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## Supplemental information

# Antibody-Fab and -Fc features promote *Mycobacterium tuberculosis* restriction

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**Supplemental Figures and Tables**

Figures S1-6

Table S1

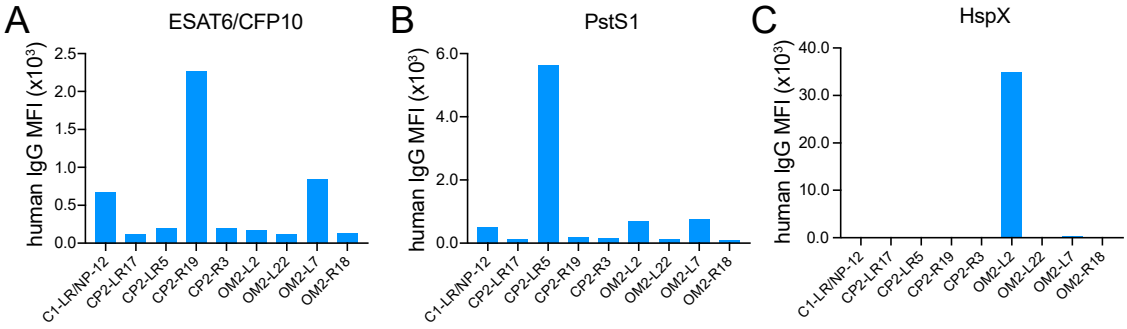


**Figure S1: Protein antigen biding profiles of novel monoclonal antibodies (Figure S1, refers to Figure 1 and Table 1).** The novel antibody clones derived from vaccination against capsular (CP) and outer membrane (OM) fractions of *Mtb* cell surface were screened for binding of *Mtb* protein antigens in a customized Luminex bead assay. Graphed are the antigens which displayed binding signal above isotype control: **(A)** Esat6 and CFP10 proteins, **(B)** Psts1 , and **(C)** HspX.

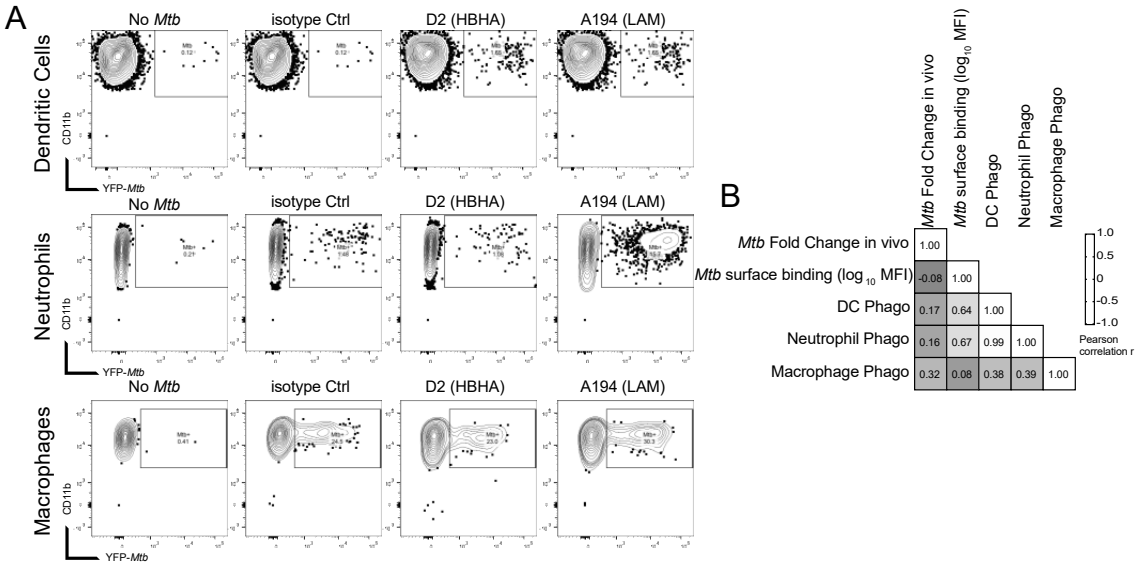


**Figure S2: Antibody mediated phagocytosis of *Mtb* and the relationship with surface binding and in vivo control (Figure S2, refers to Figure 1).** Monoclonal antibodies were combined with yellow fluorescent protein expressing *Mtb* (YFP-*Mtb*) for 1 hour to form immune complexes before combining with bone marrow-derived dendritic cells, neutrophils, and macrophages. (**A**) Gating strategy to identify and quantify the frequency of YFP-Mtb+ cells and the MFI of the YFP signal within infected cells, and phagocytosis (Phago) Scores were determined using these values. Phago Score = ((%*Mtb*$^{+}$cells x *Mtb*$^{+}$Cell mean fluorescence intensity)/100). (**B**) Pearson correlation analysis found moderate to weak linear relationships between of in vivo *Mtb* growth restriction (*Mtb* fold change in vivo), *Mtb* surface binding, DC phago, neutrophil phago, and macrophage phago. Pearson r summarized the correlation between measures.

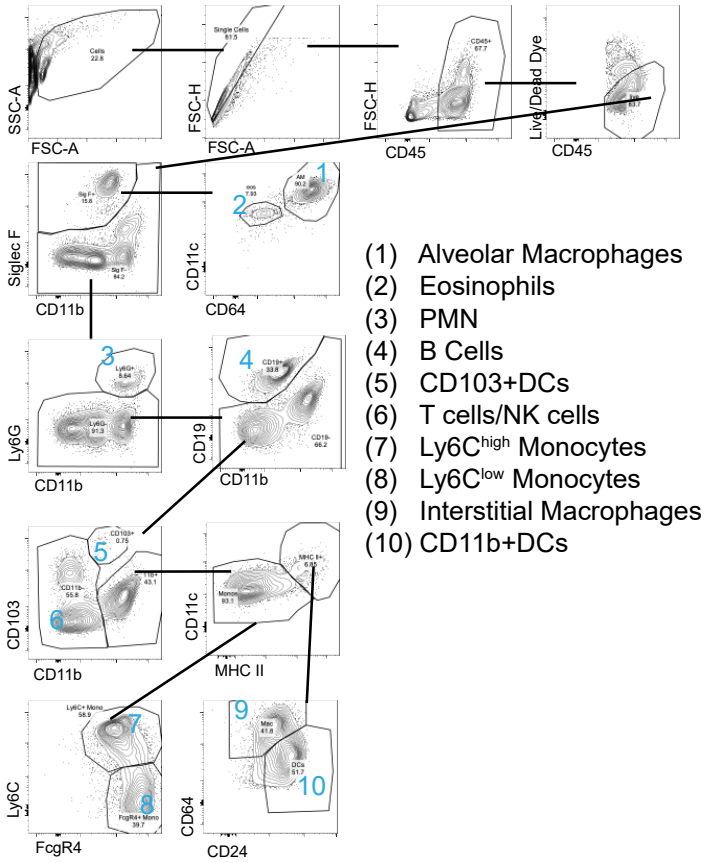


**Figure S3: Gating strategy to identify immune cell subsets in the lungs of Mtb infected mice (Figure S3, refers to Figure 3)**. Representative gates outline the strategy used to identity populations of alveolar macrophages (AM), eosinophils, neutrophils (PMN), B cells, CD103+ DC, T cells/NK cells, Ly6C$^{high}$ Monocytes, Ly6C$^{low}$ Monocytes, recruited macrophages and CD11b+ DC.

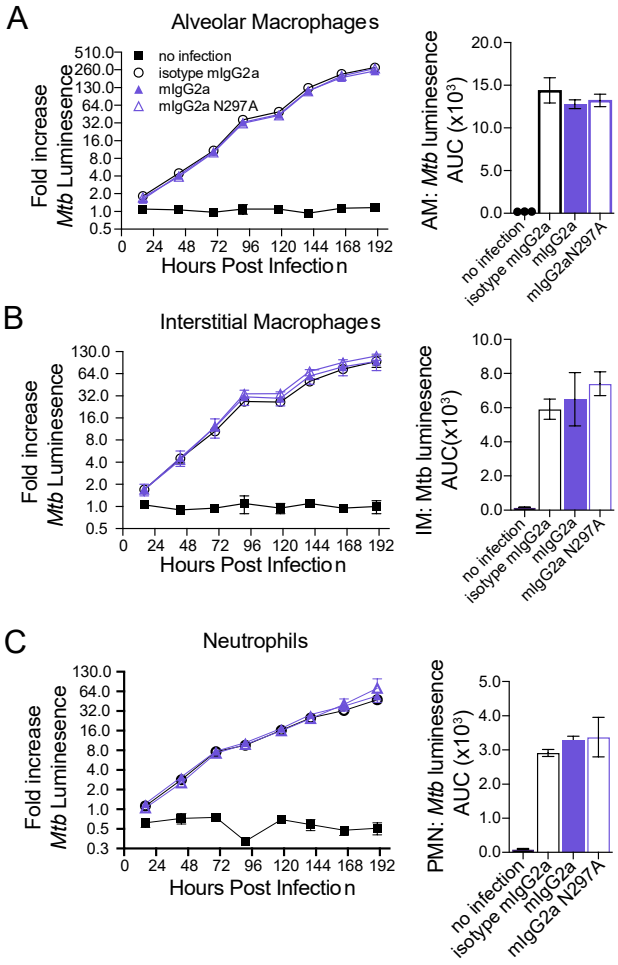


**Figure S4: αLAM Fc-variants do not promote *in vitro* restriction of *Mtb* (Figure S4 is related to Figures 3 and 4).** Growth curve of luminescent *Mtb* (lux-*Mtb*) in **(A)** alveolar macrophages, **(B)** interstitial macrophages, and **(C)** neutrophils sorted from naive mouse lung tissue were infected with *Mtb* pre-opsonized with αLAM-mIgG2a, -mIgG2a N297A variants or isotype control mAbs. x,y plots depict the fold-increase over day 1 luminescence. Bar plots depict area under the curve (AUC) of luminescence measured in the x,y, plot over 8 days. Graphs depict the mean +/- SEM. No significant differences between Fc-variants was identified for AUC in one-way ANOVA with Tukey's multiple correction.

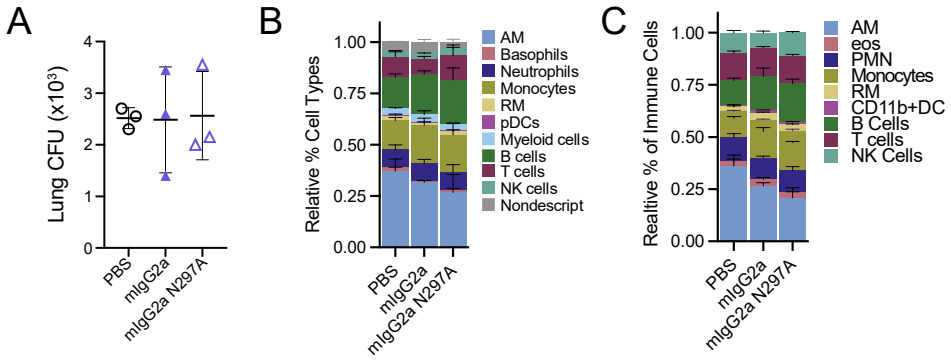


**Figure S5: Bacterial Burden and analysis of immune cells present at Day 5 post infection (Figure S5, refers to Figure 5).** C57BL/6 mice were pre-treated with PBS or 100μg of mAb mIgG2a or mIgG2a N297A αLAM mAb and infected via aerosol with ~60 Day 1 CFU of YFP-*Mtb*. **(A)** Similar levels CFUs present in the lungs treated lungs 5 days after aerosol infection with H37Rv-YFP. **(B)** Relative frequency (%) of cell types identified by scRNAseq analysis. **(C)** Relative % of immune cells identified by flow cytometric analysis (as defined in supplemental figure 4).

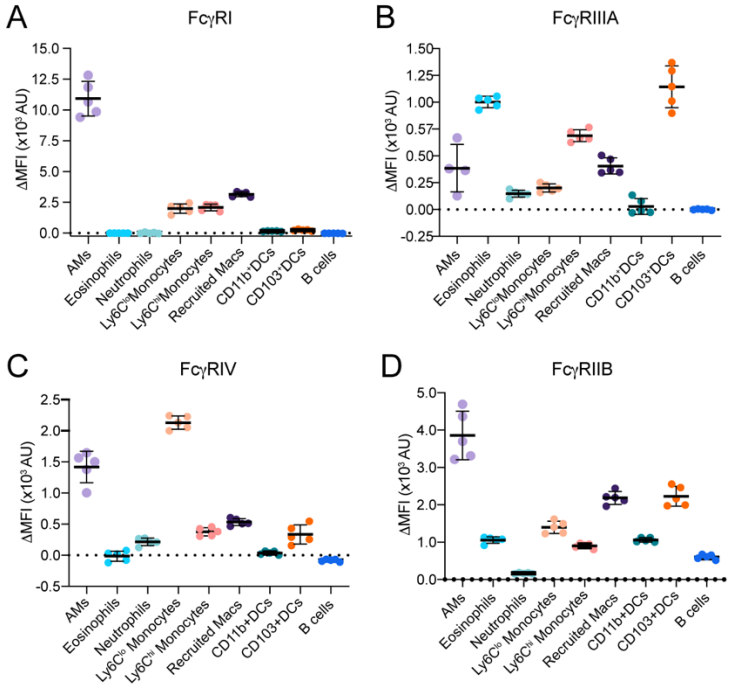


**Figure S6: FcR expression on immune cells recruited to the lung following *Mtb* infection (Figure S6 is related to Figure 3).** Relative expression of activating receptors **(A)** FcγRI, **(B)** FcγRIIIA, **(C)** FcγRIV and inhibitory **(D)** FcγRIIB on innate immune cell populations recruited to infected lungs 2 weeks post infection with ~50 *Mtb* CFU. Immune cells identified by flow cytometric analysis (as defined in supplemental figure 4).

| clone name | Western Blot: OM2 (KDa) | Western Blot: C1(KDa) | Western Blot: SOD1(KDa) | Mtb ELISA Binding* | Detergent: No Detergent ELISA Signal Ratio |
|---|---|---|---|---|---|
| C1-LR/NP-12 | 32.3 | | 46.1 | Non-Specific | 0.78 |
| CP2-LR17 | 24.3, 15.4 | 15.4 | | Specific | 0.52 |
| CP2-LR5 | 41.1 | 41.1 | | Non-Specific | 0.54 |
| CP2-R19 | | | | Non-Specific | |
| CP2-R3 | | | | Specific | 0.28 |
| OM2-L2 | 15.2 | 15.2 | | Specific | 2.84 |
| OM2-L22 | | | | Weakly Specific | 1.80 |
| OM2-L7 | | | | Specific | 0.61 |
| OM2-R18 | | | | Non-Specific | |

**Table S1: Screening of *Mtb* outer membrane and capsule antibody clones from vaccination. (Table S1, refers to Figure 1 and Table 1)** Antibodies were probed for binding to *Mtb* antigens via western blot, microscopy, and whole *Mtb* ELISA. *Western blots* performed on outer membrane preparations (OM2), capsule preparation (C1), and purified protein (SOD1), band size associated with antibody binding are listed in the above corresponding blotted *Mtb* fractions. *Mtb ELISA Binding* is defined as "Specific" for *Mtb*, "Weakly Specific" based on low binding to *Mtb* or some binding to irrelevant bacteria (*E.coli*), "Non-specific" for *Mtb* based on lack of binding or significant binding to irrelevant bacteria (*E.coli*). Finally, ELISA was performed on *Mtb* cells grown in the presence or absence of Tween-80, which removes peripherally-associated cell envelope antigens, and the ratio of signal with and without detergent was determined. Low Detergent: No Detergent binding ratios suggest mAb clones associate with Mtb-envelope associated antigen. (Table S1 refers to Figure 1)