Supplementary Material

BCG vaccination prevents reactivation of latent lymphatic murine tuberculosis independently of CD4+ T cells

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Supplementary Figures and Tables
Supplementary Figure 1: T cell numbers, serum cytokine and chemokine levels prior to *Mtb* infection. (A) Number of total CD3+, CD3+CD4+, CD3+CD8+, CD3+CD4−CD8− (DN) and CD3−NKp46+ cells (left plots), as well as numbers of total CD44+, CD44+CD69+CD103+ cells amongst CD3+CD4+ and CD3+CD8+ cells (right plots) in spleen and inguinal LN 60 days after vaccination with BCG or BCGΔBCG1419c. (B) Levels of serum cytokines and chemokines at 60 days after vaccination. Results are presented as pooled data means ± SEM from two pooled independent experiments (n = 8–10 mice per group). Statistical analyses: One-way ANOVA per analyte followed by Dunnett’s multiple comparisons test; significant differences relative to unvaccinated mice are indicated by asterisks: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Abbreviations: BCG, Bacille Calmette–Guérin; s.c., subcutaneous; i.t., intratracheal; (Supplementary to Fig. 2)
Supplementary Figure 2: Serum cytokine and chemokine levels 120 days after *Mtb* infection. (A) Levels of serum cytokines and chemokines at 120 days after *Mtb* infection. Results are presented as pooled data means ± SEM from two pooled independent experiments (n = 8–10 mice per group). Statistical analyses: One-way ANOVA per analyte followed by Dunnett’s multiple comparisons test; significant differences relative to unvaccinated mice are indicated by asterisks: * p<0.05; b.d. below detection limit. Abbreviations: BCG, Bacille Calmette–Guérin; s.c., subcutaneous; i.t., intratracheal; mAb, monoclonal antibody (Supplementary to Fig. 3)
Supplementary Figure 3: CD8+ T cell profiling after *Mtb* infection. (A, B) Frequencies of CD3+CD4+ cells in LN (A) and lung (B) at 14, 28 and 50 days following *Mtb* infection. (C, D) Frequencies of CD3+CD8+ cells in LN (C) and lung (D) at 14, 28 and 50 days following *Mtb* infection. (E, F) Total numbers of CD3+CD8+ cells in LN and lung at 14, 28 and 50 days following *Mtb* infection. (G, H) Frequencies of CD3+CD8+CD44+ cells in LN and lung at 14, 28 and 50 days following *Mtb* infection. (I, J) Total number of CD3+CD8+CD44+ cells in LN and lung at 14, 28 and 50 days following *Mtb* infection. Results are presented as individual data points from two pooled independent experiments (n = 8–10 mice per group). Statistical analyses: One-way ANOVA per time point followed by Dunnett’s multiple comparisons test; significant differences relative to unvaccinated mice are indicated by black asterisks; statistical differences relative to unvaccinated anti-CD4-treated mice are indicated by red asterisks: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. Abbreviations: BCG, Bacille Calmette–Guérin; s.c., subcutaneous; i.t., intratracheal; LN, lymph node; mAb, monoclonal antibody (Supplementary to Fig. 4)

Supplementary Figure 4: Memory CD8+ T cell profiling after *Mtb* infection. (A) Frequencies of CD44+CD62L−, CD44+CD62L+ and CD44+CD69+CD103+ cells amongst CD8+ T cells. Results are presented as pooled data means from two pooled independent experiments (n = 8–10 mice per group). Statistical analyses: Abbreviations: BCG, Bacille Calmette–Guérin; s.c., subcutaneous; i.t., intratracheal; LN, lymph node; mAb, monoclonal antibody (Supplementary to Fig. 4)
Supplementary Table 1: P-values for CFU results presented in Figure 3A-C. A one-way ANOVA per time point followed by Tukey’s multiple comparisons test was performed for CFU results shown in Figure 3A. The mean of each group was compared to the mean of every other group and p values for each combination and time point are shown. (Supplementary to Figure 3)

| Time  | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-value | p-values for each combination and time point are shown. (Supplementary to Figure 3)