Supplementary Figure 1 shows pro-inflammatory cytokines (IFN- $\gamma$ , IL-17, and IL-22) production by whole lymphocytes in  $\Delta znuA$  *B. melitensis* and RB51 vaccinated BALB/c and IFN- $\gamma^{-/-}$  mice before and after challenge with wild-type *B. melitensis* 16M.





Fig. S1. AznuA B. melitensis and RB51 vaccines stimulate enhanced IFN-y and IL-22 production by BALB/c lymphocytes and IL-17 and IL-22 production by IFN- $\gamma^{\prime}$ -lymphocytes before and after challenge with wild-type B. melitensis 16M. (A,C,E,G,I,K) BALB/c and (B,D,F,H,J,L) IFN-Y<sup>-/-</sup> mice (18/group) were nasally vaccinated with 109 CFUs of AznuA B. melitensis, RB51, or sPBS. Three wks after vaccination, whole splenic lymphocytes (pooled from 2-3 mice/culture and at least three cultures/experiment) from half of the mice were restimulated with media or 1 x 10<sup>9</sup> CFUs of heat-killed RB51 (HKRB51) for 3 days. Collected supernatants were evaluated for (A, B) IFN-y, (E, F) IL-17, and (I, J) IL-22 production using standard cytokine ELISA methods. The remaining mice were nasally challenged with 5 x 10<sup>4</sup> CFUs of wild-type B. melitensis 16M 6 wks after vaccination. At four weeks post-challenge, harvested splenic lymphocytes (pooled from 2-3 mice/culture and at least three cultures/experiment) were cultured as described above, and cell culture supernatants were evaluated for (C, D) IFN-y, (G, H) IL-17, and (K, L) IL-22 production. Results are depicted as the mean  $\pm$  SEM of triplicate cultures from two independent experiments. Significant differences in IFN- $\gamma$ , IL-17, and IL-22 production were determined: \*P  $\leq 0.001$ , \*\*P  $\leq 0.05$  (versus PBS-dosed mice); \*P < 0.001,  $^{\dagger\dagger}P < 0.05$  (versus pre-challenge level for the same vaccine group); and  $^{\ddagger}P < 0.001$ ,  $^{\ddagger}P < 0.05$  (differences between RB51- and AznuA B. melitensis-vaccinated mice); #P<0.001, ##P<0.001 (differences between BALB/c and IFN- $\gamma^{-/-}$  mice to the same vaccine). ND, none detected.