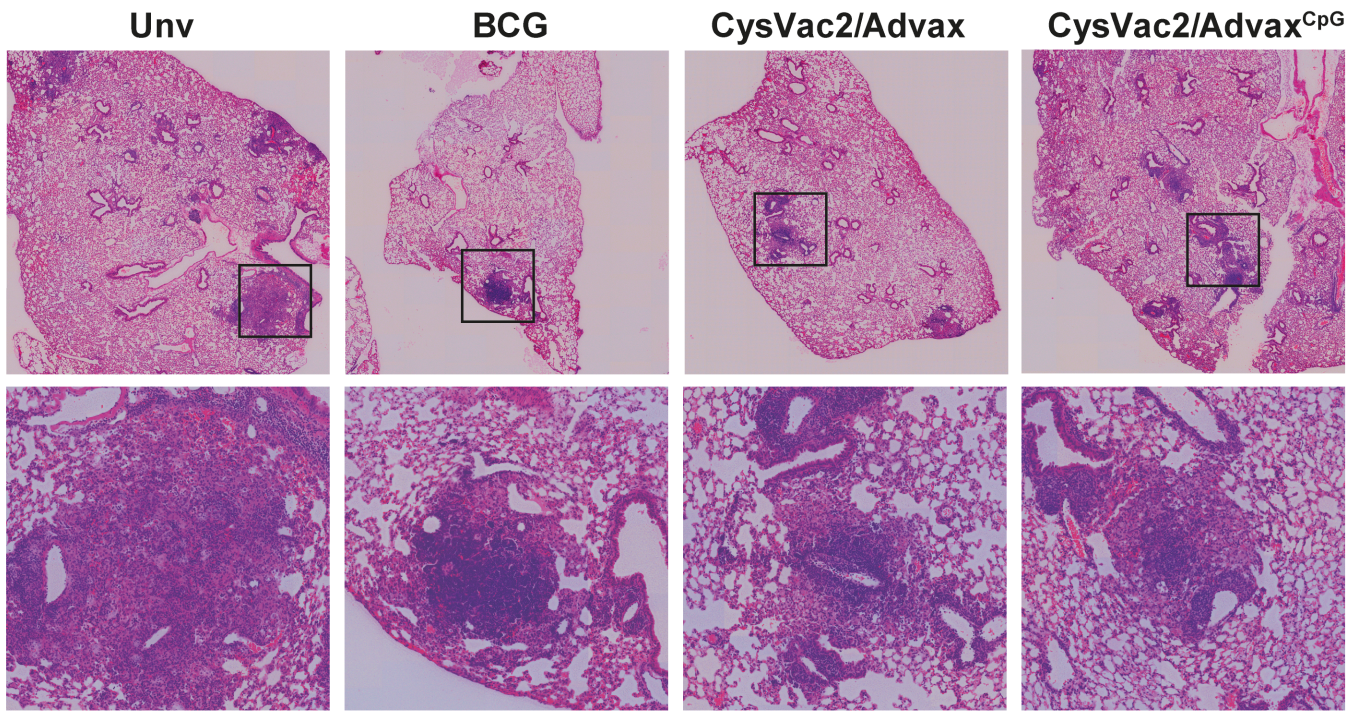


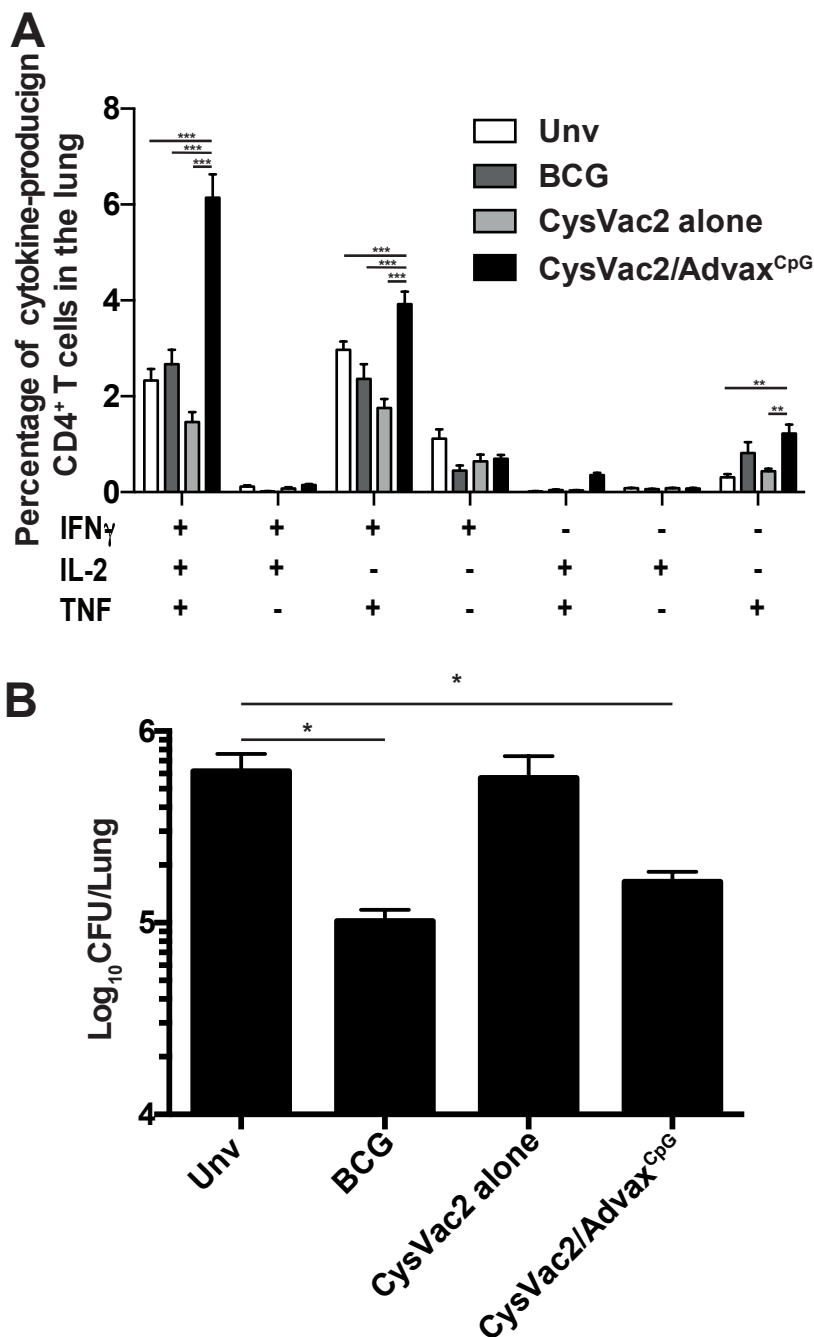
## **SUPPLEMENTARY INFORMATION**

### **Delta inulin-based adjuvants promote the generation of polyfunctional CD4<sup>+</sup> T cell responses and protection against *Mycobacterium tuberculosis* infection**

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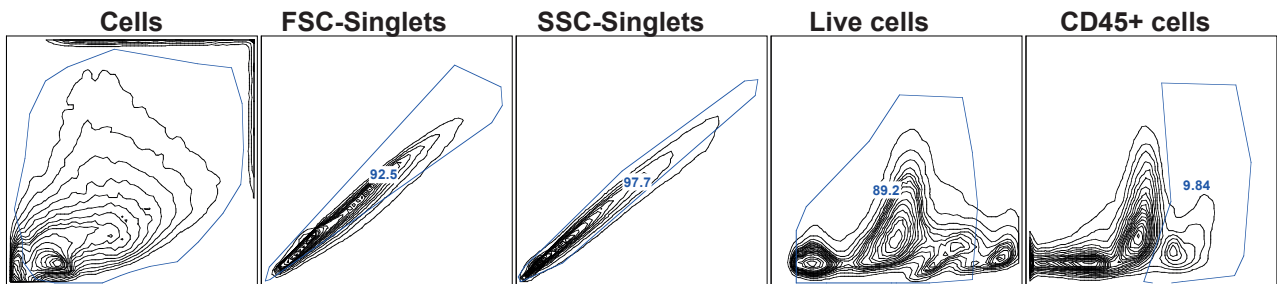


**Supplementary Figure S1: Vaccination with CysVac2/Advax vaccines reduces lung pathology.** Sections from upper right lung lobe of *M. tuberculosis*-challenged mice from figure 2 were stained with hematoxylin and eosin. Images were acquired as mosaics of multiple microscope fields at 20x (upper row) or 40x (lower row) magnification.



**Supplementary Figure S2: intradermal vaccination with CysVac2/Advax<sup>CpG</sup> protects mice against challenge with *M. tuberculosis*.**

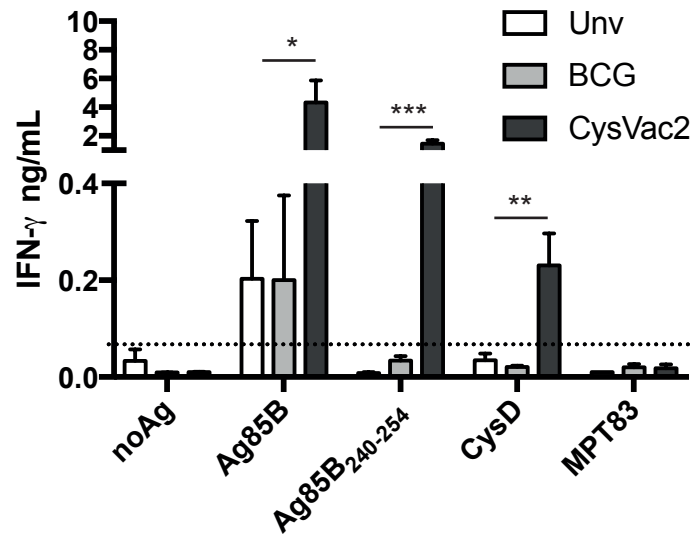
C57BL/6 mice (n=5) were vaccinated with BCG (s.c.  $5 \times 10^5$  CFU) or 3 times i.d. with 3  $\mu$ g CysVac2 formulated in either PBS or Advax<sup>CpG</sup>. Control mice were left unvaccinated (Unv). Twelve weeks after the first vaccination, the mice were challenged with approximately 100 CFU of *M. tuberculosis* by aerosol route. Four weeks later, lung cells were restimulated in vitro with CysVac2 in the presence of brefeldin A and the frequency of CysVac2-specific cytokine secreting CD4<sup>+</sup> T cells in the lung determined by flow cytometry (A). The bacterial load was assessed in the lung (B). Data (average  $\pm$  SEM) is representative of two independent experiments. Statistical significance between the groups was determined by ANOVA (\*  $P < 0.05$ , \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ).



**Supplementary Figure S3: Gating strategy for leukocytes.**

Leukocytes populations were gated by SSC-A and FSC-A plots. Single events were selected using FSC-H/FSC-A and SSC-A/SSC-H. Dead cells were excluded and viable CD45<sup>+</sup> cells were gated for further analysis.





**Supplementary Figure S4: Absence of IFN- $\gamma$  response against irrelevant recombinant mycobacterial proteins.**

C57BL/6 mice (n = 4-5 per group) were vaccinated with BCG (s.c.,  $5 \times 10^5$  CFU) or 3 times s.c. with 3  $\mu$ g CysVac2 formulated in MPL/DDA (25  $\mu$ g/250  $\mu$ g). Six weeks after the last vaccination, the secretion of IFN- $\gamma$  from splenocytes was determined by ELISA in the culture supernatants after 48 hr of *in vitro* stimulation with 10  $\mu$ g/ml of his-tagged purified Ag85B, CysD, or MPT83 (as negative control), or with the Ag85<sub>240-254</sub> synthetic peptide. The dotted lines represents the limit of detection of the ELISA. The significance of differences between the groups was determined by ANOVA (\* P<0.05, \*\* p< 0.01).