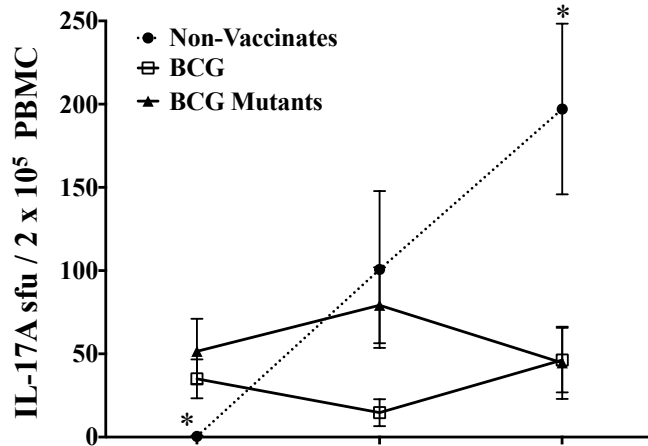
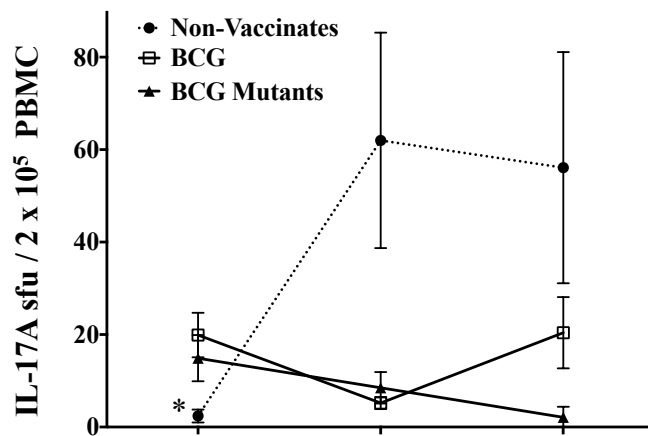


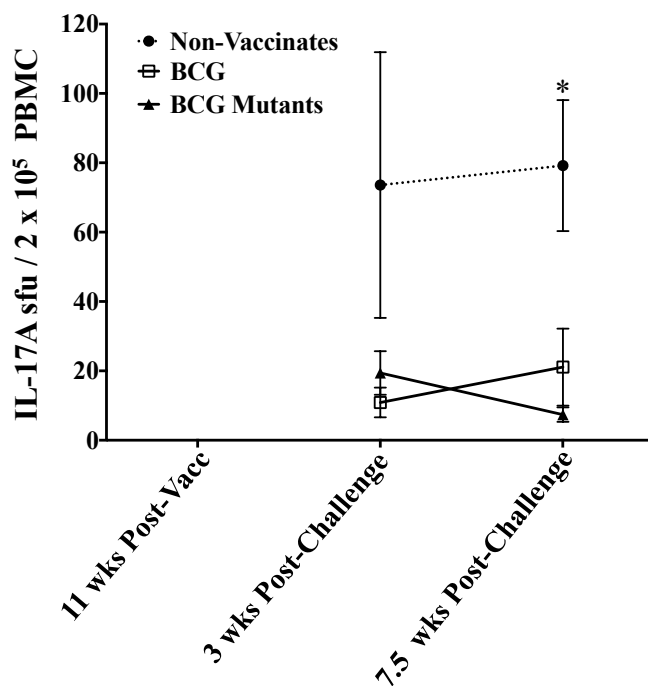
A. PPD



B. Ag85A/TB10.4



C. rESAT-6:CFP10



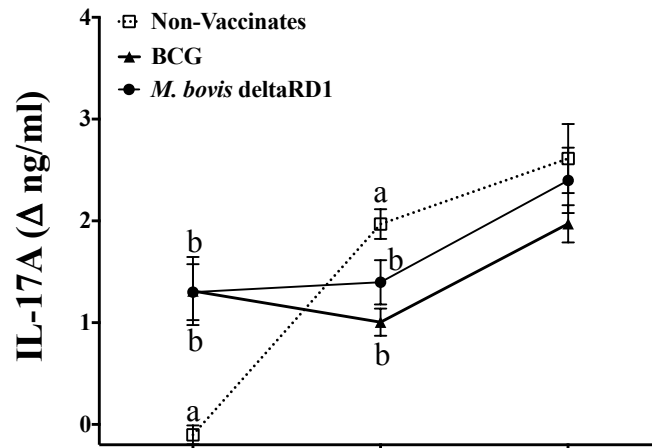
Supplemental Figure S1. IL-17A responses (protein, ELISPOT) to vaccination and subsequent challenge with virulent *M. bovis*.

Treatment groups included: non-vaccinated animals (n = 10), BCG-vaccinated animals (n = 9), and BCG mutants (i.e., BCG $\Delta fdr8$, BCG $\Delta leuCD \Delta pks16$, BCG $\Delta metA$, and BCG $\Delta mmaA4$) vaccinated animals (n = 10). Virulent *M. bovis* strain 10-7428 was administered by aerosol to all calves 3.5 months after vaccination and calves were euthanized 4.5 months after *M. bovis* challenge (Table 1). For IL-17A analysis, PBMC (2×10^5 PBMC/well) were stimulated with 5 μ g/ml *M. bovis* PPD (A), 2 μ g/ml rAg85A/TB10.4 (B), or 2 μ g/ml rESAT-6:CFP10 (C) for 18 h prior to spot development and counting.

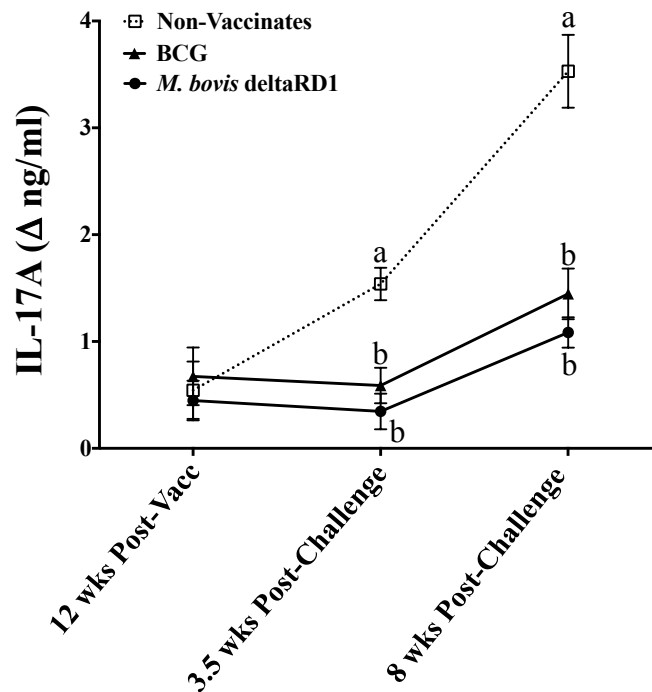
Plates were developed, as described in the Materials and Methods. Results are expressed as spot-forming units (sfu) / 2×10^5 cells (y-axis). Responses were measured 11 wks after vaccination and at 3 and 7.5 wks after *M. bovis* challenge (x-axis).

* Differs ($P < 0.05$) from BCG and BCG mutants at the respective time point.

A. PPD



B. rESAT-6:CFP10

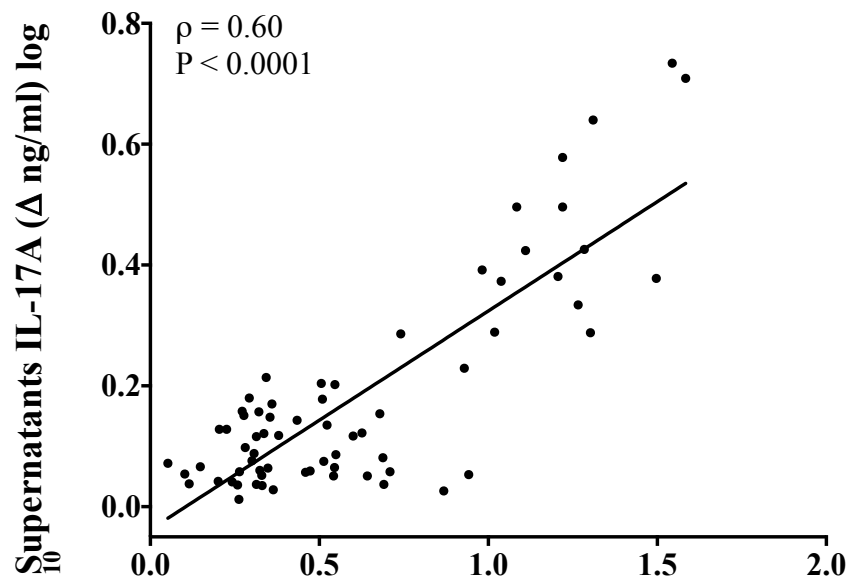


Supplemental Figure S2. Vaccine efficacy study 2007: IL-17A responses to vaccination and subsequent challenge with virulent *M. bovis*.

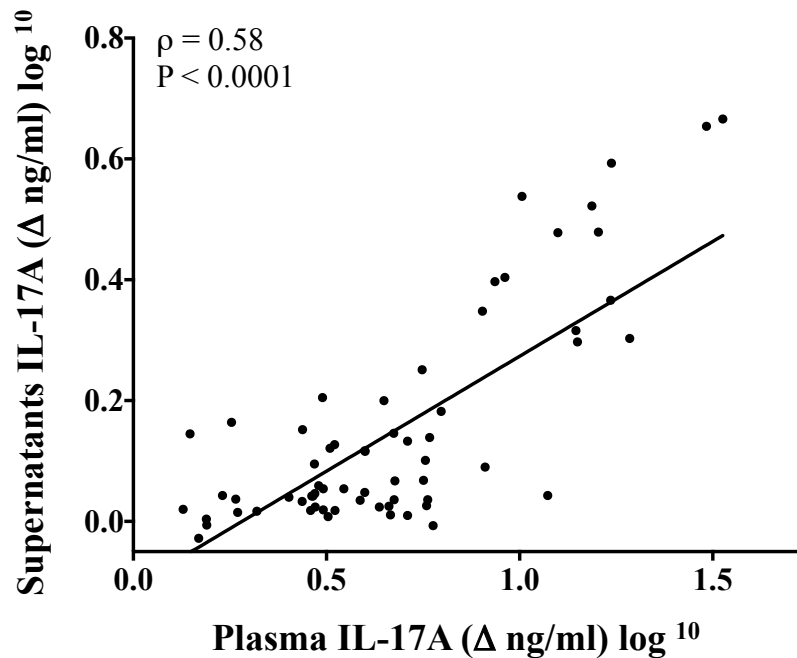
Treatment groups included: non-vaccinated animals (n = 10), BCG-vaccinated animals (n = 9), and *M. bovis* Δ RD1-vaccinated animals (n = 10). Virulent *M. bovis* strain 95-1315 was administered by aerosol to all calves 3 months after vaccination and calves were euthanized 4.5 months after *M. bovis* challenge (Table 1). PBMC (2×10^5 PBMC/well) were stimulated with 1 μ g/ml *M. bovis* PPD (Lelystad, Prionics Ag) (A), 1 μ g/ml rESAT-6:CFP10 (B) or media alone at 39°C for 16 h and supernatants harvested for IL-17A analysis by ELISA (Bovine IL-17A ELISA VetSet, Kingfisher Biotech). Responses were measured 12 wks after vaccination and at 3.5 and 8 wks after *M. bovis* challenge (x-axis).

^{a-c} Differing superscripts indicate that responses (mean \pm SEM) differ ($P < 0.05$) within the given time point.

A. PPD



B. rESAT-6:CFP10



Supplemental Figure S3. Correlation of IL-17A responses to *M. bovis* PPD (A) or rESAT-6:CFP10 (B) in whole blood versus PBMC cultures. Treatment groups included: non-infected (n = 7), 95-1315-infected (n = 8) and 10-7428-infected (n = 8) calves with the experimental design described in Table 1. IL-17A concentrations in PBMC supernatants (y-axis) or stimulated plasma from whole blood (x-axis) were determined by ELISA (Bovine IL-17A ELISA VetSet, Kingfisher Biotech). Data represent Δ ng/ml, \log^{10} (i.e., antigen stimulation minus media alone) for PBMC supernatants (y-axis) versus whole blood stimulated plasma(x-axis) for each individual animal (\bullet) and each time point (n = 69). Prior to analysis and graphing, data were transformed for positive skewness with zero values using the formula: New X = $\text{Log}^{10}(X + 1)$. ρ = Spearman's rank correlation coefficient