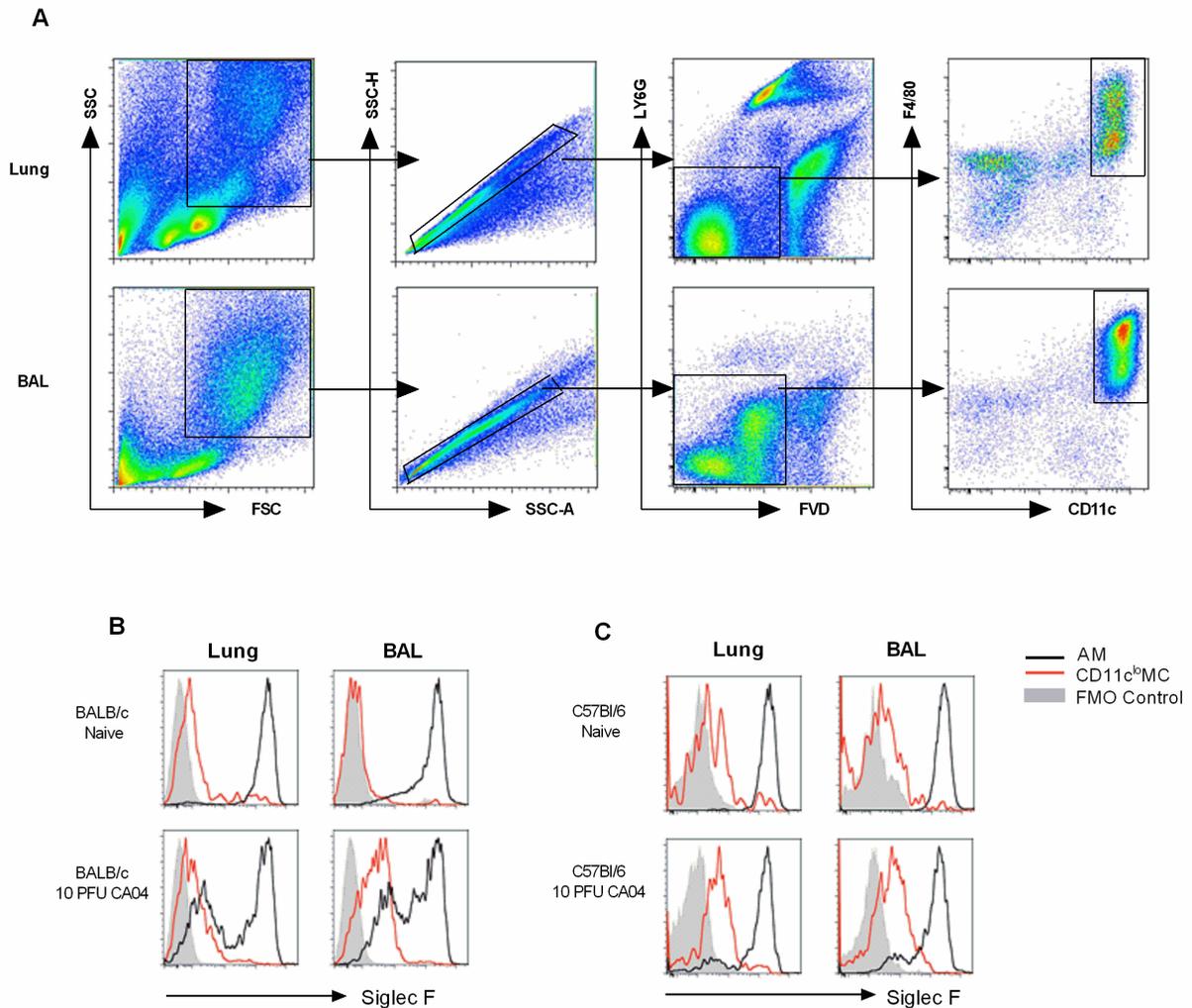


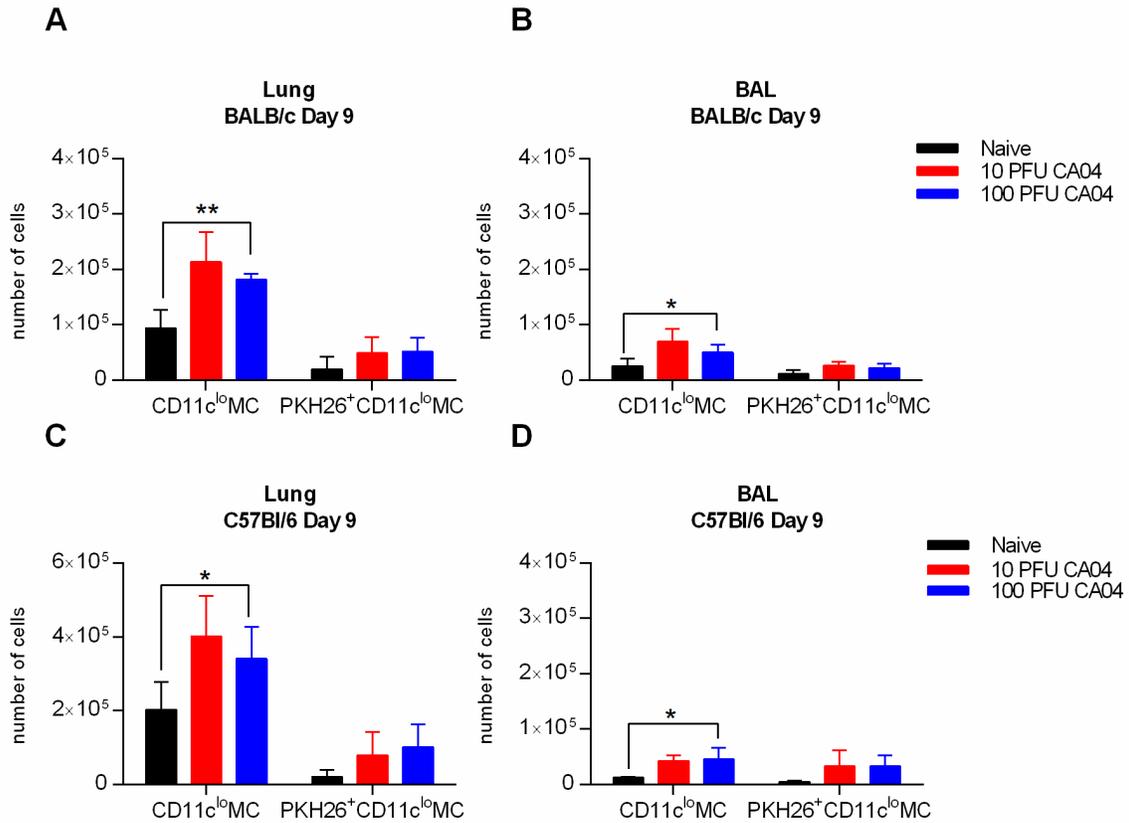
Supplemental Figure 1



1

2 **Supplemental Figure 1.** Gating strategy for flow cytometry analysis of AMs. (A) Cells were  
 3 selected based on size and granularity (FSC/SSC), followed by the exclusion of doublets (SSC-A  
 4 vs. SSC-H), dead cells and neutrophils (Ly6G vs. FVD) from the analysis. AMs were identified  
 5 as cells with high expression of CD11c and F4/80. (B) Representative plots show the expression  
 6 of Siglec F on AMs (CD11c<sup>hi</sup>F4/80<sup>+</sup>) and CD11c<sup>lo</sup>MCs (CD11c<sup>lo</sup>F4/80<sup>+</sup>) in the lung and BAL of  
 7 naïve and influenza-infected BALB/c mice. (C) Representative plots show the expression of  
 8 Siglec F on AMs (CD11c<sup>hi</sup>F4/80<sup>+</sup>) and CD11c<sup>lo</sup>MCs (CD11c<sup>lo</sup>F4/80<sup>+</sup>) in the lung and BAL of  
 9 naïve and influenza infected C57Bl/6 mice.

## Supplemental Figure 2



10

11 **Supplemental Figure 2.** Expansion of CD11c<sup>lo</sup>MCs following influenza infection. (A-B) Total

12 number of CD11c<sup>lo</sup>MCs and PKH26<sup>+</sup> CD11c<sup>lo</sup>MCs the lung (A) and BAL (B) of BALB/c mice

13 infected with 10 or 100 PFU CA04 on Day 9 post-infection. (C-D) Total number of

14 CD11c<sup>lo</sup>MCs and PKH26<sup>+</sup>CD11c<sup>lo</sup>MCs the lung (C) and BAL (D) of C57Bl/6 mice infected

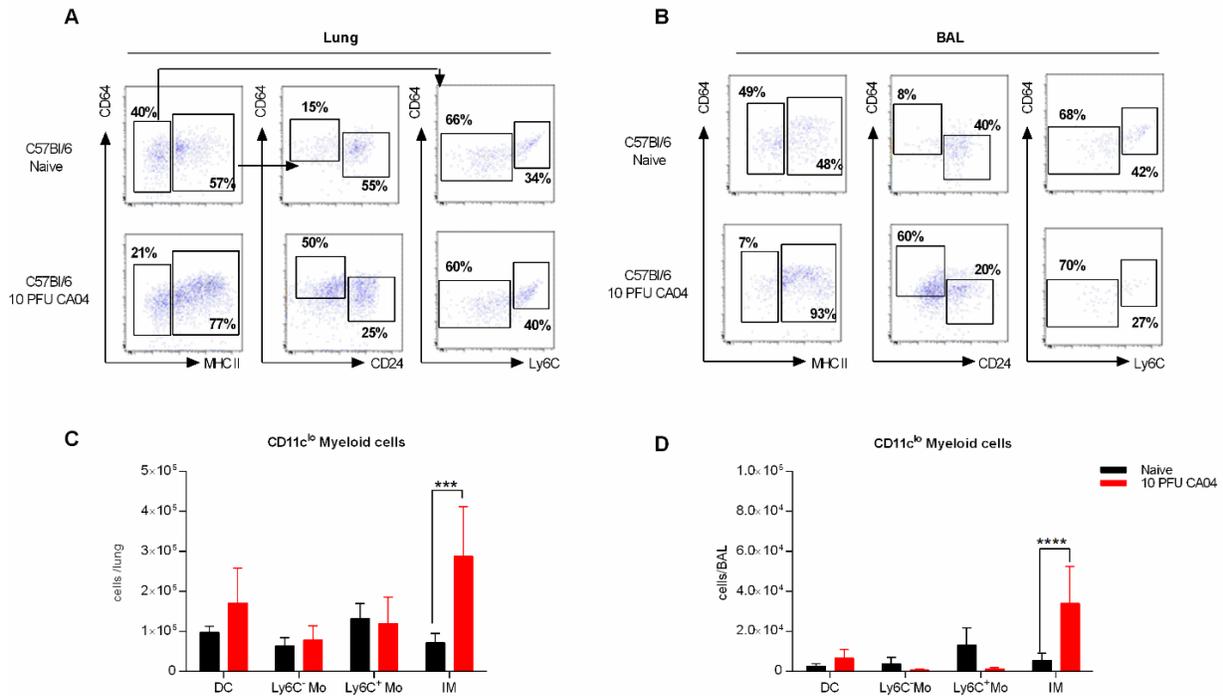
15 with 10 or 100 PFU CA04 on Day 9 post-infection. Data shown are representative of 2

16 independent experiments with 5 mice per group. Statistical analyses were performed by two-way

17 ANOVA; \* p<0.05; \*\* p<0.01.

18

Supplemental Figure 3

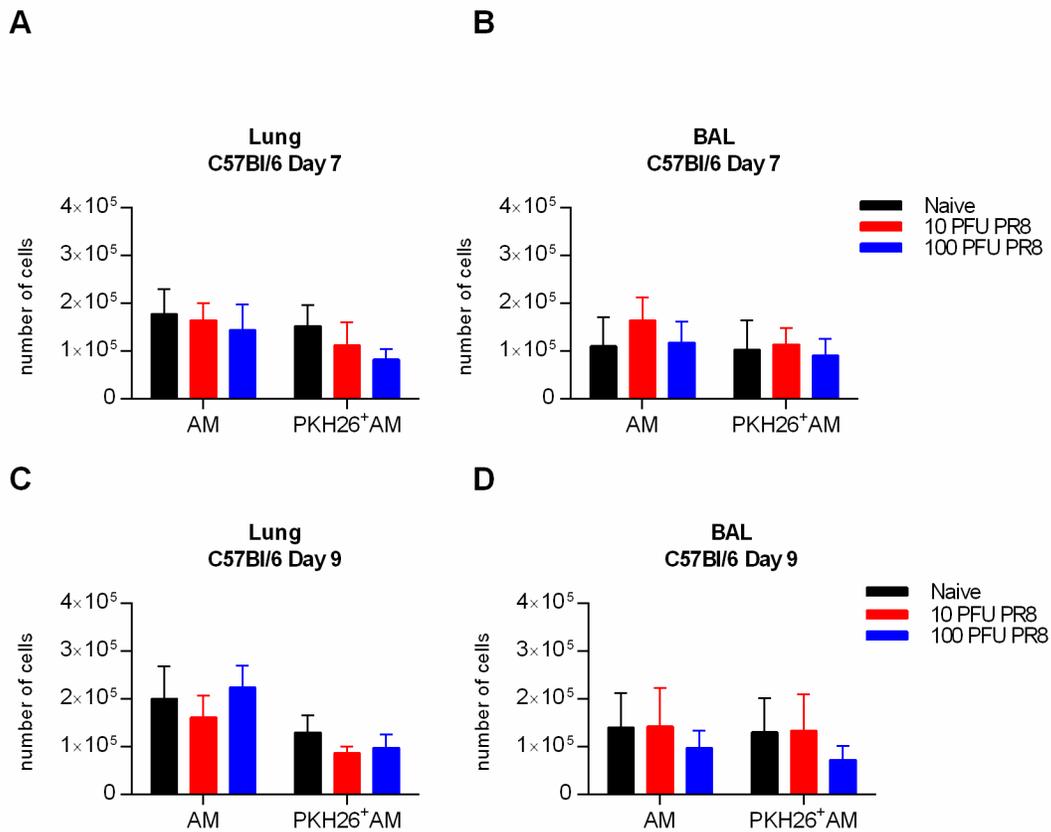


19

20 **Supplemental Figure 3.** Gating strategy for flow cytometry analysis of CD11c<sup>lo</sup>MCs. (A and  
 21 B) Representative plots show gating strategy to differentiate CD11c<sup>lo</sup>F4/80<sup>+</sup>CD11b<sup>+</sup> cells from  
 22 the lung (A) and BAL (B) of naïve and influenza infected C57Bl/6 mice using methods  
 23 described in Misharin *et al* (1). Cells were characterized as interstitial macrophages (IM) (CD64<sup>+</sup>  
 24 MHC Class II<sup>+</sup> CD24<sup>-</sup>), dendritic cells (DC) (CD64<sup>-</sup>MHC Class II<sup>+</sup>CD24<sup>+</sup>), Ly6C<sup>-</sup> monocytes  
 25 (Ly6C<sup>-</sup> Mo) (CD64<sup>+/-</sup> MHC Class II<sup>-</sup> Ly6C<sup>-</sup>) and Ly6C<sup>+</sup> monocytes (Ly6C<sup>+</sup>Mo) (CD64<sup>+/-</sup> MHC  
 26 Class II<sup>-</sup> Ly6C<sup>+</sup>). (C and D) Absolute numbers of DC, Ly6C<sup>-</sup> Mo, Ly6C<sup>+</sup> Mo, IM from the lung  
 27 (C) and BAL (D) of naïve and influenza-infected C57Bl/6 mice. Data shown are representative  
 28 of 2 independent experiments with 5 mice per group. Statistical analyses were performed by two-  
 29 way ANOVA; \*\*\* p<0.001; \*\*\*\* p<0.0001.

30 1. Misharin AV, Morales-Nebreda L, Mutlu GM, Budinger GR, and Perlman H. 2013. Flow  
 31 cytometric analysis of macrophages and dendritic cell subsets in the mouse lung. *Am J*  
 32 *Respir Cell Mol Biol* 49:503-510

## Supplemental Figure 4



33

34 **Supplemental Figure 4.** AMs are resistant to depletion following PR8 virus infection of  
35 C57Bl/6 mice. **(A-B)** Total number of AMs and PKH26<sup>+</sup>AMs the lung **(A)** and BAL **(B)** of  
36 C57Bl/6 mice infected with 10 or 100 PFU PR8 on day 7 post-infection. **(C-D)** Total number of  
37 AMs and PKH26<sup>+</sup>AMs the lung **(C)** and BAL **(D)** of C57Bl/6 mice infected with 10 or 100 PFU  
38 PR8 on day 9 post-infection. Data shown are representative of 2 independent experiments with 5  
39 mice per group. Statistical analyses were performed by two-way ANOVA.

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