## Supplementary Table SI

***Ex vivo* Panel**

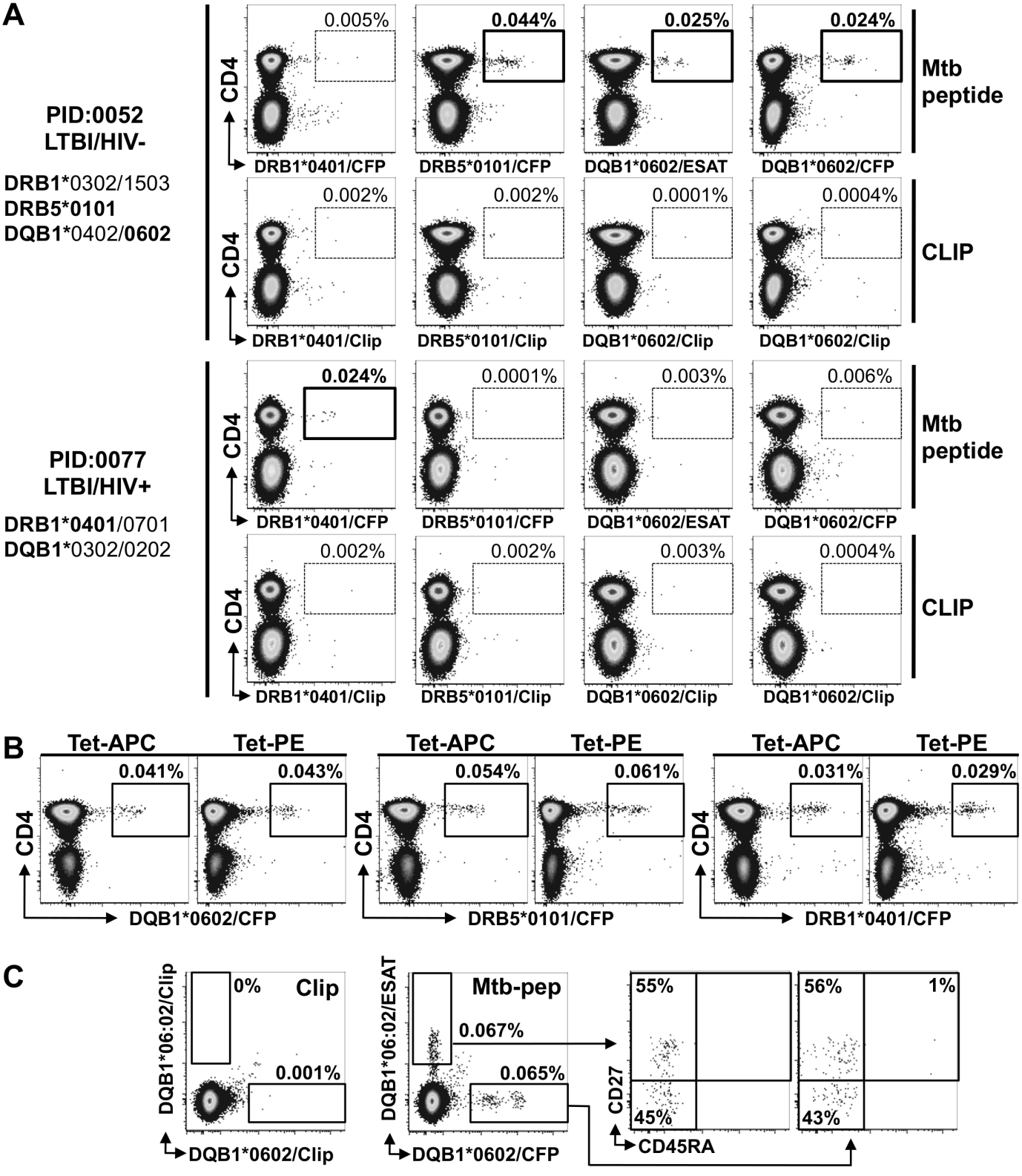
| Markers | Fluorophore | Clone | Manufacturer | Function | Staining |
|---|---|---|---|---|---|
| Viablility marker | Near Infra Red | | Invitrogen | Exclusion | Surface |
| CD14 | APC-Alexa750 | Tuk4 | Invitrogen | Exclusion | Surface |
| CD19 | APC-Alexa750 | SJ25-CI | Invitrogen | Exclusion | Surface |
| CD3 | BV650 | OKT3 | BD Biosciences | Lineage | Surface |
| CD4 | Alexa 488 | OKT4 | BD Biosciences | Lineage | Surface |
| CD45RA | PE-Tex Red | 2H4 | Beckman Coulter | Memory | Surface |
| CD27 | Alexa 700 | O323 | eBioscience | Memory | Surface |
| CCR4 | BV510 | L291H4 | Biolegend | Homing | Surface |
| CCR6 | BV605 | G034E3 | Biolegend | Homing | Surface |
| CXCR3 | PE-cy7 | 1C6/CXCR3 | BD Biosciences | Homing | Surface |
| KLRG1 | PerCP-eFluor 710 | 13F12F2 | eBioscience | Activation | Surface |
| HLA-DR | eFluor 450 | L243 | eBioscience | Activation | Surface |
| PD-1 | BV711 | EH12.2H7 | Biolegend | Activation | Surface |
| MHC class II tetramer #1 | PE | | NIH | Mtb-specific CD4 | Surface |
| MHC class II tetramer #2 | APC | | NIH | Mtb-specific CD4 | Surface |

**Functional Panel**

| Markers | Fluorophore | Clone | Manufacturer | Function | Staining |
|---|---|---|---|---|---|
| Viablility marker | Near Infra Red | | Invitrogen | Exclusion | Surface |
| CD14 | APC-Alexa750 | Tuk4 | Invitrogen | Exclusion | Surface |
| CD19 | APC-Alexa750 | SJ25-CI | Invitrogen | Exclusion | Surface |
| CD3 | BV650 | OKT3 | BD Biosciences | Lineage | ICS |
| CD4 | Alexa 488 | OKT4 | BD Biosciences | Lineage | Surface |
| CCR4 | BV510 | L291H4 | Biolegend | Homing | Surface |
| CCR6 | BV605 | G034E3 | Biolegend | Homing | Surface |
| CXCR3 | PE-cy7 | 1C6/CXCR3 | BD Biosciences | Homing | Surface |
| KLRG1 | PerCP-eFluor 710 | 13F12F2 | eBioscience | Activation | Surface |
| HLA-DR | PE | L243 | BD Biosciences | Activation | Surface |
| PD-1 | BV711 | EH12.2H7 | Biolegend | Activation | Surface |
| IFN-γ | Alexa 700 | B27 | BD Biosciences | Cytokine | ICS |
| TNF-α | eFluor 450 | MAb11 | eBioscience | Cytokine | ICS |
| IL-2 | PE/Dazzle 594 | MQ1 | Biolegend | Cytokine | ICS |
| MHC class II tetramer | APC | | NIH | Mtb-specific CD4 | Surface |

**Supplementary Table SI:** Detailed list of antibodies used in the flow cytometry panels.

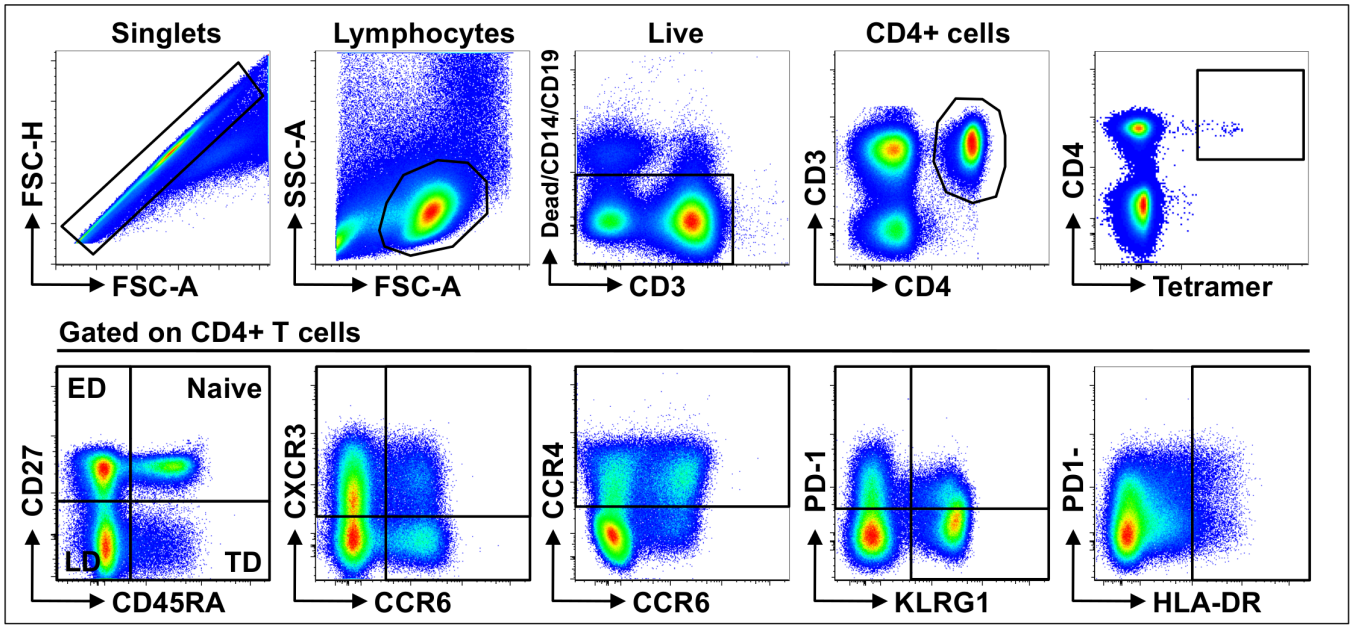
**Supplemental Figure S1**



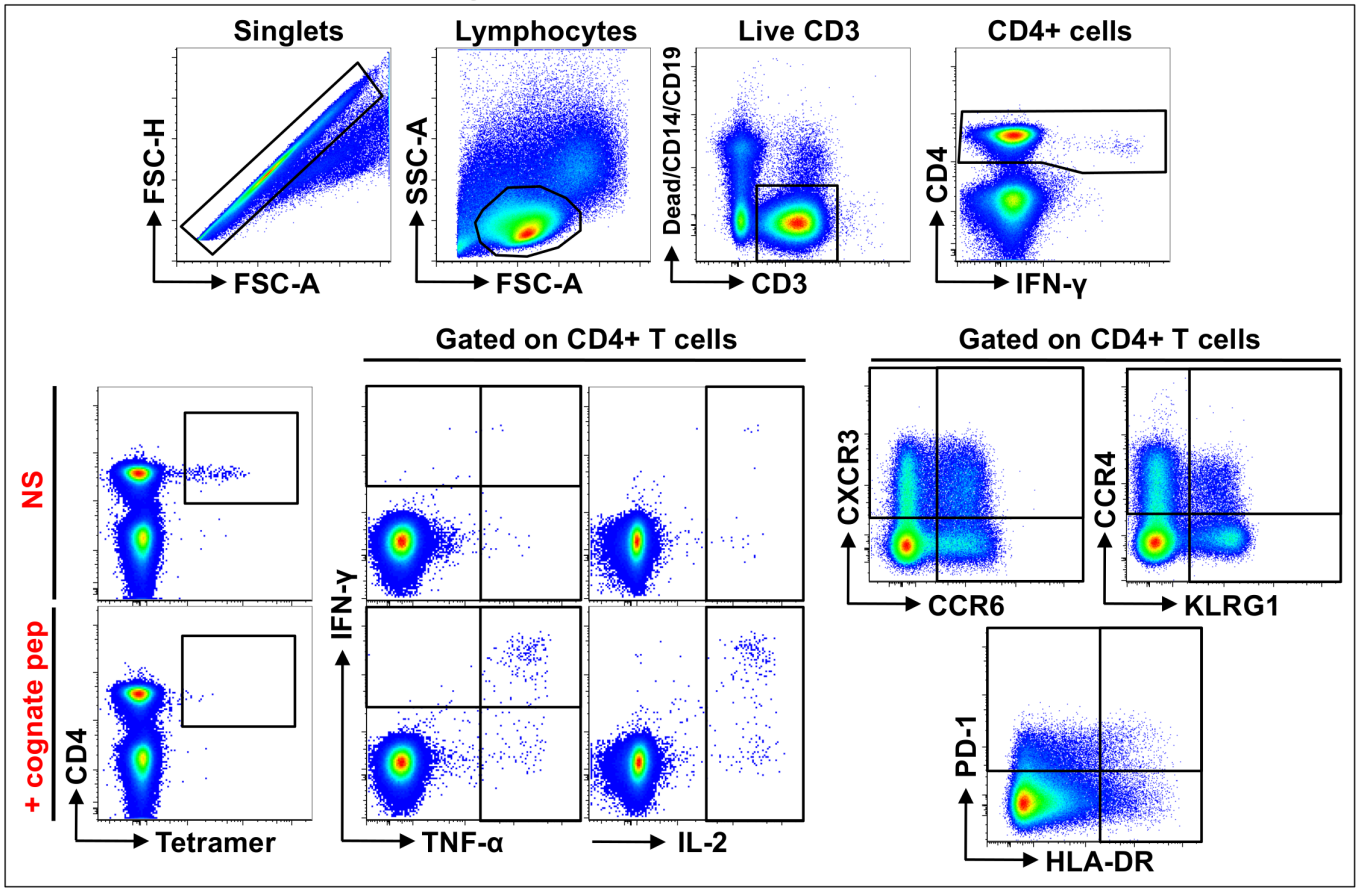

**Supplemental Figure S1: Validation of Mtb-specific MHC class II tetramer staining. A-** Example of the specificity of MHC class II tetramer labeling in two individuals. Tetramers loaded with the class II-associated invariant peptide (Clip, PVSKMRMATPLLMQA) were used as negative controls. **B-** Comparison of the performance of Mtb-specific MHC class II tetramers labeled with PE or APC. **C-** Example of dual MHC class II tetramer staining.

## Supplemental Figure S2

### A- *Ex vivo* phenotype




### B- Post-stimulation phenotype



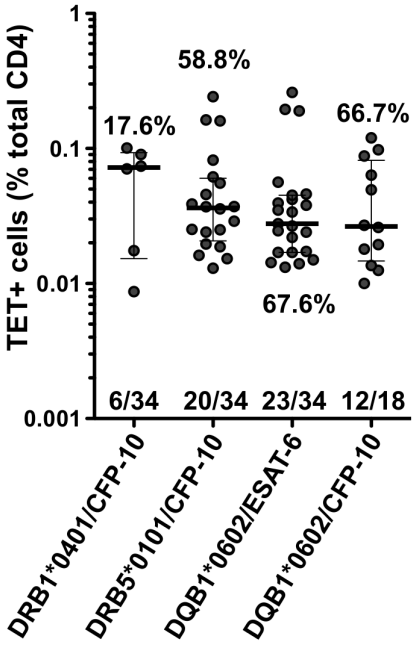

**Supplemental Figure S2: Gating strategies. A-** Gating strategy used to define the phenotype of *ex vivo* Mtb-specific MHC class II CD4+ T cells. **B-** Gating strategy used to define the functional profile and phenotype of Mtb-specific CD4+ T cells in response to cognate Mtb peptides.

# Supplemental Figure S3

**A**

| Groups | No. of screened individuals | No. TET responders | % responders | No. TET+ responses |
|---|---|---|---|---|
| LTBI/HIV- | 28 | 13 | 46.4 | 19 |
| LTBI/HIV+ | 30 | 11 | 36.7 | 19 |
| aTB/HIV- | 14 | 6 | 42.8 | 11 |
| aTB/HIV+ | 14 | 5 | 35.7 | 10 |
| **Total** | **86** | **34** | **40.0** | **59** |

**B**




**Supplemental Figure S3: Identification and frequencies of Mtb-specific MHC class II tetramer (TET) responders and responses. A-** Summary table of the number of individuals screened, number of Mtb-specific MHC class II tetramer responders and number of tetramer responses identified in each clinical group. **B-** Comparison of the magnitude of each tested Mtb-specific MHC class II tetramer (DRB1*0401/ CFP-10, DRB5*0101/CFP-10 and DQB1*0602/ESAT-6 or CFP-10). The number on the *x*-axis represents the no. of TET+ responses / no. TET+ responders identified.