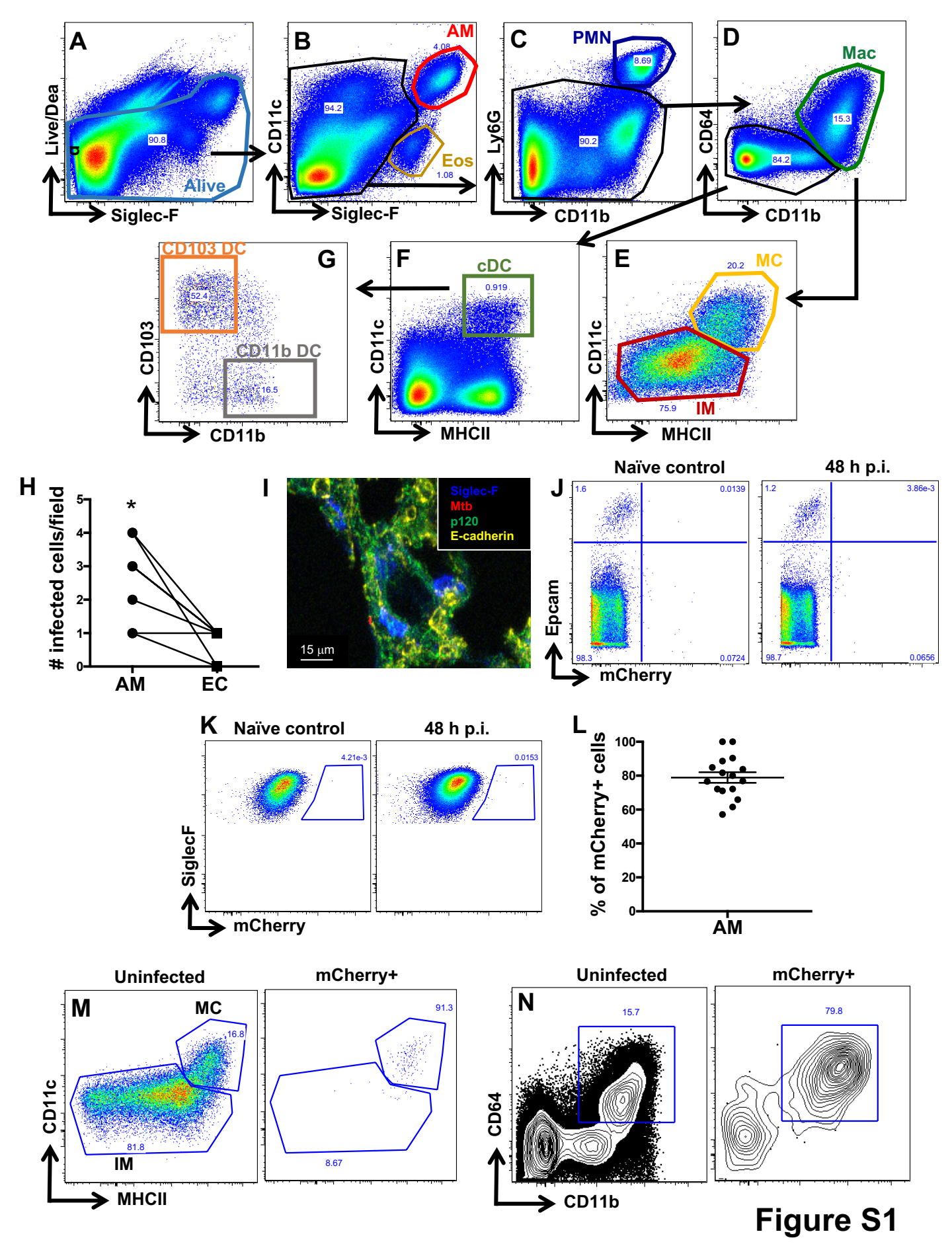
A
Live/Dea
Siglec-F
90.8
Alive
B
AM
4.08
CD11c
Siglec-F
94.2
Eos
1.08
C
PMN
8.69
Ly6G
CD11b
90.2
D
Mac
CD64
CD11b
15.3
84.2
E
MC
20.2
CD11c
MHCII
IM
75.9
F
cDC
0.919
CD11c
MHCII
G
CD103 DC
CD103
CD11b
52.4
CD11b DC
16.5
H
infected cells/field
*
AM
EC
I
Siglec-F
Mtb
p120
E-cadherin
15 µm
J
Naïve control
48 h p.i.
Epcam
mCherry
1.6
0.0139
98.3
0.0724
1.2
3.86e-3
98.7
0.0656
K
Naïve control
48 h p.i.
SiglecF
mCherry
4.21e-3
0.0153
L
% of mCherry+ cells
AM
M
Uninfected
mCherry+
MC
16.8
81.8
IM
CD11c
MHCII
91.3
8.67
N
Uninfected
mCherry+
CD64
CD11b
15.7
79.8

Figure S1

Figure S1. Related to Figure 1. Gating strategy used to identify myeloid cell subsets and epithelial cells.

(A) Dead cells were initially excluded by use of a Live/Dead Fixability dye (BioLegend). (B) AM were identified among live cells by coexpression of SiglecF and CD11c or eosinophils by SiglecF expression alone. (C) AM and eosinophil-negative cells were classified as neutrophils by CD11b and Ly6G coexpression. (D) Neutrophil-negative cells were considered macrophages by coexpression of CD11b and CD64. (E) Macrophages were further subclassified as monocyte-derived cells (MC) based on MHCII/CD11c coexpression or as interstitial macrophages (IM) based on absence of CD11c. (F) CD64-negative cells were classified as conventional DC (cDC) by coexpression of CD11c and MHCII. (G) DC were then either considered $CD11b^{+}$ or $CD103^{+}$. (H) Quantification of the number of Mtb-infected AM ($SiglecF^{+}$) and EC ($p120^{+}$E-$cadherin^{+}CD45^{-}$) observed by microscopy in 20 mm tissue sections of lungs collected 48 hours post-high dose infection (7 tissue sections, n=5 mice). (I) Representative image of an infected type I alveolar EC ($p120^{+}$E-$cadherin^{+}CD45^{-}$) 48 h post-high dose infection. (J-K) Representative flow cytometry plots of mCherry expression among (J) EC (pregated on $CD45^{-}CD31^{-}$, $Epcam^{hi/lo}$) or (K) AM (pregated on $SiglecF^{+}CD11c^{+}$) 48 h post-high dose infection. (L) Percentage of $mCherry^{+}$ lung leukocytes that are AM at day 11 p.i. with a standard dose (n=16 mice pooled from 4 experiments). (M) Representative D18 post-infection flow cytometry plots of uninfected and $mCherry^{+}$ lung leukocytes gated as in panel E. (N) Representative D21 post-infection flow cytometry plots of uninfected and $mCherry^{+}$ lung leukocytes gated as in panel D. Unpaired data are shown as means ± SEM.

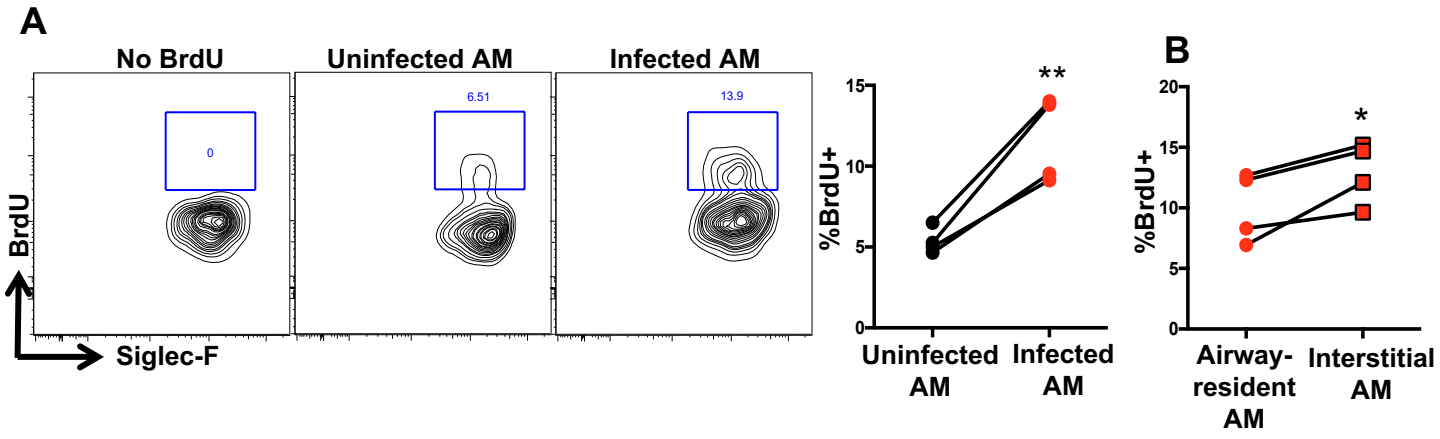

Figure S2. Related to Figure 2. Clustering of infected AM is related to local proliferation.

BrdU incorporation was evaluated in Mtb-mCherry-infected WT mice 16 days post-infection. Shown are representative FACS plots and cumulative data of BrdU incorporation by mCherry- and mCherry^{+} AM (A) and airway-resident and interstitial infected AM (B) over multiple mice (n=4 mice). Statistical differences were evaluated by paired t-test.

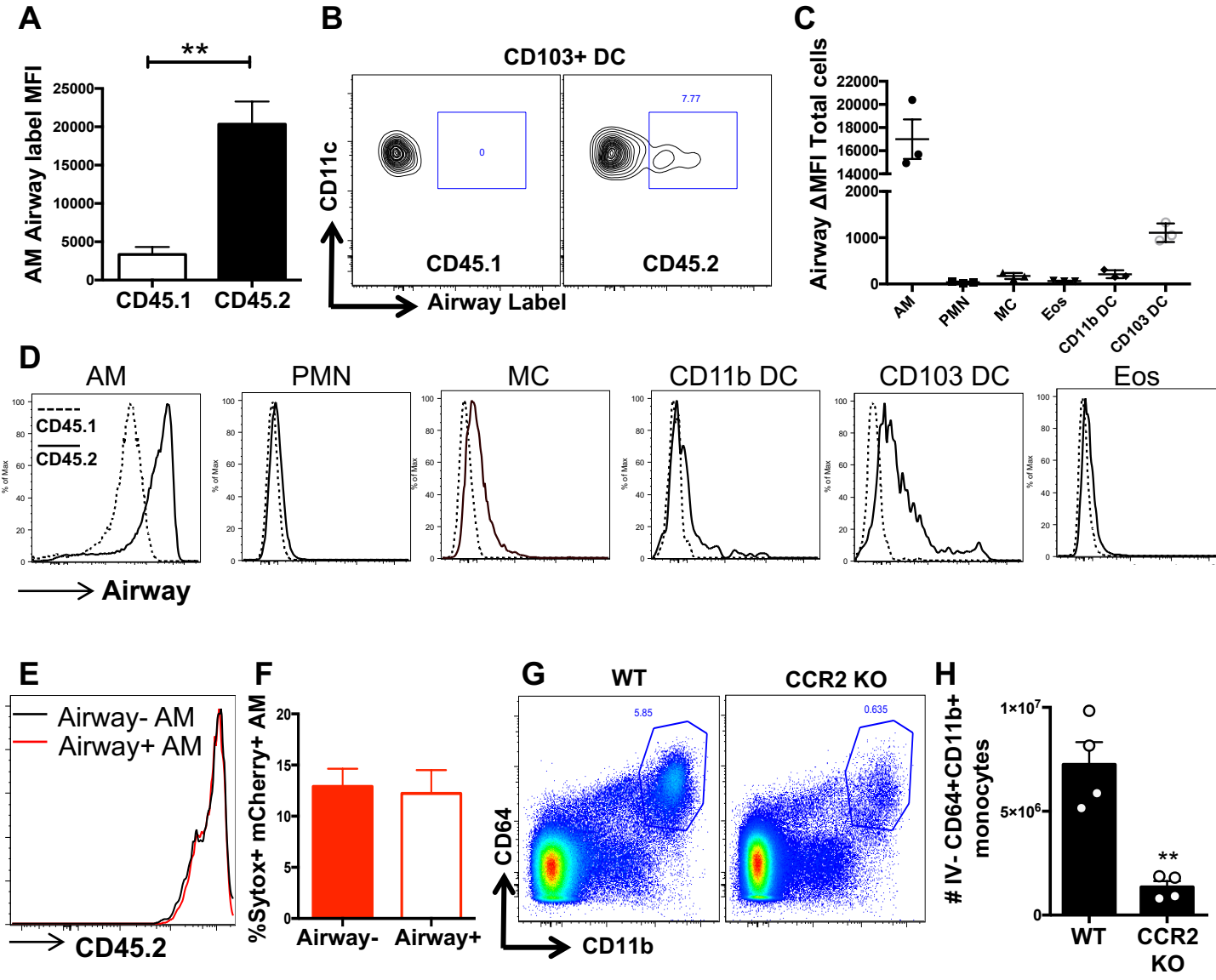
A
AM Airway label MFI
25000
20000
15000
10000
5000
0
**
CD45.1
CD45.2
B
CD103+ DC
CD11c
0
7.77
CD45.1
CD45.2
Airway Label
C
Airway ΔMFI Total cells
22000
20000
18000
16000
14000
2000
1000
0
AM
PMN
MC
Eos
CD11b DC
CD103 DC
D
AM
PMN
MC
CD11b DC
CD103 DC
Eos
CD45.1
CD45.2
% of Max
Airway
E
Airway- AM
Airway+ AM
CD45.2
F
%Sytox+ mCherry+ AM
20
15
10
5
0
Airway-
Airway+
G
WT
CCR2 KO
5.85
0.635
CD64
CD11b
H
IV- CD64+CD11b+ monocytes
1×10^7
5×10^6
0
**
WT
CCR2 KO

Figure S3

Figure S3. Related to Figure 3. Intratracheally administered antibody reliably labels airway-positive cells by FACS.

(A) Naïve C57BL/6 mice were administered either CD45.1 or CD45.2 PE-Cy7 antibody intratracheally, and mean fluorescence intensity of CD45.1 versus CD45.2 expression by AM over multiple mice (n=3 mice) is shown. (B) CD45.2 versus CD45.1 labeling by $CD103^+$ DC was also assessed by FACS. (C) The deltaMFI (CD45.2 MFI - CD45.1 MFI) of airway labeling was evaluated for each myeloid subset identified in Supplemental Figure 1. (D) Representative histograms of CD45.1 and CD45.2 expression by each myeloid subset are shown. (E) WT mice were airway labeled with CD11c antibody, and CD45 expression was evaluated on airway-positive and airway-negative $mCherry^+$ AM 15 days p.i. (F) Cell death was compared between airway-positive and airway-negative Mtb-infected AM 15 days p.i. by Sytox staining (n=5 mice). (G,H) Interstitial localization of Mtb-infected AM mice was compared between WT and CCR2 KO mice. (G) Shown are cumulative data of airway labeling of $mCherry^+$ AM at day 16 p.i. (H) Total numbers of interstitial monocytes ($IV^-CD64^+CD11b^+$) were evaluated between WT and CCR2-deficient animals (n=4 mice), and statistical differences were evaluated by unpaired t-test. Unpaired data are shown as means ± SEM.

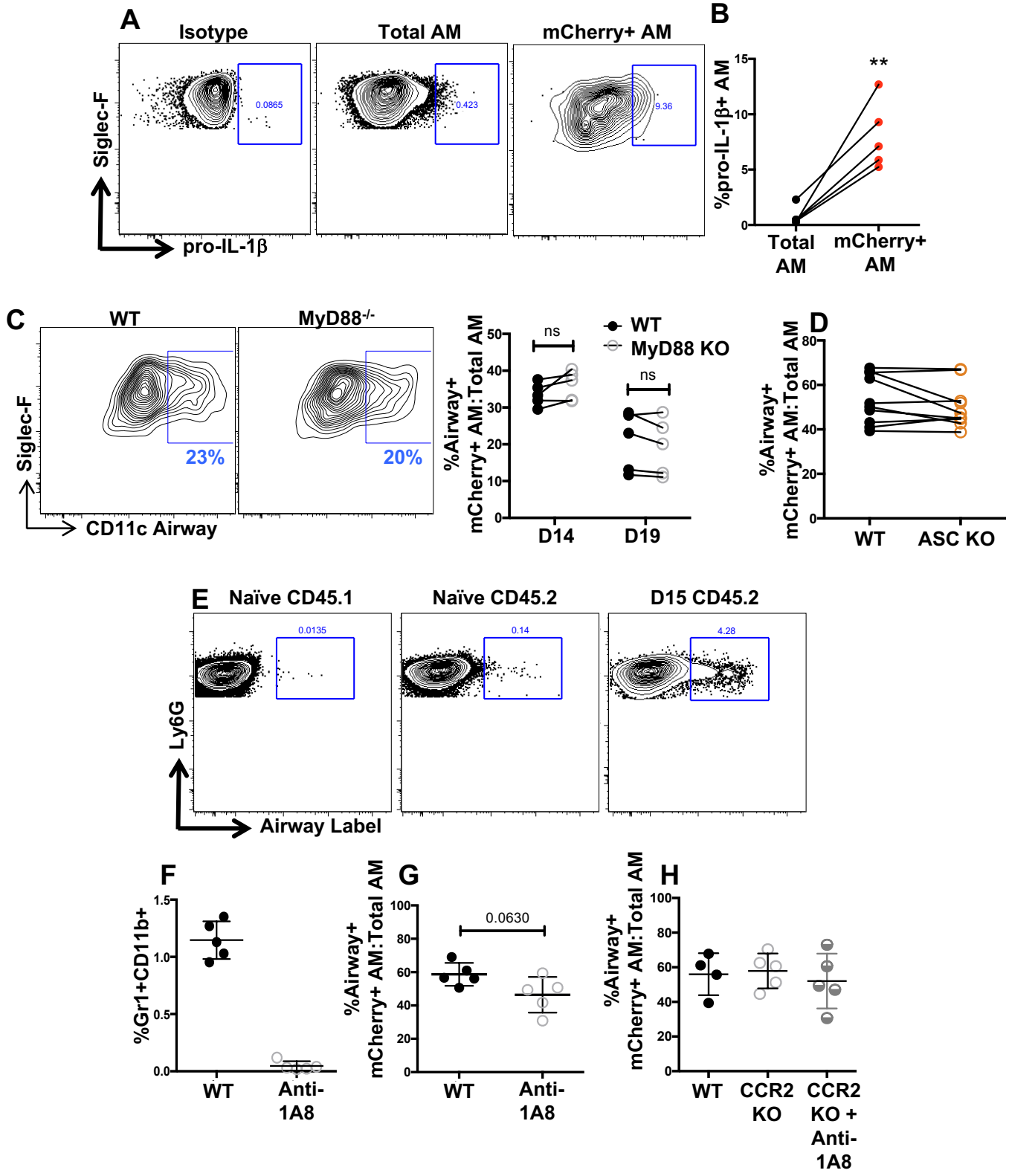
A
Isotype
Total AM
mCherry+ AM
Siglec-F
pro-IL-1β
0.0865
0.423
9.36
B
%pro-IL-1β+ AM
**
Total AM
mCherry+ AM
C
WT
MyD88-/-
Siglec-F
CD11c Airway
23%
20%
%Airway+ mCherry+ AM:Total AM
ns
ns
WT
MyD88 KO
D14
D19
D
%Airway+ mCherry+ AM:Total AM
WT
ASC KO
E
Naïve CD45.1
Naïve CD45.2
D15 CD45.2
Ly6G
Airway Label
0.0135
0.14
4.28
F
%Gr1+CD11b+
WT
Anti-1A8
G
%Airway+ mCherry+ AM:Total AM
0.0630
WT
Anti-1A8
H
%Airway+ mCherry+ AM:Total AM
WT
CCR2 KO
CCR2 KO + Anti-1A8

Figure S4

Figure S4. Related to Figure 4. Interstitial localization of AM is ESX-1 and IL-1-dependent but does not require intrinsic MyD88/ASC expression or monocyte/neutrophil recruitment.

(A,B) BL/6 mice were aerosol-infected with Mtb-mCherry, and pro-IL-1β levels were assayed by flow cytometry 15 days p.i. Shown are (A) representative FACS plots and (B) the cumulative data over 5 mice. (C) Mixed WT:MyD88$^{-/-}$ chimeras were infected with Mtb-mCherry, and airway labeling of infected AM was evaluated at d14 and d21 p.i. Shown is a representative FACS plot of airway labeling of infected AM at d14 p.i., and cumulative airway labeling data of infected WT and MyD88$^{-/-}$ AM at d14 and d21 p.i. is shown on the right (n=5 mice/group). (D) Mixed WT:ASC$^{-/-}$ bone marrow chimeras were infected with Mtb-mCherry and airway labeled with CD11c d19 p.i.. Shown are cumulative data of airway labeling of mCherry^{+} AM from two independent experiments (n=10 mice). (E) Naïve and day 15 p.i. WT mice were aerosol infected with Mtb-mCherry, and airway labeling of neutrophils was evaluated. (F-H) Mice were aerosol infected with Mtb-mCherry, and neutralizing anti-1A8 antibody was administered intraperitoneally every other day beginning day 8 through day 15. (F) Levels of neutrophils were evaluated following 1A8 depletion by measuring lung CD11b^{+}Gr1^{+} cells by FACS 15 days p.i. (G) Shown are cumulative data of airway labeling of infected AM at day 15 p.i. in the presence or absence of neutrophils. (H) Localization of D15 Mtb-infected AM was compared between WT, CCR2$^{-/-}$ mice, and CCR2$^{-/-}$ mice (n=4-5 mice/group) that were depleted of neutrophils with anti-1A8. Statistical differences were evaluated by paired (B, C, and D) or unpaired (G) t-test. Unpaired data are shown as means ± SEM.