

Supplementary Figure 1. CD154+ antigen-specific CD4 T cells are increased in rBCGvaccinated mice following stimulation ex vivo with Mtb lysate. BALB/c mice were vaccinated s.c. with  $0.5-1\times106$  CFU BCG or rBCG and the organs were aseptically removed at the indicated timepoints.  $1\times106$  cells/ml were cultured with 10 µg/ml Mtb lysate and both anti-CD154-PE and anti-CD40 at 2 µg/ml. Brefeldin A at 10 µg/ml was added for the last 2 h of a 16-h incubation according to published protocol (Kirchhoff et al., 2007). CD154+ upregulation was measured by flow cytometry on CD3+CD4+CD62L– lymphocytes. Shown are mean± SEM of two pooled experiments where n=8. \* p<0.05, \*\* p<0.01 by two-tailed Student's T test.



## Supplementary Figure 2. rBCG induces increased specific antibody production. ELISA of

mycobacterial-specific antibody in serum 28 days after (A) BCG or rBCG vaccination or (B) aerosol challenge of C57BL6 mice with MTB. Shown are mean $\pm$  SEM of three pooled experiments where n=10. \* p<0.05, \*\* p<0.01 by one-way ANOVA and Bonferroni's post-test. (C) Passive transfer of rBCG serum containing increased MTB reactive antibody fails to recapitulate increased protection following vaccination. Serum from 10 BCG or rBCG mice was collected and pooled 2-3 months following vaccination. 200ul of serum diluted 1:1 in PBS was transferred i.p into naïve mice on the day of low dose MTB aerosol infection, and additionally 3x per week for the duration of the experiment.Graph shows CFU in lung following BCG or rBCG serum treatment 14 days after infection, pooled values from 2 experiments where n=10. \* p<0.05\*p<0.01, \*\*\* p<0.001 by one tailed ANOVA followed by Bonferroni's post-test.



## Supplementary Figure 3. Dissemination to peripheral organs following adoptive transfer and Mtb infection. C57BL6 Mice were vaccinated with either BCG or rBCG, then 3 months later infected with low-dose Mtb via aerosol. CFU was measured in indicated organs 28 days later. All graphs show mean $\pm$ SEM of pooled values from two experiments where n=10. \* p<0.05, \*\*\* p<0.001 using Mann-Whitney U test.