1 **Supplemental Figure Legends**

2 **Figure S1. Validation of CD11c Itgax-DTR chimeras.** (A) Representative flow plots of

3 chimerism with Itgax DTR and host cells in uninfected, untreated chimeras. (B) Quantification

4 of chimerism with Itgax DTR and host cells in uninfected, untreated chimeras. (C)

5 Representative flow plots and quantification of total monocytes and monocyte subsets in the lung

6 10 days post infection showing lack of depletion of monocytes in DTx treated animals. (D)

7 Example staining of D14 post-infection DTx- and PBS-treated chimeras, showing CD69 and

8 CD103 staining on flu-specific CD8 T cells in the lung. (E) Number of FluNP-specific CD8 T

9 cells in the extra-vascular compartment of the lung (LEV), CD69+ CD103+ cells of the extra-

10 vascular compartment (LRM) and airways (BAL) on day 14 post infection.

11 **Figure S2. Gating strategy for pulmonary APCs.** (A)Representative flow plots and gating

12 strategy for the identification of extravascular pulmonary APC subsets of C57BL/6J mice

13 intranasally infected with x31 influenza virus. Discrimination of vascular versus extra vascular

14 cells via i.v. staining with anti-CD45.2 was performed in each experiment as shown. plots are

15 representative of 3 independent experiments with 5 mice used in each experiment.

16 **Figure S3. Number of APCs in the lung of WT and CCR2-/- mice 10 days post infection**

17 **with x31 influenza virus.** (A)Representative flow plots of APCs present in WT and CCR2-/-

18 mouse lungs on day 10 post-infection. (B) Number of extra-vascular classical and non-classical

19 monocytes (left graph) or DC subsets (right graph) in the lungs of WT and CCR2-/- mice on day

20 10 post-infection. All graphs error bars are S.E.M. Data are representative of 3 independent

21 experiments with 5 mice per time point. * $p<0.05$ n.s. $p>0.05$(two-tailed Student's t-test).

22 **Figure S4. Depletion of non-classical monocytes in CX3CR1-DTR mice has no effect upon**

23 **lung T_{RM} generation following influenza infection.** (A) Graph and representative flow plots of
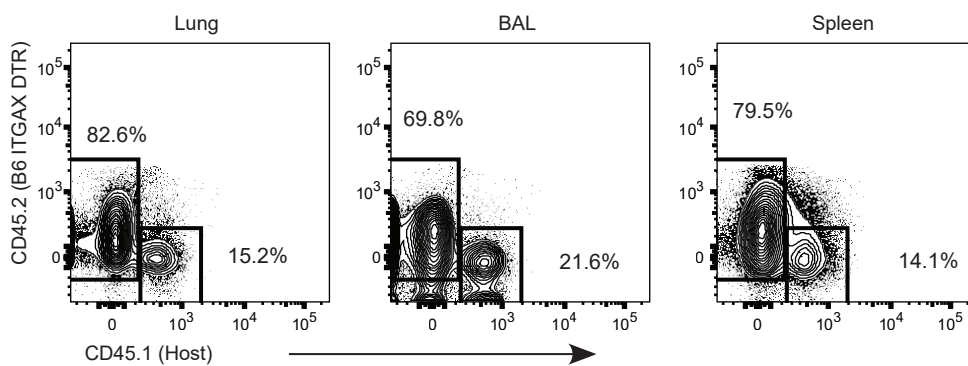
32

1   the frequency of total lymphocytes in the lung and spleen derived from donor bone marrow

2   versus host lymphocytes 6 weeks after chimera production. Gated to exclude CD45- singlet

3   lymphocytes. (B) Graph and representative flow plots of the frequency of classical and non-

4   classical monocytes in the lung and spleen of chimeras treated with DTx or PBS 10 days post

5   infection. Plots are gated on MHC-II lo, Ly6g-, CD11b+, CD11c- singlet lymphocytes.(C) Graph

6   of the number of OT-I CD8 T cells day 45 post infection in PBS and DTx treated mice in the

7   lung extra-vascular compartment (LEV), bronchoalveolar lavage (BAL), and spleen, and graph

8   of the number of CD69+ CD103+ OT-I CD8 T cells day 45 post infection in PBS and DTx

9   treated mice in the lung-extra vascular compartment and bronchoalveolar lavage. All error bars

10   are S.E.M. * $p<0.05$ n.s. $p>0.05$(two-tailed Student's *t*-test) Data are representative of two

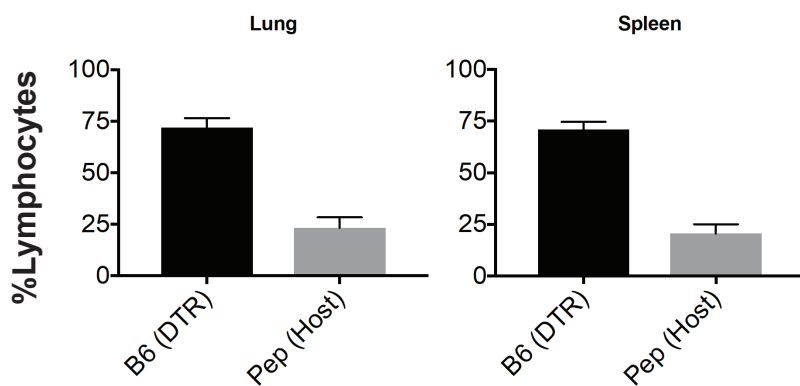11   independent experiments.

12

13   **Figure S5. Phenotype of OT-I T cells after three days of in vitro stimulation with lung APC**

14   **subsets.** (A) Representative flow plots of CD127 staining on CD8 OT-I T cells following three

15   days of *in vitro* culture with OVA peptide pulsed monocytes of indicated subtypes. (B)

16   Frequencies of CD127 positivity among highly divided CD8 OT-I T cells following three days of

17   *in vitro* culture with OVA peptide pulsed monocytes of indicated subtypes. Error bars SEM, data

18   represents 3 experiments using 3 technical replicates *in vitro* each. (C) Representative flow plots

19   of CD103+ staining on CD8 OT-I T cells following three days of *in vitro* culture with OVA

20   peptide pulsed monocytes of indicated subtypes. (D) Representative flow plots of CD69+

21   staining on CD8 OT-I T cells following three days of *in vitro* culture with OVA peptide pulsed

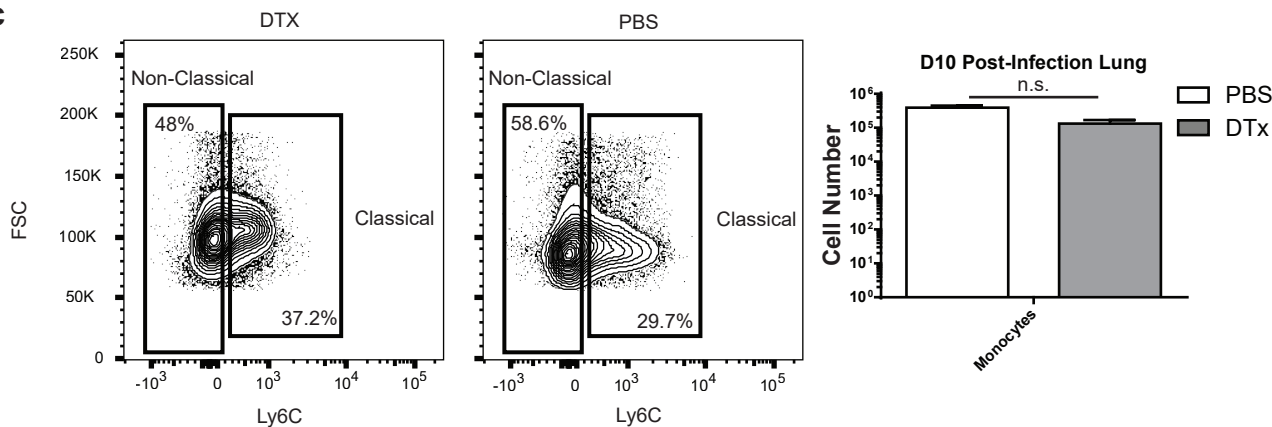22   monocytes of indicated subtypes.
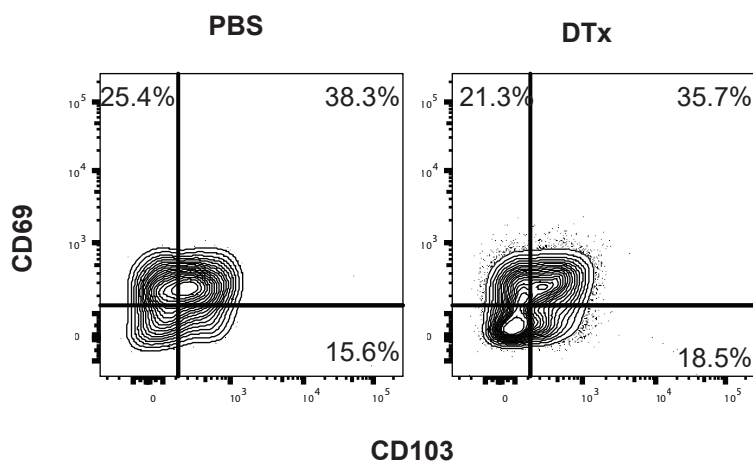
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a

D10 Chimerism Data
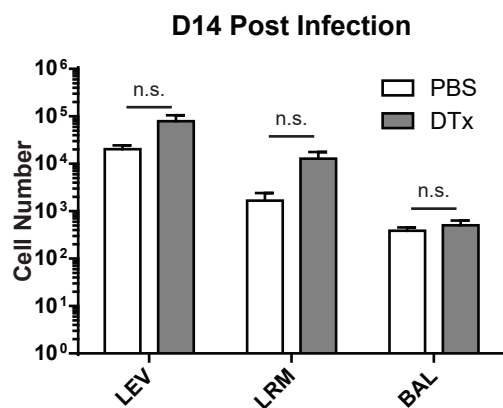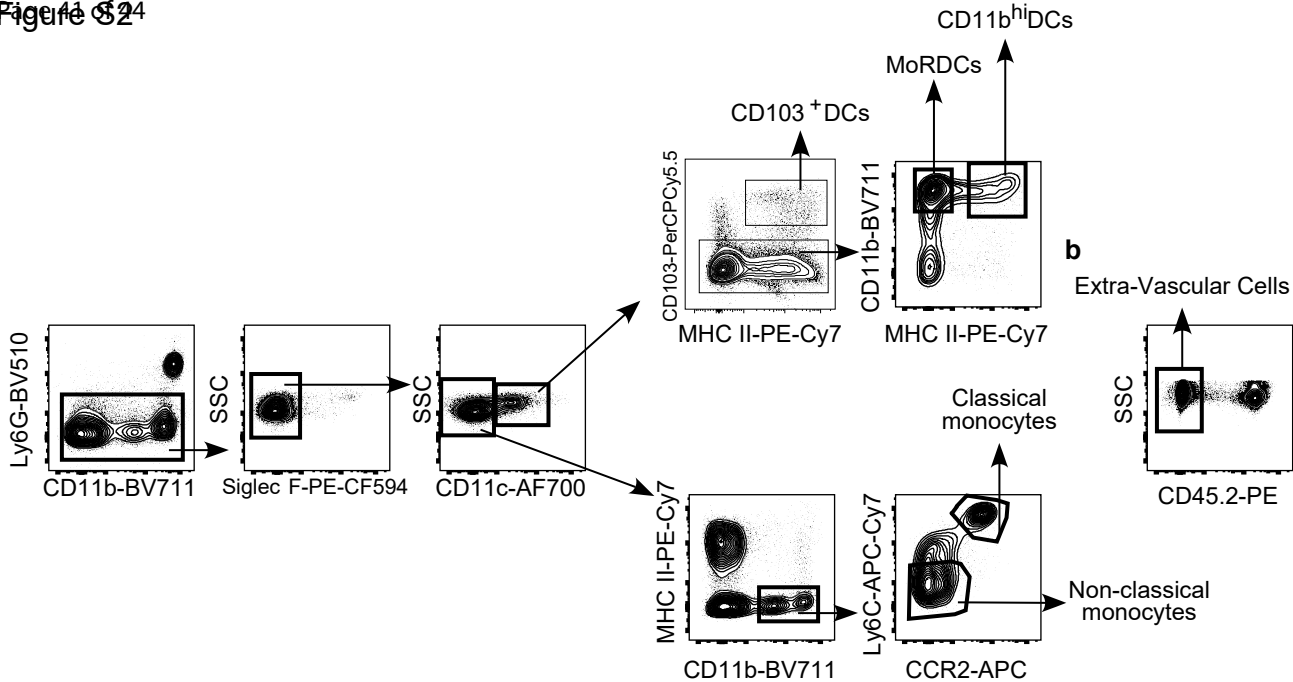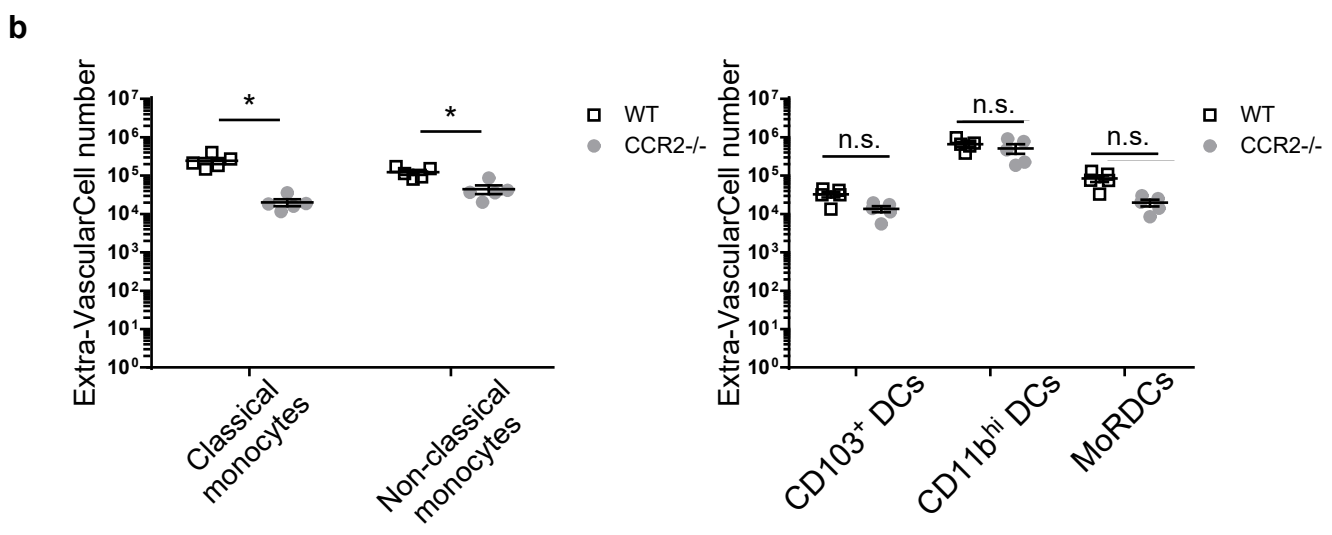

b

c

d

D14 Lungs

e

a

b

a **Chimerism CX3CR1-DTR**

b **D10 CX3CR1-DTR Depletion**

c

Figure S5

a

Classical monocytes | Non-classical monocytes

CD127-BV711

CTV

b

■ T cells only
■ T cells + classical monocytes
□ T cells + non-classical monocytes

% CD127+ among divided cells

c

CD103-PerCP-Cy5.5

T Cell Only+Antigen | Classical Monocytes | Non-Classical Monocytes

CTV

d

CD69-FITC

T Cell Only+Antigen | Classical Monocytes | Non-Classical Monocytes

CTV

e

% Parent CD62L+

T only + ag | Classical +ag | NC+Ag | DC 103+ ag | MoRDC +ag | 11b+ag

% Parent CD49a+

T only + ag | Classical +ag | NC+Ag | DC 103+ ag | MoRDC +ag | 11b+ag