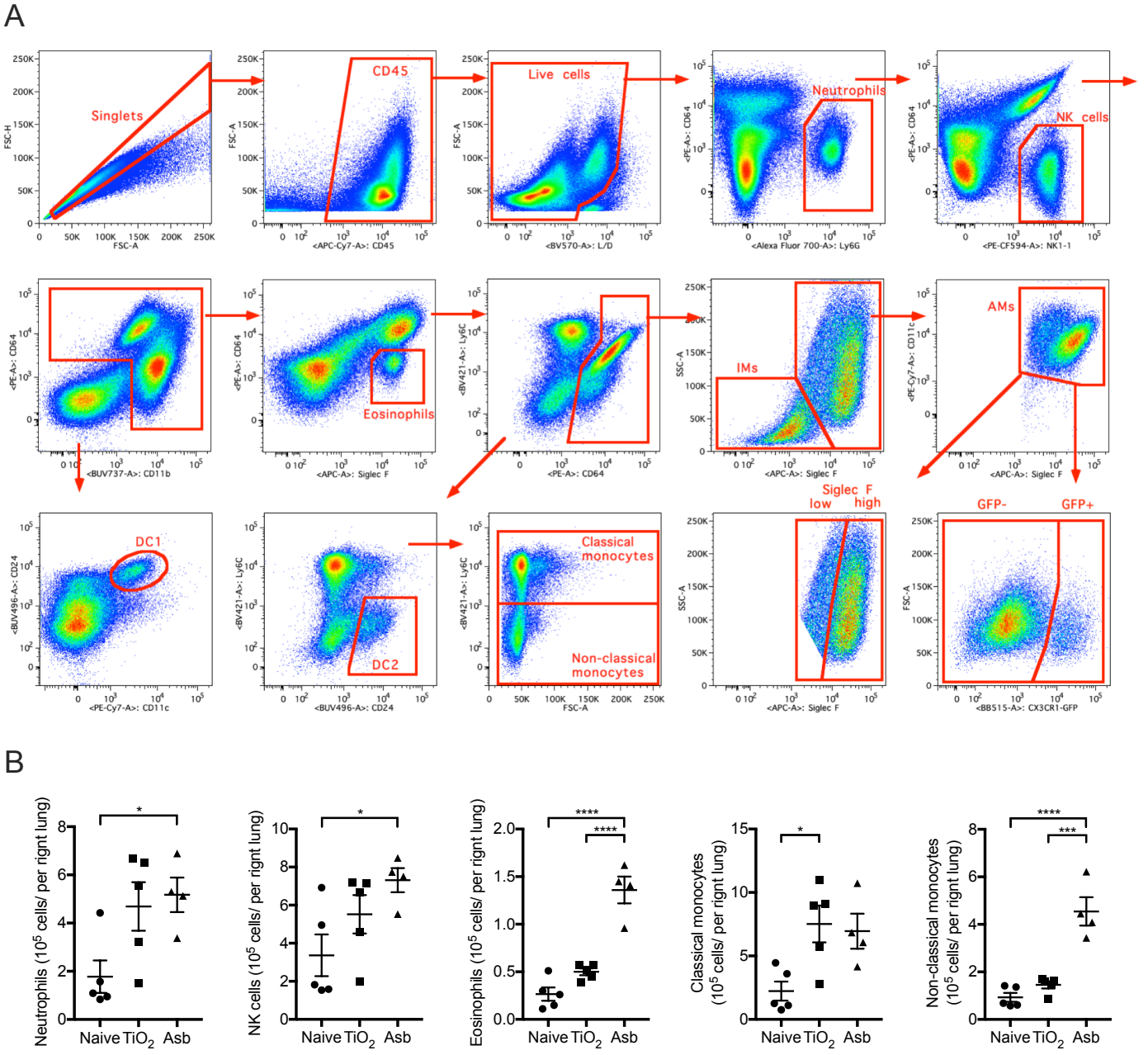

A
Singlets
CD45
Live cells
Neutrophils
NK cells
Eosinophils
IMs
AMs
DC1
DC2
Classical monocytes
Non-classical monocytes
Siglec F low high
GFP- GFP+
B
Neutrophils (10^5 cells/ per rignt lung)
NK cells (10^5 cells/ per rignt lung)
Eosinophils (10^5 cells/ per rignt lung)
Classical monocytes (10^5 cells/ per rignt lung)
Non-classical monocytes (10^5 cells/ per rignt lung)
Naive
TiO2
Asb

Figure S1 (refers to Figure 1): A. Gating strategy for quantification of myeloid populations in the lung. B. Quantification of myeloid cell populations by flow cytometry 14 days after mice were administered crocidolite asbestos or TiO2 (both at 100 g, intratracheally). All data presented as mean SEM, 4–5 mice per group, one-way ANOVA with Tukey-Kramer test for multiple comparisons; *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$. Representative data from two independent experiments is shown.

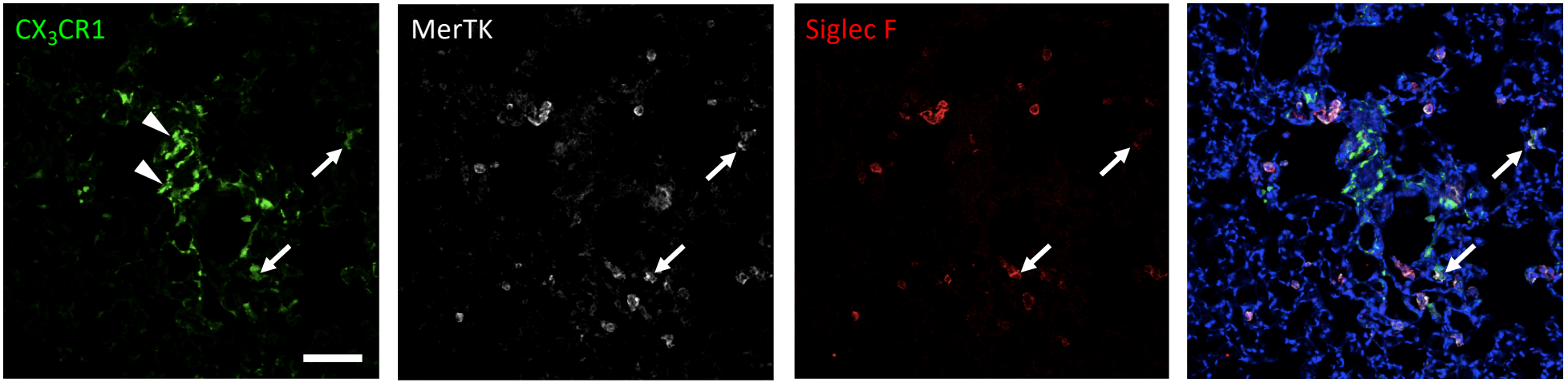
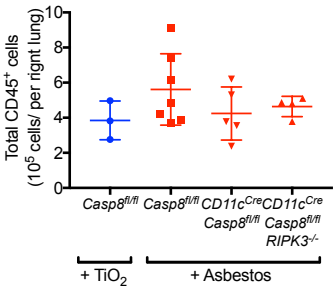
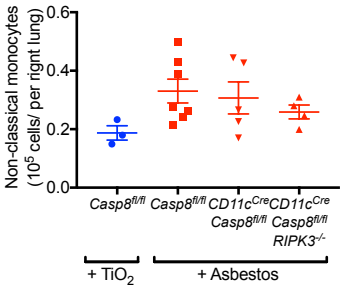
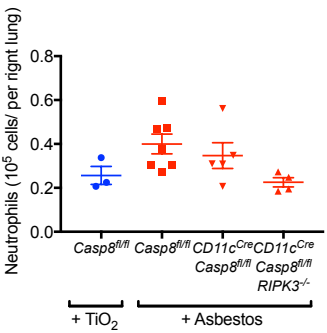

CX3CR1
MerTK
Siglec F

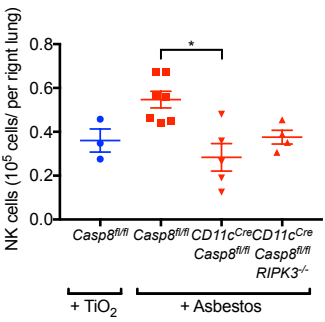
Figure S2: Monocyte-derived alveolar macrophages express canonical alveolar macrophage markers after asbestos exposure (refers to Figure 2). Representative fluorescent images showing expression of CX3CR1-GFP (green), MerTK (white), and Siglec F (red). Note, monocyte-derived alveolar macrophages are CX3CR1-GFP+MerTK+Siglec F+ (arrows), whereas peribronchial macrophages are CX3CR1-GFP+MerTK-Siglec F- (arrowheads). Scale bar 100 □m.

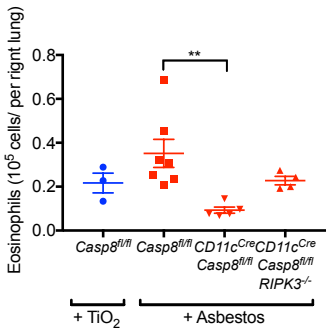
A



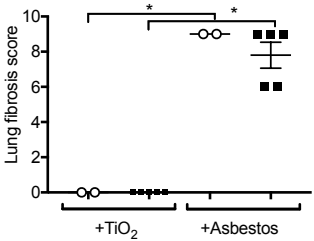


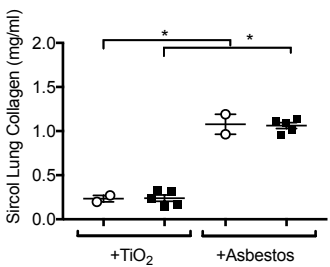






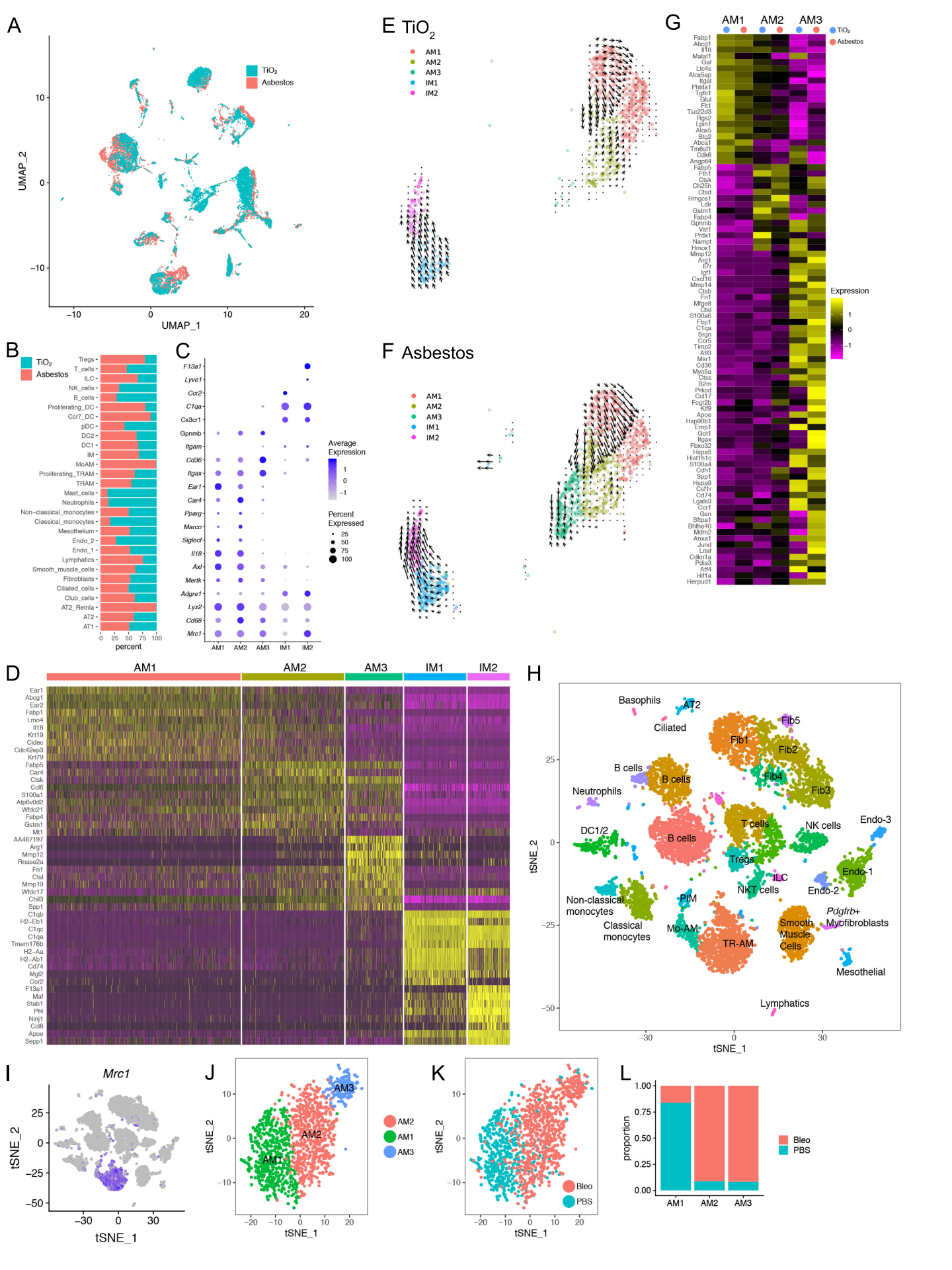
B





o PBS
■ Clodronate liposomes

Figure S3: Monocyte-derived and not tissue-resident alveolar macrophages are required for the development of asbestos-induced pulmonary fibrosis (Refers to Figure 3). A. Quantification of myeloid cell populations by flow cytometry 28 days after mice were administered crocidolite asbestos or TiO2 (both at 100 □g, intratracheally). All data presented as mean□SEM, 3–7 mice per group, one-way ANOVA, with Bonferroni correction for multiple comparisons. * $p<0.05$. B. Depletion of tissue-resident alveolar macrophages does not alter the severity of asbestos-induced fibrosis. Blinded scoring of a single longitudinal section from each mouse and quantification of soluble collagen in lung homogenates. Circles refer to controls (PBS) and squares refer to treatment with clodronate-loaded liposomes. All data presented as mean□SEM, 2–5 mice per group, one-way ANOVA, with Tukey-Kramer test for multiple comparisons. *, $p<0.05$; **, $p< 0.01$.


A
TiO2
Asbestos
UMAP_2
UMAP_1
E TiO2
AM1
AM2
AM3
IM1
IM2
G
AM1 AM2 AM3
TiO2
Asbestos
Expression
B
TiO2
Asbestos
Tregs
T_cells
ILC
NK_cells
B_cells
Proliferating_DC
Ccr7_DC
pDC
DC2
DC1
IM
MoAM
Proliferating_TRAM
TRAM
Mast_cells
Neutrophils
Non−classical_monocytes
Classical_monocytes
Mesothelium
Endo_2
Endo_1
Lymphatics
Smooth_muscle_cells
Fibroblasts
Ciliated_cells
Club_cells
AT2_Retnla
AT2
AT1
percent
C
F13a1
Lyve1
Ccr2
C1qa
Cx3cr1
Gpnmb
Itgam
Cd36
Itgax
Ear1
Car4
Pparg
Marco
Siglecf
Il18
Axl
Mertk
Adgre1
Lyz2
Cd68
Mrc1
AM1 AM2 AM3 IM1 IM2
Average Expression
Percent Expressed
F Asbestos
AM1
AM2
AM3
IM1
IM2
D
AM1 AM2 AM3 IM1 IM2
H
Basophils
AT2
Ciliated
Fib5
Fib1
Fib2
Fib4
Fib3
B cells
B cells
Neutrophils
DC1/2
B cells
T cells
NK cells
Endo-3
Tregs
ILC
NKT cells
Endo-1
Endo-2
Non-classical monocytes
PIM
Classical monocytes
Mo-AM
TR-AM
Smooth Muscle Cells
Pdgfrb+ Myofibroblasts
Mesothelial
Lymphatics
tSNE_1
tSNE_2
I
Mrc1
tSNE_1
tSNE_2
J
AM3
AM2
AM1
AM2
AM1
AM3
tSNE_1
tSNE_2
K
Bleo
PBS
tSNE_1
tSNE_2
L
proportion
AM1 AM2 AM3
Bleo
PBS

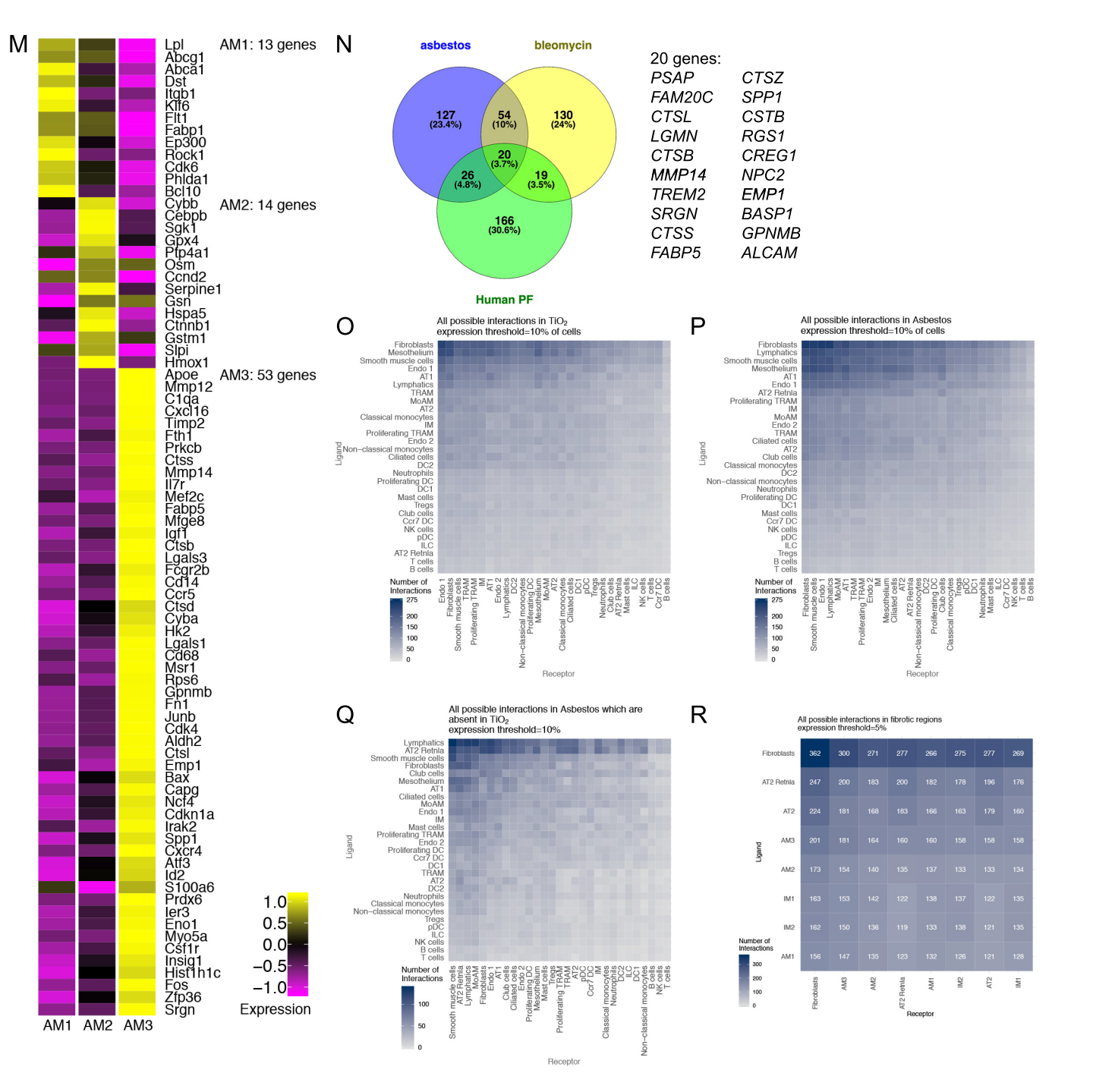

M
Lpl
Abcg1
Abca1
Dst
Itgb1
Klf6
Flt1
Fabp1
Ep300
Rock1
Cdk6
Phlda1
Bcl10
Cybb
Cebpb
Sgk1
Gpx4
Ptp4a1
Osm
Ccnd2
Serpine1
Gsn
Hspa5
Ctnnb1
Gstm1
Slpi
Hmox1
Apoe
Mmp12
C1qa
Cxcl16
Timp2
Fth1
Prkcb
Ctss
Mmp14
Il7r
Mef2c
Fabp5
Mfge8
Igf1
Ctsb
Lgals3
Fcgr2b
Cd14
Ccr5
Ctsd
Cyba
Hk2
Lgals1
Cd68
Msr1
Rps6
Gpnmb
Fn1
Junb
Cdk4
Aldh2
Ctsl
Emp1
Bax
Capg
Ncf4
Cdkn1a
Irak2
Spp1
Cxcr4
Atf3
Id2
S100a6
Prdx6
Ier3
Eno1
Myo5a
Csf1r
Insig1
Hist1h1c
Fos
Zfp36
Srgn
AM1: 13 genes
AM2: 14 genes
AM3: 53 genes
1.0
0.5
0.0
−0.5
−1.0
Expression
AM1
AM2
AM3
N
asbestos
bleomycin
Human PF
127 (23.4%)
54 (10%)
130 (24%)
20 (3.7%)
26 (4.8%)
19 (3.5%)
166 (30.6%)
20 genes:
PSAP CTSZ
FAM20C SPP1
CTSL CSTB
LGMN RGS1
CTSB CREG1
MMP14 NPC2
TREM2 EMP1
SRGN BASP1
CTSS GPNMB
FABP5 ALCAM
O
All possible interactions in TiO2 expression threshold=10% of cells
Ligand
Receptor
Number of Interactions
P
All possible interactions in Asbestos expression threshold=10% of cells
Ligand
Receptor
Number of Interactions
Q
All possible interactions in Asbestos which are absent in TiO2 expression threshold=10%
Ligand
Receptor
Number of Interactions
R
All possible interactions in fibrotic regions expression threshold=5%
Fibroblasts 362 300 271 277 266 275 277 269
AT2 Retnla 247 200 183 200 182 178 196 176
AT2 224 181 168 183 166 163 179 160
AM3 201 181 164 160 160 158 158 158
AM2 173 154 140 135 137 133 133 134
IM1 163 153 142 122 138 137 122 135
IM2 162 150 136 119 133 138 121 135
AM1 156 147 135 123 132 126 121 128
Ligand
Receptor
Number of Interactions

Figure S4: Single-cell RNA-seq identifies heterogeneous response of resident lung cellular populations during asbestos-induced pulmonary fibrosis (refers to Figure 4). A-B. UMAP plot (A) and bar plot (B) demonstrating composition of cell clusters. One mouse per library/condition. C. Dot plot demonstrating expression of selected genes in macrophage subclusters. D. Heatmap of top 10 genes (by log fold change) characterizing macrophage subclusters. E-F. RNA Velocity field projected onto UMAP-plot from Figure 4C: alveolar and interstitial macrophage subsets from (E) TiO2 and (F) asbestos-exposed lungs (derived from Figure 4C). Cluster AM3 has no outbound vectors, which suggests that it is a transcriptionally stable cell state, rather than an intermediate state between monocyte to tissue-resident macrophage differentiation. G. Heatmap of 93 genes overlapping between genes expressed in alveolar macrophages and pulmonary fibrosis-associated genes from the Comparative Toxicogenomic Database (947 genes as of February 2019) ($p = 4.561944e-06$ for cluster AM3, hypergeometric probability test). H. tSNE plot demonstrating cell clusters (10,372 cells) identified by single-cell RNA-seq 21 days after PBS or bleomycin treatment, three mice per condition. Data from Xie et al., 2018. I. Macrophages were identified using canonical lineage-restricted markers, such as Mrc1, as shown on a tSNE plot. J. Focused analysis of alveolar macrophages identifies 3 subclusters: AM1, AM2, AM3. K. tSNE and L. bar plot demonstrating composition of alveolar macrophages clusters. M. Heatmap showing 80 genes overlapping between genes expressed in alveolar macrophages in Xie et al. dataset and pulmonary fibrosis-associated genes from the Comparative Toxicogenomic Database (947 genes as of February 2019) ($p = 3.388409e-05$ for cluster AM3, hypergeometric probability test). N. Venn diagram showing overlap between the genes in cluster AM3 from asbestos dataset, cluster AM3 in Xie et al. 2018 dataset and genes from the profibrotic macrophage cluster (cluster 1) from Reyfman et al., 2018. List of 20 genes overlapping between three datasets is shown. See also Supplemental table S5. O-R. Analysis of ligand-receptor interactions emerging between the cell types during the asbestos-induced pulmonary fibrosis. All possible ligand-receptor interactions in mice exposed to TiO2 (O) and asbestos (P), and genes uniquely present only in asbestos-exposed mice (Q), only genes expressed in at least 10% cells were used for analysis. R. Analysis of the ligand-receptor pairs between the cells located in the close anatomical proximity in the distal lung parenchyma during asbestos-induced pulmonary fibrosis, only genes expressed in at least 5% of the cells were used. See Methods and online code for details.

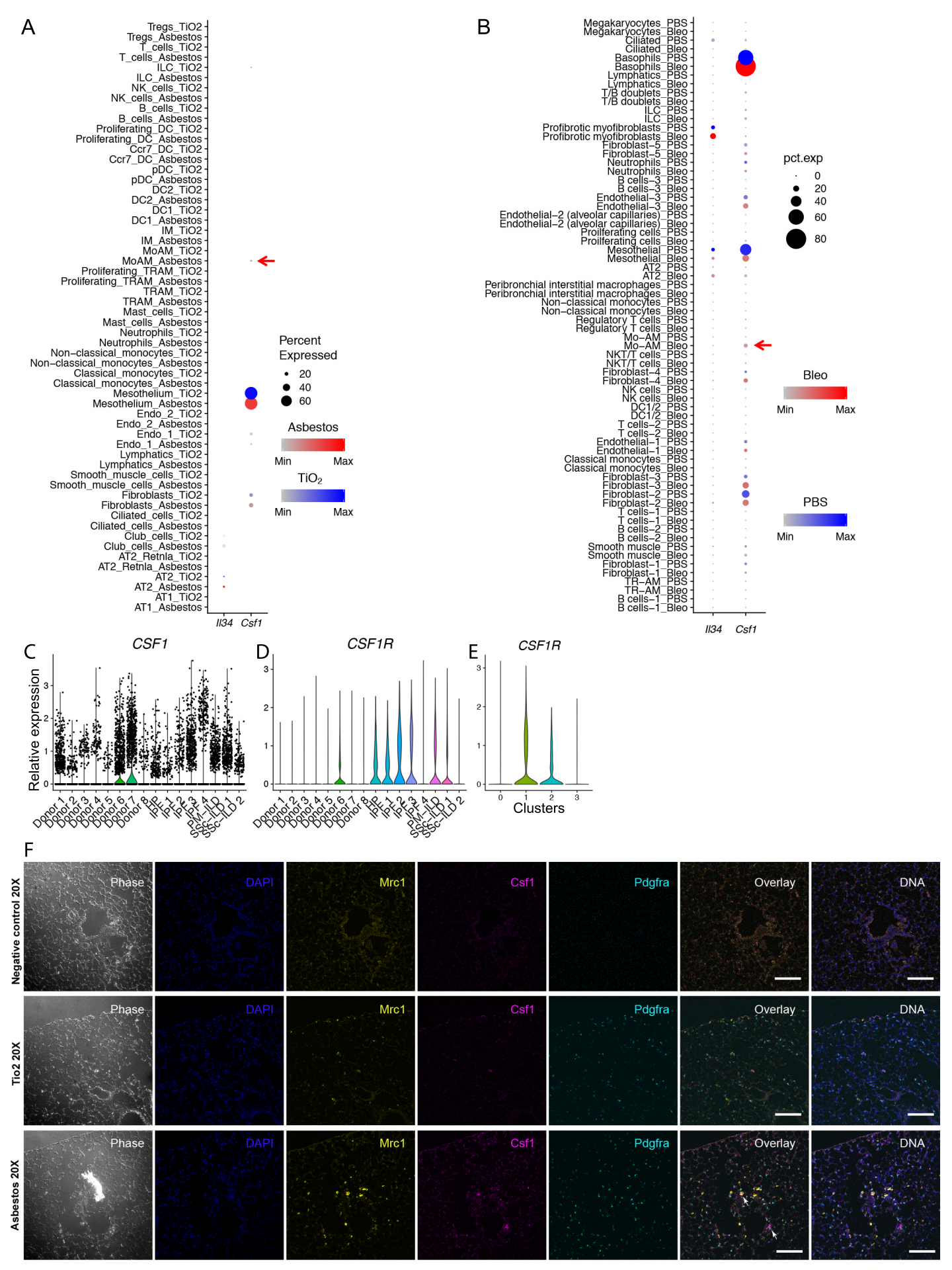

A
Tregs_TiO2
Tregs_Asbestos
T_cells_TiO2
T_cells_Asbestos
ILC_TiO2
ILC_Asbestos
NK_cells_TiO2
NK_cells_Asbestos
B_cells_TiO2
B_cells_Asbestos
Proliferating_DC_TiO2
Proliferating_DC_Asbestos
Ccr7_DC_TiO2
Ccr7_DC_Asbestos
pDC_TiO2
pDC_Asbestos
DC2_TiO2
DC2_Asbestos
DC1_TiO2
DC1_Asbestos
IM_TiO2
IM_Asbestos
MoAM_TiO2
MoAM_Asbestos
Proliferating_TRAM_TiO2
Proliferating_TRAM_Asbestos
TRAM_TiO2
TRAM_Asbestos
Mast_cells_TiO2
Mast_cells_Asbestos
Neutrophils_TiO2
Neutrophils_Asbestos
Non−classical_monocytes_TiO2
Non−classical_monocytes_Asbestos
Classical_monocytes_TiO2
Classical_monocytes_Asbestos
Mesothelium_TiO2
Mesothelium_Asbestos
Endo_2_TiO2
Endo_2_Asbestos
Endo_1_TiO2
Endo_1_Asbestos
Lymphatics_TiO2
Lymphatics_Asbestos
Smooth_muscle_cells_TiO2
Smooth_muscle_cells_Asbestos
Fibroblasts_TiO2
Fibroblasts_Asbestos
Ciliated_cells_TiO2
Ciliated_cells_Asbestos
Club_cells_TiO2
Club_cells_Asbestos
AT2_Retnla_TiO2
AT2_Retnla_Asbestos
AT2_TiO2
AT2_Asbestos
AT1_TiO2
AT1_Asbestos
Il34
Csf1
Percent Expressed
20
40
60
Asbestos
Min
Max
TiO2
Min
Max
B
Megakaryocytes_PBS
Megakaryocytes_Bleo
Ciliated_PBS
Ciliated_Bleo
Basophils_PBS
Basophils_Bleo
Lymphatics_PBS
Lymphatics_Bleo
T/B doublets_PBS
T/B doublets_Bleo
ILC_PBS
ILC_Bleo
Profibrotic myofibroblasts_PBS
Profibrotic myofibroblasts_Bleo
Fibroblast−5_PBS
Fibroblast−5_Bleo
Neutrophils_PBS
Neutrophils_Bleo
B cells−3_PBS
B cells−3_Bleo
Endothelial−3_PBS
Endothelial−3_Bleo
Endothelial−2 (alveolar capillaries)_PBS
Endothelial−2 (alveolar capillaries)_Bleo
Proilferating cells_PBS
Proilferating cells_Bleo
Mesothelial_PBS
Mesothelial_Bleo
AT2_PBS
AT2_Bleo
Peribronchial interstitial macrophages_PBS
Peribronchial interstitial macrophages_Bleo
Non−classical monocytes_PBS
Non−classical monocytes_Bleo
Regulatory T cells_PBS
Regulatory T cells_Bleo
Mo−AM_PBS
Mo−AM_Bleo
NKT/T cells_PBS
NKT/T cells_Bleo
Fibroblast−4_PBS
Fibroblast−4_Bleo
NK cells_PBS
NK cells_Bleo
DC1/2_PBS
DC1/2_Bleo
T cells−2_PBS
T cells−2_Bleo
Endothelial−1_PBS
Endothelial−1_Bleo
Classical monocytes_PBS
Classical monocytes_Bleo
Fibroblast−3_PBS
Fibroblast−3_Bleo
Fibroblast−2_PBS
Fibroblast−2_Bleo
T cells−1_PBS
T cells−1_Bleo
B cells−2_PBS
B cells−2_Bleo
Smooth muscle_PBS
Smooth muscle_Bleo
Fibroblast−1_PBS
Fibroblast−1_Bleo
TR−AM_PBS
TR−AM_Bleo
B cells−1_PBS
B cells−1_Bleo
Il34
Csf1
pct.exp
0
20
40
60
80
Bleo
Min
Max
PBS
Min
Max
C
CSF1
Relative expression
Donor 1
Donor 2
Donor 3
Donor 4
Donor 5
Donor 6
Donor 7
Donor 8
HP
IPF 1
IPF 2
IPF 3
IPF 4
PM−ILD
SSc−ILD 1
SSc−ILD 2
D
CSF1R
E
CSF1R
Clusters
F
Negative control 20X
Tio2 20X
Asbestos 20X
Phase
DAPI
Mrc1
Csf1
Pdgfra
Overlay
DNA

Figure S5: M-CSF/M-CSFR signaling is required for maintenance of monocyte-derived alveolar macrophages in fibrotic niches (refers to Figure 5). A-B. Dot plot showing expression of Csf1 and Il34 across cell types during (A) asbestos- and (B) bleomycin-induced pulmonary fibrosis. Arrows point out expression of Csf1 in monocyte-derived alveolar macrophages. C. CSF1 is expressed in human alveolar macrophages in all 8 donor lungs and 8 lungs from patients with pulmonary fibrosis. D-E. Expression of CSF1R is increased in alveolar macrophages from patients with pulmonary fibrosis, specifically, in cluster 1, containing profibrotic alveolar macrophages. Data from Reyfman et al. 2019. F. In situ RNA hybridization confirms expression of Csf1 in alveolar macrophages during pulmonary fibrosis. Analysis performed on day 28 post-TiO2 or asbestos exposure. Top row: negative controls (no primary probe), demonstrating level of autofluorescence in the tissue. Scale bar is 50 □m.

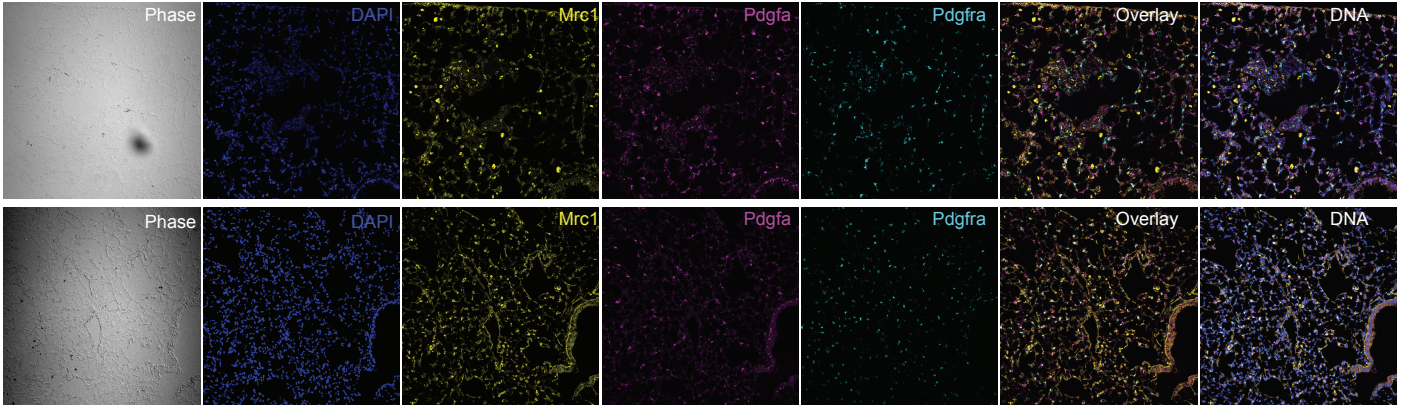

Asbestos 20X
TiO2 20X
Phase
DAPI
Mrc1
Pdgfa
Pdgfra
Overlay
DNA
Phase
DAPI
Mrc1
Pdgfa
Pdgfra
Overlay
DNA

Figure S6: Monocyte-derived alveolar macrophages express Pdgfa which is required for fibroblast proliferation (refers to Figure 6). In situ RNA hybridization confirms expression of Pdgfa in alveolar macrophages during pulmonary fibrosis, 20× magnification. Analysis performed on day 28 post-TiO_2 or asbestos exposure. Scale bar is 50 μm.

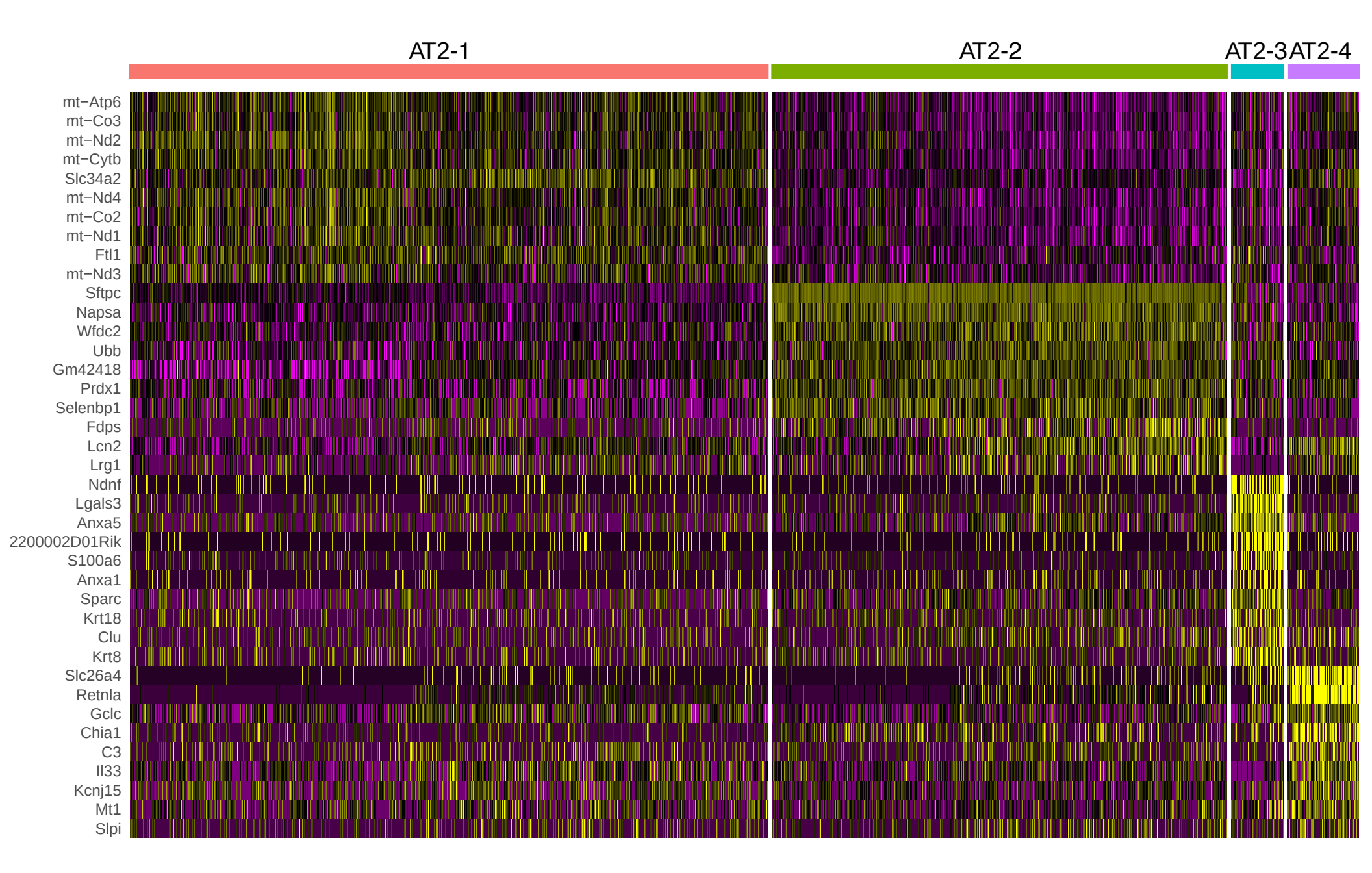

AT2-1
AT2-2
AT2-3
AT2-4
mt−Atp6
mt−Co3
mt−Nd2
mt−Cytb
Slc34a2
mt−Nd4
mt−Co2
mt−Nd1
Ftl1
mt−Nd3
Sftpc
Napsa
Wfdc2
Ubb
Gm42418
Prdx1
Selenbp1
Fdps
Lcn2
Lrg1
Ndnf
Lgals3
Anxa5
2200002D01Rik
S100a6
Anxa1
Sparc
Krt18
Clu
Krt8
Slc26a4
Retnla
Gclc
Chia1
C3
Il33
Kcnj15
Mt1
Slpi

Figure S7: Heatmap of the top 10 differentially expressing genes for sub-clusters within alveolar type II cells (refers to Figure 7).