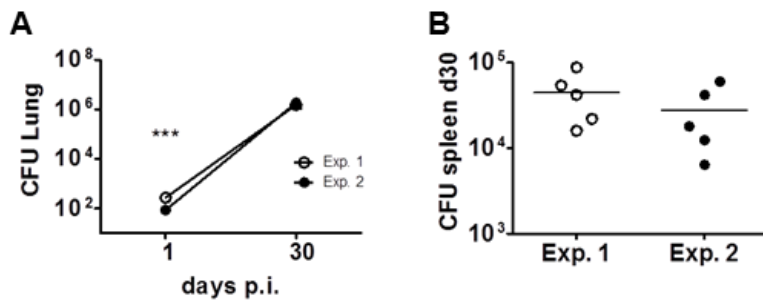
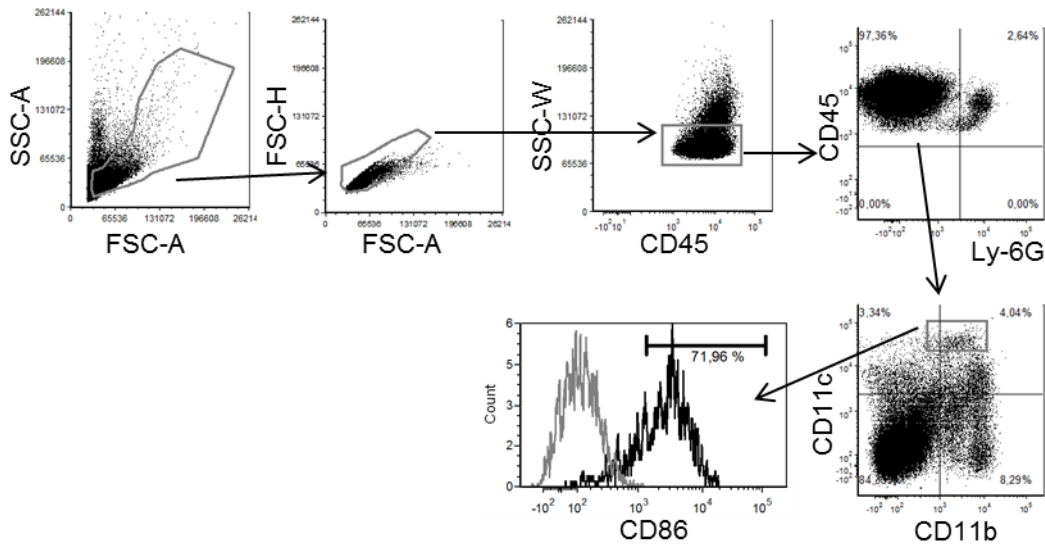


1 **SUPPORTING INFORMATION**

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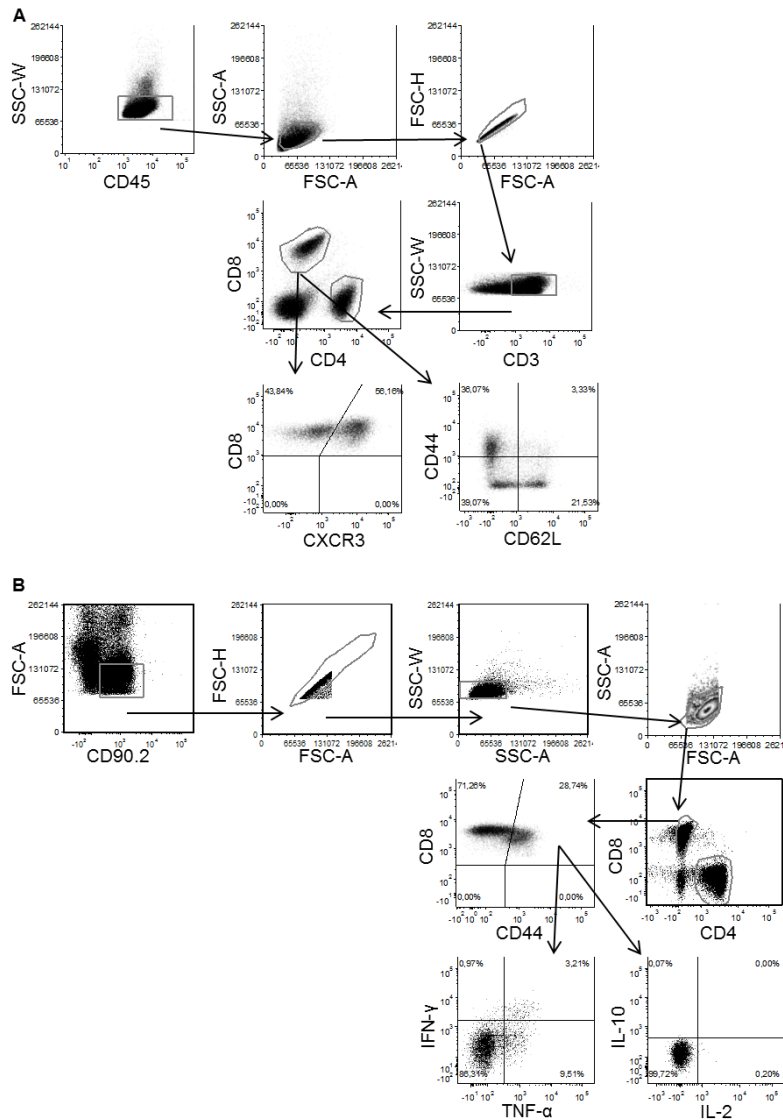
7 **Supp. Fig. A1: Lung and spleen CFU after aerosol infection of mice with *Mtb*.** C57BL/6 mice
8 were infected via the aerosol route with *M.tb* H37Rv, and after 1 and/or 30 days the lung and
9 spleen CFU was determined. Despite small but significant differences in initial uptake of *Mtb*
10 into the lungs between two independent experiments, bacterial numbers in both lung and spleen
11 are comparable on day 30 after infection, the time when mice were challenged with *PbANKA*.
12 Data from two independent experiments are shown (mean \pm SD, n = 5 for each group at each
13 time point). Exp. = experiment.



Supp. Fig. 2: Gating strategy for the analysis of cDCs in the spleen.

Gating strategy for $CD11b^{int}CD11c^{hi}$ cDCs and for the expression of MHC-II and co-stimulatory molecules shown in Figure 4: Cells were stained for CD45, Ly-6G, CD11b, CD11c, MHC-II, CD80 and CD86. FCS-A versus SSC-A, FSC-A versus FSC-C and finally CD45 versus SSC-W were used to determine an accurate $CD45^{+}$ cell population and to exclude doublets. Ly-6G versus CD45 was used to determine a $Ly-6G^{-}$ population. Gating CD11b versus CD11c was used to determine an accurate $CD11c^{hi}$ population which was $CD11b^{int}$. $CD11^{int}CD11c^{hi}$ cells were analyzed for the expression of CD-80, CD86 or MHC-II by plotting a single-parameter histogram and using gate M1 to determine positive cells and mean fluorescence intensity (MFI), respectively. In grey: FMO control. Representative histogram for CD86 is shown.

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66 **Supp. Fig. A3: Gating strategies for the analysis of T cell responses in the spleen.** A) Gating
 67 strategy for CD8⁺ and CD4⁺ T cells, effector memory T cells (CD62L⁻CD44⁺) and for the
 68 expression of CXCR3 shown in Figure 5 A and B: Cells were stained for CD45, CD3, CD4,
 69 CD8, CD44, CD62L and CXCR3. CD45 versus width of SSC was used to determine an accurate
 70 CD45⁺ cell population and to exclude doublets, followed by additional doublet exclusion by
 71 gating the CD45⁺ population in SSC-A versus FSC-A and FSC-H versus FSC-a. SSC-W versus
 72 CD3 was used to determine an accurate CD3⁺ cell population. CD8 or CD4 cells were gated and

73 single and co-expression of CD62L and CD44 was determined to distinguish between specific
74 cell phenotypes. Plotting CXCR3 versus CD8 or CD4 was used to determine an accurate CD8 or
75 CD4 and CXCR3 co-expressing cell population. Where representative scatter plots show CD8
76 gating, CD4⁺ cells were analyzed accordingly.

77 B) Gating strategy for analysis of T cell cytokine production shown in Figure 4 C and D: Cells
78 were stained for CD90.2, CD4, CD8, CD44, IFN- γ , TNF- α , IL-10 and IL-2. Area of FSC versus
79 CD90.2 was used to define a CD90.2⁺ cell population which was subjected three different gatings
80 for doublet exclusion. Gating CD8 versus CD4 was used to determine an accurate CD8⁺ and
81 CD4⁺ cell population. CD44⁺ cells were gated and assessed for their production of IFN- γ , TNF- α ,
82 IL-10 or IL-2. Where representative scatter plots show CD8 gating, CD4⁺ cells were assessed
83 accordingly.

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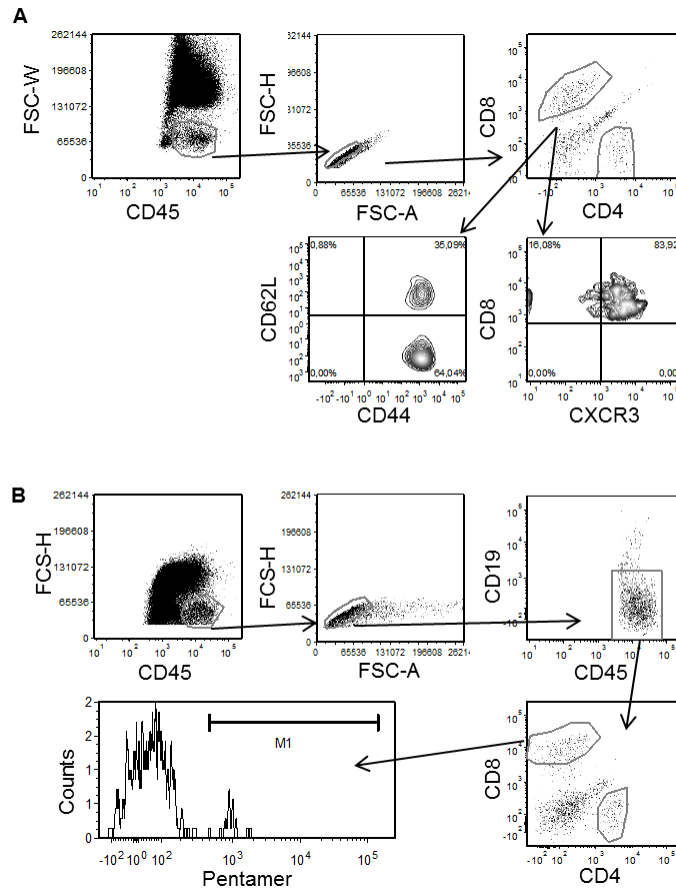
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Supp. Fig. A4: Gating strategy for the sequestration of T cells in the brain. A) Gating strategy for CD8⁺ and CD4⁺ T cells, effector memory T cells (CD62L⁻CD44⁺) and for the expression of CXCR3 shown in Figure 6 A and B: Cells were stained for CD45, CD4, CD8, CD44, CD62L and CXCR3. CD45 versus width of SSC was used to determine an accurate CD45⁺ cell population and to exclude doublets, followed by additional doublet exclusion by gating the CD45⁺ population in FSC-H versus FSC-A. Gating CD8 versus CD4 was used to determine an accurate CD8⁺ and CD4⁺ cell population. Single and co-expression of CD62L and CD44 was determined on CD8⁺ or CD4⁺ cells. Plotting CXCR3 versus CD8 or CD4 was used to determine an accurate CD8 or CD4 and CXCR3 co-expressing cell population. Where representative scatter plots show CD8 gating, CD4⁺ cells were analyzed accordingly. B) Gating

120 strategy for antigen-specific CD8⁺ T cells as shown in Figure 5 C: Cells were stained for CD45,
121 CD19, CD4, CD8 and MHC Class I Ova. CD45 versus width of SSC was used to determine an
122 accurate CD45⁺ cell population and to exclude doublets, followed by additional doublet exclusion
123 by gating the CD45⁺ population in FSC-H versus FSC-A. CD19 versus CD45 was used to
124 determine an accurate CD45⁺ CD19⁻ cell population to exclude unspecific binding of the
125 pentamer. Gating CD8 versus CD4 was used to determine an accurate CD8⁺ and CD4⁺ cell
126 population. CD8⁺ cells were analyzed for pentamer binding by plotting a single-parameter
127 histogram and using gate M1 to determine pentamer positive cells.

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