Supplementary Methods

Mice:
C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). B6.129P2-H2-K1tm1BpeH2-D1tm1Bpe/DcrJ, i.e., Kβ-/-Dβ-/- (MHC-I-/-) mice, originally purchased from the Jackson Laboratory, were a generous gift from Dr. Kenneth Rock (University of Massachusetts Medical School, MA). B6.129-H2-Ab1tm1GruN12 (MHC-II-/-) and control C57BL/6NTac mice were purchased from Taconic Biosciences (Rensselaer, NY). C7 TCR transgenic (C7) mice were a generous gift from Dr. Eric Pamer (Memorial Sloan Kettering Cancer Center, NY)53.

Bacteria:
For in vitro infections, Mtb strain H37Rv or H37Rv expressing YFP (H37Rv-YFP) was grown as previously described52, 54. Bacteria was grown to a log phase under OD600 = 1.0, opsonized with TB coat (RPMI 1640, 1% heat-inactivated FBS, 2% human serum, 0.05% Tween-80), washed again and filtered through a 5 µm filter. Bacteria was counted using a Petroff-Hausser chamber before infection. H37Rv-YFP was a generous gift from Dr. Christopher Sassetti (University of Massachusetts Medical School, MA).

Materials:
The mouse interferon-γ (IFN-γ) ELISPOT kit and AEC substrate were purchased from BD Biosciences (San Jose, CA). The following peptides used for vaccination or in vitro experiments were purchased from New England Peptides (Gardner, MA): TB10.44-11 (IMYNYPAM), Mtb3293-102 (GAPINSATAM), Ag85b240-254 (FQDAYNAAGGHNAVF) and ESAT-63-17 (EQQWNFAGIEAAASA). Mtb peptide megapool 300 was previously described23. The MojoSort negative selection kits for CD4 and CD8 enrichment were purchased from Biolegend (San Diego, CA). Zombie Aqua viability dye, LEAF purified anti-mouse CD3ε (clone 145-2C11), LEAF purified anti-mouse CD28 (clone 37.51), LEAF purified anti-mouse IL-12 (clone C17.8), LEAF purified rat IgG2a, k (clone RTK 2758) LEAF purified rat IgG1, k (clone RTK2071), and all fluorophore conjugated antibodies used for flow cytometry were purchased from Biolegend, i.e., anti-CD4 (clone-GK1.5), anti-CD8α (clone 53-6.7), anti-CD3ε (clone 145-2C11), anti-CD19 (clone 6D5), anti-CD45 (clone 30-F11), anti-F4/80 (clone BM8), anti-CD69 (clone H1.2F3) and anti-IFN-γ (clone XMG-1.2). RPMI 1640, HEPES, sodium pyruvate and L-glutamine were purchased from Invitrogen Life Technologies, ThermoFisher (Waltham, MA). Heat inactivated fetal bovine serum was purchased from GE Healthcare Life Sciences (Pittsburgh, PA). Nunc UpCell plates were purchased from ThermoFisher Scientific (Waltham, MA). Collagenase type IV was purchased from Sigma-Aldrich (St. Louis, MO). CD4 (L3T4), CD8α (Ly-2), CD90.2 and CD11b microbeads used for positive selection were purchased from Miltenyi Biotec (Germany). Peptide-loaded tetramers for ESAT-63-17 (I-Ab), Ag85b240-254 (I-Aβ), Mtb3293-102 (H2-Db), TB10.44-11 (H2-Kβ) were obtained from the National Institutes of Health Tetramer Core Facility (Emory University Vaccine Center, Atlanta, GA). Corning human AB serum, Triton-X-100 and Tween-80 were purchased from Fisher Scientific. GolgiStop and BD Cytofix/Cytoperm were purchased from BD Pharmingen (San Jose, CA). 7H11 plates were purchased from Hardy Diagnostics (Santa Maria, CA). 1400W was purchased from Cayman Chemical (Ann Arbor, MI).

Macrophages:
For isolation of murine thioglycollate-induced peritoneal macrophages (TG-PM), naïve mice were intraperitoneally injected with 2 ml of 3% thioglycollate solution. Macrophages were harvested by peritoneal lavage 4-5 days. Macrophages were CD11b-enriched as permanufacturer's instructions. Enriched macrophages were resuspended in complete media without antibiotics (RPMI 1640 supplemented with 10% fetal bovine serum, 10 mM HEPES, 1
mM sodium pyruvate and 2 mM L-glutamine) and used for Mtb-infected macrophage ELISPOT or other assays as described. Purity of the enriched macrophages was confirmed by surface-staining of cells and cells were greater than 90% CD45^+ F4/80^+.

**In vivo aerosol infection:**
Low-dose Mtb strain Erdman aerosol infections were performed as described previously. Briefly, a frozen bacterial aliquot was thawed, sonicated for 1 minute using a cup-horn sonicator and diluted in 0.9% NaCl–0.02% Tween-80 in a total volume of 5 ml used for nebulization. Infections were performed using a Glas-Col (Terre Haute, IN) full body inhalation exposure system. Mice received an inoculation dose of 25-100 CFU/mouse as determined by plating undiluted lung homogenates on 7H11 plates from a subset of infected mice within 24 hours post-infection.

**T-cell isolation from infected or vaccinated mice:**
For experiments involving pulmonary T cells, lungs were dissected from infected mice after perfusion with RPMI 1640. Single cell lung suspensions were prepared by coarse dissociation using the GentleMACS tissue dissociator (Miltenyi Biotec, Germany), followed by digestion for 30 min at 37°C in a shaker at 85 rpm with 300 U/ml of Collagenase type IV in complete media. Samples were processed again using the GentleMACS tissue dissociator, strained through a 70 µm filter, washed 1x with PBS and then strained through a 40 µm filter. CD4 or CD8 T cells were labeled with CD4 L3T4 microbeads or CD8a Ly-2 microbeads, respectively, followed by positive selection though magnetic column using AutoMACS (Miltenyi Biotec, Germany). Where indicated, CD90.2 beads were used for CD4 and CD8 T cell positive selection. T cells were resuspended in complete media without antibiotics before use. Purity of the enriched T cells was confirmed by surface staining for CD4, CD8 and CD3 and was greater than 90%.

For experiments involving splenic T cells, spleens from infected or vaccinated mice were dissociated using a syringe and filtered through a 70 µm filter. Splenocytes were washed 1x with PBS and strained through a 40 µm filter. Polyclonal CD4 or bulk T cells (CD4 and CD8 T cells) were enriched using Mojosort negative selection kits for CD4 and CD3, respectively. T cells were resuspended in complete media without antibiotics before use. Purity of the enriched T cells was confirmed by surface staining and was greater than 90%.

**T-cell lines:**
C7 CD4^+ T cells (specific for ESAT-6) and P25 CD4^+ T cells (specific for Ag85b) have been described previously. Briefly, C7 or P25 cell lines were stimulated in vitro with irradiated splenocytes pulsed with 10 µM peptide ESAT-6_3-17 (EQQWNFAGIEAAASA) or Ag85b_240-254 (FQDAYNAAGGHNAVF) in complete media containing IL-2 at 20 u/ml, in the absence of antibiotics. After the initial stimulation, the T-cell cultures were split every two days for 3-4 divisions and rested for at least three weeks post-initial stimulation before use. After the initial stimulation, the cells were cultured in complete media containing IL-2 and IL-7 and final concentrations of 20 U/ml and 10 ng/ml, respectively. T cells were used between three – six weeks post-initial stimulation. Purity of C7 T cell line was assessed by Vb10.

**BCG vaccination:**
Mice were vaccinated subcutaneously with a single dose of BCG strain SSI diluted in 0.04% Tween-80 in PBS. BCG strain SSI was generously provided by Dr. Christopher Sassetti (University of Massachusetts Medical School). The BCG stocks used for vaccination were previously frozen and thawed immediately before use, washed 2x with 0.04% Tween-80 in PBS and used at an average dose of 500,000 CFU/mouse in a final volume of 200 µl. To confirm the
dose, bacteria used for vaccination were plated on 7H10 plates. Splenic T cells were enriched at 4-5 weeks and used for the Mtb-infected macrophage ELISPOT as described above.

Supplemental References

52. Rothchild AC, Jayaraman P, Nunes-Alves C, Behar SM. iNKT cell production of GM-CSF controls Mycobacterium tuberculosis. PLoS Pathog 2014; 10(1): e1003805.

53. Gallegos AM, Pamer EG, Glickman MS. Delayed protection by ESAT-6-specific effector CD4+ T cells after airborne M. tuberculosis infection. J Exp Med 2008; 205(10): 2359-2368.

54. Mishra BB, Lovewell RR, Olive AJ, Zhang G, Wang W, Eugenin E et al. Nitric oxide prevents a pathogen-permissive granulocytic inflammation during tuberculosis. Nat Microbiol 2017; 2: 17072.
**Supplementary Figure 1.** The majority of T cells that recognize Mtb-infected macrophages express CD69+. Pulmonary CD8 and CD4 T cells were cocultured with uninfected macrophages with the respective peptides or infected macrophages as indicated and assessed for IFN-γ production by ICS at 5 hours. Data are representative of 2 independent experiments using pooled T cells from 5 mice at 5 WPI (shown) or 7.5 months post infection, analyzed in triplicates.
Supplementary Figure 2. NO inhibition does not change the frequencies of MIME+ and MIM-ICS+ T cells. Purified pulmonary CD4 and CD8 T cells were cocultured with indicated stimulations in the absence (empty bar) and the presence (filled bar) of 1400W, iNOS inhibitor. (A) Supernatants were collected from 18 hour culturing of MIME assay, and the amount of nitrites were measured using Griess assay. MIME (B) and MIM-ICS (C, D) were performed as described in the absence and presence of 1400W.