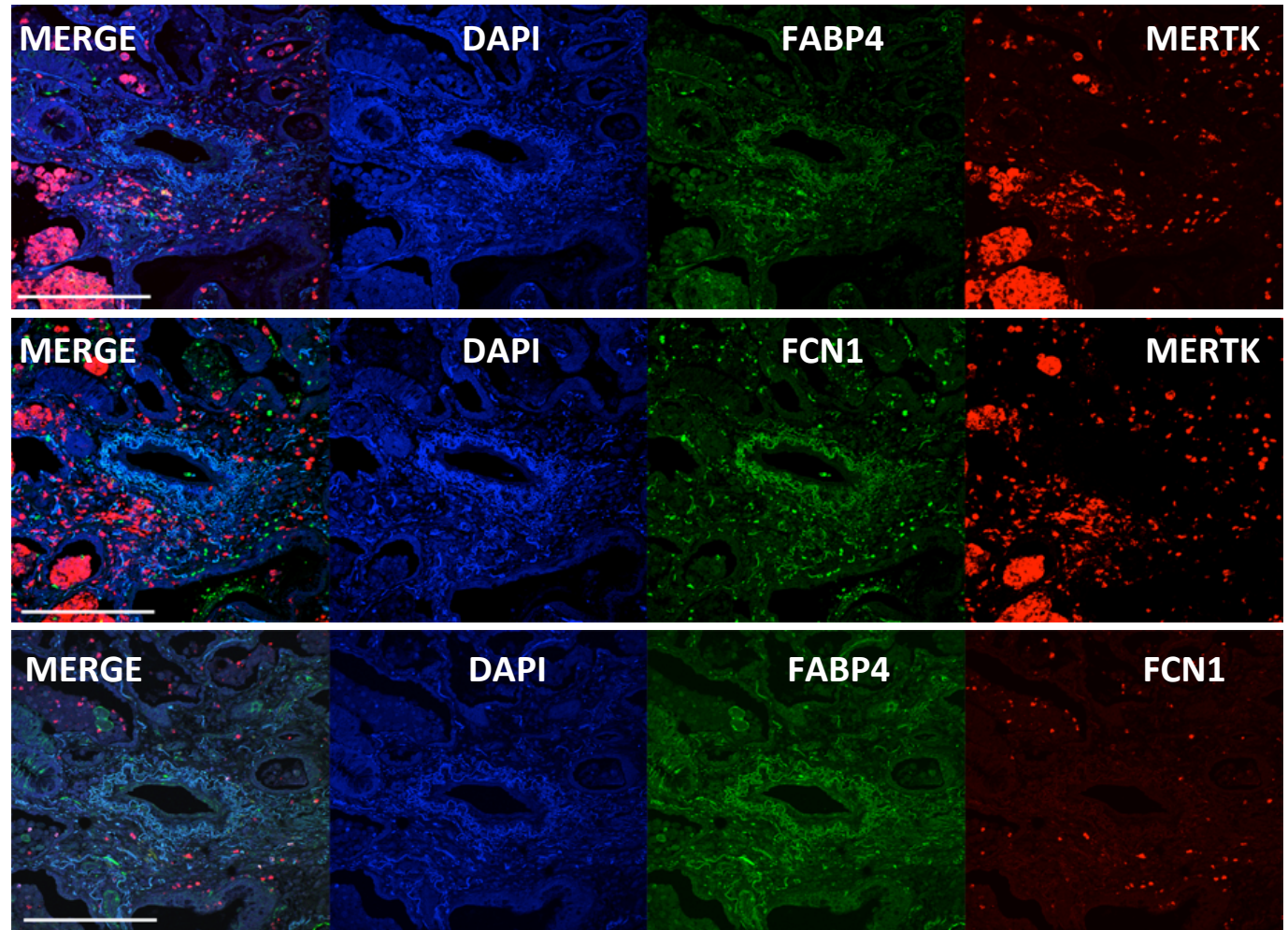


Figure S22: Merged and breakout micrographs of IF staining of serial sections of idiopathic pulmonary fibrosis lower lobe lung tissues, staining with combinations of MERTK x CD163; FABP4 x CD163; and FCN1 x CD163, as indicated. For all panels, nuclei are counterstained with DAPI (blue). Scale bar = 500 μ m.

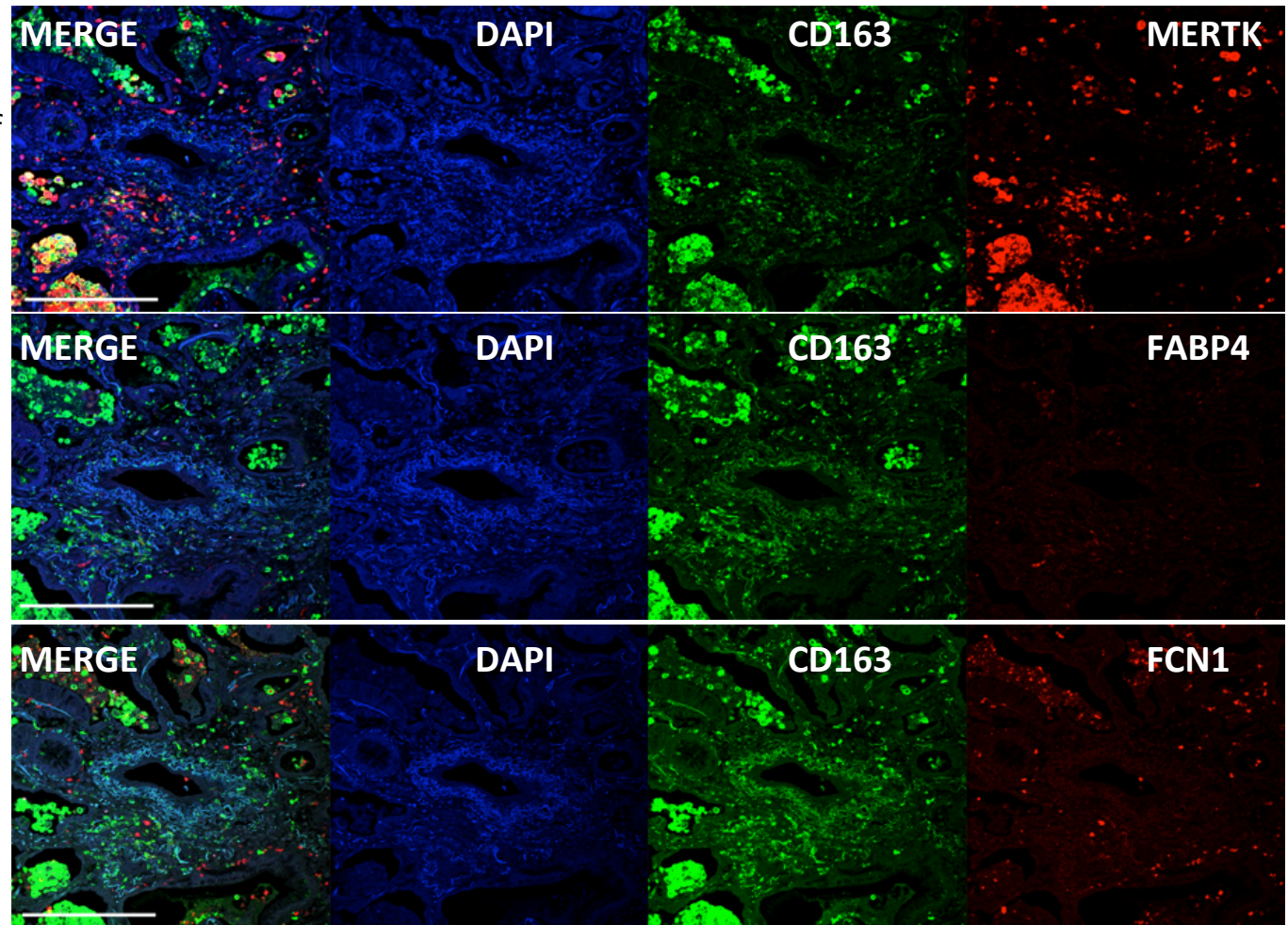
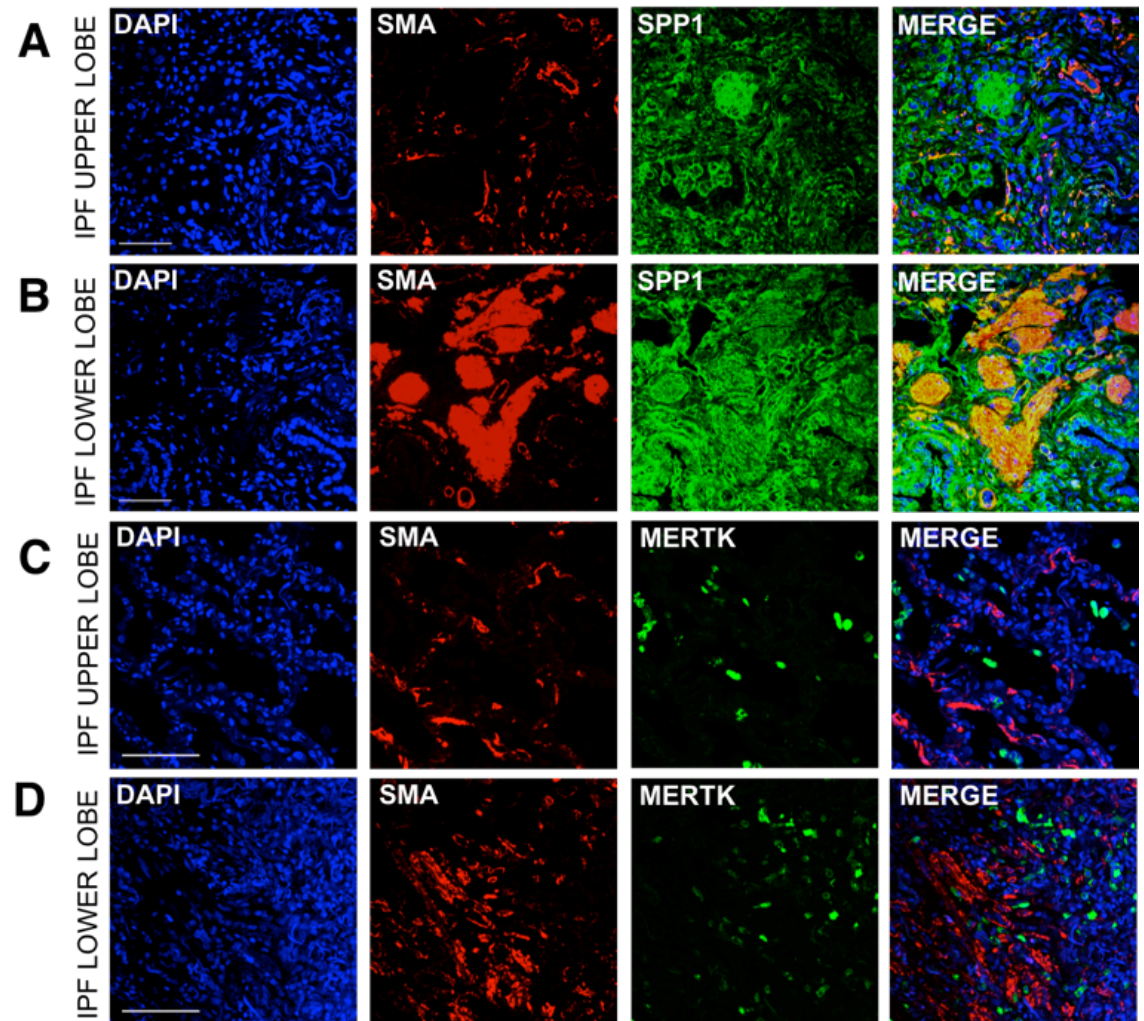


Figure S23: IF staining of SMA in both upper and lower lung tissues of idiopathic pulmonary fibrosis. (a) Double IF staining of IPF upper lobe major lung macrophage population SPP1 (green) costained with SMA (red). (b) Double IF staining of IPF lower lobe major lung macrophage population SPP1 (green) co-stained with SMA (red) dense regions of SMA indicate fibroblastic foci. (c) Double IF staining of IPF upper lobe major lung macrophage population MERTK (green) co-stained with SMA (red). (d) Double IF staining in IPF lower lobe of major lung macrophage population MERTK (green) co-stained with SMA (red) For all panels, nuclei are counterstained with DAPI (blue). Scale bar = 100 μ m. IF, immunofluorescence. IPF, idiopathic pulmonary fibrosis. SMA, smooth muscle actin.



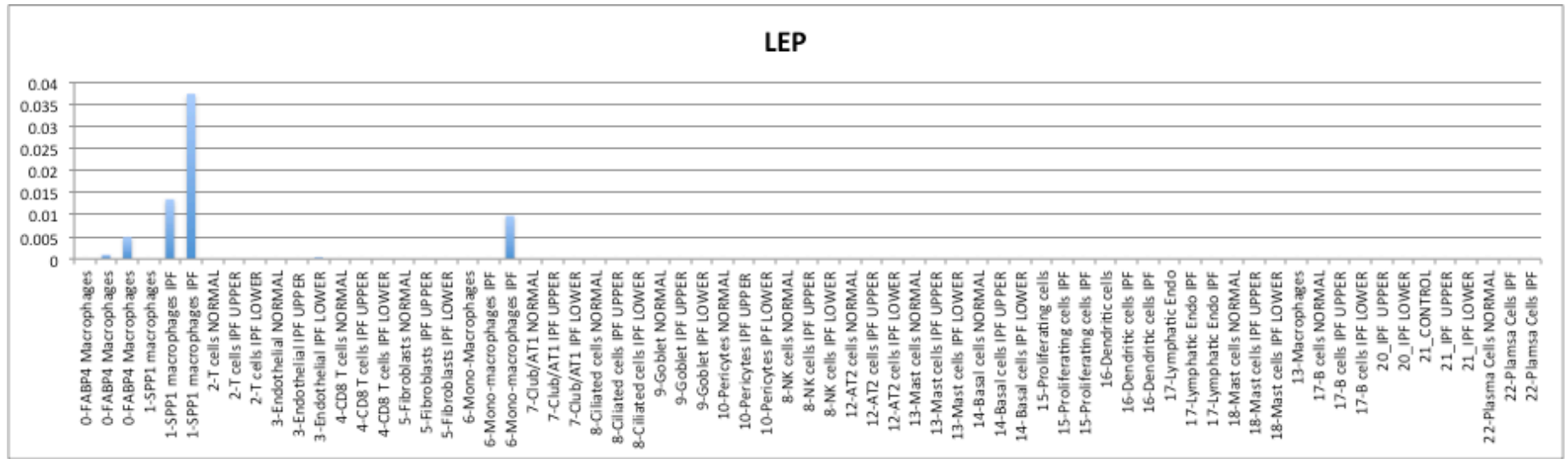
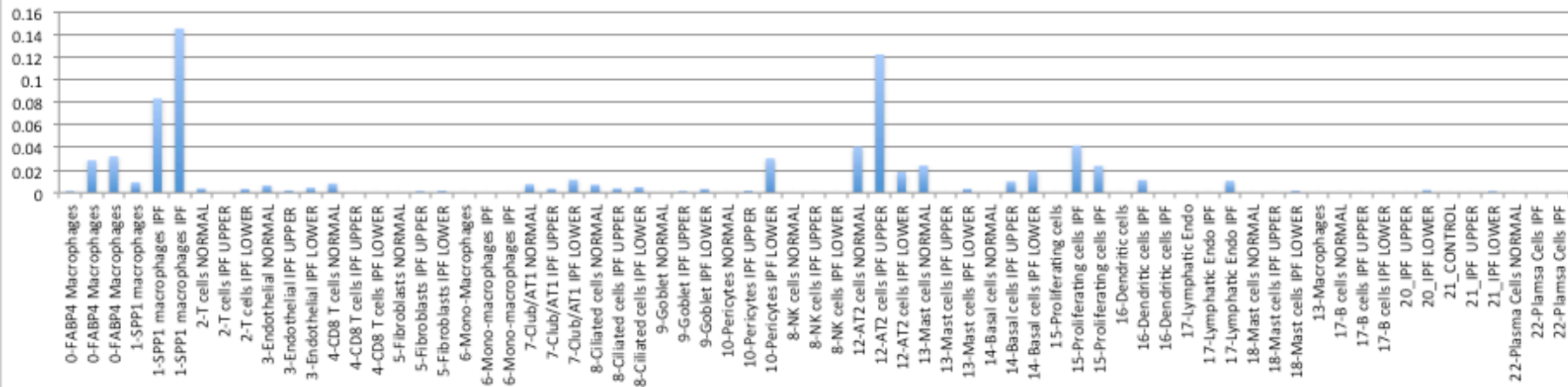
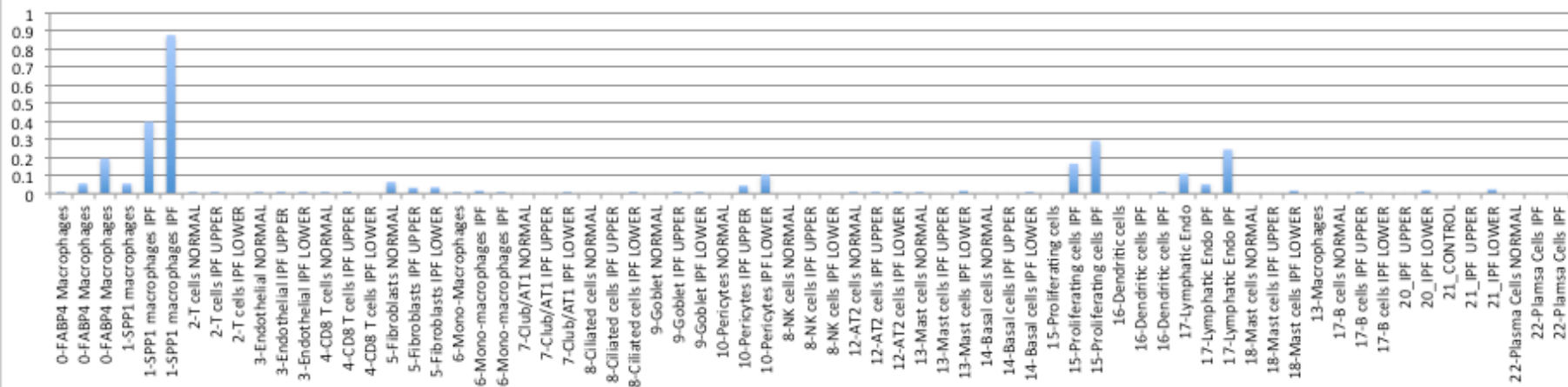


Figure S24. Genes selectively upregulated in lower lobes of IPF compared to healthy control lungs in SPP1^{hi} macrophages: LEP (nd), KCNJ5 (15.76-fold), HS3ST2 (15.58-fold), SPP1 (7.56-fold), SIGLEC15 (7.43-fold), ATP6V0D2 (7.12-fold), LGMN (6.07-fold), MERTK (4.96-fold), MMP9 (3.81-fold), and CD209 (1.86-fold).

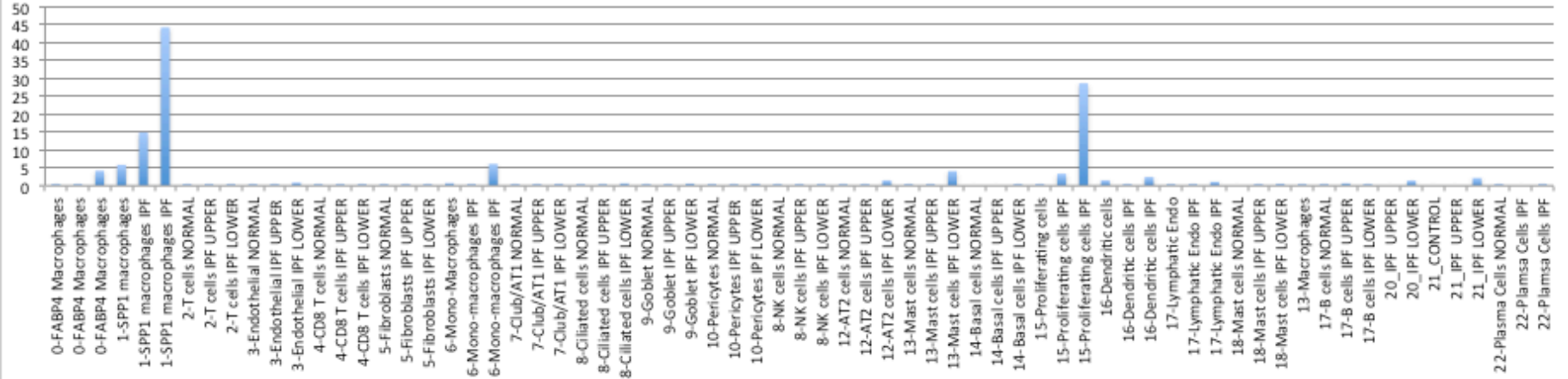
KCNJ5



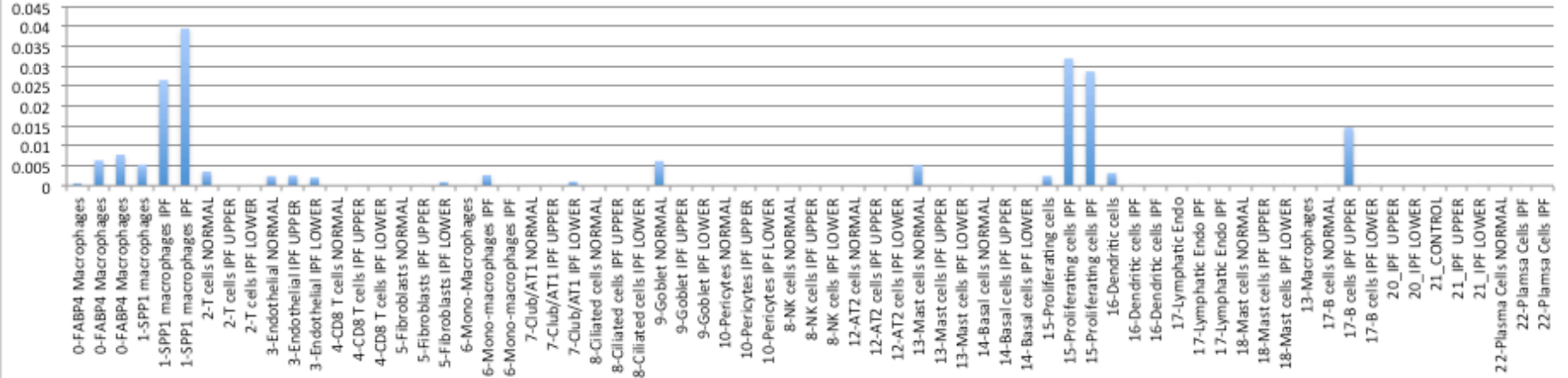
HS3ST2



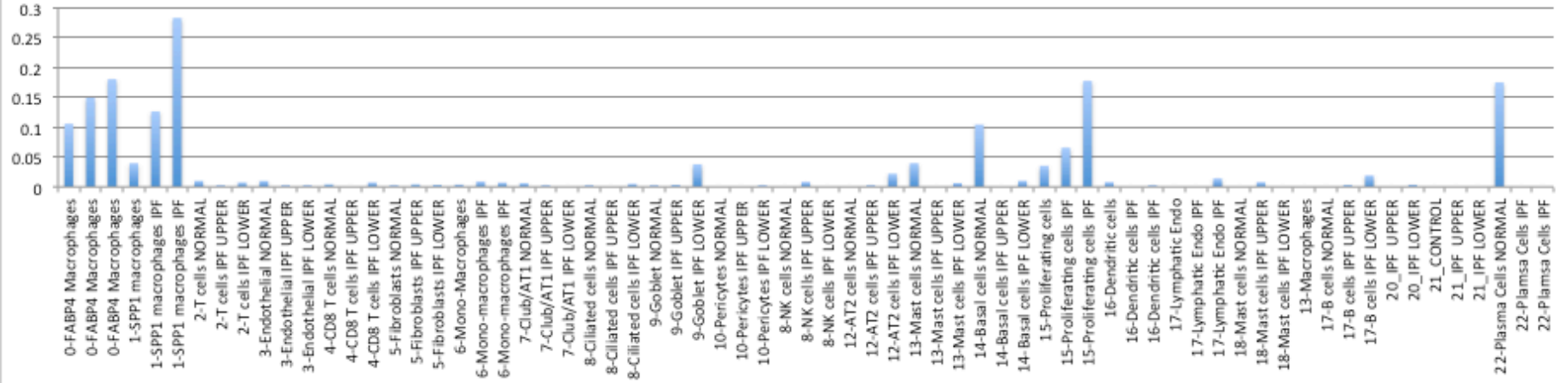
SPP1



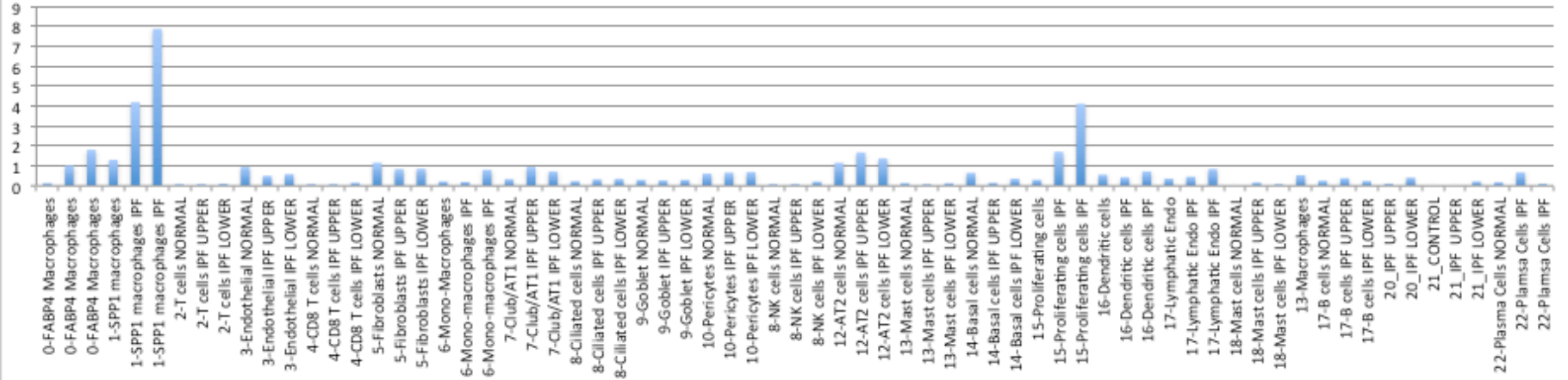
SIGLEC15



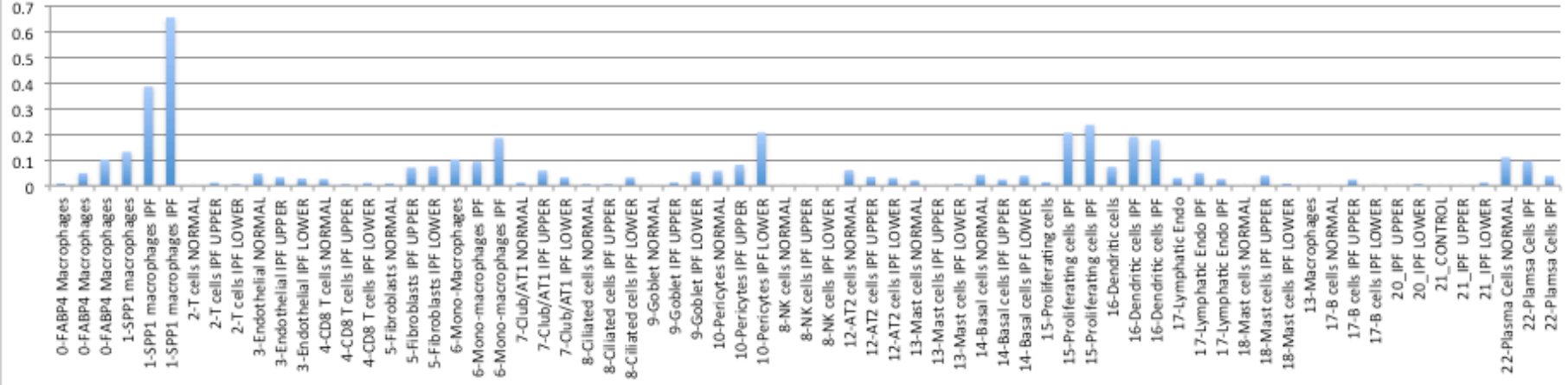
ATP6V0D2



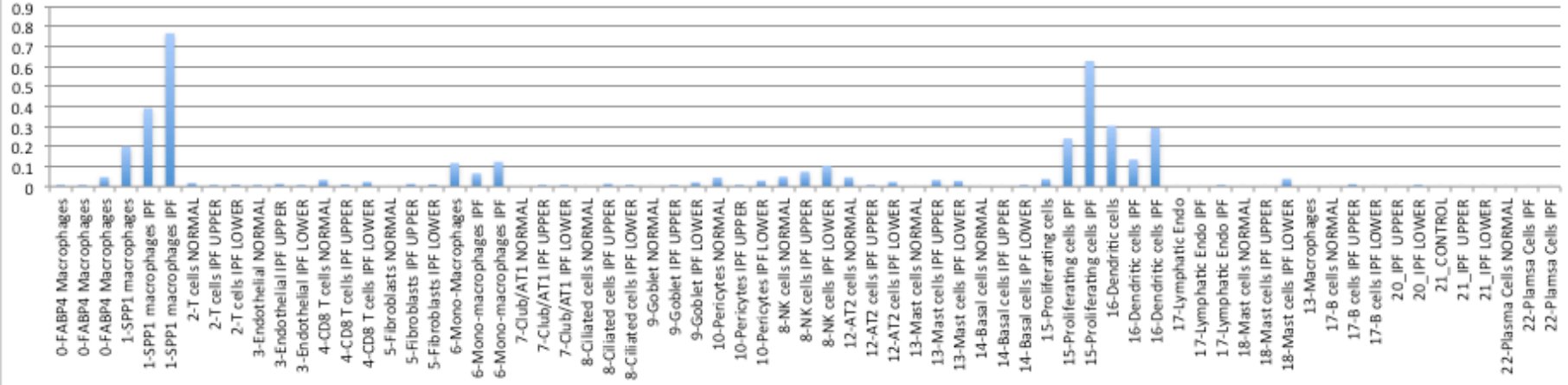
LGMN



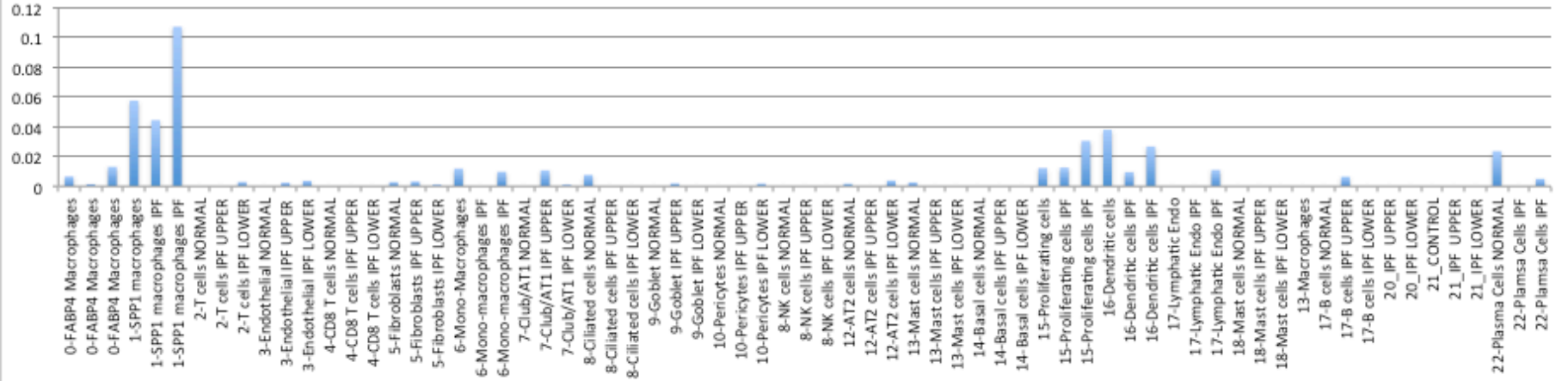
MERTK



MMP9



CD209



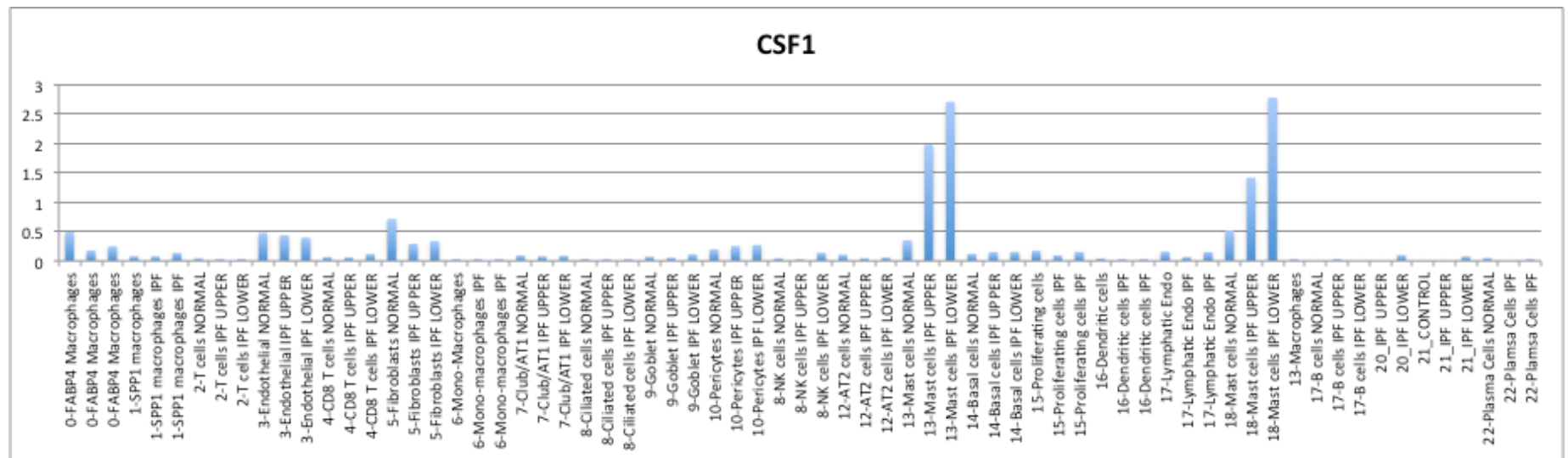
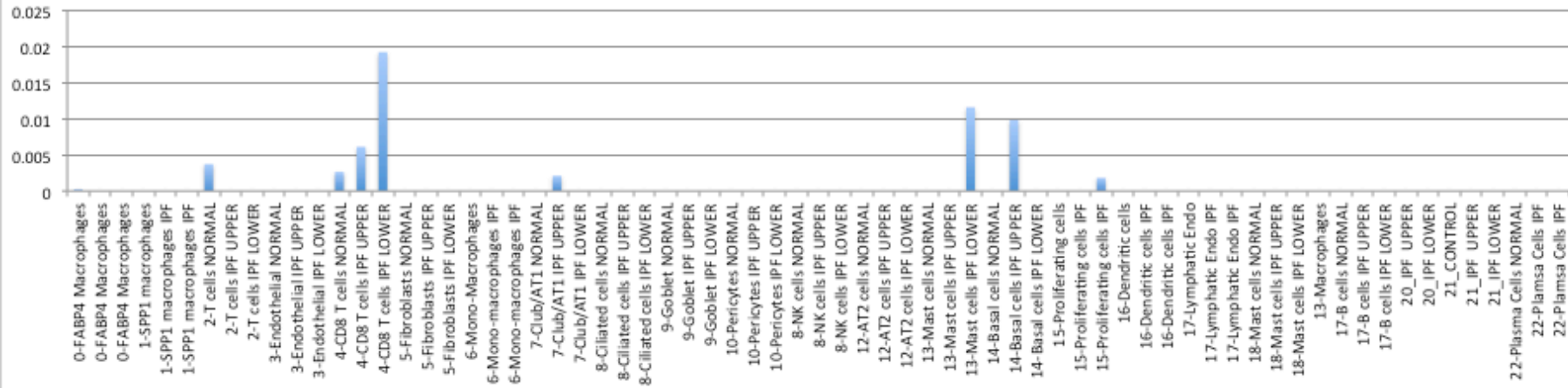
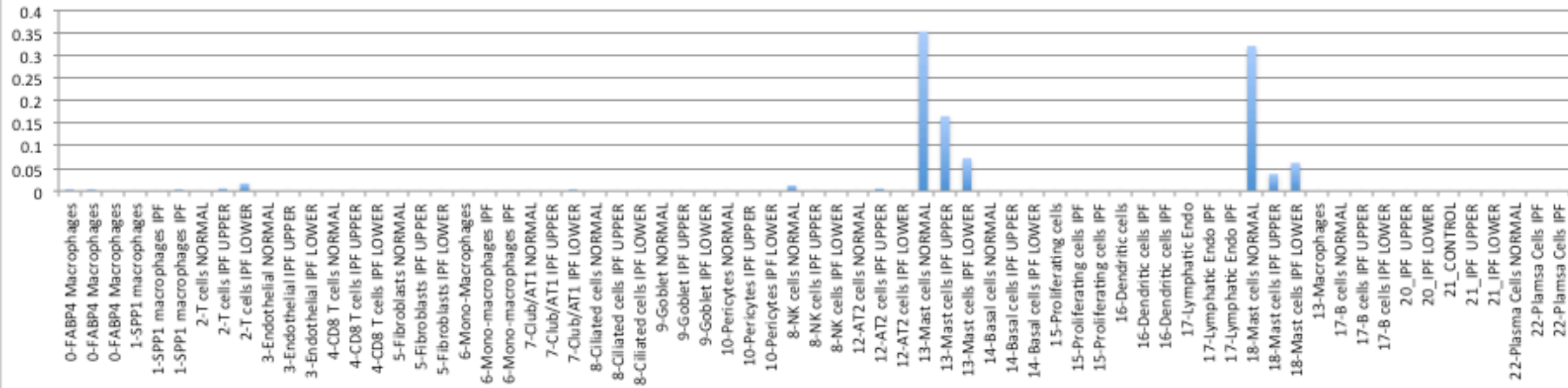


Figure S25. IL4, IL13 and CSF1 expression by cell subsets.

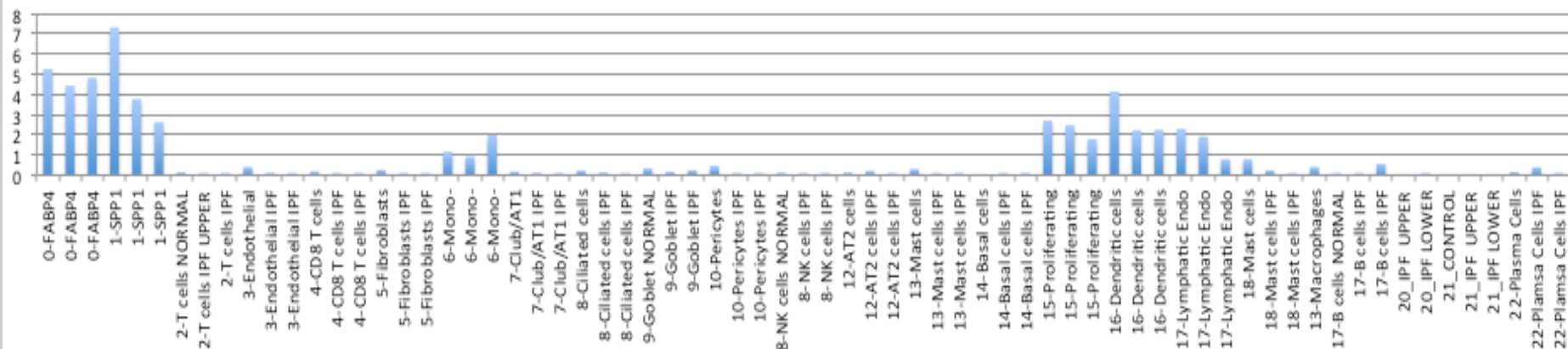
IL4



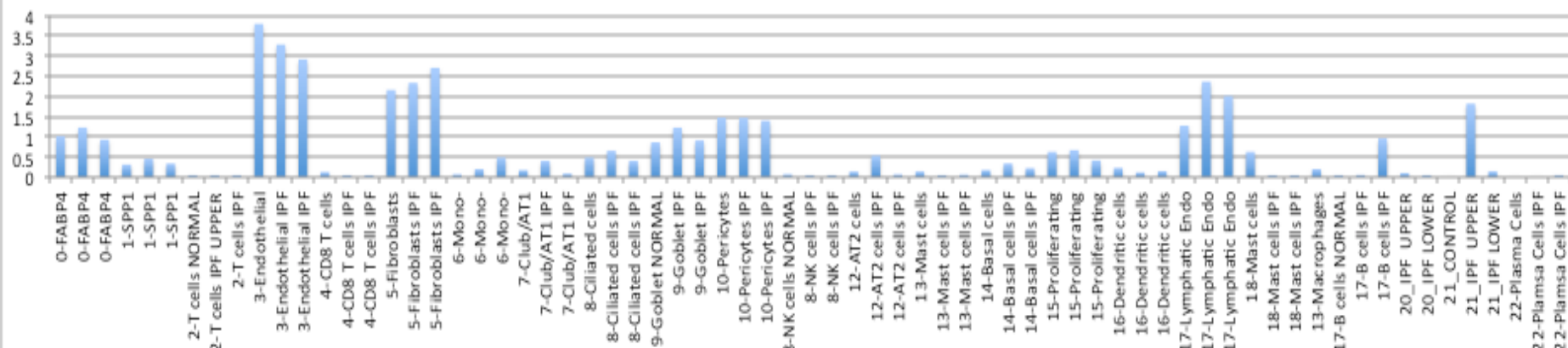
IL13

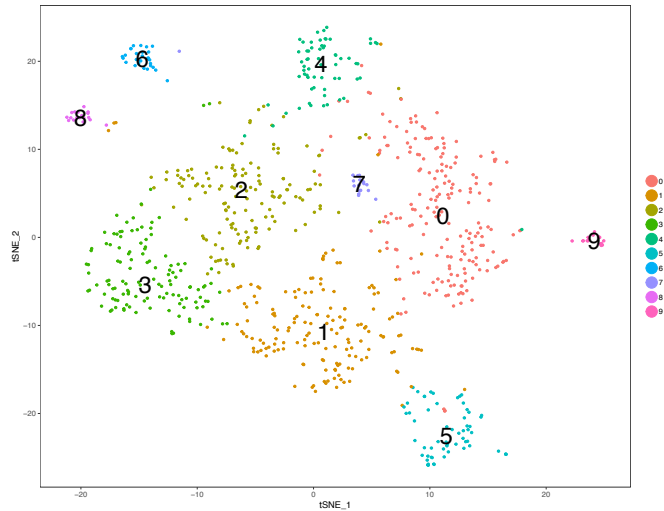


MRC1



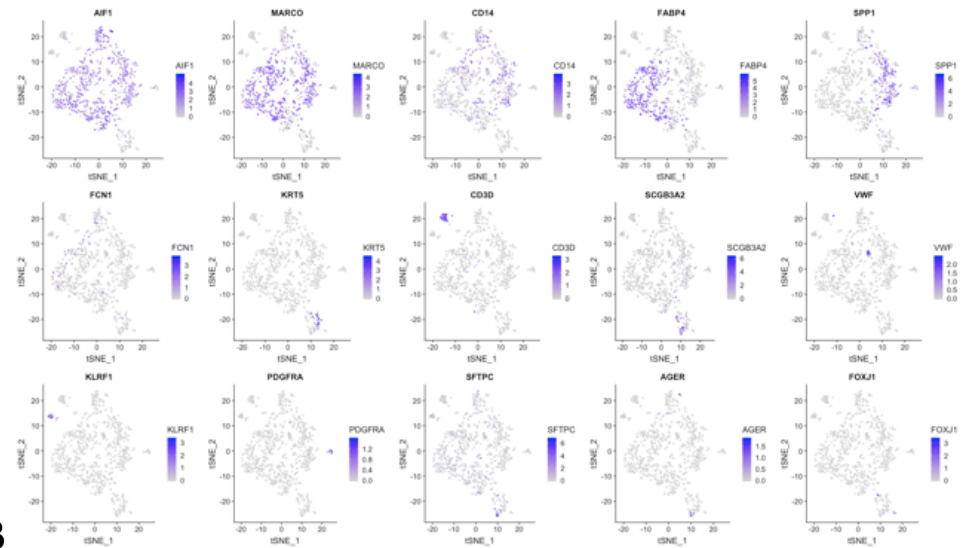
TGM2



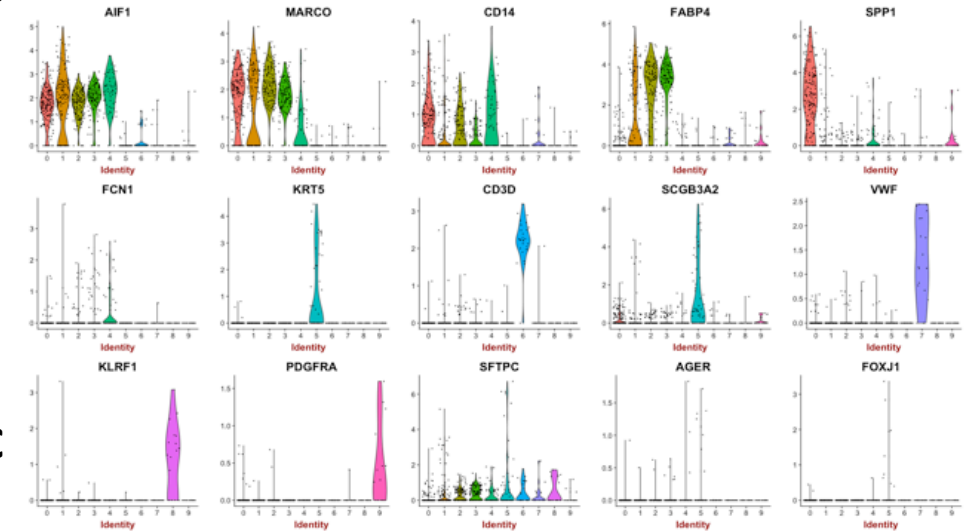


A

Figure S26. T-SNE reclustering of proliferating cells panel A, from cluster 15, Figure 2A) include SPP1^{hi} (cluster 0), FABP4^{hi} (clusters 1 and 2) and FCN1^{hi} (subcluster 4), macrophages, basal (KRT5) and club cells (SCGB3A, cluster 5), T cells (CD3D, cluster 6), endothelial cells (VWF, cluster 7), NK cells (KLRF1, cluster 8) and fibroblasts (PDGFRA, cluster 9; panels B and C). Further subclustering of proliferating epithelial cells shows rare proliferating AT2 cells (SFTPC, clusters 1 and 2), AT1 cells (AGER, cluster 2) and ciliated cells (FOXJ1; panels D and E).



B



C

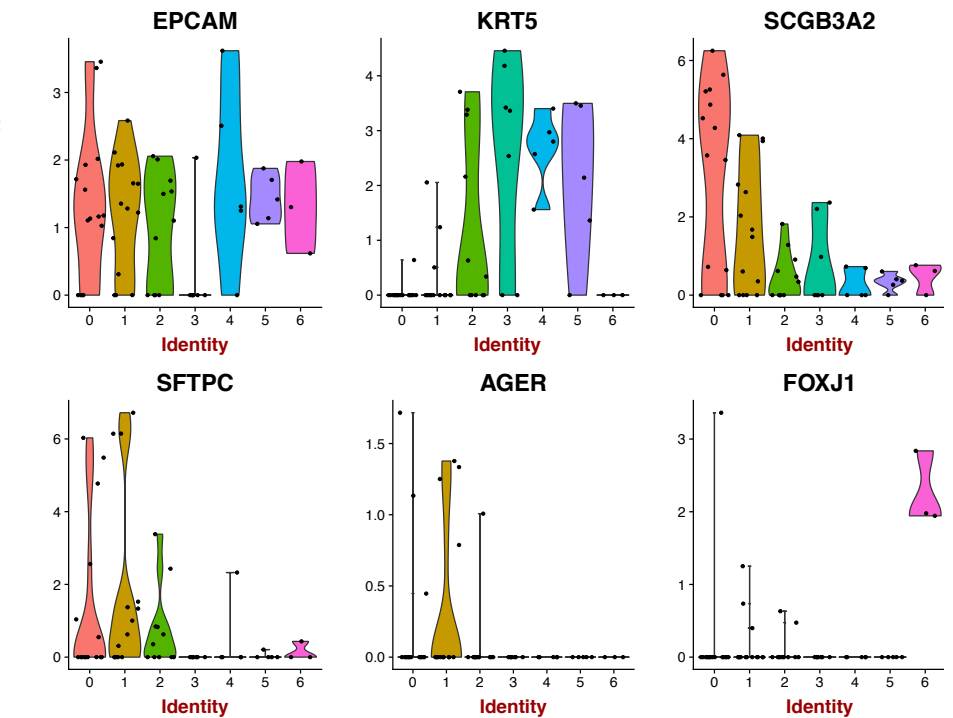
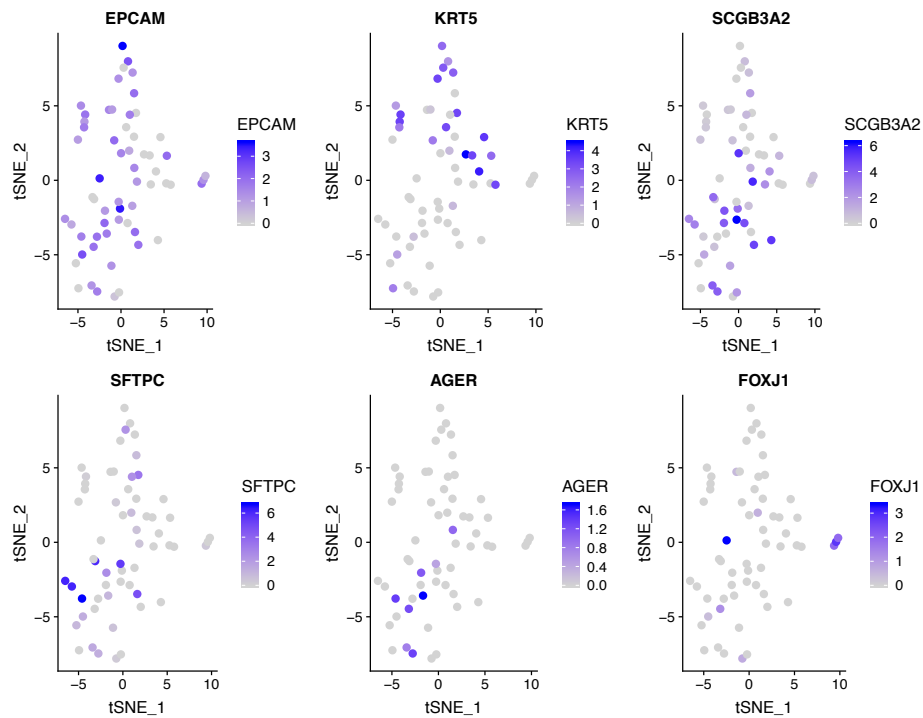


Figure S27. Reclustering of proliferating epithelial cells showed groups of proliferating basal cells (*KRT5*), club cells (*SCGBA2*), AT1 (*AGER*), AT2 (*SFTPC*), ciliated cells (*FOXJ1*).

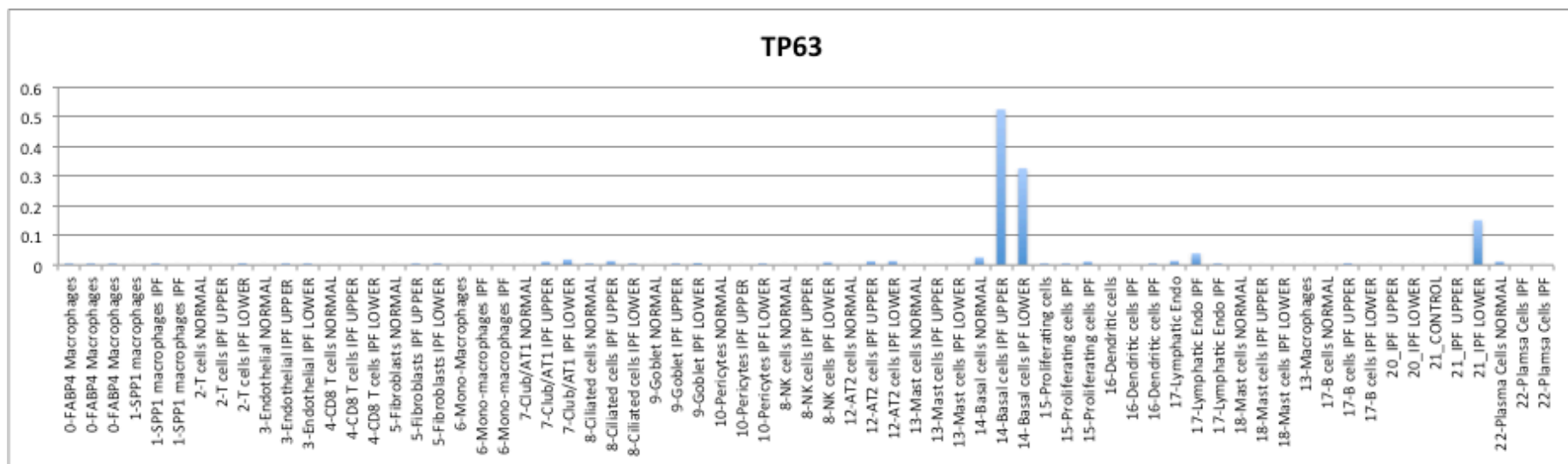
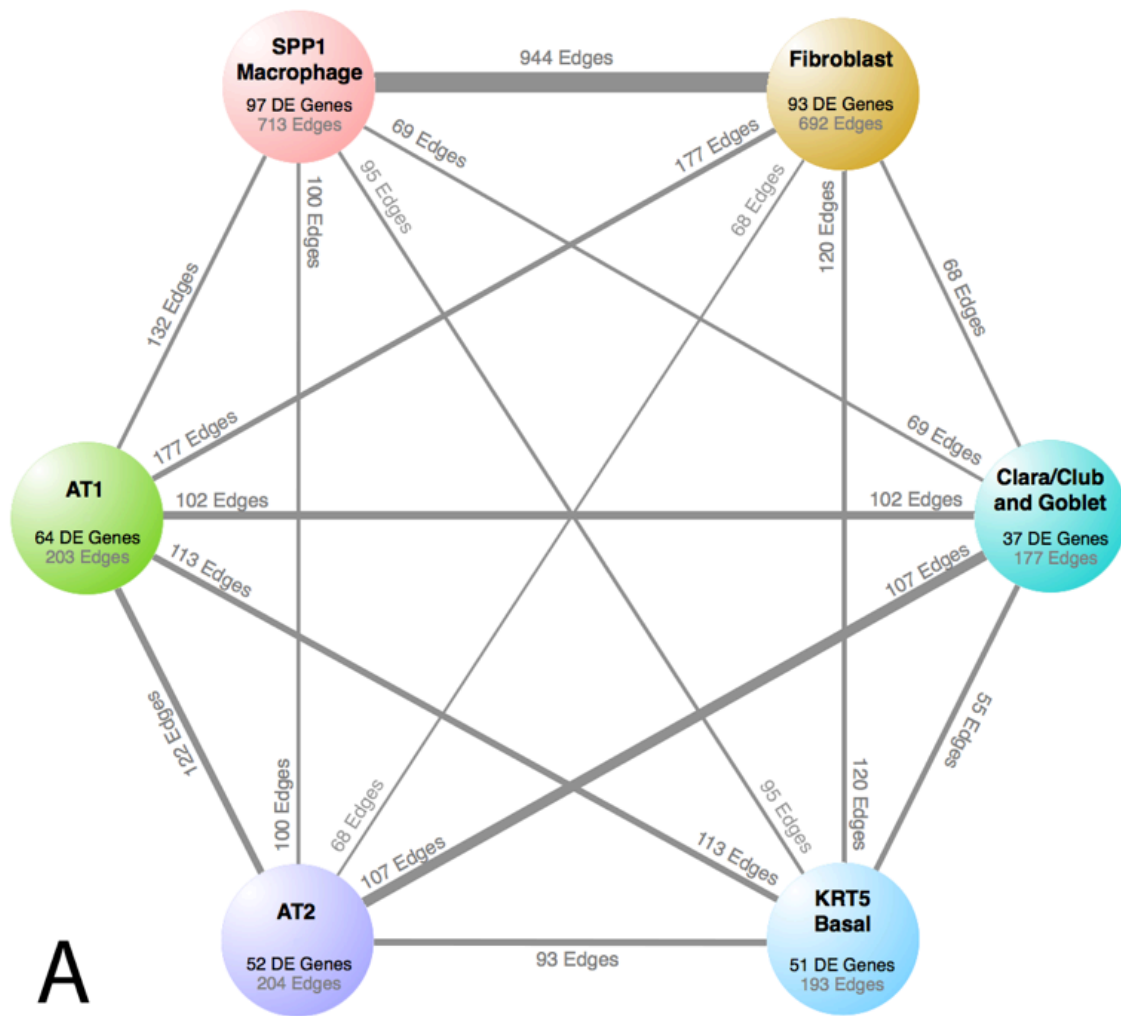


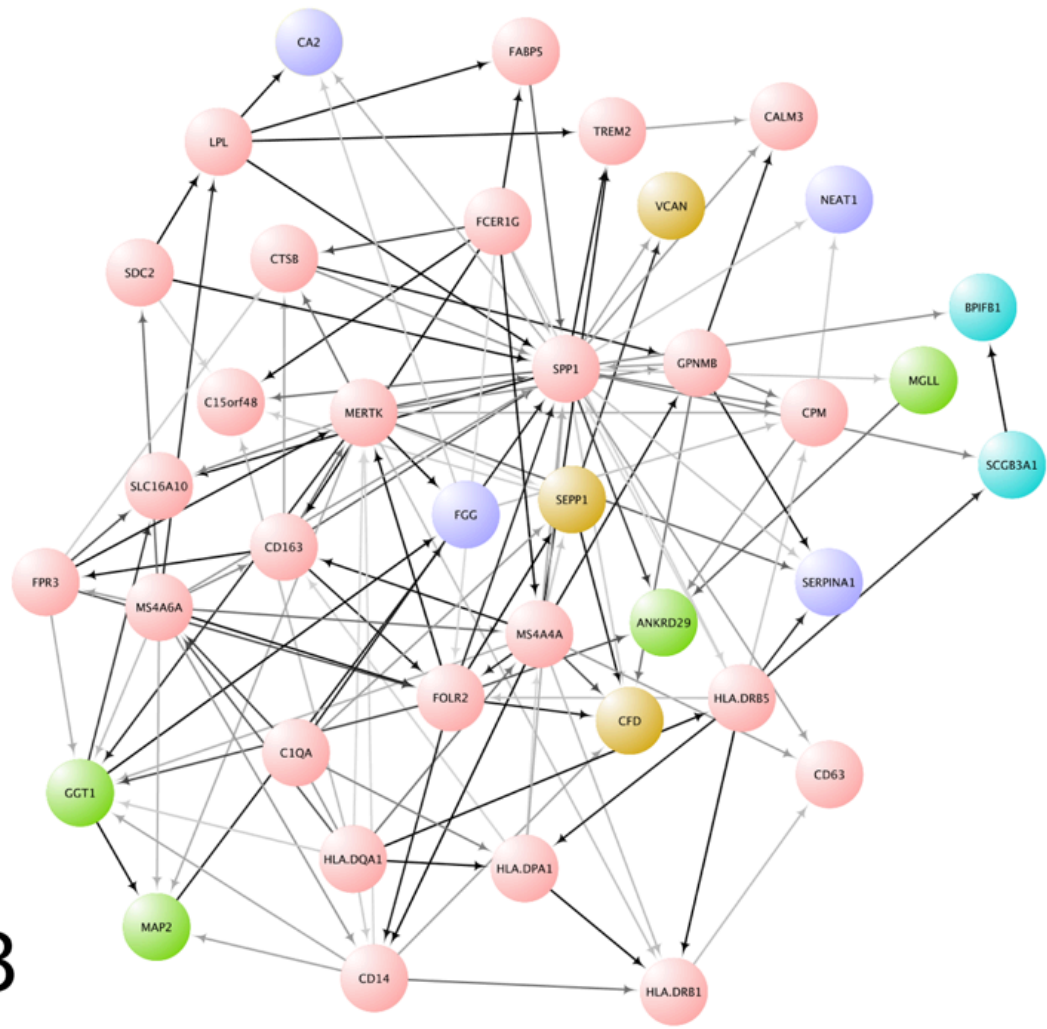
Figure S28. Basal cells in IPF lungs show highly upregulated expression of TP63

Figure S29. Causal network analysis of IPF and control lung gene expression. In summary (panel A) nodes represent the collection of genes that were differentially expressed in a given cell type. Each node is labeled with the number of differentially expressed (DE) genes that node represents as well as the number of edges among the genes represented by that node. Lines connecting nodes in this figure represent the set edges that connect pairs of genes that were differentially expressed in different cell types. These are labeled with the number of edges in the network that span the two cell types, and the line weights are proportional to the percentage of edges that appear in the network relative to the total number of possible edges. Subnetworks highlight the first neighbors of MERTK and SPP1 (panel B) and COMP (panel C). Node colors indicate the cell type in which in the gene is DE, and darker colored edges correspond to more stable connections.



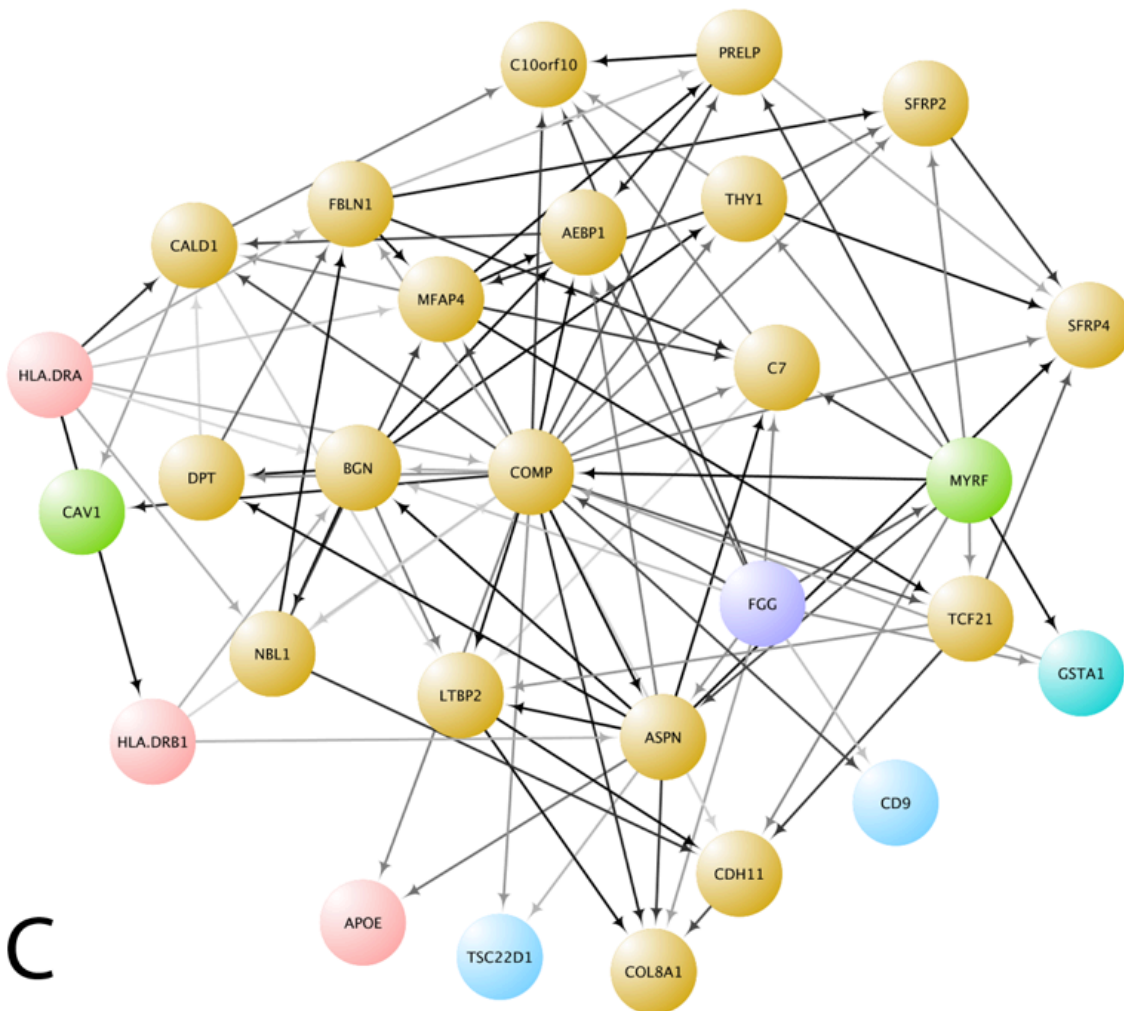
A

B



Cell Type

- SPP1 Macrophage
- Fibroblast
- AT1
- Clara/Club and Goblet
- AT2
- KRT5 Basal



- ### Cell Type
- SPP1 Macrophage
 - Fibroblast
 - AT1
 - Clara/Club and Goblet
 - AT2
 - KRT5 Basal

C