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Supplemental Data

Prime-Boost Vaccination with rBCG/rAd35 Enhances CD8+ Cytolytic T Cell Responses in Lesions from *Mycobacterium Tuberculosis*–Infected Primates

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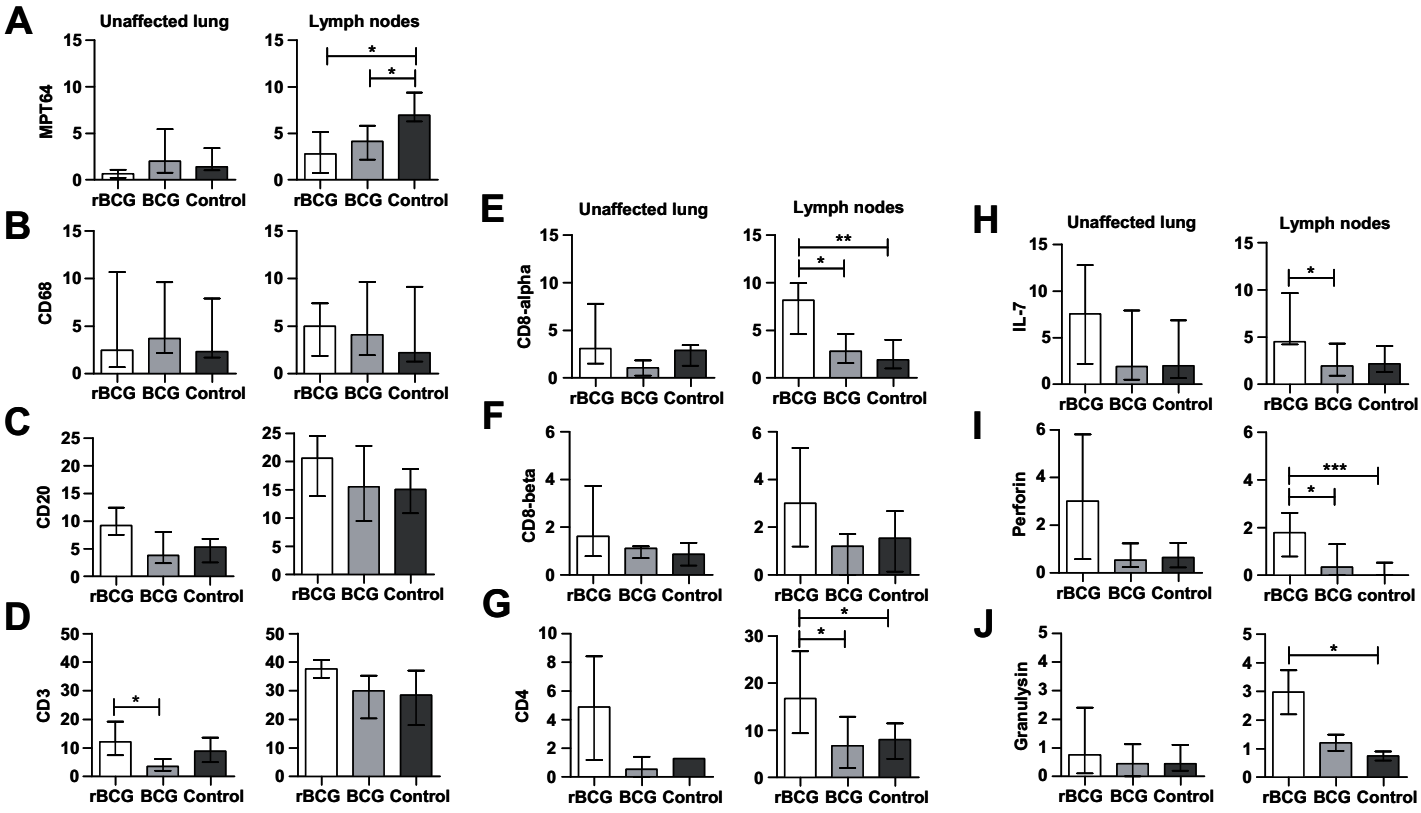
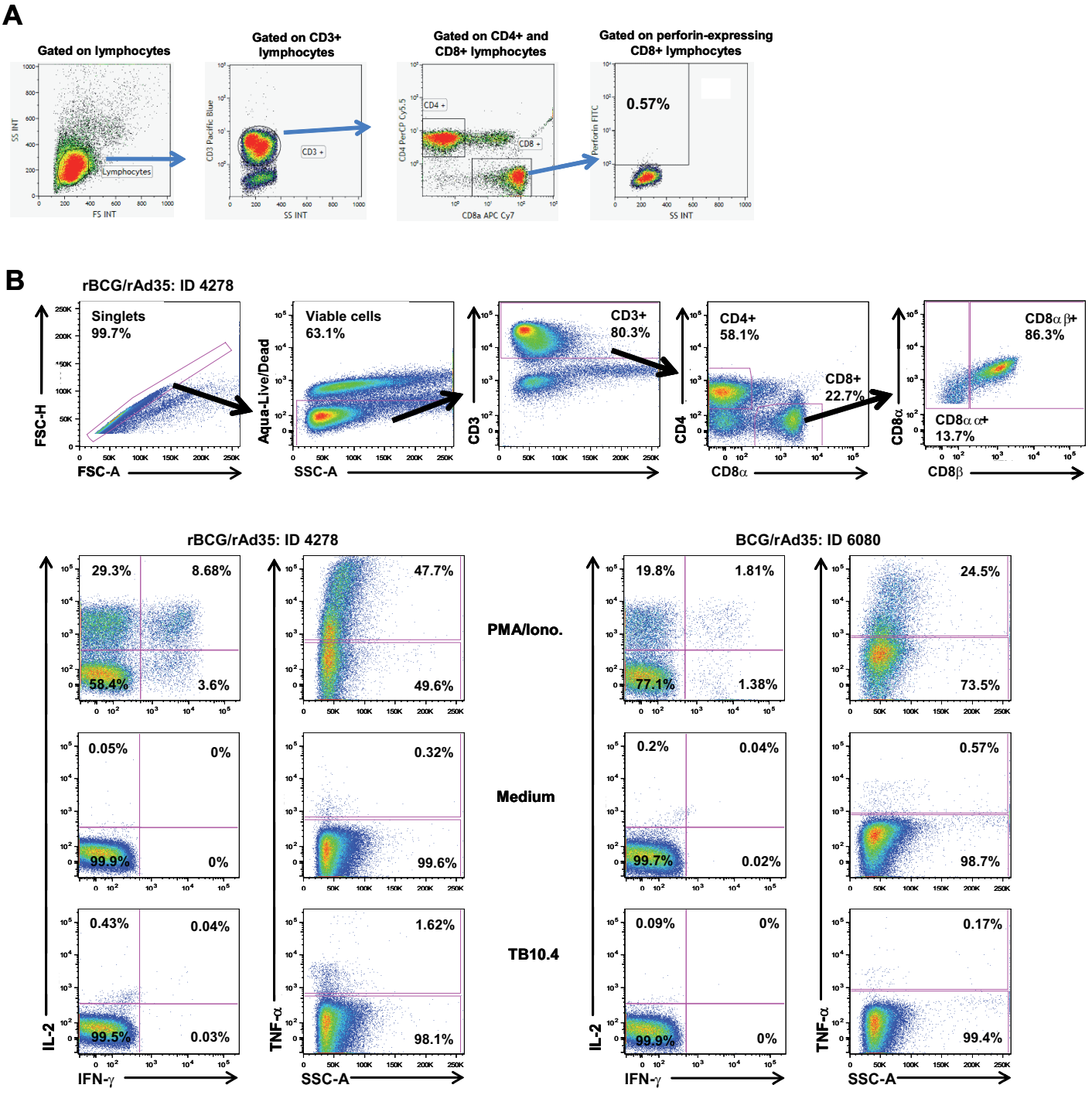


Supplementary Figure 1. Immunohistochemical analysis of unaffected lung tissue as well as axillary and hilary lymph nodes (pooled results) obtained from rBCG (rBCG/rAd35) or BCG (BCG/rAd35)-vaccinated and control (unvaccinated) animals after infection with Mtb. Protein expression of (A) MPT64, (B) CD68+ macrophages, (C) CD20+ B cells, (D) CD3+ T cells, (E) CD8α+ T cells, (F) CD8β+ T cells, (G) CD4+ T cells, (H) IL-7, (I) perforin and (J) granulysin was assessed using in situ computerized image analysis. Data are presented as % positive area of the total cell area and the median ± IQR from n=5-6 animals/group is shown. Statistical significance of differences in protein expression was determined by a non-parametric Kruskal-Wallis test.



Supplementary Figure 2. Gating strategy used in the flow cytometric analyses of (A) perforin production in the CD8+ T cell compartment (medium control) of PBMCs from a healthy NHP and (B) the different T cell compartments (CD4+, CD8αα+, and CD8αβ+ T cells) of PBMCs from rBCG/rAd35-vaccinated and Mtb-infected NHP ID4278. IL-2, IFN-γ and TNF-α cytokine production in CD4+ T cells (from rBCG/rAd35-vaccinated NHP ID4278 and BCG/rAd35-vaccinated NHP ID6080) in response to TB10.4 stimulation as well as in the negative (medium) and positive (PMA/ionomycin) controls are shown.

MATERIAL AND METHODS

Intracellular Cytokine Staining

Frozen PBMCs from vaccinated NHPs were thawed, rested overnight, and stimulated for 6 hours with peptide pools in RPMI (Gibco, Invitrogen; supplemented with 2 mM L-glutamine, 100 IU/ml penicillin, 10 mg/ml streptomycin, and 10% heat-inactivated fetal bovine serum) in the presence of 10μg/ml BFA (Sigma Aldrich). Cells stimulated with 25ng/ml phorbol 12-myristate 13-acetate (PMA) and 1μg/ml of ionomycin (Sigma Aldrich) were used as a positive control. PBMCs were stained with surface antibodies including anti-CD3 Pacific Blue (SP34-2), anti-CD4 PerCP-Cy5.5 (L200), anti-CD8α APC-Cy7 (SK1), and anti-CD8β FITC (2ST8.5H7), in the presence of an Aqua LIVE/DEAD fixable dead cell marker (Invitrogen), for 30 minutes at 4°C. Cells were washed, fixed and permeabilized using the IntraPrep Fix/Perm Kit (Beckman Coulter) and incubated for 30 minutes at 4°C with antibodies specific for intracellular cytokines including anti-IL-2 PE (MQ1-17H12), anti-interferon (IFN)-γ PE-Cy7 (B27), and anti-tumor necrosis factor (TNF)-α APC (MAb11) before flow cytometric analysis (BD FACSCanto flow cytometer; BD Biosciences). Anti-CD8β FITC (2ST8.5H7) was custom-conjugated at Beckman Coulter whereas all other antibodies were obtained from BD Biosciences.

Supplementary Table 1. Polyfunctional[a] TB10.4-specific T cells in peripheral blood 15 wks post Mtb challenge[b].

Group and animal ID	Stimulation	Average % of CD3+CD4+ cytokine-producing cells	Average % of CD3+CD8αα+ cytokine-producing cells	Average % of CD3+CD8αβ+ cytokine-producing cells
rBCG/rAd35: ID 4278	Medium control	0,23	0,64	0,25
	PMA/ionomycin	51,76	42,05	44,82
	TB10.4	0,93	2,00	1,42
BCG/rAd35: ID 6080	Medium control	0,89	1,54	0,55
	PMA/ionomycin	24,56	78,93	29,13
	TB10.4	0,43	0,65	0,46

[a] Polyfunctional T cells simultaneously producing a combination of IL-2, TNF-α, and/or IFN-γ.
[b] All stimulations were done in duplicates, and average values of duplicates are presented.