SUPPORTING INFORMATION

1. Supplemental Methods:

Intracellular cytokine staining (ICS) Both conventional ICS and Direct ICS were done as described in the text. Fresh samples were used and, if necessary, cryopreserved samples from some studied macaques were thawed and assessed in repeated or additional ICS. For ICS, 3-5×10^6 cells from PBMC or BAL samples were suspended with anti-human CD28 (BioLegend, 1µg/ml) and anti-human CD49d (BioLegend, 1µg/ml) with or without 40 ng/mL HMBPP (from Dr. Jomaa, Germany) in 150 µl final volume of medium for 1 hour at 37°C, 5% CO2 followed by an additional 5 hours in the presence of monensin (GolgiStop, BD Biosciences). Cells were stained with a cocktail of the following antibody-fluorochrome conjugates against cell surface markers: anti-CD3-PECy7 (SP34-2, BD Biosciences), anti-CD8-APC.H7 (DK25, Dako), anti-CD4-PB (OKT4, eBioscience), anti-Vγ2-FITC (7A9, Thermo). After staining cell surface markers for 15 minutes, cells were permeabilized for 45 minutes (FOXP3 Fix/Perm Buffer; eBioscience) and stained for intracellular proteins for another 45 minutes using the following antibody-fluorochrome conjugates: anti-IFN-γ-APC (B27, BioLegend) and anti-perforin-strpBV421 (Pf-344, MABTECH). Cells were washed and fixed with 2% formalin and analyzed on an LSR Fortessa flow cytometer (BD Biosciences).

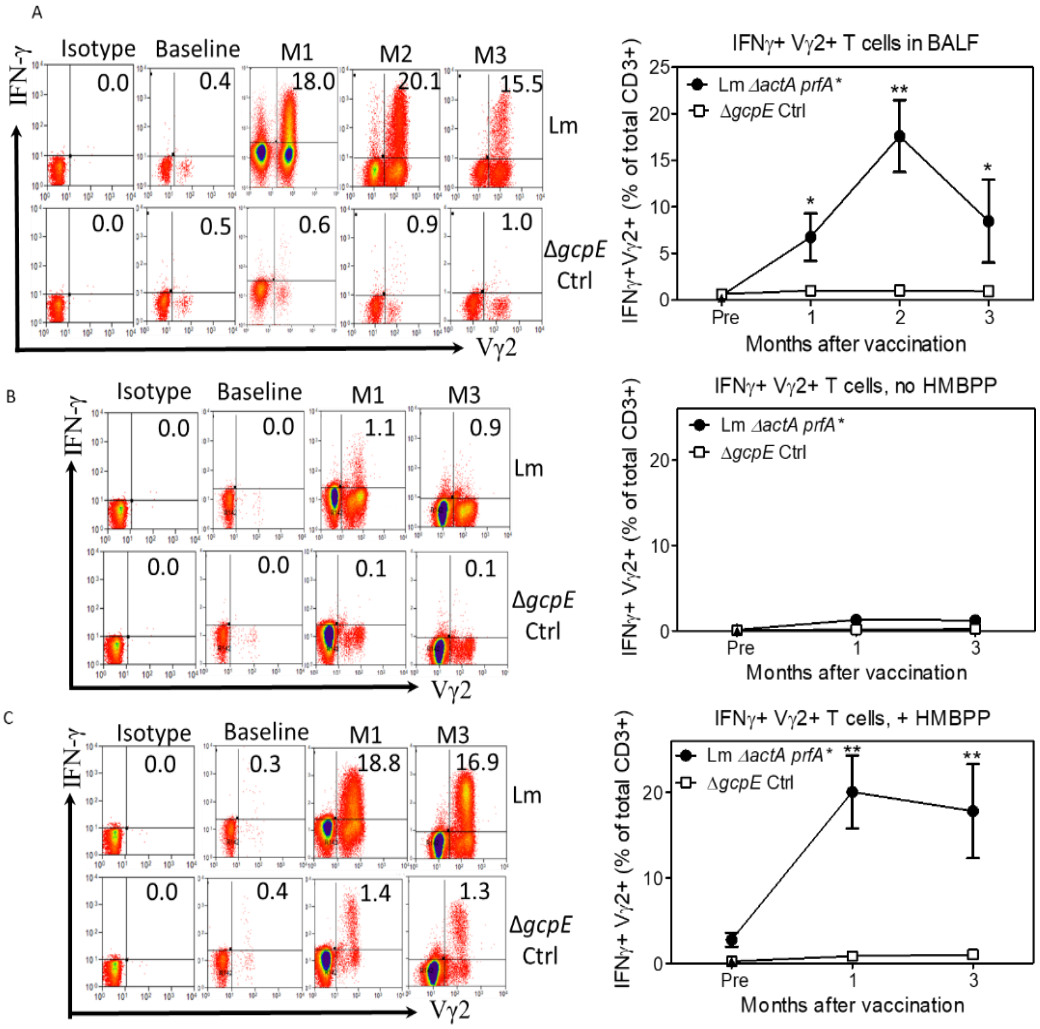
Intracellular Mtb growth inhibition assay TCR Vδ2+ cells were purified according to the manual of Dynabeads® FlowComp™ Flexi Kit (ThermoFisher, Rockford, IL) Briefly, freshly isolated splenic lymphocytes were incubated with FITC-conjugated mouse anti-human TCR Vδ2 mAb (Clone:15D, ThermoFisher) and then by desthiobiotinylated mouse anti-FITC mAb (Clone:FIT-22, Biolegend). Streptavidin-conjugated magnetic beads (Dynabeads) were then applied to isolate Vδ2+ cells via a potent binding of strepavidin-desthiobiotin, and beads were then detached by incubation with releasing buffer. Monocytes were isolated via plastic adherence after overnight culture of PBMC, and were infected with Mtb H37Rv at an MOI of 1:1 for 4 hr. Extracellular Mtb bacteria were then removed by three washes with pre-warmed (37º) PBS. Infected monocytes (target cells) were gently detached from the plate by incubation for 15 min with PBS + 2mM EDTA. The target cells were seeded into 96-well U bottom plates (1000 cells/well), and then incubated for 3 days alone or with autologous Vδ2+ cells at an effector/target cell ratio of 10:1 in triplícate unless a shortage of cells . Cells were then lysed by 0.03% SDS and serially diluted lysates were plated on 7H11 plates. CFU counts were determined after culture for 4 weeks. Inhibition data were expressed as growth index: CFU counts of moncytes plus effector cells /CFU counts of monocytes alone, as described (1).

Macroscopic and microscopic pathologic analysis of TB lesions. Macaques were euthanized at ~2.5 months post-Mtb challenge using pentobarbital (37.5 mg/kg i.v.) and immediately necropsied in a cabinet within a biosafety level 3 (BSL-3) facility. A pathologist and associates performed a blinded gross pathologic evaluation using standard procedures. Each step was documented and reported in detail and documented with photographs. Organs were collected and labeled. Multiple tissue specimens were collected from all organs whether or not they showed gross lesions.

For histopathology analysis and isolation of lymphocytes, approximately a third to a half of the tissues harvested from the right caudal, right middle, and left caudal lung lobes and liver, spleen, and kidney of necropsied macaques were collected after gross pathological analysis was completed. If there were grossly visible lesions in the lung samples, a portion containing ~50% lesional and ~50% healthy appearing tissue was taken for tissue homogenization. If no visible lesions were present, a random piece of tissue was taken.

2. Supplemental Figures and legends:

Fig. S1. Respiratory *Lm ΔactA prfA vaccination elicited sustainable increases in Th1-like Vγ2Vδ2 T cells in the lungs.**



A) Representative flow cytometry histograms (left) and graphs of data (right) show the percentages of IFNγ+ Vγ2+ T cells in CD3+ T cells in BAL fluid samples from macaques vaccinated with Lm *ΔactA*

*prfA** (Lm; top) or Δ*gcpE* (Ctrl; bottom). The data were generated by direct ICS assay without antigen stimulation in the culture.

B) and C) Representative flow cytometry histograms (left) and graphs of data (right) show the percentages of IFNγ+Vγ2+ T cells in CD3+ T cells in the blood from macaques before and after vaccination. Data are generated by the conventional ICS assay after in vitro stimulation with medium only in B), or with HMBPP in C).

*P < 0.05, **P < 0.01 (paired t test and Mann-Whitney).

Fig. S2A. Effect of respiratory Lm *ΔactA prfA immunization on TB dissemination to extra-pulmonary organs; Fig. S2B. Microscopic pathology of the lungs.**

Fig.S2A. Representative Spleen Pathology

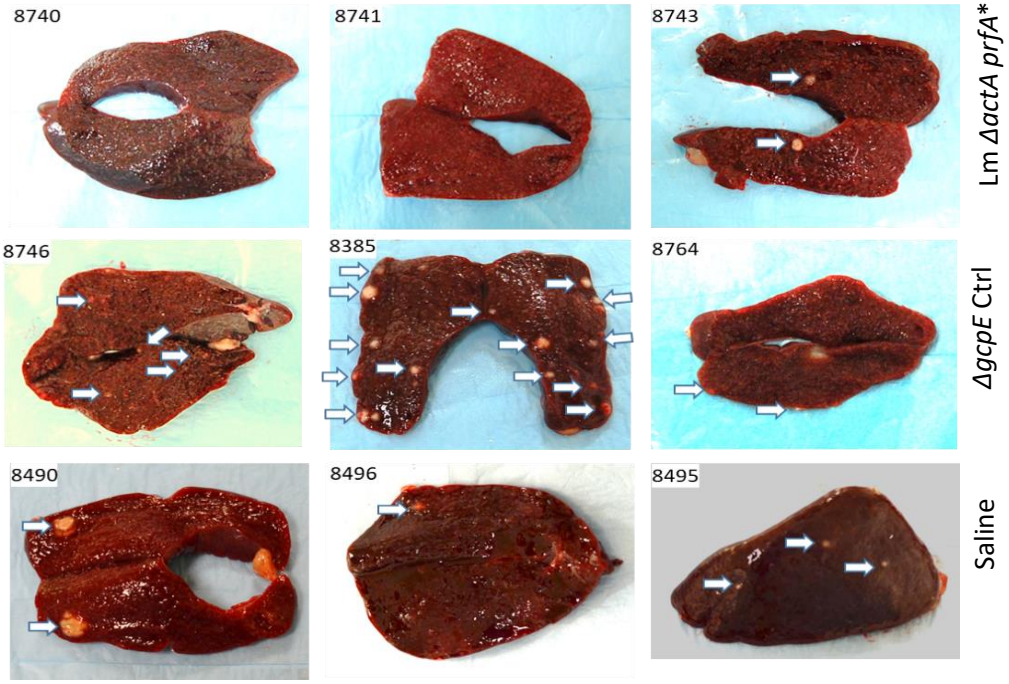


Fig. S2B. Representative Lung Histopathology

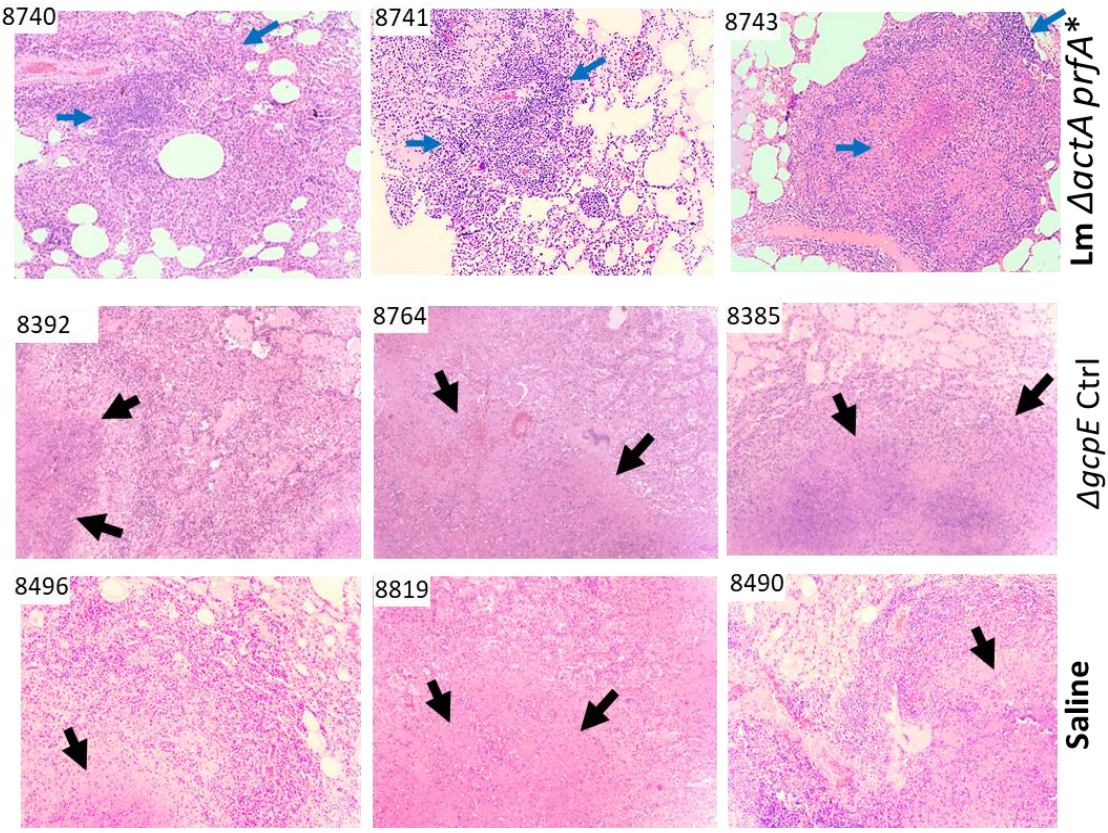
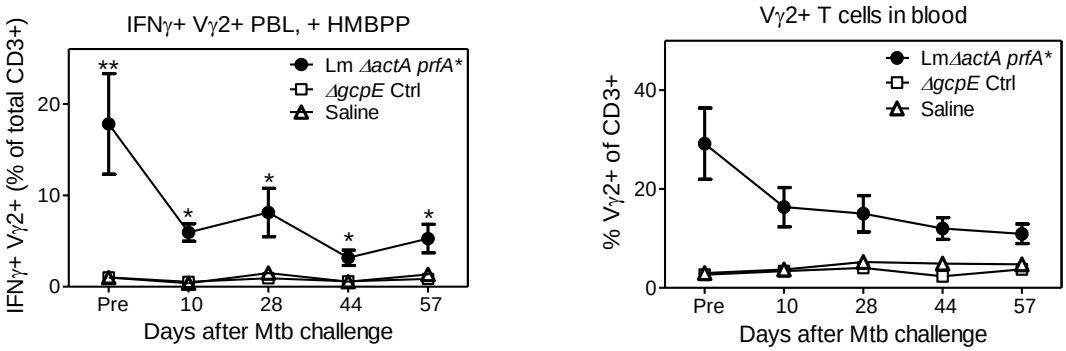


Fig.S2A photos: Gross pathology of spleens from representatives of the test and control groups as indicated on right. Spleens were collected at necropsy ~2.5 months after Mtb challenge. White arrows indicated disseminated granulomas identified in the cut or uncut sections of spleens. Pathology in livers, kidneys and spleens from each group were evaluated/scored together with lungs/chests, and presented as entire gross pathology scores in Fig.3B (see the Method section).

Fig.S2B photos: Histologic evaluation of tissue sections of right caudal lung lobes from the test and control groups. Shown are H&E stained sections taken from three representative macaques for each group, with macaque ID and magnification indicated for each image. Overall, the vector and saline controls displayed extensive necrotic TB lesions (black arrows), with inflammatory cells and edema fluid presenting in the peripheries of TB granulomas in the right caudal and other lung lobes. TB granulomas identified in the right caudal lobes from the test group usually exhibited more lymphocytic and less necrotic histopathology than those from the control groups. Mild-pathology representatives in the test group showed well-contained TB granulomas, with infiltrating lymphocytes forming cuffs (blue arrows) in the peripheries of TB granulomas.

Fig. S3. Th1-like Vγ2Vδ2 T cells in blood after Mtb challenge of Lm *ΔactA prfA-vaccinated macaques.**

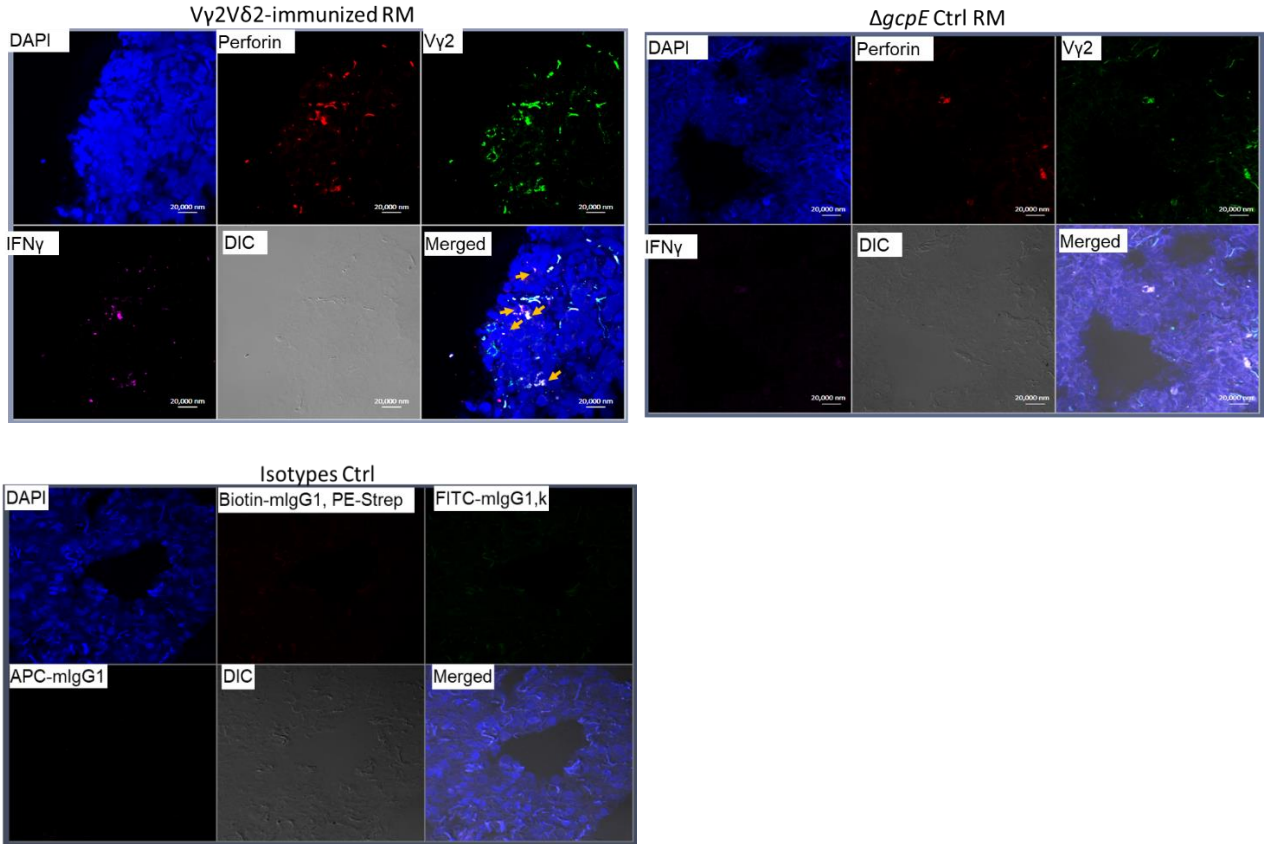


Left graph showing percentages of IFNγ+Vγ2+ T cells in CD3+ T cells in the blood from the three groups as indicated. Data are pooled from each group, and derived from the conventional ICS assay after in vitro stimulation with HMBPP. Medium alone control stimulation of PBL yielded very low levels of IFNγ+Vγ2+ T cells.

Right graph showing percentages of total Vγ2+ T cells in CD3+ T cells in blood from three groups of macaques, as indicated.

Means and standard deviations for each group are shown. *P < 0.05, **P < 0.01 (Mann-Whitney)

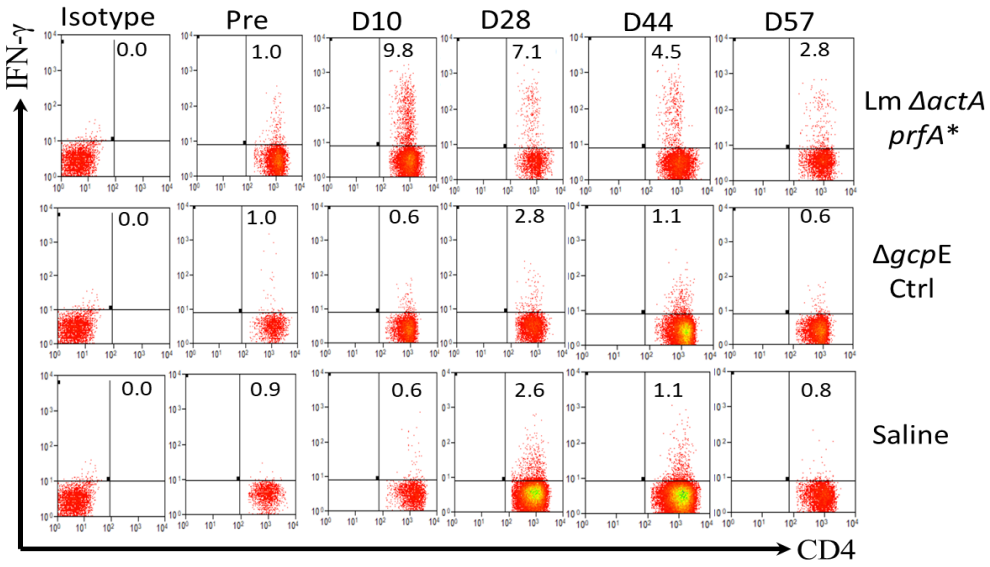
Fig. S4. V**accine-induced reduction of TB infection coincides with tissue-resident Vγ2Vδ2 T effector cells co-producing IFNγ and perforin.**



Shown in the top right panel (scale bars 20 μm as indicated) are Vγ2 (green) T effector cells co-expressing the IFNγ (pink) and perforin (red) in the merged images (co-localization marked by arrows) in tissue sections of the right caudal lung lobes from the test group receiving Lm *ΔactA prfA** immunization (Vγ2Vδ2-immunized RM). The right panel shows very few IFNγ^{+}perforin^{+} Vγ2^{+} cells in the right caudal lung section from the representative control macaque. Similar results for Vγ2 T effector cells were seen in other test and control macaques.

Bottom panel: IgG isotype controls did not give detectable staining in TB infected lung tissue sections.

Fig.S5. Rapid pulmonary Th1 responses to Mtb challenge in Lm ΔactA prfA* immunized macaques



Shown are representative flowcytometry histograms for percentages of IFNγ^{+} CD4^{+} T cells in BAL fluid samples collected after 80 CFU Mtb challenge of the three groups of macaques as indicated.

1. Yang R, *et al.* (2018) IL-12+IL-18 Cosignaling in Human Macrophages and Lung Epithelial Cells Activates Cathelicidin and Autophagy, Inhibiting Intracellular Mycobacterial Growth. *J Immunol* 200(7):2405-2417.