18 – Ciliated cells SSc 18 – Ciliated cells HC	•				•			•		•	•								•		
17 – Lymphatic endothelial cells SSc																					
17 – Lymphatic endothelial cells HC					•													•			
16 – B cells SSc	•		2																		
16 – B cells HC						$\mathbf{x}$											•				
15 – Pericytes and Smooth muscle cells SSc	•														•						
15 – Pericytes and Smooth muscle cells HC	•				•											•					
14 – Goblet cells SSc	•				•			٠			•		•								
14 – Goblet cells HC	•							•			•										
13 – Proliferating cells SSc	•				•									•							
13 – Proliferating cells HC									•		*			•				1.6			
12 – Basal cells SSc										٠	•		•								
12 – Basal cells HC													•	•							
11 – Low quality cells SSc	•				•			•			•										
11 – Low quality cells HC	•				•			•			•										
10 – Mast cells SSc	•				٠			•			•	•									
10 – Mast cells HC											٠	•								Percent Expressed	ġ.
9 – Alveolar Type 2 cells SSC					1			•		•	•									• 0 • 25	
9 – Alveolar Type 2 cells HC											•									• 50 • 75 • 100	
8 – Cillated cells and Goblet cells SSC	•							•		•	•		1						•		
8 – Cillated cells and Goblet cells HC	•							•		•									•		
7 – NK čelis SSC	•			•	*				•	*											
6 – Goblet cells and Alveolar Type 1 cells SSc				•					•	1.27											
6 – Goblet cells and Alveolar Type 1 cells HC																					
5 – Fibroblasts SSc								÷.													
5 – Fibroblasts SSC																					
4 – Endothelial cells SSC																					
4 – Endothelial cells HC																					
3 – FABP4 Mo SSc																					
3 – FABP4 Mo HC					•																
2 – T cells SSc	•			•							•										
2 – T cells HC	•			•	0				•		•										
1 - FCN1 M¢ and DC SSc	•	•	•								•										
1 - FCN1 M¢ and DC HC	•	•																20			
0 - SPP1 Mφ SSc																					
0 - SPP1 Mϕ HC																					
	SPP1	-CN1	CD1C	D3D	ABP4	VWF	1A1	B3A1	KG7	CAPS	FTPC	SAB1	<b>T17</b>	KI67	GS5	DES	34A1	VE1	orf85		
			0	0	Ę		COL	SCG	z	eatur	es	TP	K	Σ	8	-	M	C	C206		

Supplementary Figure 1. Split DotPlot of top gene markers expression per cluster in 5 lung samples (3' v3 scRNAseq 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.

Split Identity



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 coexpressed. 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 Mo 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 co-expressed are 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. SPP1 and FABP4 Mo markers.

Split Identity

	CD163	AIF1	MARCO	SPP1	MERTK	IGMN	SIGLEC10	FABP4	INHBA	Feat Feat	tures	CD1C	MKI67	CCL18	IFI27	MMP9	PLA2G7	ATF5	TFEB	
0-SPP1 Mφ HC	1	•	•	•	•	•	·	•	•	•	•			•			•	•	•	-
0-SPP1 Mφ SSc		•	•	•	•	•	•	•	•	•	•	·		•	•	•	•	•	•	
1-FCN1 M¢ and Dendritic cells HC	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
1-FCN1 M¢ and Dendritic cells SSc	•	•	•	•	•	٠	•	•	•	•	•			•	•	•	•	•	•	
2-T cells HC	•	•	•	•		•		•			•			•						
2-T cells SSc	•	•	•	•	-	•		•		•				•						
З-ҒАВР4 Мф НС	•	•	•			٠		•	•	•	•			٠			•	•	•	
З-FABP4 Mф SSc	•	٠	٠	•		•		•	•		•			•	•		•			
4-Endothelial cells HC	•	•	•	•		•		•						•	•			•		
4-Endothelial cells SSc	•	•	•	•		•		•		•				•	•			•	•	
5-Fibroblasts HC	• •	•		•		•								•	•			•		
5-Fibroblasts SSc	• •	•	•	•		•		•		•				•						
6-Goblet cells and Alveolar Type 1 cells HC	•		•																	
6-Goblet cells and Alveolar Type 1 cells SSc	• •										•			•	•					
7-NK cells HC	•	•	•	•										•						
7-NK cells SSc	•	•	•	•										•						
8-Ciliated cells and Goblet cells BSC															•					
8-Ciliated cells and Goblet cells SSc														•	•					• 75 • 100
9-Alveolar Type 2 cells HC																				• 25 • 50
9-Alveolar Type 2 cells SSc																				<ul> <li>O</li> </ul>
10-Mast cells SSC																				Dersont Expressed
11-LOW quality cells HC																				
11-Low quality cells SSc									•											
12-Basal cells HC				-								·	•							
12-Basal cells SSc	] .		•	•	·	•		•						•	•	·		•		
13-Proliferating cells HC	•	•	•	•	•	•	•			•	•	•	•	•			•	•	•	
13-Proliferating cells SSc	•	•	•	•	•	•		•	•		•		•	•	•	•	•	•	•	
14-Goblet cells HC	•	•	•	•	•	•		•	•	•			•	•	•					
14-Goblet cells SSc	•	•	•	•		•		•		•	•			•	•					
15-Pericytes and Smooth muscle cells HC	•	•	•	•		•		•		•				•			·			
15-Pericytes and Smooth muscle cells SSc	•	•	•	٠		•		•	•		•			•	•	•	•			
16-B cells HC	•	٠	•	•		•	•					•		٠	•			•	•	
16-B cells SSc	•	•	•	•	•	•		•				•		•	•	•		•	•	
17-Lymphatic endothelial cells HC	• •							•							•					
17-Lymphatic endothelial cells SSc	• •							•						•	•				•	
18-Ciliated cells HC	•	•	•	•		•		•				. T. 1		•					11	
18-Ciliated cells SSc					•		•	•	•	•		•			•	•				

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

	Homo sapiens (REF)					l l	1
GO biological process complete	<u>#</u>	<u>#</u>	expected	Fold Enrichment	<u>+/-</u>	<u>raw P value</u>	<u>FDR</u>
ightarrow very-low-density lipoprotein particle clearance	6	2	.02	> 100	+	1.93E-04	4.68E-02
ightarrow plasma lipoprotein particle clearance	39	3	.10	28.80	+	1.91E-04	4.77E-02
ightarrow 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
ightarrow neutrophil mediated immunity	493	14	1.32	10.63	+	4.19E-11	9.44E-08
ightarrow myeloid leukocyte mediated immunity	515	14	1.38	10.18	+	7.34E-11	8.91E-08
ightarrow 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
ightarrow 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
ightarrow leukocyte activation involved in immune response	630	15	1.68	8.92	+	8.36E-11	9.42E-08
ightarrow cell activation involved in immune response	634	15	1.69	8.86	+	9.12E-11	9.59E-08
ightarrow immune response	1972	18	5.27	3.42	+	2.10E-06	1.07E-03
$\rightarrow$ response to stimulus	8496	37	22.69	1.63	+	1.55E-04	4.02E-02

Supplemental Figure 6. Differentiating genes between SPP1 M $\phi$  and FABP4 M $\phi$  during analysis of only macrophages, dendritic cells, proliferating M $\phi$  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
ТТҮНЗ	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.

Split Identity

	CD163	AIF1	MARCO	SPP1	MERTK	IGMN	SIGLEC10	FABP4	INHBA	FCN1	IL1B	CD3D	NKG7	WVF	LYVE1	MS4A1	DES	RGS5	MALAT1	TPSAB1	COL1A1	MKI67	EPCAM	CD1C	HBA2	
0-FCN1 M¢ and Dendritic cells HC	•	•	•		•	•	•			•	•		•	14 .			0 . •		•				2		оч	-
0-FCN1 M¢ and Dendritic cells SSc	•	•	•	•	•	•	•			•			•		·				•					•		
1-T cells and NK cells HC		•										•	•						•							
1-T cells and NK cells SSc												•	•						•							
2-Endothelial cells HC						•		•						•	•			•	٠							
2-Endothelial cells SSc						•		•						•				•	•							
3-Epithelial cells HC						•													•		•		•			
3-Epithelial cells SSc						•													•	ð	·		•			
4-FABP4 Mφ HC	•	•	•	1.1		٠		•	•		•								•							
4-FABP4 Mφ SSc	٠	•	•			٠	•	•	•	•	٠		•	·					٠							
5-SPP1 Mφ HC	•	•	•		•	•	•	•	•	•	•		•						٠	4						
5-SPP1 Mφ SSc	•	•	•	٠	•	•	•	•		•			•						٠							
6-Fibroblasts HC					1	•													•		•					
6-Fibroblasts SSc			•		•	•			•					•				•	•		•					
7-Lymphatic endothelial cells HC					•	•		•						•	•				•							<ul> <li>50</li> <li>75</li> <li>100</li> </ul>
7-Lymphatic endothelial cells SSc						٠		•						٠	•			÷	٠							• 0 • 25
8-B cells HC							•									•			٠					83		Percent Expre
8-B cells SSc					•											•			•							
9-Pericytes and Smooth muscle cells HC																	•	•	•		•					
9-Pericytes and Smooth muscle cells SSc					•	•		•									•	•	•		•					
10-Mitochonrial related genes HC								L.				÷	•						•							
10-Mitochonrial related genes SSc												•							•	τ.,	÷		÷		÷	
11-Mast cells HC							•											÷	•	•						
11-Mast cells SSc																			•	•						
12-Proliferating cells HC	•	•	•									•	•						•			•				
12-Proliferating cells SSc	•	•	•	•	•	•	•	•		•	٠		•	•				•	•	3		•	·	·		
13-Dendritic cells HC		•				•	•				•		•						•					•		
13-Dendritic cells SSc	•	•		•		٠	•				•	•	•						•					•		
14-Hemoglobin related genes HC								90										Ŧ							•	
14 Here slabin related series 350																										

Supplementary Figure 9. Split of DotPlot 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 Μф.

14-Hemoglobin related genes SSc															•					
14-Hemoglobin related genes HC														·	•			•		
13-Dendritic cells SSc	•	•		•		•	•				•	•						•	•	
13-Dendritic cells HC		•				•	•				•	•	•	•	•	•		•	•	
12-Proliferating cells SSc	•	٠	•	•	•	•		•	•	٠		·	•	•	٠		•	٠	•	
12-Proliferating cells HC	•	•	•			•			•	•		•	•	•	•	·		•	•	
11-Mast cells SSc										•					·	•		·		
11-Mast cells HC															÷	•		•		
10-Mitochonrial related genes SSc	•														•					
10-Mitochonrial related genes HC								٠		87										
9-Pericytes and Smooth muscle cells SSc					•	•		•							٠					
9-Pericytes and Smooth muscle cells HC						•		•							•			•		
8-B cells SSc				i.	•							•						•	•	
8-B cells HC							•					•			•			•	•	Descent Everaged
7-Lymphatic endothelial cells SSc						•		•							•			•	•	• 0 • 25
7-Lymphatic endothelial cells HC					•	•		•							•			•		• 50 • 75
6-Fibroblasts SSc					·	•			•						٠		•	•	•	
6-Fibroblasts HC		*	4		*	•		٠	•			•			•			•	•	
5-SPP1 Mφ SSc	•	•	•	•	•	•	•	•		•	•			•	•		•	•	•	
5-SPP1 Mφ HC	•	•	•	•	•	•	•	•	•	٠	•			•	•	·	•	•	•	
4-FABP4 Mφ SSc	•	•	•			•		•	•	•	•	•		•	•		•	•		
4-FABP4 M¢ HC	•	•	•			•		•	•		•			•	•		•	•		
3-Epithelial cells SSc				·		•									٠			•	•	
3-Epithelial cells HC					·	•				-			•		٠			•	•	
2-Endothelial cells SSc						•		•						·	•			•		
2-Endothelial cells HC						•				•					•			•		
1-T cells and NK cells SSc																				
1-T cells and NK cells HC			÷												·			·		
0-FCN1 M¢ and Dendritic cells SSc	•	•	•	•	•	•	•			•	•	•		•	·	•	•	•	•	
0-FCN1 M¢ and Dendritic cells HC	•	•	•		•	•	•			•	•				•		•	•	•	_
	163	Ξ	0	Ę	¥	z	10	P4	BA	N1	8	2	21	.18	27	64	67	53	8	
	CD	All	MAR	SPP	MER	IGMI	SIGLEC	FABI	INH	FCI	11	CD1	MKIE	CCL	IFI	MM	PLA2	АТ	TFE	

Split Identity

Features

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 M $\phi$  and FCN1 M $\phi$  and FABP4 M $\phi$  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
ΜΑΤΚ	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
СТЅВ	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
ΟΤΟΑ	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 M $\phi$ .



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.

В

UMAP\_2



NOR

SSC

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.

		00103		WARGO	JFF I	Feat	tures	FUNT	ILID	CDIC	WIND/	
		CD163		MARCO	SDD1	EARD4	INHRA	ECN1	II 1B	CD1C	MKIEZ	00
	0-FABP4 Macrophage_000	<b>—</b>		ă l								
	0-FABP4 Macrophage SSC			•		•	•		•			
	1-SPP1 Macrophage NOR	ě.			-			•				
	1-SPP1 Macrophage SSC	ě	-									
	2-CD14 Monocyte_SSC	<b>—</b>	<b>—</b>									
	2-CD14 Monocyte SSC											
	3-Venous Endothelial MOD											
	3 Venous Endothelia											
						•						
	5-Ciliated_SSC -	•		•	•		•	*	•		•	
	6-CD8 T Cell_NOR			•		•	•	•	•		•	
	6-CD8 T Cell_SSC -		•			•	•				•	
	7-Dendritic Cell_NOR -	•		•		•	•		•	•	•	
	7-Dendritic Cell_SSC -			•	•	•	•		0	•		
	8-AT2_NOR -	•		•		•	•	•	•		•	
	8-AT2_SSC -	•	•	•	•	•	•	•				
	9-Myofibroblast_NOR -		•	•		•					•	
	9-Myofibroblast_SSC -	•	•	•	•	•	•	•	•		•	
	10-NK Cell_NOR -					•		•	•		•	
	10-NK Cell_SSC -	1.1			A.,	•	(***)			1		
	11-Capillary Endothelial_NOR -	2.4		•		•		1 A.		1.0		0.0
	11-Capillary Endothelial SSC -											-0.5
	12-Club NOR -											0.5
	12-Club SSC -				•			•				1.0
	13-Mast Cell NOR											1.5
S	13-Mast Cell_SSC -											2.0
Olit	14-Proliferating Myeloid NOR											2.5
p	14-Proliferating Myeloid SSC											Average Expression
en	15-Pericyte SMC_NOR -											• • •
tit	15-Pericyte SMC_SSC -											• 75
-												• 50
	17-Basal_NOR											• 25
												· 0
	17 Pasal SSC											Percent Expressed
	18-1 vmphatic Endothelial NOP											Demonst Comments i
	18-Lymphatic Endothelial SSC											
	19-Adventitial Fibroblast_SSC			-								
	20-Arterial Endothelial_NOR					•						
	20-Arterial Endothelial_SSC 1	·		•	•	•				· ·		
	21-AT1_NOR1			*	•		•	•	•		•	
	21-AT1_SSC -	•		•	•	•	•	•	•			
	22-B Cell_NOR	•		•		•	•				10	
	22-B Cell_SSC -		•	•	•	•					•	
	23-Plasma Cell_NOR			•							·	
	23-Plasma Cell_SSC -	•	•		•	•					÷	
	24-KBC Platelet_NOR -										•	
	24-RBC Platelet_SSC 1	•		•	•	•						
-	25-Proliterating 1 and NK Cell_NOR	•	•	•			•		•			
2S	25-Proliferating L and NK Cell_SSC -		•		•		*					
-	25 Proliferating T and NK Call SSC										-	
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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 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 M $\phi$  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 M $\phi$  to FABP4 M $\phi$ as predicted by Signac (Satija Lab) and Loupe (10X Genomics) software.

Target Gene
SPP1
MMP9
KLF6
NR1H3
SREBF1
TFEB
TFEB
SPP1
NR1H3
KLF6
ARID3A
MMP9
KLF6
MAF
BHLHE40
NR1H3
CREB3L2
TFEB
SREBF1
ARID3A
MMP9
MMP9
SPP1
MITF
NR1H3
BHLHE40
TFEB

Transcription Factor	Target Gene
CREB312	CRFB3L2
CILEBOLL	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- M¢ and FABP4-M¢ subpopulations based on cell clustering following scRNA-sequencing of the same SSc-ILD lung sample in scRNA-sequencing 3' v3 Chemistry.



with Peak

Cluster

of Cells in

Proportion (

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- M $\phi$  and FABP4-M $\phi$  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 M¢ to FABP4 M¢ 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 M¢ 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 chromatin pattern showing 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 Mo compared to FABP4 Mo 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 Md compared to SSc-ILD SPP1 Mφ.





Supplementary Figure 31. ScATACsequencing 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  $M\phi$ , 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 Μφ.



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 proximal the region to transcriptional start site and in a broad second region 3' from the gene in FABP4 Mo 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 Μф 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 M $\phi$  and FABP4 M $\phi$  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 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 FABP4 and МΦ 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 problematic noted macrophage was 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 scATACsequencing (Signac software, Seurat package, Satija Lab). Signac software (Satija Lab) recognizes SPP1 M¢ and FABP4 M¢ 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 Md and FABP4 Md subpopulations based on cell clustering following scRNA-sequencing of the SC333 healthy control lung sample in scRNAsequencing 5' v1 chemistry from the same healthy control individual. There are identified macrophage subpopulations including SPP1 Mφ, FABP4 Mφ and FCN1 Mφ for the scATACseq sample. There appear to be few SPP1  $M\phi$ 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.

