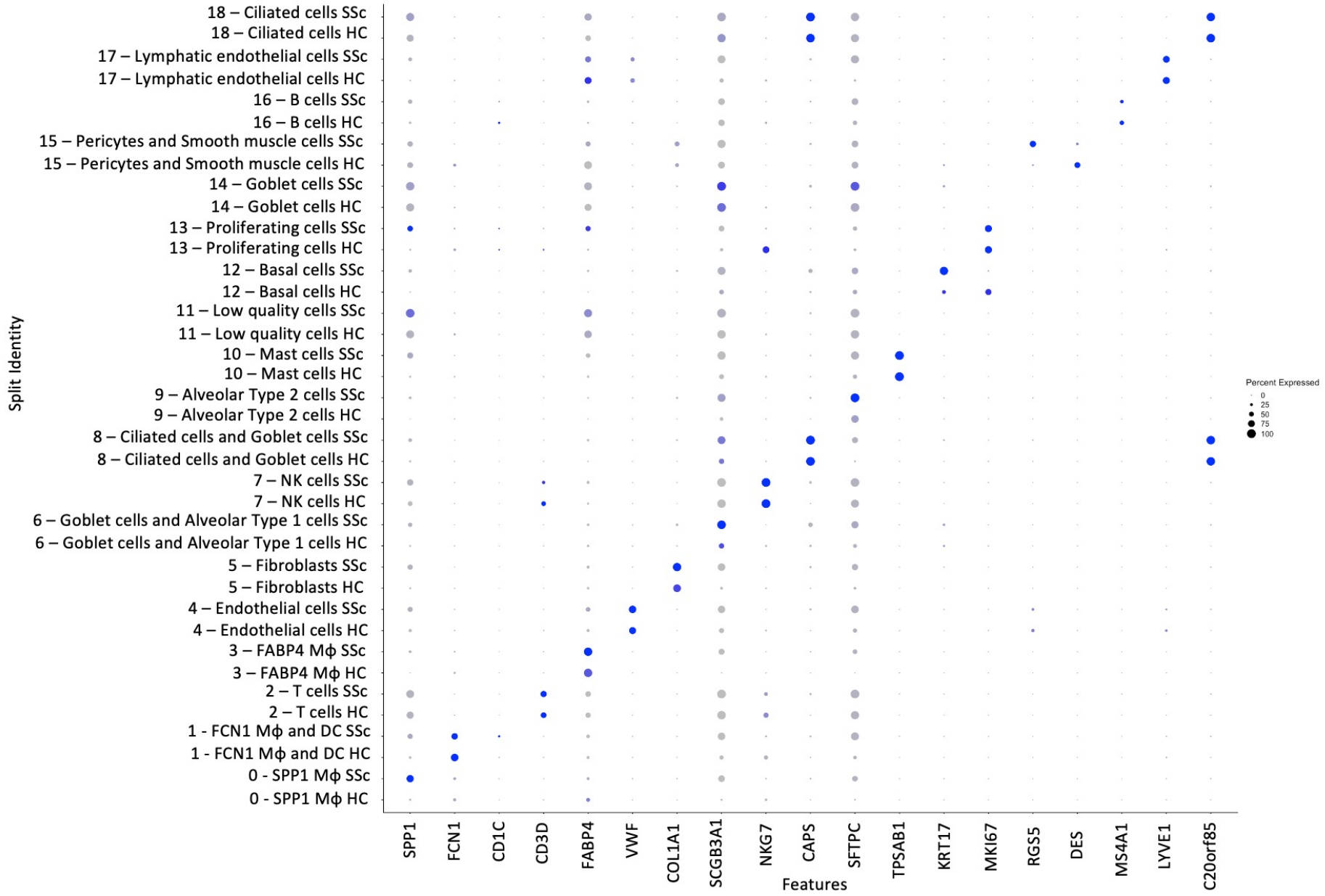
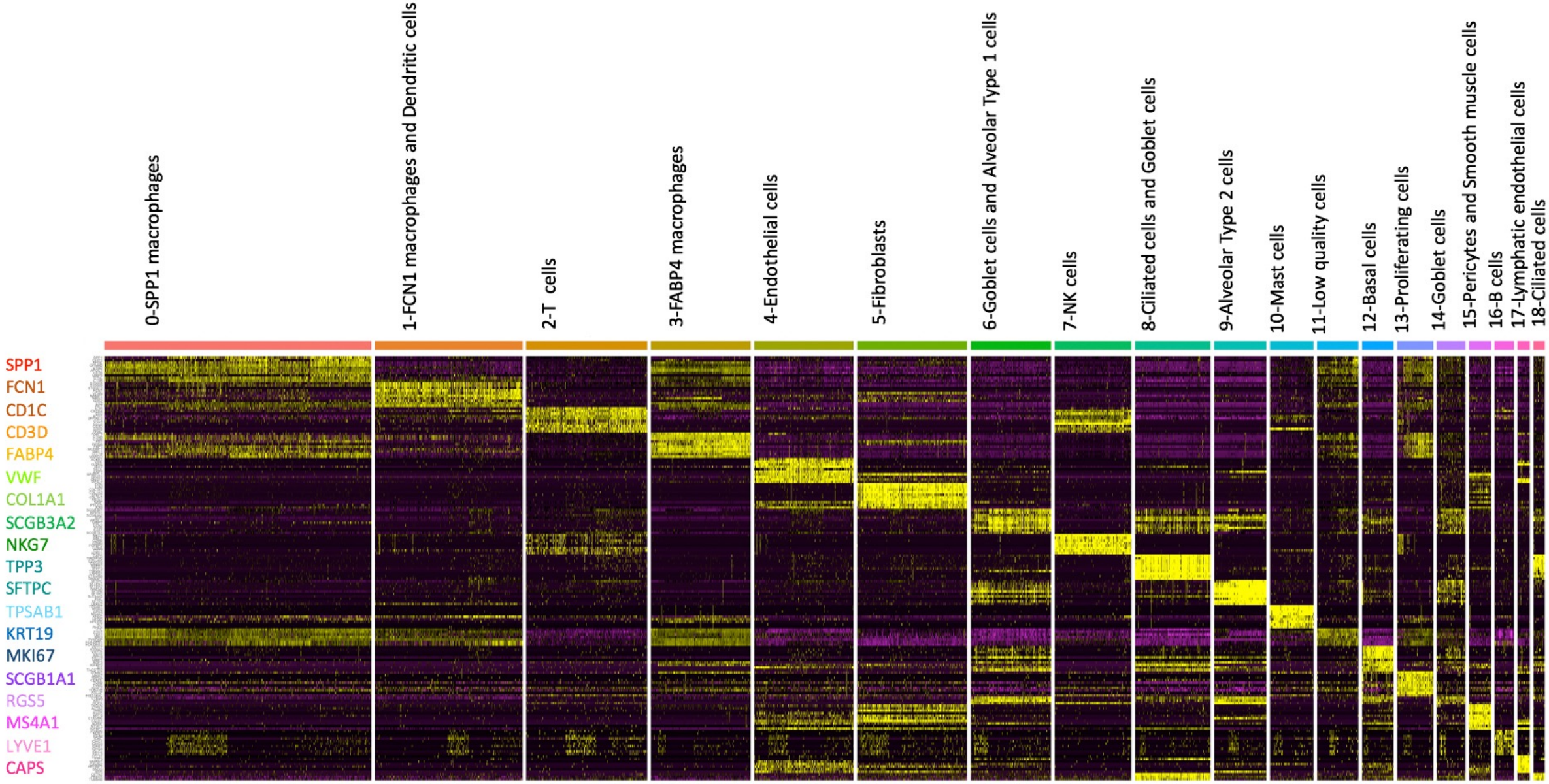
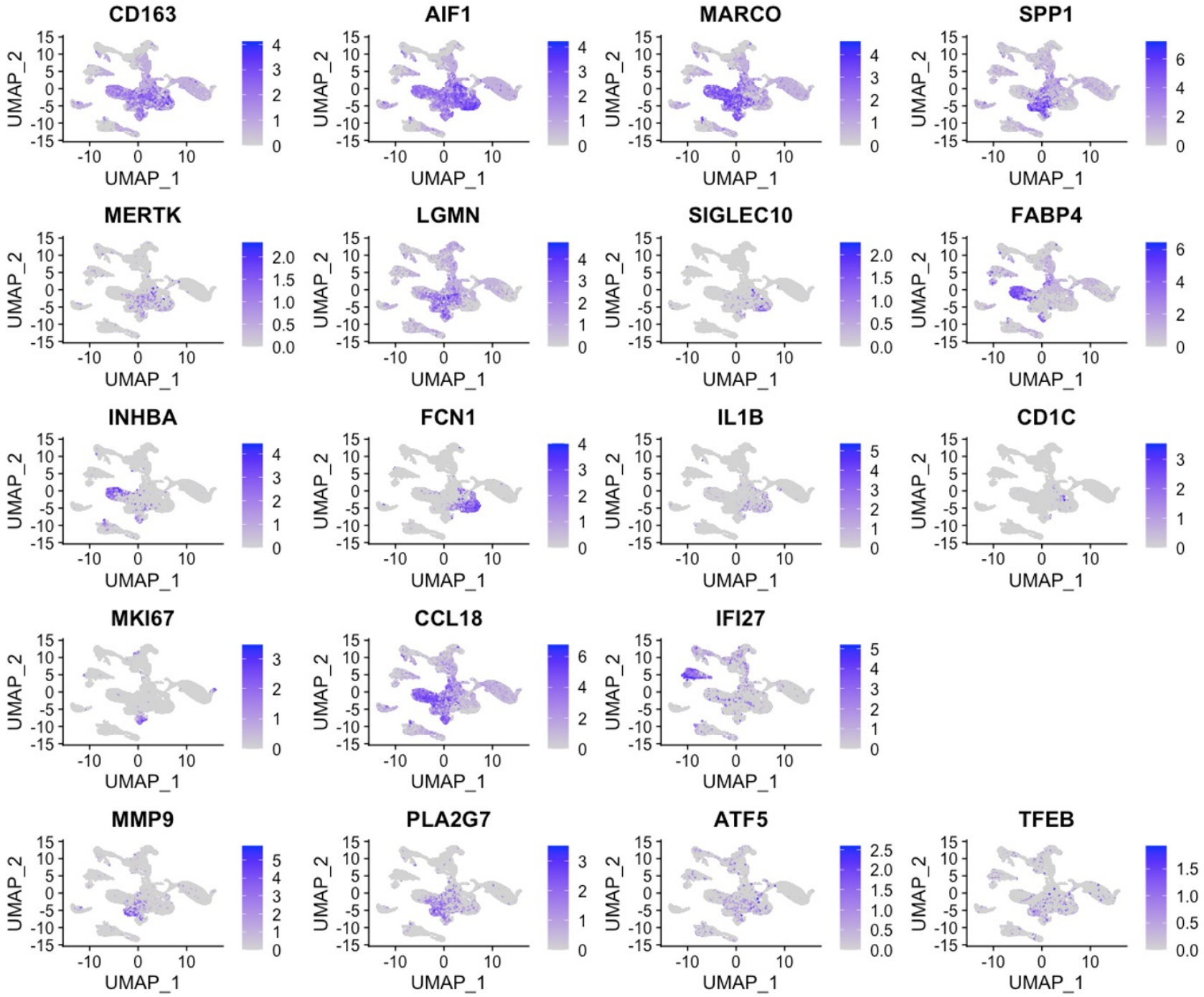
Supplementary Figure 1. Split DotPlot of top gene markers expression per cluster in 5 lung samples (3’ v3 scRNA-seq chemistry). SPP1 gene marker is upregulated by the scleroderma macrophages in respective macrophage and proliferating cells clusters. FCN1 and FABP4 gene marker is expressed by both scleroderma and normal macrophages in the respective cluster.



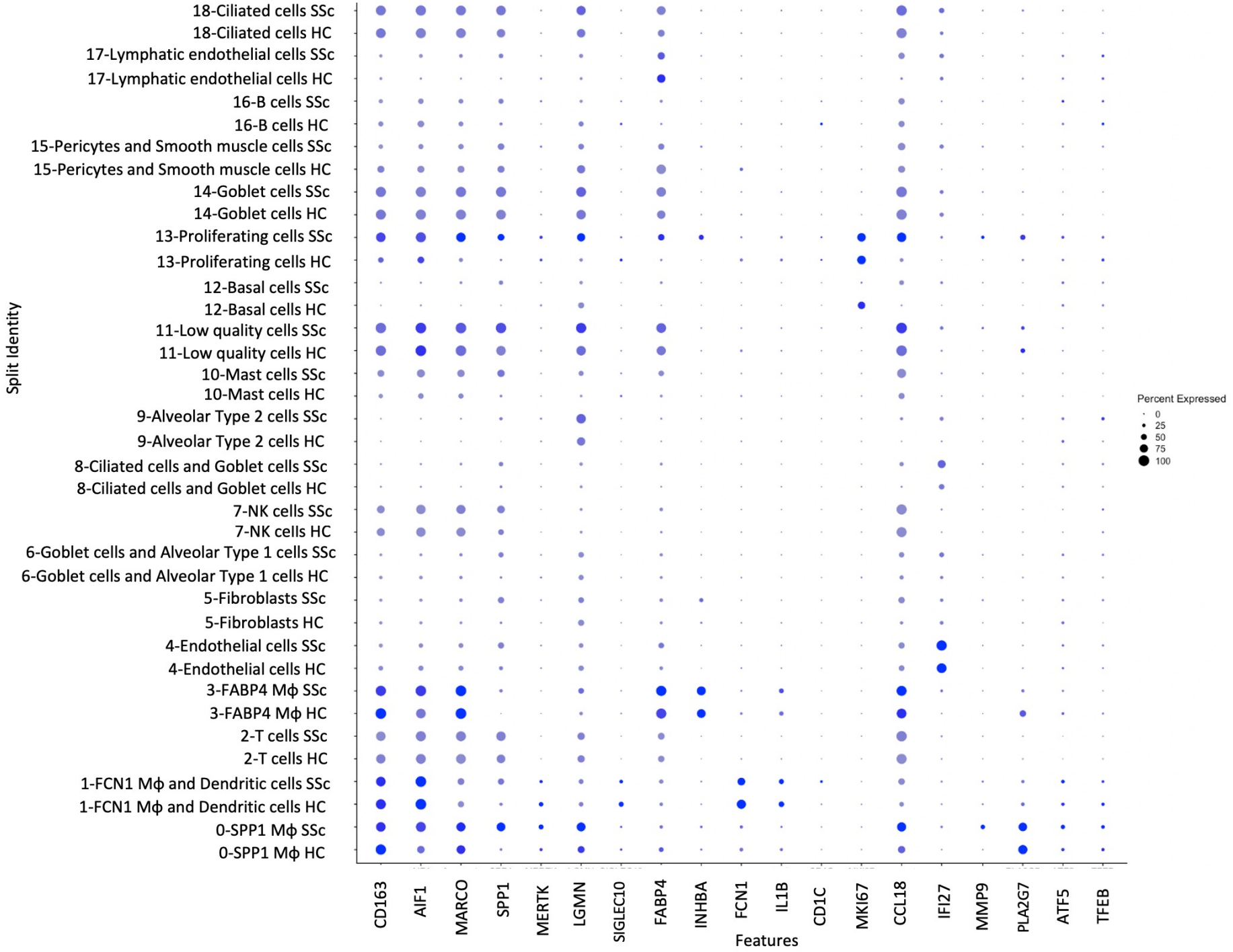
Supplementary Figure 2. Heatmap showing top 10 genes expression per cluster in 5 lung samples (3’ v3 scRNA-seq chemistry).



Supplementary Figure 3. FeaturePlot of gene markers expression for macrophages, dendritic cells, proliferating cells and other genes of interest including IFI27, CCL18, MMP9, PLA2G7, ATF5, TFEB genes in 5 lung samples (3’ v3 Chemistry). SPP1, MERTK, LGMN gene markers are expressed in the same macrophage clusters. FABP4 and INHBA gene markers are co-expressed. FCN1 and IL1B are expressed in the same macrophage cluster. Dendritic cells gene marker CD1C is expressed in the same cluster as FCN1 macrophages. Proliferating cells express both SPP1 and FABP4 macrophage markers.



Supplementary Figure 4. Split DotPlot of gene markers expression for macrophages, dendritic cells, proliferating Mϕ and other genes including IFI27, CCL18, MMP9, PLA2G7, ATF5, TFEB genes in 5 lung samples (3' v3 scRNA-seq chemistry). SPP1, MERTK, LGMN gene markers are expressed in the same macrophage cluster and are upregulated in scleroderma macrophages within the SPP1 macrophage cluster. FABP4 and INHBA gene markers are co-expressed by both scleroderma and normal macrophages within the same cluster. FCN1 and IL1B are expressed in the same macrophage cluster. Dendritic cells gene marker CD1C is expressed in the same cluster as FCN1 macrophages. Proliferating Mϕ express both SPP1 and FABP4 Mϕ markers.



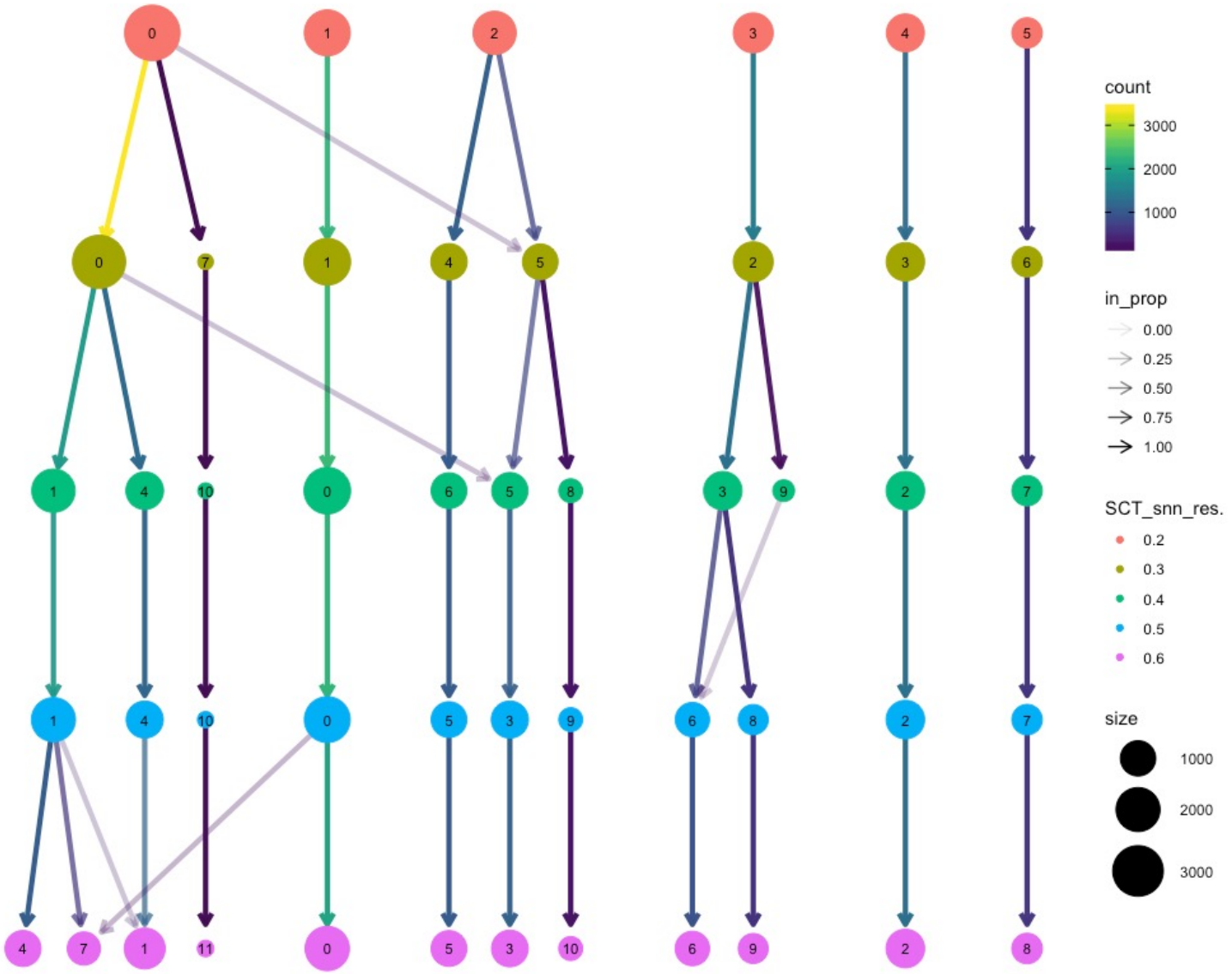
Supplemental Figure 5. Gene Ontology findings for differentiating genes between SPP1 Mϕ vs FABP4- and FCN1 Mϕ.

	Homo sapiens (REF)						
GO biological process complete	#	#	expected	Fold Enrichment	+/-	raw P value	FDR
→ very-low-density lipoprotein particle clearance	6	2	.02	> 100	+	1.93E-04	4.68E-02
→ plasma lipoprotein particle clearance	39	3	.10	28.80	+	1.91E-04	4.77E-02
→ regulation of plasma lipoprotein particle levels	75	5	.20	24.96	+	2.30E-06	1.13E-03
cholesterol efflux	25	4	.07	59.91	+	1.02E-06	5.73E-04
→ cholesterol transport	60	4	.16	24.96	+	2.54E-05	9.32E-03
→ sterol transport	76	4	.20	19.71	+	6.13E-05	2.15E-02
→ organic hydroxy compound transport	151	5	.40	12.40	+	5.92E-05	2.12E-02
steroid catabolic process	26	3	.07	43.21	+	6.22E-05	2.13E-02
→ lipid catabolic process	304	7	.81	8.62	+	1.79E-05	6.90E-03
→ lipid metabolic process	1222	15	3.26	4.60	+	5.18E-07	3.03E-04
→ steroid metabolic process	262	6	.70	8.58	+	7.69E-05	2.47E-02
neutrophil degranulation	482	14	1.29	10.88	+	3.13E-11	1.24E-07
→ neutrophil mediated immunity	493	14	1.32	10.63	+	4.19E-11	9.44E-08
→ myeloid leukocyte mediated immunity	515	14	1.38	10.18	+	7.34E-11	8.91E-08
→ leukocyte mediated immunity	755	14	2.02	6.94	+	9.25E-09	7.68E-06
→ immune effector process	1095	15	2.92	5.13	+	1.29E-07	8.45E-05
→ immune system process	2849	21	7.61	2.76	+	6.67E-06	2.92E-03
myeloid cell activation involved in immune response	524	15	1.40	10.72	+	6.67E-12	1.05E-07
→ leukocyte activation involved in immune response	630	15	1.68	8.92	+	8.36E-11	9.42E-08
→ cell activation involved in immune response	634	15	1.69	8.86	+	9.12E-11	9.59E-08
→ immune response	1972	18	5.27	3.42	+	2.10E-06	1.07E-03
→ response to stimulus	8496	37	22.69	1.63	+	1.55E-04	4.02E-02

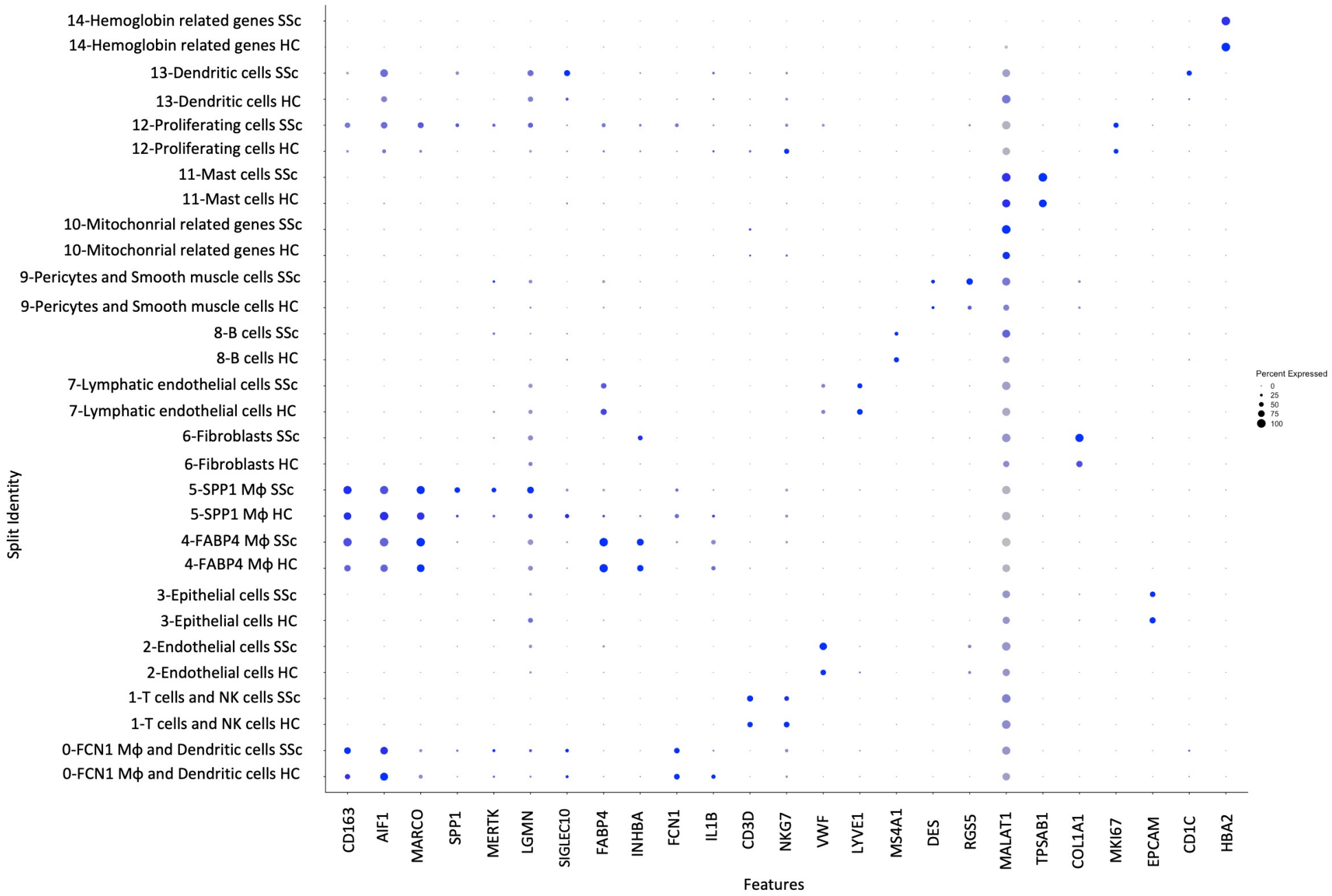
Supplemental Figure 6. Differentiating genes between SPP1 Mϕ and FABP4 Mϕ during analysis of only macrophages, dendritic cells, proliferating Mϕ from 1 control and 4 SSc-ILD lung samples in 3' v3 chemistry.

	p_val	avg_logFC	pct.1	pct.2	p_val_adj
SPP1	0	3.30283596	0.802	0.205	0
CCL2	0	1.9948943	0.459	0.077	0
LGMN	0	1.33368645	0.828	0.496	0
RGS1	0	1.19074799	0.719	0.268	0
MARCKS	0	1.16429467	0.722	0.179	0
CCL3	0	1.15787983	0.538	0.131	0
A2M	0	0.98929751	0.686	0.186	0
CTSB	0	0.96867367	1	0.995	0
EMP1	0	0.943562	0.736	0.269	0
CD84	0	0.932686	0.833	0.341	0
LILRB4	0	0.9031076	0.827	0.342	0
CALM3	0	0.88730018	0.873	0.654	0
GPR183	0	0.8415434	0.66	0.203	0
FNIP2	0	0.81529597	0.826	0.384	0
PLEKHO1	0	0.74960591	0.656	0.206	0
CD48	0	0.73841137	0.667	0.182	0
FPR3	0	0.68726643	0.72	0.317	0
SGK1	0	0.66610425	0.918	0.679	0
ABCA1	0	0.63707043	0.702	0.296	0
HM13	0	0.63642167	0.891	0.542	0
LIMS1	0	0.60535518	0.947	0.825	0
TTYH3	0	0.58333966	0.679	0.213	0
LGALS1	0	0.57432743	0.994	0.993	0
PEA15	0	0.56913487	0.601	0.182	0

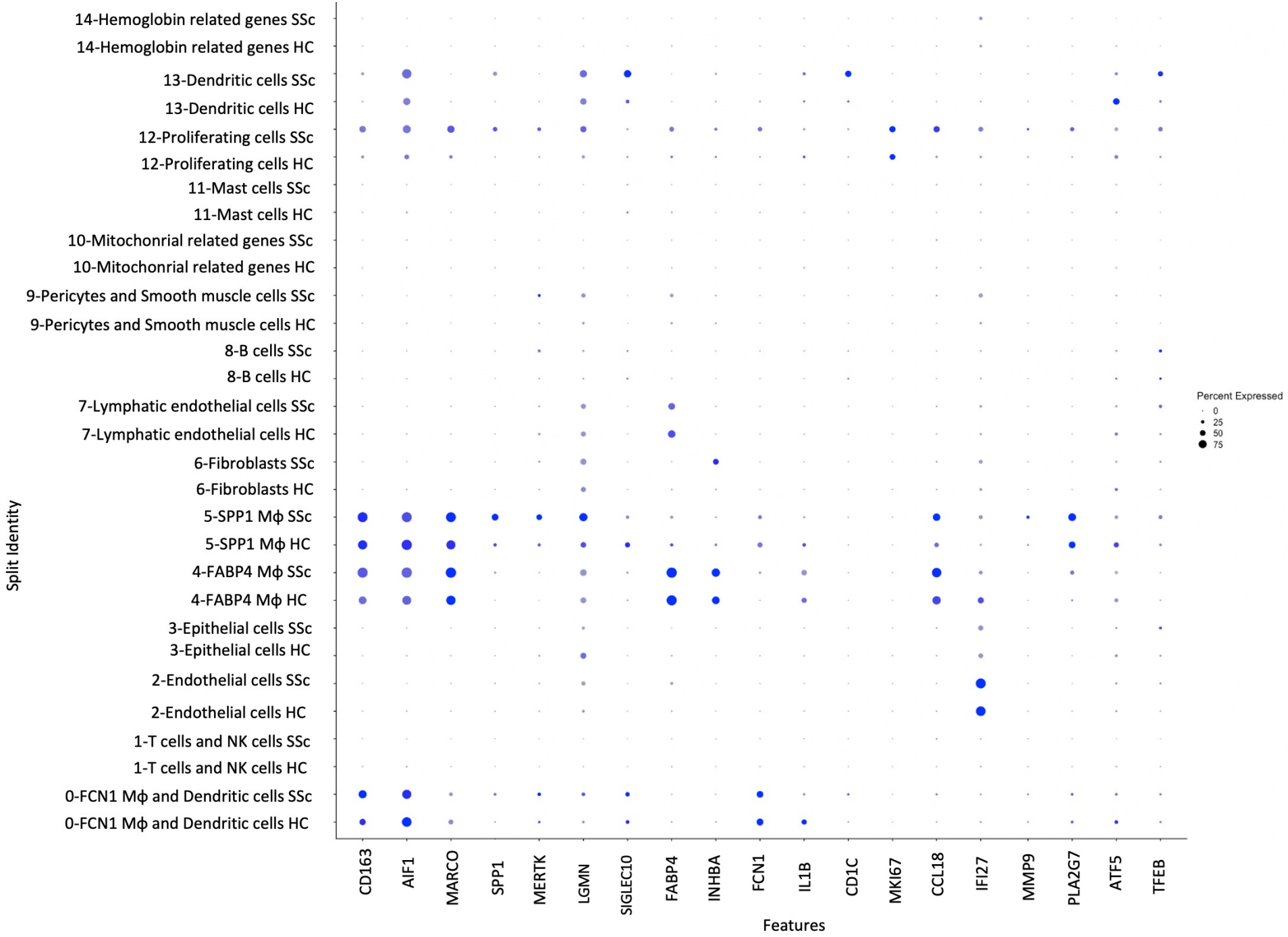
Supplemental Figure 7. Analysis of only macrophages, monocytes, dendritic cells, proliferating cells of 1 normal and 4 SSc-ILD lung samples in 3’ v3 chemistry performed using 8 Principal Components and resolution 0.5 as per clustree package (Seurat, Satija Lab).



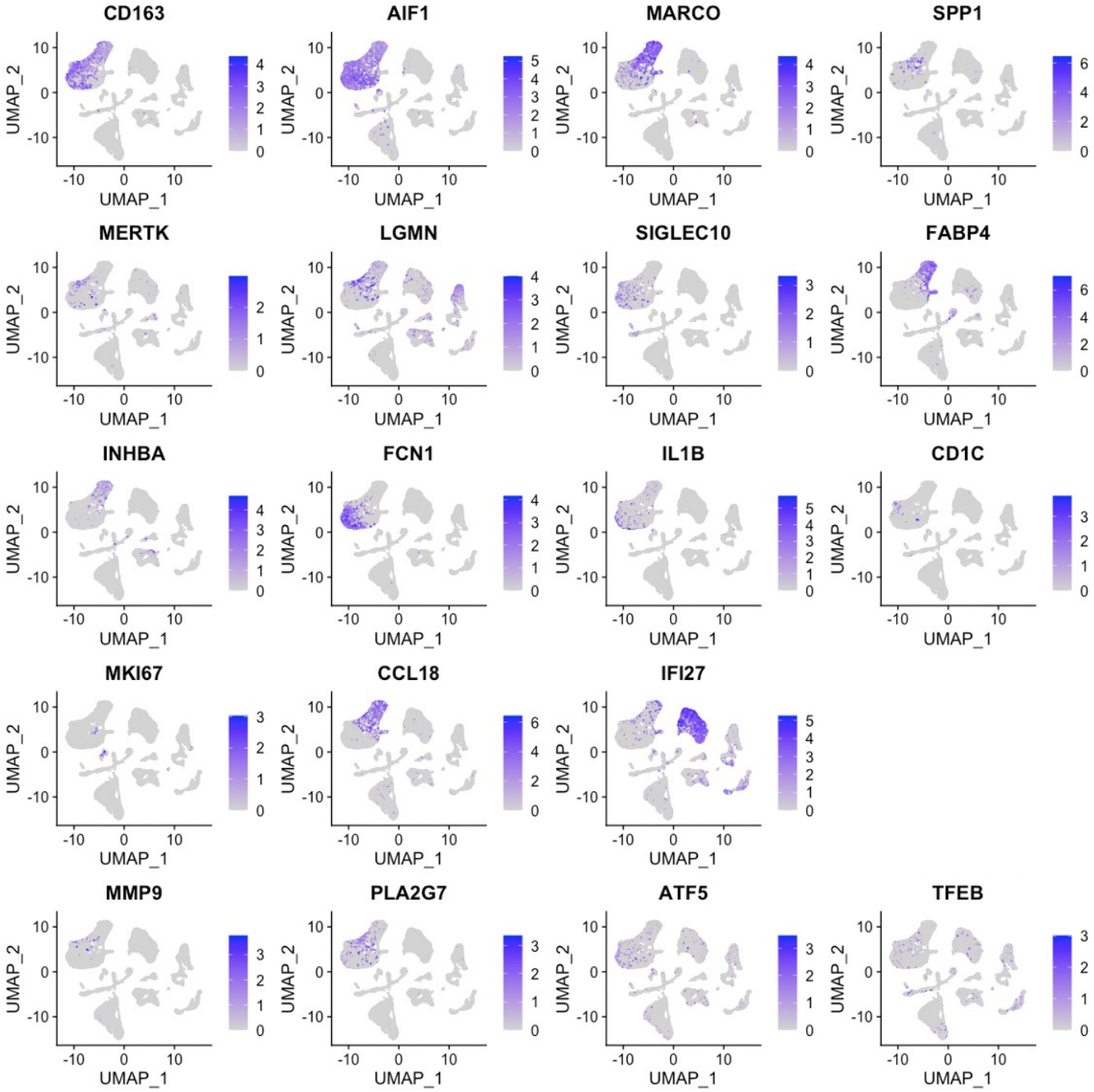
Supplementary Figure 8. Split DotPlot of top gene markers expression per cluster of 6 lungs in 5’ v1 chemistry. *SPP1* gene is upregulated in SSc-ILD macrophages and proliferating Mϕ clusters.



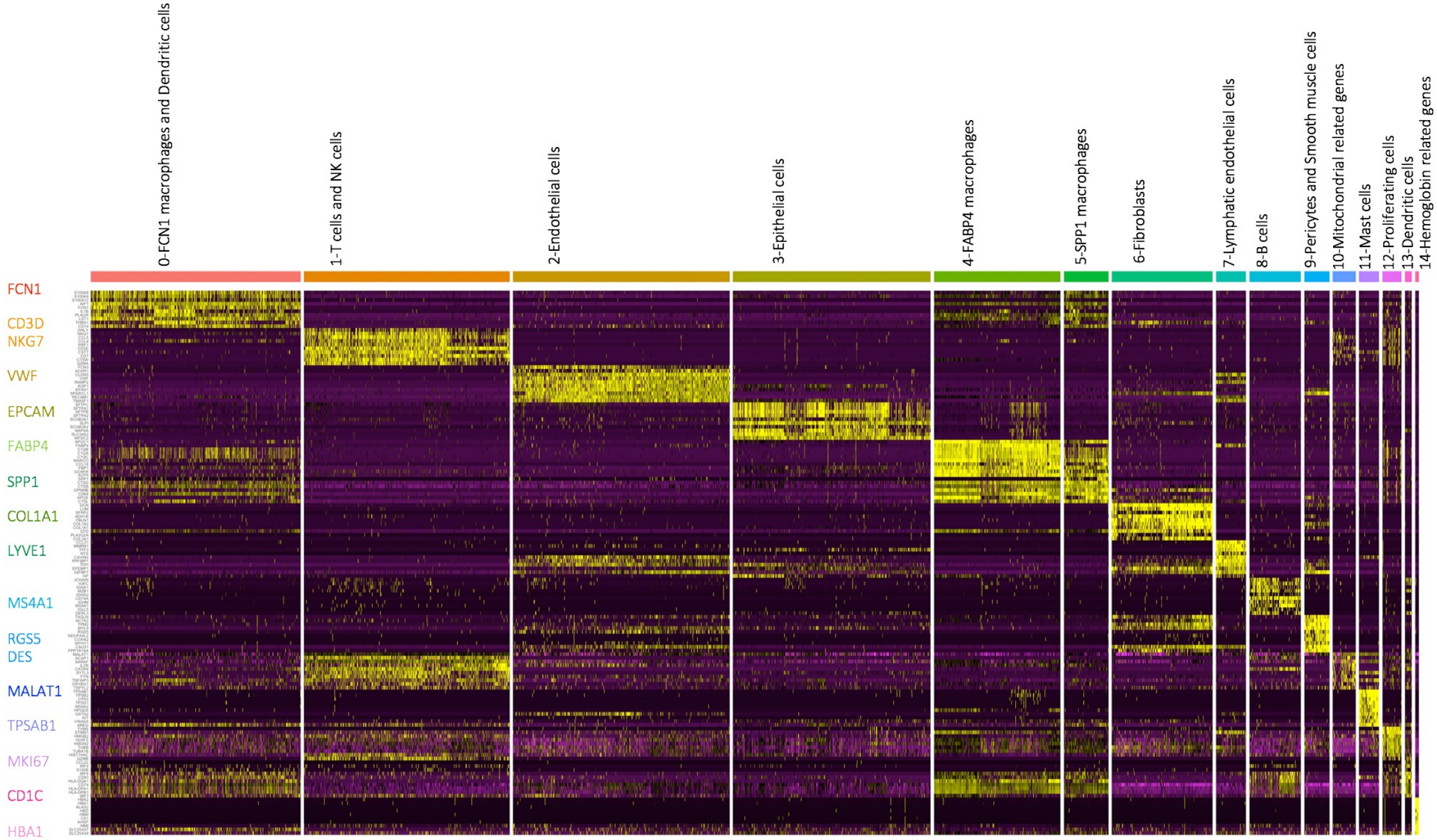
Supplementary Figure 9. Split DotPlot of macrophage subpopulations gene markers, dendritic cells, proliferating cells and other genes expression per cluster of 6 lungs in 5' v1 chemistry. *SPP1* gene is upregulated in the scleroderma macrophages in respective macrophage and proliferating cell clusters. *MMP9* is upregulated by the SSc-ILD SPP1 Mϕ.



Supplementary Figure 10. FeaturePlot of macrophage subpopulations gene markers, dendritic cells, proliferating cells and other genes expression during analysis of macrophages, dendritic cells, proliferating cells from 6 lungs in 5’ v1 chemistry. *SPP1, MERTK, LGMN* genes are co-expressed in the same macrophage cluster.



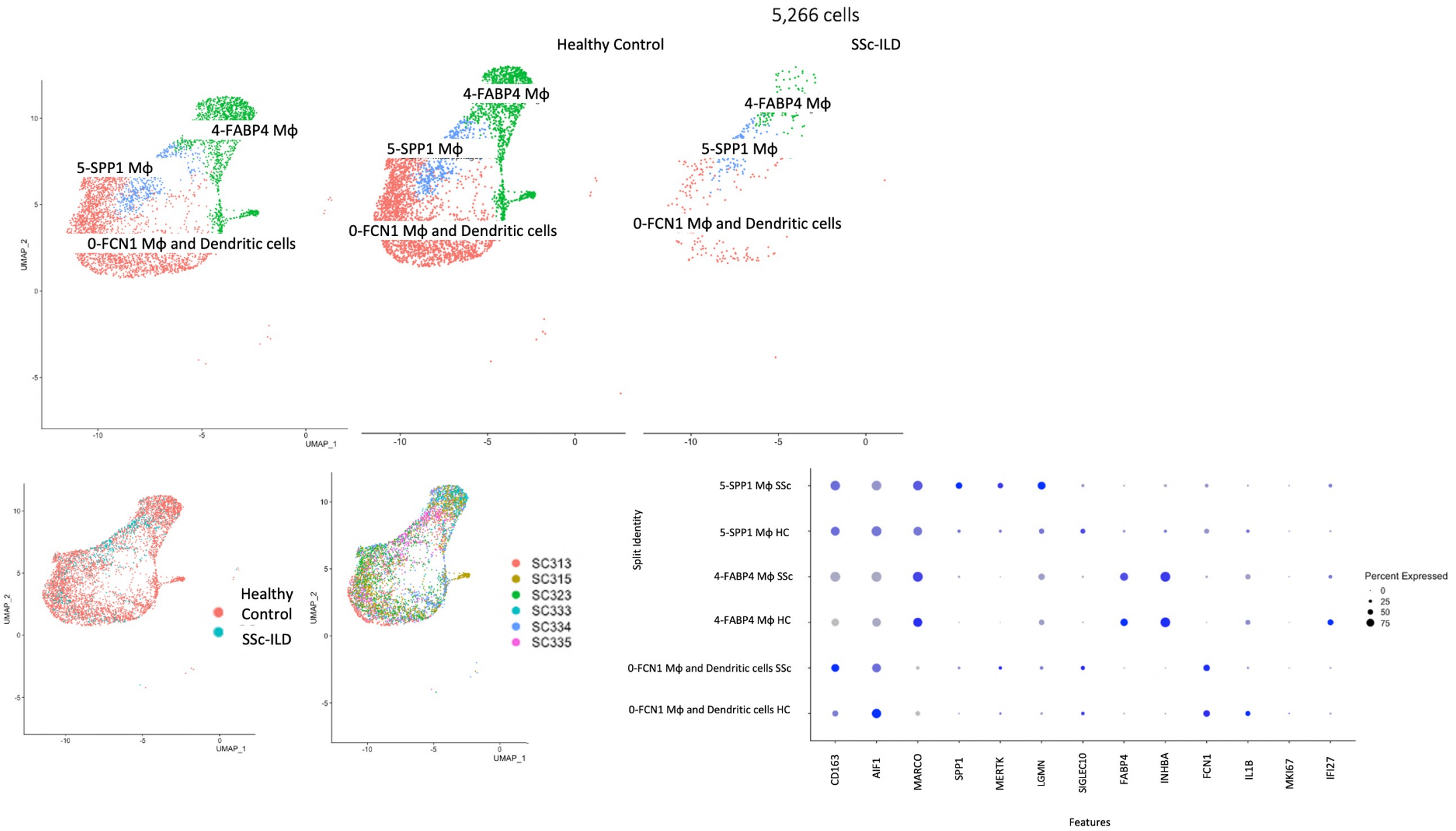
Supplementary Figure 11. Heatmap of top 10 genes expression per cluster of 6 lung samples in 5’ v1 chemistry.



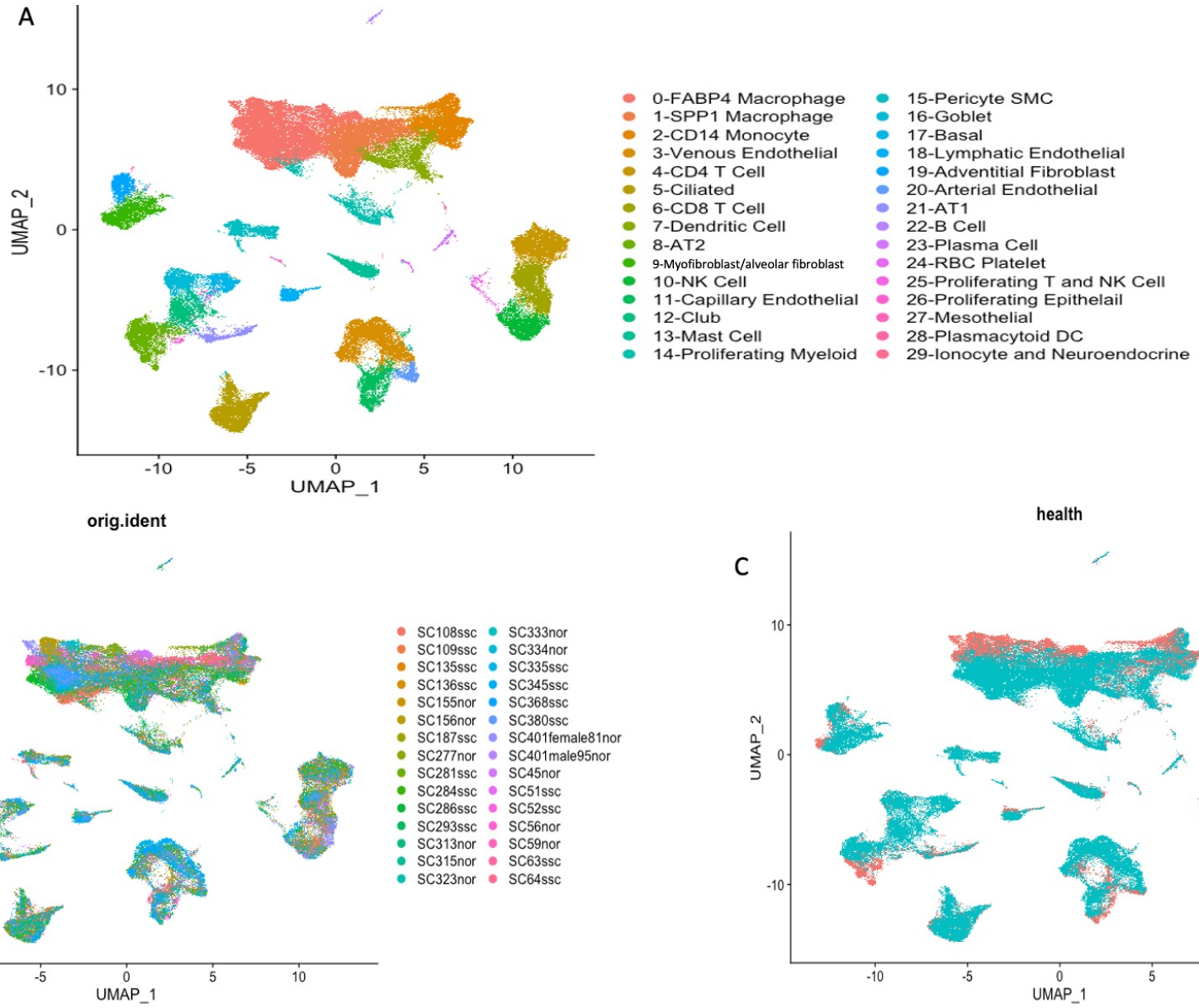
Supplementary Figure 12. Differentiating Genes between SPP1 Mϕ and FCN1 Mϕ and FABP4 Mϕ from 6 lungs in 5' v1 chemistry. *SPP1* and *PLA2G7* are some of the genes included in the list predicted by the software (Seurat, Satija Lab).

Gene Name	p_val	avg_logFC	p_val_adj
PLA2G7	7.65E-163	0.67324545	2.57E-158
GPNMB	1.21E-149	1.0804473	4.05E-145
SPP1	6.56E-143	1.99500057	2.20E-138
MATK	1.80E-125	0.37829333	6.04E-121
CTSD	1.20E-117	0.96783973	4.04E-113
PMP22	5.97E-113	0.5615302	2.00E-108
CTSB	2.53E-112	0.77837216	8.47E-108
CSTB	7.88E-108	0.75302663	2.64E-103
CD63	3.52E-96	0.59510584	1.18E-91
CCL2	1.12E-90	1.31801783	3.77E-86
DAB2	7.17E-88	0.49342676	2.41E-83
EMP1	1.24E-87	0.41768143	4.17E-83
TREM2	1.34E-87	0.78370618	4.49E-83
NPC2	1.01E-84	0.47150944	3.40E-80
LAMP1	2.42E-79	0.55574508	8.12E-75
CTSA	1.11E-78	0.50475862	3.74E-74
OTOA	4.97E-77	0.40792275	1.67E-72
ATP6V1F	7.26E-76	0.42065777	2.44E-71
FCGR2B	1.76E-75	0.3862756	5.90E-71
ACP5	5.64E-75	0.618868	1.89E-70
HM13	3.16E-74	0.4397216	1.06E-69
LHFPL2	4.53E-74	0.34128348	1.52E-69
CD68	1.82E-73	0.48546651	6.10E-69
PDXK	1.13E-69	0.34096117	3.79E-65

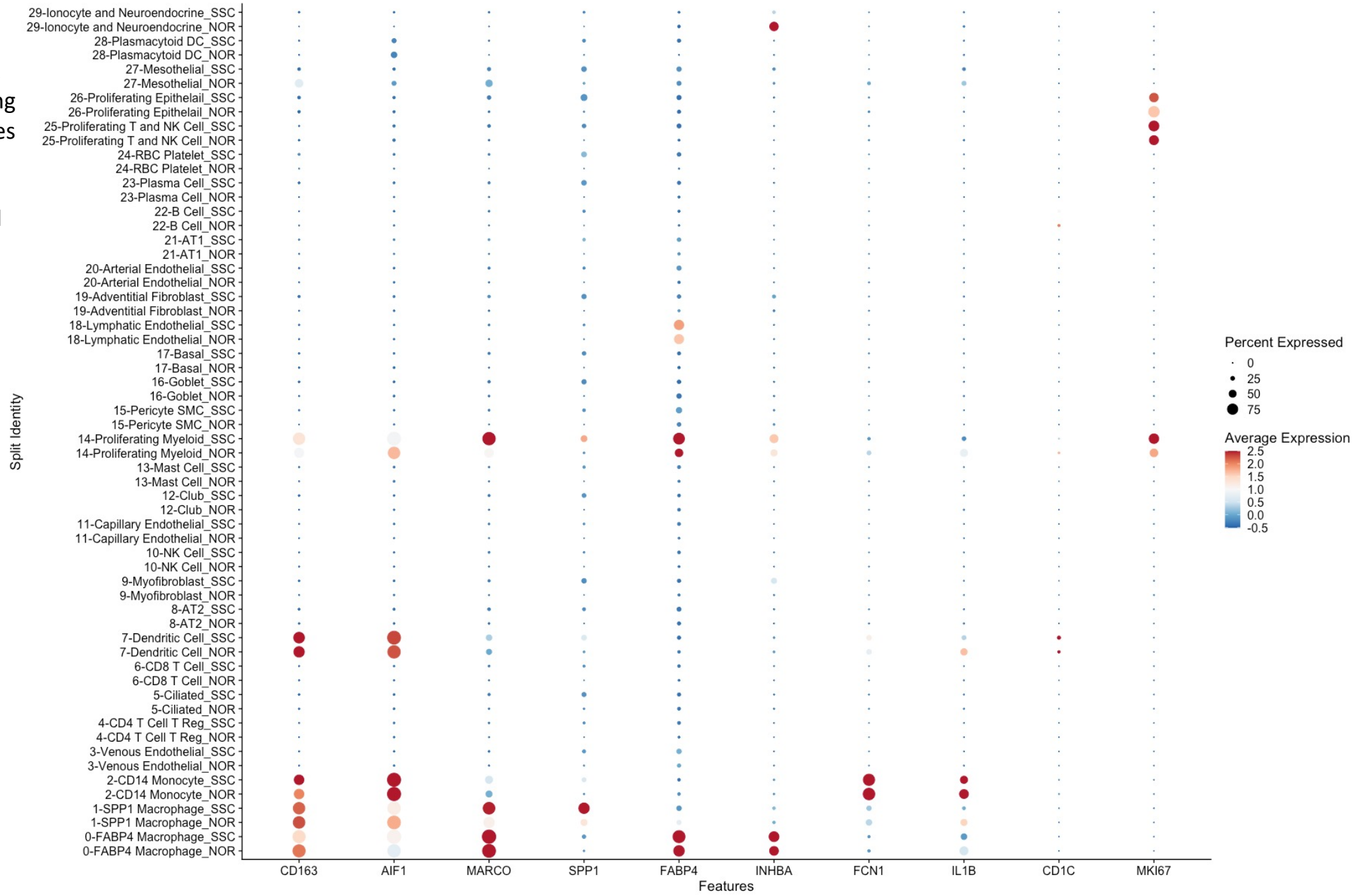
Supplementary Figure 13. Clustering of macrophages only from 6 lungs in 5' v1 chemistry as per health status and individual identity. Split DotPlot of macrophage subpopulations gene markers expression indicates *SPP1, MERTK* and *LGMN* upregulation in the SSc-ILD SPP1 Mϕ.



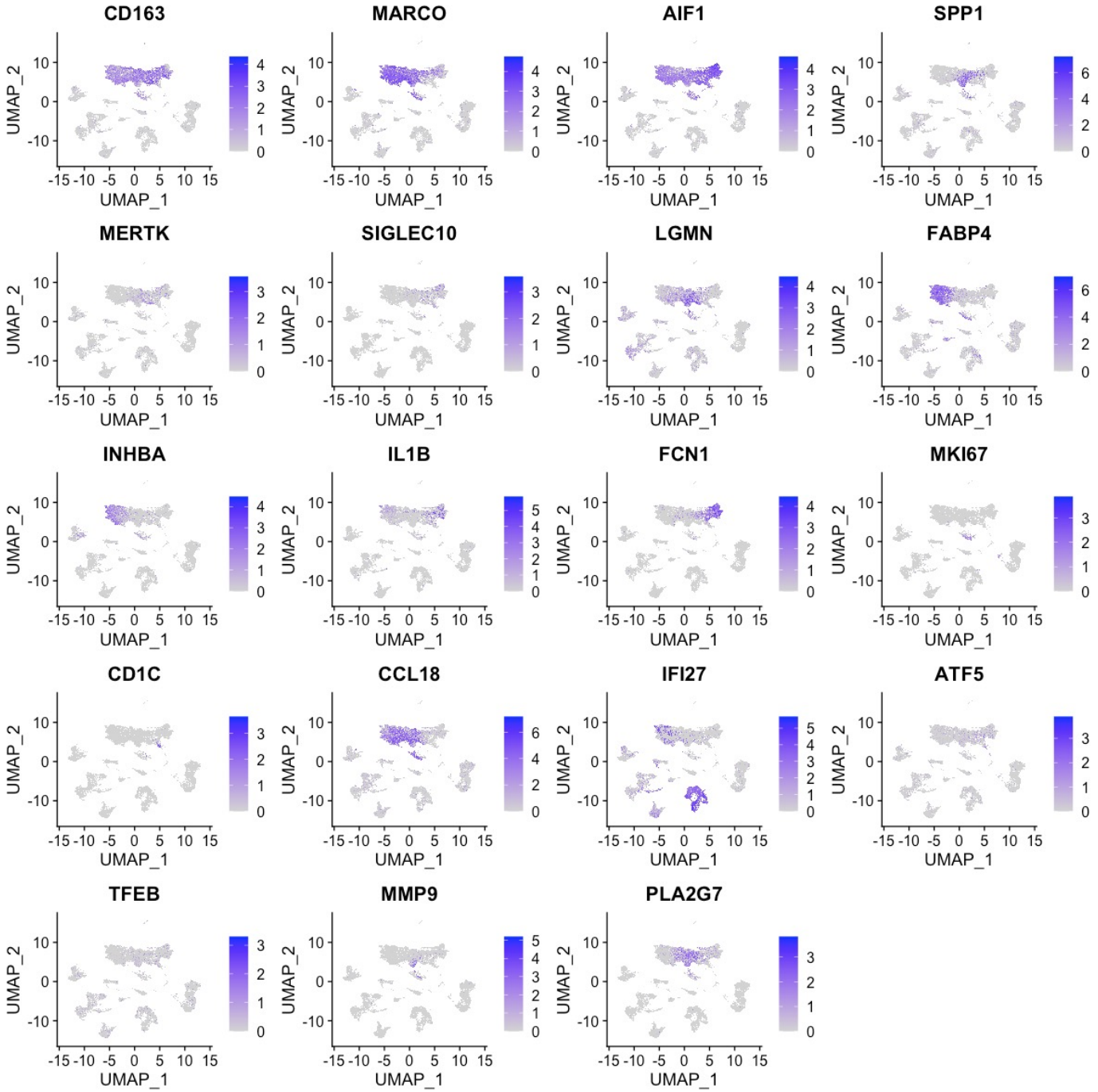
Supplementary Figure 14. Single-cell RNA-sequencing analysis of 30 lung samples in 3’ v3 and v2 chemistry as well as 5’ v1 and v2 chemistry (17 SSc-ILD and 13 HC). (A) Visualization of clustering by UMAP per cell type. (B) Visualization of clustering by UMAP per individual identity. (C) Visualization of clustering by UMAP per health status. SPP1 macrophages cluster 1 is formed primarily of macrophages from the SSc-ILD patients.



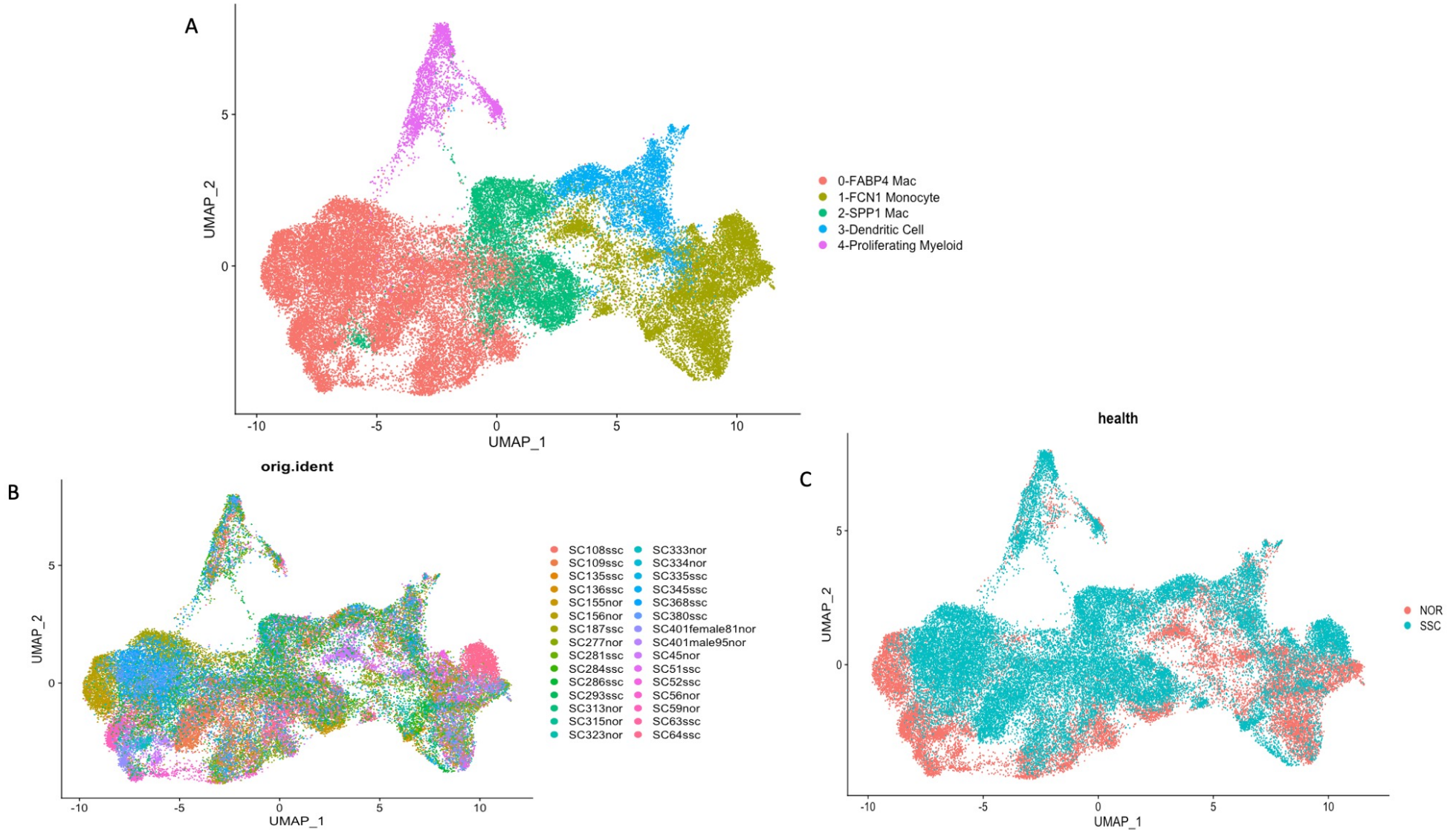
Supplementary Figure 15. Single-cell RNA-sequencing analysis of 30 lung samples in 3’ v3 and v2 chemistry as well as 5’ v1 and v2 chemistry (17 SSc-ILD and 13 HC). Visualization of gene expression by split DotPlot of macrophage subpopulation markers, dendritic cells, proliferating cells and other interesting genes.



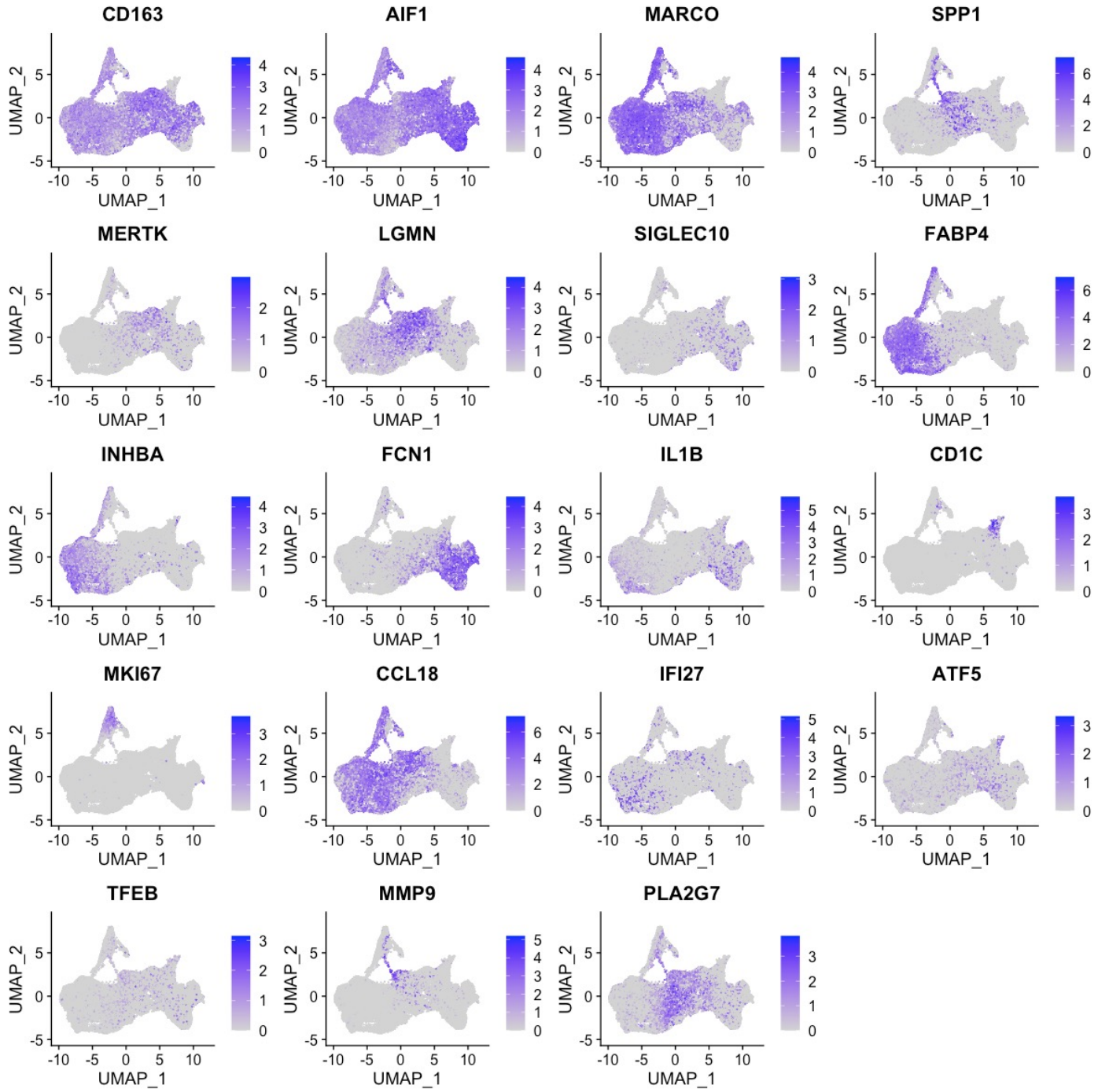
Supplementary Figure 16. Single-cell RNA-sequencing analysis of 30 lung samples in 3’ v3 and v2 chemistry as well as 5’ v1 and v2 chemistry (17 SSc-ILD and 13 HC). Visualization of gene expression by FeaturePlot of macrophage subpopulation markers, dendritic cells, proliferating cells and other interesting genes.

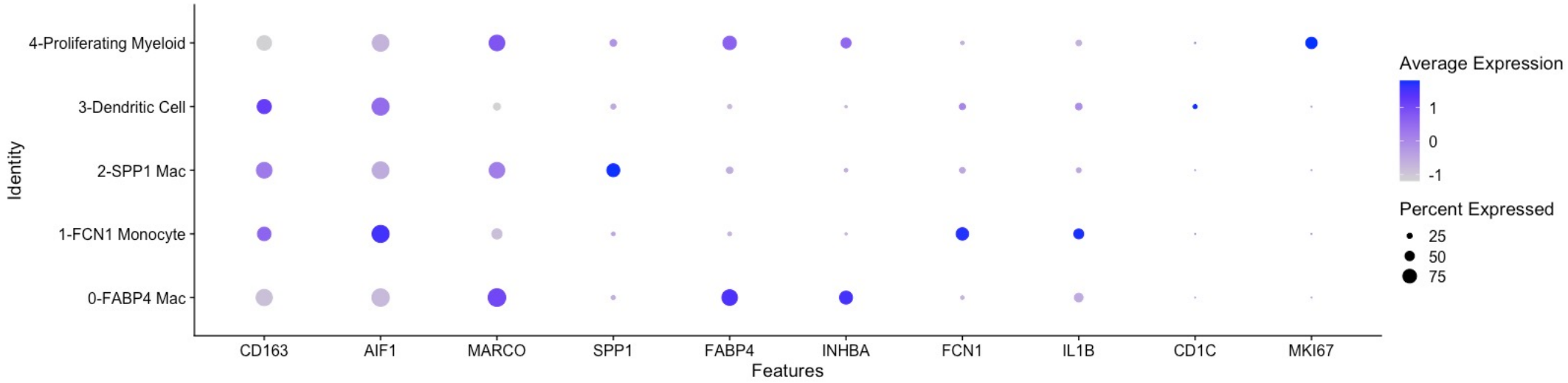


Supplementary Figure 17. Single-cell RNA-sequencing analysis of macrophage subpopulations, dendritic cells, monocytes, proliferating cells from 30 lung samples in 3’ v3 and v2 chemistry as well as 5’ v1 and v2 chemistry (17 SSc-ILD and 13 HC). (A) Visualization of clustering by UMAP per cell type. (B) Visualization of clustering by UMAP per individual identity. (C) Visualization of clustering by UMAP per health status. SPP1 macrophages cluster 1 is formed primarily of macrophages from the SSc-ILD patients.

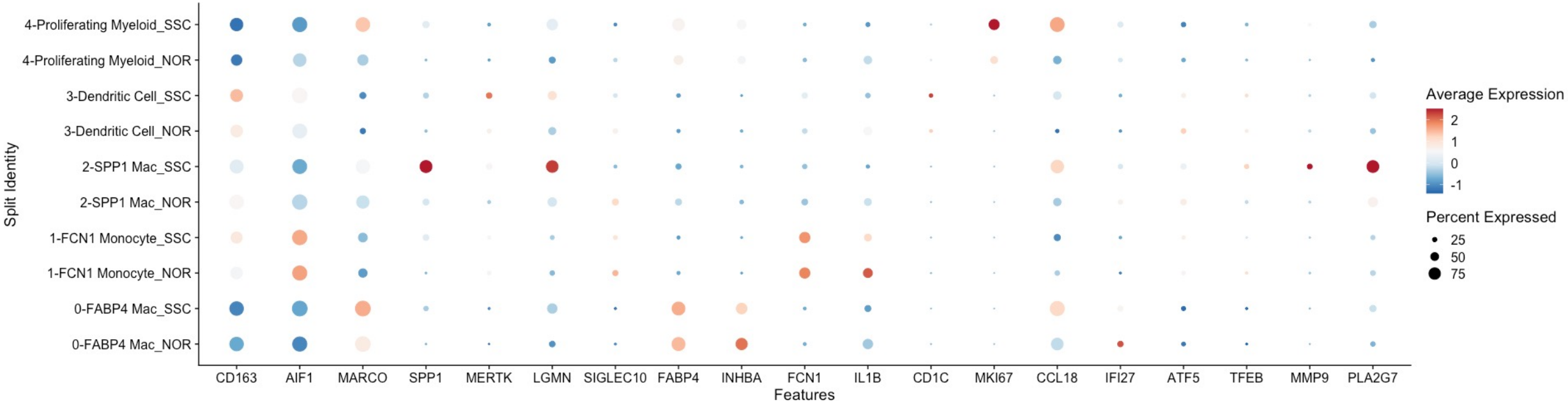


Supplementary Figure 18. Single-cell RNA-sequencing analysis of macrophage subpopulations, dendritic cells, monocytes, proliferating cells from 30 lung samples in 3’ v3 and v2 chemistry as well as 5’ v1 and v2 chemistry (17 SSc-ILD and 13 HC). Visualization of gene expression by FeaturePlot of macrophage subpopulation markers, dendritic cells, proliferating cells and other interesting genes.

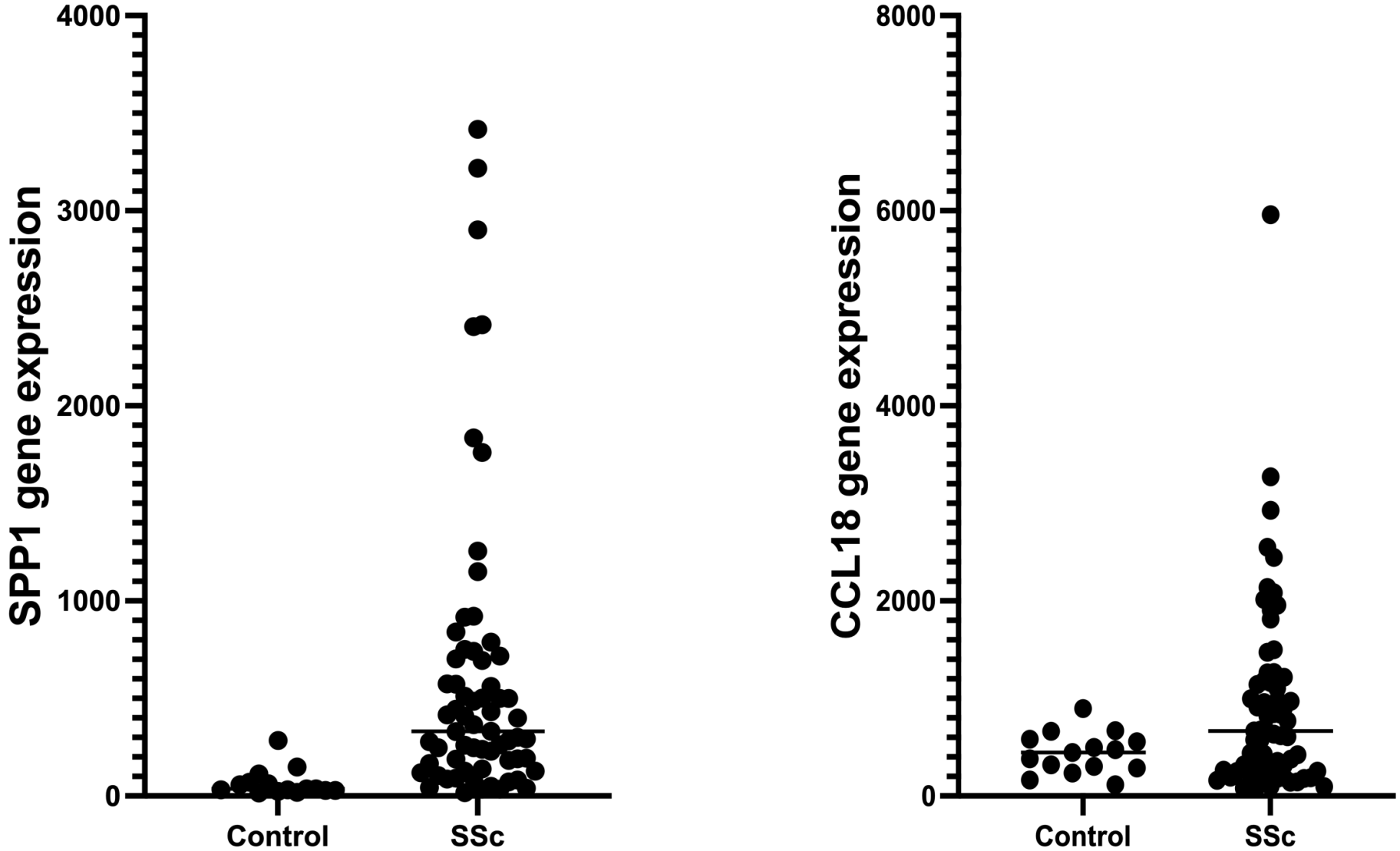




Supplementary Figure 19. Single-cell RNA-sequencing analysis of macrophage subpopulations, dendritic cells, monocytes, proliferating cells from 30 lung samples in 3' v3 and v2 chemistry as well as 5' v1 and v2 chemistry (17 SSc-ILD and 13 HC). Visualization of gene expression by DotPlot of macrophage subpopulation markers, dendritic cells, proliferating cells and other interesting genes.

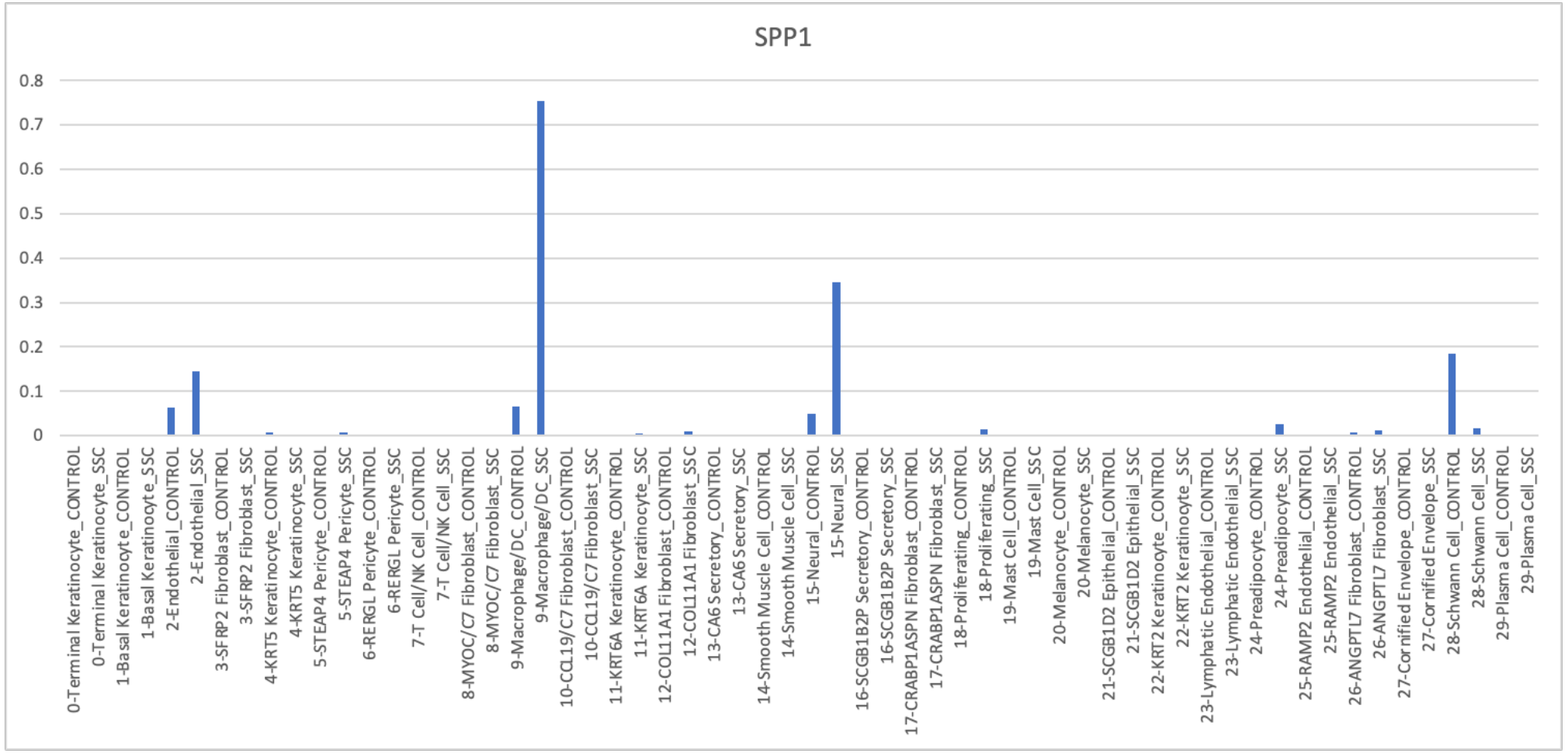


Supplementary Figure 20. Single-cell RNA-sequencing analysis of macrophage subpopulations, dendritic cells, monocytes, proliferating cells from 30 lung samples in 3' v3 and v2 chemistry as well as 5' v1 and v2 chemistry (17 SSc-ILD and 13 HC). Visualization of gene expression by split DotPlot of macrophage subpopulation markers, dendritic cells, proliferating cells and other interesting genes.

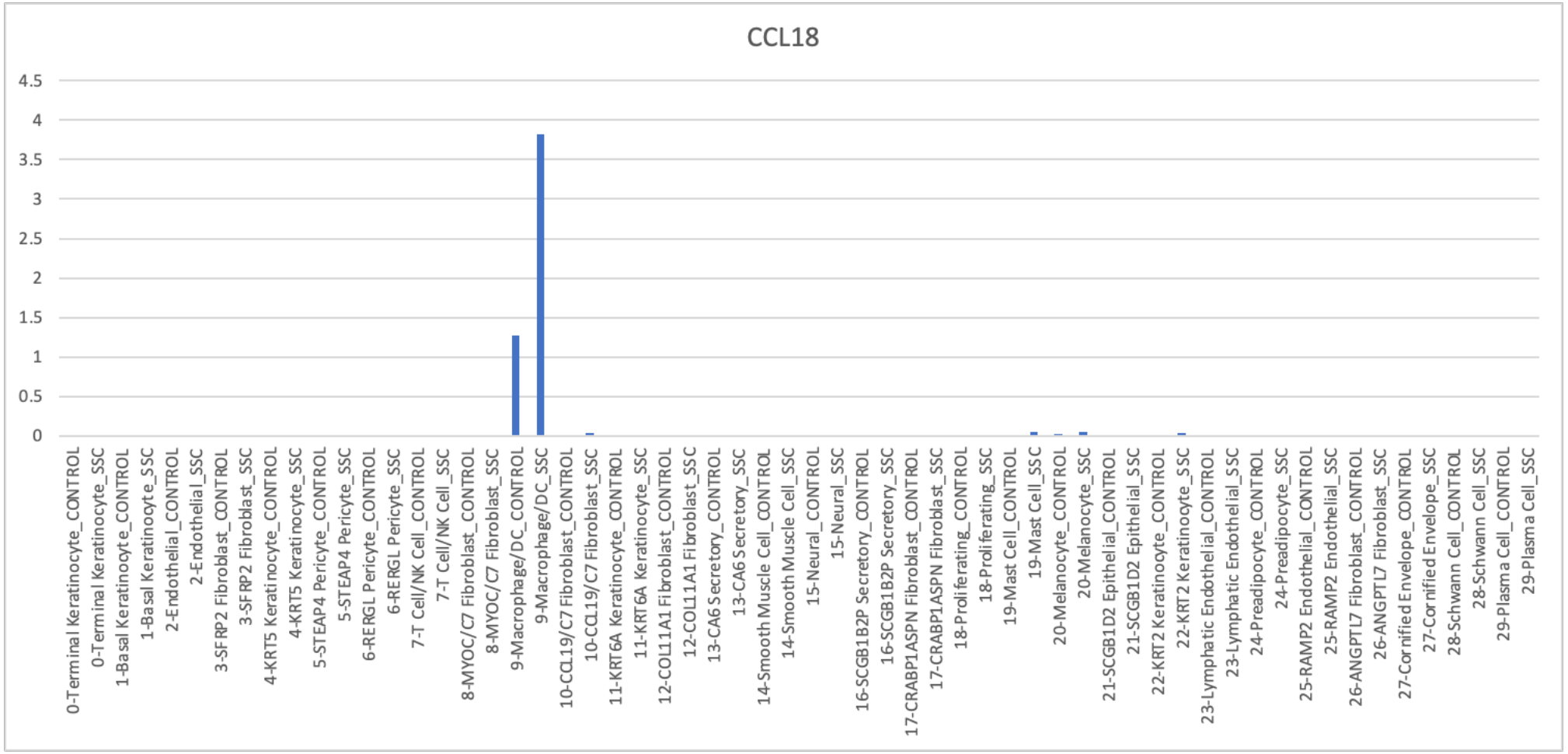


Supplementary Figure 21. Bulk gene expression of *SPP1* and *CCL18* in skin from patients with SSc (n=65) compared to healthy controls (n=15), p<0.005 for both.

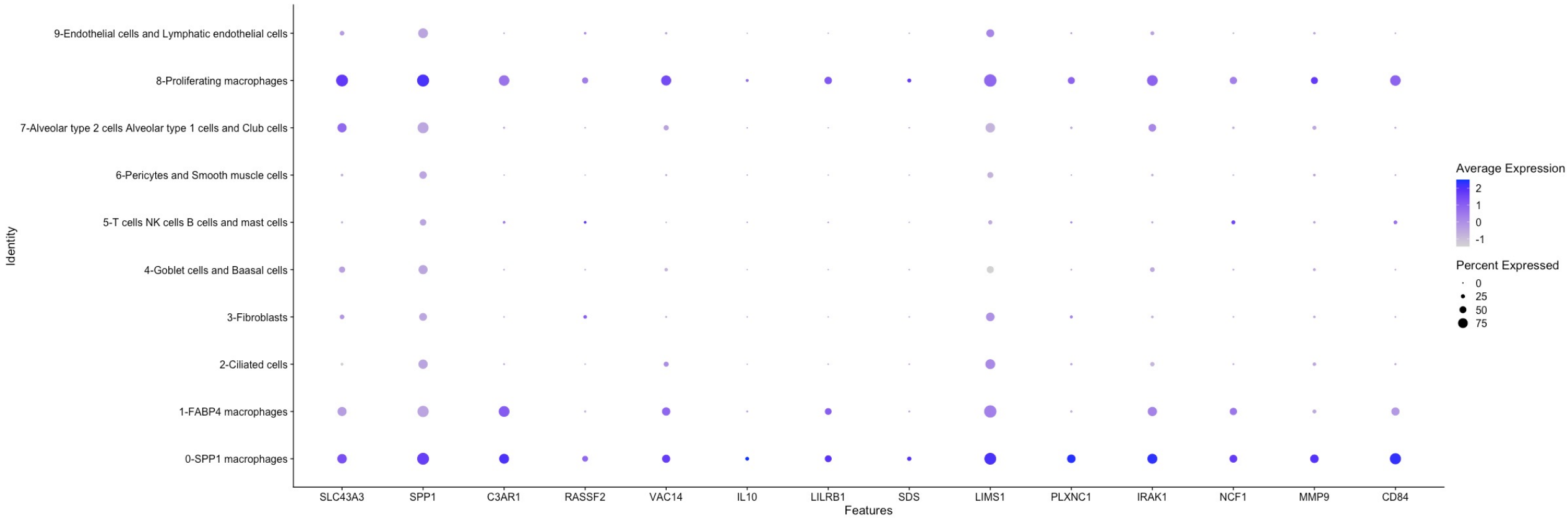
Supplementary Figure 22. Average expression of *SPP1* in SSc and control skin cell populations



Supplementary Figure 23. Average expression of *CCL18* in SSc and control skin cell populations



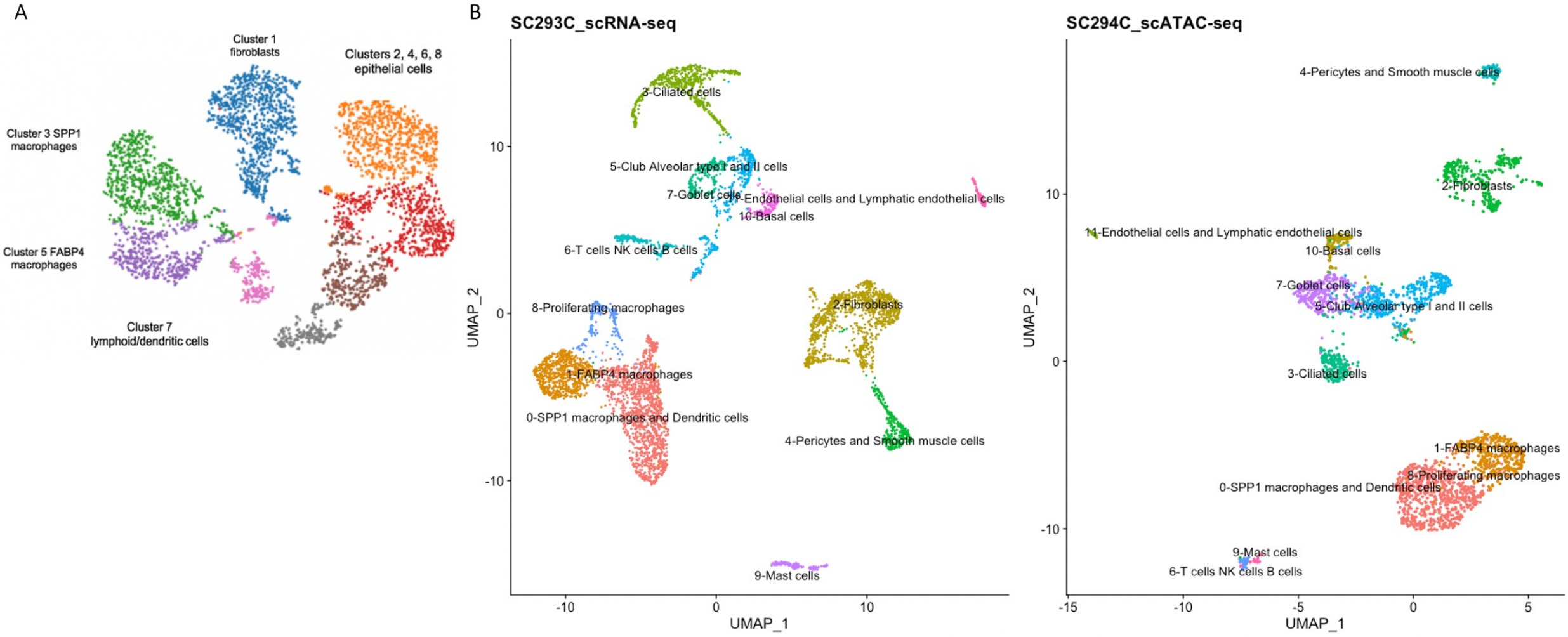
Supplementary Figure 24. Dot Plot of transcription factor ATF5 target genes expression for SPP1 Mϕ in cell types from SC293 SSc-ILD lung sample using scRNA-sequencing 3’ v3 Chemistry. ATF5 target genes include *SPP1* and were predicted using SCENIC software. *MMP9* is upregulated in SPP1 Mϕ.



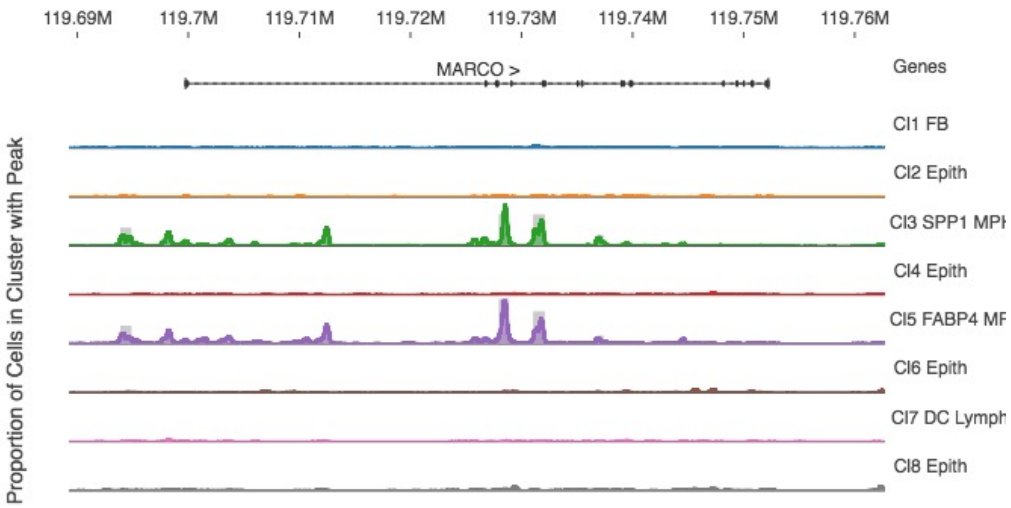
Supplementary Figure 25. Transcription factors important for SPP1 Mϕ with respective target genes as predicted by SCENIC software. *SPP1* and *MMP9* are target genes for transcription factors ATF5, TFEB, ZNF219, SREBF1, BCL11A, ETV5. The rest of the target genes are included in the accessible transcription factor motifs when comparing SPP1 Mϕ to FABP4 Mϕ as predicted by Signac (Satija Lab) and Loupe (10X Genomics) software.

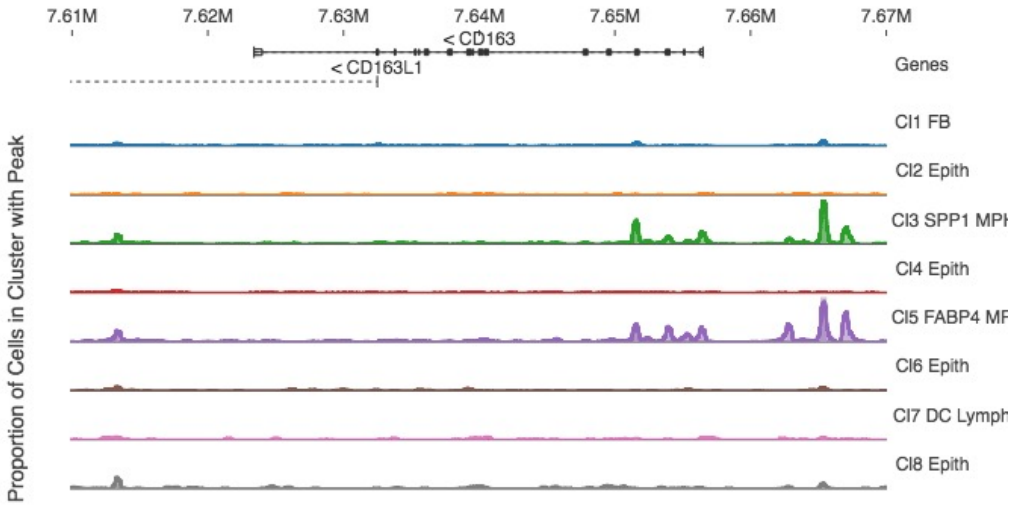
Transcription Factor	Target Gene
ATF5	SPP1
	MMP9
KLF6	KLF6
NR1H3	NR1H3
	SREBF1
	TFEB
TFEB	TFEB
	SPP1
	NR1H3
	KLF6
ZNF219	ARID3A
	MMP9
SREBF1	KLF6
	MAF
	BHLHE40
	NR1H3
	CREB3L2
	TFEB
	SREBF1
	ARID3A
	MMP9
BCL11A	MMP9
	SPP1
	MITF
	NR1H3
	BHLHE40
	TFEB

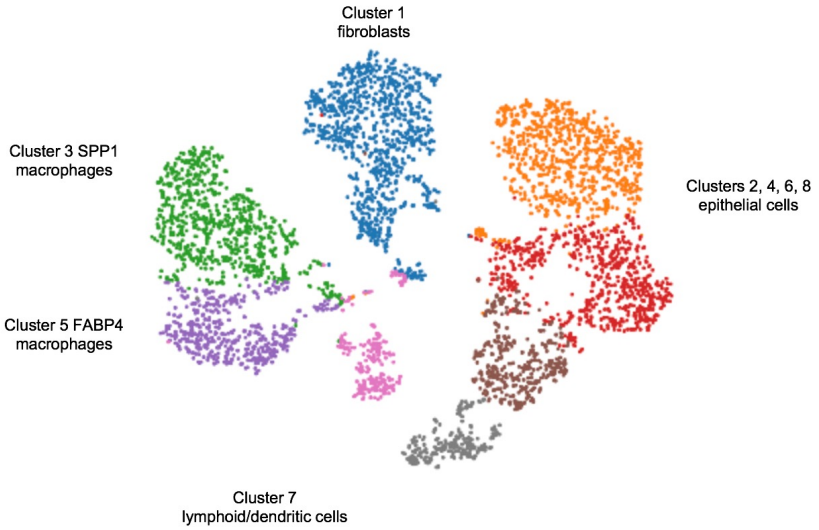
Transcription Factor	Target Gene
CREB3L2	CREB3L2
	MAF
MAFB	MAF
BHLHE40	ARID3A
MITF	KLF6
	BHLHE40
	TFEB
ARID5B	MAFB
	KLF6
ARID3A	FOSL2
	ARID3A
ETV5	ETV5
	MAF
	SPP1
	MMP9
	BHLHE40
	MAFB
	TFEB
ATF6	ATF6
	CREB3L2
FOXN3	MITF
	TFEB
	CREB3L2
	ARID3A
JUN	JUN
	KLF6

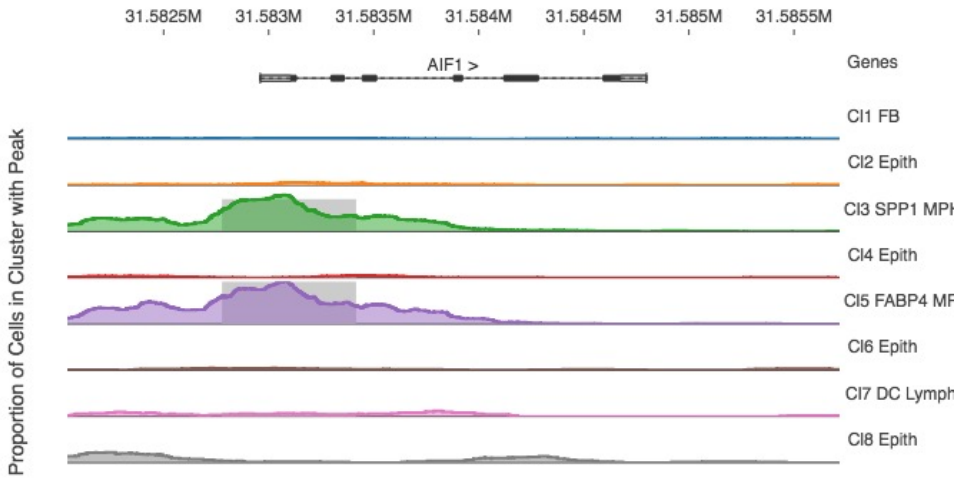


Supplementary Figure 26. Cell clusters identification during scATAC-sequencing of a SSc-ILD lung sample. (A) Cell clusters identification of SC294 SSc-ILD lung sample sequenced using scATAC-sequencing (Loupe, 10X Genomics). Loupe clusters cells based on pattern of accessible chromatin. (B) Cell clusters identification of SC294 SSc-ILD lung sample sequenced using scATAC-sequencing (Signac, Seurat package, Satija Lab). Signac recognizes all cell types including SPP1- Mϕ and FABP4-Mϕ subpopulations based on cell clustering following scRNA-sequencing of the same SSc-ILD lung sample in scRNA-sequencing 3' v3 Chemistry.

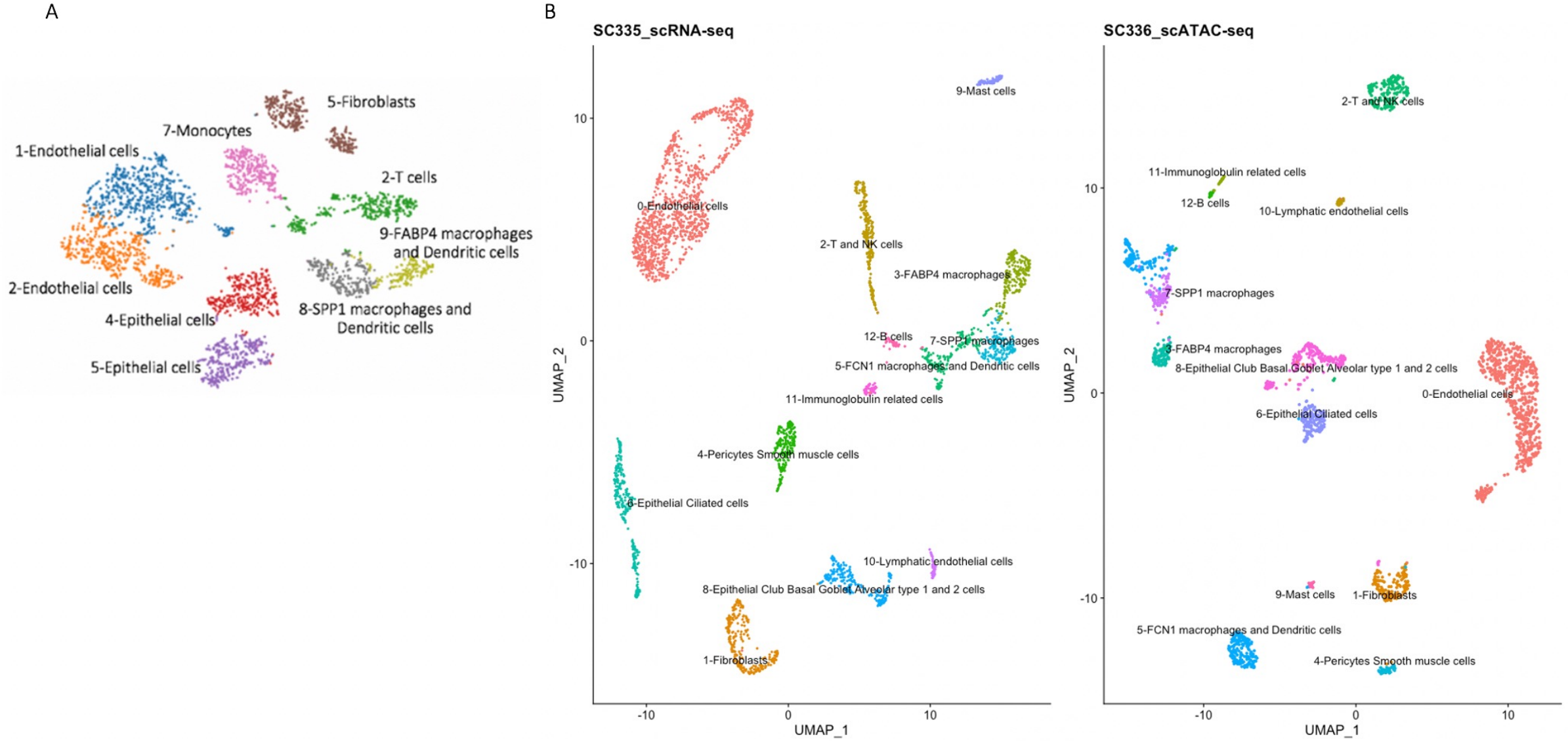








Supplementary Figure 27. Cell clusters identification during scATAC-sequencing of SSc-ILD lung sample SC294 (Loupe software, 10X Genomics). *MARCO, AIF1, CD163*- macrophage markers show open chromatin selectively in clusters 3, 5 indicating that these are macrophage clusters.

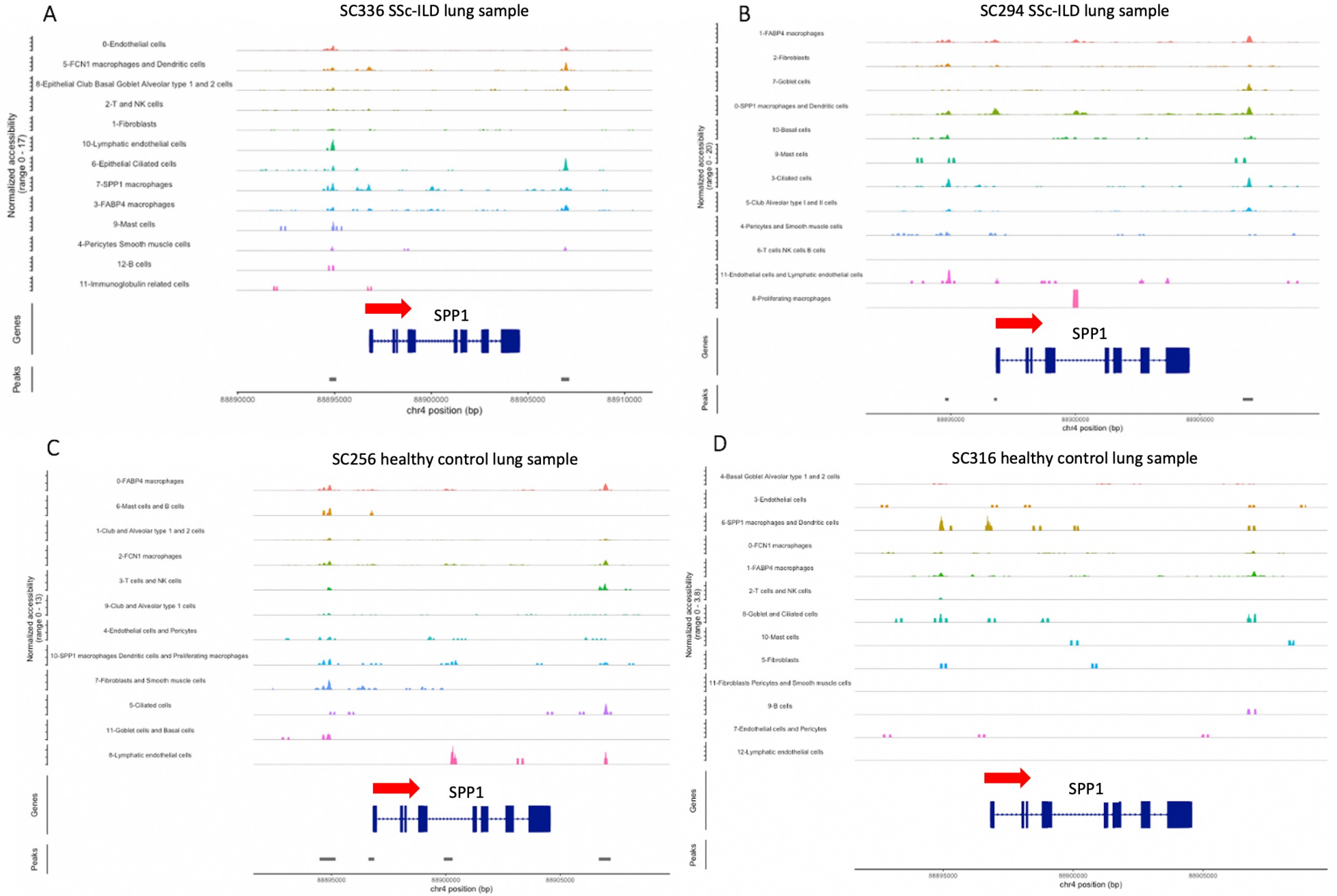


Supplementary Figure 28. (A) Cluster identification for SC336 SSc-ILD lung sample following scATAC-sequencing (Loupe software, 10X Genomics). Loupe software clusters cells based on patterns of accessible chromatin. (B) Cluster identification for SC336 SSc-ILD lung sample following scATAC-sequencing (Signac software, Seurat package, Satija Lab). Signac software (Satija Lab) recognizes SPP1- Mϕ and FABP4-Mϕ subpopulations based on cell clustering following scRNA-sequencing of the same SSc-ILD lung sample in scRNA-sequencing 5' Chemistry.

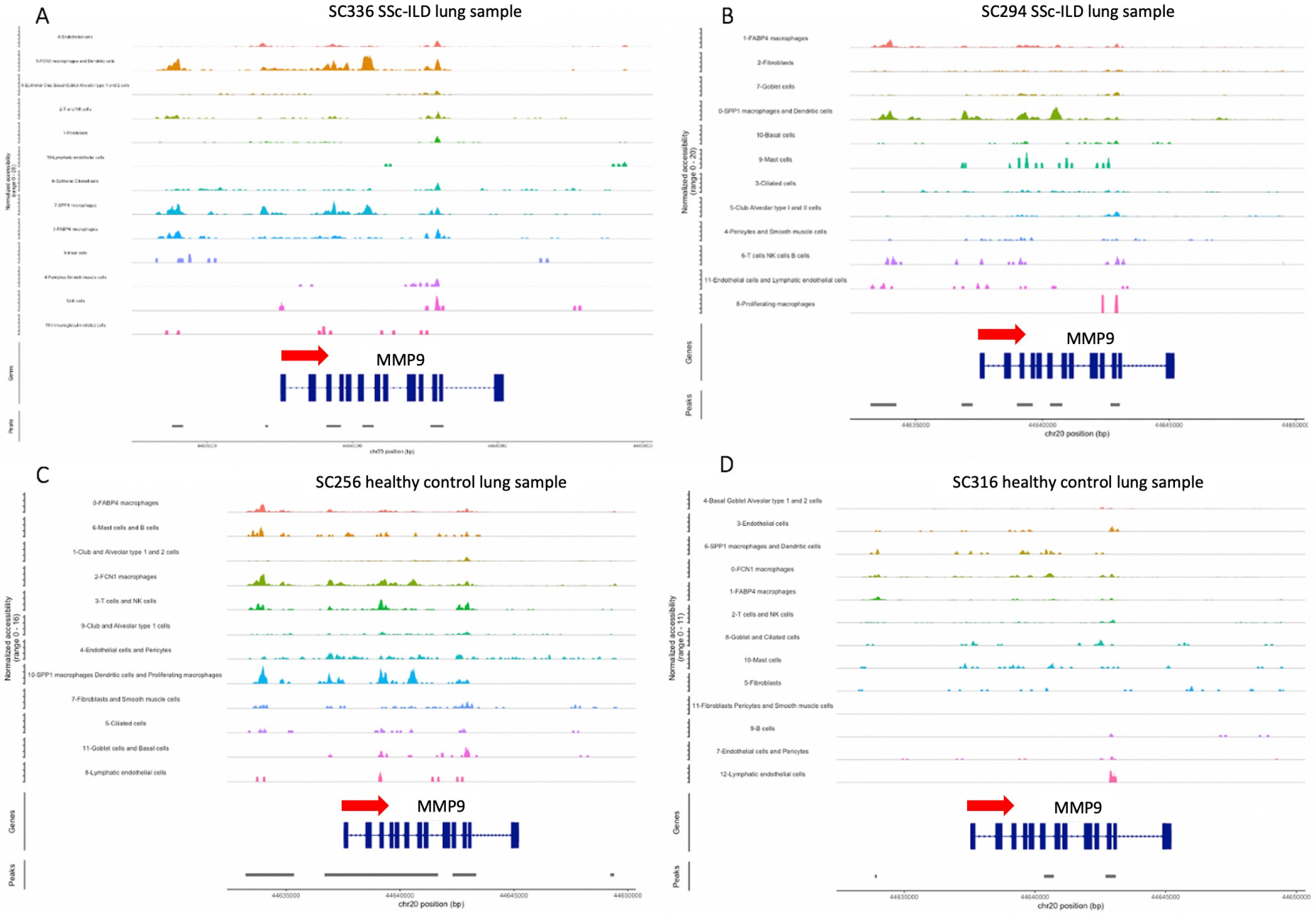
Motif Name	SPP1 macrophages Log2 Fold Change	SPP1 macrophages P-Value
SSc-ILD sample SC294		
JUNB	0.20128049	2.26E-73
FOSL2	0.20495588	2.26E-73
JDP2	0.19566932	7.78E-73
JUND	0.21575973	2.03E-68
RELA	0.18520054	7.90E-60
JUN::JUNB	0.17614251	1.27E-55
JUN(var.2)	0.15630376	3.61E-55
FOSL2::JUN	0.18803655	1.57E-54
Nr1h3::Rxra	0.02567833	0.01146031
Creb3l2	0.03804506	0.00139408
Mafb	0.03082591	0.04946388
Arid3a	0.00755867	0.41551652
SSc-ILD sample SC336		
FOSL2	0.18855657	7.16E-17
JUNB	0.18504058	2.80E-16
BHLHE40	0.04021566	0.16647644
TFEB	0.03742098	0.17654154
CREB3L2	0.03199525	0.2519222
SREBF1	0.01300544	0.80017521
ARID3A	0.006227	0.85249829
MAFB	0.00728728	0.91262936

Supplementary Figure 29. Accessible locally distinguishing transcription factor binding sites per cluster when comparing SPP1 Mϕ to FABP4 Mϕ as predicted by Loupe software, 10X Genomics for SSc-ILD samples SC294 and SC336. Transcription factors NR1H3, CREB3L2, MAFB, ARID3A, JUN and BHLHE40, TFEB, CREB3L2, SREBF1, ARID3A, MAFB for respective SSc-ILD samples SC294 and SC336 are also predicted to be important for SPP1 Mϕ by SCENIC software.

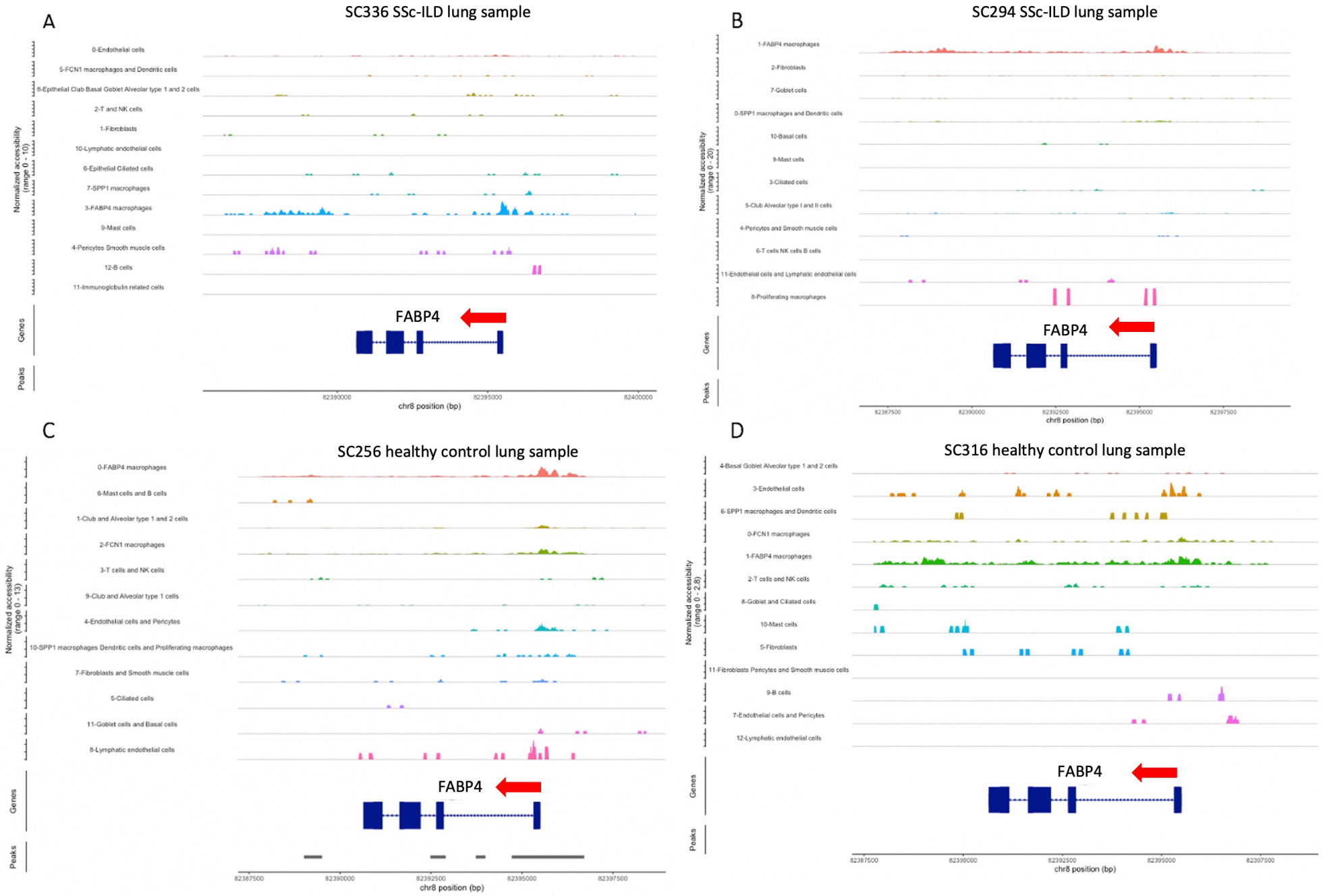
Supplementary Figure 30. ScATAC-sequencing analysis of 2 SSc-ILD lung samples (A, B) and 2 HC lungs (C, D) by Signac showing chromatin pattern changes for gene *SPP1* for all cell types. The red arrow indicates the direction of transcription. Exons are shown in blocks and introns flank exons. (A, B) *SPP1* gene showed more accessible chromatin for the SSc-ILD SPP1 Mϕ compared to FABP4 Mϕ in the region proximal to the transcriptional start site for a SSc-ILD lung, as well as regions further 5' of the promoter and in intron 4. (C, D) *SPP1* gene showed less accessible chromatin for the HC SPP1 Mϕ compared to SSc-ILD SPP1 Mϕ.



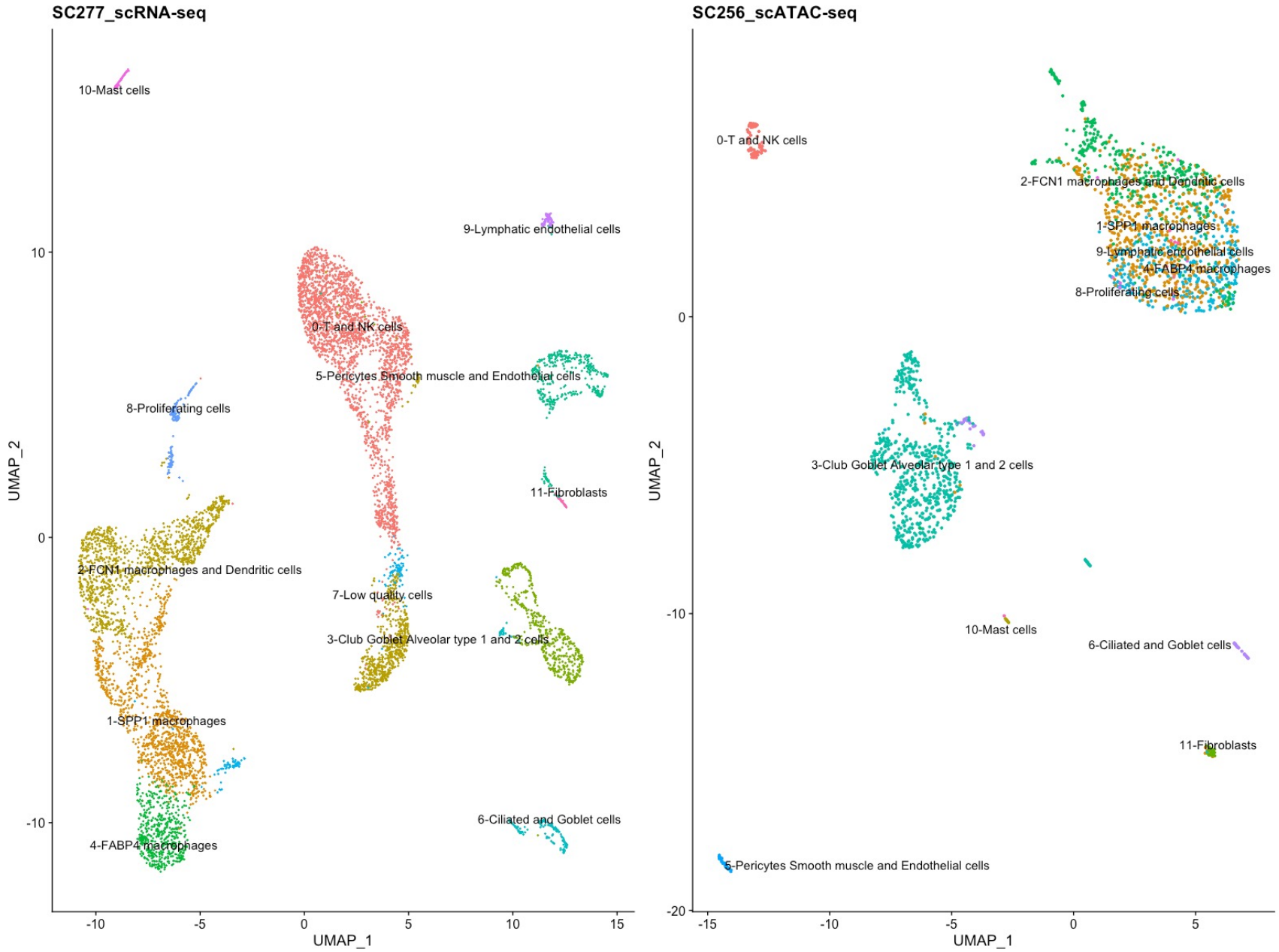
Supplementary Figure 31. ScATAC-sequencing analysis of 2 SSc-ILD lung samples (A, B) and 2 HC lungs (C, D) by Signac showing chromatin pattern changes for gene *MMP9* for all cell types. The red arrow indicates the direction of transcription. Exons are shown in blocks and introns flank exons. (A, B) *MMP9* gene showed more accessible chromatin in SPP1 Mϕ, but in this case increased accessibility was not seen around the promoter but rather in regions around exon 6, exons 9-12 and introns. (C, D) *MMP9* gene showed less accessible chromatin for the HC SPP1 Mϕ compared to SSc-ILD SPP1 Mϕ.



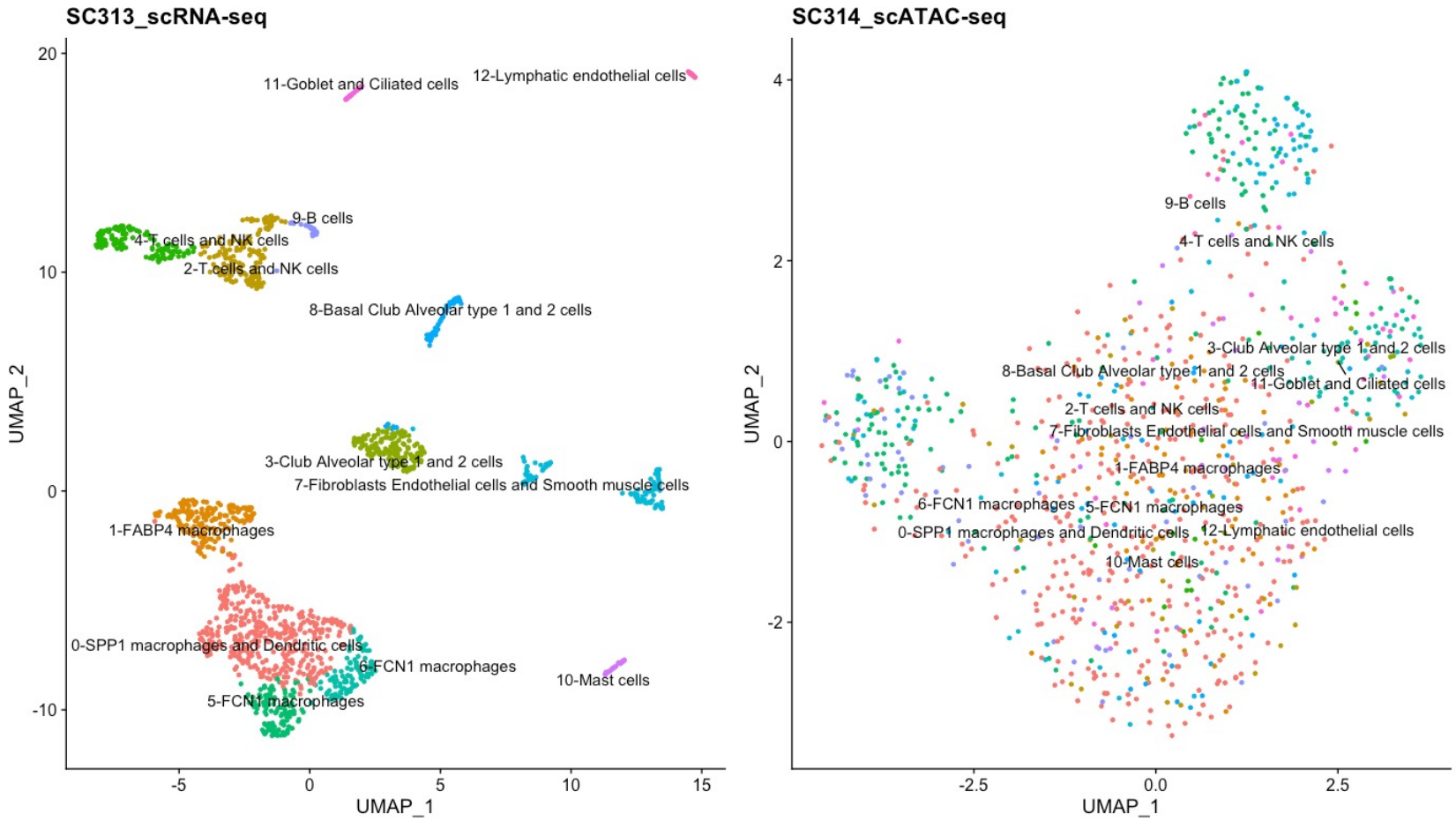
Supplementary Figure 32. ScATAC-sequencing analysis of 2 SSc-ILD lung samples (A, B) and 2 HC lungs (C, D) by Signac showing chromatin pattern changes for gene *FABP4* for all cell types. The red arrow indicates the direction of transcription. Exons are shown in blocks and introns flank exons. (A, B, C, D) *FABP4* gene showed more accessible chromatin in a region proximal to the transcriptional start site and in a broad second region 3' from the gene in FABP4 Mϕ compared to SPP1 Mϕ.



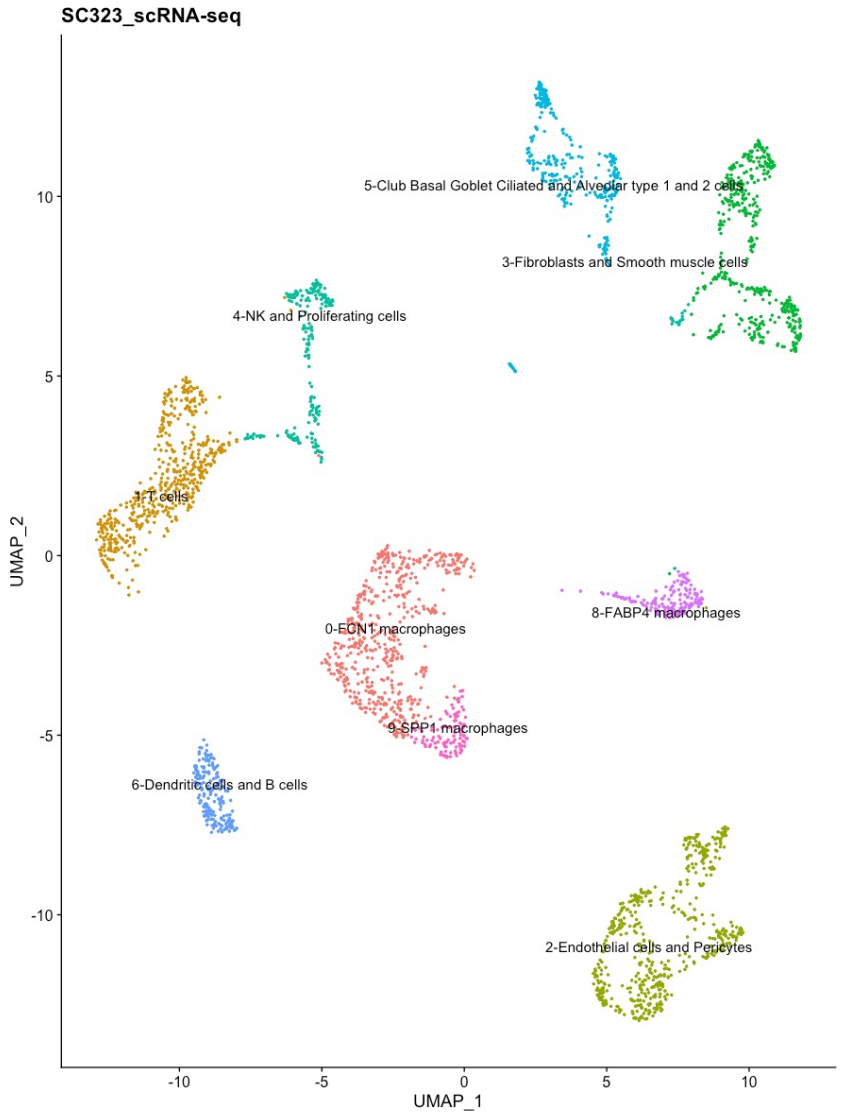
Supplementary Figure 33. Cluster identification for SC256 healthy control lung sample following scATAC-sequencing (Signac software, Seurat package, Satija Lab). Signac software (Satija Lab) recognizes SPP1 Mϕ and FABP4 Mϕ subpopulations based on cell clustering following scRNA-sequencing of the SC277 healthy control lung sample in scRNA-sequencing 3’ v3 Chemistry from a different healthy control individual. There was noted problematic macrophage subpopulations clustering for the scATAC-seq sample, as the macrophage subpopulations were not clustered distinctly from one another.

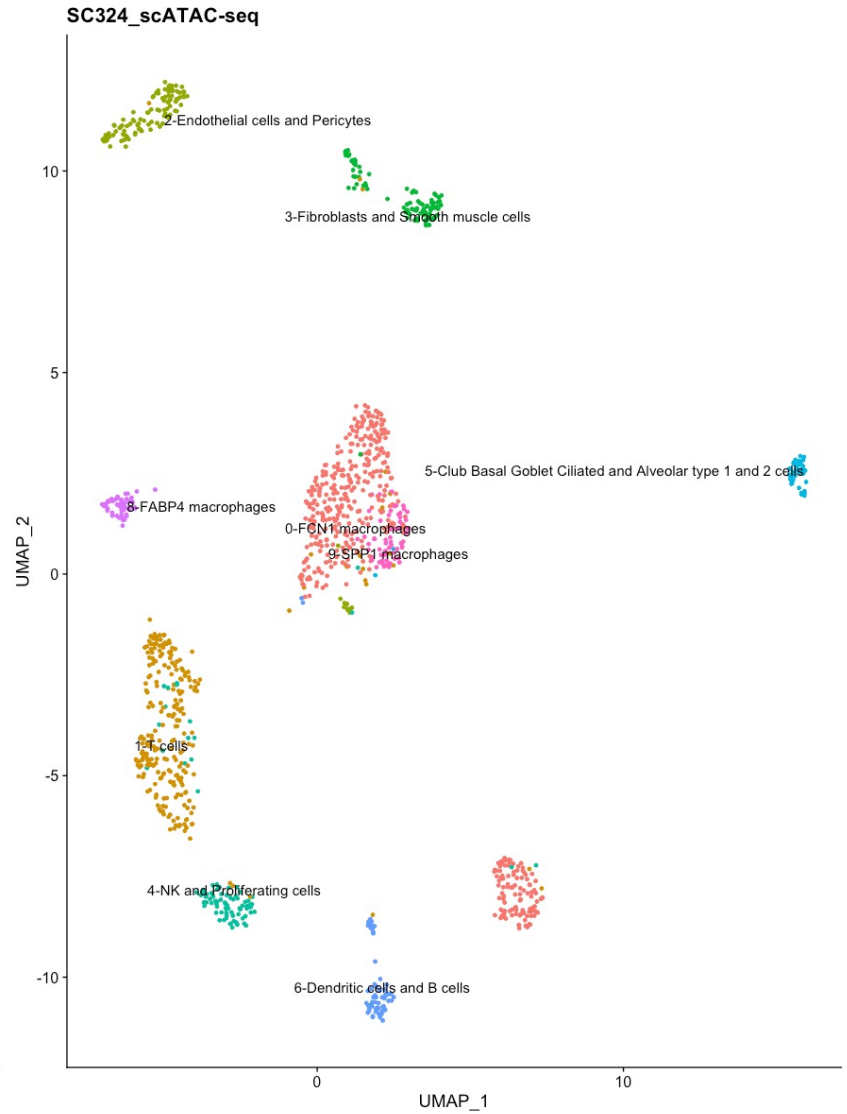


Supplementary Figure 34. Cluster identification for SC314 healthy control lung sample following scATAC-sequencing (Signac software, Seurat package, Satija Lab). Signac software (Satija Lab) recognizes SPP1 Mϕ and FABP4 Mϕ subpopulations based on cell clustering following scRNA-sequencing of the SC313 healthy control lung sample in scRNA-sequencing 5' v1 chemistry from the same healthy control individual. There was noted problematic macrophage subpopulations clustering for the scATAC-seq sample, as the macrophage subpopulations were not clustered distinctly from one another.

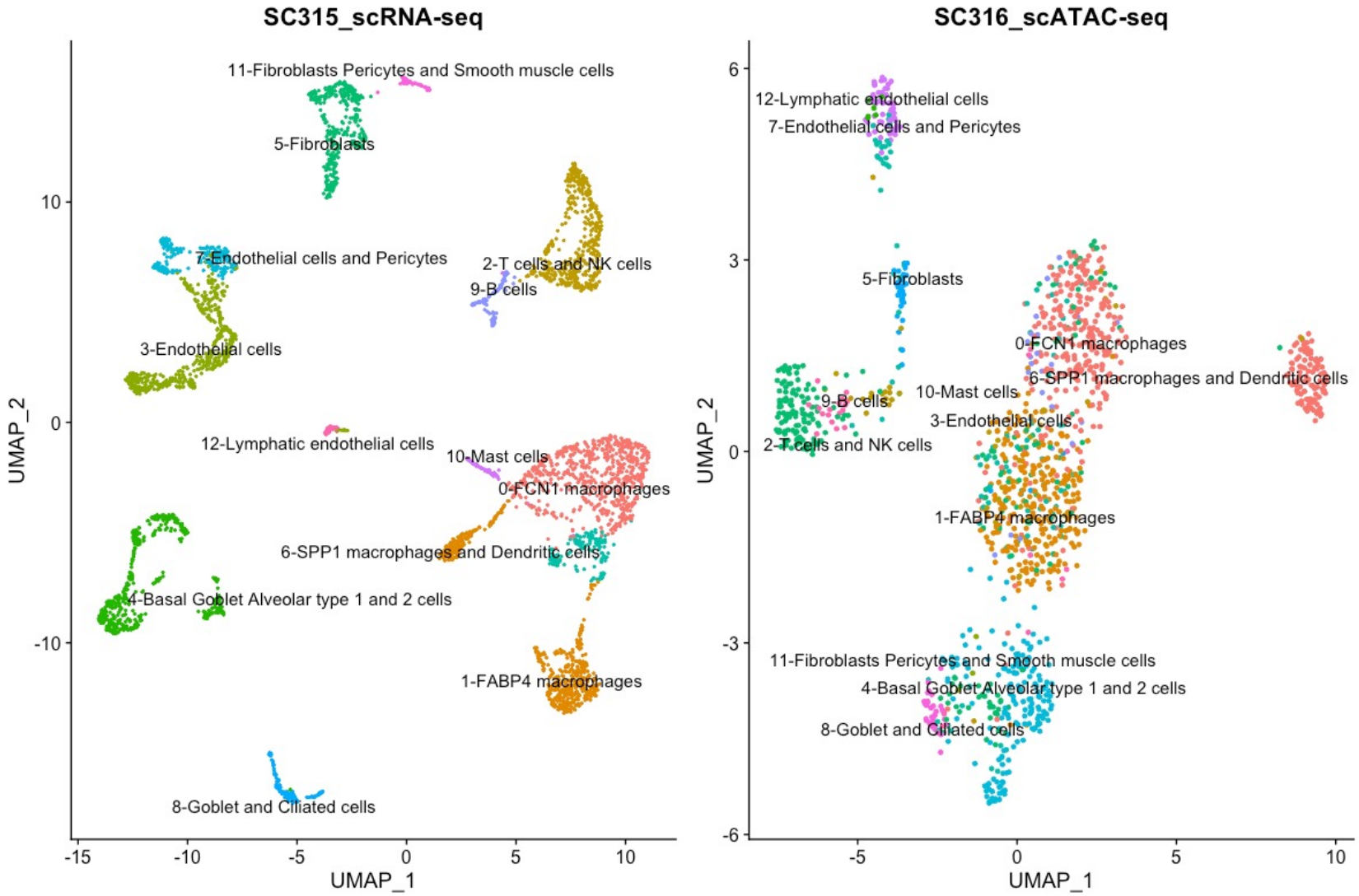


Supplementary Figure 35. Cluster identification for SC324 healthy control lung sample following scATAC-sequencing (Signac software, Seurat package, Satija Lab). Signac software (Satija Lab) recognizes SPP1 Mϕ and FABP4 Mϕ subpopulations based on cell clustering following scRNA-sequencing of the healthy control lung sample in scRNA-sequencing 5’ v1 chemistry from the same healthy control individual. There was noted problematic macrophage subpopulations clustering for the scATAC-seq sample, as the macrophage subpopulations were not clustered distinctly from one another.

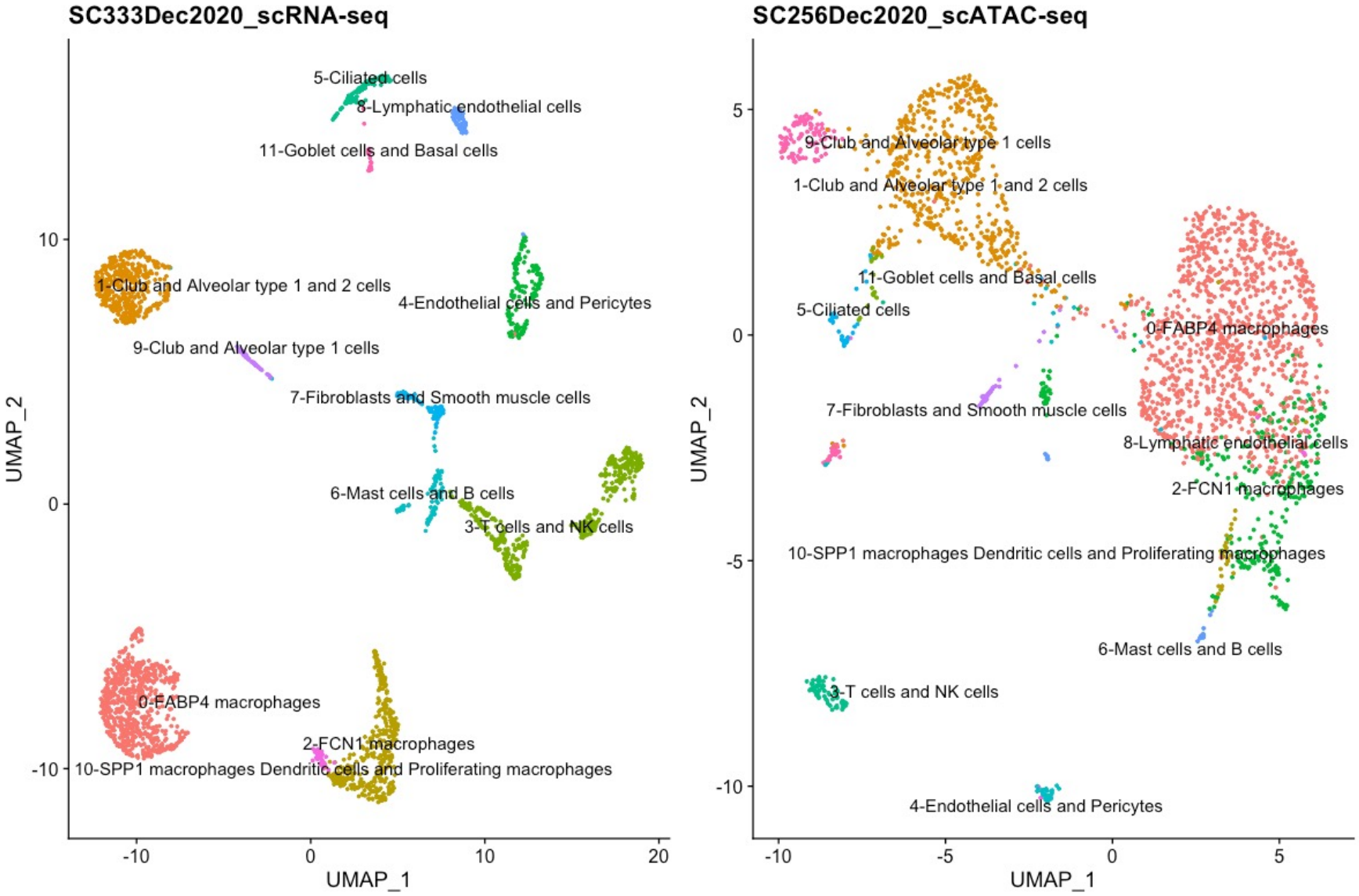


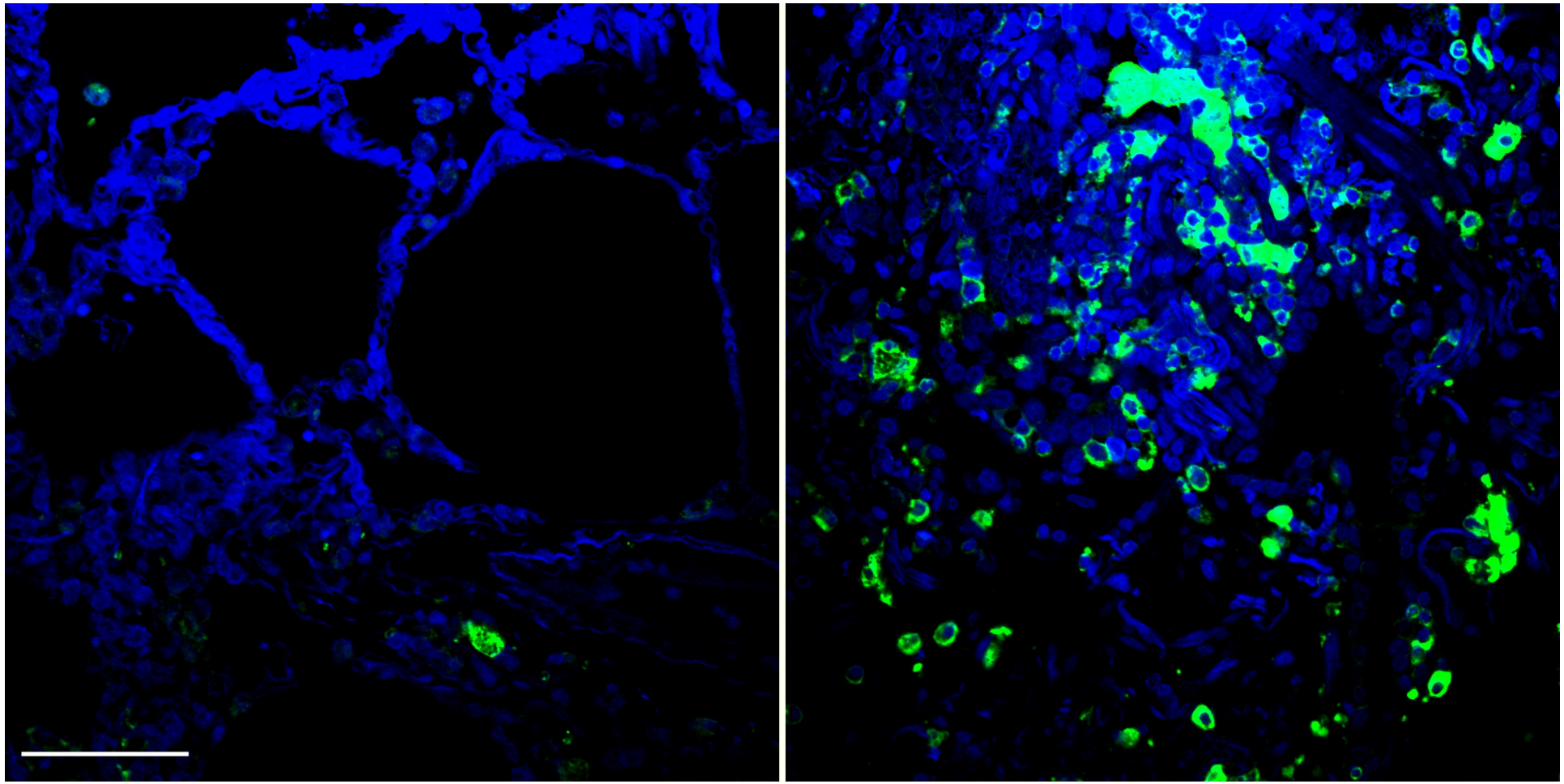


Supplementary Figure 36. Cluster identification for SC316 healthy control lung sample following scATAC-sequencing (Signac software, Seurat package, Satija Lab). Signac software (Satija Lab) recognizes SPP1 Mϕ and FABP4 Mϕ subpopulations based on cell clustering following scRNA-sequencing of the SC315 healthy control lung sample in scRNA-sequencing 5' v1 chemistry from the same healthy control individual. There was noted problematic macrophage subpopulations clustering for the scATAC-seq sample, as the macrophage subpopulations were not clustered distinctly from one another.



Supplementary Figure 37. Cluster identification for SC256 healthy control lung sample following scATAC-sequencing (Signac software, Seurat package, Satija Lab). Signac software (Satija Lab) recognizes SPP1 Mϕ and FABP4 Mϕ subpopulations based on cell clustering following scRNA-sequencing of the SC333 healthy control lung sample in scRNA-sequencing 5' v1 chemistry from the same healthy control individual. There are identified macrophage subpopulations including SPP1 Mϕ, FABP4 Mϕ and FCN1 Mϕ for the scATAC-seq sample. There appear to be few SPP1 Mϕ present in healthy control lung samples.

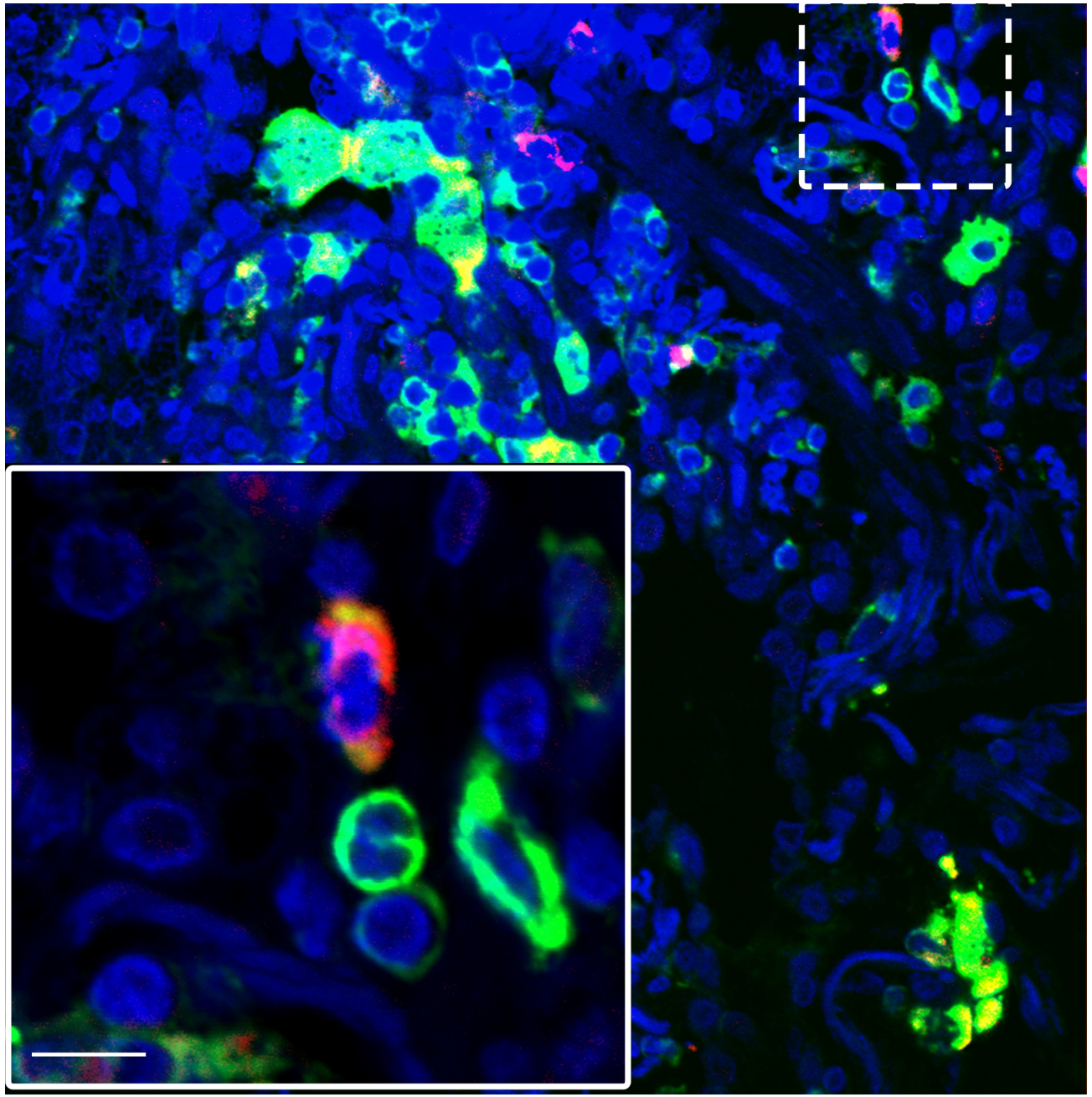




Control vs SSC-ILD MERTK (green) DAPI (blue) 40X scale bar = 100uM

Supplementary Figure 38. Immunofluorescence for MERTK antibody using HC vs SSc-ILD lung samples. MERTK antibody is in green and DAPI stain is in blue indicating the nucleus of the macrophages.

Supplementary Figure 39. Immunofluorescence for MERTK and TFEB antibodies using SSc-ILD lung sample. MERTK antibody is in green, TFEB antibody is in red and DAPI stain is in blue. The area on the lower left part of the figure is zooming in on a single cell level.



SSC-ILD MERTK (green) TFEB (red) DAPI (blue) original 40X inset scale bar = 25uM @120X

Supplementary Figure 40. Immunofluorescence for MERTK and TFEB antibodies using SSc-ILD lung sample. Zooming in on a single cell level DAPI stain is in blue, MERTK antibody is in green and TFEB antibody is in red. The area in the middle and the right part of the figure show the breakout for each stain on a single cell level.