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Supplemental Information

Pulmonary MTBVAC vaccination induces immune signatures previously correlated with prevention of tuberculosis infection

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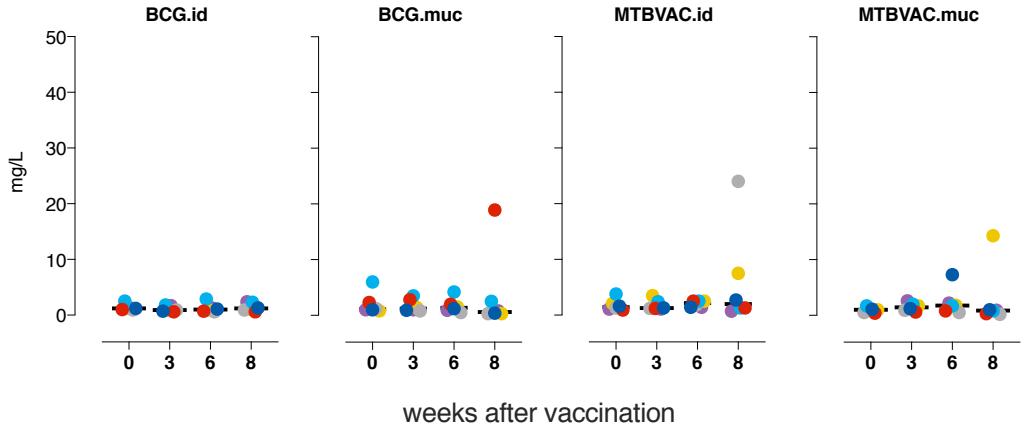
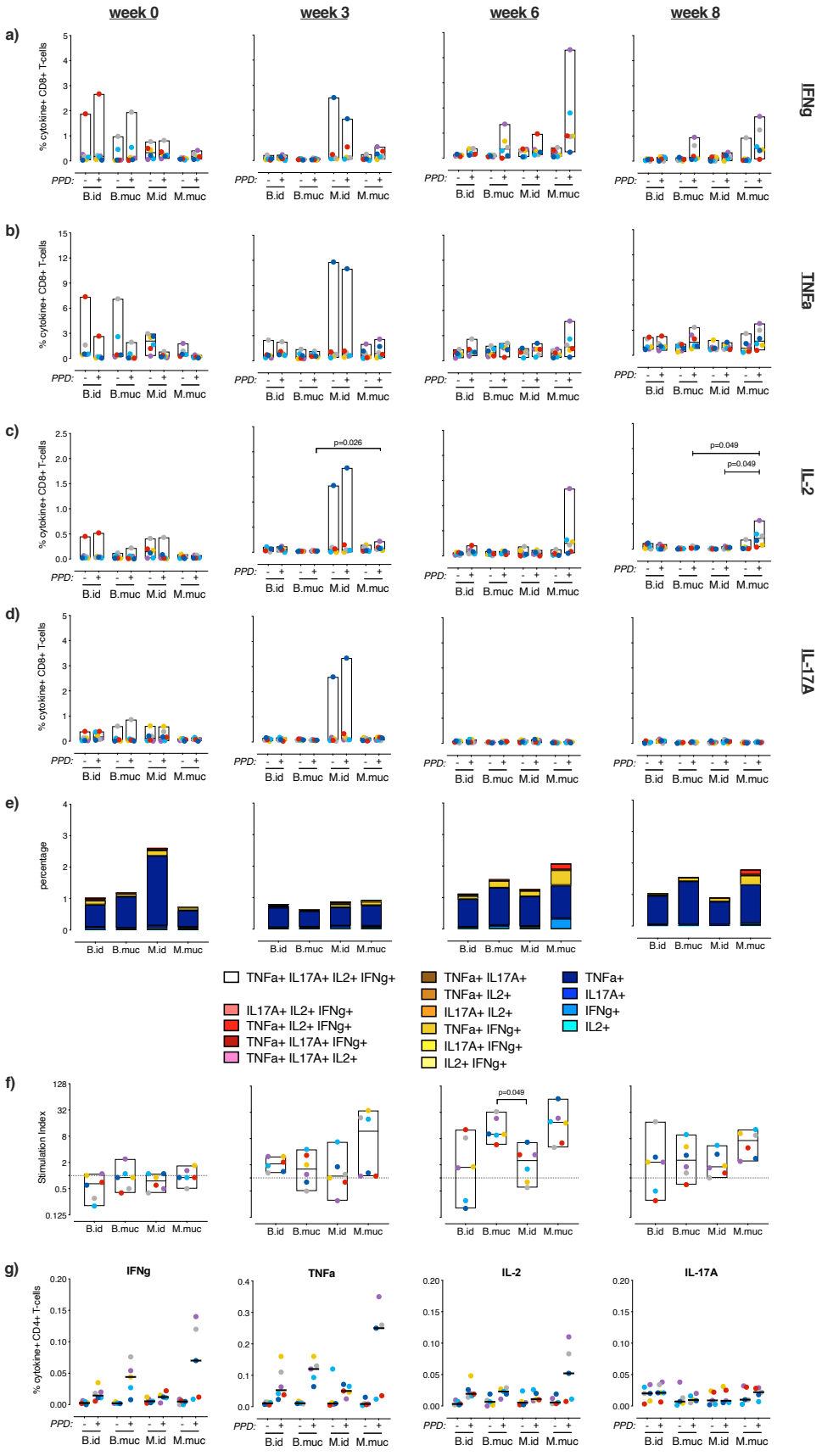
Supplemental Figure 1: Serum C-reactive protein levels post vaccination (*Relating to Figure 1*)



Levels of C-reactive protein did not reveal any adverse systemic inflammatory response after vaccination. Individual CRP values are plotted per treatment group over time, with horizontal bars indicating medians.

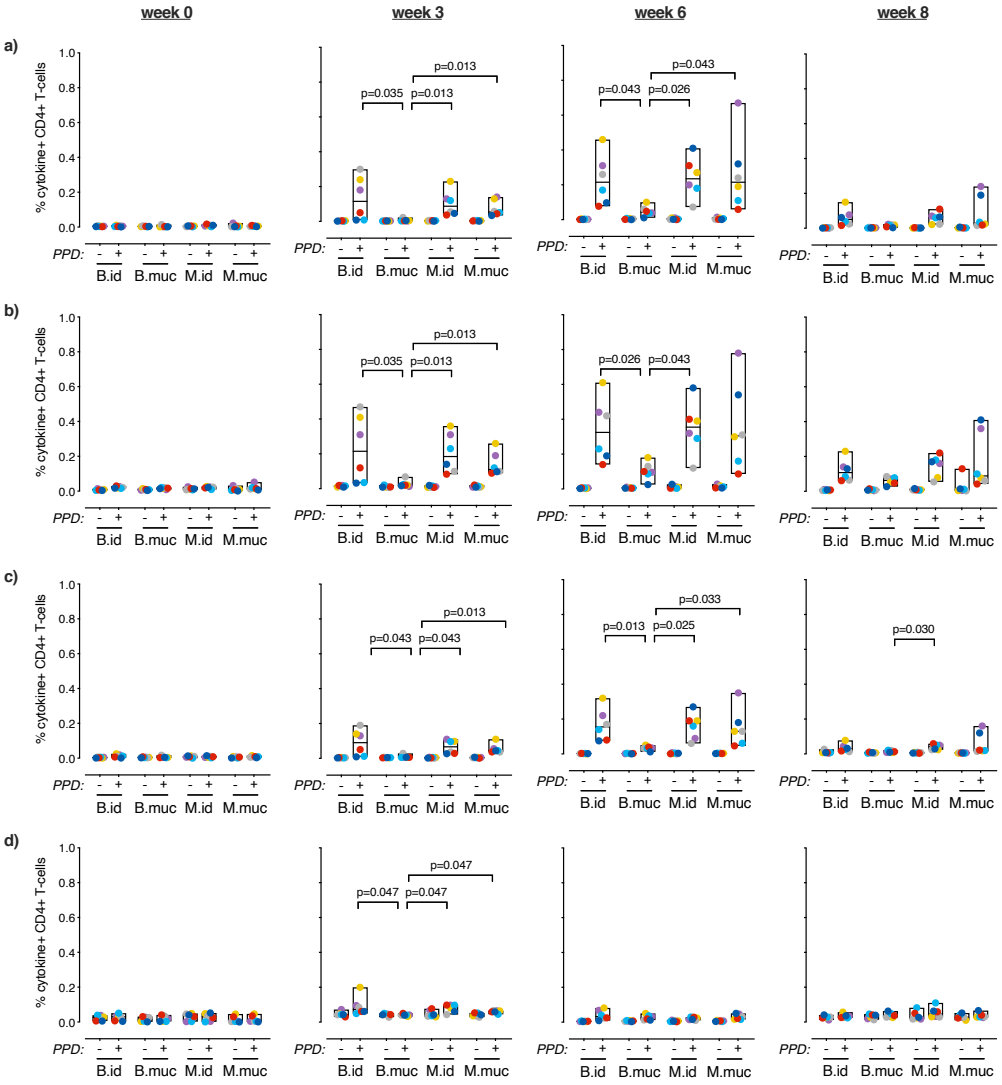
Supplemental Figure 2: BAL CD8+ T-cell responses and proliferation, and bronchoalveolar lymph node CD4+ T-cell responses (*Relating to Figure 2*)



Flow cytometric analysis of **a)** IFNγ, **b)** TNFα, **c)** IL2 and **d)** IL17A responses in CD8+ T-cells before and at various time points after vaccination, either or not upon *vitro recall* stimulation with PPD. **e)** Stacked bar graphs depicting frequencies of single and multiple cytokine producing subsets of CD8+ T-cells after PPD stimulation by median values. **f)** PPD-specific proliferation of BAL cells, depicted as the stimulation index: the ratio of antigen- over medium control-stimulated values. **g)** Production of IFNγ, TNFα, IL2 and IL17A by CD4+ T-cells from lung draining lymph nodes either or not in response to PPD, 8 weeks after vaccination.

In **a-d** and **g** "+" indicates PPD stimulated samples; "-" indicates unstimulated, culture medium-incubated samples as controls. For all graphs n=6 animals per group. Horizontal lines in bars indicate group medians. Significance of group differences was determined by two-sided Mann-Whitney test adjusted for multiple comparisons. Holms adjusted p-values ≤ 0.05 are depicted. Colour coding per individual is consistent throughout the paper including supplementals.

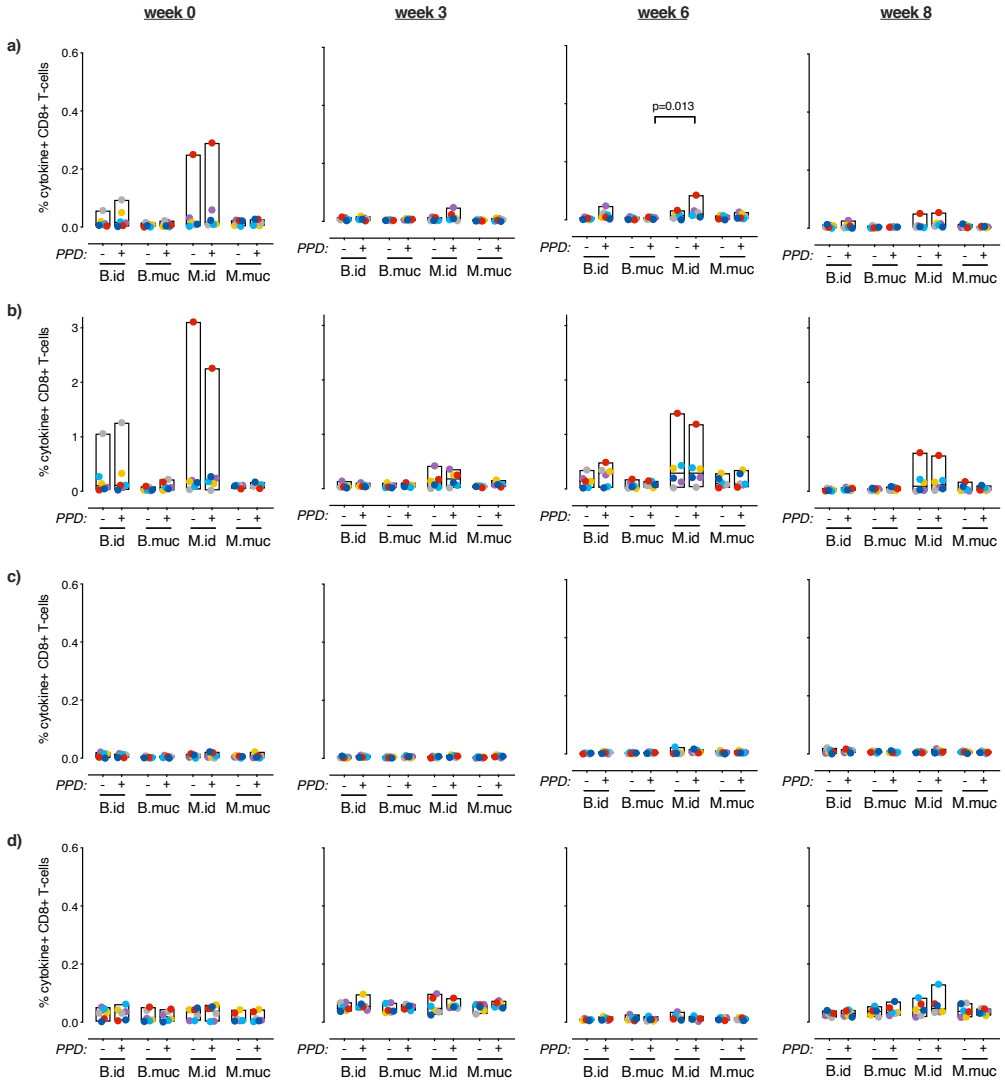
Supplemental Figure 3: Cytokine production by peripheral CD4+ T-cells (*Relating to Figure 3*)



Production of **a)** IFNγ, **b)** TNFα, **c)** IL2 and **d)** IL17A by peripheral CD4+ T-cells before and at various time points after vaccination, either or not upon *in vitro* recall stimulation with PPD.

PPD stimulated samples are indicated by "+", "-" indicates unstimulated, culture medium control samples. For all graphs n=6 animals per group. Horizontal lines indicate group medians. Significance of group differences was determined by two-sided Mann-Whitney test adjusted for multiple comparisons. Holms adjusted p-values ≤ 0.05 are depicted. Colour coding per individual is consistent throughout.

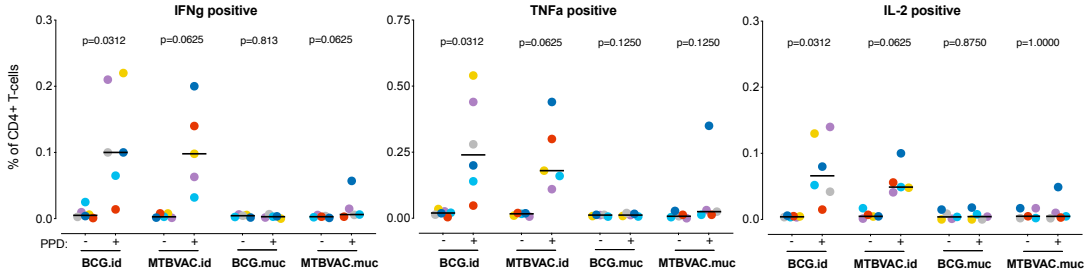
Supplemental Figure 4: Cytokine production by peripheral CD8+ T-cells (*Relating to Figure 3*)



Production of **a)** IFNγ, **b)** TNFα, **c)** IL2 and **d)** IL17A by peripheral CD8+ T-cells before and at various time points after vaccination, either or not upon *in vitro* recall stimulation with PPD.

PPD stimulated samples are indicated by "+", "-" indicates unstimulated, culture medium control samples. For all graphs n=6 animals per group. Horizontal lines indicate group medians. Significance of group differences was determined by two-sided Mann-Whitney test adjusted for multiple comparisons. Holms adjusted p-values ≤ 0.05 are depicted. Colour coding per individual is consistent throughout.

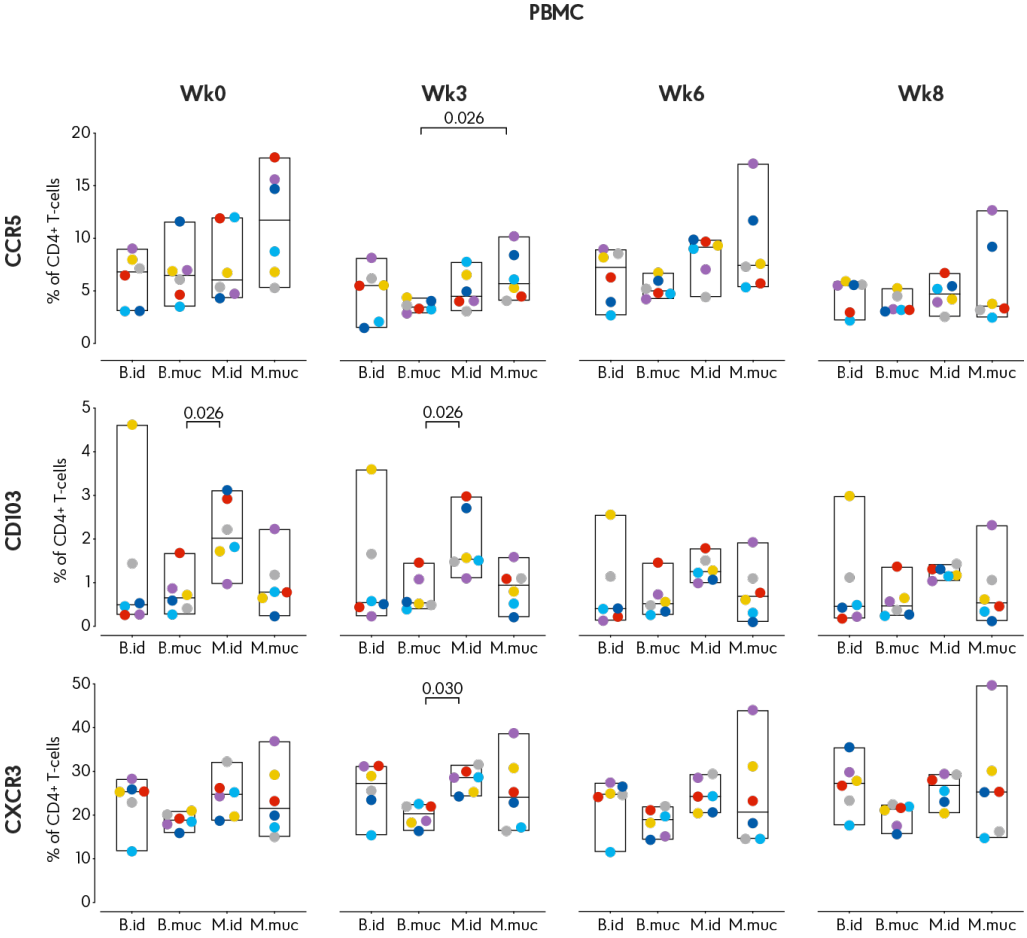
Supplemental Figure 5: Cytokine production by CD4+ T-cells in TST-DTH draining lymph nodes (*Relating to Figure 3*)



Production of IFNγ, TNFα, and IL2 by CD4+ T-cells in axillar lymph nodes draining the injection site of the diagnostic Tuberculin Skin Test (TST), which was applied at week 8 after vaccination to investigate if mucosal vaccine routing would result in a distinctive T-cell response after *in vivo* recall stimulation.

For all graphs n=5 animals per group, except for BCG.id (n=6). Horizontal lines indicate group medians. Significance of antigen-specific responses was determined by Wilcoxon matched pairs-signed rank testing. Colour coding per individual is consistent throughout.

Supplemental Figure 6: Homing marker expression by peripheral T-cells (*Relating to Figure 4*)



Frequency of CCR5+, CD103+ and CXCR3+ CD4+ T-cells in unstimulated peripheral blood mononuclear cells before and at various time points after vaccination.

For all graphs n=6 animals per group. Horizontal lines indicate group medians. Significance of group differences was determined by two-sided Mann-Whitney test adjusted for multiple comparisons. Holms adjusted p-values ≤ 0.05 are depicted. Colour coding per individual is consistent throughout.