Cell Reports, Volume *43*

Supplemental information

CpsA mediates infection of recruited lung myeloid cells by *Mycobacterium tuberculosis*

Steven J. Grigsby, G.V.R. Krishna Prasad, Joshua B. Wallach, Ekansh Mittal, Fong-Fu Hsu, Dirk Schnappinger, and Jennifer A. Philips

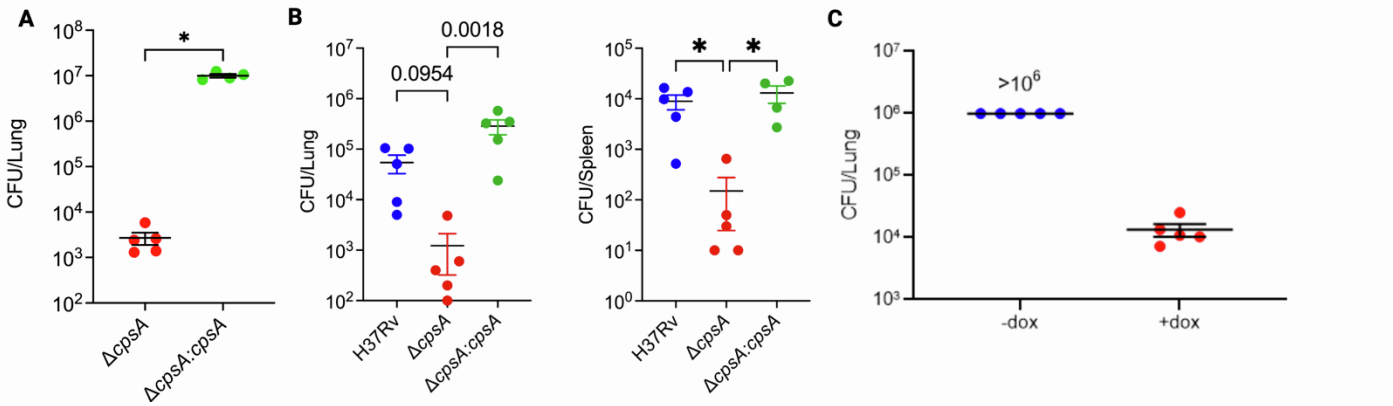

Figure S1. Complementation of *cpsA* under its native promoter or tet-regulated expression rescues the growth defect of *ΔcpsA* mutant, Related to Figure 1 and Figure 2

(A) Lung CFU 3 wpi from mice infected with ~200 CFU of the indicated strain of Mtb by aerosol. (B) Lung and spleen CFU 4 wpi from mice infected with ~30 CFU of the indicated strain by aerosol. (C) Lung CFU from mice infected with ~100 CFU of CpsA TetOFF Mtb. Mice were fed normal chow or chow containing doxycycline starting immediately post-infection, and CFU were enumerated 2 wpi. CFU for -dox group are approximate because colonies were too numerous to count. Each data point represents one mouse. Error bars indicate mean +/- SEM. ns: not significant; * $p<0.05$. Mann-Whitney test (AC); Kruskal-Wallis (B).

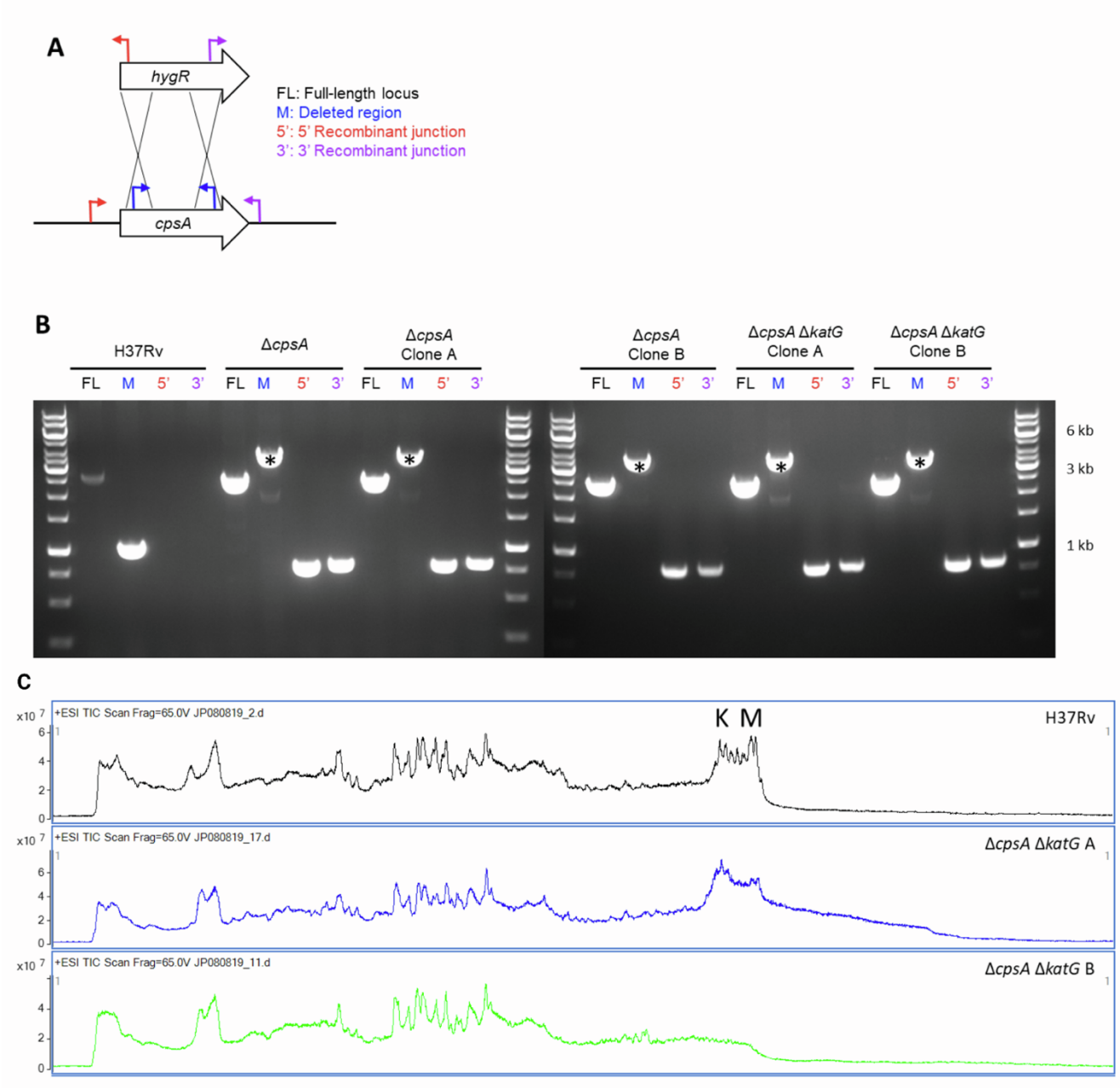

Figure S2. Construction and verification of Δ*cpsA* Δ*katG* mutant, Related to Figure 1

(A) Cloning strategy illustrating replacement of *cpsA* with a *hygR* marker in the Δ*katG* Mtb strain to generate a double mutant and the location of primers used for PCR screening of clones. (B) Gel electrophoresis of PCR products using primer pairs shown in A. (C) HPLC chromatogram of whole-cell lipid extract with marked peaks corresponding to keto- (K) and methoxy- (M) PDIM species. Δ*cpsA* Δ*katG* clone A was used for subsequent experiments. (*) nonspecific bands.

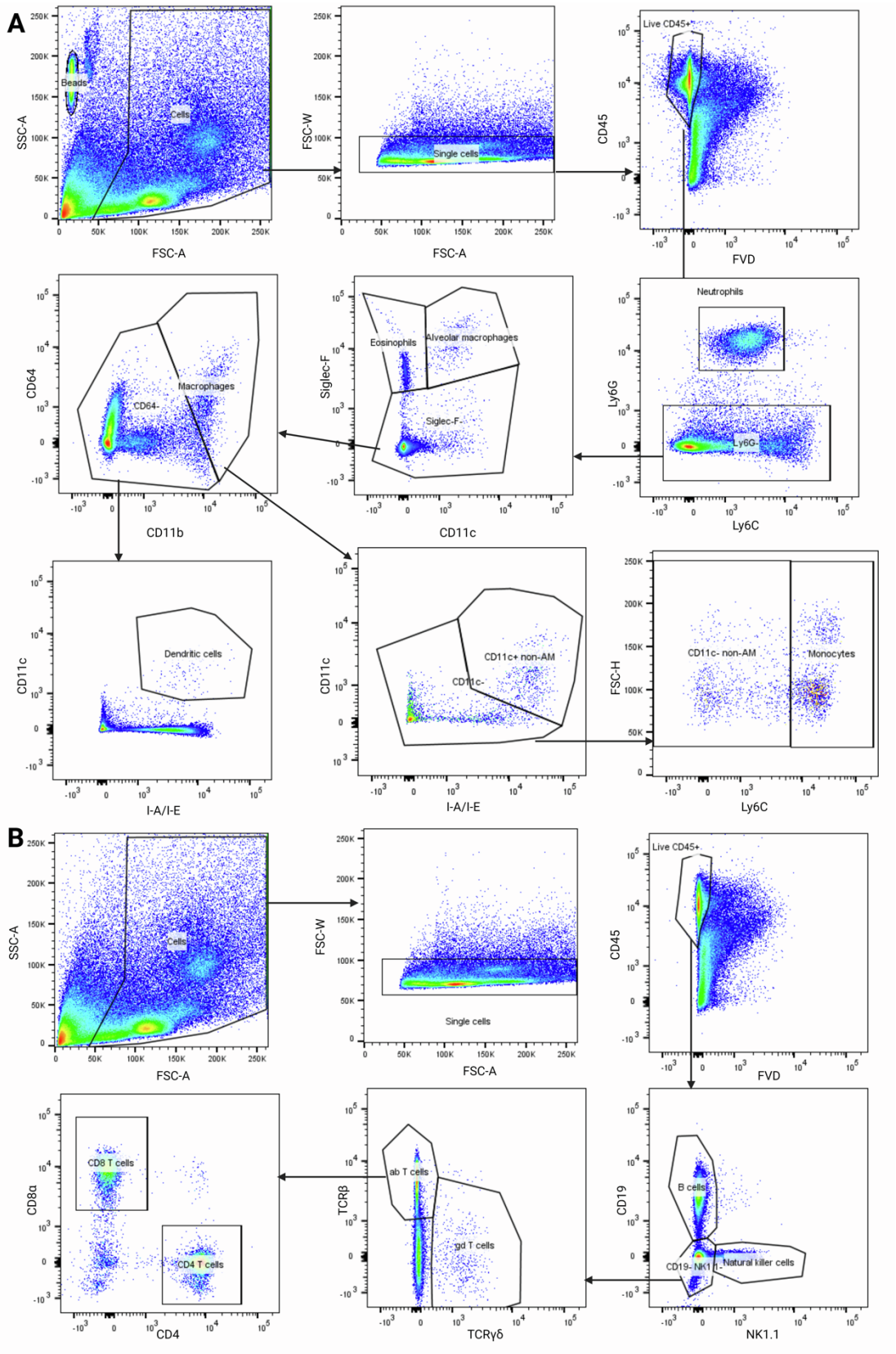

Figure S3. Gating strategy for lung cells of Mtb-infected mice, Related to Figures 3-7

Flow cytometry plots of lung cells from a naive C57BL/6J mouse to illustrate gating strategy using myeloid (A) and lymphoid (B) marker panels. FSC-A: forward scatter (area); SSC-A: side scatter (area); FSC-W: forward scatter (width); FVD: fixable viability dye.

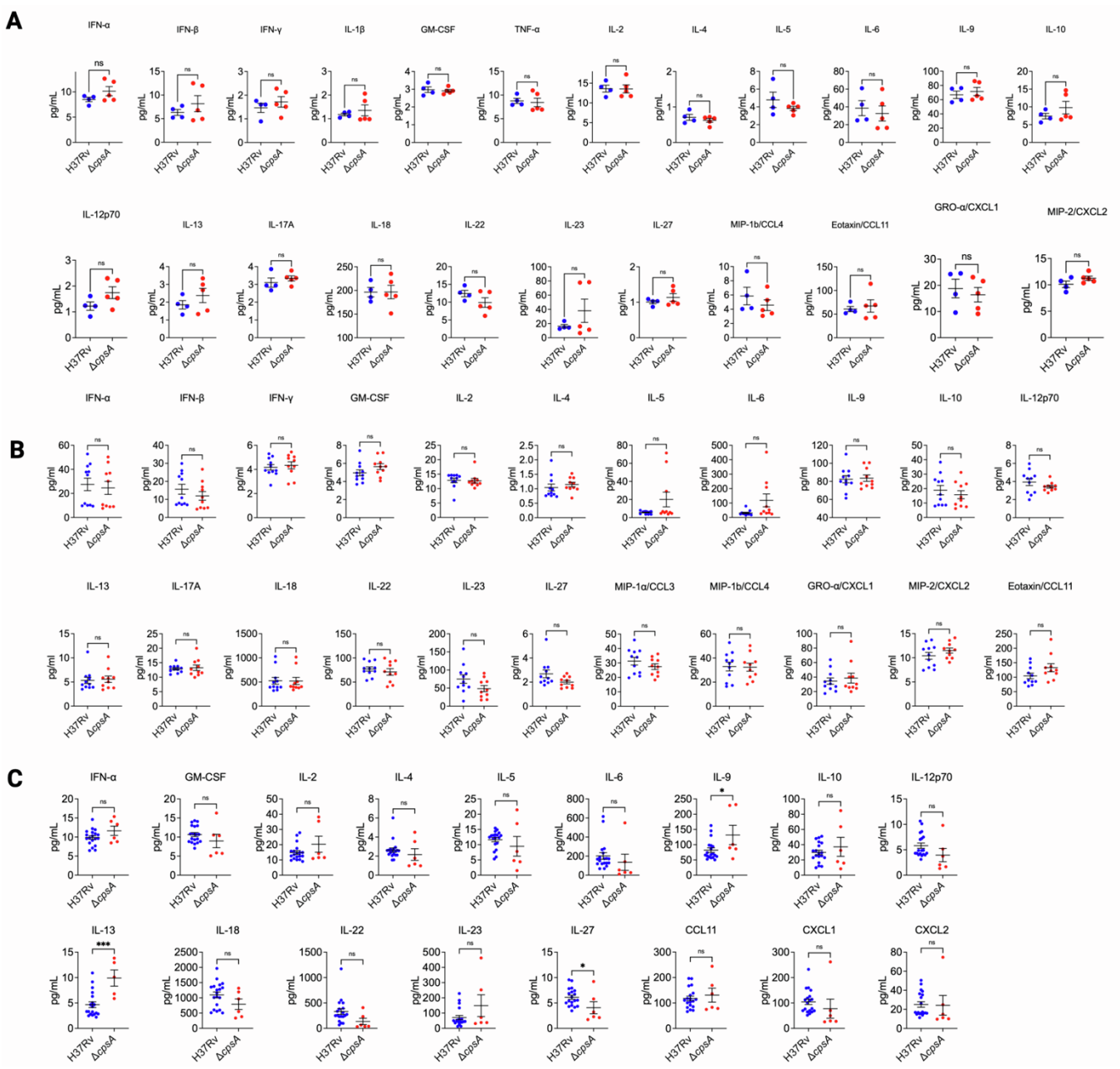

Figure S4. Cytokine responses to Δ*cpsA* Mtb infection, Related to Figure 3 and Figure 5

Cytokines and chemokines were measured by Luminex from whole-lung homogenate from mice infected with 100 CFU of H37Rv or Δ*cpsA* Mtb for 2 weeks (A), 6 weeks (B), or from mice infected with H37Rv (~1500 CFU) or Δ*cpsA* Mtb (~7000 CFU) for 3 weeks (C). (A-C) Data consists of 4-5 mice per group from 1 experiment (A), 10-11 mice from 2 independent experiments (B), or 6-16 mice per group from at least 2 independent experiments (C). Each data point corresponds to one mouse. Error bars indicate mean +/- SEM. ns: not significant; *p<0.05; ***p<0.001. Student's t test.

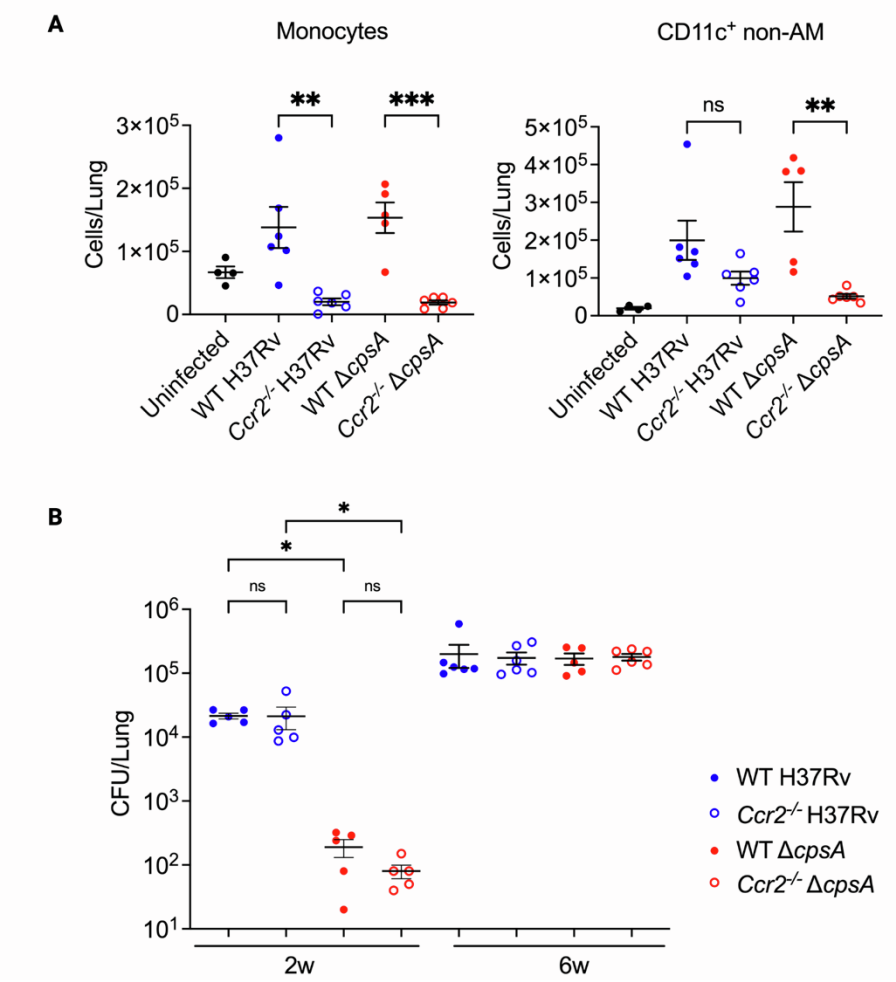

Figure S5. Recovery of Δ*cpsA* Mtb does not depend on *Ccr2*, Related to Figure 3

WT or *Ccr2*$^{-/-}$ C57BL/6J mice were infected with ~100 CFU H37Rv Mtb or Δ*cpsA* Mtb and lungs were harvested 2 and 6 wpi for CFU and flow cytometry. (A) Absolute cell counts of monocytes and CD11c+ non-AMs from uninfected and infected mice at 6 wpi. (B) Lung CFU from infected mice at 2 and 6 wpi. Each data point represents one mouse. Data consists of 4-6 mice per group from 2 independent experiments. Error bars indicate SEM. ns: not significant; * $p<0.05$; ** $p<0.01$; *** $p< 0.001$. One-Way ANOVA with Tukey's multiple comparisons test.

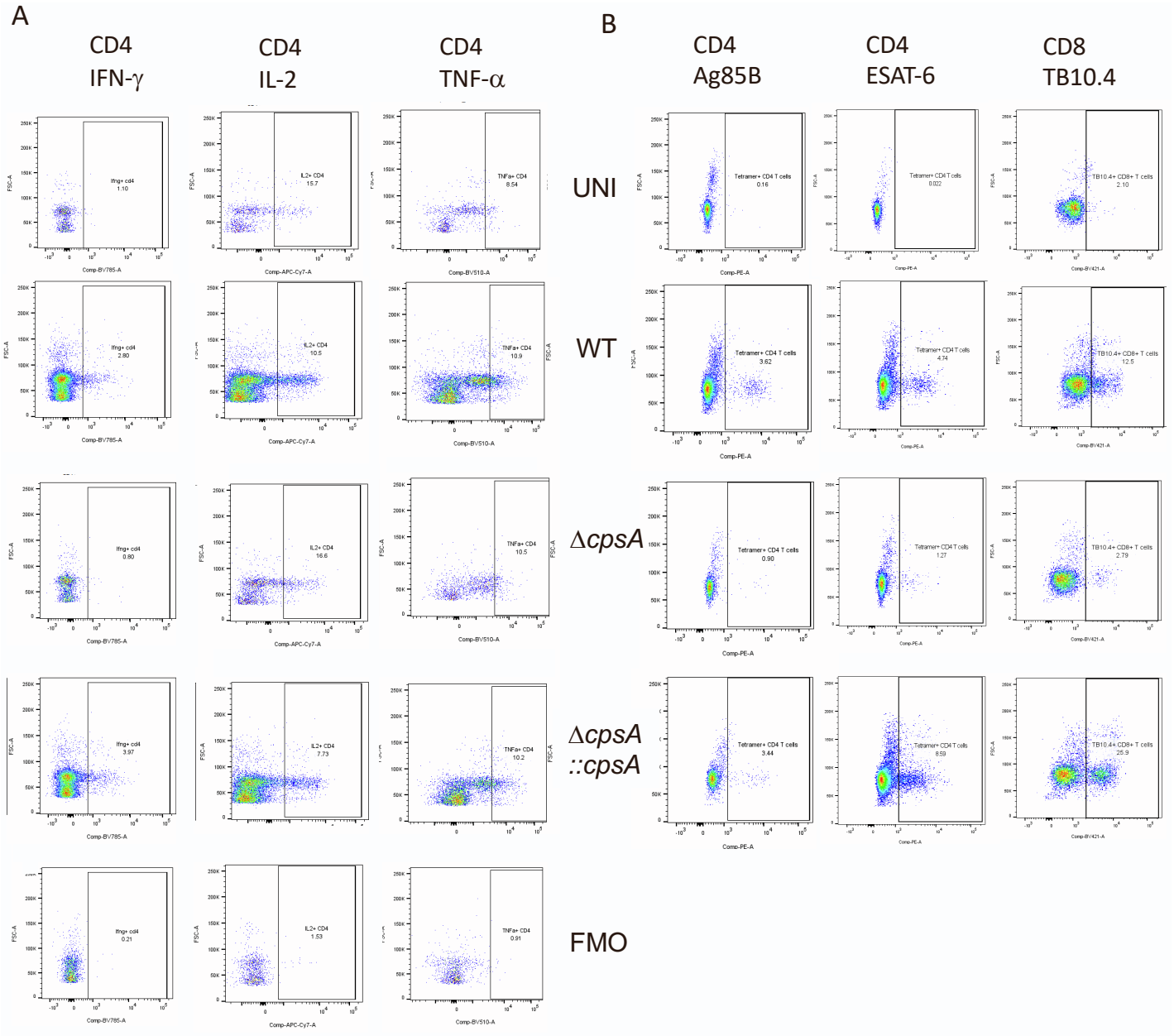

Figure S6. Flow cytometry plots showing intracellular cytokine and tetramer staining, Related to Figure 4

Examples of flow cytometry plots of CD4 or CD8 T cells from uninfected mice and mice infected with WT Mtb, Δ*cpsA* Mtb, and Δ*cpsA::cpsA* Mtb showing (A) intracellular IFN-γ, IL-2, and TNF-α staining, and (B) Ag85B, ESAT-6 and TB10.4 tetramer staining. FSC-A: forward scatter (area); UNI- uninfected; FMO: fluorescence minus one controls.

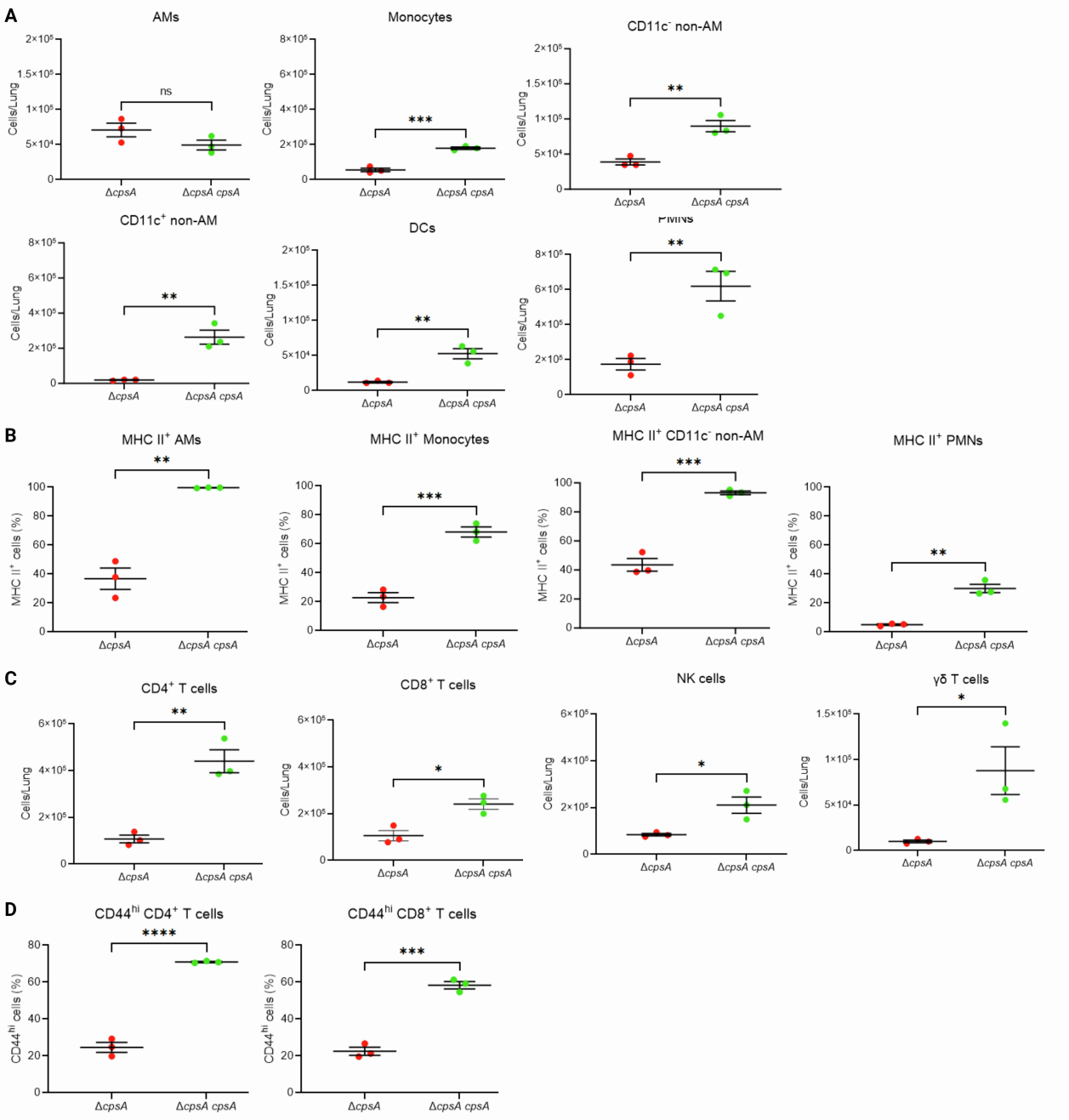

Figure S7. Complementation of Δ*cpsA* Mtb rescues the inflammatory response, Related to Figure 5

(**A-D**) WT C57BL/6J mice were infected with ~9000 CFU Δ*cpsA* Mtb or 300 CFU Δ*cpsA::cpsA* Mtb by aerosol, and lungs were harvested 3 wpi for flow cytometry.(A) Absolute cell counts of myeloid cells. (B) MHC II expression expressed as the percentage positivity of the indicated cell population. (C) Absolute counts of lymphoid cells. (D) CD44 expression in CD4 and CD8 T cells. Data consists of 3 mice per group from one experiment. Each data point corresponds to one mouse. Error bars indicate SEM. ns not significant; * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001.Student's t test.

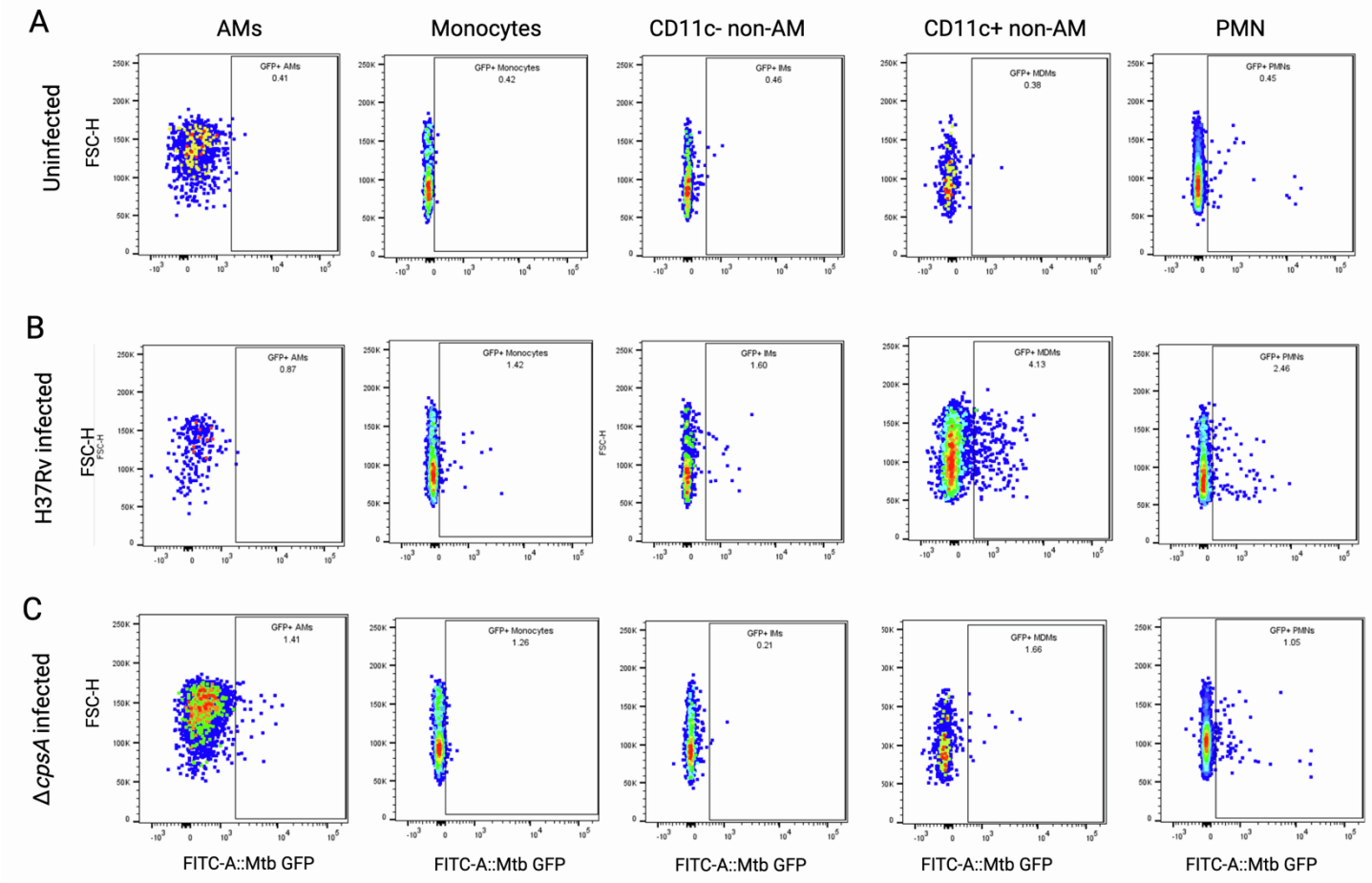

Figure S8. Gating strategy to identify infected cells, Related to Figure 6 and Figure 7.

Lung myeloid cells from mice that were (**A**) uninfected, (**B**) WT Mtb infected, and (**C**) Δ*cpsA* Mtb infected for 3 weeks prior to euthanasia. Representative flow cytometry plots demonstrate how infected myeloid cells were identified. FSC-H: forward scatter height