

**Supplementary Figure 1** 



**Supplementary Figure 2** 









**Supplementary Figure 5** 







**Supplementary Figure 8** 

## Supplementary Table 1: ICS Antibody Panels

## Table 1A: Whole blood ICS panel

## Table 1B: PBMC ICS panel

SI.No.	Antibody	Clone	Channel	Company	Cat. No.
1	CD3 BV570*	UCHT1	V-586	BioLegend	300436
2	CD4 BUV395*	SK3	UV-379	BD	563550
3	CD8 BV711*	RPA-T8	V-710	BD	563677
4	IFNγ V450*	B27	V-450	BD	560371
5	TNFα FITC*	MAb11	B-530	BioLegend	502906
6	IL-2 APC*	MQ1-17H12	R-670	BD	561054
7	IL-17A BV605*	BL 168	V-610	BioLegend	512326
8	MIP-1β PE*	D21-1351	YG-582	BD	561120
9	CD45RA APC-H7	HI100	R-780	BD	560674
10	CD56 BV650	HCD56	V-660	BioLegend	318344
11	CD27 BV785	0323	V-780	BioLegend	302832
12	γδTCR PE-CF594	B1	YG-610	BD	562511
13	CD14 BV510	M5E2	V525	BioLegend	301842

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3	CD8 BV711*	RPA-T8	V-710	BD	563677
4	IFNγ V450*	B27	V-450	BD	560371
5	TNFα FITC*	MAb11	B-530	BioLegend	502906
6	IL-2 Alexa 700*	MQ1-17H1 2	R-710	BioLegend	500320
7	IL-17A BV605*	BL 168	V-610	BioLegend	512326
8	MIP-1β PE*	D21-1351	YG-582	BD	561120
9	CD45RA APC-H7	HI100	R-780	BD	560674
10	IL-17F BV650*	033-782	V-660	BD	564264
11	IL-10 BV786*	JES3-9D7	V-780	BD	564049
12	IL-22 PE-Cy7*	22URTI	YG-780	Thermo Fisher	25-7229-4 2
13	CCR7 Alexa Fluor 647	G043H7	R-670	BioLegend	353218
14	Avid	NA	V-525	Thermo Fisher	L34966

\*Intracellular

## SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: Flow cytometry gating strategy for T-cell analysis in whole blood. Schematic representation of flow cytometry plots showing sequential gating strategy of whole blood cells for analysis of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells. All gates for non-functional markers were defined using fluorescence minus one (FMO) controls; gates for functional markers were defined using the unstimulated samples. Initial gating was done on FSC-H and FSC-A to discriminate singlets, followed by the exclusion of CD14<sup>+</sup> monocytes. Lymphocytes were gated using FSC-A and SSC-A. Within the lymphocyte gate, CD3<sup>+</sup> cells were identified, followed by CD4<sup>+</sup> and CD8<sup>+</sup> T-cells. CD27 and CD45RA expression within CD4<sup>+</sup> and CD8<sup>+</sup> T-cell subsets was used to define memory phenotypes (naïve, CM, EM and TD). To define functional markers for CD4<sup>+</sup> and CD8<sup>+</sup> T-cells, a gate was applied for each cytokine, not taking into account the co-expression of other markers. Boolean gates were then created based on these gates to identify cells expressing different combinations of markers.

**Supplemental Figure 2: TB10.4-specific CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses in whole blood before and after BCG revaccination.** Line graphs show changes in TB10.4 specific IFN-γ and/or IL-2 frequencies of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells over time in BCG vaccinated IGRA<sup>+</sup> (Group 1, N=19) and IGRA<sup>-</sup> (Group 2, N=18) subjects versus unvaccinated control IGRA<sup>+</sup> (Group 3, N=18) and IGRA<sup>-</sup> (Group 4, N=18) subjects.

**Supplemental Figure 3:** Flow cytometry gating strategy for T-cell analysis in PBMC. Schematic representation of flow cytometry plots showing sequential gating strategy of PBMCs for analysis of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells. All gates for non-functional markers were defined using fluorescence minus one (FMO) controls; gates for functional markers were defined using the unstimulated samples. Initial gating was done on FSC-H and FSC-A to discriminate singlets, followed by the exclusion of dead cells by AVID stain. Lymphocytes were gated using FSC-A and SSC-A. Within the lymphocyte gate, CD3<sup>+</sup> cells were identified, followed by CD4<sup>+</sup> and CD8<sup>+</sup> T-cells. To define functional markers for CD4<sup>+</sup> T-cells, a gate was applied for each cytokine, not taking into account the co-expression of other markers. Boolean gates were then created based on these gates to identify cells expressing different combinations of markers.

**Supplemental Figure 4: CD4<sup>+</sup> T-cell cytokine responses in PBMC to BCG stimulation.** Representative flow cytometry plots (from same donors as shown in Figure 9) show total IFN- $\gamma$ , IL-2, IL-17A, IL-17F, IL-22 and IL-10 cytokine positive CD4<sup>+</sup> T-cells in unstimulated control and after *in vitro* stimulation with BCG at T5 in Group 1 versus Group 3 subjects.

**Supplemental Figure 5: Th17 subsets in PBMC to Mtb antigens and BCG stimulation.** Representative flow cytometry plots (from same donors as shown in Figure 9) show CD4<sup>+</sup>Th17 subsets expressing either IFN-γ or IL-10 with IL-17F with or without *in vitro* stimulation of PBMC with Ag85A, BCG, LTAg at T5 in a Group 1 subject. The quadrant gates for the cytokines were positioned closer to the negative cells for these analyses to examine the Th17 double-positive cells. Since those cells are rare and the MFI of the cytokines on those cells is low, the lower position of the quadrant was necessary to include those cells.

**Supplemental Figure 6: Th17 subsets in PBMC to BCG stimulation.** Representative flow cytometry plots show CD4<sup>+</sup>Th17 subsets expressing either IFN- $\gamma$  or IL-10 with IL-17F with or without *in vitro* stimulation of PBMC with BCG at T5 in two additional Group 1 subjects.

**Supplemental Figure 7: Gating Strategy for Innate Cells.** A representative sequential gating strategy for NKT, CD56<sup>br</sup> NK, CD56<sup>dim</sup> NK and  $\gamma\delta$  T-cells in whole blood is shown. Frequencies of IFN-y expressing cells were studied in all populations.

**Supplemental Figure 8: BCG revaccination did not impact Hep B antibody titer in study participants.** Hep B antibody titer was measured in plasma of study participants at T0, T1, T2, T4 and T5. Antibody titer was measured by standard ELISA and concentration was expressed as IU/ml. N for Group 1 = 16, Group 2=10, Group 3=16 and Group 4=10. P values for longitudinal samples were calculated by comparing each time point to baseline using the Freidman test and corrected for multiple comparisons using Dunn's test. **Supplemental Table 1:** Panel of antibodies used for cell surface and intracellular markers for (A) whole blood and (B) PBMC ICS assays.

**Supplemental File 1:** Clinical details of study participants who were included in final data analysis.