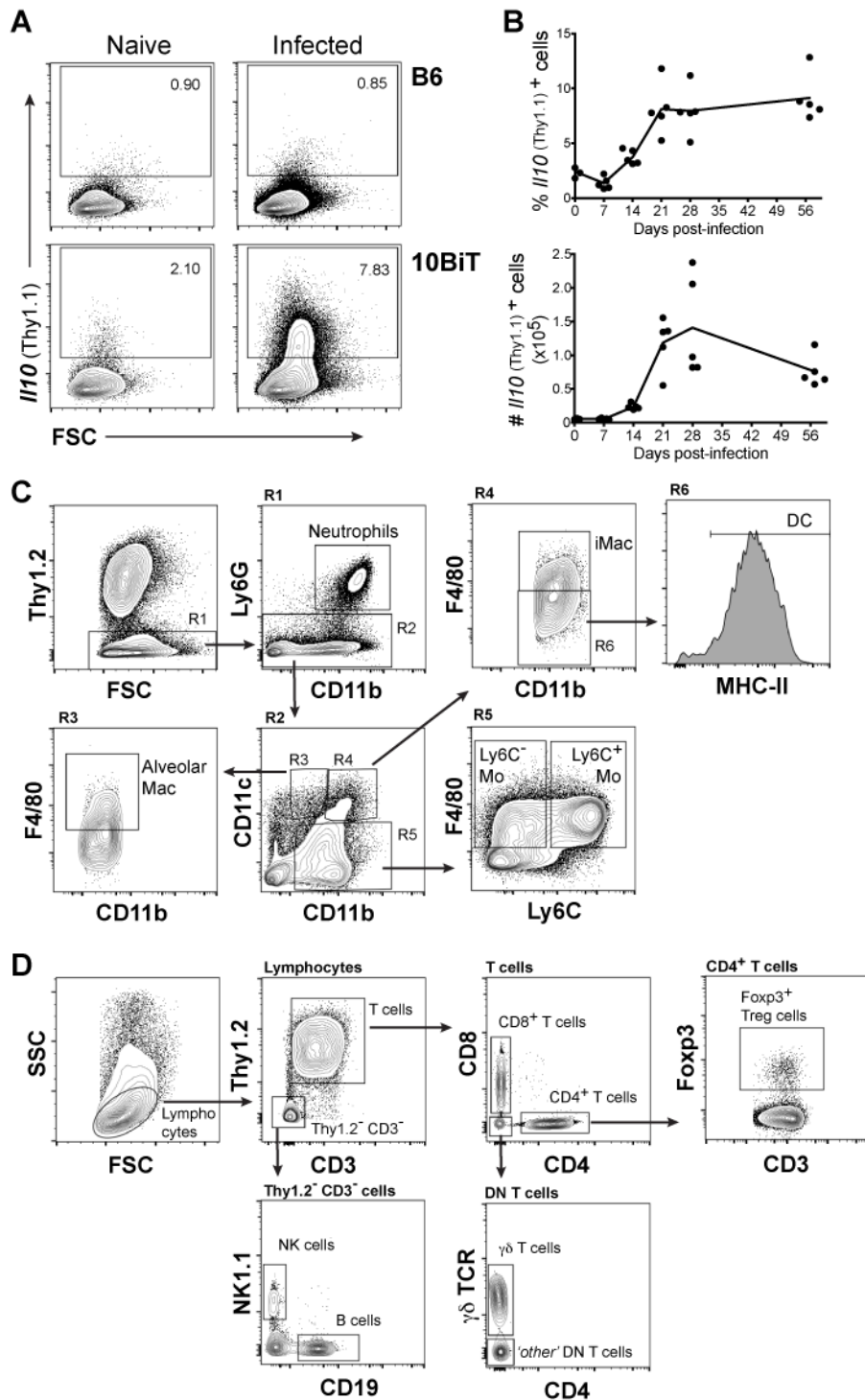


Supplemental Information

**T cell-derived IL-10 impairs host resistance to *Mycobacterium tuberculosis* infection**

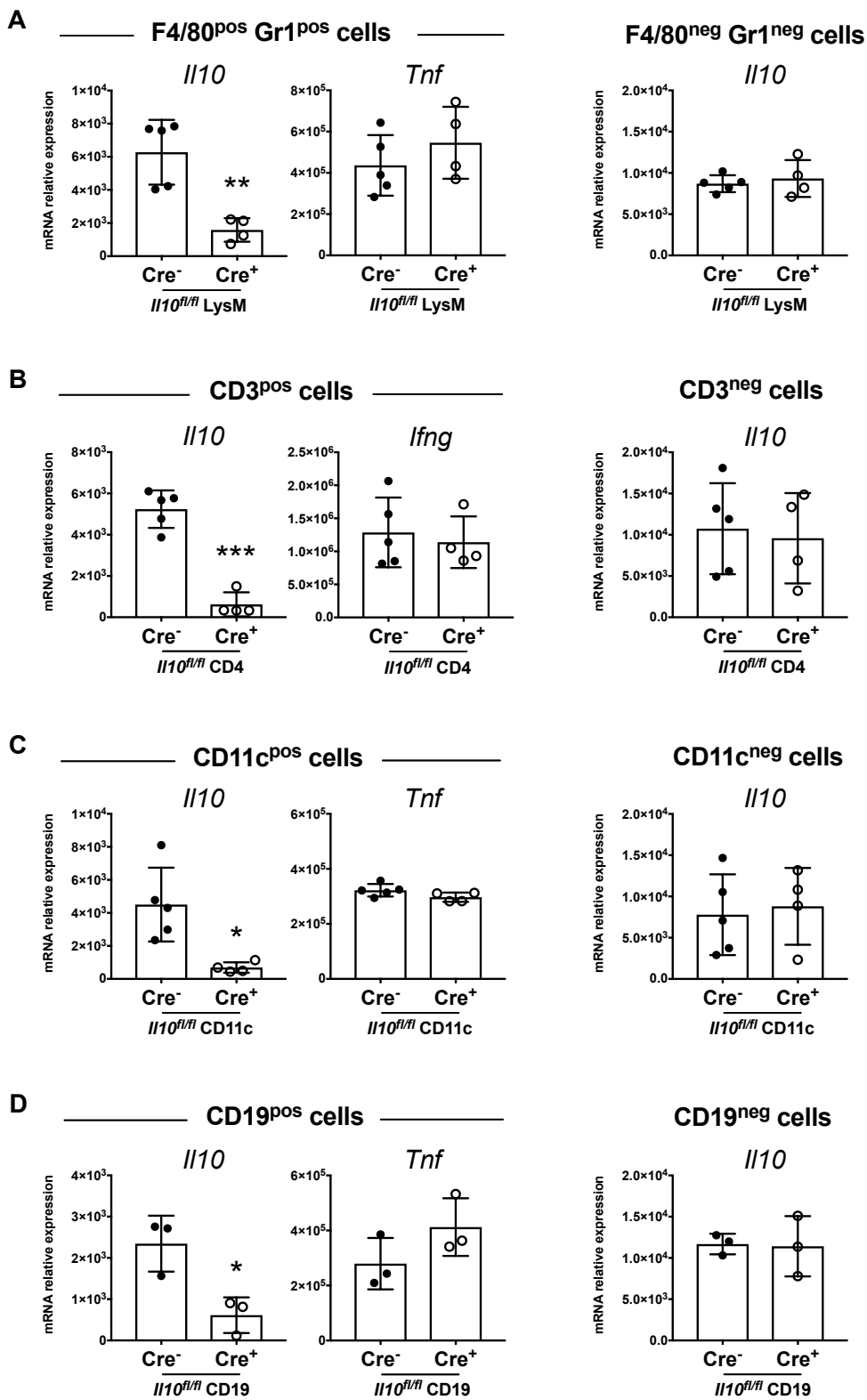
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<sup>1</sup>LMT and PSR contributed equally to this work



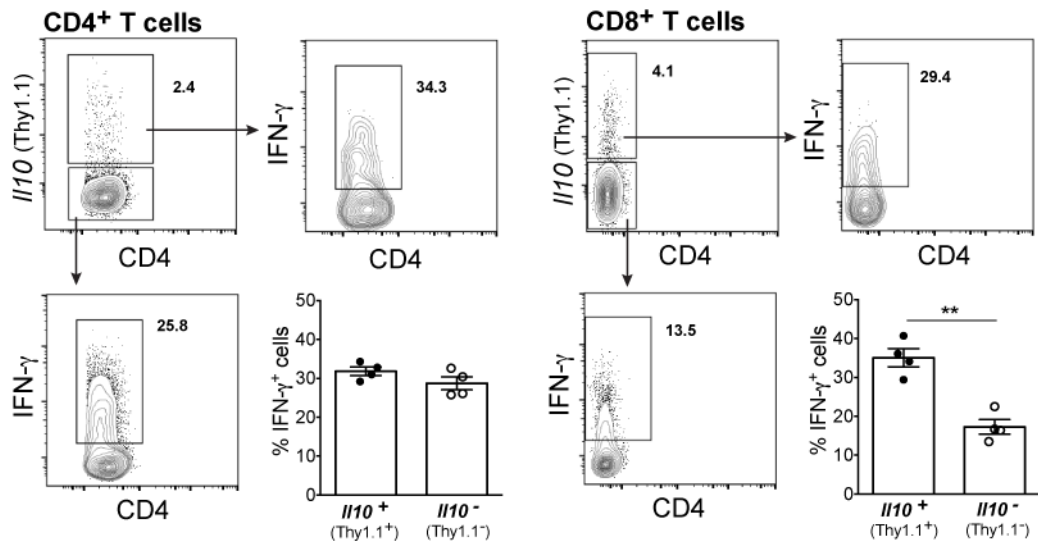
**Supplemental Figure 1**

**Supplemental Figure 1.** *Kinetic of lung IL-10-expressing cells during M. tuberculosis infection.* WT and/or 10BiT IL-10 reporter mice were infected with *M. tuberculosis* HN878. At specific days post-infection, lung cell suspensions were prepared and stained as described in Materials and Methods to detect *Il10* (Thy1.1<sup>+</sup>) expressing cells. **(A)** The gating strategy after exclusion of dead cells and duplets is shown for one representative lung from naïve or infected B6 and 10BiT mice. **(B)** The percentage (top panel) and the total number (bottom panel) of total *Il10* (Thy1.1) expressing cells in the lungs at indicated time-points are shown as mean ± SEM. Results are representative of three independent experiments with individual data points depicting individual mice (3-5 mice/time-point/experiment). **(C-F)** Gating strategy for analysis of myeloid **(C)** and lymphoid cells **(D)** in *M. tuberculosis* infected lungs. Plots show concatenated data of 5 lungs at day 28 post-infection from one representative experiment. Myeloid cells: Neutrophils (Thy1.2<sup>-</sup> CD11b<sup>+</sup> Ly6G<sup>+</sup>), Alveolar Macrophages (Thy1.2<sup>-</sup> Ly6G<sup>-</sup> CD11b<sup>int</sup> CD11c<sup>+</sup> F4/80<sup>+</sup>), Interstitial Macrophages (iMac) (Thy1.2<sup>-</sup> Ly6G<sup>-</sup> CD11b<sup>+</sup> CD11c<sup>+</sup> F4/80<sup>+</sup>), DC (Thy1.2<sup>-</sup> Ly6G<sup>-</sup> CD11b<sup>+</sup> CD11c<sup>+</sup> F4/80<sup>-</sup> MHC-II<sup>int/high</sup>) and Ly6C<sup>+</sup> or Ly6C<sup>-</sup> Monocytes (Mo) (Thy1.2<sup>-</sup> Ly6G<sup>-</sup> CD11b<sup>+</sup> CD11c<sup>-</sup> F4/80<sup>+</sup> Ly6C<sup>+</sup> or Ly6C<sup>-</sup>). Lymphoid cells: T cells (Thy1.2<sup>+</sup> CD3<sup>+</sup>), NK cells (Thy1.2<sup>-</sup> CD3<sup>-</sup> CD19<sup>-</sup> NK1.1<sup>+</sup>) and B cells (Thy1.2<sup>-</sup> CD3<sup>-</sup> CD19<sup>+</sup> NK1.1<sup>-</sup>). T cells subsets: CD4<sup>+</sup> T cells (Thy1.2<sup>+</sup> CD3<sup>+</sup> CD4<sup>+</sup> CD8<sup>-</sup>), CD8<sup>+</sup> T cells (Thy1.2<sup>+</sup> CD3<sup>+</sup> CD4<sup>-</sup> CD8<sup>+</sup>), gd T cells (Thy1.2<sup>+</sup> CD3<sup>+</sup> CD4<sup>-</sup> CD8<sup>-</sup> gdTCR<sup>+</sup>), 'other' DN T cells (Thy1.2<sup>+</sup> CD3<sup>+</sup> CD4<sup>-</sup> CD8<sup>-</sup> gdTCR<sup>-</sup>) and Foxp3<sup>+</sup> Treg cells (Thy1.2<sup>+</sup> CD3<sup>+</sup> CD4<sup>+</sup> CD8<sup>-</sup> Foxp3<sup>+</sup>).



Supplemental Figure 2

**Supplemental Figure 2.** *Cell type-specific deletion of *Il10* gene in *Il10<sup>fl/fl</sup>-Cre* mice .* *Il10<sup>fl/fl</sup> LysM-Cre<sup>+</sup>, Il10<sup>fl/fl</sup> CD4-Cre<sup>+</sup>, Il10<sup>fl/fl</sup> CD11c-Cre<sup>+</sup>, Il10<sup>fl/fl</sup> CD19-Cre<sup>+</sup>* mice and respective Cre- control mice were infected with *M. tuberculosis* HN878. At day 28 post-infection, lung cells from infected mice were isolated. (A) Lung cell suspensions from *Il10<sup>fl/fl</sup> LysM-Cre<sup>+</sup>* and Cre<sup>-</sup> mice were incubated with anti-F4/80 (REA12) and anti-Gr1 (RB6-8C5) biotin Abs. (B) Lung cell suspensions from *Il10<sup>fl/fl</sup> CD4-Cre<sup>+</sup>* and Cre<sup>-</sup> mice were incubated with anti-CD3 (17A2) biotin Ab. (C) Lung cell suspensions from *Il10<sup>fl/fl</sup> CD11c-Cre<sup>+</sup>* and Cre<sup>-</sup> mice were incubated with anti-CD11c (N418) biotin Ab. (D) Lung cell suspensions from *Il10<sup>fl/fl</sup> CD19-Cre<sup>+</sup>* and Cre<sup>-</sup> mice were incubated with anti-CD19 (6D5) biotin Ab. Cells were then incubated with anti-Biotin magnetic beads (Miltenyi Biotec) and positive and negative cell fractions sorted according to manufacturer's instructions. *Il10* and *Tnf* or *Ifng* mRNA expression was analyzed by quantitative RT-PCR and normalized against *Hprt1* mRNA levels. Data are shown as mean  $\pm$  SD of 3-5 mice per group. Differences were tested for significance by an unpaired *t* Test (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ). As seen in the figure, the levels of *Il10* mRNA were significantly reduced in the respective positive cells isolated from each of the above Cre<sup>+</sup> mice compared to Cre<sup>-</sup> mice, while similar levels of *Il10* mRNA were detected in the negative cell fractions isolated from Cre<sup>+</sup> mice and respective Cre<sup>-</sup> control mice. Similar levels of other cytokines such as *Tnf* (for *Il10<sup>fl/fl</sup> LysM-Cre<sup>+</sup>*; *Il10<sup>fl/fl</sup> CD11c-Cre<sup>+</sup>*; *Il10<sup>fl/fl</sup> CD19-Cre<sup>+</sup>*) or *Ifng* (for *Il10<sup>fl/fl</sup> CD4-Cre<sup>+</sup>*) were detected in positive cells isolated from Cre<sup>+</sup> and Cre<sup>-</sup> mice. These findings demonstrate the selective deletion of *Il10* in F4/80<sup>+</sup> and Gr1<sup>+</sup> cells (macrophages, monocytes and neutrophils) in *Il10<sup>fl/fl</sup> LysM-Cre<sup>+</sup>* mice; CD11c<sup>+</sup> cells (mostly DC and macrophages) in *Il10<sup>fl/fl</sup> CD11c-Cre<sup>+</sup>* mice; CD3<sup>+</sup> cells (T cells) in *Il10<sup>fl/fl</sup> CD4-Cre<sup>+</sup>* mice; or CD19<sup>+</sup> cells (B cells) in *Il10<sup>fl/fl</sup> CD19-Cre<sup>+</sup>* mice; validating the cell-type specific deletion of *Il10* in the conditional knockout mice.



**Supplemental Figure 3.** *IL-10-expressing T cells from M. tuberculosis infected lungs co-produce IFN- $\gamma$  upon polyclonal stimulation.* 10BiT IL-10 reporter mice were infected with *M. tuberculosis* HN878. At day 28 post-infection, lung cell suspensions were prepared and restimulated *ex vivo* with PMA (50ng/ml) plus ionomycin (500ng/ml) and Brefeldin A (10 $\mu$ g/ml) for 4h. Production of IFN- $\gamma$  by *Il10*<sup>+</sup> (Thy1.1<sup>+</sup>) or *Il10*<sup>-</sup> (Thy1.1<sup>-</sup>) cells among CD4<sup>+</sup> (left panel) or CD8<sup>+</sup> (right panel) T cells was determined by intracellular staining. Plots show concatenated data of 4 lungs from one representative experiment out of two independent experiments (4-5 mice/ experiments). The percentage of IFN- $\gamma$  expressing cells among *Il10*<sup>+</sup> (Thy1.1<sup>+</sup>) or *Il10*<sup>-</sup> (Thy1.1<sup>-</sup>) cells is shown as mean  $\pm$  SEM, with individual data points depicting individual mice. Differences were tested for significance by an unpaired Students *t* Test (\*\*,  $p < 0.01$ ).