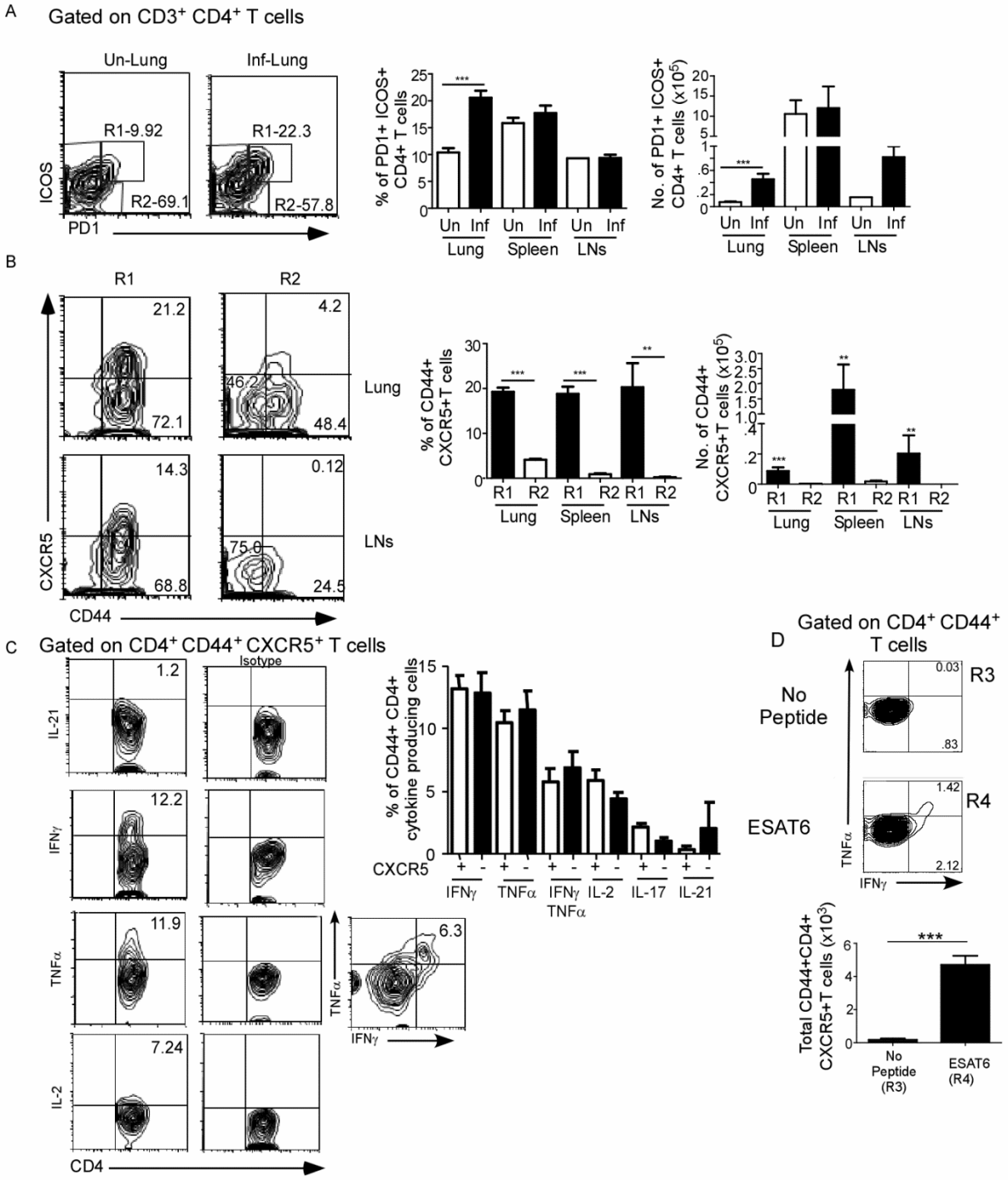

Figure S1: Pulmonary CD4+ CXCR5+ T cells produce proinflammatory cytokines. On day 25 post infection, organs from uninfected (Un) and infected (Inf) B6 mice were assessed for the percentage or number of ICOS+ PD1+ within CD4+ T cells by flow cytometry (A). The expression of CD44 and CXCR5 and number of CD44+ CXCR5+ T cells within ICOS+PD1+ gate (R1) or ICOS-PD1- gate (R2) was calculated (B). The percentage of cytokine producing cells within CD44hi CXCR5+ and CD44hi CXCR5- was determined after stimulation with PMA/Ionomycin for 5 hours followed by intracellular staining and flow cytometry (C). A typical contour plot showing cytokine specific staining (left panel) within activated CD4+ CXCR5+ T cells and relevant isotype control antibody (right panel) shown. Cells from day 25 Mtb-infected lungs were stimulated with ESAT6 peptide and expression of CXCR5 was determined on CD44 hi IFNγ+TNFα+ cells (D). The data points represent the mean (±SD) of values from 4-6 mice. (A-D). **p=0.005, ***p=0.0005. One experiment representative of two.

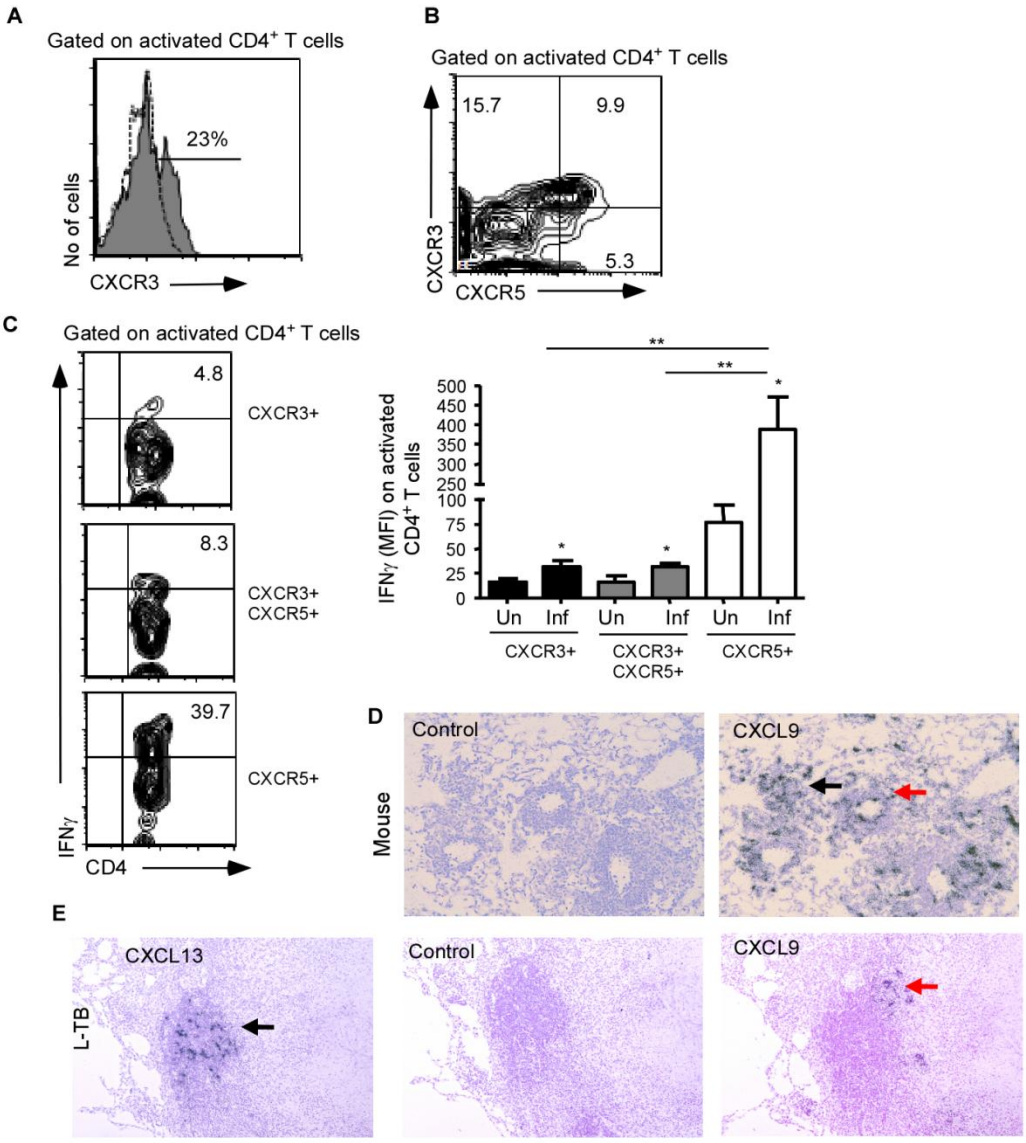

Figure S2: CD4+ CXCR5+ T cells also coexpress the chemokine receptor, CXCR3. B6 mice were infected as in Figure 3 and lungs were harvested on day 25 post infection. The expression of CXCR3 (A), CXCR3 and CXCR5 (B) on activated CD4+ cells was determined by flow cytometry. A typical histogram with CXCR3 specific staining (filled) and relevant isotype control antibody within activated CD4+ T cells is shown (open) (A). A typical contour plot showing co-expression of CXCR3 and CXCR5 on activated CD4+ T cells is shown (B). The frequency and mean fluorescent intensity of IFNγ expression within activated CD4+ CXCR5+, CXCR5+CXCR3+ and CXCR3+ T cells was determined in cells stimulated with PMA/Ionomycin for 5 hours followed by intracellular staining and flow cytometry (C). The data points represent the mean (±SD) of values from 4-6 mice. (A-C). Lung FFPE serial sections were analyzed by ISH to determine localization of CXCL9 or CXCL-13 mRNA expression in murine (D) or L-TB NHP (E). Black arrows point to mRNA localization within the lung parenchyma, while red arrows point to mRNA localization near blood vessels. *p=0.05, **p=0.005. Original magnification 100x. One experiment representative of two.

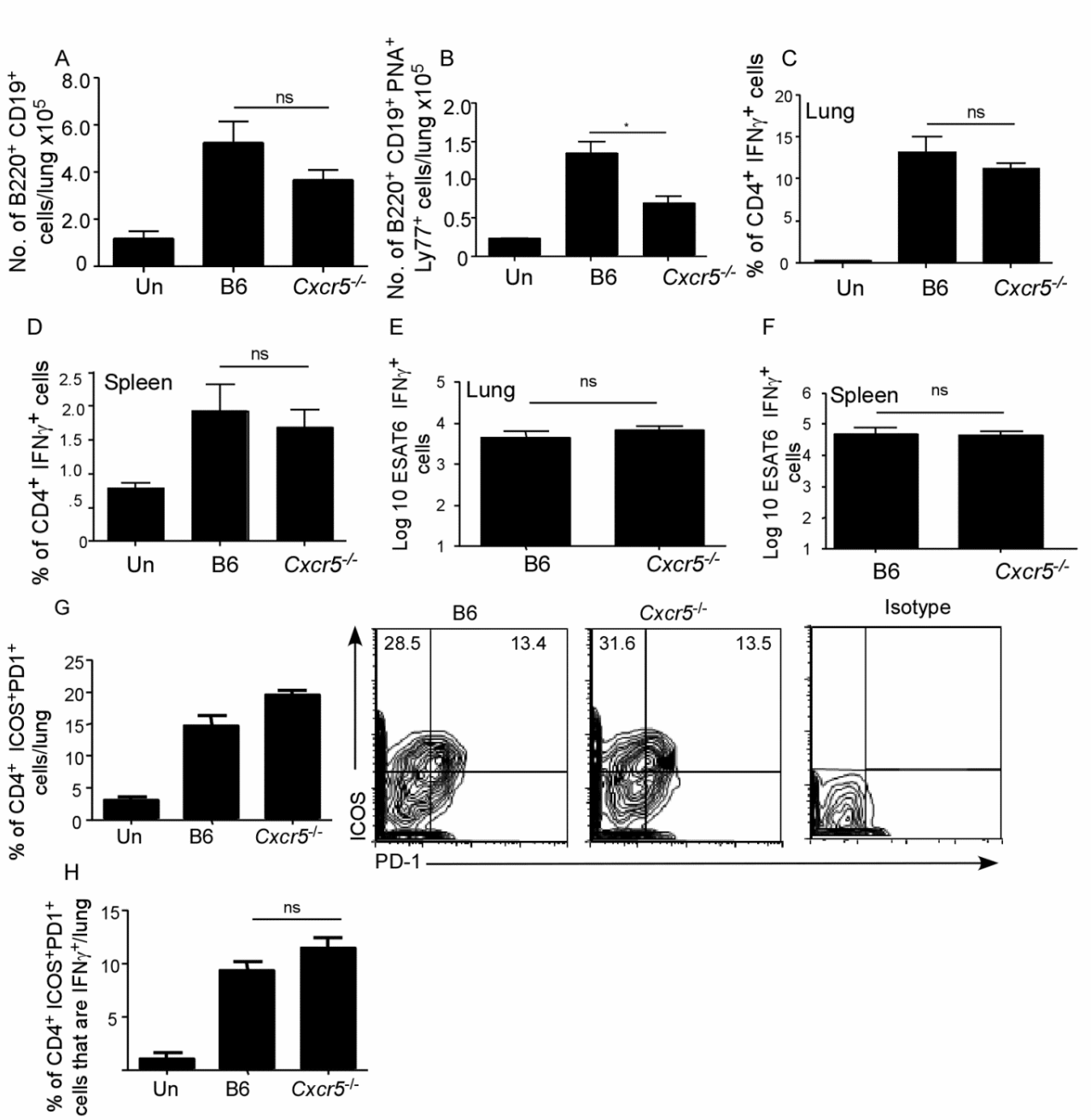

Figure S3: CXCR5 expression is not required for accumulation of cytokine-producing ICOS+ PD1+ T cells during Mtb infection. B6 and Cxcr5-/- mice were infected as in Figure 3. The number of total B cells (B220+ CD19+) (A) or GC B cells (B220+, CD19+, PNA+, and Ly77+) (B) was determined by flow cytometry in B6 and Cxcr5-/- Mtb-infected lungs on day 21 post infection. The total percentage of IFNγ producing CD4+ T cells in the lung (C) and spleen (D) were determined by intracellular staining and flow cytometry after stimulation with PMA/Ionomycin for 5 hours ex vivo. The number of ESAT-6 specific IFNγ–producing CD4+ T cells were determined in the lung (E) and spleen (F) by antigen-driven ELISpot assay. The percentage of CD4+ T cells expressing ICOS and PD1 (G) and CD4+ ICOS+ PD1+ cells expressing IFNγ (H) was determined by intracellular staining with PMA/Ionomycin. The data points represent the mean (±SD) of values from 4-6 mice (A-H). ns-not significant. One representative of two experiments is shown. *p=0.05. ns=not significant.

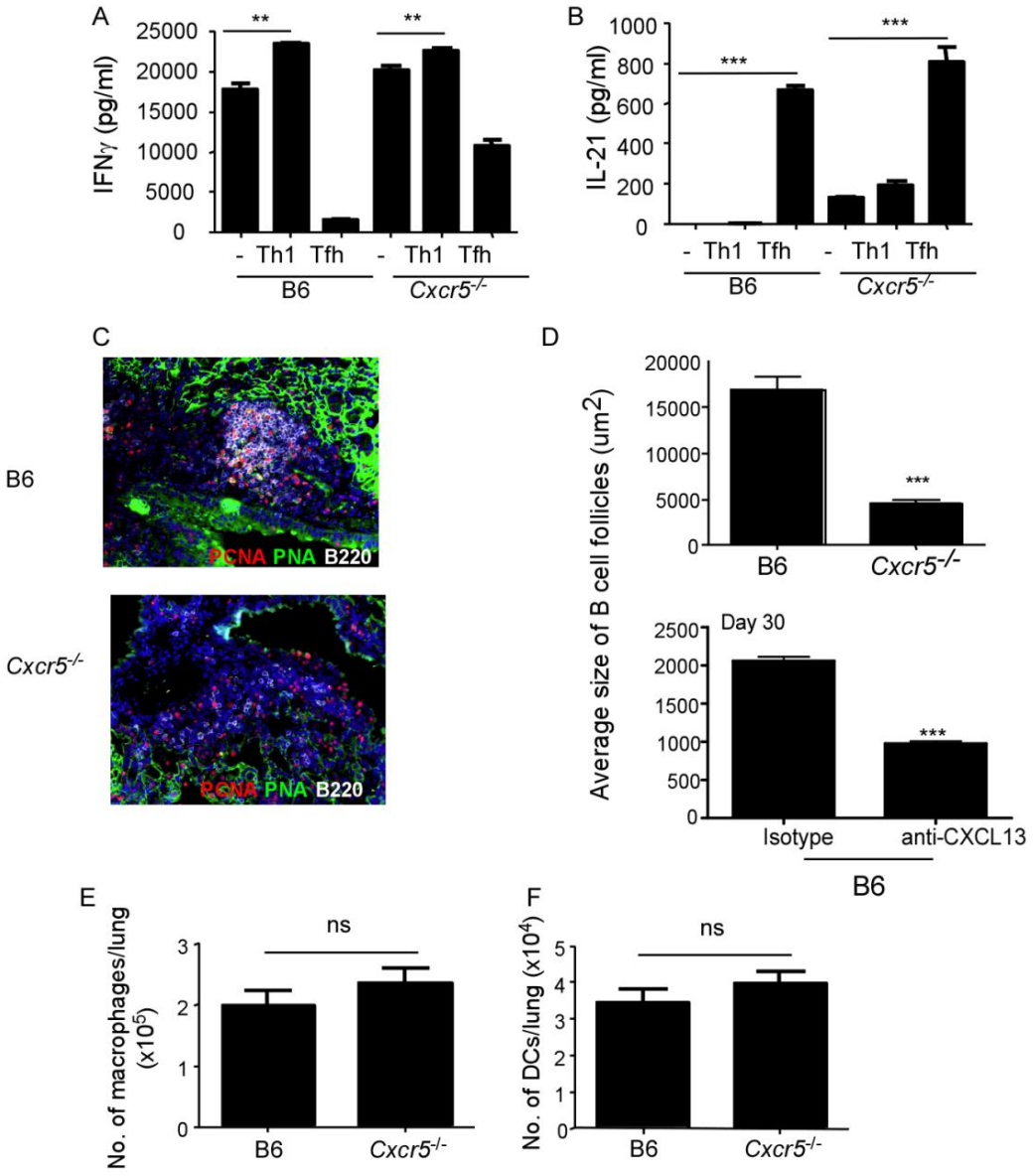

Figure S4: CXCR5 expression is required for lymphoid follicle structure formation during TB. B6 and Cxcr5-/- mice were subcutaneously vaccinated with ESAT6$_{1-20}$ peptide in adjuvant and on day 14 post vaccination, splenic CD4+ T cells were cultured in vitro along with irradiated APCs and antigen under Th1 or Tfh cell differentiation conditions for 6 days as described under methods. At the end of the culture period, the CD4+ T cells were re-stimulated with beads coated with anti-CD3/CD28 for 24 hours and culture supernatants assayed for the production of IFNγ (A) or IL-21 (B) by ELISA. Triplicates samples were assayed for the production of cytokines (A,B). B6 and Cxcr5-/- mice were infected as in Figure 3. B cell lymphoid follicles were detected with PCNA, PNA, and B220 at day50 post infection (C). The average size of B cells follicles was quantified in B6 and Cxcr5-/- Mtb-infected lungs at day 50 post infection and B6 mice treated with isotype or CXCL13 antibody using the Zeiss Axioplan microscope (D). 200X magnification shown. The number of lung macrophages (E) and dendritic cells (F) were determined in B6 and Cxcr5-/- Mtb-infected lungs by flow cytometry on day 21 post infection. The data points represent the mean (±SD) of values from 4-6 mice (C-F). One representative of two experiments is shown. **p=0.005, ***p=0.0005. ns=not significant.

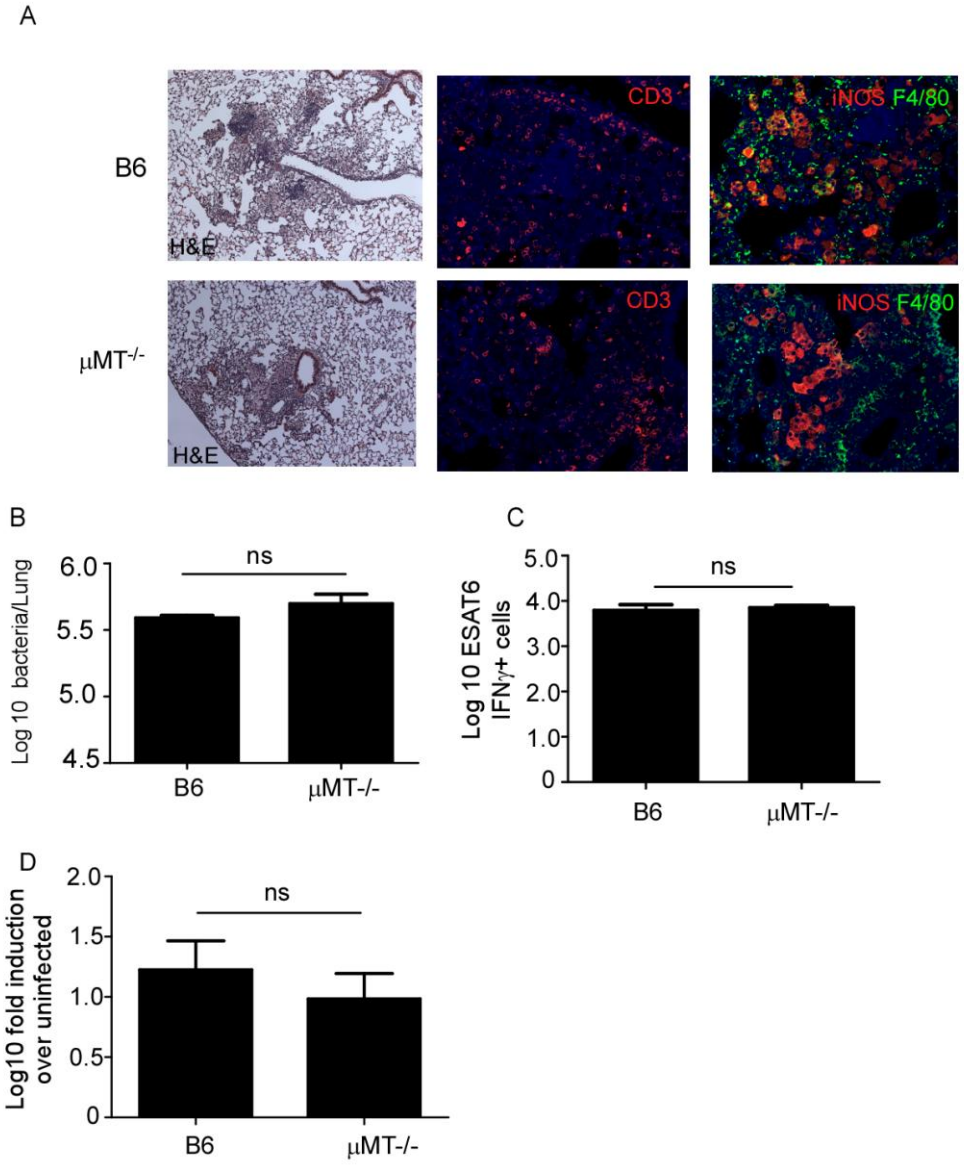

Figure S5: B cell deficient mice localize T cells within the granuloma and control Mtb. B6 and μMT-/- mice were infected as described in Figure 3 and FFPE lung sections were stained with H&E or analyzed using antibodies specific for CD3 and iNOS, F4/80 (A); Representative pictures are shown. 50X magnification-H&E images, 200X magnification-fluorescent images. At day 50 post infection, bacterial burden was determined in the lung (B). The number of ESAT-6 specific IFNγ producing CD4+ T cells were determined in the lung by antigen driven ELISpot assay (C). Log10 fold induction of iNOS mRNA in B6 and μMT-/- Mtb-infected lungs relative to levels in uninfected lungs was determined by RT-PCR (D). The data points represent the mean (±SD) of values from 4-6 mice. (A-D). ns=not significant. One experiment representative of two.

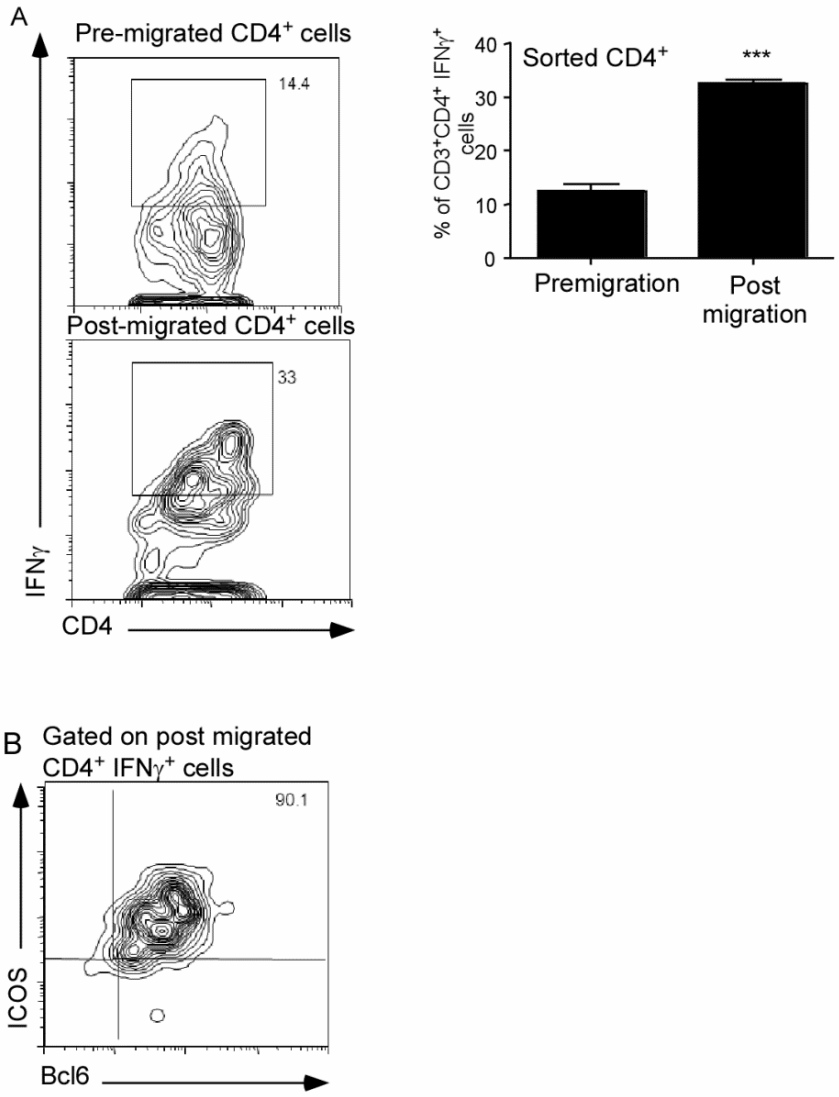

Figure S6: CD4+ T cells responsive to CXCL13 produce IFNγ. CD4+ T cells were sorted from day 21 Mtb-infected B6 lungs and assayed in vitro chemotaxis migration assay towards CXCL13 (500 ng/ml). The ability of CD4+ T cells to produce IFNγ pre- and post-migration was determined by intracellular staining and flow cytometry in PMA/Ionomycin stimulated cells (A). A representative contour plot showing surface expression of Bcl6 and ICOS on CD4+ T cells that was determined by flow cytometry (B). The data points represent the mean (±SD) of values from 3-6 samples (A-B). ***p=0.0005.

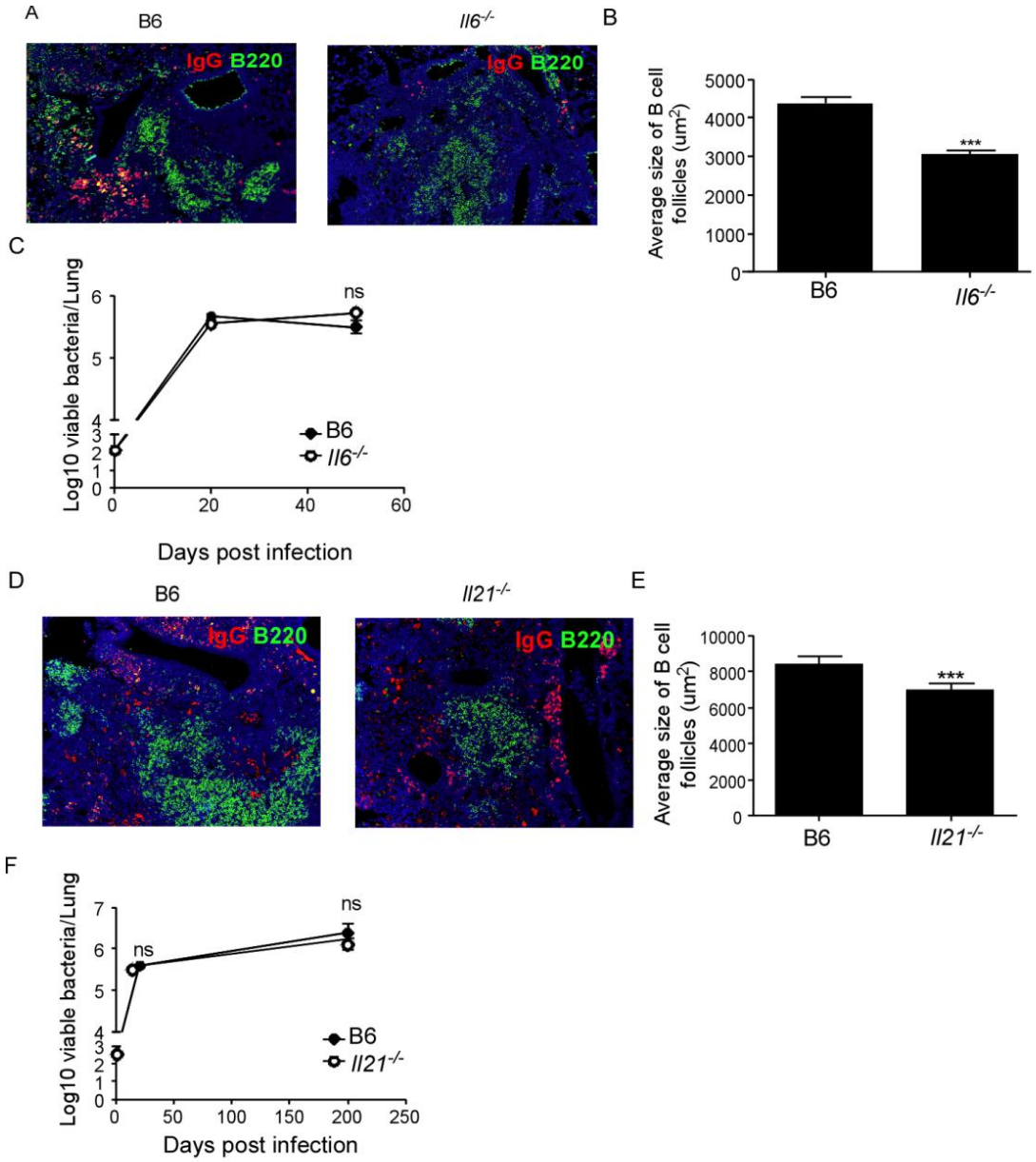

Figure S7: IL-6 and IL-21 are required for optimal B cell lymphoid follicle formation, but are not essential for Mtb control. B6 and Il6-/- mice or B6 and Il21-/- mice were infected as in Figure 3. At day 50 post infection, FFPE lung sections were assessed for B cell lymphoid follicle organization by immunofluorescence, 200X magnification (A, D). The average size of B cell lymphoid follicles (B,E) and lung bacterial burden were determined at different time points post infection (C, D). The data points represent the mean (±SD) of values from 4-6 mice (A-F). ***p=0.0005. ns=not signigicant.