Supporting Information

Reiley et al. 10.1073/pnas.1006298107

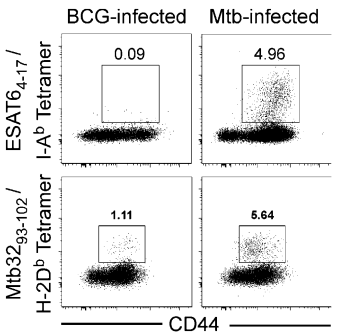

Fig. S1. ESAT6$_{4–17}$/I-A^b Tetramer specificity. Lymphocytes isolated from the lung of either bacillus Calmette–Guérin- (i.v. injected with 2×10^6 bacteria) or *Mycobacterium tuberculosis* (Mtb)-infected (aerosol with 75 CFU) mice on day 45 postinfection. Representative dot plots of CD44 and ESAT6$_{4–17}$/I-A^b tetramer staining gated on CD4 T cells or Mtb32$_{93–103}$/H-2D^b tetramer staining gated on CD8 T cells. Dot plots are from the lungs of three mice analyzed within each group.

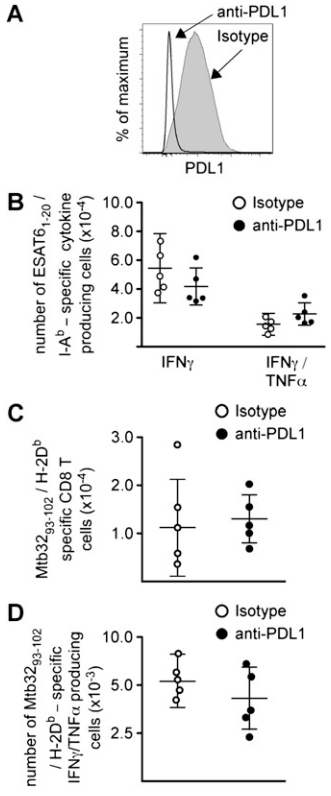

Fig. S2. Programmed death-1 (PD-1)/PD-1–programmed death ligand 1 (PDL1) blockade does not affect the number or function of antigen-specific T cells. Infected mice were administered a single injection of isotype or anti-PDL1 antibody. (*A*) PDL1 surface expression on lung lymphocytes 24 h after antibody administration, anti-PDL1 (solid black line), and isotype (shaded histogram). (*B*) Infected mice were treated with anti-PDL1, from day 30 to day 45 postinfection. Lymphocytes isolated from the lungs were stimulated with ESAT6$_{1–20}$ peptide for 5 h in the presence of brefeldin A. The number of ESAT6-specific IFNγ- and IFNγ/TNFα-producing CD4 T cells from each group is shown. (*C*) The numbers of Mtb$_{93–102}$/H-2D^b antigen-specific CD8 T cells from the lung of treated mice are shown. (*D*) Lymphocytes isolated from the lungs were stimulated with Mtb$_{93–102}$ peptide for 5 h in the presence of brefeldin A. The number of Mtb32-specific IFNγ/TNFα-producing CD4 T cells from each group is shown. The error bars indicate the SD. Data are presented from the lungs of five mice analyzed on each day. The data are representative of two experiments of similar design.

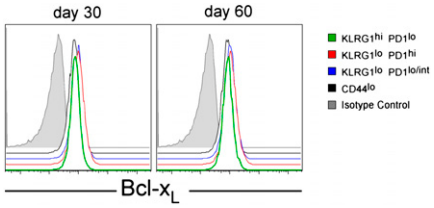

Fig. S3. The anti-apoptotic molecule Bcl-x_L is not down-regulated on PD-1–expressing cells. Representative histogram of Bcl-x_L staining on killer cell lectin-like receptor G1 (KLRG1)^{lo} PD-1^{hi}, KLRG1^{hi} PD-1^{lo}, KLRG1^{lo} PD-1$^{lo/int}$, and CD44^{lo} lung lymphocytes isolated on day 30 or day 60 postinfection. Data are presented from the lungs of five mice analyzed on each day.

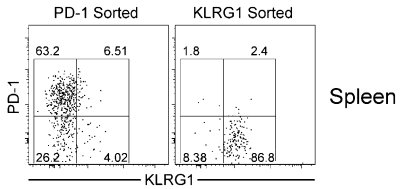

Fig. S4. Differentiation of PD-1– and KLRG1-expressing CD4 T cells. B6.SJL-*Ptprc*^{a} *Pep3*^{b}/BoyJ mice were infected, lymphocytes from the spleen, mediastinal lymph node, and lung were isolated on day 25 postinfection, and PD-1– or KLRG1-expressing cells were purified by flow cytometric cell sorting. PD-1–expressing cells (2.3×10^4) or KLRG1-expressing cells (2.5×10^4) were transferred to noninfected congenic C57BL/6 recipient mice. Representative dot plots of KLRG1 and PD-1 expression on CD4$^+$/CD45.1$^+$ donor cells in the spleen 6 d following transfer. Gates were set on the host population CD4 T cells.