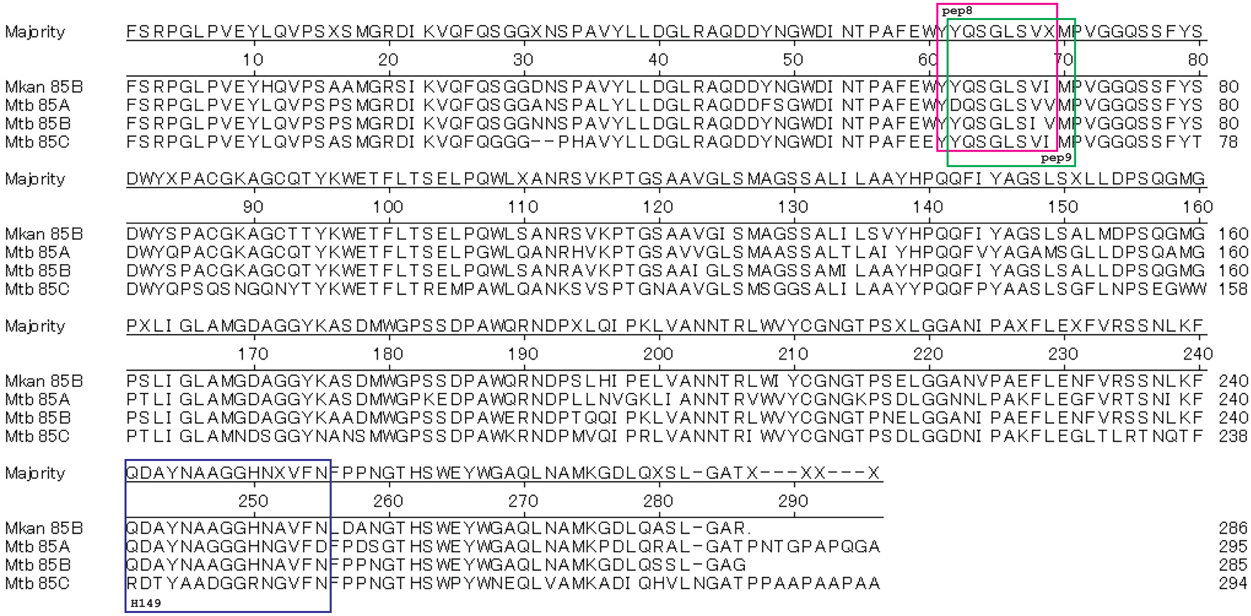
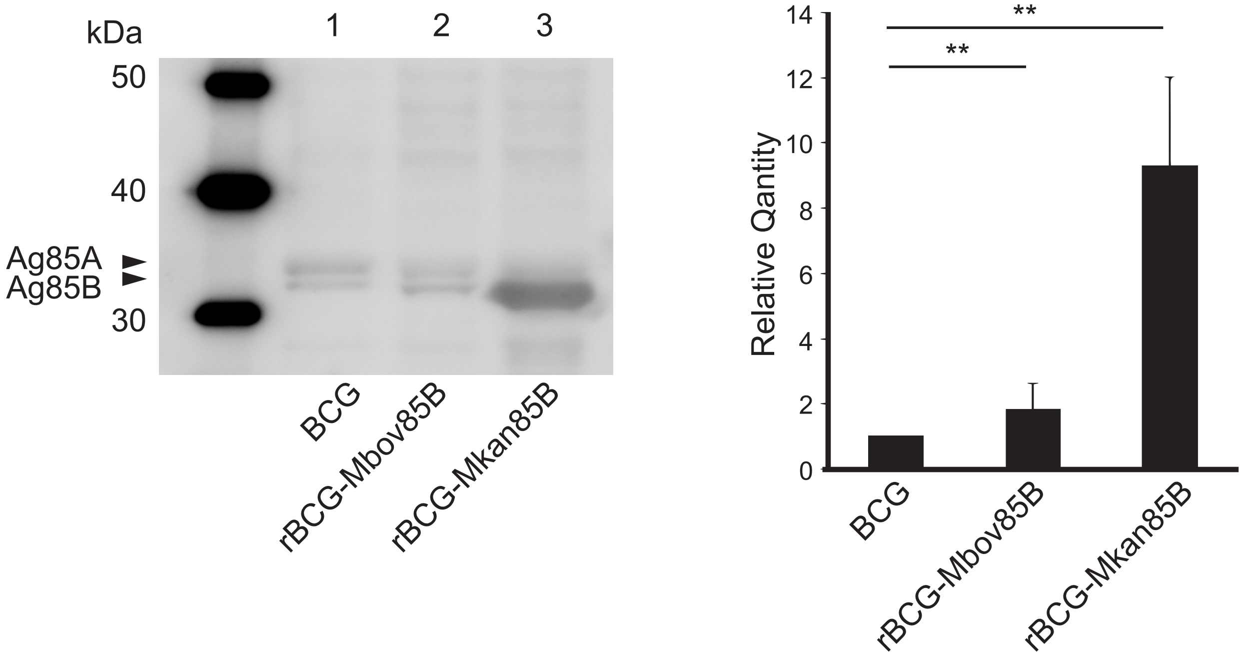
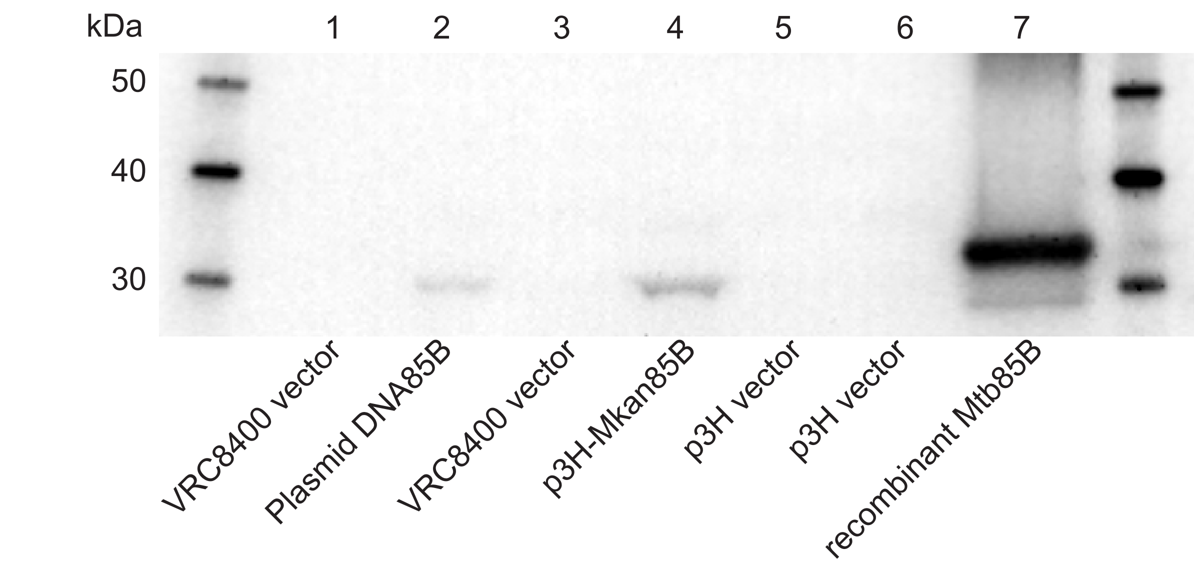


Supplemental Figure 1. Alignment of amino acid sequences of the immunodominant epitopes of the fibronectin binding proteins of the antigen 85 complex of Mtb and M. kansasii. The antigen 85 (Ag85) complex of three proteins with Ag85A, Ag85B and Ag85C of Mtb were compared by alignment with Ag85B of M. kansasii with ClustalW. Magenta and green boxed residues are pep8 (aa 61-69) and pep9 (aa 62-70) epitopes for polyfunctional CD8+ T cell-activation in rBCG85B/DNA85B-immunized H2d BALB/c or H2b/d CB6F1 mice. Blue boxed residues are H149 epitopes (aa 241-255) for CD4+ T cell-activation on the Ag85B in the immunized H2b C57BL/6 or H2b/d CB6F1 mice.

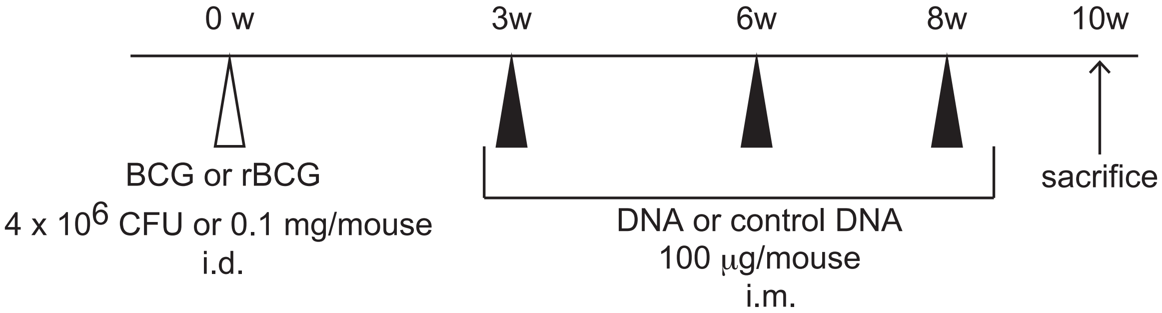
A



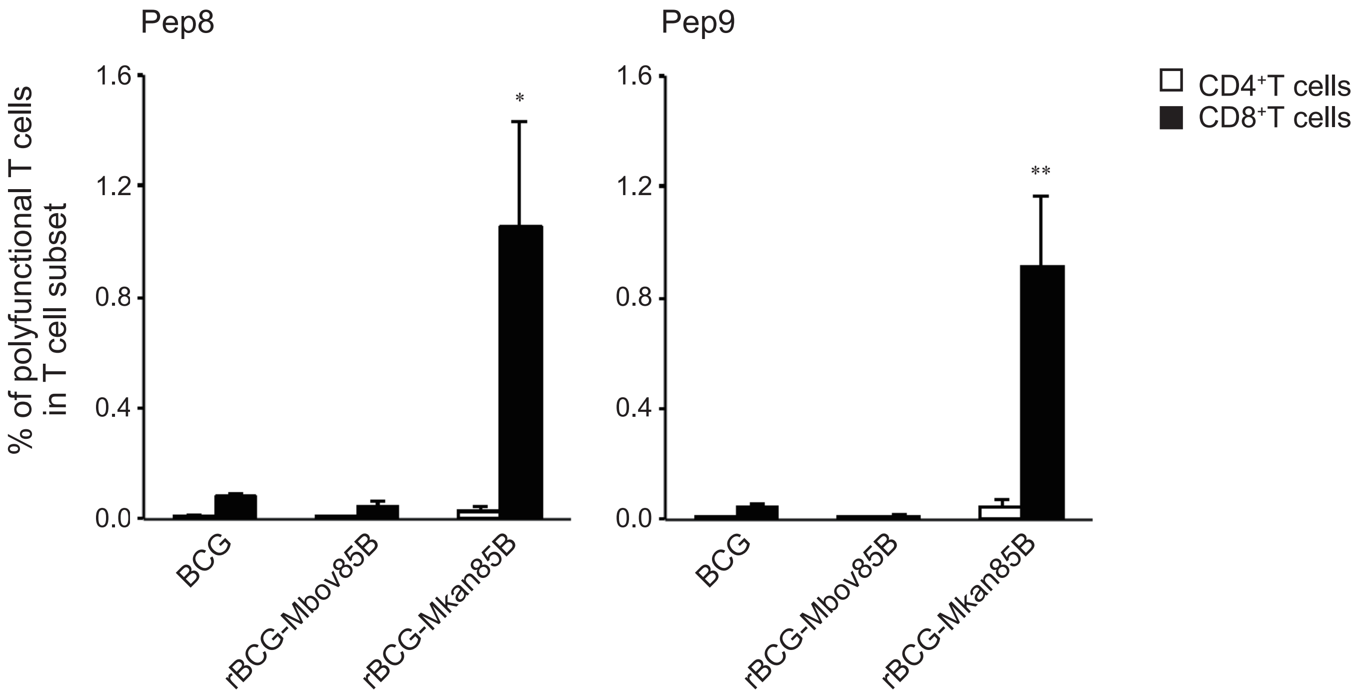
B



Supplemental Figure 2. Western blot of expression of Mkan85B protein by rBCG and in 293T transfectants. (A) rBCG-Mkan85B, rBCG-Mbov85B, and BCG were grown in Middlebrook 7H9 medium supplemented 10% ADC, 0.0625% Tween80, and 100 mg/mL kanamycin. The bacterial cell were centrifuged then lysed, and SDS solubilized lysate was run on an SDS-containing polyacrylamide gel, and blotted. The expression of Ag85B was detected using anti-Ag85B polyclonal antibody (Abcam, Cambridge, UK). Lane 1, solubilized BCG lysate; Lane 2, solubilized rBCG-Mbov85B lysate; Lane 3, solubilized rBCG-Mkan85B lysate. Graph shows densitometric value of Ag85B. Western blot represents three independent experiments. **, $p < 0.01$; one-way ANOVA test. Error bars represent SEM. (B) Expression of Ag85B protein in p3H-Mkan85B and plasmid DNA85B transfected 293T cells. p3H-Mkan85B (lane 4), Plasmid DNA85B (lanes 2), or control p3H vector lacking Mkan85B (lane 5 and 6), control VRC8400 vector lacking Mkan85B (lane 1and 2) were transfected into 293T cells as described in Materials and Methods and analyzed by SDS-PAGE, and Western Blotting, as described above. Lane 7, recombinant Mtb85B protein. Western blot represents three independent experiments.



Supplemental Figure 3. Immunization schedule. Mice were immunized with BCG vaccine, rBCG-Mkan85B or rBCG-Mbov85B at a concentration of 4×10^6 CFU or 0.1 mg of bacilli i.d., and 100 µg of plasmid DNA or control DNA in saline i.m. three times.



Supplemental Figure 4. Comparison of immunogenisity between rBCG-Mkan85B and rBCG-Mbov85B. CB6F1 mice were immunized by priming with rBCG-Mkan85B and boosting with plasmid DNA-Mkan85B (rBCG-Mkan85B/DNA-Mkan85B) or rBCG-Mbov85B and boosting with plasmid DNA-Mbov85B (rBCG-Mbov85B/DNA-Mbov85B). We studied polyfunctional $CD4^+$ or $CD8^+$ T cells after immunization followed by in vitro restimulation with pep8 (left panel) or pep9 (right panel). Data represent mean +SEM (n = 3 to 7).* $p < 0.05$, ** $p<0.01$; one-way ANOVA using Turkey-Kramer test.