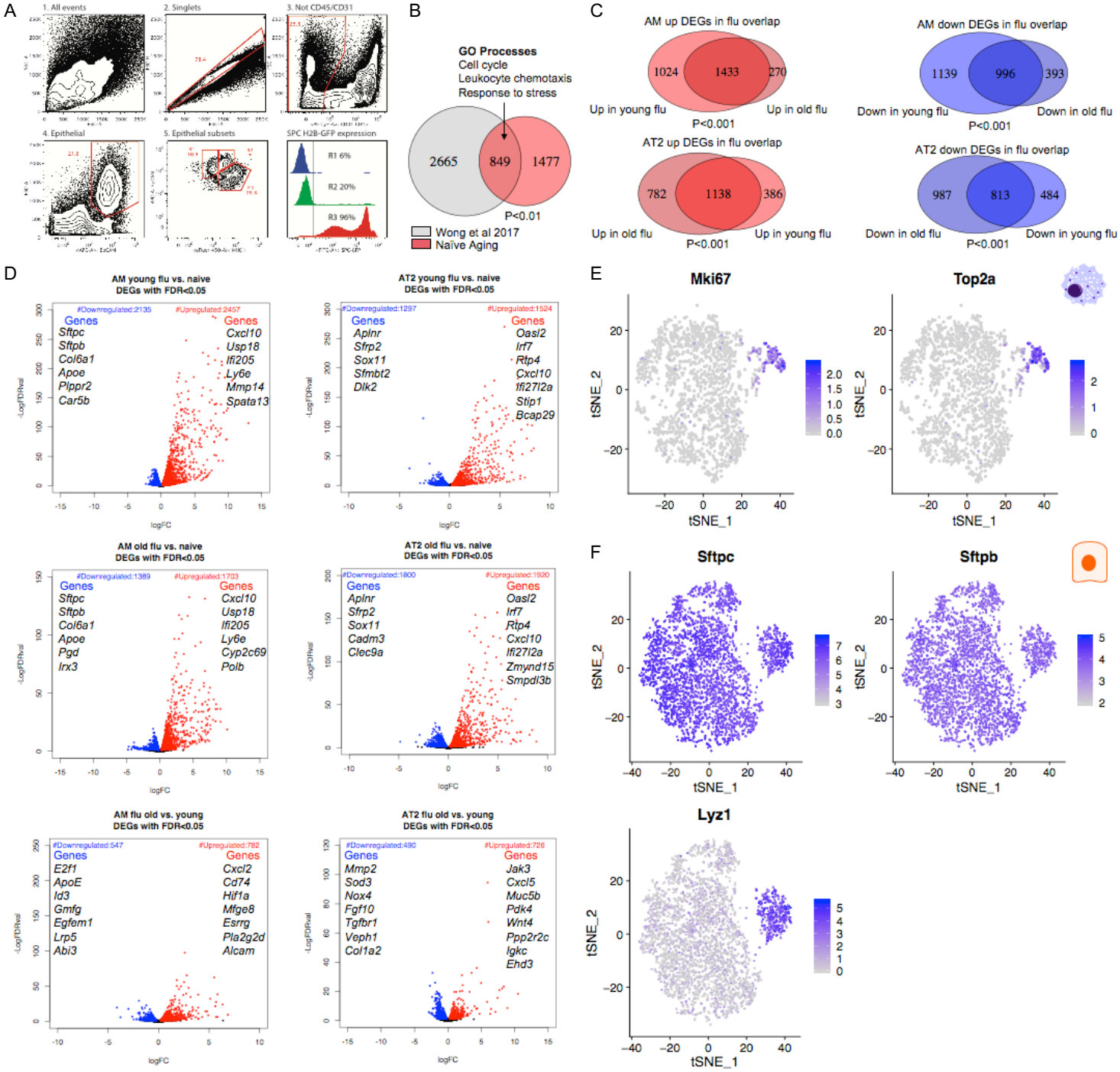
A
1. All events
2. Singlets
3. Not CD45/CD31
4. Epithelial
5. Epithelial subsets
SPC H2B-GFP expression
R1 6%
R2 20%
R3 96%
B
GO Processes
Cell cycle
Leukocyte chemotaxis
Response to stress
2665
849
1477
P<0.01
Wong et al 2017
Naïve Aging
C
AM up DEGs in flu overlap
1024
1433
270
Up in young flu
Up in old flu
P<0.001
AM down DEGs in flu overlap
1139
996
393
Down in young flu
Down in old flu
P<0.001
AT2 up DEGs in flu overlap
782
1138
386
Up in old flu
Up in young flu
P<0.001
AT2 down DEGs in flu overlap
987
813
484
Down in old flu
Down in young flu
P<0.001
D
AM young flu vs. naive
DEGs with FDR<0.05
#Downregulated:2135
#Upregulated:2457
Genes
Sftpc
Sftpb
Col6a1
Apoe
Plppr2
Car5b
Genes
Cxcl10
Usp18
Ifi205
Ly6e
Mmp14
Spata13
AT2 young flu vs. naive
DEGs with FDR<0.05
#Downregulated:1297
#Upregulated:1524
Genes
Aplnr
Sfrp2
Sox11
Sfmbt2
Dlk2
Genes
Oasl2
Irf7
Rtp4
Cxcl10
Ifi27l2a
Stip1
Bcap29
AM old flu vs. naive
DEGs with FDR<0.05
#Downregulated:1389
#Upregulated:1703
Genes
Sftpc
Sftpb
Col6a1
Apoe
Pgd
Irx3
Genes
Cxcl10
Usp18
Ifi205
Ly6e
Cyp2c69
Polb
AT2 old flu vs. naive
DEGs with FDR<0.05
#Downregulated:1800
#Upregulated:1920
Genes
Aplnr
Sfrp2
Sox11
Cadm3
Clec9a
Genes
Oasl2
Irf7
Rtp4
Cxcl10
Ifi27l2a
Zmynd15
Smpdl3b
AM flu old vs. young
DEGs with FDR<0.05
#Downregulated:547
#Upregulated:782
Genes
E2f1
ApoE
Id3
Gmfg
Egfem1
Lrp5
Abi3
Genes
Cxcl2
Cd74
Hif1a
Mfge8
Esrrg
Pla2g2d
Alcam
AT2 flu old vs. young
DEGs with FDR<0.05
#Downregulated:490
#Upregulated:726
Genes
Mmp2
Sod3
Nox4
Fgf10
Tgfbr1
Veph1
Col1a2
Genes
Jak3
Cxcl5
Muc5b
Pdk4
Wnt4
Ppp2r2c
Igkc
Ehd3
logFC
-LogFDRval
E
Mki67
Top2a
tSNE_1
tSNE_2
F
Sftpc
Sftpb
Lyz1
tSNE_1
tSNE_2

Figure S1

Figure S1. Age-related changes in alveolar macrophage transcriptomes persist during influenza A infection in mice. Relates to Figure 1.

(A) Genetic validation of gating strategy for isolation of alveolar type 2 cells (AT2) from mice. Single cell suspensions were prepared from the lungs of BAC transgenic mice expressing GFP driven by the Surfactant protein C (*Sftpc*) promoter (*Tg-SFTPC-H2B-GFP*) and analyzed via flow cytometry. Epithelial cells were identified as singlets/live/CD45-negative/CD31-negative/EpCAM-positive cells and further subdivided based on EpCAM and MHC II expression. Gate R1: EpCAM-high MHC II-negative, Gate R2: EpCAM-high MHC II-positive and Gate R3: EpCAM-intermediate MHC II-positive. Over 95% of cells in gate R3 were positive for the *Sftpc* promoter-driven GFP reporter and this gate was used to identify AT2 cells during conventional flow sorting.

(B) Venn diagram shows overlap between differentially expressed genes between young and old tissue resident alveolar macrophages in this study (Fig. 1C) and those reported by Wong et. al., 2017.

(C) Venn diagram shows overlap between differentially expressed genes between young adult and old naïve and influenza A-treated mice. A hypergeometric test was used to evaluate the statistical significance for overlap.

(D) Volcano plots showing differentially expressed genes (FDR $q<0.05$) between young adult and old mice before and after infection with influenza A virus in alveolar macrophages and AT2 cells.

(E) tSNE plot shows representative marker genes for alveolar macrophages in young adult and old mice.

(F) tSNE plot shows representative marker genes for AT2 cells in young adult and old mice.

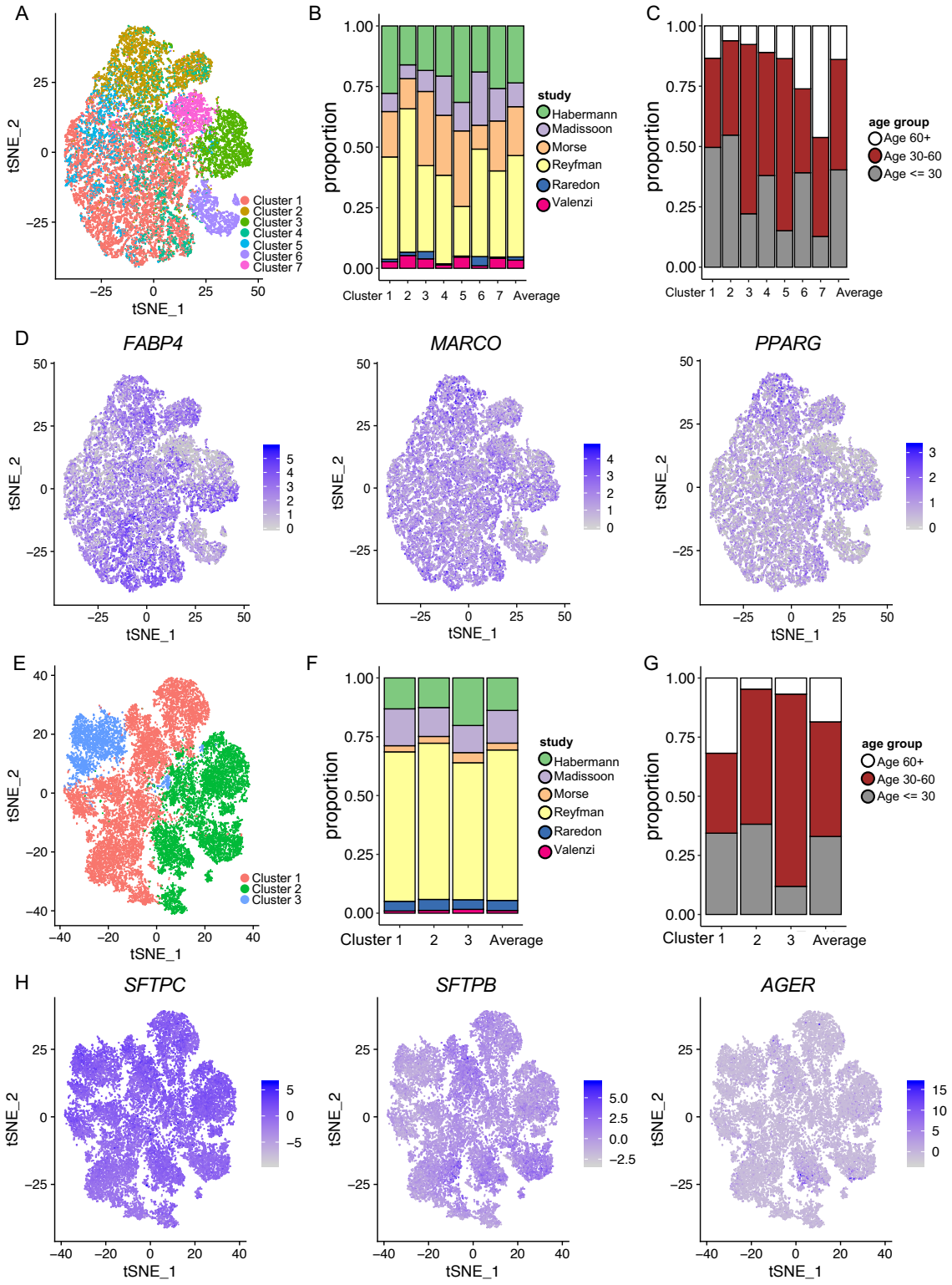
A
tSNE_2
tSNE_1
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 7
B
proportion
study
Habermann
Madissoon
Morse
Reyfman
Raredon
Valenzi
Cluster 1 2 3 4 5 6 7 Average
C
proportion
age group
Age 60+
Age 30-60
Age <= 30
Cluster 1 2 3 4 5 6 7 Average
D
FABP4
MARCO
PPARG
tSNE_2
tSNE_1
E
Cluster 1
Cluster 2
Cluster 3
F
proportion
study
Habermann
Madissoon
Morse
Reyfman
Raredon
Valenzi
Cluster 1 2 3 Average
G
proportion
age group
Age 60+
Age 30-60
Age <= 30
Cluster 1 2 3 Average
H
SFTPC
SFTPB
AGER
tSNE_2
tSNE_1

Figure S2

Figure S2. Integrated analysis of single cell RNA-Seq data obtained from the normal human lung reveals uniform changes in the transcriptome of alveolar macrophages with age. Relates to Figure 2.

(A) t-SNE plot shows clustering of alveolar macrophages from the six studies.

(B) Barplot showing contributions of alveolar macrophages from the six studies to the clusters.

(C) Barplot showing contributions of alveolar macrophages from individuals within chronological age tertiles to each cluster.

(D) Feature plots demonstrate distribution of canonical alveolar macrophage marker genes across clusters.

(E) t-SNE plot shows clustering of AT2 cells from the six studies.

(F) Barplot showing contributions ofAT2 cells from the six studies to the clusters.

(G) Barplot showing contributions of AT2 cells from individuals within chronological age tertiles to each cluster.

(H) Feature plots demonstrate distribution of canonical alveolar type 2 (*SFTPC*, *SFTPB*) and alveolar type 1 cell (*AGER*) marker genes across clusters.

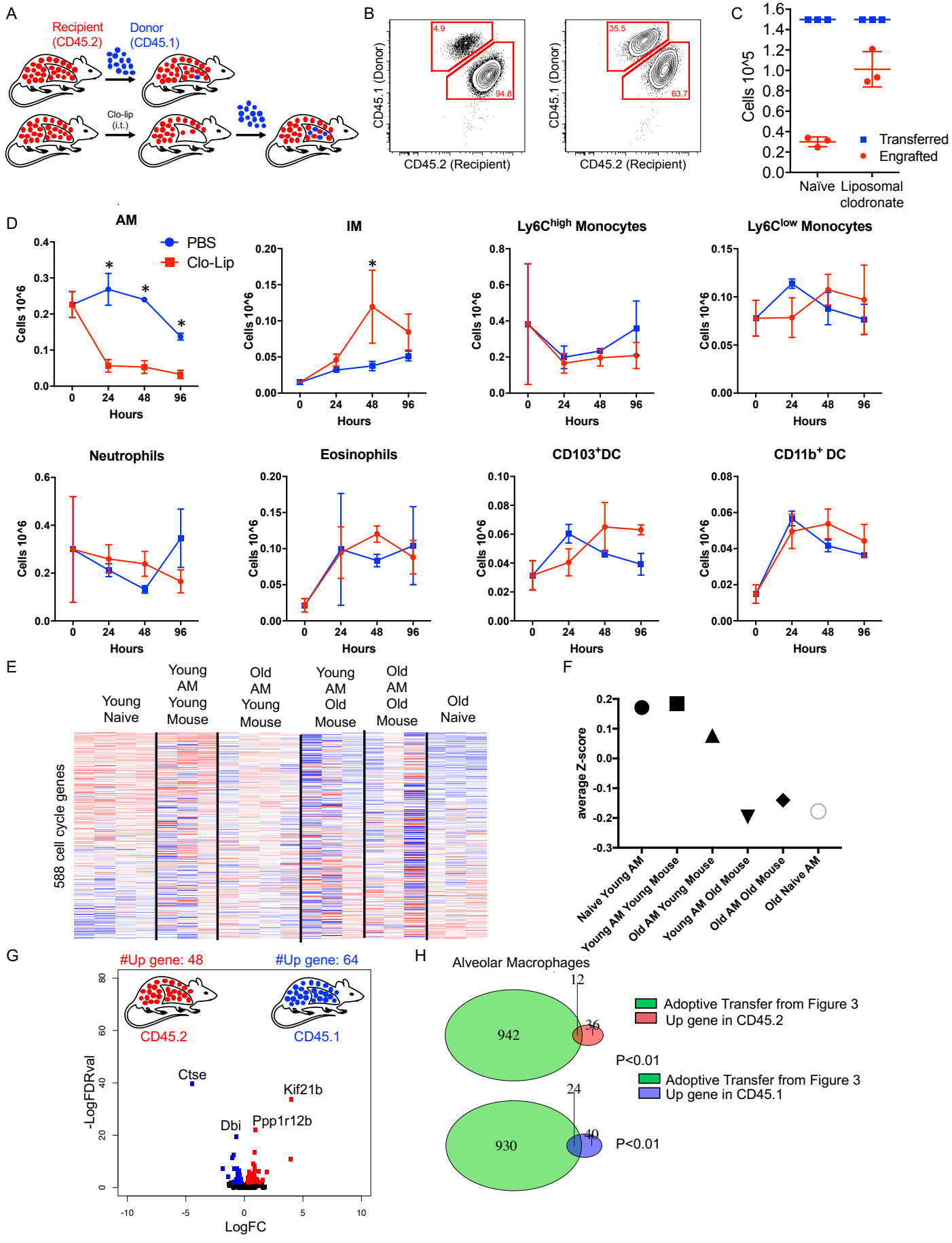
A
Recipient (CD45.2)
Donor (CD45.1)
Clo-lip (i.t.)
B
CD45.1 (Donor)
CD45.2 (Recipient)
4.9
94.8
35.5
63.7
C
Cells 10^5
Transferred
Engrafted
Naïve
Liposomal clodronate
D
AM
IM
Ly6C^high Monocytes
Ly6C^low Monocytes
Neutrophils
Eosinophils
CD103^+DC
CD11b^+ DC
PBS
Clo-Lip
Cells 10^6
Hours
E
Young Naive
Young AM Young Mouse
Old AM Young Mouse
Young AM Old Mouse
Old AM Old Mouse
Old Naive
588 cell cycle genes
F
average Z-score
Naive Young AM
Young AM Young Mouse
Old AM Young Mouse
Young AM Old Mouse
Old AM Old Mouse
Old Naive AM
G
#Up gene: 48
#Up gene: 64
CD45.2
CD45.1
Ctse
Kif21b
Dbi
Ppp1r12b
-LogFDRval
LogFC
H
Alveolar Macrophages
942
12
36
P<0.01
Adoptive Transfer from Figure 3
Up gene in CD45.2
930
24
40
P<0.01
Adoptive Transfer from Figure 3
Up gene in CD45.1

Figure S3

Figure S3. Age-related transcriptomic changes in tissue-resident alveolar macrophages are not cell autonomous.

(A) Schematic design of heterochronic adoptive transfer experiments with or without intratracheal liposomal clodronate.

(B) Representative flow cytometry plots from untreated and liposomal clodronate-treated CD45.2 mice (25 μL, intratracheally) before the adoptive transfer of $1.5x10^5$ TRAM from CD45.1 mice.

(C) Quantification of adoptively transferred TRAM 60 days after adoptive transfer (n=3 mice per group). $P<0.05$ for engrafted group. Wilcoxon test.

(D) The number of immune cells measured in the single cell suspension prepared from the lung using flow cytometry 24, 48 and 96 hours after liposomal clodronate treatment (n=2-4 mice per group). * $P<0.05$ for comparison between untreated and liposomal clodronate treated animals (Student's t-test).

(E) Heatmap showing the expression of cell cycle-related genes in TRAM 60 days after heterochronic adoptive transfer into young or old mice.

(F) Average Z scores for the cell cycle genes in each of the columns in (E).

(G) Volcano plot showing differentially expressed genes between TRAM from the lungs of CD45.1 or CD45.2 mice (FDR $q< 0.01$) (see Table S4 for full list of genes).

(H) Venn diagram shows overlap between differentially expressed genes in CD45.1/CD45.2 TRAM and genes differentially expressed in heterochronic adoptive transfer experiments (Refers to Figure 3D).

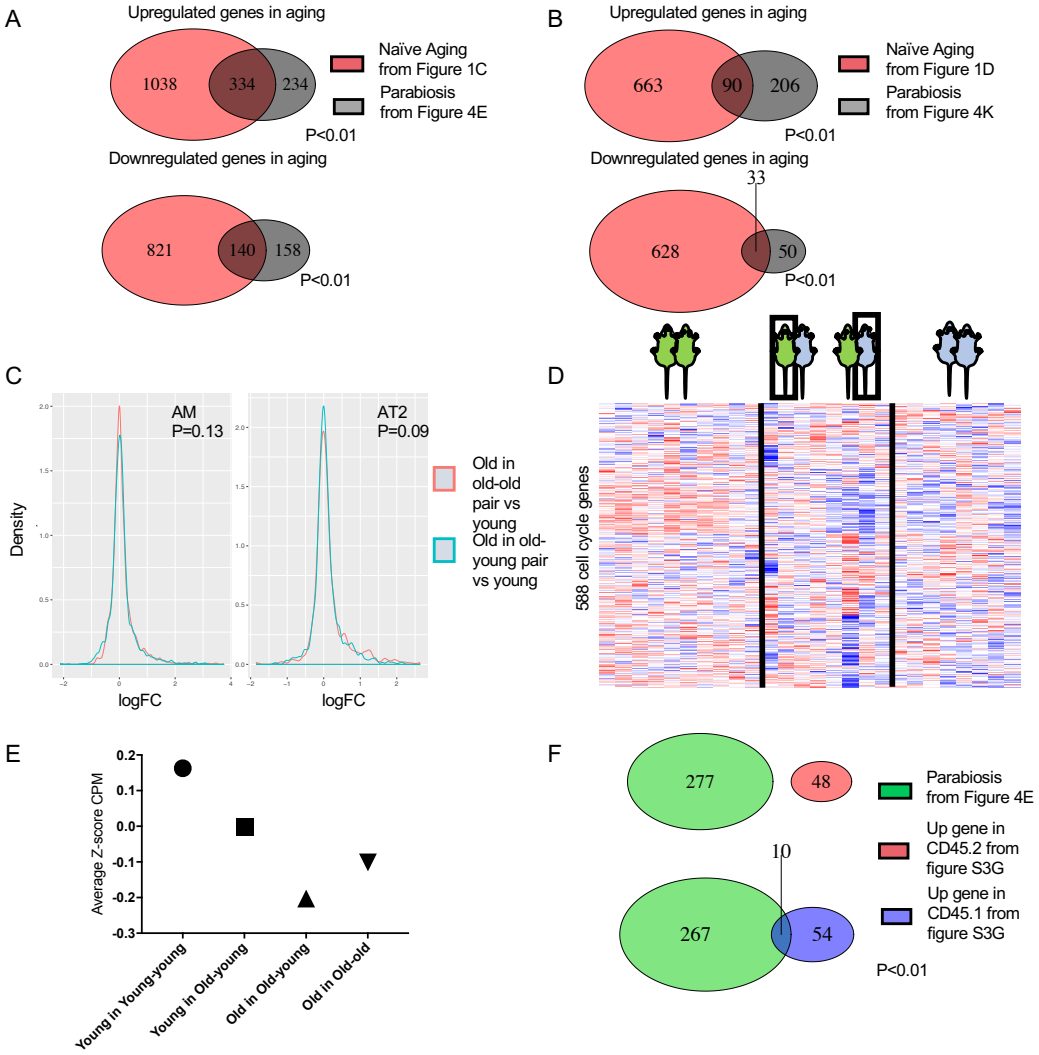
A
Upregulated genes in aging
1038
334
234
Naïve Aging from Figure 1C
Parabiosis from Figure 4E
P<0.01
Downregulated genes in aging
821
140
158
P<0.01
B
Upregulated genes in aging
663
90
206
Naïve Aging from Figure 1D
Parabiosis from Figure 4K
P<0.01
Downregulated genes in aging
33
628
50
P<0.01
C
AM
P=0.13
AT2
P=0.09
Density
logFC
logFC
Old in old-old pair vs young
Old in old-young pair vs young
D
588 cell cycle genes
E
Average Z-score CPM
0.2
0.1
0.0
-0.1
-0.2
-0.3
Young in Young-young
Young in Old-young
Old in Old-young
Old in Old-old
F
277
48
10
267
54
Parabiosis from Figure 4E
Up gene in CD45.2 from figure S3G
Up gene in CD45.1 from figure S3G
P<0.01

Figure S4

Figure S4. Heterochronic parabiosis does not reverse age-related transcriptomic changes in tissue-resident alveolar macrophages or alveolar type 2 cells.

(A) Venn diagram showing overlap of differentially expressed genes in young/young compared with old/old parabiont pairs and differentially expressed genes in TRAM identified between young adult (4-6 month) and old (18-24 month) mice (from Fig. 1C). Hypergeometric test.

(B) Venn diagram showing overlap of differentially expressed genes in young/young compared with old/old parabiont pairs and differentially expressed genes in AT2 cells (from Fig. 1D). Hypergeometric test.

(C) Distribution of log-fold change showing no significant difference ($p>0.05$ by Wilcoxon rank sum test) for differentially expressed genes in TRAM and AT2 identified between young adult and old mice (from Fig. 1) and heterochronic parabiont pairs.

(D) Heatmap showing the lack of change in expression of cell cycle-related genes (from Fig. S3E) in TRAM between old and young adult mice with isochronic or heterochronic parabiont pairs.

(E) Average Z scores for the cell cycle genes for each of the columns in (D).

(F) Venn diagram showing overlap of differentially expressed genes in TRAM from parabionts in young/young compared with old/old pairs with differentially expressed genes (with FDR <0.01) in TRAM from CD45.1 and CD45.2 mice (from Fig. S3G). Hypergeometric test.

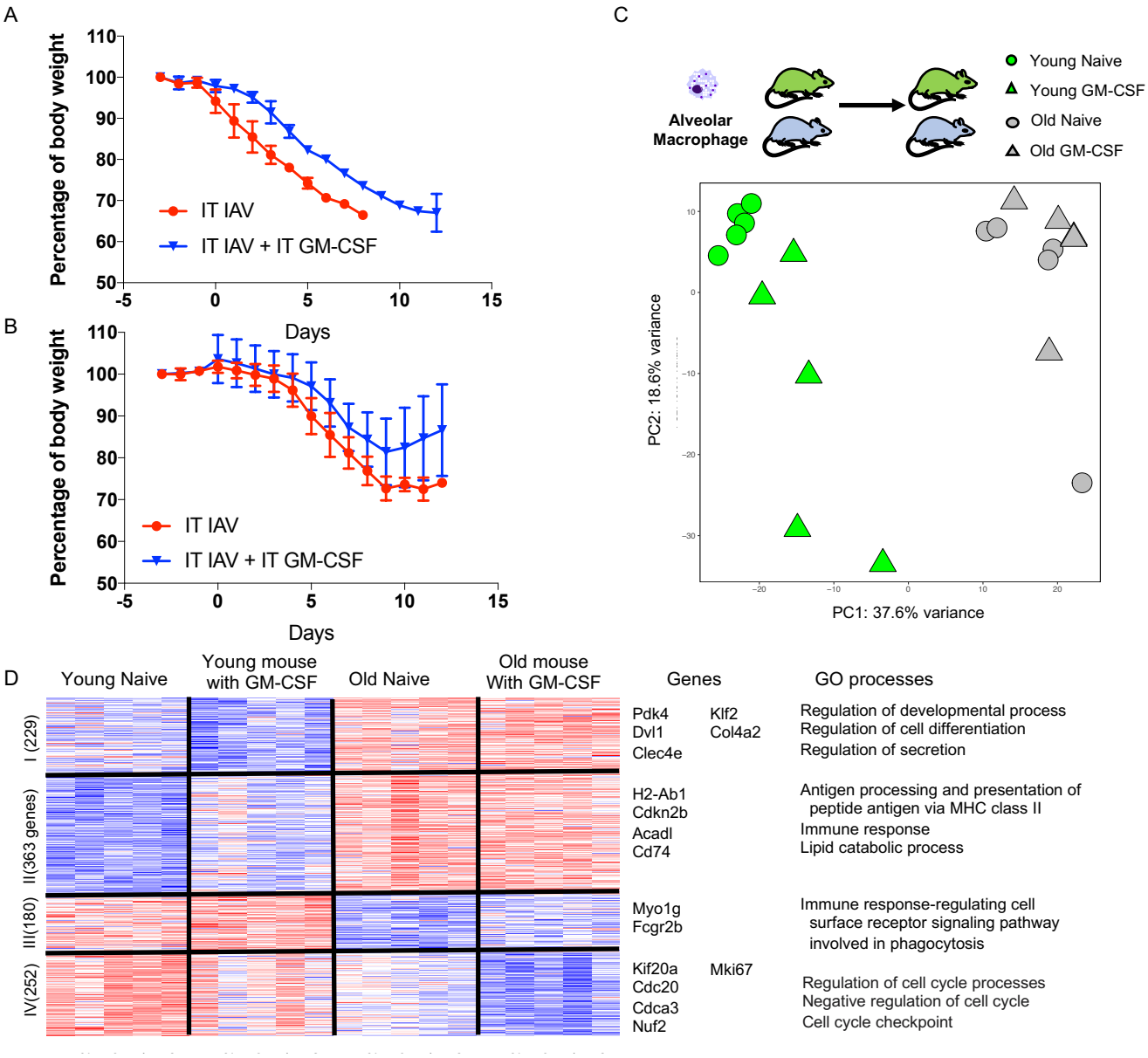
A
Percentage of body weight
IT IAV
IT IAV + IT GM-CSF
B
Days
C
Alveolar Macrophage
Young Naive
Young GM-CSF
Old Naive
Old GM-CSF
PC2: 18.6% variance
PC1: 37.6% variance
D
Young Naive
Young mouse with GM-CSF
Old Naive
Old mouse With GM-CSF
I (229)
II(363 genes)
III(180)
IV(252)
Genes
GO processes
Pdk4
Dvl1
Clec4e
Klf2
Col4a2
Regulation of developmental process
Regulation of cell differentiation
Regulation of secretion
H2-Ab1
Cdkn2b
Acadl
Cd74
Antigen processing and presentation of peptide antigen via MHC class II
Immune response
Lipid catabolic process
Myo1g
Fcgr2b
Immune response-regulating cell surface receptor signaling pathway involved in phagocytosis
Kif20a
Cdc20
Cdca3
Nuf2
Mki67
Regulation of cell cycle processes
Negative regulation of cell cycle
Cell cycle checkpoint

Figure S5

Figure S5. The aging microenvironment confers resistance to GM-CSF signaling in alveolar macrophages.

(A) Daily weight for old mice infected influenza A virus (A/WSN/33), 25 pfu/animal with (n=5) or without (n=4) intratracheally administered GM-CSF. P=0.0007 between two groups. Two-way ANOVA test.

(B) Daily weight loss curve for young adult mice with or without (both n=5) intratracheally administered GM-CSF. All mice received influenza A virus (A/WSN/33), 25 pfu/animal. P=0.15 between two groups. Two-way ANOVA test.

(C) Young adult (4-6 month) and old (18-24 month) mice were treated with exogenous GM-CSF (5 mg/kg day -3 and day 0) intratracheally and TRAM were harvested 14 days later for RNA-Seq. PCA plot of transcriptomes of TRAM from animals in the four conditions.

(D). Heatmap shows k-means clustering of differentially expressed genes in TRAM (FDR <0.01 in ANOVA-like test) between old and young mice with or without treatment with GM-CSF. Selected genes and GO biological processes for each cluster are highlighted (see also table S16).

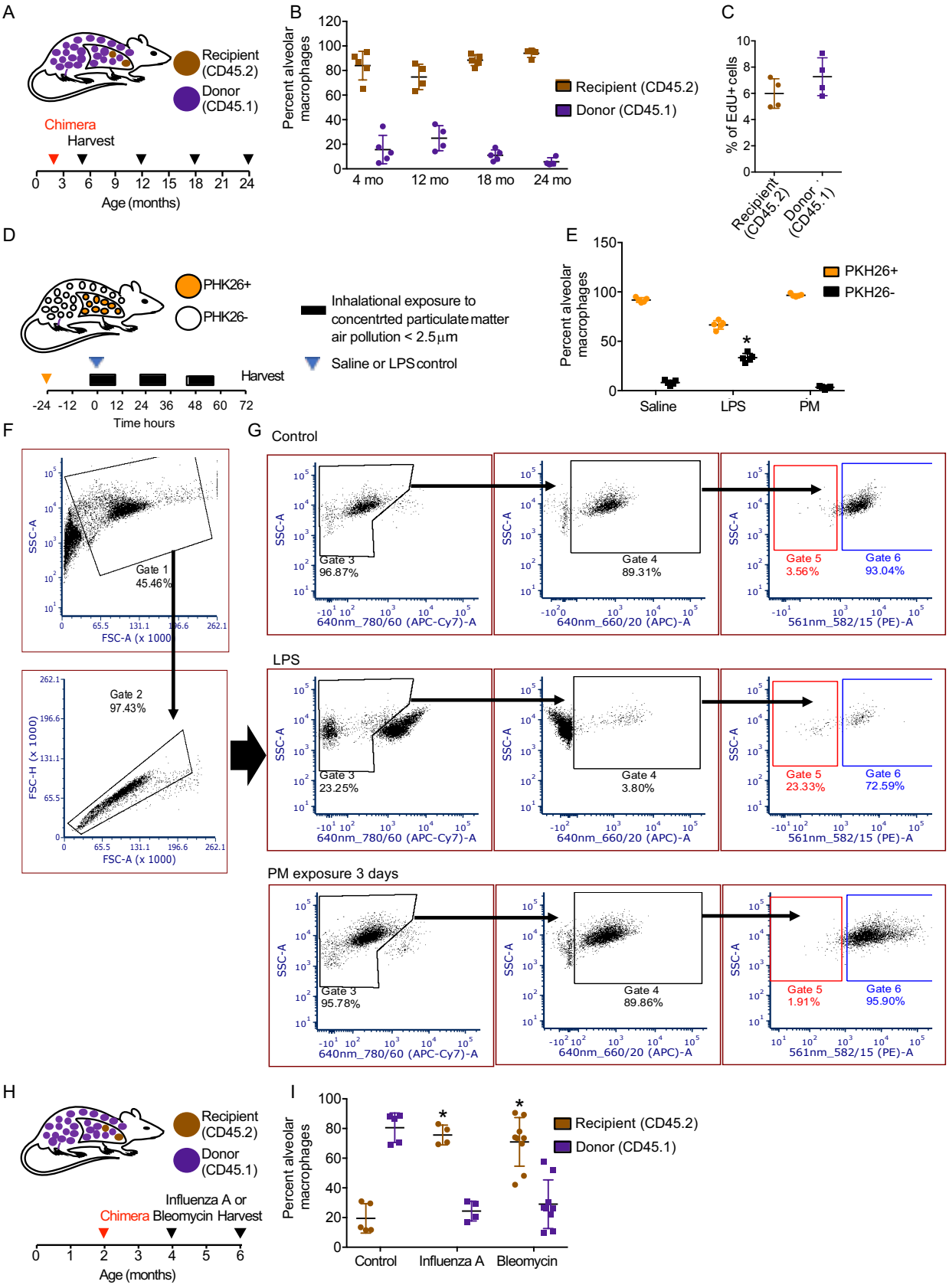
A
Recipient (CD45.2)
Donor (CD45.1)
Chimera
Harvest
0 3 6 9 12 15 18 21 24
Age (months)
B
Percent alveolar macrophages
120 100 80 60 40 20 0
4 mo 12 mo 18 mo 24 mo
Recipient (CD45.2)
Donor (CD45.1)
C
% of EdU+ cells
10 8 6 4 2 0
Recipient (CD45.2)
Donor (CD45.1)
D
PHK26+
PHK26-
Inhalational exposure to concentrted particulate matter air pollution < 2.5μm
Saline or LPS control
Harvest
-24 -12 0 12 24 36 48 60 72
Time hours
E
Percent alveolar macrophages
150 100 50 0
PKH26+
PKH26-
*
Saline LPS PM
F
SSC-A
Gate 1
45.46%
FSC-A (x 1000)
Gate 2
97.43%
FSC-H (x 1000)
FSC-A (x 1000)
G
Control
SSC-A
Gate 3
96.87%
640nm_780/60 (APC-Cy7)-A
Gate 4
89.31%
640nm_660/20 (APC)-A
Gate 5
3.56%
Gate 6
93.04%
561nm_582/15 (PE)-A
LPS
Gate 3
23.25%
Gate 4
3.80%
Gate 5
23.33%
Gate 6
72.59%
PM exposure 3 days
Gate 3
95.78%
Gate 4
89.86%
Gate 5
1.91%
Gate 6
95.90%
H
Recipient (CD45.2)
Donor (CD45.1)
Influenza A or
Chimera Bleomycin Harvest
0 1 2 3 4 5 6
Age (months)
I
Percent alveolar macrophages
100 80 60 40 20 0
*
*
Recipient (CD45.2)
Donor (CD45.1)
Control Influenza A Bleomycin

Figure S6

Figure S6. Tissue-resident alveolar macrophages represent a stable population that persists over the lifespan in the absence of severe lung injury.

(A) Schematic of the experimental design for (B,C). Bone marrow chimeric mice with thoracic shielding were generated from CD45.1 donors and CD45.2 recipients at 8 weeks of age and then harvested at the ages indicated.

(B) The percentage of monocyte-derived alveolar macrophages (MoAM, CD45.1) or TRAM (CD45.2) over the lifespan. Differences in the percentage of cells between ages were not significant (one-way ANOVA with Bonferroni correction, n=3-5 per time point).

(C) Percentage of EdU+ cells in MoAM (CD45.1) and TRAM (CD45.2) one day after a single pulse of EdU was administered (6 months of age). N=4 mice per group. $P>0.05$, student's t-test.

(D) Alveolar macrophages were labeled by intratracheal administration of PKH26 in vivo. After 24 hours, animals were treated intratracheally with PBS (50 mL), LPS (1 mg/kg in 50 mL PBS) or were exposed to concentrated particulate matter (PM) air pollution (6 hours of exposure ~100-120 mg/m^3, 6 hours daily for 3 consecutive weekdays). Concentrations of particles are estimates based on particle measures from the inlet and outlet of the concentrator and reported ambient $PM_{2.5}$ measures at a nearby Environmental Protection Agency monitoring station.

(E) Percentage of alveolar macrophages that were labeled (PHK26+) or unlabeled (PHK26-) after exposure to PBS, LPS or concentrated ambient PM air pollution via inhalation 6 hours daily for three consecutive weekdays. All mice were harvested after three days of exposure. One-way ANOVA with Bonferroni correction, n=5 per group, * indicates $P<0.05$ for comparison with control.

(F) Gating strategy to identify alveolar macrophages in bronchoalveolar lavage fluid via flow cytometry.

(G) Representative flow cytometry plots of bronchoalveolar lavage fluid from a control, LPS and PM-treated animal.

(H) Schematic showing shielded chimeric mice (4 months of age) were treated intratracheally with influenza A virus (A/WSN/33) or intratracheal bleomycin and then harvested 60 days later. (I) MoAM (CD45.1) and TRAM (CD45.2) were quantified by flow cytometry 60 days after infection with influenza A virus (A/WSN/33) or intratracheal administration of bleomycin. Control mice were not treated. * indicates $P < 0.05$ for comparison with untreated mice (paired t-test). N=4-9 mice per group.

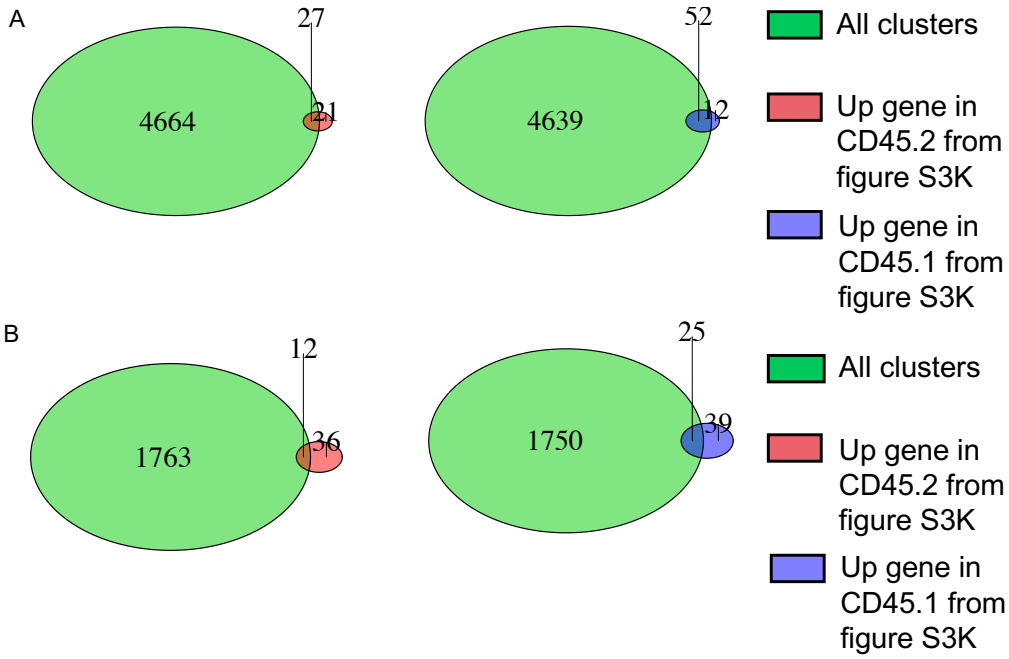
A
27
4664
21
52
4639
12
All clusters
Up gene in CD45.2 from figure S3K
Up gene in CD45.1 from figure S3K
B
12
1763
36
25
1750
39
All clusters
Up gene in CD45.2 from figure S3K
Up gene in CD45.1 from figure S3K

Figure S7

Figure S7. Differences between CD45.1/CD45.2 strains do not explain differential gene expression in tissue-resident alveolar macrophages and monocyte-derived alveolar macrophages from aged shielded chimeric mice.

(A) Venn diagram showing overlap between all differentially expressed genes in TRAM and MoAM from shielded chimeras in aging (See Fig. 6A) with those differentially expressed in TRAM from untreated CD45.1 and CD45.2 mice (from Fig. S3G). Hypergeometric test.

(B) Venn diagram showing overlap between differentially expressed genes in Clusters IV and V of Fig. 6A (comparison of TRAM and MoAM in aging shielded chimeras) with those differentially expressed in TRAM from untreated CD45.1 and CD45.2 mice (from Fig. S3G). Hypergeometric test.

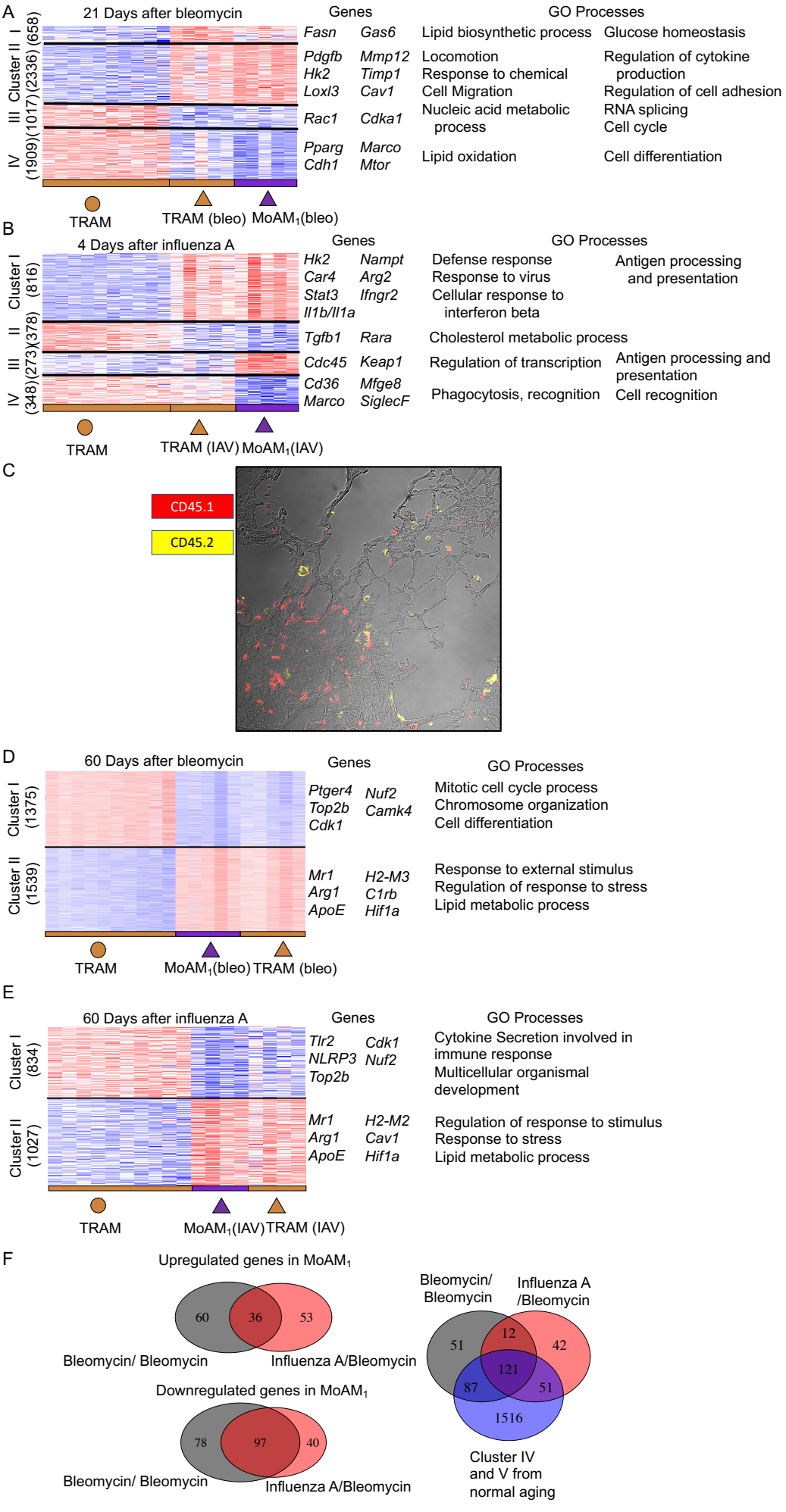
A
21 Days after bleomycin
Genes
GO Processes
Cluster I (658)
Cluster II (2336)
Cluster III (1017)
Cluster IV (1909)
Fasn Gas6
Lipid biosynthetic process
Glucose homeostasis
Pdgfb Mmp12
Hk2 Timp1
Loxl3 Cav1
Locomotion
Response to chemical
Cell Migration
Regulation of cytokine production
Regulation of cell adhesion
Rac1 Cdka1
Nucleic acid metabolic process
RNA splicing
Cell cycle
Pparg Marco
Cdh1 Mtor
Lipid oxidation
Cell differentiation
TRAM
TRAM (bleo)
MoAM1(bleo)
B
4 Days after influenza A
Genes
GO Processes
Cluster I (816)
Cluster II (378)
Cluster III (273)
Cluster IV (348)
Hk2 Nampt
Car4 Arg2
Stat3 Ifngr2
Il1b/Il1a
Defense response
Response to virus
Cellular response to interferon beta
Antigen processing and presentation
Tgfb1 Rara
Cholesterol metabolic process
Cdc45 Keap1
Regulation of transcription
Antigen processing and presentation
Cd36 Mfge8
Marco SiglecF
Phagocytosis, recognition
Cell recognition
TRAM
TRAM (IAV)
MoAM1(IAV)
C
CD45.1
CD45.2
D
60 Days after bleomycin
Genes
GO Processes
Cluster I (1375)
Cluster II (1539)
Ptger4 Nuf2
Top2b Camk4
Cdk1
Mitotic cell cycle process
Chromosome organization
Cell differentiation
Mr1 H2-M3
Arg1 C1rb
ApoE Hif1a
Response to external stimulus
Regulation of response to stress
Lipid metabolic process
TRAM
MoAM1(bleo)
TRAM (bleo)
E
60 Days after influenza A
Genes
GO Processes
Cluster I (834)
Cluster II (1027)
Tlr2 Cdk1
NLRP3 Nuf2
Top2b
Cytokine Secretion involved in immune response
Multicellular organismal development
Mr1 H2-M2
Arg1 Cav1
ApoE Hif1a
Regulation of response to stimulus
Response to stress
Lipid metabolic process
TRAM
MoAM1(IAV)
TRAM (IAV)
F
Upregulated genes in MoAM1
60
36
53
Bleomycin/ Bleomycin
Influenza A/Bleomycin
Downregulated genes in MoAM1
78
97
40
Bleomycin/ Bleomycin
Influenza A/Bleomycin
Bleomycin/ Bleomycin
Influenza A /Bleomycin
51
12
42
121
87
51
1516
Cluster IV and V from normal aging

Figure S8

Figure S8. During injury, monocyte-derived alveolar macrophages show enhanced inflammatory gene expression after influenza A infection and enhanced fibrotic gene expression after bleomycin exposure when compared with tissue-resident alveolar macrophages. Monocyte-derived alveolar macrophages and tissue-resident alveolar macrophages become increasingly similar during the resolution of lung injury.

(A) Heatmap shows k-means clustering of differentially expressed genes between naïve TRAM, TRAM 21 days after bleomycin-induced lung injury, and recruited MoAM collected at the same time points (FDR q< 0.01 in ANOVA-like test). Representative genes and GO processes are shown on the right. See also Table S20.

(B) Heatmap shows k-means clustering of differentially expressed genes between naïve TRAM, TRAM 4 days after influenza A-infection, and recruited Mo-AM at the same time (FDR q< 0.01 in ANOVA like test). Representative genes and GO processes are shown on the right. See also Table S21.

(C) Lung sections from shielded chimeric mice 21 days after the administration of bleomycin were analyzed using immunofluorescent microscopy with antibodies against CD45.2 to label TRAM (yellow) or CD45.1 to label MoAM (red). As demonstrated in the representative section, CD45.1 MoAM were disproportionately represented in areas of injury/fibrosis compared to areas that were relatively free of fibrosis.

(D) Heatmap shows k-means clustering of differentially expressed genes (FDR < 0.01) in TRAM and MoAM retained after bleomycin-induced pulmonary fibrosis. Naïve TRAM are included as a comparison. Representative genes and GO biological processes are shown on the right. See also table S22.

(E) Heatmap shows k-means clustering of differentially expressed genes (FDR < 0.01) in TRAM and MoAM retained after influenza A-induced pneumonia. Naïve TRAM are included as a comparison. Representative genes and GO processes are shown on the right. See also table S23.

(F) Venn diagram shows overlap between genes differentially expressed (FDR<0.01) between TRAM and MoAM recruited in response to historic bleomycin or influenza A infection after a second injury with bleomycin and the differences observed between TRAM and MoAM in normal aging (refers to Fig. 6A). P value for overlap <0.01. Hypergeometric test.

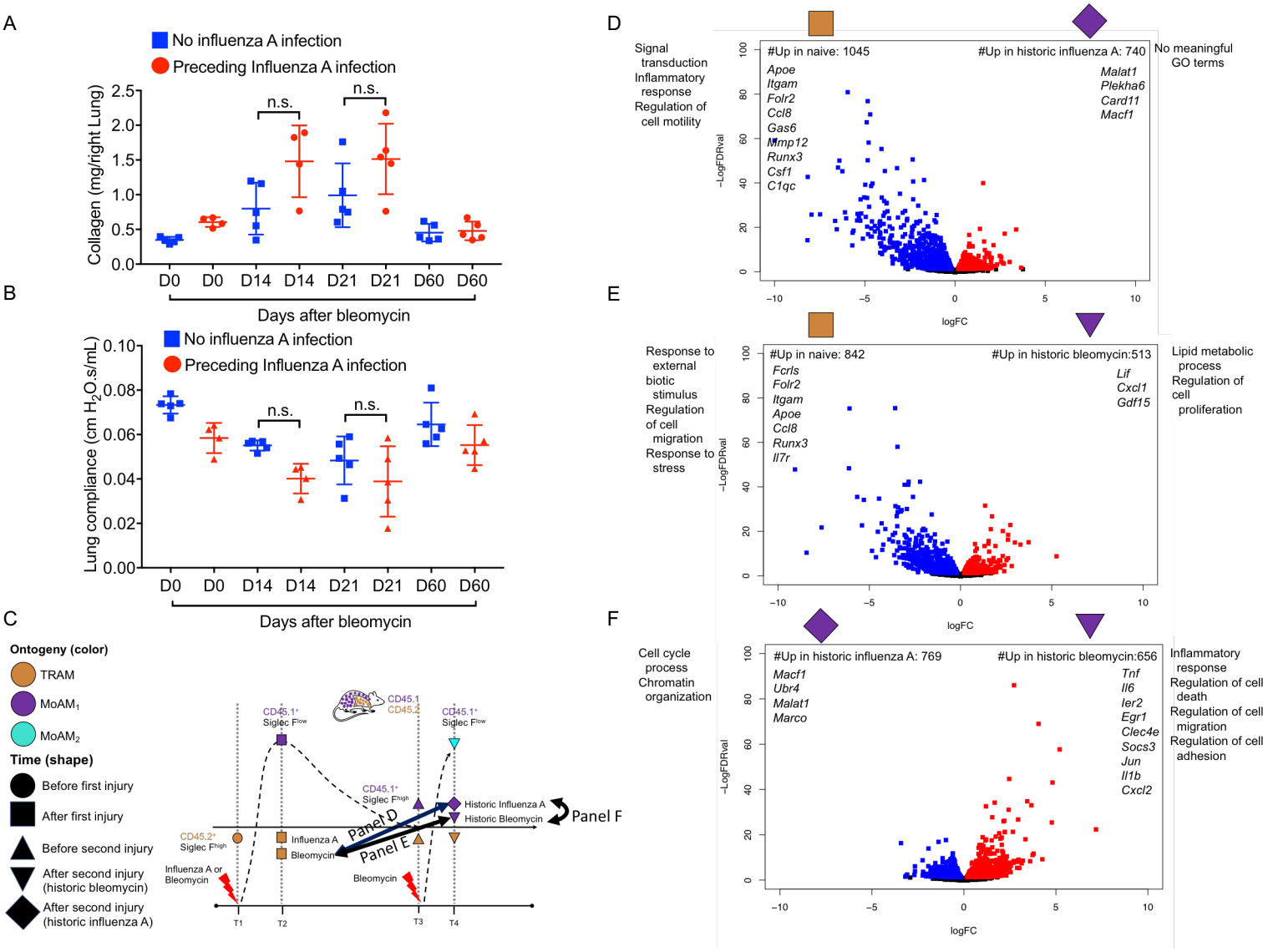
A
No influenza A infection
Preceding Influenza A infection
Collagen (mg/right Lung)
n.s.
n.s.
D0 D0 D14 D14 D21 D21 D60 D60
Days after bleomycin
B
No influenza A infection
Preceding Influenza A infection
Lung compliance (cm H2O.s/mL)
n.s.
n.s.
D0 D0 D14 D14 D21 D21 D60 D60
Days after bleomycin
C
Ontogeny (color)
TRAM
MoAM1
MoAM2
Time (shape)
Before first injury
After first injury
Before second injury
After second injury (historic bleomycin)
After second injury (historic influenza A)
CD45.1+ Siglec Flow
CD45.1 CD45.2
CD45.1+ Siglec Flow
CD45.1+ Siglec Fhigh
Historic Influenza A
Historic Bleomycin
Panel F
CD45.2+ Siglec Fhigh
Influenza A
Bleomycin
Panel D
Panel E
Influenza A or Bleomycin
Bleomycin
T1 T2 T3 T4
D
Signal transduction
Inflammatory response
Regulation of cell motility
#Up in naive: 1045
#Up in historic influenza A: 740
Apoe Itgam Folr2 Ccl8 Gas6 Mmp12 Runx3 Csf1 C1qc
Malat1 Plekha6 Card11 Macf1
No meaningful GO terms
−LogFDRval
logFC
E
Response to external biotic stimulus
Regulation of cell migration
Response to stress
#Up in naive: 842
#Up in historic bleomycin:513
Fcrls Folr2 Itgam Apoe Ccl8 Runx3 Il7r
Lif Cxcl1 Gdf15
Lipid metabolic process
Regulation of cell proliferation
−LogFDRval
logFC
F
Cell cycle process
Chromatin organization
#Up in historic influenza A: 769
#Up in historic bleomycin:656
Macf1 Ubr4 Malat1 Marco
Tnf Il6 Ier2 Egr1 Clec4e Socs3 Jun Il1b Cxcl2
Inflammatory response
Regulation of cell death
Regulation of cell migration
Regulation of cell adhesion
−LogFDRval
logFC

Figure S9

Figure S9. Tissue-resident alveolar macrophages demonstrate immune tolerance irrespective of ontogeny.

(A) Collagen levels (picrosirius red precipitation) measured in mice after a single bleomycin exposure with or without a preceding influenza infection (n=4-5 mice per group). FDR $q>0.05$ after multiple comparison adjustment.

(B) Lung compliance measured in mice after a single bleomycin exposure with or without preceding influenza A infection (n=4-5 mice per group). FDR $q>0.05$ after multiple comparison adjustment.

(C) Schematic for experimental design for panel D-F . Pairwise comparisons described in panels D through F are indicated by double arrow.

(D) Volcano plot comparing TRAM during the first injury (bleomycin) and MoAM recruited after historic infection with influenza A virus, both re-challenged with bleomycin (FDR $q<0.05$). Selected differentially expressed genes and GO biological processes are shown. See Table S24 for a full list of genes.

(E) Volcano plot comparing TRAM during the first injury (bleomycin) and MoAM recruited after historic exposure to bleomycin, both re-challenged with bleomycin (FDR $q<0.05$). Selected differentially expressed genes and GO biological processes are shown. See Table S24 for a full list of genes.

(F) Volcano plot comparing MoAM recruited after historic exposure to bleomycin or infection with influenza A virus, both re-challenged with bleomycin (FDR $q<0.05$). Selected differentially expressed genes and GO biological processes are shown. See Table S24 for a full list of genes.