iScience, Volume *25*

Supplemental information

CD47 restricts antiviral function of alveolar macrophages during influenza virus infection

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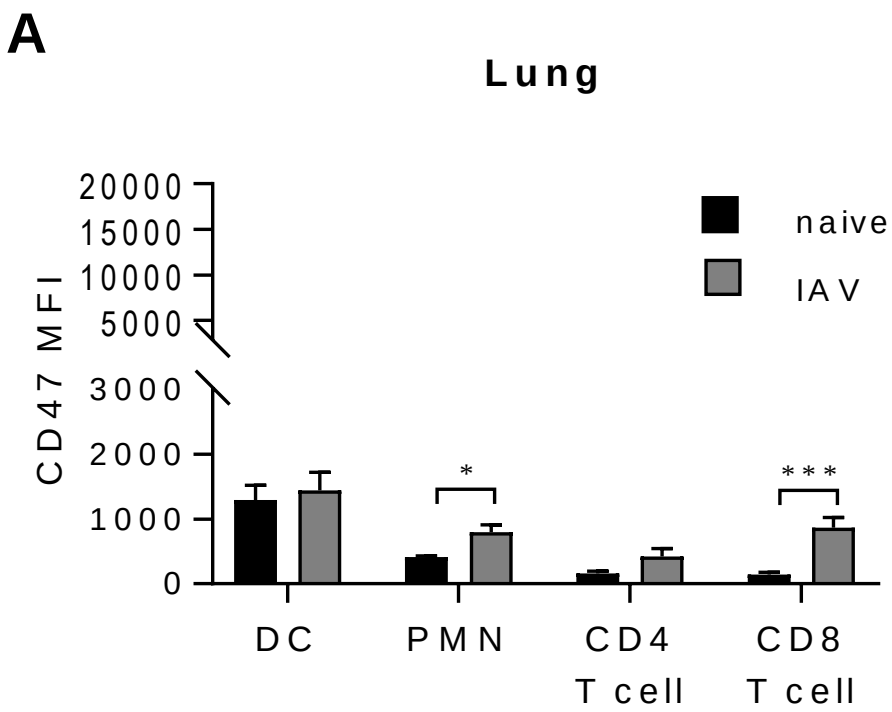
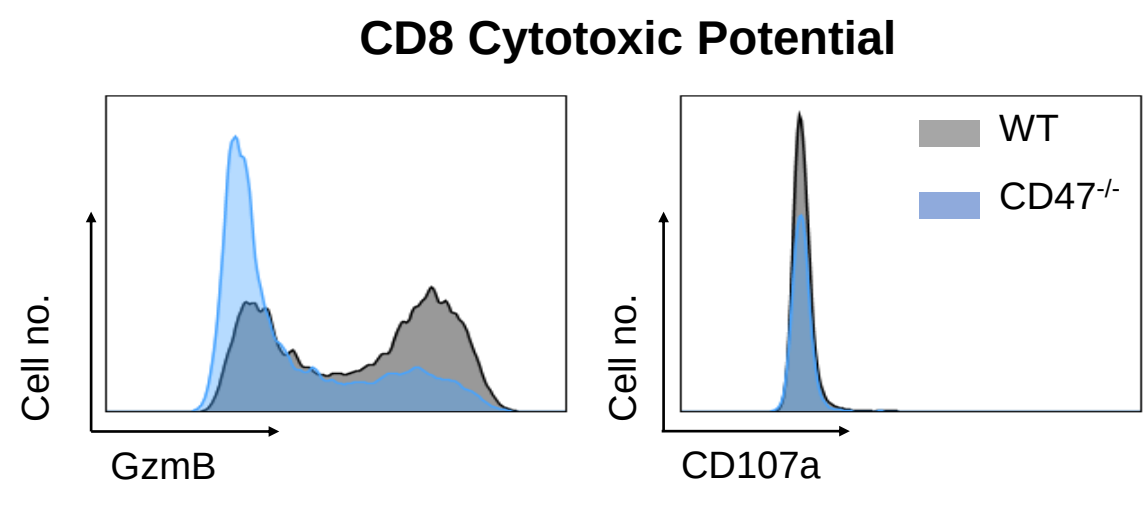
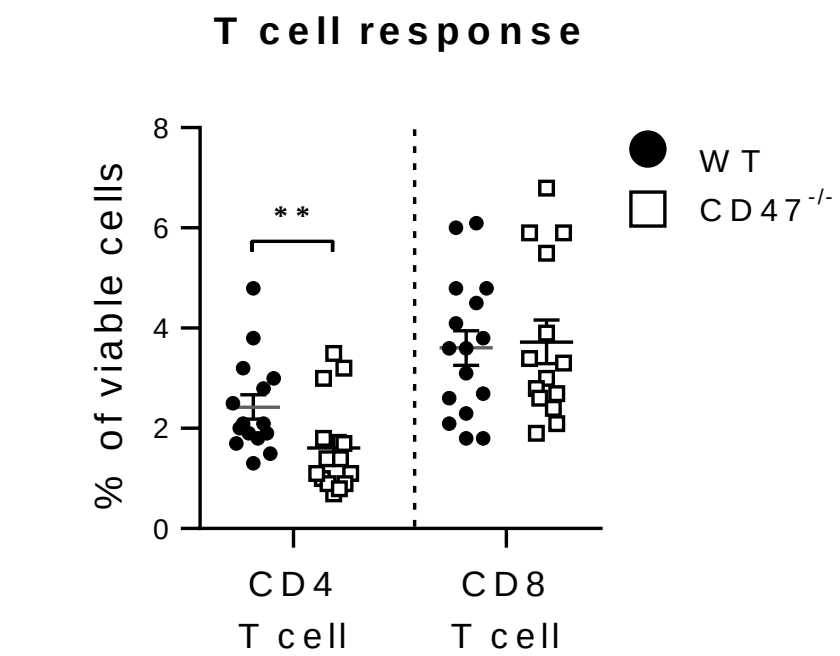
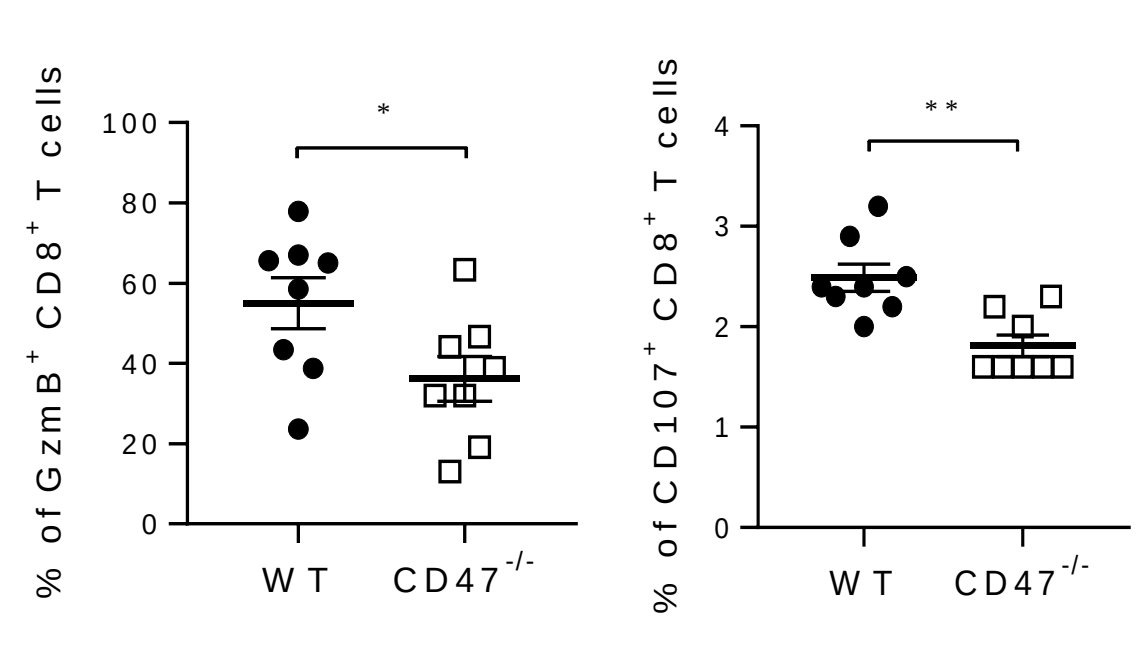
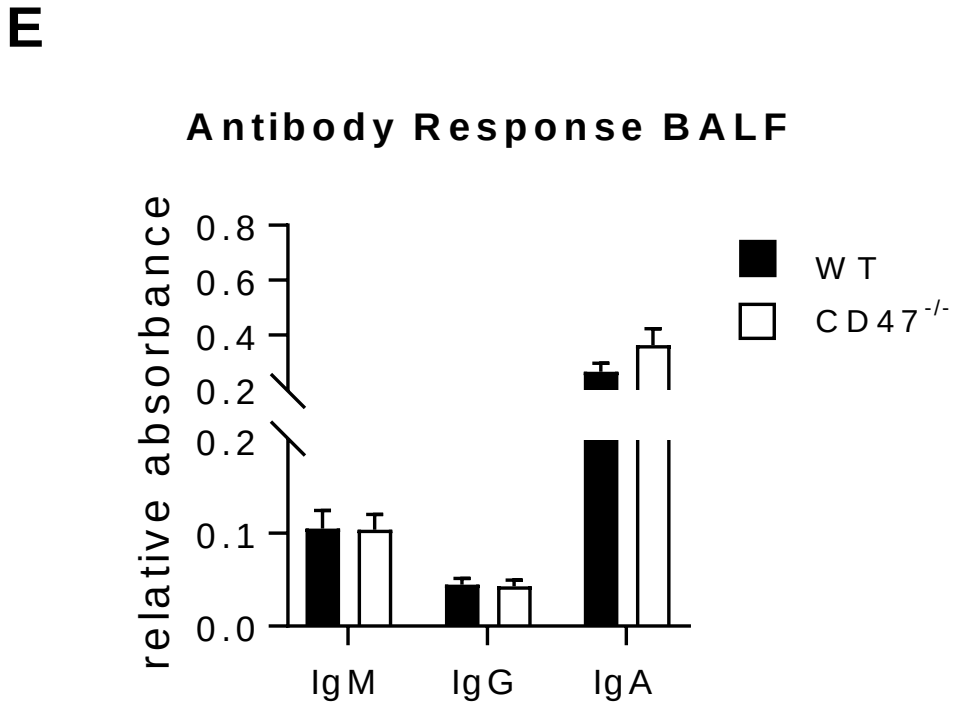
Figure S1. Deficiency of CD47 does not enhance adoptive immune responses in the lung, Related to Figure 1











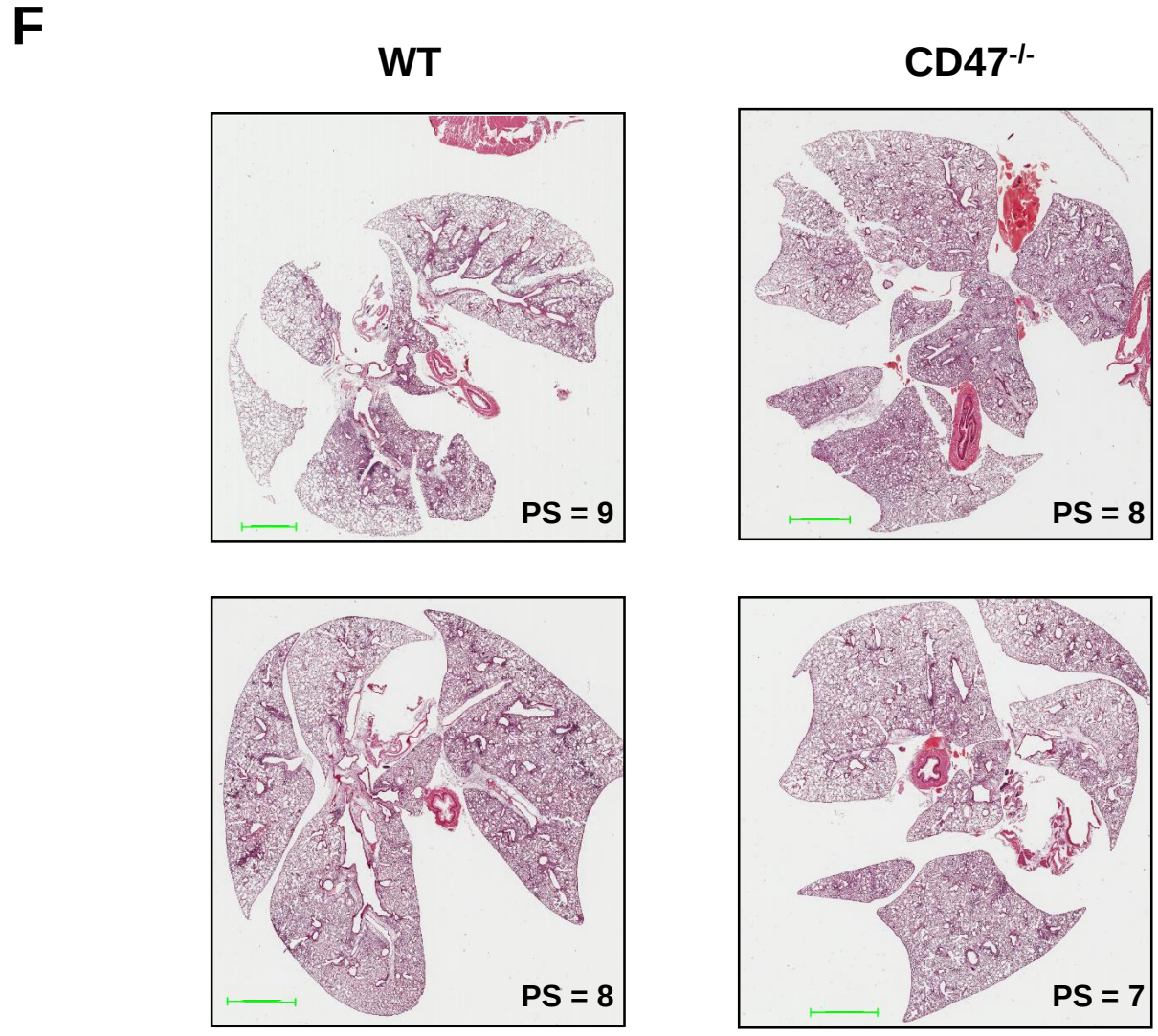


Figure S1. Deficiency of CD47 does not enhance adoptive immune responses in the lung.
A) Dendritic cells (DC), neutrophils (PMN) (both 3 dpi) as well as $CD4^+$ and $CD8^+$ T cells (7 dpi) were analysed via flow cytometry regarding the mean fluorescence intensity (MFI) of CD47 (n=9 (naive) – 12 (IAV) (IAV 75 PFU). B) Representative histograms for granzyme B (GzmB) and CD107a expression by $CD8^+$ T cells in the lung 7 dpi. C) $CD8^+$ T cells were analysed regarding the frequencies of GzmB and CD107a expressing cells upon restimulation. D) The frequencies of $CD4^+$ and $CD8^+$ T cells in the lung were examined 7 dpi. E) Influenza specific antibodies in the BALF of IAV infected mice were detected via ELISA 7 dpi (n=9). Results from two independent experiments are shown. Student's t-test was performed. * = $p<0.05$.F) Histopathology was scored (PS) after H&E staining in a blinded manner according to the inflammation markers, including inflammatory cell infiltration, cell necrosis, granulocyte infiltration and histiocytosis. Two represantive lungs per group are shown (scale bar = 2 mm).

Figure S2. Low dose infection does not lead to enhanced virus clearance, Related to Figure 1

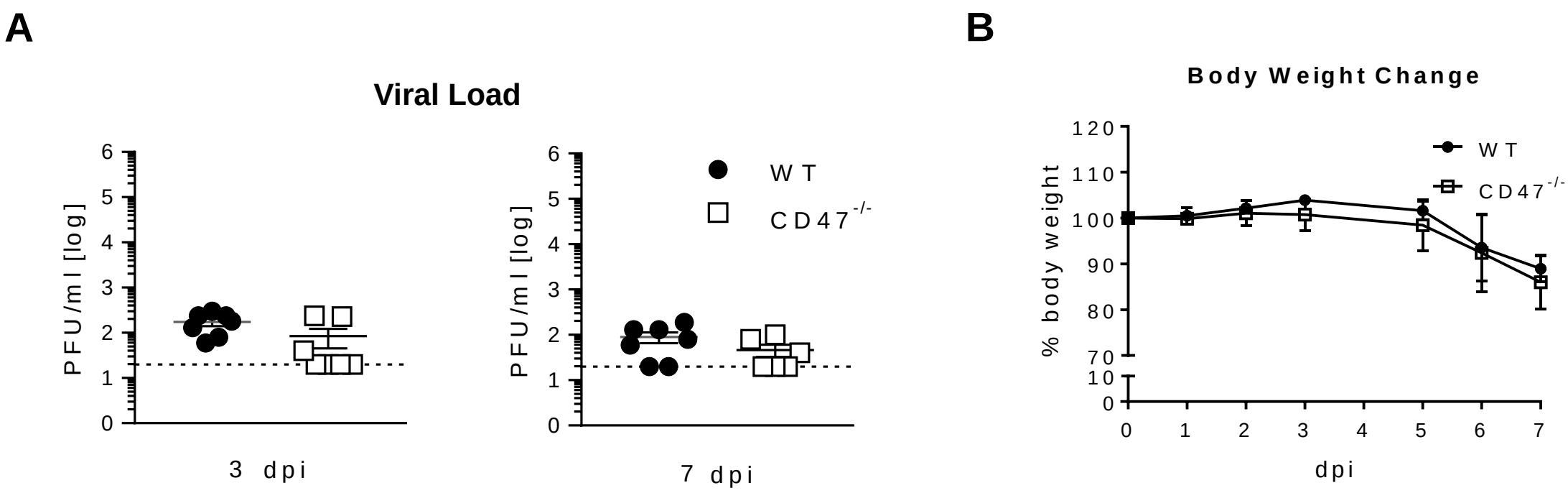


Figure S2. Low dose infection does not lead to enhanced virus clearance. Wildtype (WT) and CD47$^{-/-}$ mice were i.n. infected with 8 PFU of influenza virus A/PR8/34.A) Viral load in the lung of mice infected was determined at days 3 and 7 after infection via plaque assay. B) Body weight change after infection with 8 PFU (n=3). Results from two independent experiments are shown.

Figure S3. Flow cytometry of alveolar macrophages in the lung, Related to Figure 2

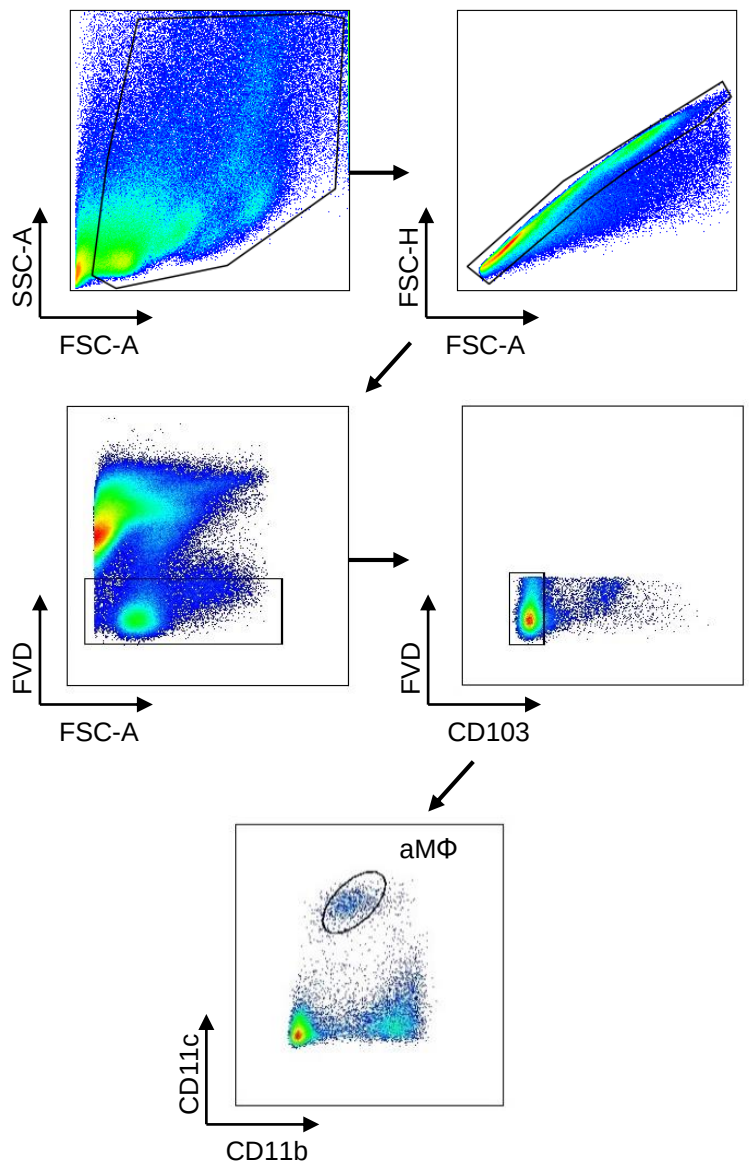


Figure S3. Flow cytometry of alveolar macrophages in the lung. aMΦ were differentiated by CD11c and CD11b expression. DCs were excluded by previously gating on $CD103^-$ cells.

Figure S4. Alveolar macrophages of CD47$^{-/-}$ mice do not show a proinflammatory phenotype, Related to Figure 4

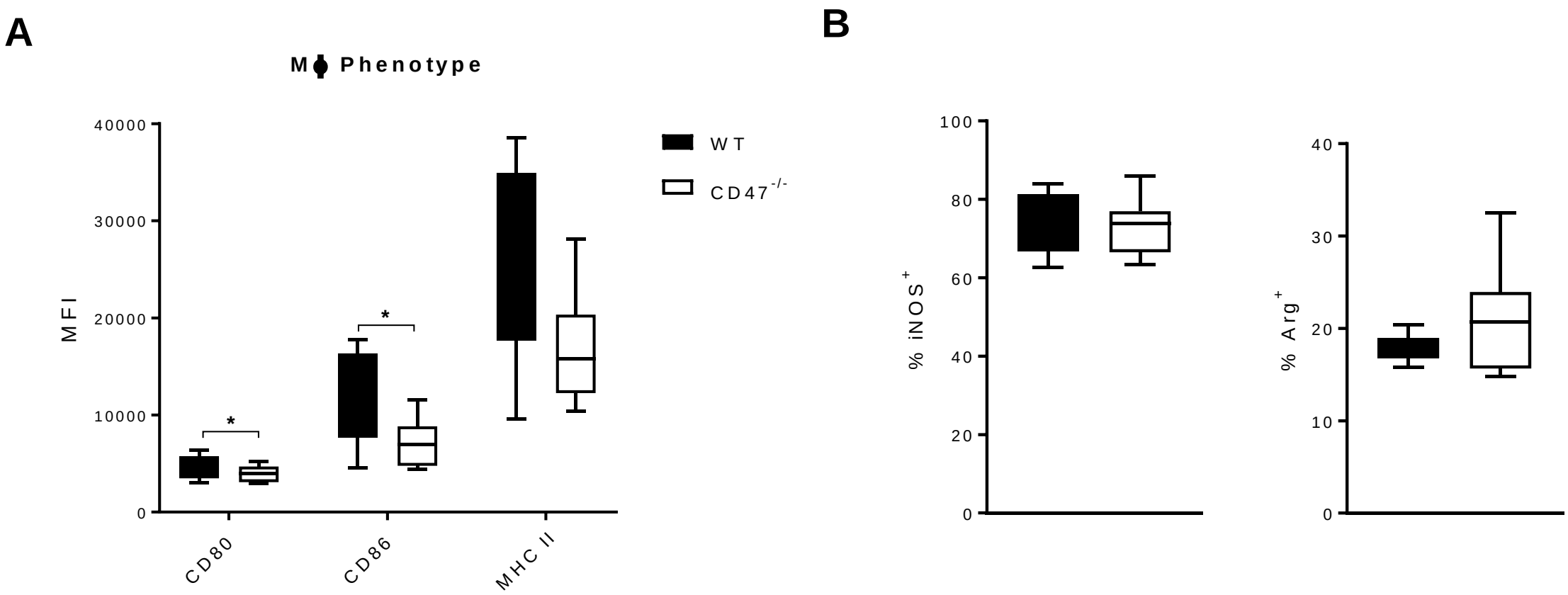


Figure S4. Alveolar macrophages of CD47$^{-/-}$ mice do not show a proinflammatory phenotype. A) Flow cytometric analysis of CD80, CD86, and MHC II expression by aMΦ from the lung 3 dpi (n=9). Mean fluorescent intensity (MFI) is shown. B) Frequencies of iNOS and arginase (Arg) expressing aMΦ in the lung 3 dpi (n=9). C) Data shown are mean ± SEM. Student's t-test and one-way ANOVA with Tukey's multiple-comparisons post-test was performed. * = $p<0.05$, ** = $p<0.01$. Results from two independent experiments are shown.

Figure S5. HB expression by immune cells in the lung, Related to Figure 6

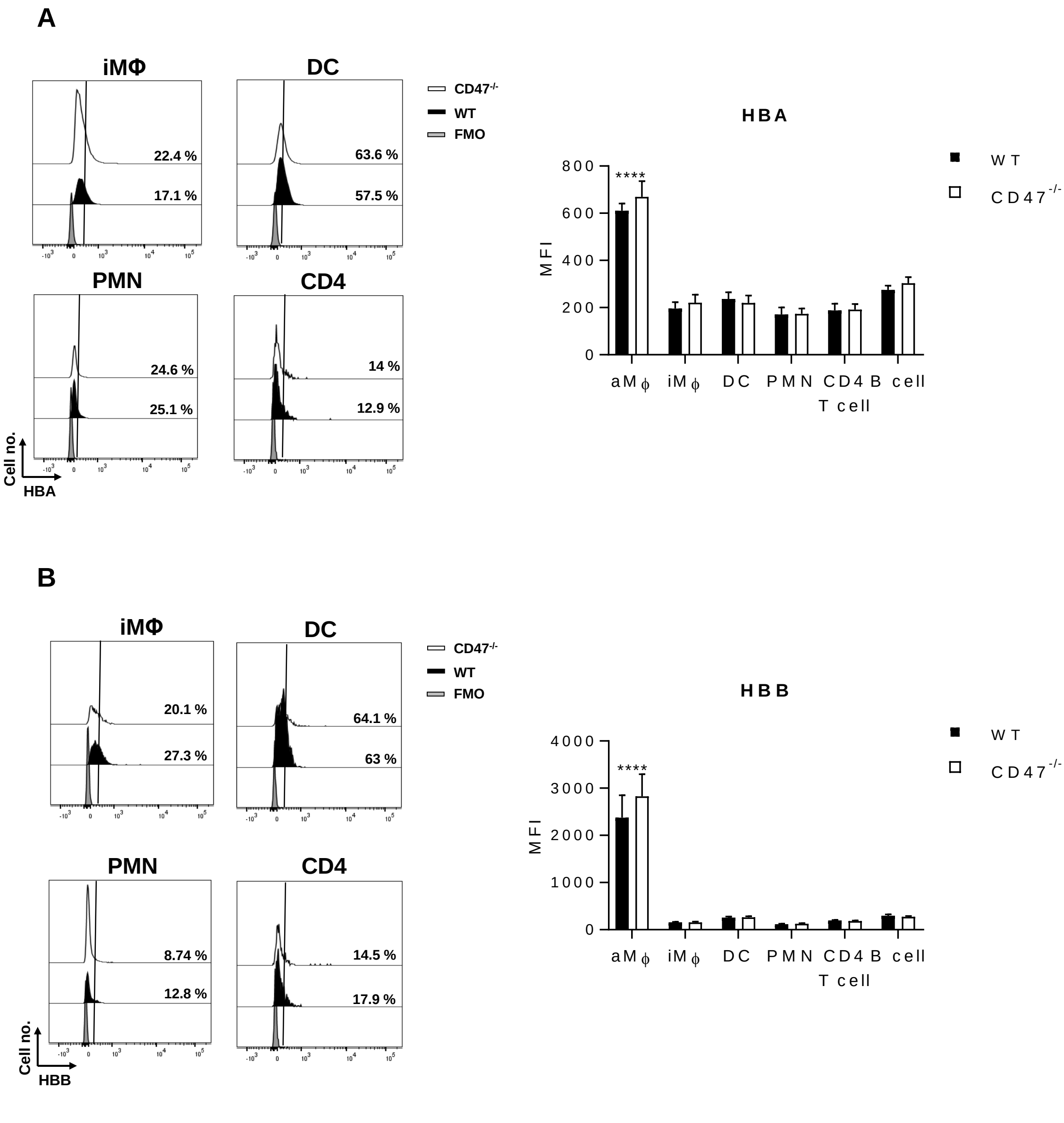


Figure S5. HB expression by immune cells in the lung. Flow cytometric analysis of hemoglobin alpha (HBA) (A) and hemoglobin beta (HBB) (B) expression by iMΦ, DC, PMN and $CD4^+$ T cells from the lung 3 dpi (n=6-8). Mean fluorescent intensity (MFI) is shown. Results from two independent experiments are shown. Data shown are mean ± SEM. Two-way ANOVA with Sidak's multiple-comparisons post-test was used. Statistical analysis between aMΦ and all other cell subsets is shown. **** = $p<0.0001$.